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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: SRA 5172 (Methamidophos): Review of a 21-Day Dermal Toxicity Study with Rabbits (82-2)

DP Barcode No. D221020 P.C. Code No. 101201
Submission No. and Case No. - Not available
Tox. Chem. No. 378 A

TO: William J. Hazel, Section Head
Special Review Section
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

FROM: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/15/95
Section I, Toxicology Branch I
Health Effects Division (7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 12/18/95
Section I, Toxicology Branch I
Health Effects Division (7509C)

Karl P. Baetcke, Branch Chief KB 1/26/96
Toxicology Branch I
Health Effects Division (7509C)

As requested by you, Section I, Toxicology Branch I, has reviewed the following study: SRA 5172 - Subacute Dermal Toxicity Study on Rabbits; K.G. Heimann and G. Nash; Bayer AG Institut fur Toxikologie, Germany; Report No. 10330; Study No. T 0010270; Completion Date: October 30, 1981. **MRID No. 00147935**

In this study, SRA 5172 was applied on both intact and abraded skin of the New Zealand strain rabbits at dose levels of 0 (vehicle control), 0.5 and 5.0 mg/kg of body weight. The applications were made 5 times per week, for 3 weeks. Each group consisted of 6 males and 6 females, 3 with intact and 3 with abraded skin.

The only treatment-related effect observed was the inhibition **of cholinesterase (ChE) activities in plasma** (males and females with intact skin), **erythrocytes** (males with abraded and females with intact skin) **and brain** (males with intact skin, and females with both intact and abraded skin). ChE activities in plasma and erythrocytes were determined before the start of

treatment with SRA 5172 and after 10th and 15th treatments (days 0, 10 and 15, respectively). Brain ChE activity was determined only at the termination of the study.

Based on the inhibition of ChE activities in plasma, erythrocytes and brain, **the NOEL and LOEL are 0.5 mg/kg/day and 5.0 mg/kg/day,** respectively.

Because of many deficiencies (detailed in the **COMMENTS** section of the review), **this study is classified as Supplementary (Not Upgradable)** and does not satisfy the requirement, Guideline No. 82-2, for a Repeated Dermal Toxicity (21-Day) in the Rabbit. (Note: At the request by Chevron Chemical Company, Toxicology Branch/HED granted a waiver for the 21-day dermal study in January, 1984. This waiver was upheld by the FIFRA '88 DCI Review Committee in January, 1991).

Although this study is classified as Supplementary and is not upgradable, it has some scientifically valid information on ChE inhibitions. Since Methamidophos exerts its pesticidal action by inhibiting ChE activities, ChE activities have always been of primary concern in many studies with Methamidophos. Therefore, the ChE data in this study may be useful for the purpose of risk assessment when occupational exposure is of concern.

Krystyna K. Locke 12/15/95

Primary Review by: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)

Secondary Review by: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C) Roger Gardner 12/18/95

DATA EVALUATION RECORD

STUDY TYPE: 82-2 Repeated Dose Dermal Toxicity (21-Day) in the Rabbit

EPA IDENTIFICATION NUMBERS:

MRID No. 00147935
EPA ID No. - Not available
Case No. - Not available
Tox. Chem. No. 378 A

DP Barcode No. D221020
Submission No. - Not available
P.C. Code No. 101201

TEST MATERIAL: SRA 5172 (Methamidophos); purity: 64.5%; batch no. 808902036; stored at room temperature (in the lab fume cabinet).

REPORT NUMBER; 10330; Study No. T 0010270

SPONSOR: Mobay Chemical Corporation

TESTING FACILITY: Bayer AG Institut fur Toxikologie, Germany.

TITLE OF REPORT: SRA 5172 - Subacute Dermal Toxicity Study on Rabbits

AUTHORS: K.G. Heimann and G. Nash

STUDY COMPLETION DATE: October 30, 1981

EXECUTIVE SUMMMARY

In this subchronic dermal toxicity study (MRID 00147935), SRA 5172 (Technical Methamidophos; a.i. content or purity: 64.5%) was applied on the back and flank skin (area: 42 cm^2) of 6 New Zealand rabbits/sex/group for 3 weeks (5 times/week), at doses of of 0 (vehicle control), 0.5 and 5.0 mg/kg of body weight. The application sites of 3 rabbits/sex/group were intact (shorn), whereas the application sites on the remaining rabbits were abraded. The test sample, prepared before each treatment, was applied in an emulsion of Cremophor EL/distilled water, using 0.5 mL of an emulsion/kg body weight. During the 6-hour exposure periods, the rabbits were kept in harness in which they could not wipe off or ingest the test material. At the end of each exposure, the application sites were cleaned with soap and water.

