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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No.: 101201. Methamidophos (racemate and enantiomers): Acute oral studies with rats and hens; studies related to Guideline No. 81-7; and a review article. 6(A)(2) data

Case Number: 819351
Submission No.: S397161
HED Project No.: 1-1405
Tox. Chem. No.: 378A

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (H7509C) Krystyna K. Locke 10/28/91

TO: Larry Schnaubelt/Robert Richards, PM 72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C) Roger Gardner 10-28-91 KB 10/25/91

Toxicology Branch/HED has completed an evaluation of the following studies:

"Can Methamidophos Cause Delayed Polyneuropathy In Man or In Test Animals?" M.K. Johnson and M. Lotti;
Mobay Report No.: 100277; November, 1989.
MRID No.: 41685801
EPA Guideline No.: None

"Methamidophos (Racemate and Enantiomers) Study for Acute Oral Toxicity to Rats." W. Flucke;
Mobay Report No.: 100278; May 4, 1990.
MRID No.: 41685802
EPA Guideline No.: 81-1

"Methamidophos (Tamaron) (Racemate and Enantiomers) Study for Acute Oral Toxicity to the Hen (Gallus domesticus)."
W. Flucke; Mobay Report No.: 100279; May 29, 1990.
MRID No.: 41685803
EPA Guideline No.: None

"Methamidophos (Racemate and Enantiomers) Study for Effects on NTE (Neuropathy Target Esterase) In Hens Following Oral Administration." W. Flucke and A. Eban;
Mobay Report No.: 100280; May 29, 1990.
MRID No.: 41685804
EPA Guideline No.: Related to 81-7

"Methamidophos (Racemate and Enantiomers) Study for OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy): Exploratory Studies on Hens." W. Flucke;
Mobay Report No. 100281; April 10, 1990.
MRID No.: 41685805
EPA Guideline No.: Related to 81-7

Each of the four studies (MRID No.: 41685802-41685805) was performed by Bayer AG, Wuppertal, West Germany. The "performing laboratories" for the review article (MRID No.: 41685801) were: 1) Medical Research Council Toxicology Unit, Carshalton, Surrey, UK and 2) Universita degli Studi di Padova, Instituto di Medicina del Lavoro, Padova, Italy.

Only the OPIDP study (MRID No.: 41685805) contains 6(A)(2) data. According to the flagging statement submitted by the registrant, this study meets or exceeds the criteria numbered 6 (Pesticide assessment guideline No. 81-7: When compared with controls, treated animals show a response indicative of acute delayed neurotoxicity).

O,S-Dimethyl phosphoramidothioate (Methamidophos, Tamaron, Monitor), an organophosphorus ester insecticide, is a racemic mixture of two stereoisomers, dextrorotary D(+) and levorotary L(-). This mixture, methamidophos (+ -), can be separated into individual isomers (enantiomers), D(+) or methamidophos (+) and L(-) or methamidophos (-). These three methamidophos compounds--methamidophos (+ -), methamidophos (+) and methamidophos (-)--were used as test materials in each of the above studies.

The following findings were most important in each study:

MRID No.: 41685802. Acute Oral (Male Wistar rats)

LD_{50} (mg/kg b.w.):
Methamidophos (+ -): 16
(+): 14
(-): 16

Methamidophos (+ -):
Toxicity Category: I
Core Classification: Minimum

2

Based on the parameters examined (clinical observation, mortality, body weight gain and gross necropsy), no major differences between the racemic mixture and the enantiomers could be determined.

MRID No.: 41685803. Acute Oral (Hens).

LD_{50} (mg/kg b.w.):
Methamidophos (+ -): 25
(+): 43
(-): 82

Methamidophos (+ -):
Toxicity Category: Not applicable
Study Classification: Acceptable

Toxic symptoms observed with each test compound were characteristic of cholinesterase (ChE) inhibition.

Above LD_{50} values show that the enantiomers potentiate in the racemic methamidophos. Hens (Lohmann LSL; original breed: white leghorn) were 9-10 months old at the start of the study.

MRID No.: 41685804. Neuropathy Target Esterase (NTE) Study in Hens.

This "study" actually consists of five studies, ranging in duration from 2 to 22 days, which were conducted between February 8, 1988, and February 21, 1989. The hens used in these studies were 8 months old. The single doses of the methamidophos compounds, administered orally in the presence of antidotes, were equivalent to 2-10 times their LD_{50} values. The following parameters were studied: 1) Inhibition of NTE activities in lymphocytes, spinal cord, brain and sciatic nerve; 2) Reactivation of the inhibited NTE activity in brain; and 3) Identification of the forms in which inhibited NTE existed, whether NTE was modified ("aged" = incapable of being reactivated) or unmodified ("unaged" = capable of being reactivated).

Based on the parameters examined, the neurotoxic potential of methamidophos (+ -), methamidophos (+) and methamidophos (-) was low.

This study is acceptable as an NTE study and is related to EPA Guideline 81-7 (acute delayed neurotoxicity).

MRID No.: 41685805. Organophosphorus Ester-Induced Delayed Polyneuropathy (OPIDP) Study in Hens.

This "study" actually consists of four studies, ranging in duration from 22 to 56 days, which were conducted between May 17,

3

1988, and September 13, 1988. In each study, single doses of the methamidophos compounds were administered orally in the presence of antidotes.

Methamidophos (+ -) induced OPIDP only at a dose of 400 mg/kg b.w. (16 times LD_{50}).

Methamidophos (+) induced OPIDP at a dose of 400 mg/kg b.w. (9.3 times LD_{50}).

Methamidophos (-) did not induce OPIDP at 400 mg/kg b.w. (4.9 times LD_{50}; only dose tested).

Based on the above data, the delayed neurotoxicity potential of methamidophos (+ -) and methamidophos (+) is low.

This study was reported as 6(A)(2) data because, according to the registrant, OPIDP had been induced under experimental conditions for the first time by methamidophos.

This study is acceptable as an OPIDP study and is related to EPA Guideline No. 81-7. However, this study does not satisfy the acceptance criteria for EPA Guideline No. 81-7.

MRID No.: 41685801. Can Methamidophos Cause Delayed Polyneuropathy in Man or in Test Animals?

This review article is based on 32 publications from the open literature and one unpublished manuscript. Most of the papers (25) were published during 1980-1989.

Based on data presented in this article, methamidophos can cause delayed polyneuropathy in humans only when ingested in high amounts, usually deliberately (suicide attempts) or, occasionally, accidentally. Adult hen, a preferred species for study of delayed neurotoxicity (polyneuropathy) in experimental animals, develops polyneuropathy only after ingesting methamidophos at doses equivalent to 12-16 times the unprotected LD_{50}. The delayed polyneuropathy potential of methamidophos is, therefore, very low.

Response to 6(A)(2) Data:

This study should be considered in assessment of the delayed neurotoxicity potential of methamidophos. However, no regulatory or other action (such as a peer review) is currently warranted.

GUIDELINE: None

Primary Review by: Krystyna K. Locke 10/28/91
Krystyna K. Locke, Review Section I, Toxicology Branch I/HED

Secondary Review by: Roger Gardner, Roger Gardner 10-28-91
Section Head, Review Section I, Toxicology Branch I/HED

DATA EVALUATION RECORD

STUDY TYPE: Review article

EPA IDENTIFICATION NOS: Tox. Chem. No.: 378A
MRID No.: 41685801

REPORT NUMBER: 100277

SPONSOR: Mobay Corporation, Kansas City, Missouri

PERFORMING LABORATORY: Medical Research Council Toxicology Unit
Carshalton, Surrey UK
and
Universita degli Studi di Padova
Instituto di Medicina del Lavoro
Padova, Italy

TITLE OF REPORT: Can Methamidophos Cause Delayed Polyneuropathy In Man or In Test Animals?

AUTHORS: M.K. Johnson and M. Lotti

REPORT ISSUED: November, 1989

CONCLUSIONS:

Based on data presented in this review article, methamidophos can cause delayed polyneuropathy in humans only when ingested in high amounts, usually deliberately (suicide attempts) or, occasionally, accidentally. Adult hen, a preferred species for study of delayed polyneuropathy in experimental animals, develops delayed polyneuropathy only after ingesting methamidophos at doses equivalent to 12-16 times the unprotected LD_{50} value. The delayed polyneuropathy (neurotoxicity) potential of methamidophos is, therefore, very low.

SUMMARY:

This review article is based on 32 papers from the open literature and 1 unpublished manuscript (Attachment I). These papers were published during 1961-1989, but most (25) were published during 1980-1989. Other studies submitted with this review article (MRID 416858-02, -03, -04 and -05) are discussed in the article, but are excluded from the list of references.

