



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

MEMORANDUM

DEC 28 1983

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Isofenphos, EPA Reg.#3125-326. Acute Delayed Neurotoxicity of Technical Isofenphos In Hens; Mobay's Report#80678 by E.J. Hixson, April 19, 1982; submitted on 12/5/83. Accession#251884 CASWELL#447AB

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

FROM: Amal Mahfouz, Toxicologist  
Section V, Toxicology Branch  
Hazard Evaluation Division (TS-769)

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

*Amal Mahfouz*  
*12/28/83*  
*Jeffery DC*

*WLB*

Synonyms: Oftanol, Amaze, Isofenphos, and SRA 12869.

Action Requested:

On 12/5/83, pursuant to Section 6(a)(2) of FIFRA as amended, Mobay Chemical Company submitted for review the following neurotoxicity studies in hens (Report # 80678, 4/19/83):

- A. An acute delayed neurotoxicity study in hens, performed by Mobay and the University of Kansas in Manhattan, Department of Pathology, study #255, 11/6/81.
- B. A neurotoxic esterase (NTE) activity study in hens, performed at Bayer AG Institut fur Toxikologie Wupertal-Elberfeld, study #3007376, 11/3/82.

Additional supportive literature authored by M.K. Johnson were also included in this submission.

NOTE: Previous neurotoxicity studies (Mobay's Report #34025, 3/30/72 by Bayer AG - Institut Fur Toxikologie, and Report #53881, 7/19/72 by Huntington Research Center; Accession #096657) were submitted by the registrant with PP#8G2025 and reviewed by Bill Greear in 3/16/78; these studies did not reflect a delayed neurotoxic effect with Isofenphos use.

### Recommendation and Discussions:

A. No delayed neurotoxicity was reflected in these studies:

1. The new acute neurotoxicity study in hens (report #80678; study #255, 1981) did not reflect clinical symptoms or histopathological lesions indicative of OP delayed neurotoxicity.

In this study, 30 adult Leghorn hens were orally administered a single dose of technical Isofenphos, 32 mg/kg b.w. (36 mg/kg b.w. as calculated by this reviewer), and protected with 50 mg/kg atropine.

However, this study is classified as Core Supplementary because the surviving birds (13/30 hens) were not challenged with a second dose of Isofenphos upon 21 days of survival.

2. The neurotoxic esterase (NTE) activity study in hens (report #80678; study #300376, 1982) did not reflect a level of NTE inhibition indicative of delayed neurotoxicity, with the exception of hen #25 where 76.3% NTE inhibition was observed in the sciatic nerve after one day of the initial exposure and hen #45 where 84.4% inhibition was observed in the sciatic nerve after three days of the initial exposure.

In this study, 49 birds were orally treated with a single dose of technical Isofenphos, 32 mg/kg b.w., and protected with 50 mg/kg b.w. atropine. The activity of NTE was determined in 4 different hens at each of the following intervals 1, 2, 3, 7, 10, 14 days after the initial treatment.

However this study is classified as Core Invalid due to the following issues:

a. NTE activities for only 24/49 birds were determined; however, the method used to select the birds for these determinations was not identified.

b. Five birds died during the first 24 hours and six additional birds died within the next 3 days. However, 14/49 birds remained unaccounted for in this study.

c. Individual data for control and treated birds were not provided i.e., both the clinical symptoms and the actual level of NTE activity per gram brain tissues per hour for each bird were not reported.

This study may be upgraded to supplementary upon receipt and review of the registrant's response to the above issues discussed in section a, b and c.

B. We have reason to believe that Isofenphos causes delayed neurotoxicity in hens based on the preliminary data discussed by Barry W. Wilson of the University of California in his November 21, 1983 memo to Rex Magee of the California Department of Food and Agriculture (CDFA), see our memo of 12/1/83. Consequently, a NOEL for this effect should be determined.

In addition, the Isofenphos neurotoxicity studies sponsored by Mobay in 1972 (Reports #34025/53881) did not reflect any delayed neurotoxic symptoms at oral dosages of 74 or 100 mg/kg b.w. for atropinized hens, while Wilson's studies in California (1983) reflected a mild delayed neurotoxic effect at an oral dosage of 75 mg/kg b.w. and a stronger response at a subcutaneous dosage of 100 mg/kg b.w. These differences between Mobay's data and Wilson's finding need to be addressed by the registrant.

Also, the oral LD<sub>50</sub> for unprotected hens is 20.9 (17.2-24.9) mg/kg as determined by Mobay (Report #34025, 3/30/72), while the oral LD<sub>50</sub> for hens as determined by B. Wilson is 5 mg/kg (see Wilson's memo of 11/21/83). This 4x difference in the oral LD<sub>50</sub> needs to be explained by the registrant.

