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

AUG 3 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA ID No.: 101201. Evaluation of 3-Month Delayed Neurotoxicity Study in Hens with SRA 5172 (Methamidophos Technical)

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Krystyna K. Locke 7/13/89

TO: Jay S. Ellenberger/Ruby Whiters PM/RM Team No. 50
Generic Chemical Support Branch
Special Review/Reregistration Division (H7508C)

THRU: Robert Zendzian, ~~Acting~~ Section Head
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Record No.: 240734

Tox. Chem. No.: 378A
HED Project No.: 9-0965

Toxicology Branch I/IRS has completed an evaluation of the following study:

3-Month Subchronic Delayed Neurotoxicity Study with SRA 5172 (C.N. Methamidophos); RCC Umweltchemie AG; Switzerland; No. 94213/064293; May 15, 1987.
MRID/Accession No.: 409852-02.
EPA Guidelines No.: 82-5.

SRA 5172, administered by gavage 5 times per week (Monday through Friday) for 3 months, did not cause delayed neurotoxicity in white leghorn hens (age at first dosing: 12 months). The dose levels tested were 0, 0.3, 1 and 3 mg/kg body weight.

NOEL (General Toxicity): 0.3 mg/kg
LEL: 1 mg/kg [Inhibition of plasma butyrylcholinesterase (BuChE) and spinal cord neurotoxic esterase (NTE) activities]

Treatment-related findings observed in the 3 mg/kg group included somnolence, emaciation, weight loss (22%) and inhibition of BuChE activity in plasma (48%) and NTE activity in brain (17%) and spinal cord (42%).

Ataxia, abnormal motor activities or histopathological changes in brain, spinal cord and peripheral nerves, generally regarded as indicators of delayed neurotoxicity, were not observed in any hen on the study.

Classification of Study: Core-Guideline

The above 90-day delayed neurotoxicity study was not required because an acceptable (Core-Guideline) acute delayed neurotoxicity study with Monitor Technical (No. 79 ANH01, 7/29/79, MRID 00041317) was available. In that study, no signs of delayed neurotoxicity or spinal cord lesions were observed at the 50.63 mg/kg level (highest tested), in the presence of atropine sulfate (50 mg/kg body weight), with or without redosing. White leghorn hens, 9-18 months old, were used in the acute study.

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Reviewed by: Krystyna K. Locke Krystyna K. Locke 7/4/89
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Robert Zendzian, Acting Section Head 7/5/89
Section I, Tox. Branch I/IRS (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 82-5. Subchronic Delayed Neurotoxicity: 90 Days (Hen)

Tox. Chem No.: 378A
MRID No.: 409852-02

TEST MATERIAL: SRA 5172; Purity: 76%; Batch No.: 808 526 298; Light yellowish crystals; Expiration Date: 9/4/86.

SYNONYMS: Methamidophos Technical, Monitor

STUDY NUMBER(S): 94213/064293

SPONSOR: Bayer AG, Institut fur Toxikologie, Wuppertal, Switzerland
Submitted by: Valent (Chevron)

TESTING FACILITIES: KFM Kleintierfarm Madoerin AG
CH 4414 Fuellinsdorf/Switzerland
(housing),

RCC Research and Consulting Company AG
CH 4452 Itingen/Switzerland,

RCC UMWELTCHEMIE AG
CH 4452 Itingen/Switzerland

TITLE OF REPORT: 3-Month Subchronic Delayed Neurotoxicity Study with SRA 5172 (C.N. Methamidophos)

AUTHOR(S): K. Sachsse et al.

REPORT ISSUED: May 15, 1987

CONCLUSIONS:

Based on negative results of the forced motor activity tests and microscopic examinations of brain, spinal cord and peripheral nerves, SRA 5172 (Methamidophos Technical) did not induce delayed neurotoxicity in hens under the conditions of this study. Based on the results of a preliminary study, the highest dose of SRA 5172 tested was 3 mg/kg body weight.

