3-18-95

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

#378A 41170

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methamidophos (Monitor): 81-8 SS Acute Neurotoxicity Studies and Related Data

DP Barcode Nos. D198115, D199019 and D206974
Submission Nos. S456136, S457625 and S472569

EPA ID No. 101201
Rereg. Case No. 0043
CAS Registry No. 10265-92-6

P.C. Code No. 101201
Case No. 819351
Tox. Chem. No. 378 A

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)
Krystyna K. Locke 3/8/95

TO: Larry Schnaubelt / Robert Richards, PM Team No. 72
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)
Roger Gardner 3/10/95 3/18/95

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

81-8 SS An Acute Oral Neurotoxicity Screening Study with Technical Grade Methamidophos (MONITOR®) in Rats. B.F. Hamilton; Miles Inc., Agricultural Division, Stilwell, Kansas, Missouri; Report No. 105053 and 92-412-QL; Date of Report: November 5, 1993. MRID No. 43025001

81-8 SS An Acute Oral Neurotoxicity Screening Study with Technical Grade Methamidophos (MONITOR®) in Rats. L.P. Sheets; Miles Inc., Agricultural Division, Stilwell, Kansas, Missouri; Report No. 105053-1 and 94-412-YW; Date of Report: August 12, 1994. MRID No. 43345801

81-8 SS and 82-5 b Historical Control and Method Validation Studies in Rats for the Acute and Subchronic Neurotoxicity Screening Battery. L.P. Sheets, B.P. Stuart and S.G. Lake; Miles Inc., Agricultural Division, Stilwell, Kansas, MO.; Report No. 103979; Date of Report: March 31, 1993. MRID No. 42770301

In the first study (MRID No. 43025001), Methamidophos was administered in a single gavage dose to male and female Sprague-Dawley rats at 0, 1, 3 and 8 mg/kg (nominal levels; analytical levels were 0, 0.9, 3.3 and 9.0 mg/kg, respectively). These rats were assessed for neurobehavioral functions; cholinesterase activities in serum, erythrocytes and brain; various hematological and clinical chemistry parameters; and also examined grossly and histopathologically. Because cholinergic toxicity was observed at all levels tested, a second study was undertaken to determine a NOEL.

In the second study (MRID No. 43345801), Methamidophos was administered in a single gavage dose to male and female Sprague-Dawley rats at 0, 0.3 and 0.6 mg/kg (nominal levels; analytical levels were 0, 0.3 and 0.7 mg/kg, respectively). Relative to the control values, Methamidophos at the 0.3 mg/kg dose had no effect on any of the parameters examined. Compared with the controls, the 0.7 mg/kg dose had no effect on the neurobehavioral parameters tested, but cholinesterase activities in plasma, erythrocytes and brain were inhibited singificantly.

The third submission (MRID No. 42770301) was not reviewed separately. Only the historical control and method validation data for the acute neurotoxicity screening battery were reviewed and the findings included in the evaluation of the first study (MRID No. 43025001).

Considered individually, neither of the above studies meets the guideline requirement for an acute neurotoxicity screen (81-8 SS). Considered together, this requirement has been satisfied. Also, the first study considered together with the second study, and vice versa, can be classified as Core-Minimum. Based on the results of both studies, the NOEL and LOEL for neurobehavioral effects are 0.7 mg/kg and 0.9 mg/kg (analytical values), respectively, for both sexes. The NOEL and LOEL for cholinesterase activities (brain, erythrocytes and plasma) are 0.3 mg/kg and 0.7 mg/kg, respectively, for both sexes.

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Krystyna K. Locke 3/8/95

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

Roger Gardner 3/10/95

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

DATA EVALUAATION RECORD

STUDY TYPE: 81-8 (SS) Acute Neurotoxicity Screening Battery

EPA IDENTIFICATION NUMBERS:

MRID No. 43345801
DP Barcode No. D206974
Submission No. S472569
P.C. Code No. 101201
CAS Registry No. 10265-92-6

Rereg. Case No. 0043
Case No. 819351
I.D. No. 101201
Tox. Chem. No. 378 A

TEST MATERIAL: Methamidophos Technical (O,S-Dimethyl phosphoramidothioate); Purity: 75.6%; Batch No. 0-06-7009; Clear, colorless liquid; stored at freezer conditions, at which it is stable for at least 3 years.

SYNONYM(S): Monitor

SPONSOR: Miles Inc., Agricultural Division, Kansas City, MO

STUDY NUMBERS: 105053-1 and 94-412-YW

TESTING FACILITY: Miles Inc., Agricultural Division, Toxicology, Stilwell, Kansas

TITLE OF REPORT: An Acute Oral Neurotoxicity Screening Study with Technical Grade Methamidophos (MONITOR®) in Rats

AUTHOR: L.P. Sheets

STUDY COMPLETION DATE: August 12, 1994

EXECUTIVE SUMMARY: In an acute (supplemental) neurotoxicity screening study (MRID 43345801), Methamidophos (75.6% a.i.) was administered in a single gavage dose to 18 male and 18 female Sprague-Dawley rats at 0, 0.3 and 0.7 mg a.i./kg (analytical values; nominal values: 0, 0.3 and 0.6 mg a.i./kg, respectively). Twelve rats/sex/dose were assessed for neurobehavioral functions at about 2 hours postdosing and on days 7 and 14. Six rats/sex/dose were used for the determination of cholinesterase (ChE) activities in plasma and erythrocytes at about 2 weeks before dosdosing, and in plasma, erythrocytes and brain at about 2 hours after dosing. Other parameters examined were clinical observations and body weights. Gross necropsy and histopathology were not performed because nothing remarkable was observed in another (main) rat acute neurotoxicity screening study at 9.0 mg of Meth-

amidophos (a.i.)/kg (MRID 43025001).

Relative to the control values, Methamidophos at the 0.3 mg/kg dose had no effect on any of the parameters examined. The 24% inhibition of plasma ChE activity for the females in this group was statistically insignificant, was due to an unusually high control value and was, therefore, regarded by the testing facility (and Tox. Branch/HED) as biologically insignificant.

Relative to the control values, the 0.7 mg/kg dose had no effect on the neurobehavioral parameters examined, but the ChE activities were inhibited significantly ($p \leq 0.05$) in males and females (M/F) as follows: erythrocytes (21/26%), plasma (27/25%) and brain (15/26%).

This study should be considered together with another acute neurotoxicity study (MRID 43025001) in which a NOEL for neurobehavioral effects was not determined. Based on the results of both studies, a NOEL for neurobehavioral effects is 0.7 mg/kg and a LOEL is 0.9 mg/kg (analytical value; nominal value = 1.0 mg/kg), for males and females. The NOEL and LOEL for ChE activities are 0.3 mg/kg and 0.7 mg/kg, respectively. This study, considertogether with the first study, is classified as Core-Minimum and satisfies the guideline requirement for an acute neurotoxicity screen (81-8 SS).

EXPERIMENTAL PROCEDURES:

The current study, regarded by the registrant as supplemental to the original (main) study, was started on April 11, 1994 and terminated on April 28, 1994. The main study (MRID 43025001) was completed on November 5, 1993.