5

Most of the article is concerned with clinical reports (case histories) of poisoning with methamidophos and with laboratory investigations of delayed neuropathic potential of methamidophos. The latter include a possible mechanism of delayed neuropathy, neuropathy tests in hens with racemic methamidophos, neuropathy tests in hen with resolved isomers of 99.5% pure racemic methamidophos, and various in vitro studies with hen and human brain. In the latter, the inhibition constant, I_{50}, of methamidophos and isomers for acetylcholine esterase (AChE) and Neuropathy Target Esterase (NTE) were calculated.

The following information/findings might be of interest:

1. The key enzymes in the development of OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy) are AChE and NTE. The development of OPIDP is not possible without severe or potentially fatal acute intoxication which results from an inhibition of AChE. Methamidophos is a more potent inhibitor of AChE than NTE in both humans and hens.

2. Based on studies in which hens were acutely exposed to high doses of methamidophos, about 70-80% inhibition of NTE activity in the nervous tissue, as well as aging of the enzyme complex (modification; incapability of being reactivated) was necessary to trigger OPIDP. As far as chronic exposure to methamidophos is concerned, there is no evidence that high inhibition of NTE is necessary to initiate OPIDP.

3. Considering points 1. and 2., and the fact that only high doses of methamidophos cause severe inhibition of NTE and trigger OPIDP, it is unlikely that agricultural workers, using normal precautions required when applying methamidophos or any anticholinesterase insecticide, would develop OPIDP.

4. Agricultural workers who use a variety of organophosphorus and organochlorine pesticides, or are exposed to n-hexane or some higher hydrocarbons, might be more susceptible to acute neuropathic effects, being exposed for long periods to sub-toxic doses of these chemicals. However, there is no evidence in the literature that pre-exposure alters the outcome in cases of acute poisoning.

5. Ingestion of alcohol prior to or at the same time as poison is not uncommon in suicide attempts. However, there is no clear evidence that this potentiates the neuropathic potential of organophosphorus pesticides.

6. Toxic effects of methamidophos are due to an active ingredient (a.i.) of a formulation. Studies conducted in

hens with purified methamidophos (95.5-99.5% a.i.) and with technical grade methamidophos (73-75% a.i.) showed that their effects on the severity of acute toxic responses; NTE activity in brain, spinal cord and sciatic nerve; reactivation of inhibited NTE; and mortality were comparable. It is, therefore, unlikely that any of the impurities found in technical methamidophos inhibit NTE or AChE. However, at 50 and 65 mg/kg a.i., the acute response in each group was severe and/or prolonged in the case of purified methamidophos and was "very severe with some fatalities in spite of therapy" in the case of technical methamidophos.

7. Several cases of OPIDP had been reported. The usual cause was a deliberate or accidental ingestion, but at least 2 or 3 were related to spraying operations. In Sri Lanka, for example, men and women who wanted to commit suicide (ages 14-33) drank 30-80 ml of Tamaron. All had cholinergic symptoms. Four people recovered in 6 weeks-2 years.

   In another instance, a 73-year old woman ingested (suicide attempt) an unknown but apparently large amount of Tamaron formulation containing 195 mg a.i./ml. Thirty-six hours after ingestion, her methamidophos blood level was 6 µg/ml and erythrocyte AChE, lymphocyte NTE and plasma butyryl cholinesterase (BuChE) were markedly inhibited (she died).

8. OPIDP may be caused by a single dose of an organophosphorus pesticide, but, regardless of dose, there is always a delay of 8-15 days in hen and 2-5 weeks in man before typical signs develop. The early symptoms of OPIDP in man are tingling and weakness of the extremities; degeneration of the distal ends of some motor and sensory neurons; and muscle wasting and ataxia in severe cases.

9. The target for initiation of OPIDP is NTE in the neural tissue. However, the estimation of NTE in lymphocytes is a practical way of determining neuropathic effects which might result from exposure to organophosphorus NTE inhibitors.

10. Methamidophos was also implicated in organophosphorus neurotoxicity intermediate syndrome which is characterized by paralysis of proximal limb muscles, neck flexors, motor cranial nerves and respiratory muscles, and occurs 24-96 hours after the cholinergic phase.

11. The target tissues in delayed polyneuropathy are brain, spinal cord and peripheral nerves. Based on <u>in vitro</u> studies, there seems to be no substantial differences between hens and humans (two most sensitive species) in AChE and NTE inhibition and spontaneous reactivation, and in aging of inhibited NTE. However, racemic methamidophos and

7

isomers displayed different potentials to inhibit target enzymes (AChE and NTE), as is evident from the I_{50} values (Attachment II). Spontaneous reactivation of inhibited AChE occurred in both species, but no spontaneous reactivation of NTE was observed.

Aging of inhibited NTE can be monitored by determining reactivation in the presence of KF (Attachment III). In studies with brain homogenates of hens and humans, virtually complete reactivation was achieved by methamidophos (+ - ; racemate) and methamidophos (+), indicating that NTE inhibited by these compounds was not "aged" or modified. However, little reactivation was obtained with methamidophos (-), suggesting that NTE inhibited by this isomer was "aged" or modified.

12. Detailed studies of the mechanism of delayed neuropathy had been carried out with hens, less extensively with other animals, including sheep and cats, and also with target enzyme (NTE) in man. Based on studies with hens, a mechanism was proposed which is detained in Attachment IV. Briefly, phosphorylation (or phosphonylation) of NTE inhibits its activity. This is followed by "aging" in which a R-O-P bond is cleaved leaving an ionized phosphoryl residue which is believed to be the ultimate deleterious agent (moiety) bound to the neuronal membrane. Both the primary attack on NTE and aging are essential to initiate OPIDP by organophosphates and phosphonates.

Attachment I

METHAMIDOPHOS

Page _____ is not included in this copy.

Pages 10 through 18 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

GUIDELINE: 81-1

Primary Review by: Krystyna K. Locke, Review Section I, Toxicology Branch I/HED

Secondary Review by: Roger Gardner
Section Head, Review Section I, Toxicology Branch I/HED

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral (Male Rats)

EPA IDENTIFICATION NOS: Tox. Chem. No.: 378A
MRID No.: 41685802

TEST MATERIAL: Methamidophos: racemic mixture and enantiomers, as follows:

Test Material*	Batch No.	Purity (a.i.), w/w
Methamidophos (+ -)	Racemic	95.3%
Methamidophos (+)	GSS 307-3	98.5%
Methamidophos (-)	GSS 306-4	97.8%

Each was a white, water-soluble powder, stored at 22° C in the absence of light.

SYNONYMS: Tamaron, Monitor

REPORT NUMBER: 100278

SPONSOR: Mobay Corporation, Kansas City, Missouri.

TESTING FACILITY: Bayer AG, Wuppertal, West Germany.

TITLE OF REPORT: Methamidophos (Racemate and Enantiomers): Study for Acute Oral Toxicity to Rats

AUTHOR: W. Flucke

REPORT ISSUED: May 4, 1990

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*Methamidophos, an organophosphorus ester insecticide, is a racemic mixture of two stereoisomers, dextrorotary D (+) and levorotary L (-). This mixture can be separated (purified) into individual isomers (enantiomers), D (+) or methamidophos (+) and L (-) or methamidophos (-).

CONCLUSIONS:

LD_{50} values and (95% confidence intervals) were:

Methamidophos (+ -): 16 (13.3-19.2) mg/kg b.w.
Methamidophos (+) : 14 (12.8-15.7) mg/kg b.w.
Methamidophos (-) : 16 (13.1-20.0) mg/kg b.w.

Slope functions were:

Methamidophos (+ -): 1.292
Methamidophos (+) : 1.158
Methamidophos (-) : 1.340

Classification of study: Core-Minimum

Toxicity category: I

Each test compound was very toxic to male rats in this study. Toxic signs observed were characteristic of cholinesterase inhibition. Based on the parameters examined (clinical observation, mortality, body weight gain and gross necropsy), no major differences between the racemic mixture and the enantiomers could be determined.

EXPERIMENTAL PROCEDURES

The purpose of this study was to determine whether significant differences exist between the racemic methamidophos and the enantiomers with regard to acute toxic effects in a mammalian species (rat). The study was conducted during July 25-August 15, 1988.

Unfasted, male SPF-bred Wistar rats, 5 per dose, were treated (by gavage) with single doses of the test materials, as follows:

Test Material	Doses (mg/kg b.w.)
Methamidophos (+ -)	10, 14, 16, 20 and 31.5
Methamidophos (+)	10, 12.5, 13.2, 14, 16, 20 and 31.5
Methamidophos (-)	10, 12.5, 14, 16, 20 and 31.5

The test materials were dissolved in demineralized water in the morning before administration and complete solubility was observed. The homogeneity and stability of the test materials in solutions were, therefore, not determined. All test materials were administered simultaneously in the volume of 10 ml/kg of body weight. It was not reported how doses were selected.