C. We also need a clarification from Mobay relative to the following issues:

1. The previously submitted neurotoxicity studies (see Mobay's reports #34025 & 53881, dated 1972) reflected an oral LD<sub>50</sub> of 74(60-91) mg/kg for atropinized hens. The newly submitted neurotoxicity study (Mobay's report #80678, dated 4/19/82; study #233) reflected a much higher acute toxicity in atropinized hens than the value reported above in the 1972 data i.e., in the new study, 17/30 atropinized hens died within 24 hours when treated with 32 mg/kg technical isofenphos. The registrant should provide an explanation for these controversial findings.

2. In the recently submitted report # 80678 dated 4/19/83, the number of hens that died in study #255, were 17/30 hens, while the number of hens that died in study #3007376, were 11/49 hens. The first study reflected data indicative of an oral LD<sub>50</sub> lower than 32 mg/kg; the second study reflected an oral LD<sub>25</sub> higher than 32 mg/kg. Although these 2 studies were performed in 2 different laboratories, this reviewer believes that the large difference in the acute oral toxicity in these newly submitted studies should be explained by the registrant.

Thus, due to the above discussed findings (see above Section A, B, and C) the Agency requests from the registrant to submit the following studies/data:

- °All individual data for the newly submitted NTE activity study.
- °A new Acute Delayed Neurotoxicity Study in Hens (using an appropriate dosage of Isofenphos).
- °A Subchronic 90-Day Neurotoxicity Study in Hens.
- °Any additional information available to, or in possession of the registrant relative to Oftanol neurotoxicity.

#### REVIEW

A. Study Type: Acute Delayed Neurotoxicity Study in Hens

Accession No.: 251884

Sponsor: Mobay Chemical Corporation, Ag. Chem. Div.,  
Box 4913, Hawthorne Road, Kansas City,  
Missouri 64120

Report No.: 80678, study # 255

Test Facilities: Department of Pathology, College of  
Veterinary Medicine, Kansas State  
University, Manhattan, Kansas 66506  
and  
Mobay Chemical Corporation, Environ-  
mental Health Research Inst. Corporate,  
Toxicology Dept., 17745 South Metcalf,  
Stillwell, Kansas 66085

Study Personnel: D.W. Lamb (Lab. Management), E.J. Hixson  
(Study Director), H.E. Hoss (Pathology),  
and R.S. Schroeder (Quality Assurance).

Study Period: From October 16, 1981 to November 6, 1981;  
Report completed on April 19, 1982.

Date Submitted: 12/5/83

Test Substance: Technical Isofenphos, 91.9% a.i. (Ref. 47898),  
Batch #0005281, a clear colorless liquid  
supplied by Mobay. The test substance was  
stored at room temperature, and a sample was  
stored in the freezer for archiving.

Protocol: Fifty adult white Leghorn hens, 12 months old, weighing 1.30 to 2.27 kg were used. The hens were supplied by the Kansas State University Poultry Department, and they were held in quarantine for 2 weeks before treatment.

The hens were randomly assigned (by drawing cage numbers from a hat) to 3 groups: an Isofenphos group of 30 birds, a positive control group (Tri-o-tolyl phosphate) of ten birds and a control group which consisted of five hens treated with 50 mg/kg water and 5 hens untreated. The hens were indentified by leg bands, individually housed and supplied with KSU hen laying ration.

Each bird in the Isofenphos group received a single oral dose (gavage) of 32 mg/kg\*. No vehicle was used and the doses were calculated assuming, 1 ul of test material weighs 1 mg. All hens in this group received 50 mg/kg atropine intramuscularly at the time of Isofenphos administration. Each bird in the positive control group received 500 mg/kg TOTP.

All hens were observed daily after dosing for signs of toxicity; and they were weighed on the day of dosing and twice weekly thereafter. The surviving hens were sacrificed after 21 days of treatment by intraarterial infusion of formalin after being anesthetized with CO<sub>2</sub>.

After gross examination, the brain, spinal cord (cervical, thoracic and lumbar) and sciatic nerve were collected, fixed in 10% buffered neutral formalin, and histologically processed. Sections were prepared from the cerebellum (stem), cervical and thoracic spinal cord (3 cross sections and one longitudinal for each of the two areas), lumber spinal cord (2 cross sections, one longitudinal, and a section of the glycogen body) and sciatic nerve (one cross section and one longitudinal section).

Hematoxylin and eosin were used to stain one set of slides, and luxol fast blue was used for the other set.

The 17 hens that died during the first 24 hours of the study were not processed for a histopathological examination.

\*This reviewer was informed (orally by the PM) on 12/20/83 that the specific gravity of Isofenphos technical (90% a.i. or above) is 1.135 at 20°C. Hence, the actual dosage as calculated by this reviewer is 36 mg/kg technical Isofenphos.