Fasted male and female Sprague-Dawley rats, 18/sex/dose, received single doses of Methamidophos by gavage as follows: 0 (vehicle), 0.3 and 0.7 mg/kg (analytical values; the nominal values were 0.3 and 0.6 mg/kg). Methamidophos was administered in 0.5% (w/v) methylcellulose with 0.4% (w/v) Tween 80 in deionized water, in a volume of 10 ml/kg. Twelve rats/sex/dose were used for neurobehavioral testing and the remaining rats (6/sex/dose; satellite group) for cholinesterase determinations.

The doses used in the current study were based on the results obtained in an earlier study (main study; MRID 43025001), in which analytically-confirmed doses of 0.9, 3.3 and 9.0 mg/kg were tested (the nominal doses were 1, 3 and 8 mg/kg). In that study, the low dose of 0.9 mg/kg produced inhibitions of cholinesterase activities in serum, erythrocytes and brain, and sightly reduced motor/locomotor activity, each in males and females. In the current study, the rats were:

(1) Obtained from Sasco, Inc., St. Louis, MO.;

(2) Acclimated for at least 6 days prior to treatment;

(3) Nine weeks old at the time of treatment;

(4) Housed individually in suspended stainless steel wire-mesh cages, at temperature of 18-26°C, relative humidity of 40-70% and 12-hour light/dark cycle;

(5) Assigned to groups on the weight basis, using software from INSTEM Computer Systems;

(6) Identified by cage cards and tailmarks; and

(7) Fed unrestricted amounts of food (Purina Rodent Laboratory Chow 5001-4) and water (automatic system).

The following parameters were examined:

(1) Clinical observations: at least once daily;

(2) Body weight: weekly;

(3) Motor/locomotor activity (figure-eight maze): one week prior to treatment, about 90 minutes after administration of the test material (Day 0), and at 7 and 14 days after treatment;

(4) Functional observational battery (FOB; home cage observations, observations during handling, open field observations, reflex / physiological observations, landing foot splay and grip strength): at the same time intervals as motor activity; and

(5) Cholinesterase activity (satellite group): approximately 2 weeks before dosing in plasma and erythrocytes, and approximately at 2 hours after dosing (Day 0) in plasma, erythrocytes and brain. This time was selected to provide a measure of cholinesterase activity when rats underwent neurobehavioral evaluation and corresponded to cholinesterase determinations in the main study.

Rats used for neurobehavioral testing were sacrificed after the FOB and motor activity tests (14-15 days after dosing). Rats in the satellite group were sacrificed at approximately 2 hours after dosing. Gross necropsy and histopathology were not performed because nothing remarkable was observed in the main study at dose of 9.0 mg/kg (HDT).

The statistical analyses, the procedures used in motor activity and FOB testing, and the validation of these procedures, were the same as those in the main study. All of these procedures, but cholinesterase determinations, were referenced in the current submission. According to the review of the main study (p. 13), cholinesterase determinations were made spectrophotometrically using modifications of the Ellman method, but the procedure was neither described nor referenced.

A Quality Assurance Statement and a GLP Statement, each signed and dated August 8, 1994, were included in the currently submitted report.

RESULTS:

Clinical Observations

Treatment-related clinical signs were not observed. Perianal stain was observed in one control male and red nasal stain, in one male from the 0.6 mg/kg group. In the case of females, one rat in the 0.3 mg/kg group had urine stains and another rat in the same group had red lacrimal stains. There were no unscheduled deaths.

Body Weight

Body weight gains of the female rats were not affected by treatment and the decreases observed in the male rats, when compared with the controls, were statistically insignificant. At the termination of the study, female rats in the control, 0.3 mg/kg and 0.6 mg/kg groups gained 34, 30 and 33 g, respectively. The corresponding weight gains for the male groups were 77, 72 and 60 g, respectively. Relative to the control value, male rats in the treated groups gained 7% and 22%, respectively, less weight than did the controls.

Motor/Locomotor Activities

Motor/locomotor activities were not affected by treatment. These data (Tables 5 and 6 of the submitted report) are in Attachment I of this review.

Functional Observational Battery (FOB)

Treatment-related effects were not observed in these tests. The findings for the 90-minute interval (Day 0) are in Attachments II and III (Tables 3 and 4, respectively, of the submitted report) of this review. Table 3 contains data concerned with the home cage observations, observations during handling, open field observations and reflex / physiological observations. Table 4 contains the grip strength and footsplay data.

Cholinesterase Activities

Relative to the control values, statistically significant decreases in erythrocyte, plasma and brain cholinesterase activities (RChE, PChE and BChE, respectively) were observed only in the 0.6 mg/kg group, in both males and females. The decreases in cholinesterase activities, observed in the 0.3 mg/kg group, were statistically insignificant. The results of cholinesterase determinations are shown below.

Nominal Dose # (mg/kg)	Pretreatment		Day 0		
	RChE	PChE	RChE	PChE	BChE
MALE RATS					
0.3	-3	+4	-5	-6	+1
0.6	-4	+25	-21*	-27*	-15*
FEMALE RATS					
0.3	+4	-11	-8	-24	-6
0.6	-3	+8	-26*	-25*	-26*

The above table appears on page 23 of the submitted report (MRID 43345801). Values in the table represent percent (%) differences from the control values. Cholinesterase activities are in Attachment IV of this review.

Analytical doses were 0.3 and 0.7 mg/kg. * Statistically different from control: $p \leq 0.05$. Based on N=6/sex/dose.

With the exception of the BChE inhibition in the high-dose males, the remaining statistically significant cholinesterase inhibitions in the high-dose group were considered by the testing facility to be biologically significant. Because of the small magnitude of the difference (<20%), the 15% BChE inhibition was not considered to be biologically significant.

The 24% inhabition of PChE activity for the low-dose females was not considered by the testing facility to be biologically significant for the following reasons: **(1)** Lack of statistical significance; **(2)** The Methamidophos-related inhibition of PChE activity on day 0 was actually only 13% [the difference between pretreatment (-11%) and day 0 (-24%)]; and **(3)** The mean value for PChE activity, for the controls, on day 0 was increased substantially by the results for one female (2.27 IU/ml) which exceeded the group mean (1.32 ± 0.34 IU/ml) for the remaining 5 controls

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by 2.8 standard deviations (S.D.). While the probability was 99.5% that this value did not belong to the same population, this result was included because it was less than 3.0 S.D., the criterion used by the testing facility to define an outlier. However, the inclusion of this female skewed the results toward a higher mean value for the control group, thereby accentuating the difference in PChE activity for the control females relative to the treated animals. Toxicology Branch I/HED accepts the above explanations.

COMMENTS:

The results of an earlier acute neurotoxicity screening study with Methamidophos indicated that the lowest dose tested, 0.9 mg/kg, resulted in a cholinergic toxicity in male and female Sprague-Dawley rats (MRID 43025001). The purpose of the present study was, therefore, to test lower doses of Methamidophos in order to establish a NOEL and this was accomplished. Review of the submitted report and supporting data indicated that the design and conduct of the present study were adequate. Minor deficiencies in the design and reporting of the study included the following:

(1) Determinations of cholinesterase activities were done only at 2 weeks before dosing and at 2 hours after dosing, at which time the rats (satellite group; 6/sex/dose) were sacrificed. No cholinesterase determinations were performed during the remaining 13 days when the rats (12/sex/dose) underwent neurobehavioral testing.

