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Caswell 012311

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

SEP - 4 1997

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ISOFENPHOS: Review of Acute Toxicity Studies and Reproductive Toxicity Study

EPA Identification Nos.: Tox Chem Code: 109401
MRID No 42030001 (§81-2), 41609901 (§81-3), 41609911 (§81-4), 41609904 (§81-5), 41609902 (§83-4)
Caswell No.: 447AB
DP Barcode: D238594
Submission No.: S529123

FROM Robert F. Fricke, Ph.D. Robert F. Fricke Sept 2, 1997
Reregistration Branch II
Health Effects Division (HED) (7509C)

THRU: Alan Nielsen
Branch Senior Scientist
Reregistration Branch II, HED

TO: Ruby Whiters
Chemical Review Manager
Special Review and Reregistration Division (7508C)

To support the reregistration of Isofenphos by Bayer Corporation, acute neurotoxicity studies and a reproductive toxicity study were reviewed. The results of these studies are summarized as follows:

1. ACUTE DERMAL TOXICITY STUDY:

Citation: Bomann, W. (1991) SRA 12869 Techn. (c.n.: Isofenphos) Study for acute dermal toxicity in the rat. Bayer AG, Wuppertal, Federal Republic of Germany. Report No.: 101274, Study No.: T 3037229. May 10, 1991. MRID 42030001. Unpublished.

Executive Summary: In an acute dermal toxicity study (MRID 42030001), SPF-bred Wistar rats (5/sex/dose) were dermally exposed for 24 hours to SRA 12869 (92.4%) at dose levels of 25,100,160, 250,355, or 500 mg/kg in males and 5, 25,50, or 100 mg/kg in females. SRA 12869

was applied to shorn intact dorsal skin (approx. 10% of the total body surface area). Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing. Signs of cholinergic toxicity were observed up 9 days posts-dosing. No treatment-site dermal irritation was observed. Treatment- and dose-related effects on body weight were observed in animals from both sexes. Gross necropsy of decedent animals revealed ulcerous foci in the glandular stomach; distended, dark, and/or patchy lungs; dark livers; pale and/or small spleens; empty gastrointestinal tracts; pale kidneys; and an esophagus engorged with shavings. No treatment-related effects were observed upon necropsy of animals sacrificed after 14 days.

Dermal LD_{50}: Males = 191 (143-256) mg/kg (95% C.I.)
Females = 70 mg/kg (estimated)
Combined = Approximately 100 mg/kg (observed)

TOXICITY CATEGORY = I

2. ACUTE INHALATION TOXICITY STUDY

Citation: Pauluhn, J., (1988), SRA 12869 (Common Name: Isofenphos) Study of the acute inhalation toxicity in accordance with OECD Guidelines No. 403. Bayer AG, Wuppertal, Federal Republic of Germany. Laboratory report No.: 99261, Study No.: T 4027654. MRID 41609901. Unpublished.

Executive Summary: In an acute inhalation toxicity study (MRID 41609901), groups of five or ten young adult SPF-bred Wistar rats/sex were exposed by nose-only inhalation to SRA 12869 Technical (91.7%) at concentrations ranging from 0.0679 to 0.998 mg/kg for 4 hours. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postexposure.

Deaths occurred within 8 days in females exposed at 0.172 mg/L and higher and in males exposed at 0.293 mg/L and higher. Clinical signs, consistent with acute cholinergic toxicity, were observed up to 6 days in males and 10 days in females. Clinical signs included: bradypnea, shortness of breath, excessive salivation, unpreened hair coat, reduced activity, stiff-legged gait, piloerection, difficulty breathing, prostration, tremors, atony, and bloody nose.

At day 3, body weights were decreased in males dosed at 0.293 mg/L and higher and females dosed at 0.167 mg/L and higher. By day 14, the body weight gains by males were comparable to control values, while body weight gains of 0.253 and 0.468 mg/L females were still decreased.

Gross pathological examination of animals which died during the study revealed pale livers with lobular patterns; reddened and/or ulcer-like foci of the glandular stomach; abnormal contents of the gastrointestinal tract; pale spleens; distended, hepatoid foci, and/or edematous lungs; pale kidneys; and reddened renal pelvis. At terminal sacrifice, with the exception of distended lungs in animals dosed at 0.293 mg/L and higher, no other treatment-related gross pathological changes were observed.

At the lowest dose tested (0.068 mg/L), cholinesterase activities were markedly inhibited at 30 to 40 min (> 81% plasma, >67%, RBC) and 20 hours (>58% plasma, >49% RBC) post-dosing.

Inhalation LC_{50} Males = 0.525 mg/L (estimated)
Females = 0.273 (0.199-0.374) mg/L (95% C.I.)
Combined = Approximately 0.468 mg/L (observed)

TOXICITY CATEGORY = II

This study is classified **acceptable (§81-3)** and satisfies the guideline requirement for an acute inhalation study in the rat.

3. PRIMARY EYE IRRITATION STUDY

Citation: Sheets, L. (1990) Primary eye irritation study with technical grade Isofenphos in rabbits. Mobay Corporation, Stilwell, KS. Laboratory Study Nos. 100269 and 90-335-EP. July 30, 1990. MRID 41609911. Unpublished.

Executive Summary: In a primary eye irritation study (MRID 41609911), 0.1 mL of technical-grade Isofenphos (90.8%) was instilled into the conjunctival sac of the left eye of three adult New Zealand White rabbits/sex. The treated eyes were not rinsed. The animals were observed for up to 72 hours following treatment, and eye irritation was scored using a modified Draize scheme.

Ocular irritation was greatest in the treated eyes 1 hour following instillation, and included slight conjunctival redness, very slight conjunctival chemosis, and severe conjunctival discharge in 6/6 treated eyes. Two male animals died from acute cholinergic toxicity prior to the 24-hour observation interval. In the remaining four animals, slight conjunctival redness persisted in 4/4 eyes at 24 hours and 2/4 eyes at 48 hours. No corneal or iridial changes were observed during the study, and conjunctival redness completely subsided by 72 hours.

TOXICITY CATEGORY III

This study is classified **acceptable (§81-4)** and satisfies the guideline requirement for a primary eye irritation study in the rabbit.

4. PRIMARY SKIN SENSITIZATION STUDY

Citation: Sheets, L. (1990) Primary dermal irritation study with technical grade Isofenphos in rabbits. Mobay Corporation, Stilwell, KS. Laboratory Study Nos. 100185 and 90-325-EM. May 29, 1990. MRID 41609904. Unpublished.

Executive Summary: In a primary dermal irritation study (MRID 41609904), New Zealand White rabbits (3/sex) were dermally exposed (6 cm^2 site/animal) to 0.5 mL of Isofenphos (90.8%) for 4 hours. Animals were observed for dermal irritation for up to 72 hours following application, and irritation was scored by the Draize scale.

Very slight to well-defined erythema was observed at up to 5/6 sites between 1 and 24 hours following patch removal. No other dermal irritation was observed during the study. The primary

dermal irritation index was 0.42.

TOXICITY CATEGORY IV

This study is classified **acceptable (§81-5)** and satisfies guideline requirements for a primary dermal irritation study in the rabbit.

5. TWO-GENERATION REPRODUCTION STUDY IN THE RAT

Citation: Holzum, B. (1989), SRA 12869 (C.N. Isofenphos) 2 Generation Study in Rats. Department of Toxicology, Bayer AG, West Germany. Laboratory Project Report Number 99801, January 10, 1989. MRID 41609902. Unpublished.

Executive Summary: In this two-generation, two litter reproduction study (MRID 41609902) SRA 12869 (92.9%) was administered to Bor strain:WISW (SPF Cpb) rats (25/sex/dose) at dietary levels of 0, 1, 5, or 25 ppm (achieved doses of 0, 0.08-0.16, 0.44-0.69, or 2.21-3.92 mg/kg/day). Exposure to F_0 animals began at 5 weeks of age and lasted for 13 weeks prior to mating the first time to produce F_{1a} pups. F_0 animals were mated a second time to produce F_{1b} pups. At 4 weeks of age, F_{1b} pups were selected to become parents of the F_{2a} and F_{2b} generations and were given the same concentration of SPA 12869 in their diets as their dam. The F_{1b} parental animals were given test diets for approximately 12 weeks prior to mating the first time to produce the F_{2a} pups. Exposure of the test material to all animals was continuous in the diet throughout the study.

Parental toxicity was characterized at the mid-dose as reductions in cholinesterase activity in plasma (18.5-31.9%, $p \leq 0.01$, both sexes) and in erythrocytes (7.1%, $p \leq 0.05$, females only). At the high-dose, treatment-related reductions in cholinesterase activity in the brain (27.0%, males; 31.8%, females; $p \leq 0.01$), plasma (16.5-26.4%, $p \leq 0.01$, both sexes), and RBC (53.7-80.7%, $p \leq 0.01$, both sexes) were noted. In addition at the high-dose, treatment-related increases in mortality (12%, F_0 females) and increases in absolute ovarian weights (F_0, 9%; F_{1b}, 12%; $p \leq 0.05$) were noted.

No treatment-related clinical findings or changes in body weights, body weight gains, food consumption, or reproductive indices were noted in either sex of either generation throughout the study.

The LOEL for systemic toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on reductions in plasma and RBC cholinesterase activities. The systemic NOEL is 1 ppm (0.08-0.16 mg/kg/day).

