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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **SRA 12869 TECHNICAL (ISOFENPHOS):** Toxicology Review of a 90-Day Subchronic Dermal Toxicity Study [Action: 627 Core Data Submission]

TO: Kathryn Davis, PM 52/Ruby Whiters
Reregistration Division
SRRD

FROM: SanYvette Williams-Foy, D.V.M.
Toxicology Branch II
HED (7509C)

THROUGH: Susan L. Makris, M.S. 8/31/94
Acting Section Head, Review Section IV
Toxicology Branch II
HED (7509C)

and

Marcia van Gemert, Ph.D., Chief 9/1/94
Toxicology Branch II
HED (7509C)

STUDY IDENTIFICATIONS: **Submission: 8449072**
Case #: 816229
DP Barcode: D195383
PC Codes: 010904

Registrant: Bayer AG

ACTION REQUESTED: Review of a 90-day subchronic dermal toxicity study [MRID 428917-02] for acceptability for guideline §82-3 and to fulfill data requirements for the reregistration of SRA 12869 Technical (Isofenphos).

RESPONSE: The submitted study has been reviewed by Toxicology Branch II and found to be classified as core minimum. The executive summary is given below. The Data Evaluation Report [DER] for this study is attached.

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EXECUTIVE SUMMARY: The effects of Isofenphos (SRA 12869), were evaluated after repeated 6-hour daily (5 days/week) dermal application to the skin of male and female rabbits (10/sex/group) at doses of 0, 2, 10 or 50 mg/kg bw/day for 90 days.

Based on the results of this study, Isofenphos (SRA 12869), did not cause any dermal effects after repeated application. Statistically significant decreases in plasma, red blood cell and brain cholinesterase levels were seen in test animals at 10 and 50 mg/kg/day.

The no-observed-effect level (NOEL) for local dermal effects is 50 mg/kg bw per day in both sexes. The no-observed-effect level for cholinesterase inhibition in the plasma, red blood cells and brain is 2 mg/kg bw per day.

Reviewed by: SanYvette Williams-Foy, D.V.M. 8-31-94
Sec. IV, Tox. Branch II (7509C)
Secondary reviewer: Susan L. Makris, M.S., Acting Section Head
Section IV, Tox. Branch II (7509C) 8/31/94

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Subchronic Dermal Toxicity Study - Rabbits

GUIDELINE: #82-3 **MRID #:** 428917-02

PC Code: 10904
DP BARCODE: D195383
SUBMISSION: S449072
CASE #: 816229

TEST MATERIAL: SRA 12869 Technical

SYNONYM: Isofenphos

STUDY TITLE: "SRA 12869 TECHNICAL: SUBCHRONIC DERMAL TOXICITY STUDY ON RABBITS"

AUTHOR: L. Diesing, M. Driest, and B. Schilde

LAB PROJECT ID NO: 102688

LAB REPORT #: 21362

TESTING FACILITY: Bayer AG Dept. of Toxicology, F.R. GERMANY

SPONSOR: Bayer AG, Wuppertal 1, F.R. Germany

STUDY COMPLETED : May 13, 1992

Executive Summary: The effects of Isofenphos (SRA 12869), were evaluated after repeated 6-hour daily (5 days/week) dermal application to the skin of male and female rabbits (10/sex/group) at doses of 0, 2, 10 or 50 mg/kg bw/day for 90 days.

Based on the results of this study, Isofenphos (SRA 12869), did not cause any dermal effects after repeated application. Statistically significant decreases in plasma, red blood cell and brain cholinesterase levels were seen in test animals at 10 and 50 mg/kg/day.

The no-observed-effect level (NOEL) for local dermal effects is 50 mg/kg bw per day in both sexes. The no-observed-effect level for cholinesterase inhibition in the plasma, red blood cells and brain is 2 mg/kg bw per day.