(2) There were certain ambiguities in reporting the cholinesterase data. For example, in the Results section, the 2-hour time interval when cholinesterase activities were determined was referred to as Day 0. However, in Appendix X, the same time interval was referred to as Day 1 (for male rats) and Day 2 (for female rats).

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Methamidophos TOXR# 011470

Page_____ is not included in this copy.

Pages 9 through 28 are not included.

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.

DATA EVALUATION REPORT

Methamidophos

Study Type: Acute Neurotoxicity Screening Battery

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Carrie Rabe, Ph.D. Date 4/22/94

Independent Reviewer William McLellan, Ph.D. Date 4/22/94

QA Reviewer Carol Maczka, Ph.D. Date 4/22/94

11/6/95

[NOTE: **Text was altered by TB-I subsequent to preparation of draft by Clement International Corp. following termination of contract with HED.**]

Contract Number: 68D10075
Work Assignment Number: 3-69
Clement Numbers: 273, 274
Project Officer: Caroline Gordon

EPA Reviewer: Linnea Hansen, Ph.D.
Review Section IV, Toxicology Branch I
Health Effects Division (7509C)

Signature: Linnea J. Hansen
Date: 1/6/95

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I
Health Effects Division (7509C)

Signature: Marion Copley
Date: 2/3/95

DATA EVALUATION REPORT

STUDY TYPE: Acute oral neurotoxicity screening battery in rats (Guideline series 81-8ss)

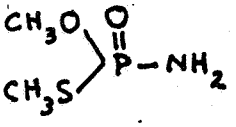
TOX. CHEM. NUMBER: 378A

P.C. CODE: 101201

MRID NUMBER: (1) 430250-01 (main study)
(2) 427703-01 (historical control and method validation)

TEST MATERIAL: Methamidophos

SYNONYMS: Monitor; *O,S*-Dimethyl phosphoramidothioate



STUDY NUMBER: (1) 105053, report no.; 92-412-QL, study no. (main study)
(2) 103979, report no.; 90-992-HN and -IF, 90-912-IL, 91-992-KT, -LO and LR, study nos. (historical control and method validation)

SPONSOR: Miles Inc.
Agricultural Division
Kansas City, Missouri

TESTING FACILITY: Miles Inc. Agricultural Division, Toxicology
Stilwell, Kansas

TITLE OF REPORT: (1) An acute oral neurotoxicity screening study with technical grade Methamidophos (MONITOR®) in rats
(2) Historical control and method validation studies in rats for the acute and subchronic neurotoxicity screening battery

AUTHORS: (1) L.P. Sheets and B.F. Hamilton
(2) L.P. Sheets, B.P. Stuart, and S.G. Lake

REPORT ISSUED: (1) November 5, 1993
(2) March 31, 1993

Executive Summary: In an acute neurotoxicity screening study, methamidophos (~76% a.i.) was administered in a single gavage dose to 24 male and 24 female Sprague-Dawley rats at 0, 1, 3 or 8 mg a.i./kg. Twelve rats/sex/dose were assessed for neurobehavioral functions at about 2 hours postdosing and on days 7 and 14. Six/sex/dose were used for cholinesterase determinations at 2 hours postdosing and 6/sex/dose for hematology and clinical chemistry on day 1.

At 1 mg/kg, males had slightly decreased motor/locomotor activities (-23 to -25% less than controls; not statistically significant) and one male had clinical signs (increased sitting/lying; urine, oral and nasal staining). Females showed slightly reduced motor activity during the first interval (-26%). At 3 mg/kg, markedly decreased motor/locomotor activity (-84 to-96%), repetitive chewing, uncoordinated gait, muscle fasciculations, impaired righting reflex, decreased forelimb grip strength (80% of control), decreased activity and rearing, increased ease of removal from cage and decreased body temperature were observed. Males at 3 mg/kg also had ataxia and reduced approach or touch response and increased SGOT activity (143% of controls), and females had increased lateral recumbency and tremors and decreased triglycerides (56% of control). At 8 mg/kg, salivation, flattened posture, reduced clicking sound or tail pinch responses and increased SGPT (170-181% of control) were observed. Males also had tremors and increased serum cholesterol (130% of control) and females had decreased hindlimb grip strength (71% of control), reduced approach and touch responses and increased SGOT (670% of control). **The study LOEL is 1 mg/kg, based on slightly reduced motor/locomotor activity in males and females and clinical signs in one male consistent with neurotoxicity secondary to cholinesterase inhibition. The study NOEL is ≤ 1 mg/kg.**

Cholinesterase in serum, erythrocytes and brain was inhibited at all doses. At 1 mg/kg, activity was -24% to -39% less than controls, increasing to -67% to -81% at 3 mg/kg and -82 to -92% at 8 mg/kg. **The ChE LOEL is 1 mg/kg, based on inhibition of all measured activities. The ChE NOEL is ≤ 1 mg/kg.**

This study is classified as core-minimum and satisfies the guideline requirement for an acute neurotoxicity screen (81-8ss). Although NOELs were not determined, a second rat acute neurotoxicity study on methamidofos (MRID 433458-01) demonstrated a NOEL = 0.7 mg/kg (and threshold ChE NOEL = 0.3 mg/kg).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test Material: Methamidophos (technical grade)

Description: Clear, colorless liquid

Lot/Batch #: 0-06-7009

Purity (as analyzed on the indicated dates): 76.7% a.i. (4/92), 75.5% a.i. (11/92), 75.8% a.i. (6/93), 75.6% a.i. (10/93); impurities not reported

Stability of compound: Stable for at least 3 years at warehouse conditions

CAS #: 10265-92-6

2. Vehicle and/or positive control: The test material was administered in 0.5% (w/v) methylcellulose with 0.4% (w/v) Tween 80 in deionized water. No concurrent positive controls were used.

3. Test animals: Rat

Strain: Sas:CD(SD)BR Sprague Dawley

Age and weight at study initiation: approximately 9 weeks; males weighed 233-293 g and females weighed 155-191 g (fasted weights).

Source: Sasco, Inc., St. Louis, Missouri

Housing: Individually in stainless steel wire mesh cages

Environmental conditions:
Temperature: 18-26°C
Humidity: 40-70%
Air changes: Not reported
Photoperiod: 12-hour light-dark cycle

Acclimation period: at least 6 days prior to placement in the study

B. STUDY DESIGN

1. Animal assignment

Animals with body weights within the range defined as "mean body weight for each sex ± 20%" were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Dose (mg/kg)	Neurobehavioral Tests[a] male	Neurobehavioral Tests[a] female	Cholinesterase Measurements[b] male	Cholinesterase Measurements[b] female	Clinical Chemistries male	Clinical Chemistries female
Vehicle	0	12	12	6	6	6	6
Low	1	12	12	6	6	6	6
Mid	3	12	12	6	6	6	6
High	8	12	12	6	6	6	6

[a]Motor activity and FOB testing. Of these animals, 6/sex/dose were used for gross and microscopic pathology examinations 15 or 16 days after dosing
[b]Animals added after sacrifice of other animals in study

Rats were administered test compound by gavage in a total volume of 10 ml/kg. The amount administered per animal was based on the animal's fasted body weight. Animals were dosed in groups staggered over a 4-day period (balanced for dose level and sex).