The rats were:

1. Obtained from Winkelmann Animal Breeders, Borchen, Kreis Paderborn, West Germany.

2. Acclimated for at least seven days.

3. Seven to eight weeks old at the start of the study. Their mean initial weight was 180 g (165-196 g).

4. Housed 5/cage, all in one room, at temperature of 22° ± 2° C, relative humidity of about 50%, light/dark cycle of 12 hours and air changes of at least 10 times/hour.

5. Allowed unlimited food (Altromin 1324 Maintenance Diet for Rats and Mice) and tap water (provided in polycarbonate bottles).

6. Assigned to treatment groups and dose levels by a computer system.

7. Identified by cage cards and by individual markings with saturated aqueous picric acid solution.

8. Observed daily for toxic signs and mortality, for 14 days following treatment.

9. Weighed prior to treatment and on the observation days 4, 8 and 15.

10. Sacrificed by inhalation of diethyl ether and examined grossly at the end of the observation period. The animals which did not survive the study were also examined.

The LD_{50} values were calculated by the procedure of J.T. Litchfield and F. Wilcoxon (J. Pharmacol. Exper. Therap. 96, 99-113, 1949).

RESULTS

Toxic Signs

Irrespective of the test material used, the initial symptoms (trembling spasms) occurred at all dose levels shortly after treatment, as follows: 12-22 minutes (racemic methamidophos); 29-52 minutes (D (+) isomer) and 9-18 minutes (L (-) isomer).

Irrespective of the test material, the main symptoms observed were trembling spasms, palmospasms, salivation, lacrimation, labored breathing, bristling fur and apathy. In the surviving animals, the spasms subsided after 24 hours to 4 days,

salivation and lacrimation after 24 hours, labored breathing after 24 hours to 4-5 days, bristling fur after 2-9 days, and apathy after 4-11 days.

Mortality

Irrespective of the test material, no mortality occurred in the 10 mg/kg and 12.5 mg/kg groups, whereas all animals died in the 31.5 mg/kg group. All animals also died in the 20 mg/kg group treated with methamidophos (+). In other groups, dose-related mortality ranged from 20% to 80%. Mortalities occurred 21-23 minutes to 3-4.5 hours after treatment.

Body Weights

Dose-dependent weight losses were observed on study day 4, but weight gains in all groups occurred thereafter. Relative to the mean body weights before treatment, the mean percent weight losses ranged from 6.2 to 19.3 in the methamidophos (+ -) group, from 11.6 to 22.6 in the methamidophos (+) group and from 9.8 to 25.3 in the methamidophos (-) group. Relative to the mean body weights before treatment, the dose-unrelated mean percent weight gains on day 15 were 14.0-23.5, 10.1-20.3 and 5.0-21.4 in the methamidophos (+ -), methamidophos (+) and methamidophos (-) groups, respectively.

Gross Pathological Findings

Irrespective of the test material and dose, animals which did not survive the study had distended, patchy lungs and dark livers. One rat in the 14.0 mg/kg methamidophos (+ -) group, one in the 13.2 mg/kg methamidophos (+) group and three in the methamidophos (-) group (two at 14.0 mg/kg dose and one at 20.0 mg/kg dose) also had fluid in lung tissue. The following findings were observed only in the 16.0 mg/kg methamidophos (-) group: 1) patchy spleen and distended, empty intestinal tract (one rat) and 2) patchy spleen and slightly reddened proventriculus (one rat).

No abnormalities were observed in animals sacrificed at the termination of the study.

COMMENTS

This study was reported in a clear, detailed manner, but it was not stated how doses were selected for each test compound. The results of analysis of the test materials for purity (active ingredient) were also submitted.

The study methods conformed to OECD Guidelines for Testing of Chemicals (February 24, 1987) and to EPA Pesticide Assessment Guidelines (November, 1984), with two exceptions: fasted animals

were not used and only males were used. Since the objective of this study was to establish differences among a compound and its isomers, the use of fasted animals and both sexes was considered unnecessary. Toxicology Branch/HED agrees.

According to the registrant (Mobay Corporation), this study was not conducted in compliance with the Good Laboratory Practice Standards of 40 CFR part 160 (FIFRA). Nevertheless, it meets these standards, with two exceptions:

1. No GLP inspections were performed by the quality assurance unit.

2. The test substances were not analyzed for homogeneity, stability and concentration in the test solutions.

Since the test materials were solutions which were used immediately after preparation, the lack of data on homogeneity and stability should not affect the experimental results. However, the unavailability of other information/data precluded this study from being classified as a Core-Guideline study.

A statement of no claim of data confidentiality was submitted.

GUIDELINE: 81-0

Primary Review by: Krystyna K. Locke, Review Section I, Toxicology Branch I/HED — Krystyna K. Locke 10/28/91

Secondary Review by: Roger Gardner, Section Head, Review Section I, Toxicology Branch I/HED — Roger Gardner 10-28-91

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral (Hen)

EPA IDENTIFICATION NOS: Tox. Chem. No.: 378A
MRID No.: 41685803

TEST MATERIAL: Methamidophos: racemic mixture and enantiomers, as follows:

Test Material*	Batch No.	Purity (a.i.), w/w
Methamidophos (+ -)	Racemic	95.3%
Methamidophos (+)	GSS 307-3	98.5%
Methamidophos (-)	GSS 306-3	96.5%

Each was a white, water-soluble powder, stored at 22° C in the absence of light.

SYNONYMS: Tamaron, Monitor

REPORT NUMBER: 100279

SPONSOR: Mobay Corporation, Kansas City, Missouri.

TESTING FACILITY: Bayer AG, Wuppertal, West Germany.

TITLE OF REPORT: Methamidophos (Tamaron) (Racemate and Enantiomers): Study for Acute Oral Toxicity to the Hen (Gallus domesticus)

AUTHOR: W. Flucke

REPORT ISSUED: May 29, 1990

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*Methamidophos, an organophosphorus ester insecticide, is a racemic mixture of two stereoisomers, dextrorotary D (+) and levorotary L (-). This mixture can be separated (purified) into individual isomers (enantiomers), D (+) or methamidophos (+) and L (-) or methamidophos (-).

CONCLUSIONS:

LD_{50} values and (95% confidence intervals) were:

Methamidophos (+ -): 25 (19.4-32.7) mg/kg b.w.
Methamidophos (+) : 43 (31.3-60.5) mg/kg b.w.
Methamidophos (-) : 82 (56.9-118.7) mg/kg b.w.

Slope functions were:

Methamidophos (+ -): 2.45
Methamidophos (+) : 2.05
Methamidophos (-) : 1.48

Classification of study: Acceptable

Toxic signs observed with each test material were characteristic of cholinesterase inhibition. Although the test materials exerted the same type of effects, there were quantitative differences between the enantiomers and between the racemic methamidophos and the enantiomers in the LD_{50} values, time of occurrence of mortality, duration of toxic signs and duration of the recovery period.

With an LD_{50} of 82 mg/kg b.w., methamidophos (-) was less acutely toxic than methamidophos (+). However, the toxic signs and the recovery period lasted longer and the mortalities occurred later with methamidophos (-) than with methamidophos (+).

In the case of methamidophos (+ -), the racemate, mortalities occurred faster with racemate than with the enantiomers, but the duration of symptoms was comparable with that of methamidophos (-).

On the basis of the LD_{50} values for the enantiomers (43 and 82 mg/kg b.w.), the expected theoretical LD_{50} value for the racemic methamidophos, assuming additive effects, would be 63 mg/kg b.w. Based on the experimentally determined LD_{50} value of 25 mg/kg b.w., the racemic methamidophos was, therefore, 2.5 times more toxic than the enantiomers. The testing laboratory concluded from these findings that the acute toxic effects of the enantiomers potentiate in the racemic methamidophos. Toxicology Branch/HED agrees with this conclusion.

EXPERIMENTAL PROCEDURES

The purpose of this study was to obtain LD_{50} values for acute delayed neurotoxicity studies which might be conducted at a later date. The study was conducted during June 22-August 30, 1988.

Unfasted Lohmann LSL strain laying hens (Lohmann Selected Leghorn; original breed: white leghorn), 5 per dose, were treated (by gavage) with single doses of the test materials, as follows:

Test Material	Doses (mg/kg b.w.)
Methamidophos (+ -)	15, 22.5, 33.8 and 50.7
Methamidophos (+)	4, 10, 15, 22.5, 33.8, 40 and 50.7
Methamidophos (-)	33.8, 50.7, 76, 100 and 160

The test materials were dissolved in demineralized water just before administration and complete solubility was observed. The homogeneity and stability of the test materials in solutions were, therefore, not determined. All test materials were administered simultaneously in the volume of 5 ml/kg of body weight. It was not reported how doses were selected.