Reproductive toxicity was demonstrated at 5 ppm as treatment-related increases in the number of litters with small to very small pups (F_{1b}) and emaciated pups (F_{2b}). For the F_{1b} mid-dose litters, treatment-related reductions were noted in the lactation index (34.9% vs. 63.5% for controls, $p \leq 0.01$) and in mean litter sizes for days 14-28 (47%, $p \leq 0.01$). The lactation index was also decreased for the mid-dose F_{2b} litters (71.2% vs. 89.6% in controls, $p \leq 0.01$).

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At 25 ppm, treatment-related increases in the numbers of litters with small to very small pups (F_{1a}

and F_{1b}), cold pups (F_{1b} and F_{2b}), and emaciated pups (F_{2b}) were observed. For the high-dose F_{1a} and F_{1b} litters, treatment-related increases were noted in the number of deaths between days 5-28 and related reductions were observed in mean litter sizes on days 14-28 (F_{1a}, 47%, $p \leq 0.01$) or 7-28 (F_{1b}, 34-60%, $p \leq 0.01$ or ≤ 0.05), number of pups alive by day 28, and lactational indices (F_{1a}: 47.1% vs. 88.1% for controls, $p \leq 0.01$; F_{1b}: 11.8% vs. 63.5% for controls, $p \leq 0.01$). In addition for the F_{1b} litters, a treatment-related reduction in the viability index was noted (75.8% vs. 96.6% for controls, $p \leq 0.01$). For the high-dose F_{2b} litters, treatment-related reductions in the viability index (91.5% vs. 99.1% for controls, $p \leq 0.01$) and lactation index (70.0% vs. 89.6%, $p \leq 0.01$) were observed. For both generations, the total number of pups born was reduced at the high-dose; this was because of increased mortality of the F_0 dams and their offspring (only 9 F_{1b} females were available for mating) resulting in a smaller number of females which gave birth. A treatment-related reduction in pup body weights during lactation was also noted at the high-dose (F_{1a}, 11-19% $p \leq 0.01$ or 0.05; F_{1b}, 23-29%, $p \leq 0.01$).

There were no treatment-related findings at the low-dose.

The LOEL for reproductive toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (reductions in the lactation indices and mean litter sizes). The reproductive NOEL is 1 ppm (0.08-0.16 mg/kg/day).

The reproductive study in the rat is classified **acceptable** and satisfies the guideline requirements for a 2-generation reproductive study (**§83-4**) in rat.

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DATA EVALUATION RECORD

ISOFENPHOS

Study Type: §83-4; Two-Generation Reproductive Toxicity Study in Rats

Work Assignment No. 3-15 (MRID 41609902)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Primary Reviewer: Sandra Daussin	Signature: Sandra Daussin Date: 8/6/97
Secondary Reviewer: Steven Brecher, Ph.D.	Signature: Steven Brecher Date: 8/11/97
Project Manager: Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 8/11/97
Quality Assurance: William Spangler, Ph.D.	Signature: William J. Spangler Date: 08/11/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Robert Fricke, Ph.D.
Reregistration Branch II (7509C)

Robert F. Fricke 26 Aug 97.

EPA Work Assignment Manager: Jess Rowland, M.S.
Science Analysis Branch (7509C)

Jess Rowland 8/26/97

DATA EVALUATION RECORD

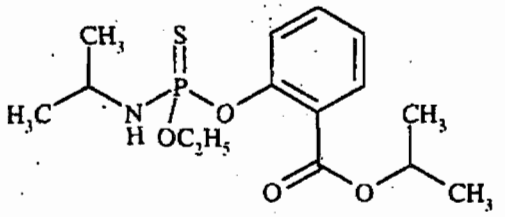
STUDY TYPE: Two-generation Reproduction Study - Rat

OPPTS Number: 870.3800 OPP Guideline Number: §83-4

DP BARCODE: None SUBMISSION CODE: None
P.C. CODE: 109401 TOX. CHEM. NO.: 447AB

TEST MATERIAL (PURITY): SRA 12869 (92.9%.).

SYNONYMS: Isofenphos, Isopropyl salicylate o-ester with o-ethyl isopropyl-phosphoramidothioate (CAS)



CITATION: Holzum, B. (1989), SRA 12869 (C.N. Isofenphos) 2 Generation Study in Rats. Department of Toxicology, Bayer AG, West Germany. Laboratory Project Report Number 99801, January 10, 1989. MRID 41609902. Unpublished.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

EXECUTIVE SUMMARY: In this two-generation, two litter reproduction study (MRID 41609902) SRA 12869 (92.9%) was administered to Bor strain:WISW (SPF Cpb) rats (25/sex/dose) at dietary levels of 0, 1, 5, or 25 ppm (achieved doses of 0, 0.08-0.16, 0.44-0.69, or 2.21-3.92 mg/kg/day). Exposure to F_0 animals began at 5 weeks of age and lasted for 13 weeks prior to mating the first time to produce F_{1a} pups. F_0 animals were mated a second time to produce F_{1b} pups. At 4 weeks of age, F_{1b} pups were selected to become parents of the F_{2a} and F_{2b} generations and were given the same concentration of SPA 12869 in their diets as their dam. The F_{1b} parental animals were given test diets for approximately 12 weeks prior to mating the first time to produce the F_{2a} pups. Exposure of the test material to all animals was continuous in the diet throughout the study.

Parental toxicity was characterized at the mid-dose as reductions in cholinesterase activity in plasma (18.5-31.9%, $p \leq 0.01$, both sexes) and in erythrocytes (7.1%, $p \leq 0.05$, females only). At the high-dose, treatment-related reductions in cholinesterase activity in the brain (27.0%, males; 31.8%, females; $p \leq 0.01$), plasma (16.5-26.4%, $p \leq 0.01$, both sexes), and RBC (53.7-80.7%, $p \leq 0.01$, both sexes) were noted. In addition at the high-dose, treatment-related increases in mortality (12%, F_0 females) and increases in absolute ovarian weights (F_0, 9%; F_{1b}, 12%;

$p \leq 0.05$) were noted.

No treatment-related clinical findings or changes in body weights, body weight gains, food consumption, or reproductive indices were noted in either sex of either generation throughout the study.

The LOEL for systemic toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on reductions in plasma and RBC cholinesterase activities. The systemic NOEL is 1 ppm (0.08-0.16 mg/kg/day).

Reproductive toxicity was demonstrated at 5 ppm as treatment-related increases in the number of litters with small to very small pups (F_{1b}) and emaciated pups (F_{2b}). For the F_{1b} mid-dose litters, treatment-related reductions were noted in the lactation index (34.9% vs. 63.5% for controls, $p \leq 0.01$) and in mean litter sizes for days 14-28 (47%, $p \leq 0.01$). The lactation index was also decreased for the mid-dose F_{2b} litters (71.2% vs. 89.6% in controls, $p \leq 0.01$).

At 25 ppm, treatment-related increases in the numbers of litters with small to very small pups (F_{1a} and F_{1b}), cold pups (F_{1b} and F_{2b}), and emaciated pups (F_{2b}) were observed. For the high-dose F_{1a} and F_{1b} litters, treatment-related increases were noted in the number of deaths between days 5-28 and related reductions were observed in mean litter sizes on days 14-28 (F_{1a}, 47%, $p \leq 0.01$) or 7-28 (F_{1b}, 34-60%, $p \leq 0.01$ or ≤ 0.05), number of pups alive by day 28, and lactational indices (F_{1a}: 47.1% vs. 88.1% for controls, $p \leq 0.01$; F_{1b}: 11.8% vs. 63.5% for controls, $p \leq 0.01$). In addition for the F_{1b} litters, a treatment-related reduction in the viability index was noted (75.8% vs. 96.6% for controls, $p \leq 0.01$). For the high-dose F_{2b} litters, treatment-related reductions in the viability index (91.5% vs. 99.1% for controls, $p \leq 0.01$) and lactation index (70.0% vs. 89.6%, $p \leq 0.01$) were observed. For both generations, the total number of pups born was reduced at the high-dose; this was because of increased mortality of the F_0 dams and their offspring (only 9 F_{1b} females were available for mating) resulting in a smaller number of females which gave birth. A treatment-related reduction in pup body weights during lactation was also noted at the high-dose (F_{1a}, 11-19% $p \leq 0.01$ or 0.05; F_{1b}, 23-29%, $p \leq 0.01$).

There were no treatment-related findings at the low-dose.

The LOEL for reproductive toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (reductions in the lactation indices and mean litter sizes). The reproductive NOEL is 1 ppm (0.08-0.16 mg/kg/day).

The reproductive study in the rat is classified **acceptable** and satisfies the guideline requirements for a 2-generation reproductive study (**§83-4**) in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SRA 12869
   Description: Clear yellowish liquid
   Lot/Batch #: Fl. no. 2358/355
   Purity: 92.9% a.i.
   CAS #: 25311-71-1

2. Vehicle: Dietary admix

3. Test animals: Species: rat
   Strain: Bor strain: WISW (SPF Cpb)
   Age at start of dosing: (F_0) approximately 5 wks, (F_1) approximately 3 wks (at weaning)
   Weight at start of dosing:
   (F_0) Males: 60-84 g Females: 54-78 g
   (F_1b) - Data not reported for mated animals selectively
   Source: Winkelmann, Borchen, Germany
   Housing: Makrolon® type II or type III cages; individually except during the mating period or when with pups during lactation
   Diet: Altromin® 1321, *ad libitum*
   Water: Tap water, *ad libitum*
   Environmental conditions
   Temperature: 22±2°C
   Humidity: ≥50%
   Air changes: ≥10/hour
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): Not reported

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group until sperm or a copulation plug were observed in the vaginal tract. Each mating period lasted approximately 18-19 days. The F_0 generation animals were mated twice to produce F_{1a} and F_{1b} litters. The F_{1b} generation animals were mated twice to produce F_{2a} and F_{2b} litters. Sibling matings within the F_{1b} generation were avoided.