Dose selection and timing for analysis of effects on the day of dosing was based on the results of an acute range-finding study (study was not identified or provided for review) in which rats (3/sex/dose) receiving a single oral gavage dose of methamidophos exhibited repetitive chewing, muscle fasciculations, ataxia, decreased activity, and tremors at 4.2 mg/kg and 7.6 mg/kg. In addition, at 7.6 mg/kg rats exhibited salivation, urine staining, and lacrimation. The NOEL was 1.7 mg/kg. The time to onset of effects was approximately 1 hour. No information was provided regarding the time to peak effects or inhibition of cholinesterase activities.

2. Validation of methodology

Data were submitted from studies conducted with untreated and positive controls. These data established the reproducibility of baseline values and the ability of the functional observational battery, motor activity tests, and neuropathological examinations to detect treatment-related changes (see Appendix 1 of DER).

3. Preparation and analysis of dosing solutions

Technical methamidophos was dissolved in vehicle (0.5% [w/v] methylcellulose with 0.4% [w/v] Tween 80 in deionized water) at concentrations of 0, 0.1, 0.3 and 0.8 mg/ml. Stability and actual concentration in the vehicle were verified using gas chromatography. Homogeneity was assessed by visual inspection.

Results

Homogeneity analysis: Not reported (study report stated that solutions were visually inspected to verify absence of obvious suspended test material).

Stability analysis: The test material was stable (<10% loss of a.i.) for at least 8 days at 0.2 and 1.0 mg/ml.

Concentration analysis: Actual dosing concentrations ranged between 91%-112% of nominal.

TABLE 2. ACHIEVED DOSING CONCENTRATIONS

Nominal Concentration (mg/ml)	Measured Concentration[a] (mg/ml)	Measured Concentration[b] (mg/ml)
0	0	0
0.1	0.09	0.09
0.3	0.33	0.31
0.8	0.90	0.89

[a]Doses administered to rats undergoing the FOB and clinical chemistry analyses
[b]Doses administered to rats undergoing the cholinesterase determinations

4. Diet - Animals were given food (Purina Certified Rodent Chow #5001-4 "etts" form) and received water *ad libitum*.

5. Statistics - Body weight, clinical chemistry, and cholinesterase data were analyzed for homogeneity of variance using Bartlett's test ($p \leq 0.001$) and data were transformed if necessary to achieve homogeneity. Data with homogeneous variances were evaluated using a one-way analysis of variance followed by a Dunnett's t-test. Continuous FOB, motor activity, and locomotor activity data were analyzed first using a Repeated Measures analysis of variance, followed by a one-way analysis of variance. These tests were followed by a Dunnett's test to determine which groups were different from control. Categorical FOB data were analyzed using General Linear Modeling and Categorical modeling, followed by Dunnett's test and an Analysis of Contrasts, respectively. Statistical significance was identified where $p \leq 0.05$ for all tests except Bartlett's.

6. A signed and dated quality assurance statement was present. A signed and dated GLP statement was present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected once daily for signs of *toxicity*, and twice daily (once daily on weekends and holidays) for *morbidity* and *mortality*.

Results - No deaths occurred prior to termination of the study.

Clinical signs associated with cholinesterase inhibition (muscle fasciculations, tremors, ataxia, urine staining, salivation, and/or staining around the eyes, nose, or mouth) were observed in males at all doses and in females at the mid and high doses (Table 3). Severity of the signs was not indicated. The incidence of males with one or more of these signs was 1/12, 12/12 and 12/12 at the low, mid, and high doses, respectively. The incidence of females with one or more of the above signs was 5/12 and 12/12 at the mid and high doses, respectively. Most signs were observed only on the day of dosing, and all were resolved by study day 5. Staining sometimes persisted for several days.

2. Body Weight

Animals were weighed once each week as part of the functional observational battery.

Results - No treatment-related effects on body weight were observed.

3. Food Consumption

Food consumption was not determined.

4. Functional Observational Battery (FOB)

A functional observational battery was conducted using 12 rats/sex/dose at one week prior to dosing, 90 minutes-2 hours after dosing, and on days 7 and 14. Animals were evaluated without knowledge of the treatment group to which they belonged.

TABLE 3. Selected Clinical Signs for Rats Receiving a Single Oral Gavage Dose of Methamidophos[a]

Parameter	Incidence of Clinical Signs by Dose Level (mg/kg)			
	0	1	3	8
		Males		
Muscle fasciculations	0/12[b]	0/12	7/12	12/12
Tremors	0/12	0/12	0/12	2/12
Ataxia	0/12	0/12	1/12	6/12
Urine stain	0/12	1/12	9/12	12/12
Salivation	0/12	0/12	0/12	1/12
Oral Stain	0/12	1/12	3/12	8/12
Lacrimal stain, red	0/12	0/12	1/12	2/12
Nasal stain, red	0/12	1/12	10/12	6/12
		Females		
Muscle fasciculations	0/12	0/12	0/12	7/12
Tremors	0/12	0/12	0/12	0/12
Ataxia	0/12	0/12	0/12	2/12
Urine stain	0/12	0/12	5/12	12/12
Salivation	0/12	0/12	0/12	1/12
Oral stain	0/12	0/12	0/12	7/12
Lacrimal stain, red	0/12	0/12	0/12	0/12
Nasal stain, red	0/12	0/12	2/12	6/12

[a] Data extracted from Study No. 92-412-QL, Table 1 (p. 35).
[b] Values indicate the total no. animals affected during the study/total no. animals per dose. Data was apparently not analyzed statistically.

Home Cage Observations	Open Field Observations
X Posture*	X Posture*
X Tremors*	X Gait*
X Convulsions*	X Arousal*
X Bizarre or stereotypic behavior*	Circling*
Fecal composition*	X Stereotypy*
X Gait*	X Convulsions*
	X Tremors*
Manipulative Observations	X Urination*
X Ease of removal from cage*	X Defecation*
X Ease of handling*	Head position
Position of hindlimbs when held by tail	X Number of rears*
X Pupillary size*	X Respiration*
X Lacrimation*	X Piloerection*
X Staining of eyes, nose, mouth*	X Vocalization*
X Exophthalmos*	Response Observations
X Palpebral closure*	X Auditory response*
X Salivation*	X Approach response*
X Fur appearance*	Catalepsy withdrawal
	X Righting reflex*
Physiological measures	Placing reflex
Body tone*	X Pupil response*
X Body weight*	X Touch response*
X Rectal temperature*	Eye blink response
X Muscle tone*	X Pain response*
	Neuromuscular tests
	Hindlimb extensor strength
	X Forelimb grip strength*
	X Hindlimb grip strength*
	X Hindlimb footsplay*

*Recommended by Subdivision F (March 1991) Guidelines

Most FOB parameters were scored as being either present or absent or were scored using graded categories to denote differences from controls. Fecal pellets and urine pools were counted. Fore- and hindlimb grip strength were measured in kilograms using a protocol described only as "SOP E-201." Hindlimb footsplay was measured in mm. Body temperature was obtained using a rectal thermistor. The relationship to treatment was determined by comparison with both pretest and control data. Low incidence findings, not reasonably expected to be observed in controls, were considered to reflect a treatment-related effect.