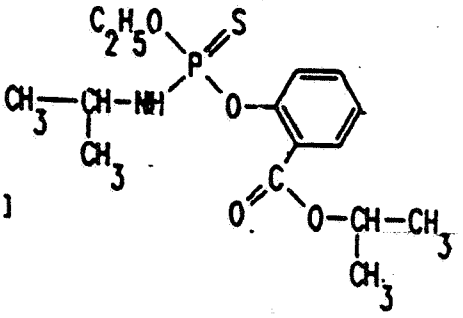
This study is classified as Core Minimum, and satisfies minimum guideline requirements [#82-3] for a 90-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the experimental procedures and results of a 90-day dermal toxicity study in rabbits with SRA 12869 technical (Isofenphos).

II. MATERIALS

1. Test Material



Chemical Name: SRA 12869 tech.
Common Name: Isofenphos
Purity: 92.1%
Batch: 808816825
Description: Yellow-brown liquid
pH: 6.0 [2% in 0.1% NaCl solution]

2. Test Animals

Species: Rabbit
Strain: New Zealand White, HC:NZ strain
Source: Interfauna UK Lim, Huntingdon, ENGLAND
Sex: Males and Females
Weight: ♂ 3.15 kg; ♀ 3.01 kg
Age: 13-18 weeks
Acclimation: 27 days
Identification: ear tattoos, color markings, cage cards

3. Animal Husbandry

Housing: Individual
Food: Ssniff® rabbit diet *ad libitum*
Water: Tap *ad libitum*

4. Environment:

Test animals were maintained under adequate environmental conditions [Temp.: 22± 2°C; Hum.: 50%; 12 hr light/12 hr dark cycle; Air exchange: 10 times/hr]

III. METHODS

A. Study Period

Test animals were treated once a day, 5 days/week for 13 weeks or for a total of 66 [0 + 2 mg/kg bw; males], 67 [10 + 50 mg/kg bw; males], 68 [0 + 2 mg/kg bw; females], or 69 [10 + 50 mg/kg bw; females] days.

B. Dose Selection

Doses were selected based on results from two pilot range-finding studies, T1039540 and T7039690. Study T7039540 used doses of 0, 1, 10, or 100 mg/kg bw. Treatment-related changes

included inhibition of plasma cholinesterase (CHE) at doses of 10 and 100 mg/kg bw, and inhibition of RBC and brain cholinesterase at doses of 100 mg/kg bw. Study T7039690 used doses of 0, 2.5, 5 or 7.5 mg/kg bw. Treatment-related changes included plasma CHE inhibition at doses of 5 and 7.5 mg/kg bw, and inhibition of RBC CHE at doses of 7.5 mg/kg bw.

Based on the results of these two dose range-finding studies, doses used for the main study were 0, 2, 10 or 50 mg/kg bw.

C. Test Material Formulation and Application

SRA 12869 tech was formulated with 2% v/v Cremophor® FL in sterile physiological saline. The stability (65 hours, unspecified storage conditions) and homogeneity of Isofenphos in the treatment formulations were analytically verified prior to study initiation (the homogeneity of the lowest concentration was only verified during the study itself).

The test material was stable (0-1% variability) for 65 hours. For the 1% and 50% dose groups adequate homogeneity was demonstrated. Concentration analyses conducted on samples from the concurrent study revealed levels of test substance that were between 7.4 and 11.6% below nominal.

D. Study Period

Test animals were treated on 5 weekdays/week for 13 weeks. The treatments were also performed on the last weekend prior to study termination.

E. Treatment Method

The day before study initiation, the backs and flanks of the rabbits were shaved to expose at least 10% of body skin area. The test substance, at a volume of 2 ml/kg bw, was distributed over a gauze patch placed on the back of each rabbit using a blunted needle syringe. The gauze patch was turned over, then fixed with tape for a 6-hour period. After patch removal, each treatment area was cleaned with soap and water.

F. Clinical observations

Observations were made once a day for behavior and appearance.

G. Ophthalmic examination

Examinations were performed prior to treatment in all animals, and on all control and high-dose animals at termination.