Twelve-month old hens, 16/group, were treated with SRA 5172 (mg/kg body weight) as follows: 0 (Group 1), 0.3 (Group 2), 1 (Group 3) and 3 (Group 4). The test substance was administered as aqueous solutions by gastric intubation, 5 times/week, Monday through Friday, for 3 months. The following parameters were examined in all hens on the study: clinical signs, mortality, forced motor activity and body weights. The following parameters

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were also examined in 10 hens from each group: plasma butyryl-cholinesterase (BuChE) activity, necropsy and histopathology of brain, spinal cord and peripheral nerves. Neuropathy target esterase (neurotoxic esterase, NTE), considered by the testing laboratory to be a key enzyme in delayed neurotoxicity (threshold inhibition of 45-65% initiates severe neuropathy), was determined in brains and spinal cords of 6 hens/group; these hens were examined neither grossly nor microscopically.

NOEL (General toxicity): 0.3 mg/kg
LEL: 1 mg/kg (Inhibition of plasma BuChE and spinal cord NTE activities)

Treatment-related findings observed in the 3 mg/kg group included somnolence, emaciation, weight loss (22%) and inhibition of BuChE activity in plasma (48%) and NTE activity in brain (17%) and spinal cord (42%).

Ataxia, abnormal motor activities or histological changes in brain, spinal cord and peripheral nerves, generally regarded as indicators of delayed neurotoxicity, were not observed in any hen on the study.

CLASSIFICATION OF STUDY: Core-Guideline

EXPERIMENTAL PROCEDURES

Dosing for this study was started on April 17, 1986, and terminated on July 9, 1986 (A-animals), or July 2, 1986 (B-animals).

Domestic White Leghorn hens, 16/group, were assigned randomly to four groups and treated with SRA 5172 (mg/kg body weight) as follows: 0 (Group 1), 0.3 (Group 2), 1 (Group 3) and 3 (Group 4). The test material was administered as aqueous solutions (2 mL/kg body weight), by gavage, 5 times/week, Monday through Friday. In each group, 10 hens (A-animals) were used for determination of plasma butyrylcholinesterase (BuChE) activity and 6 hens (B-animals) for determination of neuropathy target esterase activity (neurotoxic esterase, NTE) in brain and spinal cord. BuChE activity was determined at pretest and monthly intervals during the study (weeks 4, 8 and 12), using butyrylthiocholine as substrate. NTE activity was determined at necropsy, using phenyl valerate as substrate[1,2]. All hens were observed for clinical signs and mortality twice daily; forced motor activity was observed twice weekly; and individual body weights were recorded weekly. Forced motor activity was assessed on the scale of 0-4 (Attachment I).

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[1]M.K. Johnson. Arch. Toxicol. 37, 113-115, 1977.
[2]M.K. Johnson and R.J. Richardson. Neurotoxicology 4, 311-320, 1983.

At the termination of the study, all hens were anesthetized by intravenous injection of sodium pentobarbitone. Those hens of Allocation group A (A-animals) were perfused with buffered 4% formaldehyde solution. After perfusion, brain (cerebrum, cerebellum and medulla oblongata), spinal cord (cervical, thoracic and lumbosacral regions) and peripheral nerves (sciatic and tibial) were examined microscopically. Tissue sections were stained with hematoxylin-eosin (standard stains), luxol fast blue (for myelin sheath) and Bodian's colloidal silver method (for axons). All of the Allocation group A hens, including the nonsurvivors, were also examined grossly.

The hens of Allocation group B (B-animals) were examined neither grossly nor microscopically. After removal of brains and spinal cords for NTE determination, the carcasses were discarded.

Hens used in this study were: 1) Obtained from a supplier in West Germany (Lohmann Tierzucht GmbH); 2) Acclimated for one week; 3) Identified by cage and wing numbers; 4) Housed 4/cage at 18-22° C, relative humidity of 45-75%, and 12-hour light/dark cycle; and 5) Fed unrestricted amounts of food and water. At the initiation of the study, the hens were 12 months old and weighed 1.6-2.2 kg.