The only treatment-related effect observed was the inhibition of cholinesterase (ChE) activities in the 5.0 mg/kg group. Relative to the pretreatment (Day 0) values, the percent inhibitions () were as follows: **in plasma** - males (23-35) and females (23) with intact skin; and **in erythrocytes** - males (15-31) with abraded skin and females (26) with intact skin. Similar results are obtained when ChE activities in the SRE 5172-treated rabbits are related to control values. Relative to the control values, ChE activity **in brain** was inhibited 12-14%. Cholinesterase activities in plasma and erythrocytes were determined before the start of treatment with SRA 5172 and after 10th and 15th treatments (days 0, 10 and 15, respectively). Brain ChE activity was determined only at the termination of the study.

Based on the inhibition of ChE activities in plasma, erythrocytes and brain, **the NOEL and LOEL are 0.5 mg/kg/day and 5.0 mg/kg/day,** respectively.

Because of many deficiencies (detailed in the **COMMENTS** section of the review), **this study is classified as Supplementary (Not Upgradable)** and does not satisfy the requirement, Guideline No. 82-2, for a Repeated Dermal Toxicity (21-Day) in the Rabbit. (Note: At the request by Chevron Chemical Company, Toxicology Branch/HED granted a waiver for the 21-day dermal study in January, 1984. This waiver was upheld by the FIFRA '88 DCI Review Committee in January, 1991).

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EXPERIMENTAL PROCEDURES

This study was carried out from May to June, 1981. SRA 5172 was applied to the backs and flanks (area: about 6 cm x 7 cm) of 6 white New Zealand rabbits/sex/group for 15 consecutive workdays (Monday through Friday), at doses of 0 (vehicle control), 0.5 and 5 mg/kg of body weight. The application sites of 3 rabbits/sex/group were intact ("shorn of hair 48 hours before the start of treatment"), whereas the application sites on the remaining rabbits were abraded with sandpaper (which produced redness and slight swelling) 24 hours before the start of the test. The hair which grew during the test was reshorn twice weekly from the areas under treatment. The test sample, prepared before each treatment, was applied in an emulsion of Cremophor EL/distilled water, using 0.5 mL of the emulsion/kg of body weight. The concentration of SRA 5172 in the in the emulsion used for the 0.5 mg/kg group was 0.1% and in that used for the 5 mg/kg group, 1.0%. During the 6-hour exposure periods, the rabbits were kept in harness in which they could not feed or drink. Also, it was apparently impossible for them to wipe off or ingest the test substance. At the end of each exposure, the application sites were cleaned with soap and water. **The rabbits were:**

(1) Obtained from the breeder, Hacking and (name illegible), Huntingdon, England. At that time, the rabbits weighed 2.5-3.0 kg. The acclimation period, if any, was not reported.

(2) Housed singly at temperatures of 19-23°C and 12 hours light/12 hours dark cycles. Humidity was not reported.

(3) Assigned to groups randomly and given free access to food (Z 222 rabbit feed) and water.

The following parameters were examined for all rabbits on the study:

(1) **Clinical Observations:** The appearance and behavior of the animals were inspected daily.

(2) **Body Weights:** Were obtained before the initiation of treatment and at the end of each week during the treatment.

(3) **Local Skin Irritation:** The application sites were examined for symptoms of inflammation (redness and swelling) before the start of the study and at the end of each exposure period. The redness was evaluated according to the guidelines published in the US Federal Register 38, 187, 27049 (1973) or Draize procedure (1959), as follows:

Redness	Score
None	0
Slight, barely perceptible	1
Slight to moderate, easily perceptible	2
Moderate to strong	3
Severe, partially eroded	4

To evaluate the swelling, the skin fold on the back, in the center of the exposed area, was measured with a Cutimeter (Skinfold Caliper, Holtain Ltd., Crosswell, England).