Results:

The administration of a single Isofenphos oral dose of 32 mg/kg b.w. to adult hens (a dosage greater than the unprotected oral LD<sub>50</sub>)\* did not result in an acute delayed neurotoxic response. However 17/30 hens died within 24 hours of treatment, but no histopathology was performed on these birds. The Isofenphos survivors reflected a higher body weight loss than both the control and the TOTP treated groups during the first 10 days of the study period. However, the Isofenphos hens recovered gradually by the end of the observation period (21 days) while the TOTP treated birds continued to lose weight.

Locomotor ataxia and paralysis were observed in all Isofenphos survivors during the first 5 to 6 days of treatment, however the TOTP bird exhibited these symptoms from days 14 or later, until termination. Histopathological examinations of the nerve tissues indicated that no delayed neurotoxic effects occurred in the Isofenphos treated hens, while typical effects of organophosphate-induced delayed neurotoxicity were noted in the TOTP treated birds i.e., degeneration, demyelination, axonal swelling and macrophage accumulation in the spinal cord of the 10 TOTP treated birds. In addition to the TOTP induced lesions in the spinal cord one of these TOTP birds reflected minimal effects in the brain and another hen reflected minimal effects in the sciatic nerve.

\*The unprotected oral LD<sub>50</sub> for hens was not determined in this study. Previous data in Mobay's Report #34025, 3/30/72 reflected an oral LD<sub>50</sub> value of 20.9 (17.2-24.9) mg/kg b.w.

Core Classification: Supplementary Data.

°The surviving hens were not challenged by a second dosage of the test compound after 21 days of the initial treatment.

B. Study Type: Neurotoxic Esterase Activity in Hens

Accession No.: 251884

Sponsor: Mobay Chemical Corporation

Report No.: Mobay's Report #80678; testing facility  
study #3007376

Test Facilities: Bayer Institut fur Toxikology Wupertal-Elberfeld

Study Performed: J. Thyssen and A. Eben

Date of Study: 11/3/82 (however it was indicated that the study  
was performed in November and December of 1982)

Date Submitted: 12/5/83

Test Substance: Technical Isofenphos 91.9%, Batch #808116803

Test Animals: Adult White Leghorn hens supplied by Brunkschulte  
Senden/Minister, FRG. The birds were housed in an air-conditioned  
house (20°C) with free access to open pen with natural ground.  
Feed (standardized food pptt, LVK) and water were available  
ad libitum.

Dosage: Liquid isofenphos was administered once by gavage at a  
dose level of 32 mg/kg b.w. (assuming 1 ul of test substance  
weighs 1 mg). The birds received 50 mg/kg b.w. atropine  
intramuscularly at the time of Isofenphos administration; the  
control birds received only atropine.

Apparently a group of 49 hens were treated with the chemical  
and 6 additional hens were used as a control group. Investiga-  
tion of the NTE activity in brain, spinal cord and sciatic  
nerve were performed at 1, 2, 4, 7, 10 and 14 days after the  
compound administration; four hens were used at each interval.

The surviving hens and in some cases moribund hens were  
killed by decapitation, bled, and the brain, spinal cord and  
sciatic nerve collected and homogenized. The NTE activity was  
determined according to the method of Johnson, M.K., Arch.  
Toxicol. 37, 113, 1977.

### Results:

Mortality: 11/49 treated birds died, 5 birds died within one day of treatment and 6 birds died in the following 3 days.

Symptoms: It was stated by the author that all treated birds displayed typical signs of ChE inhibition.

NTE determination: All data reflected some NTE inhibition in the brain, spinal cord and sciatic nerve i.e. 20 to 59% in brain, 11 to 48% in the spinal cord and 6.5 to 84.4% in the sciatic nerve during the first 7 days of dosing; the level of inhibition in these tissues decreased by the end of the study to 0-22%.

However it is well known that a NTE inhibition level of 80% or above during the first 24 to 40 hours of exposure is required before an OP compound can be considered to cause delayed neurotoxicity. In this study, only hen #45 (1/4 birds tested at the 3 day interval) reflected 84.4% NTE inhibition, and hen #25 (1/4 birds tested after one day of exposure) reflected 76.3% inhibition; these 2 high levels of NTE inhibition were only observed in the sciatic nerve homogenates of these 2 hens.

### Conclusions:

This reviewer cannot adequately assess the NTE activity from the above results due to the following issues:

1. Individual NTE activity/ gram brain/ hour, and individual clinical symptoms for each hen were not reported.
2. Apparently 24/49 birds had their NTE activity determined. No information was provided on the remaining 14 survivors.
3. No explanation was provided for the selection of hens for NTE determinations.
4. Johnson method of 1977 indicated that 0.6 mg of the brain tissue should be used in the determination of the NTE activity (second page of Johnson 1977 publication, third line under the paragraph headed "simplified assay of neurotoxic esterase"). This reviewer needs to verify the actual amount of brain tissues used for these determinations in this study.

Core Classification: Invalid

The study may be upgraded to Core Supplementary when a satisfactory answer is provided relative to the 4 issues discussed above in our Conclusions section.