The hens were:

1. Obtained from Brinkschulte Breeders, Senden, West Germany.

2. Acclimated for at least seven days.

3. Nine months and 16 days to ten months and 26 days old at the start of the study. Their mean initial weight was 1.18-1.85 kg.

4. Housed 5/floor-based cage, all in one room with a heated floor, at temperature of 22° ± 8° C, relative humidity of about 50%, and light/dark cycle of 12 hours. Only during the initial 24 hours following treatment were the hens individually housed in standard hen cages.

5. Allowed unlimited food (Ssniff-LVK whole wheat feed for laying hens) and tap water (provided through an automatic system).

6. Assigned to treatment groups and dose levels using "randomizing lists".

7. Identified by consecutively numbered wing tabs.

8. Observed daily for toxic signs and mortality, for 28 days following treatment.

9. Weighed prior to treatment and at weekly intervals thereafter.

10. Sacrificed by intravenous injection of a 20% sodium hexobarbital solution and examined grossly at the end of the observation period. The hens which did not survive the study were also examined.

The LD_{50} values were calculated using an HP 3000 computer system and/or dose-mortality curves. The references on which these calculations were based appear in Attachment I.

RESULTS

Toxic Signs

Irrespective of the test material and doses used, the main symptoms were apathy, ruffled feathers, staggering gait, respiratory disturbances (rapid, shallow breathing), diarrhea, salivation, lacrimation, random wingbeats and flat or lateral prostration. A dry-appearing and limp comb was observed in the surviving hens, mostly in the methamidophos (+ -) and methamidophos (-) groups.

Irrespective of the test material, most of the symptoms appeared within 5-30 minutes after treatment and, in the surviving hens, subsided in about 6 days in the methamidophos (+) group or in about 16 days in the remaining two groups. In some hens treated with methamidophos (+ -) or methamidophos (-), toxic signs persisted throughout the duration of the study.

Mortality

In the methamidophos (+ -) group, dose-dependent mortality ranged from 20 to 100% and deaths occurred within 50 minutes to 5 hours after treatment. In the methamidophos (+) group, dose-independent mortality occurred within 1 hour to 2 days after treatment. Mortality was also dose-independent in the methamidophos (-) group; it ranged from 20 to 80% and occurred within 1-5 days after treatment.

Body Weights

Following treatment with the test materials, the hens in all dose groups lost weight. Relative to the mean body weights on day 1, the mean percentage weight losses on day 8 were:

Methamidophos (+ -) : 4, 18, and 22.1 (one hen)* in groups 1, 2, and 3, respectively. (All hens died in group 4 shortly after treatment.)

Methamidophos (+) : 11.1, 4.0, 8.3, 8.8, 8.6, 5.5 and 33.5 (one hen)* in groups 1, 2, 3, 4, 5, 6 and 7, respectively.

Methamidophos (-) : 13.5, 13.9, 12.8, 17.0 (one hen)* and 14.6 (one hen)* in groups 1, 2, 3, 4 and 5, respectively.

The initial weight losses persisted in most hens through day 15 and in 1-2 hens/dose through days 22 or 29. Relative to the mean body weights on day 1, the mean percentage weight gains at

the termination of the study (day 29) were:

Methamidophos (+ -) : 2.8, 1.4 and 5.8 (one hen)* in groups 1, 2 and 3, respectively.

Methamidophos (+) : 6.9, 8.0, 6.2, 8.1, 8.6 and 10.3 in groups 1, 2, 3, 4, 5 and 6, respectively. The only surviving hen in group 7 had a weight loss of 9.2%.

Methamidophos (-) : 5.2, 8.7, 14.9, 8.5 (one hen)* and 13.9 (one hen)* in groups 1, 2, 3, 4 and 5, respectively.

Gross Pathological Findings

Irrespective of the test material and dose, hens which did not survive the study had distended crops, occasionally filled with fluid; distended, fluid-filled lungs; pale spleen and kidneys; dark livers; reddened intestinal tract mucosa, in some cases; ulcer-like foci in proventriculus; and occasional heminthasis.

Irrespective of the test material and dose, hens which were sacrificed at the termination of the study had occasional instances of pale liver, kidneys and spleen; reddened duodenum and egg concentration in abdominal cavity, the latter observed only the methamidophos (+ -) and methamidophos (-) groups. According to the testing laboratory, only the reddening of the duodenal mucosa could be indirectly associated with the treatment as a symptom of stress.

COMMENTS

This study was reported in a clear, detailed manner, but it was not stated how doses were selected for each test compound. The results of analysis of the test materials for purity (active ingredient) were also submitted.

The methods employed were based on the OECD Guidelines for Testing of Chemicals (February 24, 1987) and on EPA Pesticide Assessment Guidelines (November, 1984).

According to the registrant (Mobay Corporation), this study was not conducted in compliance with the Good Laboratory Practice Standards of 40 CFR part 160 (FIFRA). Nevertheless, it meets these standards, with two exceptions:

1. No GLP inspections were performed by the quality assurance unit.

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*Means that one hen was left in that group because the remaining four hens had died.

2. The test substances were not analyzed for homogeneity, stability and concentration in the test solutions.

Since the test materials were solutions which were used immediately after preparation, the lack of data on homogeneity and stability should not affect the experimental results.

A statement of no claim of data confidentiality was submitted.

Attachment I

REFERENCES USED IN CALCULATING LD_{50} VALUES

ROSIELLO, A.P., J.M. ESSIGMANN und G.N. WOGAN
Rapid and accurate determination of the median lethal dose (LD_{50}) and its error with small computer.
J. Tox. and Environ. Health 3, 797-809 (1977).

PAULUHN,J.
Über die computergestützte Abschätzung der LD_{50}/LC_{50}
BAYER AG Bericht-Nr.: 11835 vom 18.5.1983.

BLISS, C.I.
The determination of the dosage-mortality curve from small numbers.
Q.J. Pharm. Pharmacol. 11, 192-216 (1938).

BLISS, C.I.:
The calculation of the dosage-mortality curve.
Ann. Appl. Biol. 22, 134 (1935).

BAIRD, J.B. and R.L. BALSTER:
Analysis of Nominal Dose-Effect Data with an Advanced Programmable Calculator.
Neurobehaviour. Tox. 1, 73-77 (1979).

GUIDELINE: Related to 81-7

Primary Review by: Krystyna K. Locke, Review Section I, Toxicology Branch I/HED Krystyna K. Locke 10/28/91

Secondary Review by: Roger Gardner, Section Head, Review Section I, Toxicology Branch I/HED Roger Gardner 10-28-91

DATA EVALUATION RECORD

STUDY TYPE: Neuropathy Target Esterase (NTE) - Hen

EPA IDENTIFICATION NOS: Tox. Chem. No.: 378A
MRID No.: 41685804

TEST MATERIAL: Methamidophos: racemic mixture and enantiomers, as follows:

Test Material*	Batch No.
Methamidophos (+ -)	Racemic
Methamidophos (+)	307-1, 307-2, 307-5, 307-6 and Fr. II
Methamidophos (-)	306-1, 306-2, 306-6 and Fr. I

The purity of racemic methamidophos was 95.5%, but the purity of the enantiomers was not reported, except for a statement that they were optically pure. Each test compound was a white, water-soluble powder, stored at 22° C in the absence of light.

SYNONYMS: Tamaron, Monitor

REPORT NUMBER: 100280

SPONSOR: Mobay Corporation, Kansas City, Missouri.

TESTING FACILITY: Bayer AG, Wuppertal, West Germany.

TITLE OF REPORT: Methamidophos (Racemate and Enantiomers): Study for Effect on NTE (Neuropathy Target Esterase) in Hens Following Oral Administration

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*Methamidophos, an organophosphorus ester insecticide, is a racemic mixture of two stereoisomers, dextrorotary D (+) and levorotary L (-). This mixture can be separated (purified) into individual isomers (enantiomers), D (+) or methamidophos (+) and L (-) or methamidophos (-).

AUTHORS: W. Flucke and A. Eben

REPORT ISSUED: May 29, 1990

SUMMARY AND CONCLUSIONS:

Classification of study: Acceptable

This submission contains the results of five studies, ranging in duration from 2 to 22 days, which were conducted during February 9, 1988, and February 21, 1989. The test materials, administered orally as single doses to 8-month old hens were methamidophos (+ -) or racemic methamidophos: 50 mg/kg b.w.; methamidophos (+): 50, 100 or 400 mg/kg b.w.; methamidophos (-): 50, 200 or 400 mg/kg b.w.; TOCP (positive control): 100 or 300 mg/kg b.w.; and demineralized water (vehicle and negative control). The methamidophos compounds were administered in the presence of antidotes (atropine sulfate and 2-PAM chloride) because the doses used were otherwise lethal. Doses higher than 400 mg/kg b.w. could not be used, even under antidote protection, since no hen would have survived the treatment. Furthermore, the enantiomers could only be produced in limited amounts, a fact which also determined/limited the scope of the studies (number of hens, test times and dose levels).