(4) **Hematology:** The following determinations were performed before the start of treatment and at the termination of treatment: erythrocytes, leucocytes, hemoglobin, hematocrit, and thrombocyte and differential blood count. Mean corpuscular erythrocyte volume, mean hemoglobin content of erythrocytes and mean cell hemoglobin concentration were also calculated at the same time intervals.

(5) **Clinical Chemistry:** The following determinations were performed before the start of treatment and at the termination of treatment: aspartate aminotransferase (GOT), alanine aminotransferase (GPT), alkaline phosphatase (ALP) and cholinesterase activities; and plasma urea, blood sugar (glucose ?) and creatinine concentrations. Cholinesterase activity was also determined after the 10th treatment with SRA 5172. The procedure of Ellmann (Biochem. Pharmacol. 7, 88, 1961) was used for the determination of cholinesterase activities.

(6) **Urinalysis:** The following determinations were performed before the treatment was started and at the termination of the study, using urine collected from each rabbit over a 16-hour period (overnight): protein, pH, sugar (glucose ?), blood (hemoglobin) and urobilinogen. The deposit, obtained by centrifuging urine for 5 min. at 2000 g, was evaluated microscopically for bacteria, epithelia, erythrocytes, leucocytes, amorphous salts, triple phosphate (triphosphate ?) and calcium oxalate.

(7) **Necropsy:** Twenty-four to 48 hours after the last treatment, the rabbits were anesthetized with sodium hexobarbitone, exsanguinated by the vena caudalis and grossly examined by dissection.

(8) **Organ Weights:** The following organs were weighed: heart, lung, liver, spleen, kidneys, adrenals, testicles, ovaries and thyroid. These organs, and also epididymides, uterus, and treated and untreated dorsal

skin, were fixed in Bouin's solution or in formol calcium (liver and one kidney). Brain was not weighed, but samples were taken for cholinesterase determination.

(9) **Histopathology:** The following tissues were examined histologically: heart, lung, liver, spleen, kidneys, adrenals, thyroid, testicles, epididymides, uterus, ovaries and skin (treated and untreated). The fixed organs/tissues were embedded in Paraplast, sectioned and stained with hemalum (hematoxylin ?) - eosin or by the Periodic Acid Schiff (PAS) reaction (additional sections of kidneys only). Sections of liver were also stained for fat with Oil Red O.

RESULTS

Clinical Observations

Nothing remarkable was observed in any group. SRA 5172 (Methamidophos) had no effect on the appearance and behavior during the treatment period. There were no mortalities.

Body Weights

The body weight data are somewhat difficult to interpret. At the termination of the study, there was a dose-related decrease in the meam body weight gain for the male rabbits with an intact skin, whereas the reverse was true for the male rabbits with an abraded skin: the control male group gained the least weight and the high-dose male group (5 mg/kg) gained the most weight.

Comparing the initial and final body weights, the female rabbits in the low-dose group (0.5 mg/kg), both with intact and abraded skin, lost weight, whereas those in the high-dose group gained weight. The group mean weight loss in the low-dose group was attributed to the weight loss by two rabbits. According to the submitted report (MRID 00147935), page 10, " The distinct weight loss in female rabbits nos. 19 and 30 (0.5 mg/kg, shorn and abraded skin respectively), which lowers the mean body weights accordingly, is considered a non-relevant chance finding, as it is not specific to the group ". However, female no. 19 was in the control (intact skin) group and not in the low-dose group (page 30 of the submitted report). Also, this animal gained weight during the course of the study. The weight gain data are shown below.

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TABLE 1 Group Mean Body Weight Gains (g) During the Study

SRA 5172 mg/kg	Intact Skin		Abraded Skin	
	Males	Females	Males	Females
0	310	100	50	100
0.5	140	- 10	80	- 80
5.0	90	70	180	220

The above table is based on Table 1, page 11, of the submitted report (MRID 00147935). Weight gain = Final body weight minus body weight before the start of treatment. - Sign before the number denotes weight loss. Weekly group mean body weights and weekly individual body weights are in Attachment I of this review.