H. Body weights

Body weights were determined before study initiation, and at weekly intervals thereafter.

I. **Food consumption**

Individual food intake was determined on a weekly basis and used to calculate mean daily food intake.

J. **Dermal evaluation**

Treatment areas were examined before study initiation and once a day throughout the study for reddening of the skin. This was done 24 hours after initiation of the previous treatment and on days following a treatment-free day. Reddening was graded based on the Draize test and on USDA guidelines.

Swelling was evaluated before study initiation and two times a week by measuring skinfold thickness of the back at the center of the treatment area using a skin fold caliper. Two measurements were made at two different points and the mean was calculated for the individual results. Gross findings were also recorded.

K. **Clinical Laboratory Tests**

Blood was withdrawn from fasted test animals before treatment was initiated, on Day 28/29 (from non-fasted animals to determine CHE activity), and at termination. Enzyme assays of liver samples and brain CHE activity determinations were performed following necropsy.

Plasma and red blood cell CHE activities were determined at the end of the treatment period using blood samples withdrawn 5.5 hrs after initiation of the final treatment.

1. Hematology

The following parameters were measured pre-treatment and post-treatment: Hemoglobin, MCHC, reticulocyte counts, MCH, Heinz bodies, MCV, hematocrit, platelet count, RBC count, total and differential WBC counts, RBC morphology.

2. Clinical chemistry

The following parameters were measured pre-treatment and post-treatment: alkaline phosphatase, calcium, phosphorus, urea, chloride, sodium, potassium, glucose, alanine aminotransferase, aspartate aminotransferase, creatinine, total bilirubin, protein, albumin, cholesterol, acetylcholinesterase [brain, serum, plasma, and erythrocytes].

3. Liver tissue

The following parameters were measured: N-demethylase, O-demethylase, cytochrome P-450, and triglycerides

4. Pathology

a. At death or termination of the study all animals were sacrificed and necropsied. Macroscopic examinations were performed on each animal.

b. The kidneys, brain, thyroid, heart, lung, liver, adrenals, spleen, ovaries and testes were weighed.

c. Microscopic evaluations were performed on the adrenal glands, kidneys, liver, heart, lung, spleen, pituitary, thyroid, pancreas, thymus, salivary gland, mesenteric lymph nodes, aorta, sciatic nerve, brain, testes, epididymides, prostate, seminal vesicle, ovaries, uterus, vagina, trachea, esophagus, stomach, intestines [duodenum, jejunum, ileum, cecum and rectum], urinary bladder, gallbladder, sternum, skeletal muscle, mandibular lymph nodes, and treated and untreated skin.

L. Statistical Analyses

Methods of statistical analysis are presented in Appendix 1, appended from page 22-23 of the study.

M. Regulatory Compliances

A signed statement of No Data Confidentiality Claim was dated 06/01/92.

A signed statement dated 02/27/92 indicated that this study was conducted according to the promulgation of the principles of EPA's Good Laboratory Practice Standard [40 CFR Part 160].

A signed Quality Assurance Statement was dated 05/12/92.

No flagging statement was included.

IV. RESULTS:

A. Test Material Formulation

The analytical results for the stability and homogeneity of the 1% and 50% test substance formulations were adopted from another study, in which the batch of test substance used was different from that in this study.

The test material was stable (0-1% variability) for 65 hours. For the 1% dose and 50% dose groups adequate homogeneity was demonstrated. Concentration analyses conducted on samples from the concurrent study revealed levels of test substance that were between 7.5 and 11.6% below nominal.

B. Clinical Observations and Mortality

No treatment-related changes were observed, and no treatment-related deaths occurred during the study. Two male rabbits (one each in the 2 and 10 mg/kg dose levels) were sacrificed due to hindlimb paralysis resulting from spinal injury; a replacement animal was added to the 2 mg/kg group.

C. Body weights

There were no statistically significant effects on body weights of males or females throughout the study period.