Dose levels of SRA 5172 used in the 3-month delayed neurotoxicity study were based on the results obtained in the 10-day range-finding study (No. 064282; not submitted). In the range-finding study, five 12-month old hens received daily oral administrations of SRA 5172 (5 mg/kg body weight). The results (drowsiness, body weight loss and marked cholinesterase inhibition) indicated, according to the testing laboratory, that a prolonged treatment with 5 mg/kg would be detrimental to the hens in a subchronic study.

Dosing solutions (SRA 5172 dissolved in distilled water) were prepared immediately prior to each dosing and stored at room temperature. The stability of Methamidophos in dosing solutions was determined at the initiation of the study by analyzing these solutions after a storage time of 2 and 48 hours at room temperature. The concentration of Methamidophos in dosing solutions was determined monthly, starting with the first preparation.

Body weights and BuChE and NTE activities were analyzed statistically as detailed in Attachment II.

RESULTS

Stability and Concentrations of SRA 5172 (Methamidophos) in Dosing Solutions

Methamidophos was degraded slowly in dosing solutions, as follows:

Group	Storage Time (hrs)	Percent Decrease*
2	2	7.7
	48	10.9
3	2	2.7
	48	5.3
4	2	5.3
	48	13.2

*Calculated as follows: Methamidophos concentration in solution right after preparation (reported as % of nominal) - methamidophos concentration after 2- or 48-hour storage at room temperature (reported as % of nominal) = % decrease. The above table is based on Table 2, page 80, of the submission.

The mean concentrations of Methamidophos in freshly prepared dosing solutions for Groups 2, 3 and 4 were 99.5, 99.0 and 100.1%, respectively, of the nominal concentrations. These concentrations were determined monthly and individual values for the treated groups ranged from 87.6 to 107.4% of nominal concentrations.

Mortality

There were four deaths during the study, two in Group 1 (days 36 and 57) and two in Group 4 (both on day 75). Both hens in Group 4 were motionless and prostrate shortly before death, but their deaths were not regarded by the testing laboratory as treatment-related. One death in each group was attributed to laying problems. One Group 4 hen had minimal (Grade 1) gliosis in medulla oblongata and abdominal cavity filled with 40 mL of watery-clear fluid. No abnormalities were found macroscopically and microscopically in the remaining three hens.

Clinical Signs

In Group 4, somnolence and slight emaciation were noted from treatment day 10 onwards. No clinical signs or symptoms were observed in Groups 1, 2 and 3.

Forced Motor Activity

No signs of ataxia or abnormal motor activity were observed in any hen on the study, including the nonsurvivors.

Body Weights

Hens in Groups 1, 2 and 3 gained weight during the study, whereas those in Group 4 lost weight. Comparing body weights on study days 1 and 85, the mean weight gain in Groups 1, 2 and 3 was 8.9, 7.4 and 6.2%, respectively, and the mean weight loss in Group 4 was 21.8% ($p < 0.01$). Group 4 hens began losing weight significantly ($p < 0.01$) every week, from study week 3 until the end of treatment.

Plasma Butyrylcholinesterase Activity (BuChE)

BuChE activity, reported as µmol-SH/mL, was inhibited significantly only in Groups 3 and 4, as follows:

Group	2	3	4
SRA 5172 (mg/kg)	0.3	1.0	3.0
Test Week	Percent Inhibition of BuChE Activity Relative to Controls (Group 1)[a]		
4	3	23	48*
8	7	27*	51*
12	0	17	44*
Average of the Above Means	5	22	48*

[a]This table is based on CLINICAL BIOCHEMISTRY Tables (pages 31 and 33-35) of the submission; SRA 5172 (Methamidophos Technical).

*$p < 0.05$

According to the above data, the inhibition of plasma BuChE activity was treatment-related and dose-dependent.

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Neuropathy Target Esterase (Neurotoxic Esterase; NTE) in Brain and Spinal Cord at Test Week 12

NTE activity, reported as µg phenol/mL reaction mixture, was inhibited significantly only in Group 3 (spinal cord) and Group 4 (brain and spinal cord), as follows:

Group	2	3	4
Tissue	Percent Inhibition of NTE Activity Relative to Controls (Group 1)[a]		
Brain	0	2	17*
Spinal cord	6	22*	42*

[a]This table is based on CLINICAL BIOCHEMISTRY tables (pages 31 and 36) of the submission.