2. Study schedule: Starting at approximately 5 weeks of age, F_0 animals were given test diets for a 13 week premating period and then mated to produce the F_{1a} pups. Five days after birth, the F_{1a} pups were culled to 8 animals, the excess pups were sacrificed. The remaining pups were reared to 4 weeks of age, at which time they were sacrificed. F_0 animals were mated again 22 days after rearing the F_{1a} pups to produce the F_{1b} generation. At 4 weeks of age, 25 randomly selected F_{1b} (1 pup/sex/dose) were selected from each litter for further

treatment. After 15 weeks (approximately 12 weeks of exposure), the F_{1b} were mated twice, as described above, to produce the F_{2a} and F_{1b} generation pups. Excess pups were sacrifice. The F_0 and F_{1b} parental animals were sacrifice after weaning the second litter. Exposure of the test material to all animals was continuous in the diet throughout the study and all pups were given the same concentration of the test diet as their dam.

3. Animal assignment: F_0 and F_{1b} animals were randomly assigned to test groups as seen in Table 1.

Table 1. Animal assignment

Test Group	Dose in Diet[a] (ppm)	Animals/group			
		F_0 Males	F_0 Females	F_{1b} Males	F_{1b} Females
Control	0	25	25	25	25
Low (LDT)	1	25	25	25	25
Mid (MDT)	5	25	25	25	25
High (HDT)	25	25	25	7 b	9 b

a Diets were administered from the beginning of the study until sacrifice.
b The high-dose F_{1b} generation only consisted of 7 males and 9 females because of high pup mortality at the high-dose after the second mating of the F_0 animals.

4. Dose selection rationale: In a range-finding study summarized in the current submission, SRA 12869 (% a.i. not reported) was administered in the feed to rats for 4 weeks at doses of 0, 3, 15, or 45 ppm. At the high-dose, effects on body weight gain were noted. At doses ≥3 ppm, plasma cholinesterase activity was inhibited and at dose levels of ≥15 ppm, cholinesterase activity in erythrocytes and the brain were inhibited. No other details of the study were reported.

Based upon the results of this range-finding study, the subsequent full developmental toxicity study in rats was conducted using a high-dose of 25 ppm, a mid-dose of 5 ppm, and a low-dose of 1 ppm.

5. Dosage preparation and analysis: The study report states that animals were fed with new diets weekly and that the target concentrations were confirmed at approximately 3-month intervals throughout the study. Prior to the start of the study, the stability of the test substance in the diet (1 and 50 ppm diets) was evaluated for a period of 14 days at an unspecified temperature. Homogeneity (front left, back left, and back right of a rectangular tray) was also evaluated prior to the start of the study for 1 and 50 ppm diets.

Results - Homogeneity Analysis: 96-108% of nominal

Stability Analysis: Test diets prepared at 1 and 50 ppm were stable for up to 14 days were

within 94 and 83% of nominal, respectively.

Concentration Analysis: 1 ppm diet: 84-130% of nominal; 5 ppm diet: 92-124% of nominal; 25 ppm diet: 96-115% of nominal

The report does not state how often new test diets were prepared. However, the concentration of the test diets were adequately confirmed throughout the study. The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the test substance was demonstrated to be stable in the test diets for 2 weeks. The report also states that feed mixes were not dispensed beyond the reliable period of use.

C. OBSERVATIONS

1. Parental animals

a. Clinical Observations: Animals were observed for mortality and for clinical signs twice daily. A detailed examination of general well-being, behavior, state of the coat and body orifices, and changes in excretory products was performed at the weekly weighings.

b. Body Weights and Food Consumption: The F_0 and F_{1b} males were weighed weekly throughout the study. The F_0 females were weight weekly through mating; on gestational days 1, 6, 15, and 20; and again weekly after the birth of the pups. The F_{1b} females were weighed weekly throughout mating; on gestational days 0, 7, 14, and 21 (first mating) or 20 (second mating); on days 0, 7, 14, 21, and 28 of lactation; and weekly after the pups had been weaned. For the F_0 animals, weekly food consumption was recorded until the first mating and from weeks 29-35 for males or up to week 32 for females. For the F_{1b} animals, weekly food consumption was recorded throughout the study.

c. Plasma, RBC and Brain Cholinesterase Activities: Ten F_{1b} rats/sex/group (randomly selected) from the 0, 1, and 5 ppm dose groups and all high-dose animals (7 males and 9 females) were chosen for determining cholinesterase levels in plasma and erythrocytes. Animals were bled from the retro-orbital venous plexus on two occasions at 3-day intervals after the second mating (males) or after the F_{2b} pups had been weaned (females). In addition, cholinesterase activity was determined in the brain at necropsy for these animals.

d. Menstrual cycle: For the F_{1b} females, the state of the menstrual cycle was determined using vaginal smears during a 2 week period before the first mating and during the first and second mating period up until insemination.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 3).

Table 3. F_{1a} and F_{1b}/F_{2a} and F_{2b} Litter Observations.[a]

Observation	Time of observation (lactation day)						
	Day 0	Day 5[b]	Day 5[c]	Day 7	Day 14	Day 21	Day 28
Number of live pups	X	X	X	X	X	X	X
Pup weight [d]	X	X	X	X	X	X	X
External alterations	X	X	X	X	X	X	X
Number of dead pups [e]	X	X	X	X	X	X	X
Sex of each pup (M/F)	X						

a Data extracted from the study report page 19.
b Before standardization (culling).
c After standardization (culling).
d For the F_{1a} generation, weights of the individual pups were not determined, rather only total litter weights were recorded.
e The number of pups that died was recorded only on days 5, 7, 14, and 21, and 28 for the F_{1a} generation; for the F_{1b}, F_{2a}, and F_{2b} generation, these values were recorded daily.

On day 5 postpartum, litters were standardized to a maximum of 8 pups/litter. With the exception of the F_{1a} pups and F_{1b} pups culled at day 5, all pups were subjected to a gross necropsy at sacrifice. In addition, F_{2a} and F_{2b} pups which died were also evaluated grossly.

c. Postmortem observations

1. Parental animals: All parental rats which died or were sacrificed moribund were examined grossly at necropsy. All surviving F_0 and F_{1b} parents were sacrificed after the F_{1b} and F_{2b} pups were weaned, respectively, and examined grossly at necropsy. The following organs (X) were fixed in a buffered 10% formaldehyde solution and selected organs (XX) were weighed

XX Ovaries	X Epididymides
X Uterus	X Prostate
X Vagina	X Seminal vesicles
X Mammary gland	XX Testes
XX Brain [a]	X Spleen
X Pituitary	XX Kidneys
X Lesions	XX Liver

a Brain was weighed only for F_{1b} rats in which cholinesterase activity was determined.

2. Offspring: The F_{1b} offspring not selected as parental animals and all F_{1a}, F_{2a}, and F_{2b} offspring were sacrificed at 28 days of age. The F_{2a} and F_{2b} pups sacrificed on day 5 and the F_{1b}, F_{2a}, and F_{2b} pups sacrificed at 4 weeks of age were subjected to a gross necropsy; F_{1a} generation pups were discarded. In addition, F_{2a} and F_{2b} pups found dead were examined by gross necropsy. All body cavities were opened and particular attention was given to the reproductive organs. Gross lesions were fixed in a buffered 10%

formaldehyde solution.

D. DATA ANALYSIS

1. Statistical analyses: All collected data were subjected to routine appropriate statistical procedures.

2. Indices

a. Reproductive indices: The following reproductive indices were calculated for the F_0 and F_{1b} adults:

$$\text{Insemination Index (\%)} = \frac{\text{No. of inseminated females}}{\text{No. of mated females}} \times 100$$

$$\text{Fertility Index (\%)} = \frac{\text{No. of Females with Pups}}{\text{No. of Inseminated Females}} \times 100$$

$$\text{Gestation Index (\%)} = \frac{\text{No of Females with Surviving Pups}}{\text{No. of Females with Pups}} \times 100$$

$$\text{Gestation Index (\%)} = \frac{\text{No. of Females with Live Pups}}{\text{No. of Pregnant Females}} \times 100$$

b. Offspring viability indices: The following viability indices were calculated for the F_1 and F_2 litters:

$$\text{Viability Index (\%)} = \frac{\text{No. of Surviving Pups after 5 Days (before cull)}}{\text{No. of Surviving Pups at Birth}} \times 100$$

$$\text{lactation Index (\%)} = \frac{\text{No. of Surviving Pups after 4 Weeks}}{\text{No. of Surviving Pups after 5 Days (after cull)}} \times 100$$

c. Historical control data: Historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: No treatment-related clinical findings were noted in the F_0 and F_{1b} males and females throughout the study.

At 25 ppm, three F_0 females died or were sacrificed moribund at day 27 of the first pregnancy, day 22 of the second pregnancy, or on the second day of the second mating (12% group mortality). In all three animals, a dilated uterine cornua was found at necropsy. Other findings in these animals included focal metritis, focal liver necrosis, dilated renal tubules, an increase in extramedullary hematopoiesis in the spleen, greenish-yellow fluid in the abdominal cavity, and/or a lack of lymphoid cells in the spleen. In the controls and the mid-dose groups, no F_0 females died. One F_0 low-dose female died of an adenoma of the mammary gland with concomitant mastitis. As only one low-dose animal died of an adenoma of the mammary gland, this death is not considered treatment-related. The increase in mortality in the high-

dose females may be due to treatment with SRA 12869. There were no deaths of the F_0 males at any dose level.