Local Skin Irritation

No redness (as an indication of inflammmation) was observed on rabbits with intact skin. On the rabbits with abraded skin, including the controls, redness (score 1.7-3.0) was observed only during the first 3 treatment days. The results of the skin fold measurements (summarized below) did not indicate alterations caused by the test material.

TABLE 2 Skin Fold Thickness (mm) After Treatment Days 1-15

SRA 5172 mg/kg	Intact Skin		Abraded Skin	
	Males	Females	Males	Females
0	2.6-3.6	1.9-2.2	3.4-3.9	2.0-3.3
0.5	2.4-3.4	1.9-2.3	2.5-3.7	2.1-3.0
5.0	2.8-3.6	2.1-2.8	3.5-4.1	2.2-3.0

The above table is based on Table 3, page 14, of the submitted report (MRID 00147935).

Hematology

The test material had no effect on any of the parameters examined.

Clinical Chemistry

With the exception of cholinesterase activities, the test material had no effect on any of the parameters examined. The results obtained for cholinesterase activities in plasma, erythrocytes and brain are summarized in TABLES 3 and 4 below.

TABLE 3 **Percent Decreases in Cholinesterase Activities, Relative to the Control Values, on Days 0, 10 and 15, Respectively**

SRA 5172 mg/kg	Intact Skin			Abraded Skin		
	Male Rabbits					
	Plasma					
0.5	0	0	0	0	15	24
5.0	10	20	44	0	18	32
	Erythrocytes					
0.5	0	19	0	0	4	0
5.0	0	30	18	0	40	0
	Brain					
0.5	-	-	0	-	-	0
5.0	-	-	14	-	-	0
	Female Rabbits					
	Plasma					
0.5	11	5	14	9	12	15
5.0	13	25	35	17	22	35
	Erythrocyte					
0.5	9	5	5	24	0	4
5.0	9	11	30	15	13	19
	Brain					
0.5	-	-	0	-	-	0
5.0	-	-	12	-	-	12

This table is based on Table 8, page 21, of the submitted report (MRID 00147935). The above inhibitions were calculated by the toxicologist who reviewed this study (KKL); the actual values for cholinesterase (ChE) activities are in Attachment II of this review. Cholinesterase activities were determined before the treatment with SRA 5172 and after the 10th and 15th treatments (days 0, 10 and 15, respectively, in the above table). Zero (0) decrease means that the ChE activity was the same or greater than the control value.

According to the above data, with possibly one exception, ChE activities were not inhibited in the 0.5 mg/kg male and female rabbits with intact and abraded skin. Only plasma ChE

activity appears to be slightly inhibited by SRA 5172 in the male and female rabbits with abraded skin, after the 15th treatment.

In the case of the 5.0 mg/kg group, the following ChE inhibitions were observed: in plasma (male and female rabbits with intact and abraded skin); in erythrocytes (males and females with intact skin); and in brain (males and females with intact skin and females with abraded skin). The highest inhibitions occurred in plasma and the lowest in the brain.

A slightly different profile of ChE inhibitions is obtained when ChE activities in plasma and erythrocytes on days 10 and 15 are compared with those on day 0 (pretreatment values), an approach used by the authors of this study. These data are summarized in TABLE 4.

TABLE 4 **Percent Decreases in Cholinesterase Activities on Treatment Days 10 and 15 When Related to the Pretreatment Values (Day 0)**

	Intact Skin		Abraded Skin	
Treatment Days	10	15	10	15
SRA 5172 (mg/kg)	**Male Rabbits**			
	Plasma			
0	13	0	0	0
0.5	18	9	18	15
5.0	23	35	16	19
	Erythrocytes			
0	0	0	0	0
0.5	15	17	0	6
5.0	12	20	31	15
	Female Rabbits			
	Plasma			
0	8	0	9	0
0.5	0	0	12	0
5.0	23	23	15	17
	Erythrocytes			
0	12	4	21	14
0.5	9	0	0	0
5.0	14	26	19	18

The above table is based on Table 8, page 21, of the submitted report (MRID 00147935). The decreases in ChE activities were calculated by the toxicologist who reviewed this study (KKL); the actual values for ChE activities are in Attachment II of this review. Zero (0) inhibition means that the ChE activity was the same or greater than the pretreatment value.