Note: Footsplay measurements were not made on 3 high-dose males and 2 high-dose females on test day 0 because it was determined that they could not position their feet in such a way upon landing to give meaningful results.

Results - Treatment-related effects consistent with cholinesterase inhibition were observed in the incidences of a variety of findings in mid- and/or high-dose rats at the time of peak effect (Tables 4a and 4b). Evidence of muscarinic stimulation (autonomic effects) included salivation, urine staining, and repetitive chewing. Neuromuscular effects included impaired gait, impaired righting reflex, muscle fasciculations, and decreased forelimb and hindlimb grip strength. CNS effects included flattened body position, tremors, sluggishness, increased ease of handling, decreased rearing, and failure to respond to stimuli in the approach, touch, auditory (click) and tail pinch tests. In addition, decreased core body temperature was observed in mid- and high-dose rats. In general, the incidence and/or severity of these effects was dose-dependent.

In addition, the study authors noted that a single low-dose male sat/lay (rather than standing) during the open field portion of the FOB. Because all of the control animals stood during the open field assessments, the study authors concluded that this male was exhibiting a treatment-related effect. No other evidence of treatment was observed in this male or in any other low-dose animal during the FOB. However, the affected male [#1004] was the only low-dose animal to exhibit treatment-related clinical signs during daily clinical observations (see above) and also showed pronounced decreases in motor and locomotor activities.

5. Motor Activity

Motor activity and locomotor activity were measured in automated figure-8 mazes equipped with 8 photobeams located around the perimeter of each apparatus. Motor activity counts (number of photobeam interruptions) and locomotor activity counts (number of photobeam interruptions eliminating consecutive counts for a given beam) were measured in 10-minute intervals over a 90-minute period. Motor activity and locomotor activity were assessed 30 minutes after completion of the FOB on Days 0, 7 and 14.

Results - Overall motor and locomotor activities were significantly decreased in mid- and high-dose rats on Day 0 (Table 5) but not at days 7 or 14. The activities observed in mid- and high-dose rats were markedly lower (72 - 98%) than controls. In addition, the study authors considered the approximately 25% decreases in overall motor and locomotor activities observed in low-dose males to be treatment related even though they were not significantly different than controls. This conclusion was based on evidence from historical controls indicating that differences from control of 20% or less probably reflect variability of the test system. Interval data (Appendices 2 and 3) also show occasional statistically significant decreases relative to controls in both males and females at low dose (motor activity: males by 22 - 50%

TABLE 4a. Functional Observation Battery (FOB) Data at Peak Effect on Day 0 for Male Rats Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

	FOB Data (Incidence)[c] by Dose Level (mg/kg)			
Parameter	0	1	3	8
Autonomic Effects				
–Repetitive Chewing				
home cage - peak effect	0/12	0/12	2/12 (1.0)	0/12
open field - peak effect	0/12	0/12	4/12 (1.0)	8/12* (1.3)
–Salivation				
peak effect	0/12	0/12	0/12	9/12* (1.8)
–Urine Staining				
peak effect	0/12	0/12	0/12	7/12* (1.0)
Neuromuscular Effects				
–Incoordinated Gait				
home cage - peak effect	0/12	0/12	8/12* (1.3)	11/12* (1.7)
open field - peak effect	0/12	0/12	12/12* (1.3)	12/12* (1.8)
–Muscle Fasciculations				
home cage - peak effect	0/12	0/12	10/12* (1.8)	12/12* (2.0)
open field - peak effect	0/12	0/12	12/12* (1.9)	12/12* (2.0)
–Impaired Righting Reflex				
peak effect	1/12	1/12	8/12*	11/12*
–Forelimb Grip Strength [Mean (kg) ± S.D.]				
peak effect	1.01±0.15	1.01±0.16 (100)	0.81±0.14* (80)	0.31±0.11* (31)
–Hindlimb Grip Strength [Mean (kg) ± S.D.]				
peak effect	0.46±0.08	0.48±0.07 (104)	0.48±0.15 (104)	0.34±0.07 (74)
CNS Effects				
–Flattened Posture				
home cage - peak effect	0/12	0/12	0/12	4/12
open field - peak effect	0/12	0/12	0/12	6/12
–Tremors				
home cage - peak effect	0/12	0/12	0/12	6/12* (1.5)
open field - peak effect	0/12	0/12	0/12	7/12* (1.4)
–Sluggishness				
peak effect	1/12	1/12	12/12*	12/12*
–Number of Rears (Mean ± S.D.)				
peak effect	2.5±2.0	4.4±2.1*	0.5±0.8*	0.2±0.6*
–Approach Response - no reaction				
peak effect	0/12	0/12	2/12	5/12*
–Touch Response - no reaction				
peak effect	0/12	0/12	2/12	7/12*
–Auditory Response - no reaction				
peak effect	0/12	0/12	0/12	4/12
–Tail Pinch - no reaction				
peak effect	0/12	0/12	0/12	6/12
–Increased Ease of Removal From Cage				
peak effect	8/12	3/12	12/12	11/12
Physiological Effects				
–Body Temperature [Mean (°C) ± S.D.]				
peak effect	37.3±0.4	37.8±0.8	34.4±1.0*	33.4±0.9*

[a] Data extracted from Study No. 92-412-QL, Table 3 (pp. 40-43).
[b] Numbers in parentheses indicate average severity, where 1 = slight and 2 = moderate-to severe.
[c] The incidence is presented for each parameter unless a continuous effect is measured.

* Significantly different from control, $p \leq 0.05$

TABLE 4b. Functional Observation Battery (FOB) Data at Peak Effect on Day 0 for Female Rats Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