There were no treatment-related deaths at any dose level in the F_{1b} animals. No F_{1b} females died in the 5 and 25 ppm dose groups. Two low-dose F_{1b} females died; one of unknown causes and one of peritonitis caused by gastritis with a mucosal ulcer. One F_{1b} male died in the mid-dose group and a partial dysplasia of the cerebellum was noted at necropsy. No other F_{1b} males died.

2. Body weight and food consumption: Selected data for body weights, gains, and food consumption are summarized in Table 3. There were no treatment-related effects on body weights or body weight gains at any dose level in the F_0 generation males or females during the pre-mating interval. Body weight gains were calculated by the reviewer and no statistical analyses were performed on these data.

Food consumption was unaffected by treatment in the F_0 males and females at all dose levels during the pre-mating interval. In the high-dose F_0 females, overall food consumption during the pre-mating interval was 11.6% higher than the controls. The study authors concluded this may have been a treatment-related increase; however, the difference from controls was not statistically significant and it is not considered biologically important by the reviewer.

There were no treatment-related effects on body weights at any dose level in the F_{1b} generation males or females during the pre-mating interval. Body weight gains were calculated by the reviewer and no statistical analyses were performed on these data. Bodyweight gains were reduced in the high-dose F_{1b} females at weeks 4-5 (30%) and body weights were reduced at week 6 by 8% ($p \leq 0.05$). For all other weeks in the pre-mating interval, however, body weights and body weight gains were comparable to the controls. The 4% reduction in overall body weight gains (weeks 4-16) for the high-dose F_{1b} females is due to the initial 30% decrease in gains at weeks 4-5. As the reduction in body weight and body weight gain was a transient finding only during the initial treatment period, it is not considered treatment-related. In addition, the reviewer notes that the number of animals in the F_{1b} high-dose female group was only 9, verses 25 for the controls. Therefore, it is difficult to make an accurate comparison of these two groups. Body weight gains were comparable to the controls for the low- and mid-dose females and for the males in all treatment groups. Food consumption values were unaffected by treatment at all dose levels in the F_{1b} males and females.

There were no treatment-related changes in body weights or body weight gains noted in either parental generation during gestation and lactation. Food consumption for the gestation and lactation periods were not reported. At the high-dose in the F_{1b} generation, body weights were occasionally lower than controls (16% on day 21 of the first pregnancy; 8% on day 14 of second lactation, $p \leq 0.01$). However, as these differences were seen sporadically and a comparison of the F_{1b} high-dose female group to the controls is complicated by the differences in sample size (9 high-dose rats vs. 25 controls), these are not considered biologically important reductions in body weight.

Table 3: .Mean Body Weights (g), Body Weight Gains (g), and Food Consumption (g/animal/day) for F_0 F_{1a}, and F_{1b} Generation Males and Females during the Pre-mating Period.[a]

Observation	Week	Dose Group (ppm)			
		0	1	5	25
F_0 Generation Males					
Mean body weight	0	72	71	72	72
	13	319	322	325	321
Mean weight gain[b]	0-13	247	251	253	249
Mean food consumption	0-13	21	20	21	21
F_0 Generation Females					
Mean body weight	0	68	67	67	67
	13	194	195	191	192
Mean weight gain[b]	0-13	126	128	124	125
Mean food consumption	0-13	17	17	17	19
F_{1b} Generation Males					
Mean body weight	4	57	61	64	53
	16	341	362	355	334
Mean weight gain[b]	4-16	284	301	291	281
Mean food consumption	4-16	28.6	28.1	26.3	28
F_{1b} Generation Females					
Mean body weight	4	54	56	63	54
	16	200	203	210	195
Mean weight gain[b]	4-16	146	147	147	141
Mean food consumption	4-16	23.3	25.3	22.8	23.5

a Data extracted from the study report pages 29, 41, 67-76, and 89-95.
b Calculated by the reviewer from the mean data presented in the study report.

3. Test Substance Intake: Based on food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the first pre-mating periods are presented in Table 4. The values are considered to be representative of the test substance intake for the entire study.

Table 4: Test substance intake (mean mg/kg body weight/day).[a]

Dose Level (ppm)					
1	5	25	1	5	25
Male			Female		
F_0 Generation					
0.08	0.44	2.21	0.11	0.56	3.04
F_{1b} Generation					
0.11	0.54	2.96	0.16	0.69	3.92

a Data extracted from the study report pages 29 and 43.

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4. Reproductive function:

a. Estrous cycle length and periodicity: The study report stated that the state of the menstrual cycle for the F_{1b} females was determined using vaginal smears during a 2 week period before the first mating and during the first and second mating period up until insemination. However, these data were not reported. There was no indication of treatment-related changes in female fertility at any dose level.

b. Sperm measures: No sperm parameter observations were made in this study. However, there were no indications of treatment-related male fertility abnormalities during the study.

c. Sexual maturation (F_1): No observations were made pertaining to the sexual maturation rates of the F_1 or F_2 litters.

d. Reproductive performance: Reproductive performance results are presented in Table 5. There were no treatment-related changes noted in the insemination, fertility, or gestation indices or in the gestation periods at any dose levels for either generation. For the F_{1b} generation, the fertility index at the first mating was lower than the controls at the high-dose (77.8 vs. 100% for controls). However, as the difference was not statistically significant and the F_{1b} fertility index at the second mating was comparable to the controls at the high-dose (100 vs. 87% for controls), this is not considered a treatment-related finding.

5. Parental postmortem results

a. Organ weights: Absolute and relative tissue weights for parental animals are presented in Table 6. At 25 ppm the absolute ovarian weights were increased for the F_0 and F_{1b} generations (9 and 12%, respectively, $p \leq 0.05$). For both generations, no histopathology was noted in ovaries and relative ovarian weights were comparable to the controls at all dose levels. The changes in ovarian weights are considered treatment-related as they occurred in both generations at the high-dose. For the high-dose F_{1b} animals, the absolute (9%, $p \leq 0.05$ in females) and relative (6%, $p \leq 0.05$ in males) kidney weights were reduced. There was no treatment-related histopathology noted in the kidneys of either generation at any dose level. The study author concluded that the changes in kidney weights were treatment-related. However, the changes in kidney weights were noted only in the second generation and the absolute and relative weights were not consistently affected for either sex. In addition, the sample size of the high-dose F_{1b} generation was considerably smaller than the controls (n=7 males and 9 females vs. 25 controls/sex). Therefore, the changes in kidney weights are not considered treatment-related. There were no treatment-related changes in liver or testicular weights in either generation or in brain weights for the F_{1b} generation at any dose level.

Table 5: Reproductive Performance.[a]

Observation	Dose Group (ppm)			
	0	1	5	25
F_0 Generation - Litter F_{1a}				
Insemination Index	96.0	100	100	100
Fertility Index	95.8	84.0	100	83.3
Gestation Index	100	100	100	100
Gestation Period (Days)	22.5	22.5	22.5	22.3
Mated females	25	25	25	25
F_0 Generation - Litter F_{1b}				
Insemination Index	100	100	100	100
Fertility Index	88.0	88.0	96.0	86.4
Gestation Index	100	90.9	100	100
Gestation Period (Days)	22.1	22.3	22.3	22.2
Mated females	25	25	25	23
F_{1b} Generation - Litter F_{2a}				
Insemination Index	100	96.0	100	100
Fertility Index	100	91.7	88.0	77.8
Gestation Index	100	100	100	100
Gestation Period (Days)	22.0	22.1	21.9	21.7
Mated females	25	25	25	9
F_{1b} Generation - Litter F_{2b}				
Insemination Index	92.0	91.7	92.0	100
Fertility Index	87.0	95.5	87.0	100
Gestation Index	100	100	95.0	100
Gestation Period (Days)	22.0	22.0	22.2	22.1
Mated females	25	24	25	9

a Data extracted from the study report pages 30, 44, 77 and 99.

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Table 6: Absolute and Relative (in parentheses) Tissue Weights of F_0 and F_{1b} Generation Parents.[a]

Generation	Sex	Tissue	Dose Group (ppm)			
			0	1	5	25
F_0	Males	Kidney	2433 (635)	2370 (622)	2416 (629)	2377 (620)
	Females	Kidney	1565 (679)	1547 (666)	1541 (656)	1512 (656)
		Ovaries	145 (63)	149 (64)	153 (65)	158* (69)
F_{1b}	Males	Kidney	2452 (613)	2477 (581*)	2562 (596)	2351 (579*)
	Females	Kidney	1641 (711)	1712 (731)	1692 (670)	1493* (642)
		Ovaries	128 (56)	134 (57)	139 (55)	143* (62)

a These data are extracted from the study report pages 56 and 57.
* $p < 0.05$

b. Pathology

1. Macroscopic examination: There were no treatment-related macroscopic findings for the F_{1b} parental generation at any dose level or in the low- and mid-dose groups of the F_0 generation. The study authors concluded that there were no treatment-related macroscopic findings at any dose level in either generation. However, for the high-dose F_0 animals, three females died or were found dead during the study. As no deaths occurred in the controls or mid-dose and only one death in the low-dose group of the F_0 females, this increase in mortality in the high-dose may be due to treatment with SRA 12869. In all three animals, a dilated uterine cornua was found at necropsy and this finding may be attributed to treatment with the test substance.