D. Food consumption

Statistically significant [$p \leq 0.05$ or $p \leq 0.01$] increases in food consumption were seen in males, only. These increases were seen, in Table 1, at the 2 mg/kg bw dose on Week 6, at the 10 mg/kg bw dose on Weeks 1, 4, 7, and 10-13, and at the 50 mg/kg bw dose on Weeks 5, 8, 12, and 13. These findings were not considered to be treatment-related.

Table 1. Male Mean Daily Food Consumption/Animal (g)

Week	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
1	111	106	113*	111
4	111	115	117*	113
5	108	111	110	113*
6	106	110*	109	110
7	107	109	112*	111
8	107	110	108	111*
10	106	107	110**	108
11	108	106	113**	112
12	107	104	114**	110*
13	94	93	98**	99**

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from page 26 of the study.

E. Local skin findings

Skin fold thickness was statistically significantly ($p < 0.05$) different in the mid-dose (10 mg/kg) animals from that in control animals on treatment Days 20, 2[illegible], and 90 for males and Day 44 for females (Study report page numbers 35-36). Because no dose-related changes were seen, this finding was not

considered to be treatment-related. Other localized dermal observations occurred sporadically among control and treated groups and were not attributed to treatment (report page numbers 35-36).

F. Hematology

Pretreatment hematological evaluations (Table 2) revealed significant differences ($p \leq 0.05$ or $p \leq 0.01$) between the groups designated for control of test substance application.

Post-treatment evaluations [Table 3], revealed statistically significant increases in thrombocytes and pseudoeosinophils, and decreases in lymphocytes of males in the 2 mg/kg bw dose group as compared to controls. For males at the 10 mg/kg bw treatment level, thrombocytes were increased and WBC's were decreased; and a decrease in monocytes was observed in 50 mg/kg bw males.

Statistically significant findings for females included: at the 2 mg/kg bw dose a decrease in MCHC, at the 10 mg/kg bw dose a decrease in reticulocyte counts, and at 50 mg/kg bw dose an increase in MCHC, pseudoeosinophils, basophils, and a decrease in lymphocytes and reticulocytes.

Because these values were generally within +/- 2 standard deviations of the mean historical control value (Appendix 2) and no dose-relationships were shown, and because many of these differences were also apparent at the pre-treatment hematological evaluation, they were not considered to be treatment-related.

Table 2. Pre-Treatment Hematology Parameters

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Males				
WBC 10^9/L	8.2	6.6	6.4*	6.3*
Lymphocytes %	66.6	66.6	66.4	52.8**
Pseudoeosin. %	25.9	25.6	26.6	39.6**
Females				
RBC 10^{12}/L	6.00	6.03	6.17	6.38*
HCT L/L	0.368	0.358	0.370	0.379*
HGB g/L	123	120	126	130*
MCHC g/L RBC	335	336	341	343*
Pseudoeosin. %	38.7	37.5	48.2	56.9*
Lymphocytes %	52.5	53.9	45.5	37.2*

* = $p \leq 0.05$
** = $p \leq 0.01$.
Table excerpted from pages 38 & 39 of the study.

Table 3. Post-Treatment Hematology Parameters

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Males				
WBC 10^9/L	6.6	6.8	5.4*	6.1
Thrombocytes 10^9/L	324	395*	385*	341
Lymphocytes %	68.8	54.5**	65.3	62.0*
Monocytes %	1.1	1.1	0.2	0.3*
Pseudoeosin. %	25.5	39.0**	27.1	32.7
Females				
MCH pg	20.1	19.0*	19.9	19.8
Reticulocytes %	34	33	26**	27*
Lymphocytes %	61.4	60.1	61.4	42.3**
Pseudoeosin. %	35.7	37.3	34.2	52.0**
Basophils %	2.8	2.4	4.3	5.1*

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from pages 40 & 41 of the study.