The objective of these studies was to assess the neurotoxic potential of methamidophos by determining the following: 1) effects of the methamidophos compounds (racemate and enantiomers) on Neuropathy Target Esterase (NTE); 2) restoration (reactivation) potential of inhibited NTE; and 3) forms in which inhibited NTE occurs, whether the enzyme is modified (aged = incapable of being reactivated) or unmodified (unaged = capable of being reactivated). Point 1) was studied in brain, spinal cord, sciatic nerve and lymphocytes, and points 2) and 3) only in brain. According to the authors of this submission, more than 70-80% inhibition of NTE, as well as aging of the enzyme complex, is presumed necessary to trigger OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy or delayed neurotoxicity).

Methamidophos (+ -) and each enantiomer had low neurotoxic potential, even when ingested in lethal doses, for the following reasons:

1. Methamdiophos (+ -) at 50 mg/kg b.w. (twice the LD_{50} value of 25 mg/kg b.w.) inhibited NTE activity in brain homogenates 66% at 24 and 48 hours after treatment; 89% of the inhibited NTE could be reactivated; and most of the enzyme complex was present in the unmodified (unaged) form.

2. Methamidophos (+) at 400 mg/kg b.w. (about ten times the LD_{50} value of 43 mg/kg b.w.) inhibited NTE activity 97% at 24 hours and 98% at 48 hours after treatment; 86% of the

inhibited NTE could be reactivated; and most of the enzyme complex was present in the unmodified form.

3. Methamidophos (-) at 400 mg/kg b.w. (about five times the LD_{50} value of 82 mg/kg b.w.) inhibited NTE activity 58% at 24 hours and 84% at 48 hours after treatment; only a small fraction (about 27%) of the inhibited NTE could be reactivated at 48 hours after treatment; and most of the inhibited NTE (73%) was modified (aged). Based on these findings, this compound might be a possible trigger of OPIDP. According to the authors of this submission, because NTE inhibition of 84% and modification of the enzyme complex were observed only at a dose equivalent to five times the LD_{50} value, the neurotoxic potential of methamidophos (-) must be regarded as low.

TOCP (tricresyl phosphate, mixture of isomers; 300 mg/kg b.w.) inhibited NTE activity 97-100% at 24 hours after treatment; inhibited NTE could not be reactivated; and unchanged NTE was essentially absent.

EXPERIMENTAL PROCEDURES

Five experiments (studies) were conducted with methamidophos (racemate and enantiomers) in order to determine their effects on neuropathy target esterase (NTE) in the nervous tissue (brain, spinal cord and sciatic nerve) and lymphocytes of hens.

Unfasted Lohmann LSL strain hens (Lohmann Selected Leghorn; original breed: white leghorn) were treated by gavage with single doses of methamidophos compounds, demineralized water (control) or tricresyl phosphate (mixture of isomers; TOCP; positive control), as follows:

Study Number	Test Material	Dose (mg or ml/kg b.w.)	Number of Hens Treated	Study Period
T 3027743	Water Methamidophos (+ -) TOCP	5 ml* 50 300	6 6 4	Feb. 9-11, 1988
T 3029543	Water Methamidophos (+) Methamidophos (-) TOCP	5 ml 50 50 100	6 6 6 2	Mar. 21-23, 1988

T 2029722	Water Methamidophos (+) Methamidophos (-) TOCP	5 ml 400 400 300	11 13 13 2	May 17- June 7, 1988
T 4030335	Water Methamidophos (+)	5 ml 400	3 6	Oct. 18- 19, 1988
T 6032001	Water Methamidophos (+) Methamidophos (-)	5 ml 100 200	9 9 9	Feb. 14- 21, 1989

*Values represent mg/kg b.w. unless specified "ml".

The methamidophos test compounds, dissolved in demineralized water, were administered in the presence of antidotes, atropine sulfate and the combination of atropine sulfate and 2-PAM, as is detailed in Attachment I. The antidotes, also dissolved in demineralized water, were injected subcutaneously. The administration volume of the methamidophos compounds and TOCP was 5 ml/kg b.w. and of the antidotes 0.5 ml/kg b.w. The dose levels of the methamidophos compounds used were 2-10 times greater than their LD_{50} values.

NTE activity was determined at 24 hours, 48 hours and at 7 days (one study only) after treatment, using 3 hens/dose in the case of methamidophos compounds and water (control), and 1 or 2 hens/dose in the case of TOCP (positive control). In two studies, NTE determinations were performed in lymphocytes, brain, spinal cord and sciatic nerve, whereas in three studies only brain was studied for NTE activity, as follows:

Study Number	Determination of NTE Activity			
	Before Study Initiation	24 h	48 h	7 d
T 3027743 Lymphocytes Brain Spinal cord Sciatic nerve	+	+ + + +	+ + + +	
T 3029543 Brain		+	+	
T 2029722 Brain		+	+	
T 4030335 Brain		+		

T 6032001				
Lymphocytes	+	+	+	+
Brain		+	+	+
Spinal cord		+	+	+
Sciatic nerve		+	+	+

The hens for the NTE determination were selected from the groups in numerical order. However, the hens exhibiting the most severe toxic symptoms and not expected to survive were selected first.

In one high-dose study (T 2029722; 400 mg/kg b.w. dextrorotary and 400 mg/kg b.w. levorotary methamidophos), more hens were used than were required for the scheduled determination of the NTE activity. The hens not used for NTE determination were observed after treatment for the appearance of organophosphorus ester-induced delayed polyneuropathy (OPIDP). The results of these tests are described in another report (MRID 41685805; Report No. 100281).

NTE activity was determined in freshly prepared (unfrozen) lymphocytes and tissue homogenate, as is described briefly in Attachment II.

Reactivation of inhibited NTE and determination of unmodified inhibited NTE (unaged enzyme = could be reactivated) and modified inhibited NTE (aged enzyme = could not be reactivated) were performed in brain tissue homogenates, as is referenced in Attachment II.

The hens were:

1. Obtained from Brinkschulte Breeders, Senden, West Germany.

2. Acclimated for at least seven days.

3. At least 8 months old at the start of the study. Their mean initial weight was 1.26-1.88 kg.

4. Housed by test group in the floor-based cages, in a room with a heated floor, at temperature of 22° ± 8° C, relative humidity of about 25-95%, and light/dark cycle of 12 hours. Only during the initial 24 hours following treatment were the hens individually housed in standard hen cages.

5. Allowed unlimited food (pelletized Ssniff-LVK whole wheat feed for laying hens) and tap water (provided through an automatic system).

6. Assigned randomly to treatment groups.

7. Identified by consecutively numbered wing tabs.

8. Weighed prior to treatment and before sacrifice.

9. Sacrificed by decapitation and desanguinated before the removal of organs for the NTE determination.

No reference was made to the observations of hens for toxic signs and none were reported.

RESULTS

Mortality

Two hens died in study T 2029722 shortly after treatment with enantiomers at the 400 mg/kg level: one hen received methamidophos (+) and another methamidophos (-).

Body Weights

Relative to the body weights prior to treatment, hens treated with racemic methamidophos or enantiomers lost weight, whereas those treated with TOCP and controls lost little or no weight, as follows:

Test material (mg/kg/b.w.)	Mean percent weight loss at 48 hours after treatment*
Water (controls)	0-1.3
TOCP: 100	0
300	0-3.3
Methamidophos (+ -):	
50	9.0
Methamidophos (+):	
50	10.8
100	11.1
400	13.2
Methamidophos (-):	
50	7.2
200	14.5
400	13.1

*Based on Tables 9.1.1-9.1.5, pages 51-56 of the submission (Project No. 100280; MRID 41685804)

Inhibition of Neuropathy Target Esterase (NTE)

These data were reported as individual and mean values, in terms of activities (nMol phenylvalerate/min/g of tissue) and as percent inhibitions. Since only 3 hens/group were used (1 or 2 with TOCP), statistical analyses were not performed. The NTE inhibitions after 24 and 48 hours, and after 7 days (in one study only) of treatment are summarized below.