2) Microscopic examination: There were no treatment-related microscopic findings in any treatment group of the F_{1b} parental generation or in the low- and mid-dose groups of the F_0 generation. Again, the study authors concluded that there were no treatment-related microscopic findings at any dose level in either generation. However, histopathology noted in the three high-dose F_0 females that died included focal metritis, distension of the uterus, focal liver necrosis, dilated renal tubules, an increase in extramedullary hematopoiesis in the spleen, and/or a lack of lymphoid cells in the spleen.

c. Plasma, RBC and brain Cholinesterase Activities: Cholinesterase activity values in plasma, erythrocytes and brain were determined for the F_{1b} parental animals only and these data are presented in Table 7. At 5 ppm, treatment-related reductions were noted in cholinesterase activity in plasma (18.5-31.9%, $p \leq 0.01$, both sexes) and in erythrocytes (7.1%, $p \leq 0.05$, females only). At 25 ppm, treatment-related reductions in cholinesterase activity in the brain were noted (27.0%, males; ↓31.8%, females; $p \leq 0.01$), in addition to reductions in the plasma (16.5-26.4%, $p \leq 0.01$, both sexes) and erythrocytes (53.7-80.7%, $p \leq 0.01$, both sexes). At 5 ppm, cholinesterase activity in the brain was increased in males (↑8%, $p \leq 0.05$), however, this increase is not considered biologically significant.

Table 7: Plasma, RBC and Brain ChE Activities of F_{1b} Animals[a]

Sex	Dose (ppm)	ChE Activity, Mean (% Inhibition)[b]		
		Plasma (kU/L)	RBC (kU/L)	Brain (U/g)
Male	0	0.54	2.79	1.78
	1	0.56 (0)	2.74 (2)	1.84 (0)
	5	0.44** (19)	2.64 (5)	1.92* (0)
	25	0.25** (54)	2.33** (16)	1.30** (27)
Female	0	1.10	2.27	1.51
	1	1.25 (0)	2.27 (0)	1.65 (0)
	5	0.80* (27)	2.11* (7)	1.44 (5)
	25	0.22** (80)	1.67** (26)	1.03** (32)

a Data are extracted from the study report page 53.
b Cholinesterase activity in plasma and erythrocytes were determined on two occasions at 3-day intervals after the second mating (males) or after the F_{2b} pups had been weaned (females); the second determination is listed parenthetically.
* $p \leq 0.05$, ** $p \leq 0.01$

B. OFFSPRING

1. Viability and clinical signs: Mean litter size and viability results from F_{1a}, F_{1b}, F_{2a} and F_{2b} pups during lactation are summarized in Tables 8a and 8b. At the high-dose, a treatment-related increase in the numbers of litters with small to very small pups was seen in the F_{1a} and F_{1b} litters. In addition, an increase relative to the controls in the numbers of cold pups was noted in the high-dose F_{1b} litters. At the mid-dose there was an increase in the number of F_{1b} litters with small to very small pups. No treatment-related clinical findings were noted at the low-dose for the F_{1a} or F_{1b} generations. There was an increase in the number of low-dose F_{1b} pups that were small to very small (18 pups vs. 9 controls, p. 465 of study); however, as there were no similar findings in the low-dose groups of the F_{1a}, F_{2a}, or F_{2b} litters, this is no considered a treatment-related finding.

No treatment-related clinical findings were noted in the F_{2a} generation at any dose level. For the F_{2b} generation, however, a higher percentage of emaciated F_{2b} pups were observed at the mid- and high-doses (controls: 3.7%; 5 ppm: 9.1%; 25 ppm: 13.8%), and an increased incidence of cold pups was noted at the high-dose (13.4% relative to the controls). No treatment-related clinical findings were noted at the low-dose for the F_{2a} or F_{2b} generations.

For the high-dose F_{1a} and F_{1b} litters, treatment-related increases were noted in the number of deaths between days 5-28 and related reductions were observed in mean litter sizes on days 14-28 (F_{1a}, 47%, $p \leq 0.01$) or 7-28 (F_{1b}, 34-60%, $p \leq 0.01$ or ≤ 0.05), number of pups alive by day 28, and lactational indices (F_{1a}: 47.1% vs. 88.1% for controls, $p \leq 0.01$; F_{1b}: 11.8% vs. 63.5% for controls, $p \leq 0.01$). In addition for the F_{1b} litters, a treatment-related reduction in the viability index was noted at the high-dose (75.8% vs. 96.6% for controls, $p \leq 0.01$).

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For the mid-dose F_{1a} generation, mean litter size and viability values were comparable to the controls. For the F_{1b} mid-dose litters, however, treatment-related reductions were noted in the lactation index (34.9% vs. 63.5% for controls, $p \leq 0.01$) and in mean litter sizes for days 14-28 (47%, $p \leq 0.01$).

At the low-dose F_{1a} generation, mean litter size and viability values were comparable to the controls. At 1 ppm, a statistically significant reduction was noted in the lactation index for the F_{1b} litters (51.3% vs. 63.5% for controls, $p \leq 0.05$). This reduction is not treatment-related because the lactational indices for the low-dose F_{1a}, F_{2a}, and F_{2b} generations were either comparable to the controls or not affected in a dose-related manner. In addition, mean litter sizes at the low-dose were comparable to the controls for all litters throughout the 28 day observation period.

For the F_{2a} litters, no treatment-related changes were noted in litter size, numbers of live pups, numbers of deaths, or survival indices at any dose level. Statistically significant changes in mean litter sizes and lactation indices noted in the low- and mid-dose F_{2a} litters were not dose-dependent findings. For the high-dose F_{2b} litters, however, treatment-related reductions in the viability index (91.5% vs. 99.1% for controls, $p \leq 0.01$) and lactation index (70.0% vs. 89.6%, $p \leq 0.01$) were observed. At the mid-dose, the lactation index was also decreased for the F_{2b} litters (71.2% vs. 89.6% in controls, $p \leq 0.01$). No changes were noted in mean litter size and viability indices for the low-dose F_{2b} litters.

For all litters, the total number of pups born was reduced at the high-dose. This was because of increased mortality of the F_0 dams and their offspring (only 9 F_{1b} females were available for mating) resulting in a smaller number of females which gave birth. There were no treatment-related changes noted in the sex ratios of the pups for any generation at any dose level.

2 .Body weight: At the high-dose, a treatment-related reduction of body weights was noted in the F_{1a} (↓11-19%, lactational days 5, 7, and 14; $p \leq 0.01$ or 0.05) and F_{1b} generations (↓23-29%, lactational days 5 and 7; $p \leq 0.01$). Body weights were also reduced at 5 ppm on lactational days 21 and 28 ($p \leq 0.05$ or 0.01) in the F_{1b} and F_{2b} generation; however, these are not considered treatment-related reductions because body weights at the 25 ppm were comparable to the controls for these days in these generations. Selected mean pup body weight data are presented in Table 9.

Table 8a: Mean Litter Size and Viability for F_1 Generation Animals [a]

Observation	Dose Group (ppm)			
	0	1	5	25
F_{1a} Generation				
Mean litter size				
Day 0	11.0	10.0	10.7	10.9
Day 5[b]	10.3	9.6	10.0	10.1
Day 5[c]	7.7	7.2	7.6	7.6
Day 7	7.5	7.2	7.4	7.3
Day 14	6.8	6.9	6.2	3.6**
Day 21	6.8	6.9	6.2	3.6**
Day 28	6.8	6.9	6.2	3.6**
Number live pups				
Day 0	257	216	270	220
Day 5[b]	237	201	250	202
Day 5[c]	177	152	190	153
Day 28	156	144	154	72
Number deaths[d]				
Days 0-5[b]	20	15	20	18
Days 5[c]-28	21	8	38	81
Survival indices				
Viability index	94.1	95.3	93.3	92.7
Lactation index	88.1	94.7	81.1	47.1**
F_{1b} Generation				
Mean litter size				
Day 0	10.6	10.2	10.6	10.9
Day 5[b]	10.2	9.6	10.0	8.7
Day 5[c]	7.7	7.5	7.8	7.6
Day 7	7.4	7.0	7.2	4.9**
Day 14	5.8	4.2	3.1**	2.6*
Day 21	5.7	4.5	3.0**	2.3**
Day 28	5.7	4.5	3.1**	2.3**
Number live pups				
Day 0	240	222	257	209
Day 5[b]	225	192	240	157
Day 5[c]	170	150	186	136
Day 28	108	77	65	16
Number deaths[d]				
Days 0-5[b]	15	30	17	52
Days 5[c]-28	62	73	121	120
Survival indices				
Viability index	96.6	94.6	94.5	75.8**
Lactation index	63.5	51.3*	34.9**	11.8**

a Data extracted from the study report pages 31, 33, 34, 77-79, and 150-153.
b Before standardization (culling); values calculated by the reviewer from the mean litter size data.
c After standardization (culling)
d Calculated by the reviewer.
* p 0.05, **p 0.01

Table 8b: Mean Litter Size and Viability for F_2 Generation Animals.[a]