	FOB Data (Incidence)[c] by Dose Level (mg/kg)			
Parameter	0	1	3	8
Autonomic Effects				
–Repetitive Chewing				
home cage - peak effect	0/12	0/12	0/12	4/12* (1.0)
open field - peak effect	0/12	0/12	6/12* (1.0)	10/12* (1.1)
–Salivation				
peak effect	0/12	0/12	0/12	8/12* (1.8)
–Urine Staining				
peak effect	0/12	0/12	1/12 (1.0)	5/12 (1.0)
Neuromuscular Effects				
–Incoordinated Gait				
home cage - peak effect	0/12	0/12	5/12* (1.0)	11/12* (1.7)
open field - peak effect	0/12	0/12	11/12* (1.1)	12/12* (1.9)
–Muscle Fasciculations				
home cage - peak effect	0/12	0/12	11/12* (1.7)	12/12* (2.0)
open field - peak effect	0/12	0/12	11/12* (1.9)	12/12* (2.0)
–Impaired Righting Reflex				
peak effect	0/12	1/12	8/12*	12/12*
–Forelimb Grip Strength [Mean (kg) ± S.D.]				
peak effect	0.90±0.15	0.88±0.12 (98)	0.75±0.15* (83)	0.35±0.16* (39)
–Hindlimb Grip Strength [Mean (kg) ± S.D.]				
peak effect	0.38±0.09	0.43±0.09 (113)	0.41±0.08 (108)	0.27±0.07* (71)
CNS Effects				
–Flattened Posture				
home cage - peak effect	0/12	0/12	0/12	2/12
open field - peak effect	0/12	0/12	0/12	7/12
–Tremors				
home cage - peak effect	0/12	0/12	2/12 (1.0)	3/12 (1.7)
open field - peak effect	0/12	0/12	1/12 (1.0)	9/12* (1.3)
–Sluggishness				
peak effect	0/12	0/12	11/12*	12/12*
–Number of Rears (Mean ± S.D.)				
peak effect	5.4±3.0	4.4±3.1	1.7±1.2*	0.1±0.3*
–Approach Response - no reaction				
peak effect	0/12	0/12	0/12	8/12*
–Touch Response - no reaction				
peak effect	0/12	0/12	1/12	11/12*
–Auditory Response - no reaction				
peak effect	0/12	0/12	0/12	6/12*
–Tail Pinch - no reaction				
peak effect	0/12	0/12	1/12	10/12*
–Increased Ease of Removal From Cage				
peak effect	5/12	5/12	11/12	12/12
Physiological Effects				
–Body Temperature [Mean (°C) ± S.D.]				
peak effect	38.0±0.7	37.9±0.6	34.9±0.7*	32.7±0.7*

[a] Data extracted from Study No. 92-412-QL, Table 3 (pp. 53-56).
[b] Numbers in parentheses indicate average severity, where 1 = slight and 2 = moderate-to-severe.
[c] The incidence is presented for each parameter unless a continuous effect is measured.

* Significantly different from control, $p \leq 0.05$

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TABLE 5. Overall Motor and Locomotor Activity for Rats Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

Type of Activity/ Day of Testing	Activity Scores by Dose Level (mg/kg)			
	0	1	3	8
		Males		
Motor Activity Score -Day 0	327 ± 119	252 ± 105 (77)	51 ± 31* (16)	90 ± 86* (28)
Locomotor Activity Score -Day 0	134 ± 42	100 ± 47 (75)	6 ± 5* (4)	11 ± 29* (8)
		Females		
Motor Activity Score -Day 0	516 ± 178	442 ± 99 (86)	78 ± 27* (15)	99 ± 76* (19)
Locomotor Activity Score -Day 0	187 ± 79	161 ± 32 (86)	21 ± 8* (11)	3 ± 4* (2)

[a] Data extracted from Study No. 92-412-QL, Table 5 (pp. 71-72) and Table 6 (pp. 73-74).
[b] Numbers in parentheses indicate percent of control activity.

* Significantly different from control, $p \leq 0.05$

during the first 4 intervals; females by 27%, only during first interval). The reviewers agreed with the study authors that the reduced activity in low-dose animals probably represent a threshold treatment-related effect, although some of the reduction in males was due to a single animal with markedly lower activity.

6. Cholinesterase Activity

Serum, erythrocyte, and brain cholinesterase activities were measured in 6 rats/sex/dose at approximately 2 hours after dosing. No information was provided regarding the timing of peak inhibition or recovery of cholinesterase activity. The method of blood and brain collection was not reported. Cholinesterase determinations were made using modifications of the Ellman method in which the reaction product, thionitrobenzoic acid, was measured spectrophotometrically.

Results - Statistically significant, dose-related decreases in serum, erythrocyte, and brain cholinesterase activities were observed in both males and females at all doses (Table 6).

7. Clinical pathology

Blood for hematology and clinical chemistry was collected from the orbital plexus of animals (6 females/dose) at 2 weeks prior to dosing and then at 20-24 hours after dosing (6/sex/dose). Pretest blood samples were inadvertently not collected from males. Animals were not fasted prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpusc. HGB conc. (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	X Reticulocyte count
Blood clotting measurements	X Red cell morphology
(Thromboplastin time)	
(Clotting time)	
(Prothrombin time)	

Results - No treatment-related effects were observed.

TABLE 6. Cholinesterase Activity Two Hours After Rats Received a Single Oral Gavage Dose of Methamidophos[a,b]

Parameter	Cholinesterase Data by Dose Level (mg/kg)			
	0	1	3	8
		Males		
Serum Cholinesterase (IU/mL)	0.57 ± 0.13	0.35 ± 0.05* (61)	0.11 ± 0.02* (19)	0.05 ± 0.01* (9)
Erythrocyte Cholinesterase (IU/mL)	1.21 ± 0.10	0.82 ± 0.11* (68)	0.33 ± 0.08* (27)	0.10 ± 0.06* (8)
Brain Cholinesterase (IU/g tissue)	15.1 ± 0.6	10.1 ± 0.8* (67)	4.5 ± 0.5* (30)	2.7 ± 0.2* (18)
		Females		
Serum Cholinesterase (IU/mL)	1.08 ± 0.20	0.82 ± 0.10* (76)	0.36 ± 0.05* (33)	0.12 ± 0.05* (11)
Erythrocyte Cholinesterase (IU/mL)	1.27 ± 0.08	0.86 ± 0.13* (68)	0.41 ± 0.07* (32)	0.18 ± 0.06* (14)
Brain Cholinesterase (IU/g tissue)	16.1 ± 0.7	11.5 ± 1.0* (71)	4.5 ± 0.3* (28)	2.5 ± 0.3* (16)

[a] Data extracted from Study No. 92-412-QL, Table CHE1-SUM (pp. 334-335).
[b] Numbers in parentheses indicate percent of control activity.

* Significantly different from control, $p \leq 0.05$

N=6 for all groups

b. Clinical Chemistry

Electrolytes	Other
X Calcium	X Albumin
X Chloride	X Blood creatinine
Magnesium	X Blood urea nitrogen
X Phosphorus	X Cholesterol
X Potassium	X Globulins
X Sodium	X Glucose
Enzymes	X Total bilirubin
X Alkaline phosphatase	X Total serum protein
X Cholinesterase (see above)	X Triglycerides
X Creatine phosphokinase	X Serum protein electrophoresis
X Lactic acid dehydrogenase	X Phospholipids
X Gamma glutamyl transferase	X Uric acid

X Serum alanine aminotransferase (also SGPT)
X Serum aspartate aminotransferase (also SGOT)

Results - Statistically significant treatment-related increases in serum aspartate- and alanine-aminotransferase (SGOT and SGPT, respectively) activities were observed in both males and females at the highest dose tested (Table 7). In addition, males at the mid dose had significantly increased SGPT activity. Other effects considered to be treatment-related included slight (statistically significant) increases in serum cholesterol in high-dose males and decreases in serum triglycerides in mid- and high-dose females. The study authors suggested that the increases in SGOT and SGPT activities represented damage to the liver or skeletal muscle. The physiological basis for the effects on serum cholesterol and triglycerides was unknown.