According to the above data, treatment-related inhibitions of ChE activities in plasma and erythrocytes were not observed in the low-dose (0.5 mg/kg) male and female rabbits. In the 5.0 mg/kg group, plasma ChE activity was inhibited only in the rabbits with intact skin. The inhibition was slight to distinct in the males and distinct in the females. Erythrocyte ChE activity was inhibited slightly to distinctly in the males with abraded skin and in the females with intact skin.

Urinalyses

The test material had no effect on any of the parameters examined.

Necropsy

According to the submitted report (MRID 00147935), " Gross pathology revealed no changes in the experimental animals specific to a group. The findings of dissections largely corresponded to the norm for conventionallu kept rabbits of this age and origin". No other data were submitted.

Organ Weights

SRA 5172 had no effect on the absolute and relative (organ/body weight ratio) organ weights. Although, relative to the control values, changes in the absolute and relative weights were observed for every organ and in every group, they were either **(a)** small (1-10%); **(b)** dose-unrelated; **(c)** resulted from one unusually high (or low) value obtained for a group; or **(d)** were due to some factors, other than treatment (example: 26% decrease and 12% increase in the relative weight of spleen in the low-dose and high-dose females with abraded skin, respectively).

Histopathology

According to the authors of this study (MRID 00147935), SRA 5172 did not induce any changes in the tissues examined. The histopathological findings were reported for each rabbit in the study, but every entry in the tables (pages 62-67) was zero (0). Zero was defined in the List of abbreviations, page 61, as

follows: " Finding within normal variability corresponding in particular in the species and age of the experimental animals and their conventional conditions of accomodation. These findings include minor consequences of sacrifice (pulmonary emphysema) and in particular non-specific consequences of inflammation in lung, heart, thyroid, liver, epididymes and kidneys in single animals in all groups, which can be best attributed to parasitic invasion. The possible cause of non-suppurative nephritis in the rabbit is, in our experience, invasion of encephalitozoon (nosema) cuniculi. The non-specific inflammatory kidney and liver alterations appeared macroscopically as spots, cysts, knots, lobules and scars. Occasionally juvenile testicles and epididymes and focal atrophy in the testicles were observed in single animals ". With this manner of reporting of the experimental findings, the reader has no idea which rabbit had what.

COMMENTS

This study has many deficiencies in conduct and reporting, and does not meet the December 24, 1989 EPA ACCEPTANCE CRITERIA for a Repeated Dermal Toxicity (21-day) in the Rat (82-2). These deficiencies are listed below.

1. A lot of data in the report is obscured by stamping CONFIDENTIAL PROPERTY OF MOBAY CHEMICAL CORPORATION randomly on every page of the report.

2. Although there were 6 rabbits/sex/group, 3 rabbits/sex/group had their skin abraded. Since rabbits with the intact and abraded skin were not exactly "the same", not enough rabbits were available for statistical analyses, for example, of the cholinesterase data.

3. Only 2 test groups (0.5 and 5.0 mg/kg) and a control group were used, and it was not reported how these doses were selected.

4. SRA 5172 (Methamidophos) was applied on the skin in a Cremophor LE/water emulsion. It was not reported what Cremophor LE is or why it was used, since Methamidophos is readily water-soluble.

5. Food consumption was not determined.

6. The following clinical chemistry parameters, required in the cited above EPA ACCEPTANCE CRITERIA, were not determined: bilirubin, total protein, albumin, inorganic phosphate, calcium and sodium.

7. It was not reported if the rabbits were fasted before the blood and urine collection.

8. The group mean weight loss in the low-dose (0.5 mg/kg; intact skin) female group was attributed in the report to the weight loss by rabbit number 19. However, this rabbit was in the control group and not in the low-dose group. (Details are on page 5 of this review).

9. The histopathology data were inadequately reported. (Details are on pages 9 and 10 of this review).

10. Concentration and stability of SRA 5172 in the dosing medium were not determined.

11. Compliance statements were not included in the submission.

Methamidophos TOXR # 11779

Page_____ is not included in this copy.

Pages 14 through 19 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.