Observation	Dose Group (ppm)			
	0	1	5	25
F_{2a} Generation				
Mean litter size				
Day 0	10.9	11.4	11.9*	9.3
Day 5[b]	10.6	11.2	11.8*	9.3
Day 5[c]	8.0	8.0	8.0	7.4
Day 7	7.9	7.8	8.0	7.1
Day 14	7.5	7.3	7.5	6.4
Day 21	7.5	7.3	7.5	6.4
Day 28	7.5	7.2	7.5	6.4
Number live pups				
Day 0	277	256	266	68
Day 5[b]	266	246	260	65
Day 5[c]	199	176	176	52
Day 28	188	152	165	45
Number deaths[d]				
Days 0-5[b]	11	10	6	3
Days 5[c]-28	11	24	11	7
Survival indices				
Viability index	97.8	98.0	99.2	100.0
Lactation index	94.5	86.4*	93.7	86.5
F_{2b} Generation				
Mean litter size				
Day 0	10.7	11.5	11.0	10.4
Day 5[b]	10.6	11.4	10.7	9.6
Day 5[c]	7.7	7.8	7.7	7.8
Day 7	7.6	7.7	7.5	7.0
Day 14	6.9	7.2	5.9	6.1
Day 21	6.9	7.3	5.8	6.1
Day 28	6.9	7.3	5.8	6.1
Number live pups				
Day 0	216	242	220	95
Day 5[b]	212	239	203	86
Day 5[c]	154	163	146	70
Day 28	138	145	104	49
Number deaths[d]				
Days 0-5[b]	4	3	17	9
Days 5[c]-28	16	18	42	21
Survival indices				
Viability index	99.1	99.2	97.1	91.5**
Lactation index	89.6	89.0	71.2**	70.0**

a Data extracted from the study report pages 31, 33, 34, 77-79, and 150-153.
b Before standardization (culling); values calculated by the reviewer from the mean litter size data.
c After standardization (culling)
d Calculated by the reviewer.
* p<0.05, **p<0.01

Table 9: Mean Pup Body Weights (g) for F_1 and F_2 Generation Animals.[a]

Lactation Day	Dose Group (ppm)			
	0	1	5	25
F_{1a} Generation				
0	5.9	6.0	6.0	5.8
5[b]	9.2	9.5	9.2	8.1*
5[c]	9.2	9.6	9.2	8.2*
7	11.8	12.3	11.7	9.6**
14	21.6	21.7	22.7	19.3*
21	32.2	31.7	33.8	29.9
28	50.6	50.4	53.1	47.5
F_{1b} Generation				
0	5.72	5.75	5.79	5.63
5[b]	9.33	9.31	9.50	7.20**
5[c]	9.35	9.36	9.57	7.14**
7	11.21	10.93	10.91	7.94**
14	23.91	23.89	25.82	24.02
21	36.32	39.21	39.95*	36.82
28	54.85	59.24	62.05**	55.24
F_{2a} Generation				
0	5.69	5.72	5.67	5.81
5[b]	9.43	9.24	9.38	9.30
5[c]	9.47	9.22	9.40	9.29
7	12.42	12.24	12.62	11.90
14	23.54	23.92	24.16	22.15
21	35.05	35.83	36.89	34.20
28	53.90	56.17	56.92	55.09
F_{2b} Generation				
0	5.79	5.81	5.89	5.70
5[b]	9.30	9.36	9.30	8.56
5[c]	9.26	9.35	9.24	8.60
7	12.16	12.56	12.08	11.29
14	24.43	24.07	26.20	22.85
21	36.21	35.52	40.65*	33.80
28	48.25	48.70	57.04**	49.83

a Data extracted from pages 81, 82, 102, and 107.
b Before standardization (culling)
c After standardization (culling)
* $p<0.05$, ** $p<0.01$

3. Offspring postmortem results

a. Organ weights: Organ weights were not recorded for the pups of either generation.

b. Pathology

1) Macroscopic examination: There were no treatment-related macroscopic findings in the F_{1b}, F_{2a}, or F_{2b} pups at any dose level. Gross necropsies were not performed on the F_{1a} pups.

2) Microscopic examination: There were no treatment-related microscopic findings in the F_{1b}, F_{2a}, or F_{2b} pups at any dose level. Histopathology was not performed on any tissues from the F_{1a} pups.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that parental toxicity was noted at 5 and 25 ppm primarily as inhibition of cholinesterase activity. In addition, the increased food intake and mortality observed in the female F_0 animals at 25 ppm may have been treatment-related. At 25 ppm, increased ovarian weights were also noted in the F_0 and F_{1b} generations and kidney weights were reduced in the F_{1b} generation. The LOEL for parental toxicity was 5 ppm and the parental NOEL was 1 ppm.

At the high-dose, pup body weights and viability during lactation were reduced and a greater number of small and cold pups were observed. At the mid-dose, the lactation indices (F_{1b} and F_{2b}) were reduced and the number of emaciated pups was increased (F_{2b}). The reproductive LOEL was 5 ppm and NOEL was 1 ppm.

B. REVIEWER'S DISCUSSION: Over the course of the 2-generation reproduction study, SRA 12869 was administered continuously in the diet to Bor strain:WISW (SPF Cpb) rats at dose levels of 0, 1, 5, or 25 ppm (achieved doses of 0, 0.08-0.16, 0.44-0.69, or 2.21-3.92 mg/kg/day). Exposure to F_0 animals (25/sex/dose) began at 5 weeks of age and lasted for 13 weeks prior to mating the first time to produce F_{1a} pups. F_0 animals were mated a second time to produce F_{1b} pups. At 4 weeks of age, F_{1b} pups were selected to become parents of the F_{2a} and F_{2b} generations and were given the same concentration of SRA 12869 in their diets as their dam. The F_{1b} parental animals were given test diets for approximately 12 weeks prior to mating the first time to produce the F_{2a} pups. Exposure of the test material to all animals was continuous in the diet throughout the study.

The report does not state how often fresh test diets were prepared. However, the concentration of the test diets were adequately confirmed throughout the study. The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the test substance was demonstrated to be stable in the test diets for 2 weeks. The report also states that feed mixes were not dispensed beyond the reliable period of use.

1. Parental Toxicity. Parental toxicity was characterized in the F_{1b} generation at the mid-dose as reductions in cholinesterase activity in plasma (18.5-31.9%, $p \leq 0.01$, both sexes) and in erythrocytes (7.1%, $p \leq 0.05$, females only). At the high-dose, treatment-related reductions in cholinesterase activity in the brain (27.0%, males; 31.8%, females; $p \leq 0.01$), plasma (16.5-26.4%, $p \leq 0.01$, both sexes), and erythrocytes (53.7-80.7%, $p \leq 0.01$, both sexes) were noted in the F_{1b} generation. In addition at the high-dose, treatment-related increases in mortality and absolute ovarian weights were noted as described below.

Three high-dose F_0 females died or were sacrificed moribund at day 27 of the first pregnancy, day 22 of the second pregnancy, or on the second day of the second mating (12% group mortality). In all three animals, a dilated uterine cornua was found at necropsy. Other findings in these animals included focal metritis, focal liver necrosis, dilated renal tubules, an increase in extramedullary hematopoiesis in the spleen, greenish-yellow fluid in the abdominal cavity, and/or a lack of lymphoid cells in the spleen.

A treatment-related increase in absolute ovarian weights was also noted at the high-dose for both generations (9%, F_0; 12%, F_{1b}; $p \leq 0.05$). For the high-dose F_{1b} animals, the absolute (9%, $p \leq 0.05$ in females) and relative (6%, $p \leq 0.05$ in males) kidney weights were reduced. There was no treatment-related histopathology noted in the kidneys of either generation at any dose level. The study author concluded that the changes in kidney weights were treatment-related. However, the changes in kidney weights were noted only in the second generation and the absolute and relative weights were not consistently affected for either sex. In addition, the sample size of the high-dose F_{1b} generation was considerably smaller than the controls (n=7 males and 9 females vs. 25 controls/sex). Therefore, the changes in kidney weights are not considered treatment-related.

No other parental toxicity was noted at any dose level. There were no treatment-related deaths of the F_0 males or the F_{1b} males of females at any dose level. No treatment-related clinical findings or changes in body weights, body weight gains, or food consumption were noted in ether sex of either generation throughout the study. In the high-dose group F_0 females, overall food consumption during the pre-mating interval was 11.6% higher than the controls, however, this difference was not statistically significant and is not considered biologically important. The insemination, fertility, and gestation indices and the gestation periods for both parental generations were unaffected by treatment at all dose levels. There were no treatment-related changes in liver or testicular weights in either generation or in brain weights for the F_{1b} generation at any dose level.

The LOEL for systemic toxicity is established at 5 ppm based on reductions in plasma and RBC cholinesterase activity, the systemic NOEL was established at 1 ppm.

2. Reproductive Toxicity. Reproductive toxicity was demonstrated at 5 and 25 ppm.

At the high-dose, several clinical signs of toxicity were noted as was increased pup mortality. Treatment-related increases in the numbers of litters with small to very small pups (F_{1a} and F_{1b}), cold pups (F_{1b} and F_{2b}), and emaciated pups (F_{2b}) were observed. For the F_{1a} and F_{1b} litters, treatment-related increases were noted in the number of deaths between days 5-28 and related reductions were observed in mean litter sizes on days 14-28 (F_{1a}, 47%, $p \leq 0.01$) or 7-28 (F_{1b}, 34-60%, $p \leq 0.01$ or ≤ 0.05), number of pups alive by day 28, and lactational indices (F_{1a}: 47.1% vs. 88.1% for controls, $p \leq 0.01$; F_{1b}: 11.8% vs. 63.5% for controls, $p \leq 0.01$). In addition for the F_{1b} litters, a treatment-related reduction in the viability index was noted (75.8% vs. 96.6% for controls, $p \leq 0.01$). For the high-dose F_{2b} litters, treatment-related reductions in the viability index (91.5% vs. 99.1% for controls, $p \leq 0.01$) and lactation index

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(70.0% vs. 89.6%, $p \leq 0.01$) were observed. For all litters, the total number of pups born was reduced at the high-dose. This was because of increased mortality of the F_0 dams and their offspring (only 9 F_{1b} females were available for mating) resulting in a smaller number of females which gave birth.