Mean Percent Inhibitions of NTE Activities Relative to Control Values*

Study No. and test material (mg/kg b.w.)	24 hours				48 hours			
	LYM	BR	SPC	SN	LYM	BR	SPC	SN
T 3027743								
Meth. (+ -): 50	60.8	66.0	57.7	84.7	29.0	65.7	60.0	71.3
TOCP : 300	98.8	99.7	97.9	100.0	53.2	91.0	87.5	95.4
T 3029543								
Meth. (+): 50		53.5				79.0		
Meth. (-): 50		23.5				18.0		
TOCP : 100		89.6				87.0		
T 2029722								
Meth. (+): 400		97.3				98.2		
Meth. (-): 400		57.6				83.7		
TOCP : 300		97.3				91.1		
T 4030335								
Meth. (+): 400		96.6						
T 6032001								
Meth. (+): 100	73.2	82.7	75.0	78.6	75.0	90.7	89.1	95.0
Meth. (-): 200	17.9	36.0	19.6	6.0	32.1	45.1	47.1	55.5
					7 days			
					0	52.1	45.4	45.8
					0	43.0	38.0	21.8

*This table is based on Table 1 (p. 23), Table 7 (p. 31), Table 9 (p. 34), Table 11 (p. 36) and Table 13 (p. 39) of the submission (Report No. 100280; MRID 41685804)

Meth. = Methamidophos LYM = lymphocytes; BR = brain
SPC = spinal cord; SN = sciatic nerve

38

According to the above data, at 24 and 48 hours after treatment with 50 mg/kg of racemic methamidophos, the highest inhibition of the NTE activity occurred in sciatic nerve, whereas the NTE activities were somewhat similarly inhibited in brain and spinal cord. The inhibition of NTE activity in lymphocytes was similar initially to that observed in spinal cord and brain, but halved 24 hours later.

In the case of the enantiomers, methamidophos (+) at 100 mg/kg dose level was a more potent inhibitor of NTE activity in all tissues studied than metamidophos (-) at 200 mg/kg dose level. At 48 hours after treatment with either enantiomer, the lowest inhibition of NTE activity was observed in the lymphocytes, whereas the NTE activities were similarly inhibited in brain, spinal cord and sciatic nerve. At 7 days after treatment with either enantiomer, NTE activity was no longer inhibited in lymphocytes, but was still inhibited in other tissues, to a lesser degree in most instances. Racemic methamidophos was not studied at the 100 or 200 mg/kg b.w. doses (hens would probably not live very long).

The inhibition of brain NTE activity by methamidophos (+) or methamidophos (-) was dose-related and was higher with methamidophos (+), especially at 24 hours after treatment.

At the 50 mg/kg dose, the only dose used for all three methamidophos compounds, methamidophos (+ -) or methamidophos (+)--depending on the time interval tested--inhibited NTE activity most, whereas methamidophos (-) inhibited NTE activity least, irrespective of when determinations were made.

TOCP (positive control) inhibited NTE activity in all tissues. A comment was made that TOCP, administered without antidotes, was tolerated without symptoms.

Reactivation of Inhibited NTE

These studies were performed by incubating treated brain homogenates with potassium fluoride or potassium chloride. NTE activities obtained with potassium fluoride represented reactivations, whereas those obtained with potassium chloride were considered as control values. The results of these studies are summarized below.

Mean Restored NTE Activities Expressed as Percentages of NTE Activities of Untreated Brain Homogenates*

Time after treatment	24 hours		48 hours	
Study No. and test material (mg/kg b.w.)	With KCl	With KF	With KCl	With KF
T 3027743				
Methamidophos (+ -): 50	30.6	86.7	32.1	89.3
TOCP : 300	6.4	7.3	14.9	17.9
T 3029543				
Methamidophos (+): 50	41.8	87.9	22.0	93.4
Methamidophos (-): 50	87.2	90.7	84.8	78.2
TOCP : 100	13.0	19.2	22.7	23.3
T 2029722				
Methamidophos (+): 400	2.9	86.4	1.4	82.4
Methamidophos (-): 400	40.3	48.7	15.9	26.9
TOCP : 300	7.6	9.9	15.2	17.8
T 4030335				
Methamidophos (+): 400	3.7	86.0	--	--
T 6032001				
Methamidophos (+): 100	16.3	84.3	9.5	100.0
Methamidophos (-): 200	59.7	67.4	54.6	62.9
			7 days	
			51.2	99.0
			55.9	62.7

*This table is based on Table 6 (p. 28), Table 8 (p. 32), Table 10 (p. 35), Table 12 (p. 37) and Table 19 (p. 45) of the submission (Report No. 100280; MRID 41685804).

Comparing NTE activities in brain homogenates incubated with potassium chloride or potassium fluoride, most of the inhibited NTE could be restored (reactivated) in the case of methamidophos (+ -) and methamidophos (+), but very little NTE activity could be restored in the case of methamidophos (-) and positive control (TOCP).

Unmodified Inhibited (UI) NTE and Modified Inhibited (MI) NTE*

These two forms of NTE, UI (unaged or capable of being reactivated) and MI (aged or incapable of being reactivated) were calculated by the authors of this submission, from the data obtained in the NTE reactivation studies. The calculations were performed because, according to these authors, more than 70-80% inhibition of NTE, as well as aging of the enzyme complex, is presumed necessary to trigger OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy or delayed neurotoxicity). The findings obtained are summarized below.

Mean Percent Distribution of MI-NTE and UI-NTE in Brain Homogenate*

Time after treatment	24 hours		48 hours	
Study No. and test material (mg/kg b.w.)	MI-NTE	UI-NTE	MI-NTE	UI-NTE
T 3027743				
Methamidophos (+ -): 50	13.3	56.1	10.7	57.2
TOCP : 300	92.8	0.9	82.2	3.0
T 3029543				
Methamidophos (+): 50	12.1	46.1	6.6	71.4
Methamidophos (-): 50	9.3	3.5	21.8	0
TOCP : 100	80.8	6.2	76.7	0.6
T 2029722				
Methamidophos (+): 400	13.6	83.5	17.6	81.0
Methamidophos (-): 400	51.3	8.4	73.1	3.3
TOCP : 300	90.1	2.3	82.2	2.6
T 4030335				
Methamidophos (+): 400	14.0	82.3	--	--
T 6032001				
Methamidophos (+): 100	15.7	68.0	0	90.5
Methamidophos (-): 200	32.6	7.7	37.1	8.3
			7 days	
			1.0	47.8
			37.3	6.8

*This table is based on Table 6 (p. 28), Table 8 (p. 32), Table 10 (p. 35), Table 12 (p. 37) and Table 19 (p. 45) of the submission (Report No. 100280; MRI 41685804).

41

According to the above data, brain homogenates treated with methamidophos (+ -) or methamidophos (+) had more of the unmodified inhibited NTE than did those treated with methamidophos (-) or TOCP, in which inhibited NTE occurred chiefly in a modified form.

COMMENTS

This submission contains results of five individual experiments (studies), ranging in duration from 2 to 22 days, and is rather involved. The studies were conducted between February 9, 1988, and February 21, 1989. The test materials used were methamidophos (+ -), methamidophos (+) and methamidophos (-); the last two compounds were obtained by purification of methamidophos (+ -). Although data in most instances are presented clearly and English translation of the original German version is provided, some procedures (like those used in the reactivation experiments) are not described, but only referenced. Since these tests are currently not required by EPA, the absence of experimental details makes the understanding and interpretation of the reported findings somewhat difficult.

According to the registrant (Mobay Corporation), this study was not conducted in compliance with the Good Laboratory Practice Standards of 40 CFR part 160 (FIFRA). Nevertheless, it meets these standards, with two exceptions:

1. No GLP inspections were performed by the quality assurance unit.

2. The test substances were not analyzed for homogeneity, stability and concentration in the test solutions.

Since this study is specialized and not routinely submitted in support of regulatory actions, the lack of GLP inspections by Quality Assurance Unit is not critical to accepting the reported data as valid.

Since the test materials were solutions which were used immediately after preparation, the lack of data on homogeneity and stability should not affect the experimental results.

A statement of no claim of data confidentiality and a flagging statement were also submitted. According to the latter, this study neither meets nor exceeds any of the applicable criteria.

Missing Data

Page 24 of the submission, containing Table 2 (activity and inhibition of NTE in brain homogenates: individual data) is missing.

4/2

Attachment I

METHAMIDOPHOS

Page 44 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Attachment II

METHAMIDOPHOS

Page 46 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

GUIDELINE: Related to 81-7

Primary Review by: Krystyna K. Locke, Review Section I, Toxicology Branch I/HED

Secondary Review by: Roger Gardner
Section Head, Review Section I, Toxicology Branch I/HED

DATA EVALUATION RECORD

STUDY TYPE: OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy) - Hen

EPA IDENTIFICATION NOS: Tox. Chem. No.: 378A
MRID No.: 41685805

TEST MATERIAL: Methamidophos: racemic mixture and enantiomers, as follows:

Test Material*	Batch No.
Methamidophos (+ -)	Racemic
Methamidophos (+)	307-1, 307-2 and 307-3
Methamidophos (-)	306-1, 306-2 and 306-4

The purity of racemic methamidophos was 95.5%, but the purity of the enantiomers was not reported, except for a statement that they were optically pure. Each test compound was a white, water-soluble powder, stored at 22° C in the absence of light.