8. Sacrifice and Pathology

At termination of the study, 6 rats/sex/dose (first 6 from each group of randomly selected animals used for neurobehavioral testing) were anesthetized with sodium pentobarbital and perfused via the left ventricle with phosphate-buffered sodium nitrate followed by a solution of 4% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer. These animals underwent a complete gross pathological examination and brain weights were obtained. The remaining surviving rats from the main study groups were sacrificed by carbon dioxide asphyxiation and also were examined grossly.

Tissues collected from perfused rats for post-fixation in 10% buffered formalin included the entire brain and spinal cord, both eyes (with optic nerves), bilateral peripheral nerves (sciatic, tibial, sural), gasserian ganglia and gastrocnemius muscle.

TABLE 7. Clinical Chemistry Data from Rats 24 Hours After Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

Parameter	Clinical Chemistry Data by Dose Level (mg/kg)			
	0	1	3	8
		Males		
Cholesterol (mg/dL)	43 ± 8	46 ± 9 (107)	49 ± 5 (114)	56 ± 7* (130)
Triglycerides (mg/dL)	108 ± 27	143 ± 42 (132)	146 ± 42 (135)	94 ± 34 (87)
AST (U/L)	86 ± 18	95 ± 37 (110)	123 ± 21* (143)	515 ± 261* (599)
ALT (U/L)	60 ± 10	67 ± 11 (112)	59 ± 8 (98)	102 ± 39* (170)
		Females		
Cholesterol (mg/dL)	49 ± 7	50 ± 8 (102)	47 ± 9 (96)	46 ± 12 (94)
Triglycerides (mg/dL)	118 ± 33	82 ± 27 (69)	66 ± 39* (56)	51 ± 15* (43)
AST (U/L)	76 ± 5	74 ± 13 (97)	106 ± 45 (139)	509 ± 235* (670)
ALT (U/L)	52 ± 3	45 ± 5* (87)	50 ± 5 (96)	94 ± 25* (181)

[a] Data extracted from Study No. 92-412-QL, Table CC1-SUM (pp. 324-332).
[b] Numbers in parentheses indicate percent of control value.

* Significantly different from control, $p \leq 0.05$

N=6 for all groups

Brain (6 levels - olfactory region, forebrain, midbrain, pons, medulla oblongata, and cerebellum) and spinal cord (cross and longitudinal sections of the cervical, thoracic, and lumbar regions) were embedded in paraffin and sections were stained with hematoxylin and eosin or Luxol Fast Blue/Cresyl violet and Sevier-Munger stains. Skeletal muscle, dorsal root ganglia, cauda equina, eyes, gasserian ganglia, and tissues from the hippocampus and cerebellar cortex were embedded in glycol methacrylate and sections were stained with Modified Lee's. Peripheral nerves were embedded in Epon and sections were stained with Toluidine blue. Tissues from the control and high-dose animals were examined histologically.

Results - No treatment-related effects were observed at the gross or histopathological examination of tissues.

E. DISCUSSION

Review of the final report and supporting data indicates that the design and conduct of the study were adequate. Minor deficiencies in the design and conduct of the study are reported below.

Administration of methamidophos in a single gavage dose to Sprague-Dawley rats resulted in a dose-related inhibition of cholinesterase activities (serum, erythrocyte and brain) and incidence of related signs of neurotoxicity at all doses tested. At mid dose in males and high dose in both sexes, all animals showed some signs of neurotoxicity (TB-I considers cholinergic signs to represent neurotoxicity and therefore designated the NOEL/LEL accordingly. The study authors did not consider cholinergic effects to be neurotoxicity). At low dose, only 1 male showed clinical signs, and males and females showed minimal effects on motor/locomotor activity. Approximately 2 hours after dosing, serum, erythrocyte and brain cholinesterase activities were inhibited by 24-39% at 1 mg/kg, 67-81% at 3 mg/kg and 82-92% at 8 mg/kg. Cholinesterase activity inhibition was marginally greater in males than in females.

Behavioral evidence of neurotoxicity consistent with inhibition of cholinesterase activity at 90 minutes-2 hours after dosing was observed primarily in mid- and high-dose rats. Effects observed during the FOB on the day of dosing included dose-related increases in the incidence of autonomic effects (salivation, urine staining and repetitive chewing), neuromuscular effects (impaired gait, impaired righting reflex, muscle fasciculations, decreased grip strength), CNS effects (flattened body position, tremors, increased ease of handling, sluggishness, decreased number of rears, failure to respond when approached, touched, pinched or after a loud click), decreased body temperature and decreased motor and locomotor activities in mid- and high-dose animals. Reasonably good agreement was obtained between the daily clinical observations and the FOB on the day of dosing. In addition, the daily observations

demonstrated that most effects occurred only on the day of dosing and all had resolved by study day 5.

With the exception of one low-dose male with minimal clinical evidence of neurotoxicity, individual low-dose animals did not show significant clinical evidence of neurotoxicity. The low dose was not considered a NOEL, however, because slight decreases in motor and locomotor activity were also observed in low-dose males as a group. Although some of this reduction was due to sharply reduced activity in the male with clinical signs, the reviewers agreed that the reduction probably was a threshold treatment-related effect. In addition, low-dose females showed slight decreases in motor activity only during the first 10-minute interval. The low dose is considered a threshold LEL for motor activity effects and cholinergic clinical signs in more sensitive animals. This is supported by the fact that all males and most females showed clinical signs and sharply reduced motor activity at mid dose (3 mg/kg).

Additional effects observed in treated rats included increased SGPT and SGOT activities in high-dose males and females, increases in cholesterol in high-dose males, decreases in triglycerides in high-dose females, and increases in SGOT in mid-dose males. The increased SGPT and SGOT activities were suggested to have been due to either transient muscle (secondary to cholinergic effects) or liver damage. Histopathological analyses showed no skeletal muscle damage and liver was not evaluated. Thus, the reason for the clinical pathology findings is unclear.

Based on the above information, the LOEL for cholinesterase inhibition and clinical signs of neurotoxicity is 1 mg/kg. The NOEL for cholinesterase inhibition and neurotoxicity is less than 1 mg/kg. However, a subsequently submitted rat acute neurotoxicity study on methamidofos (MRID 433458-01) demonstrated a systemic toxicity/ neurotoxicity NOEL of 0.7 mg/kg and a threshold ChE NOEL of 0.3 mg/kg (study not reviewed at this time; based on preliminary examination of data by TB-I).

F. STUDY DEFICIENCIES are as follows:

- NOEL not determined.
- Pretest blood samples were accidentally not taken from males.
- The timing for the FOB and motor activity analyses in the current study was determined based on information regarding onset of effects in a rangefinding study rather than the timing for peak effects.
- Blood cholinesterase measurements not taken during observation period.

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Appendix 1: Validation of Methods

UNTREATED RATS: STUDY NOs. 90-992-HN and 90-992-IF. Untreated rats examined for motor activity (not fasted) and clinical signs/FOB at single or multiple times (0, 4, 8, 13 weeks) demonstrated acceptable reproducibility of results and provided a historical control data base. Animals demonstrated habituation to mazes during 90-min test sessions and with repeated testing.