*$p < 0.05$

According to the above data, the inhibition of brain and spinal cord NTE activity was treatment-related and dose-dependent.

Necropsy

No abnormalities were observed in hens sacrificed at the termination of the study and in one (Group 4) nonsurvivor. Macroscopic findings of the three other hens which died during the study consisted of the following: 1) prolapsed rectum and blood-stained oviducts (Group 1); 2) blood-stained anal area (Group 1); and 3) watery-clear fluid, 40 mL, in abdominal cavity (Group 4).

Microscopic Findings

The following findings were observed in all groups, including the control group:

1. Inflammatory cell foci: focal accumulation of lymphocytes and, less frequently, histiocytes and plasma cells -- in brain (medulla oblongata), all segments of spinal cord, and peripheral nerves.

2. Gliosis: abnormal focal accumulations of glial cells -- in gray matter of brain (medulla oblongata) and spinal cord (thoracic and lumbar regions).

3. Myelin fragmentation: vacuolated remnants of myelin sheaths -- in peripheral nerves;

4. Myelin fragmentation (no description) -- in spinal cord (cervical region).

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5. Vacuolation -- in spinal cord (lumbar region).

6. Axonal swelling -- in spinal cord and peripheral nerves.

7. Axonal degeneration -- in isolated fibers of sciatic nerve.

8. Neuritis -- in tibial nerve.

The above findings occurred at a low incidence (generally 1/group), with a minimal degree of severity (Grade 1) and were not attributed to treatment (see Attachment III for details).

No abnormalities were found in 3 nonsurvivors. The fourth nonsurvivor (Group 4) had only minimal (Grade 1) gliosis in medulla oblongata.

COMMENTS

Besides the EPA Pesticide Assessment Guidelines, this study was performed in compliance with various international guidelines and a lot of emphasis was placed on NTE activity as a key enzyme in delayed neurotoxicity. According to page 22 of the submission, "Both the cholinesterase and NTE were inhibited in groups 3 (1 mg/kg) and 4 (3 mg/kg) in a dose-related fashion, whereas for prolonged chronic dosing the threshold of NTE-inhibition that initiates severe neuropathy in hens is in the region of 45-65% (Johnson, M.K. (1982), Rev. Biochem. Toxicol. 4, 141-212). The observed treatment-related NTE inhibition is under this threshold and the negative clinical and histopathological findings concerning delayed onset organophosphorus ester induced neurotoxicity are congruous."

Based on negative results of the forced motor activity tests and microscopic examinations, Toxicology Branch I/HED agrees with testing laboratories that SRA 5172 (Methamidophos Technical) did not induce delayed neurotoxicity in this study. However, EPA does not currently have guidelines or policies governing testing for NTE activity in delayed neurotoxicity studies.

Positive control was not used in this study and food consumption was not determined.

This study was inspected eight times during 3/27/86 - 5/14/87 by Quality Assurance Unit.

Attachment I

FORCED MOTOR ACTIVITY

Motor co-ordination of individual hens was checked twice weekly. Each hen was removed from its cage, and placed on the floor. Locomotion was induced for two to three minutes to ascertain any abnormality of gait (specifically for locomotor ataxia and/or paralysis). The hens were assessed on the following scale:

0 = No sign of ataxia; motor activity and with normal coordinated locomotion.

1 = Motor activity slightly reduced; occasionally uncoordinated, particularly after phases of increased motor activity.

2 = Motor activity markedly reduced; stilted gait as well as occasional stumbling, clumsy landing (with the help of the tail) after jumping from a height of 0.5 to 1 meter.

3 = Motor activity considerably reduced; animal sitting on hocks, only shuffling gait possible.

4 = Animal unable to stand; total paralysis, lying on abdomen or side with spread wings.

Attachment II

Statistical Analysis

The following statistical methods were used to analyze the body weights and clinical laboratory data :

Univariate one-way analysis of variance was used to assess the significance of intergroup differences.

If the variables could be assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups.

The Steel-test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.

Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

Individual values, means, standard deviations and statistics were rounded off before printing. For example, test statistics were calculated on the basis of exact values for means and pooled variances and then rounded off to two decimal places. Therefore, two groups may display the same printed means for a given parameter, yet display different test statistics values.

References:

C.W. Dunnett: A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Statist. Assoc. 50, 1096-121 (1955).

R.G. Miller: Simultaneous Statistical Inference, Springer Verlag, New York (1981).

Attachment III

RCC-PROJECT 064293
SRA 5172 (c.n. METHAMIDOPHOS)

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064293-FIK
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PATHOLOGY REPORT
SUMMARY TABLES

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX (KO)

ORGAN/FINDING	DOSE GROUP:	1		2		3		4	
	SEX:	M	F	M	F	M	F	M	F
	NO. ANIMALS:	10		10		10		10	
MEDULLA OBLONGATA	NO. EXAM. :	10		10		10		10	
- INFLAMM. CELL FOCI		1							
- GLIOSIS								1	
SPINAL CORD-CERVIC.	NO. EXAM. :	10		10		10		10	
- INFLAMM. CELL FOCI						1			
- AXONAL SWELLING				1					
- MYELIN FRAGMENTATION						1		1	
SPINAL CORD-THOR.	NO. EXAM. :	10		10		10		10	
- INFLAMM. CELL FOCI		1						1	
- AXONAL SWELLING				1		1			
- GLIOSIS						1			
SPINAL CORD-LUMBAR	NO. EXAM. :	10		10		10		10	
- INFLAMM. CELL FOCI				2					
- AXONAL SWELLING				1					
- GLIOSIS						1			
- VACUOLATION								1	
SCIATIC NERVE	NO. EXAM. :	10		10		10		10	
- AXONAL DEGENERATION		1							
- MYELIN FRAGMENTATION		1							
- INFLAMM. CELL FOCI		1							
- AXONAL SWELLING		1						2	
TIBIAL NERVE	NO. EXAM. :	10		10		10		10	
- MYELIN FRAGMENTATION		1							
- INFLAMM. CELL FOCI		1							
- NEURITIS						1		1	
						1			

END OF REPORT SECTION

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SRA 5172 (c. n. METHAMIDOPHOS)

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PATHOLOGY REPORT

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EXPLANATION OF SYMBOLS

Codes and Symbols used at Animal Level:

1	= male
2	= female
K0	= animal assigned to terminal sacrifice group K0
K1-K9	= animal assigned to interim sacrifice groups K1-K9
R1-R9	= assignment to recovery/post-treatment groups R1-R9
+	= incidental death or sacrificed in moribund state
K0/+	= assigned sacrifice group K0/incidental death
K1-K9/+	= assigned sacrifice groups K1-K9/incidental death
R1-R9/+	= assigned sacrifice groups R1-R9/incidental death

Codes and Symbols used at Organ Level:

A	= severe autolysis, evaluation not possible
G	= gross finding evaluated histologically
R	= recut required
O	= tissue not present/not sufficiently cut
'	= histologic examination not required
:	= tissue not preserved for examination
-	= organ examined, no pathologic finding noted
!	= gross finding
?	= re-examination required
*	= comment in text of individual animal data
(	= only one of paired organs examined/present

Codes and Symbols used at Finding Level:

Grade 1	= minimal / very few / very small
Grade 2	= slight / few / small
Grade 3	= moderate / moderate number / moderate size
Grade 4	= marked / many / large
Grade 5	= massive / extensive number / extensive size
P	= finding present, severity not scored
T0	= neoplasm, not qualified
T1	= neoplasm, definitely incidental
T2	= neoplasm, probably incidental
T3	= neoplasm, probably fatal
T4	= neoplasm, definitely fatal
B, S, N	= benign, semimalignant, malignant neoplasm; statistical indices 0, 1,2,3,4 as given under T
M00	= metastasis, primary neoplasm not available for examination
M01/M99	= metastasis, site of prim. neoplasm indicated by organ code in text of gross and microscopic findings
(	= unilateral finding in paired organs
*	= comment in text of individual animal data

For abbreviations of microscopic findings used in tables see text of gross and microscopic findings.

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