SYNONYMS: Tamaron, Monitor

REPORT NUMBER: 100281

SPONSOR: Mobay Corporation, Kansas City, Missouri.

TESTING FACILITY: Bayer AG, Wuppertal, West Germany.

TITLE OF REPORT: Methamidophos (Racemate and Enantiomers): Study for OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy): Exploratory Studies on Hens

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*Methamidophos, an organophosphorus ester insecticide, is a racemic mixture of two stereoisomers, dextrorotary D (+) and levorotary L (-). This mixture can be separated (purified) into individual isomers (enantiomers), D (+) or methamidophos (+) and L (-) or methamidophos (-).

AUTHORS: W. Flucke

REPORT ISSUED: April 10, 1990

SUMMARY AND CONCLUSIONS:

Classification of study: Acceptable as an OPIDP study, related to EPA Guideline No. 81-7.

Not Acceptable as an acute delayed neurotoxicity study to satisfy regulatory data requirements for EPA Guideline No. 81-7.

This study (actually four studies ranging in duration from 22 to 56 days each) was submitted under EPA Guideline No. 81-7, but it does not meet the requirements of this guideline. Rather, it is a special study.

The methamidophos compounds were administered (under antidote protection) as single doses to the 8-month old hens and the hens were observed for 3-4 weeks for signs of OPIDP development. It was found that: 1) methamidophos (+ -) induced OPIDP only at a dose equivalent to 16 times the LD_{50} value; 2) methamidophos (+) was more neurotoxic than racemic methamidophos and determined, therefore, the delayed neurotoxic potential of racemic methamidophos; and 3) methamidophos (-) did not have delayed neurotoxic potential (based on limited data). These findings are summarized below:

Test Materials	Number of Hens*	Dose (mg/kg b.w.)	OPIDP**	LD_{50}*** (mg/kg b.w.)	Dose/LD_{50} (Factor)
Methamidophos (+ -)	9/10 3/10	200 400	0 +	25	> 8 16
Methamidophos (+)	5/5 5/5 4/13	100 200 400	0 (+) ++	43	> 2.3 ≥ 4.6 9.3
Methamidophos (-)	2/23	400	0	82	> 4.9

*Number of hens observed after treatment/Number of hens treated

**Degree of severity:
0 = Negative
(+) = Low, reversible
\+ = Low to moderate
++ = High

***From Report No. 100279, MRID 41685803

4/8

Based on the above data, the testing facility and the registrant concluded that methamidophos (+ -), that is, racemic methamidophos, methamidophos, Monitor or Tamaron, has very low potential for neuropathy (delayed neurotoxicity). Toxicology Branch/HED agrees. However, it should be noted that these studies were not performed with technical grade methamidophos (73-75% a.i.), but with highly purified compound (95.5% a.i.).

EXPERIMENTAL PROCEDURES

Four experiments (studies) were conducted with methamidophos (racemate and enantiomers) in order to assess their neurotoxic potential in terms of clinical monitoring of OPIDP development.

Unfasted Lohmann LSL strain hens (Lohmann Selected Leghorn; original breed: white leghorn) were treated by gavage with single doses of methamidophos compounds, demineralized water (control) or tricresyl phosphate (mixture of isomers; TOCP; positive control), as follows:

Study Number	Test Material	Dose (mg or ml/kg b.w.)	Number of Hens Treated	Study Period
T 3029958	Methamidophos (+)	100* 200	5 5	July 6-Aug. 30, 1988
T 2029722**	Water Methamidophos (+) Methamidophos (-) TOCP	5 ml 400 400 300	11 13 13 2	May 17-June 7, 1988
T 2029957	Methamidophos (-)	400	10	Aug. 2-30, 1988
T 1029956	Methamidophos (+ -)	200 400	10 10	Aug. 2-Sept. 13, 1988

*Values represent mg/kg b.w. unless specified "ml".

**Hens in study T 2029722, 6/group, and both of the TOCP-treated hens, were used for NTE (neuropathy target esterase) activity determination (Report No. 100280; MRID 41685804). The remaining hens (5 in the control group and 7 in each of the methamidophos groups) were observed after treatment for the appearance of OPIDP and the results are described in this submission. Although hens in the NTE study were sacrificed in numerical order, those with most severe toxic signs and not expected to live were sacrificed first.

The methamidophos test compounds, dissolved in demineralized water, were administered in the presence of antidotes, atropine sulfate and the combination of atropine sulfate and 2-PAM, as is detailed in Attachment I. The antidotes, also dissolved in demineralized water, were injected subcutaneously. The administration volume of the methamidophos compounds was 5 ml/kg b.w. and of the antidotes 0.5 ml/kg b.w. The dose levels of the methamidophos compounds used were 2.3-16 times greater than their LD_{50} values.

The hens were:

1. Obtained from Brinkschulte Breeders, Senden, West Germany.

2. Acclimated for at least five days.

3. At least 8 months old at the start of the study. Their mean initial weight was 1.27-1.73 kg.

4. Housed by test group in the floor-based cages, in a room with a heated floor, at temperature of 22° ± 8° C, relative humidity of about 25-95%, and light/dark cycle of 12 hours. Only during the initial 24 hours following treatment were the hens individually housed in standard hen cages.

5. Allowed unlimited food (pelletized Ssniff-LVK whole wheat feed for laying hens) and tap water (provided through an automatic system).

6. Assigned randomly to treatment groups.

7. Identified by consecutively numbered wing tabs.

8. Weighed prior to treatment (day 1) and at weekly intervals thereafter. The hens of study T 2029722 were also weighed on study days 2 and 3.

9. Observed for appearance, behavior and mortality several times on the day of treatment and at least once daily thereafter. The post-treatment observation period was 28 days or 21 days (study T 2029722).

In order to assess the motor coordination, with special emphasis on ataxia and paresis, the hens were individually driven around in a stall area of about 100 m^2 for about 2-3 minutes at least twice weekly, and scored according to the following scale:

0 = normal
1 = slightly abnormal gait
2 = ataxia / disturbance in motor coordination
3 = pronounced ataxia / paresis (frequent buckling of legs, collapse of the animal)
4 = complete paralysis (inability to run)

The hens were sacrificed on day 29 and all were necropsied. The nonsurvivors were also necropsied.

RESULTS

Toxic Symptoms

The following symptoms, regarded as acute intoxication, were observed in hens treated with the methamidophos compounds: apathy, ruffled feathers, staggering gait, diarrhea; rapid, shallow breathing; flat/lateral prostration, spasms, salivation, labored breathing, dry and limp comb; and occasional random wingbeats (only with the levorotary enantiomer). In most instances, all of these symptoms were observed in all groups and were classified as moderate (staggering gait, rapid shallow breathing and spasms) or severe (remaining symptoms). The onset of symptoms ranged from 20 min. to 1 hour after treatment and their duration ranged from 4 to 22 days, as follows:

Test Material (mg/kg b.w.)	Toxic Symptoms	
	Onset (min.)*	Last Observed (day)*
Methamidophos (+ -):		
200	20	10
400	60	13
Methamidophos (+):		
100	20	4
200	25	13
400**	35	22
Methamidophos (-):		
400	30	17
400**	45	10

*Based on Table 1, page 16 of this submission (No. 100281; MRID 41685805).
**Hens in these groups are from the NTE determination study T 2029722 (Report No. 100280; MRID 41685804).

Hens in the control group behaved normally and no changes in appearance could be detected.

Mortality

With the exception of the control and two of the methamidophos (+) groups, deaths occurred in all of the remaining groups, despite intensive antidote treatment, as follows:

51

Study Number	Test Material (mg/kg b.w.)	Total Dead*	Percent	Time of Death*
T 1029956	Methamidophos (+ -):			
	200	2/10	20	3d and 28d**
	400	7/10	70	1-4 d
	Methamidophos (+):			
T 3029958	100	0/5	0	--
	200	0/5	0	--
T 2029722	400	3/13	23	7h - 5d
	Methamidophos (-):			
T 2029722	400	6/13	46	2h - 5d
T 2029957	400	9/10	90	2-6d
	Water (Control group):			
T 2029722	5 ml/kg b.w.	0/11	0	--

*Based on Table 1, page 16 of the submission (No. 100281; MRID 41685805).
**This mortality was regarded by the testing laboratory as intercurrent and was not included in Table 1. h = hour; d = day.