CHLORPROMAZINE AND TRIADIMEFON. STUDY NO. 90-912-IL. Rats were tested for motor activity after intraperitoneal administration of 200 mg/kg triadimefon, 2 mg/kg chlorpromazine, 5 ml saline vehicle/kg or no treatment. Pronounced increased motor activity was observed in animals treated with triadimefon (about 2X higher; statistically significant) and decreased activity was observed in animals treated with chlorpromazine (about 50% lower; statistically significant only for female early interval measurements) when compared to controls. Motor activity was decreased in all groups on the day of dosing due to fasting.

ACRYLAMIDE. STUDY NO. 91-992-KT. Clinical/FOB examinations and neurohistopathology were performed on rats after intraperitoneal administration of 0, 25, or 50 mg/kg acrylamide in 1 ml/kg saline. Clinical/functional effects included ataxia, piloerection, muscle fasciculations, tremors, and urine or oral stains (data not analyzed statistically). Statistically significant increases in peripheral neuropathy and axonal degeneration in the spinal cord were observed.

TRIPHENYLTIN. STUDY NO. 91-962-LO. Rats were administered 0 or 12 mg/kg triphenyltin intraperitoneally in 1 ml saline/kg and neural tissues were processed for histopathological examination. Statistically significant increases in the incidences of neuronal necrosis in the olfactory tract, piriform cortex, and hippocampus; chromatolysis of large neuronal soma in the pons, medulla, spinal cord, dorsal root ganglia, and gasserian ganglia; axonal or nerve fiber degeneration in the spinal cord and several peripheral nerves; and digestion chamber in dorsal root ganglia, gasserian ganglia, spinal cord, and sciatic nerve were observed.

CARBARYL. STUDY NO. 91-962-LR. FOB and clinical examinations were performed on rats after intraperitoneal administration of 0, 15, or 30 mg/kg carbaryl in 1 ml 2% [illegible] EL/kg. Effects observed in treated animals were characteristic of carbamate poisoning and included urine, oral, nasal, and perianal staining, ataxia, decreased touch and approach reactions, repetitive chewing, muscle fasciculations, and tremors. Results were not analyzed statistically.

APPENDIX 2. Day 0 Interval Motor Activity Scores (No. Consecutive Beam Breaks) for Rats Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

Interval	Activity Scores (No. Beam Breaks per 10-min. interval) by Dose Level			
	0 mg/kg	1 mg/kg	3 mg/kg	8 mg/kg
		Males		
Motor Activity Score (Day 0)				
-Interval 1	142 ± 42	109 ± 45 (77)	7 ± 5* (5)	20 ± 38* (14)
-Interval 2	73 ± 21	57 ± 27 (78)	6 ± 11* (8)	15 ± 29* (21)
-Interval 3	45 ± 30	25 ± 20* (56)	8 ± 14* (18)	6 ± 8* (13)
-Interval 4	22 ± 31	11 ± 17 (50)	3 ± 7 (14)	9 ± 12 (41)
-Interval 5	15 ± 25	17 ± 37	4 ± 4	3 ± 5
-Interval 6	15 ± 29	6 ± 12	5 ± 8	26 ± 46
-Interval 7	7 ± 9	3 ± 8	7 ± 9	2 ± 5
-Interval 8	7 ± 16	6 ± 16	6 ± 10	8 ± 11
-Interval 9	1 ± 2	18 ± 21*	5 ± 6	1 ± 3
		Females		
Motor Activity Score (Day 0)				
-Interval 1	166 ± 28	122 ± 27* (73)	20 ± 14* (12)	27 ± 30* (16)
-Interval 2	96 ± 28	87 ± 29 (91)	4 ± 7* (4)	16 ± 26* (17)
-Interval 3	81 ± 31	63 ± 13 (78)	3 ± 4* (4)	13 ± 18* (16)
-Interval 4	54 ± 42	59 ± 30 (109)	9 ± 6* (17)	10 ± 15* (19)
-Interval 5	34 ± 31	36 ± 31	6 ± 8*	6 ± 9*
-Interval 6	25 ± 37	16 ± 21	8 ± 10	7 ± 10
-Interval 7	24 ± 33	27 ± 31	8 ± 15	8 ± 13
-Interval 8	17 ± 25	18 ± 21	15 ± 18	5 ± 6
-Interval 9	20 ± 25	14 ± 21	5 ± 12	6 ± 9

[a] Data extracted from Study No. 92-412-QL, Table 7 (pp. 76 and 80).
[b] Numbers in parentheses indicate percent of control activity.

* Significantly different from control, $p \leq 0.05$.

APPENDIX 3. Day 0 Interval Locomotor Activity Scores (No. Non-consecutive Beam Breaks) for Rats Receiving a Single Oral Gavage Dose of Methamidophos[a,b]

	Locomotor Activity Scores (No. Non-consecutive Beam Breaks) on Day 0 by Dose Level			
Interval	0 mg/kg	1 mg/kg	3 mg/kg	8 mg/kg
		Males		
Locomotor Activity Score				
-Interval 1	65 ± 14	52 ± 20 (80)	3 ± 1* (5)	5 ± 12* (8)
-Interval 2	30 ± 10	23 ± 11 (77)	0 ± 0* (0)	3 ± 9* (10)
-Interval 3	16 ± 11	10 ± 8 (63)	0 ± 0* (0)	1 ± 1* (6)
-Interval 4	9 ± 13	5 ± 8 (56)	0 ± 0* (0)	1 ± 3* (11)
-Interval 5	5 ± 7	3 ± 6	0 ± 1	1 ± 2
-Interval 6	5 ± 9	2 ± 4	0 ± 0	0 ± 1
-Interval 7	3 ± 4	1 ± 2	0 ± 0	1 ± 1
-Interval 8	1 ± 4	3 ± 7	1 ± 2	1 ± 2
-Interval 9	0 ± 0	3 ± 6	1 ± 2	0 ± 0
		Females		
Locomotor Activity Score				
-Interval 1	66 ± 10	55 ± 10* (83)	9 ± 4* (14)	2 ± 1* (3)
-Interval 2	35 ± 9	34 ± 11 (97)	1 ± 2* (3)	0 ± 0* (0)
-Interval 3	30 ± 12	22 ± 8* (73)	1 ± 2* (3)	0 ± 0* (0)
-Interval 4	18 ± 19	20 ± 12 (111)	2 ± 1* (11)	0 ± 0 (0)
-Interval 5	11 ± 13	11 ± 10	1 ± 1*	0 ± 0*
-Interval 6	8 ± 12	5 ± 7	2 ± 3	0 ± 0
-Interval 7	9 ± 13	7 ± 10	2 ± 4	0 ± 1
-Interval 8	5 ± 9	3 ± 6	3 ± 4	0 ± 0
-Interval 9	6 ± 9	4 ± 5	1 ± 2	1 ± 3

[a] Data extracted from Study No. 92-412-QL, Table 8 (pp. 84 and 88).
[b] Numbers in parentheses indicate percent of control activity.

* Significantly different from control, $p \leq 0.05$.