In addition at 25 ppm, a treatment-related reduction on body weights was noted in the F_{1a} (11-19%, lactational days 5, 7, and 14; $p \leq 0.01$ or 0.05) and F_{1b} generations (23-29%, lactational days 5 and 7; $p \leq 0.01$).

At the mid-dose, treatment-related increases in the number of litters with small to very small pups (F_{1b}) and emaciated pups (F_{2b}) was observed. For the F_{1b} mid-dose litters, treatment-related reductions were noted in the lactation index (34.9% vs. 63.5% for controls, $p \leq 0.01$) and in mean litter sizes for days 14-28 (147%, $p \leq 0.01$). The lactation index was also decreased for the F_{2b} litters (71.2% vs. 89.6% in controls, $p \leq 0.01$). Pup body weights were unaffected by treatment at the mid-dose.

At the low-dose, no treatment-related changes were noted in mean litter size, pup viability, pup body weights, or clinical findings for any generation. At all dose levels there were no treatment-related macroscopic or microscopic findings and no changes in the sex ratios of the pups for any generation.

The LOEL for reproductive toxicity was established at 5 ppm based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (reductions in the lactation indices and mean litter sizes). The reproductive NOEL was established at 1 ppm.

C. STUDY DEFICIENCIES: There were no deficiencies with the submitted 2-generation reproduction study in the rat.

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DATA EVALUATION RECORD

Isofenphos

Study Type: Acute Dermal Toxicity (§81-2)

Work Assignment No. 3-15A (MRID 42030001)

Prepared for

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

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 7-22-97

Quality Assurance:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 7/23/97

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 07/24/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch II (7509C)

Robert F. Fricke 26 Aug 97

EPA Work Assignment Manager: Jess Rowland, M.S.
Science Analysis Branch (7509C)

Jess Rowland 8/26/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat

OPPTS Number: 870.1200

OPP Guideline Number: §81-2

DP BARCODE: None

SUBMISSION CODE: None

P.C. CODE: 109401

TOX. CHEM. NO.: 447AB

TEST MATERIAL (PURITY): SRA 12869 Technical (92.4% isofenphos)

SYNONYMS: 1-Methylethyl 2-[[ethoxy[(1-methylethyl)amino] phosphinothioyl]oxy]benzoate

CITATION: Bomann, W. (1991) SRA 12869 Techn. (c.n.: Isofenphos) Study for acute dermal toxicity in the rat. Bayer AG, Wuppertal, Federal Republic of Germany. Report No.: 101274, Study No.: T 3037229. May 10, 1991. MRID 42030001. Unpublished.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, MO.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 42030001), SPF-bred Wistar rats (5/sex/dose) were dermally exposed for 24 hours to SRA 12869 (92.4%) at dose levels of 25,100,160, 250,355, or 500 mg/kg in males and 5, 25,50, or 100 mg/kg in females. SRA 12869 was applied to shorn intact dorsal skin (approx. 10% of the total body surface area). Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing. Signs of cholinergic toxicity were observed up 9 days posts-dosing. No treatment-site dermal irritation was observed. Treatment- and dose-related effects on body weight were observed in animals from both sexes. Gross necropsy of decedent animals revealed ulcerous foci in the glandular stomach; distended, dark, and/or patchy lungs; dark livers; pale and/or small spleens; empty gastrointestinal tracts; pale kidneys; and an esophagus engorged with shavings. No treatment-related effects were observed upon necropsy of animals sacrificed after 14 days.

Dermal LD_{50}: Males = 191 (143-256) mg/kg (95% C.I.)
Females = 70 mg/kg (estimated)
Combined = Approximately 100 mg/kg (observed)

TOXICITY CATEGORY = I

This study is classified **acceptable** and satisfies the guideline requirement (**§81-2**) for an acute dermal study in the rat.

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COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: SRA 12869, Technical
   Description: Yellow-brown liquid
   Lot/Batch #: 808816825
   Purity: 92.4%
   pH: 6.0 (2% in 0.1% NaCl solution)
   CAS #: 25311-71-1

2. Vehicle: None employed

3. Test animals: Species: Rat
   Strain: SPF-bred Wistar [Bor:WISW(SPF-Cpb)]
   Age: 9-10 weeks, males; 14 to over 16 weeks, females (estimated from body weights)
   Weight: 215-267 g males; 207-247 g females
   Source: Winkelmann, Borchen, District of Paderborn, Federal Republic of Germany
   Acclimation period: ≥5 Days
   Diet: Altromin 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: 5/cage
   Environmental: Room Temperature: 22 ± 2°C, Humidity: approximately 50%, Air Changes: approximately 10/hr, Light/dark cycle: 12 hr/12 hr

B. STUDY DESIGN and METHODS:

1. In-life dates: July 3-August 21, 1990

2. Animal assignment and treatment: Males (5/dose) were treated at 25, 100, 160, 250, 355, or 500 mg/kg, while females (5/dose) were treated at 5, 25, 50 or 100 mg/kg.

   Fur from the entire trunk area of each animal was clipped at least 1 day prior to dermal administration of SRA 12869 Technical. The test substance was weighed, as received, into a piece of aluminum foil, the foil was then applied to a 30- to 31-cm^2 site (equivalent to approximately 10% of the total body surface area) and secured with an occlusive dressing (not further described). After 24 hours, the coverings were removed, and the test sites were cleaned

with soap and water. The rats were observed for signs of toxicity, dermal irritation, and/or mortality several times on the day of dosing, and at least once daily thereafter for up to 14 days. Body weights were recorded at 0 (prior to dosing), 3, 7, and 14 days. At 14 days, the surviving animals were sacrificed, and all animals were necropsied (upon death) and examined for gross pathological changes.

3. Statistics: The dermal LD_{50} value (with 95% C.I.) for male animals was calculated using the following citations: Rosiello, A., et al., J. Tox. and Environ. Health 3: 797-809 (1977); Pauluhn, J., Bayer AG Bericht-NR. 11835 (1983); Bliss, C., Ann. Appl. Biol. 22: 134 (1935); Bliss, C., Q. J. Pharm. Pharmacol. 11: 192-216 (1938); and Baird, J. and R. Balster, Neurobehaviour. Tox. 1: 73-77 (1979). The LD_{50} value for female animals was estimated.

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1.

TABLE 1. Study Results[a]

Dose, mg/kg	Animals, No. Dead/No. Treated		
	Males	Females	Combined
5	--	0/5	--
25	0/5	0/5	0/10
50	--	1/5	--
100	0/5	4/5	4/10
160	2/5	--	--
250	4/5	--	--
355	4/5	--	--
500	5/5	--	--
LD_{50}, mg/kg (95% C.I.)	**191 (143-245)**	**70 estimated**	**100 approximate**

[a] Data summarized from table on page 17 of the study.

B. Clinical observations: No signs of toxicity were observed in males from the 25 mg/kg dose group and females from the 5 mg/kg dose group. Clinical signs of cholinergic toxicity were observed at 100 mg/kg and higher in males and 25 mg/kg in females. Signs included apathy, palmospasm, labored breathing, piloerection, periodic tremors, reduced mobility, spastic gait, lacrimation, increased salivation, poor reflexes, facial edema, and/or red ocular discharge. Effects subsided from all

surviving animals by day 10. No treatment-site dermal irritation was observed.

C. Body Weight: Treatment- and dose-related effects on body weight were observed in male and female rats. Compared to the day 1 body weights, the overall(day 1-15) body weight gains were 13% at 25 mg/kg and decreased in a dose-dependent manner to 2% at 500 mg/kg. Surviving females exhibited overall changes of 3.7% at 5 and 25 mg/kg, 1.6% at 50 mg/kg , and -1.4% for the single survivor from the 100 mg/kg dose group.

D. Necropsy: Gross necropsy of decedent animals revealed ulcerous foci in the glandular stomach (18/20); distended (18/20), dark (10/20), and/or patchy (7/20) lungs; dark livers (13/20); pale (9/20) and/or small (5/20) spleens; empty gastrointestinal tracts (3/20); pale kidneys (1/20); and esophagus engorged with shavings (1/20). In 7/20 animals, many of the organs were autolytic and could not be assessed.

E. Deficiencies: There were no deficiencies that affected the validity of the study results.

DATA EVALUATION RECORD

Isofenphos

Study Type: Acute Inhalation Toxicity (§81-3)

Work Assignment No. 3-15B (MRID 41609901)

Prepared for

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

Prepared by

Pesticide Health Effects Group
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Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 7-22-97

Quality Assurance:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 7/23/97

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 07/24/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: R. Fricke, Ph.D.
Reregistration Branch II (7509C)

Robert F. Fricke 26 Aug 97

EPA Work Assignment Manager: Jess Rowland, M.S.
Science Analysis Branch (7509C)

Jess Rowland 8/26/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat

OPPTS Number: 870.1300 OPP Guideline Number: §81-3

DP BARCODE: None SUBMISSION CODE: None
P.C. CODE: 109401 TOX. CHEM. NO.: 447AB

TEST MATERIAL (PURITY): SRA 12869 Technical (91.7%)

SYNONYMS: 1-Methylethyl 2-[[ethoxy[(1-methylethyl)amino] phosphinothioyl]oxy]benzoate

CITATION: Pauluhn, J., (1988), SRA 12869 (Common Name: Isofenphos) Study of the acute inhalation toxicity in accordance with OECD Guidelines No. 403. Bayer AG, Wuppertal, Federal Republic of Germany. Laboratory report No.: 99261, Study No.: T 4027654. MRID 41609901. Unpublished.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, MO.