G. Clinical Chemistry:

Pre-treatment clinical chemistry results are shown in Table 4. Results from animals at the end of treatment are shown in Table 5. Pretreatment clinical chemistry evaluation revealed significant differences between the groups designated for control or test substance application. Statistically significant findings in males following 13 weeks of treatment included an increase in phosphorus in the 2 mg/kg bw dose group, and decreases in alanineaminotransferase, chloride, and potassium at doses of 10 and 50 mg/kg bw dose. Females dosed at 50 mg/kg bw had significantly decreased total bilirubin.

Because the post-treatment values were within ± 2 standard deviations of the mean historical control value (Appendix 2) and no dose-relationships were shown, they were not considered to be treatment-relat..

Table 4. Pre-Treatment Clinical Chemistry

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Males				
Alanineamin. (u/L)	23.2	23.3	16.6*	19.8
Creatinine (mcmol/L)	92	[illegible]1*	92	94
TBilirubin (mcmol/L)	0.8	1.0*	1.0	0.9
Potassium (mmol/L)	4.1	4.1	4.3	4.6*
Females				
TBilirubin (mcmol/L)	1.1	1.0	1.0	0.9*
Albumin (g/L)	39.2	39.2	41.8**	41.1*
Cholesterol (mmol/L)	1.35	1.42	2.10*	1.94*
Calcium (mmol/L)	3.26	3.21	3.18*	3.09**
Phosphorus (mcmol/L)	1.75	1.81	1.98*	2.03**
Protein (g/L)	61.9	64.0	67.4*	65.8*

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from pages 43 & 44 of the study.

Table 5. Clinical Chemistry at the End of Treatment

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Males				
Alanineamino. (u/L)	33.6	26.9	23.2*	24.1*
Chloride (mmol/L)	104	104	100**	100**
Potassium (mmol/L)	4.2	3.9	3.8*	3.8*
Phosphorus (mcmol/L)	1.17	1.33**	1.26	1.25
Females				
TBilirubin (mcmol/L)	1.3	1.2	1.1	1.1*

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from pages 45 & 46 of the study.

H. Cholinesterase Activity

Blood cholinesterase (CHE) samples taken on Day 28/29 show, in Table 6, statistically significant decreases in plasma CHE levels in males at doses of 10 and 50 mg/kg bw and in females at the 50 mg/kg bw dose. Red blood cell cholinesterase values were statistically significantly decreased at the 50 mg/kg bw dose in males and at doses of 10 and 50 mg/kg bw in females. All values at 50 mg/kg, however, were below the historical control range (± 2 S.D. of the mean value) as seen in Appendix II.

Blood CHE samples taken at the end of the study (Day 89) showed, in Table 7, dose-related decreases in both plasma and RBC CHE levels. These decreases became statistically significant at doses of 10 and 50 mg/kg bw in both males and females. As seen in Appendix II, plasma CHE levels and RBC CHE levels for 50 mg/kg bw females were within +/- 2 S.D. of the mean historical control value.

Table 6. Blood Cholinesterase on Day 28 (males)/Day 29 (females)

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Plasma Cholinesterase kU/L				
Males	0.60	0.54	0.39*	0.24*
Females	0.57	0.53	0.50	0.22*
RBC Cholinesterase kU/L				
Males	0.80	0.95	0.65	0.17*
Females	1.00	0.94	0.56*	0.26*

* = $p \leq 0.05$
Table excerpted from page 50 of the study.

Table 7. Blood Cholinesterase at the End of Study (Day 89)

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Plasma Cholinesterase kU/g				
Males	0.57	0.51	0.36*	0.24*
Females	0.53	0.47	0.42*	0.20*
RBC Cholinesterase kU/g				
Males	1.06	1.21	0.72*	0.31*
Females	1.03	0.96	0.54*	0.22*

* = $p \leq 0.05$
Table excerpted from page 51 of the study.

Results in Table 8 show a decreasing trend in brain cholinesterase activity that became statistically significant ($p < 0.05$) at doses of 10 and 50 mg/kg bw in males and at doses of 50 mg/kg bw in females. When compared to historical controls in Appendix II, these values were generally within ± 2 S.D. of the mean historical value.