Body Weights

Relative to the body weights prior to treatment (day 1), hens treated with the methamidophos compounds lost weight, in a dose-unrelated manner, during the first week after treatment. In some groups, hens regained their weight by the third week and, in other groups, at the termination of the study (week 4). Hens in the control group neither lost nor gained weight during the course of the study. The weight losses and gains of the methamidophos-treated hens are summarized below.

Test Material (mg/kg b.w.)	Mean Percent Weight Loss (↓) or Gain (↑) during the Post-Treatment Day Shown*		
	8 or 9	22	29
Methamidophos (+ -):			
200	28 ↓	6 ↓	2 ↓
400	27 ↓	0**	5 ↑
Methamidophos (+):			
100	16 ↓	0**	3 ↑
200	8 ↓	12 ↑	15 ↑
400	19 ↓	15 ↓#	--
Methamidophos (-):			
400##	17 ↓	3 ↓#	--
400##	29 ↓	8 ↑	7 ↑

*This table is based on Tables 10.1.1 - 10.1.4, pages 30-34 of the submission (No. 100281; MRID 41685805).

**Body weight of day 1 was regained.

#Hens in these groups were from study T 2029722 (NTE inhibition study;

52

Report No. 100280; MRID 41685804) which was terminated 22 days after treatment.

##Weight changes in these groups are for one hen only because the remaining hens either died by day 7 or were sacrificed for NTE determination.

Clinical Neurotoxicity Examination
Forced activity (driving of experimental animals)

The development of OPIDP (delayed neurotoxicity) was assessed in terms of motor coordination, with special emphasis on ataxia and paresis (frequent buckling of the legs and collapse of the hen). These examinations were performed on study days 1, 4, 8, 11, 15, 18, 22, 25 and 28 (in some groups). The following findings were observed:

Methamidophos (+):
(mg/kg b.w.)

100 - No evidence of OPIDP.
200 - Slightly abnormal gait during days 14-25 and none on day 28 in 4 out of 5 hens studied.
400 - Distinct signs of OPIDP, starting with day 8 were observed. On day 18, 2 of the surviving 4 hens were totally paralyzed; another hen had marked ataxia and paresis; and the 4th hen was ataxic and had distinct motor coordination disturbances.

Methamidophos (-):
(mg/kg b.w.)

400 - No evidence of OPIDP was observed in the 2 surviving hens out of the 23 hens treated (13 in study T 2029722 and 10 in study T 2029957). However, hens with the most severe toxic symptoms were sacrificed for the determination of NTE activity in study T 2029722.

Methamidophos (+ -):
(mg/kg b.w.)

200 - No evidence for development of OPIDP.
400 - Developing OPIDP was observed starting on day 18 and continuing until the termination of the study. Of the 3 surviving hens, 2 had slightly abnormal gait, and the 3rd hen had distinct ataxia and disturbance in motor coordination.

No developing OPIDP was observed in hens treated with demineralized water (control group).

Necropsy Findings

The following hens, which died before the termination of the study, were not examined or were only partially examined because of tissue autolysis ("were putrid") or cannibalism:

Methamidophos (+ -):

200 mg/kg b.w. - Of the 2 nonsurvivors in study T 1029956, 1 had autolysis of the GI tract and another was cannibalized.

Methamidophos (-):

400 mg/kg b.w. - In study T 2029722, 3 of the 6 nonsurvivors were autolyzed. In study T 2029957, 2 of the 9 nonsurvivors were autolyzed and 7 had autolysis of the GI tract.

No abnormalities were observed in the untreated hens (control group) and generally in the treated hens sacrificed at the termination of the study. However, abnormalities were observed in some of the survivors, as follows:

Methamidophos (+ -):

In study T 1029956, 4 of the 8 survivors in the 200 mg/kg b.w. group and the 3 survivors in the 400 mg/kg b.w. group had pale livers, lobulated and patchy in some cases.

Methamidophos (+):

In study T 3029958, in the 200 mg/kg b.w. group, 1 of the 5 survivors had slightly pale liver and another survivor had lobulated liver and enlarged kidneys permeated with fluid-filled blisters about 2 mm in diameter.

The following abnormalities were observed in hens which died during the post-treatment observation period:

1. Distended, fluid-filled lungs; reddened duodenal mucosa; and pale spleen, patchy in some cases; ---- in hens treated with methamidophos (+ -), methamidophos (+) or methamidophos (-).

2. Pale or dark liver, lobulated, occasionally friable or patchy; and distended crop; ---- in hens treated with methamidophos (+ -) or methamidophos (-).

3. Ulcer-like foci in stomach; ---- in hens treated with methamidophos (+ -).

4. Fluid-filled pericardium; and helminthic gizzard; ---- in hens treated with methamidophos (+).

5. Kidneys occasionally dark or pale and patchy; and egg concentrations in abdominal cavity; ---- in hens treated

with methamidophos (-).

COMMENTS

This submission contains results of four individual experiments (studies), ranging in duration from 22 to 56 days. The studies were conducted between May 17, 1988, and September 13, 1988. The test materials used were methamidophos (+ -), methamidophos (+) and methamidophos (-); the last two compounds were obtained by purification of methamidophos (+ -). In most instances, results are presented clearly and accurately, and English translation of the original German version is provided.

According to the registrant (Mobay Corporation), this study was not conducted in compliance with the Good Laboratory Practice Standards of 40 CFR part 160 (FIFRA). Nevertheless, it meets these standards, with two exceptions:

1. No GLP inspections were performed by the quality assurance unit.

2. The test substances were not analyzed for homogeneity, stability and concentration in the test solutions.

Since this is a specialized study and not one that would be routinely submitted in support of regulatory actions, the lack of GLP inspections by Quality Assurance Unit is not critical to accepting the reported data as valid.

Since the test materials were solutions which were used immediately after preparation, the lack of data on homogeneity and stability should not affect the experimental results.

A statement of no claim of data confidentiality and a flagging statement were also submitted. According to the latter, this study meets or exceeds the criteria numbered 6 (Pesticide assessment guideline No. 81-7: When compared with controls, treated animals show a response indicative of acute delayed neurotoxicity).

This study (actually results of four studies) was submitted as data requirement for EPA Guideline No.: 81-7. However, this study does not meet several of the December 24, 1989 acceptance criteria for Guideline No.: 81-7, Acute Neurotoxicity in the Hen (see Attachment II). Although some of these criteria are supplemental (not required for every study) and some were almost met, the use of technical form of the active ingredient and histopathology performed on all animals are required criteria and were not met. Histopathology was not performed in this study and highly purified test materials were used.

55

Inaccurate Data

1. It is shown on page 12 of this submission that TOCP (tricresyl phosphate; mixture of isomers) was used in study T 2029722 at a dose of 100 mg/kg b.w. Actually the dose used was 300 mg/kg b.w. (see Report No. 100280, page 15; MRID 41685804).

2. According to page 26 of this submission, hens treated with methamidophos (+ -) which died during the post-treatment observation period had, among other symptoms, water bubble in abdominal cavity. According to page 43 of this submission, water bubble in abdominal cavity was observed in one hen sacrificed at the termination of the study (day 29).

56

Attachment I

METHAMIDOPHOS

Page 58 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Attachment II

MRID NO.: 416838-03
Study No.: 100281
Study Date: 4/10/90

Subdivision F
Guideline Ref. No. 81-7
December 24, 1989

81-7 Acute Neurotoxicity in the Hen

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. ✓ Study performed on an organophosphate cholinesterase inhibiting compound.
2. N Technical form of the active ingredient tested.
3.* N Positive control utilized.
4. ✓ Species utilized, domestic laying hen 8-14 months of age.
5. ✓ Dosing oral by gavage or capsule (dermal or inhalation may be used).
6. ✓ An acute oral LD_{50} is determined.
7. N Dose tested equal to an acute oral LD_{50} or a limit test of 5000 mg/kg.
8.* ✓ Dosed animals may be protected with atropine and/or 2-PAM.
9. ✓ Sufficient test animals so that at least 6 survive.
10. N Negative (vehicle) control group of at least 6 hens
11.* N Positive control of at least 4 hens. (if used)
12. — Test dose repeated if no signs of delayed neurotoxicity observed by 21 days after dosing.
13. ✓ Observation period 21 days after each dose.
14. ✓ Individual daily observations.
15. ✓ Individual body weights.
16.* ✓ Individual necropsy not required.
17. N Histopathology performed on all animals. Tissue to be fixed *in situ* preferably using whole animal perfusion techniques. At least three sections of each of the following tissues:
    ____brain, including medulla oblongata
    ____spinal cord; upper cervical, mid-thoracic and lumbro-sacral regions
    ____tibial nerve; proximal regions and branches
    ____sciatic nerve

None of the hens was examined microscopically.

Criteria marked with a * are supplemental and may not be required for every study.

60