Table 8. Brain Cholinesterase on Day 89 U/g

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Males	3.02	3.15	1.86*	1.29*
Females	3.89	2.82	2.56	1.48*

* = $p \leq 0.05$
Table excerpted from page 52 of the study.

I. Liver Homogenate Tests

A statistically significant increase in cytochrome p-450 was seen, in Table 9, at the 10 mg/kg bw dose and in triglycerides at doses of 2, 10, and 50 mg/kg bw in males. The study author attributed the changes to the low control figures in males when compared to those in the females. Because a dose-relationship was not evident, these findings were not considered to be treatment-related.

Table 9. Liver Tissue of Males

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Cytochrome P450 (nmol/g)	43.9	48.5	55.6*	43.0
Triglyceride (mcmol/g)	5.52	7.04*	6.78**	6.89*

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from p.54 of the study.

J. Ophthalmic examinations

No treatment-related findings were reported.

K. Necropsy findings

At necropsy, discoloration (light red to yellow) of the adipose tissue was noticed in 4, 4, and 8 animals in the 2, 10, and 50 mg/kg/day dose groups, respectively. Although this finding appeared to be related to treatment, no histopathological correlation was found.

10. Organ weights

As shown in Table 10, absolute and relative adrenal weights of males treated at doses of 50 mg/kg bw dose were statistically significantly ($p < 0.05$, $P < 0.01$) increased when compared to control males. The study author attributed the changes to "incipient stress-related functional hypertrophy of the renal cortex as a reaction to the marked inhibition of the CHE activities in this dose group."

Although a dose-relationship was not evident, the absolute liver weight of males in the 50 mg/kg bw dose group were statistically significantly elevated.

Table 10. Organ Weights in Male Rabbits

	0 mg/kg/d	2 mg/kg/d	10 mg/kg/d	50 mg/kg/d
Absolute				
Adrenals (g)	0.408	0.367	0.394	0.524**
Liver (g)	70.9	65.2	76.3	76.2*
Relative				
Adrenals (mg)	13	12	12	16**

* = $p \leq 0.05$
** = $p \leq 0.01$
Table excerpted from pages 58 & 59 of the study.

11. Histopathology

No treatment-related findings were observed. The pituitaries were only removed from 10 animals and examined histopathologically. Since no findings were observed in the pituitaries of high-dose (50 mg/kg/day) animals, this error was not judged to compromise the validity of the study.

IV. DISCUSSION:

There were no treatment-related clinical signs, deaths, effects on food consumption or body weight. No treatment-related skin reactions or histopathological findings were observed.

Although statistically significant changes were seen in several routine hematology and clinical chemistry parameters at 10 and 50 mg/kg/day, when compared to historical control parameters were within ± 2 S.D. of the historical mean value and were not considered to be treatment-related.

Dose and treatment-related decreases in mean plasma, red blood cell, and brain cholinesterase activities were seen at doses of 10 and 50 mg/kg bw.

Analysis of liver homogenates did not demonstrate a treatment-related effect and significant but non-dose dependent increases in mean liver or adrenal gland weights at 50 mg/kg/day were not considered to be indicative of a direct treatment-related effect.

IV. CONCLUSION

Based on the results of this study, Isofenphos (SRA 12869), did not cause any dermal effects after repeated application. Statistically significant treatment-rolated decreases in plasma, red blood cell and brain cholinesterase levels were seen at 10 and 50 mg/kg/day.

The no-observed-effect level (NOEL) for local dermal effects is 50 mg/kg bw per day in both sexes. The no-observed-effect level for cholinesterase inhibition in the plasma, red blood cells and brain is 2 mg/kg bw per day.

This study is classified as Core Minimum, and satisfies minimal guideline requirements [#82-3] for a 90-day dermal toxicity study.

Tox # 011204

Page ___ is not included in this copy.

Pages 18 through 21 are not included in this copy.

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