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 41609901), groups of five or ten young adult SPF-bred Wistar rats/sex were exposed by nose-only inhalation to SRA 12869 Technical (91.7%) at concentrations ranging from 0.0679 to 0.998 mg/kg for 4 hours. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postexposure.

Deaths occurred within 8 days in females exposed at 0.172 mg/L and higher and in males exposed at 0.293 mg/L and higher. Clinical signs, consistent with acute cholinergic toxicity, were observed up to 6 days in males and 10 days in females. Clinical signs included: bradypnea, shortness of breath, excessive salivation, unpreened hair coat, reduced activity, stiff-legged gait, piloerection, difficulty breathing, prostration, tremors, atony, and bloody nose.

At day 3, body weights were decreased in males dosed at 0.293 mg/L and higher and females dosed at 0.167 mg/L and higher. By day 14, the body weight gains by males were comparable to control values, while body weight gains of 0.253 and 0.468 mg/L females were still decreased.

Gross pathological examination of animals which died during the study revealed pale livers with lobular patterns; reddened and/or ulcer-like foci of the glandular stomach; abnormal contents of the gastrointestinal tract; pale spleens; distended, hepatoid foci, and/or edematous lungs; pale kidneys; and reddened renal pelvis. At terminal sacrifice, with the exception of distended lungs in animals dosed at 0.293 mg/L and higher, no other treatment-related gross pathological changes were observed.

At the lowest dose tested (0.068 mg/L), cholinesterase activities were markedly inhibited at 30 to 40 min (> 81% plasma, >67%, RBC) and 20 hours (>58% plasma, >49% RBC) post-dosing.

Inhalation LC_{50} Males = 0.525 mg/L (estimated)
Females = 0.273 (0.199-0.374) mg/L (95% C.I.)
Combined = Approximately 0.468 mg/L (observed)

TOXICITY CATEGORY = II

This study is classified **acceptable (§81-3)** and satisfies the guideline requirement for an acute inhalation study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: SRA 12869 Technical
   Description: Clear, colorless liquid
   Lot/Batch #: 808716841/TOX 2203-00
   Purity: 91.7%
   Vapor pressure: 4.4×10^{-6} mBar (25 °C)
   Saturated Vapor Concentration: 0.06 mg/m^3 (25 °C)
   pH: 6.5 (2% in 0.1% NaCl solution)
   Molecular Weight: 345.4
   CAS #: 25311-71-1

2. Vehicle: Polyethylene glycol E 400 (Lutrol):ethanol (1:1 mixture) was used as a test substance vehicle. The nominal vehicle concentration (5.0 µL/L Lutrol as aerosol and 5.0 µL/L ethanol as vapor) was constant for all exposures.

3. Test animals: Species: Rat
   Strain: SPF-bred Wistar [Bor:WISW(SPF-Cpb)]
   Age: Young adult (2 to 3 months old)
   Weight: 170-209 g males; 174-200 g females
   Source: Winkelmann, Borchen, District of Paderborn, Federal Republic of Germany
   Acclimation period: ≥5 Days
   Diet: Altromin 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Five animals/cage
   Environmental: Room Temperature: 22 ± 2°C, Humidity: approximately 50%, Air Changes: approximately 10/hr, Light/dark cycle: 12 hr/12 hr

B. STUDY DESIGN and METHODS:

1. In-life dates: February 24-April 28, 1988

2. Exposure conditions: A cylindrical dynamic-flow exposure chamber (20 L) constructed of PVC or stainless steel was used in the study. The chamber was equipped with radial ports for attachment of individual Plexiglass exposure tubes for nose-only exposure.

   Test atmosphere was generated by pumping test solution via a Braun infusion pump equipped with a ground glass syringe into a Rhema binary nozzle using dry, compressed air. The resultant aerosol was elutriated through a 1.95-L baffling chamber and diluted with additional air prior to entering the top of the exposure chamber. For all exposures, the total airflow through the chamber (exposure plus baffling) was maintained at 10

L/min (equivalent to approximately 30 chamber turnovers/hour), and the time required for 95% equilibration was reported as 6 minutes.

The nominal test atmosphere concentration was determined at the end of each exposure period by dividing the total amount of test material delivered to the chamber by the total air volume that passed through the chamber during the exposure time. The actual test atmosphere concentration was determined analytically at the beginning (following equilibration), middle, and end of each exposure period. Samples (approximately 10 L) were drawn from the breathing zone of the animals through glass tubes packed with Florisil. Isofenphos was eluted from the Florisil with acetone, and aliquots of the extracts were analyzed by GC in conjunction with nitrogen-specific detection. The actual test concentration was calculated based on the 99.5% analytical standard (the data were not converted to the test compound sample, 91.7% purity). The nominal and average analytically-determined test concentrations are presented in Table 1.

TABLE 1.: Exposure conditions

Nominal Conc. (mg/L)	Mean Actual Conc. (mg/L)	MMAD (µm)	GSD (µm)	% Particles ≤5 µm
0.50	0.0679	1.28	1.38	100
1.00	0.0942	1.27	1.39	100
1.35	0.167	1.29	1.38	100
1.80	0.172	1.23	1.39	100
2.50	0.253	1.28	1.38	100
2.50	0.293	1.24	1.35	100
5.00	0.468	1.26	1.37	100
7.50	0.998	1.33	1.38	100

Particle size was determined apparently once during each exposure period using an Aerodynamic Particle Sizer with Laser Velocimeter (TSI-APS 3300). Samples were collected for 30 seconds from the breathing zone of the animals at an unspecified time during exposure. The average mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and the percentage of particles ≤5 µm were calculated; results are presented in Table 1.

During all exposure periods, temperatures ranged from 21 to 24 °C and the mean relative humidity was 15%. Although not monitored, the air flow was sufficiently high to ensure an oxygen level of ≥19%.

3. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 2. Rats were exposed to SRA 12869 Technical via nose-only inhalation for 4

hours. An additional group of 10 animals/sex were exposed to the vehicle only and served as controls.

Table 2: Animal Assignment

Mean Conc. (mg/L)	Animals Assigned	
	Male	Female
0	10	10
0.0679	10	10
0.0942	5	5
0.167	10	10
0.172	5	5
0.253	5	5
0.293	10	10
0.468	5	5
0.998	10	10

4. Observations: The animals were observed for signs of toxicity and/or mortality several times following exposure (day 0), and on days 1, 2, 3, 7, and 14. Body weights were recorded at 0 (prior to exposure), 3, 7, and 14 days.

5. Cholinesterase Determination: The cholinesterase activity in the erythrocytes and plasma was determined for all animals in the 0.0679 mg/L exposure group prior to exposure, and 30-40 minutes and 20 hours following exposure; blood was obtained from the retro-orbital venous plexus.

6. Gross Pathology: After 14 days, surviving animals were sacrificed, and all animals were necropsied (upon death) and examined for gross pathological changes.

7. Statistics: The inhalation LC_{50} value (with 95% C.I.) for female animals was calculated using the following citations: Rosiello, A., et al., J. Tox. and Environ. Health 3: 797-809 (1977); Pauluhn, J., Bayer AG Bericht-NR. 11835 (1983); and Bliss, C., Q. J. Pharm. Pharmacol. 11: 192-216 (1938). The LD_{50} value for male animals was estimated.

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 2. Mortality occurred in 25/35 females exposed at ≥0.172 mg/L and in 13/25 males exposed at ≥0.293 mg/L within 8 days.

B. Clinical observations: No signs of toxicity were observed in males dosed at 0.167 mg/L and lower and females dosed at 0.0942 mg/L and lower. At the higher dose levels, clinical signs of cholinergic toxicity were evident and included: respiratory abnormalities (bradypnea, shortness of breath, difficulty breathing), excessive salivation, reduced activity,

stiff-legged gait, piloerection, prostration, tremors, atony, and bloody nose. Effects subsided in males by day 7 and in females by day 11. Individual animal results were not provided.

Table 2.: Number of Animals Dead/Treated, LC_{50}, and 95% Confidence Intervals (C.I.)[a]

Mean Conc. (mg/L)	Male	Female	Combined
0.0	0/10	0/10	0/20
0.0679	0/10	0/10	0/20
0.0942	0/5	0/5	0/10
0.167	0/10	0/10	0/20
0.172	0/5	4/5	4/10
0.253	0/5	1/5	1/10
0.293	1/10	6/10	7/20
0.468	2/5	4/5	6/10
0.998	10/10	10/10	20/20
LC_{50}, mg/L (95% C.I.)	**0.525 estimated**	**0.273 (0.299-0.374)**	**0.468 approximate**

[a] Data summarized from table on page 27 of the study.

C. Body Weight: Compared to the pre-exposure body weights, males dosed at 0.293 and 0.486 mg/L had body weight gains of -4.5 and -13.3%, respectively, on Day 3. By Day 14, surviving animals had body weight gains comparable to control values. For females dosed at 0.167 mg/L and higher had body weight gains ranging from -3.2 to -26.8% on Day 3. At Day 14, the body weight gains of females dosed at 0.253 and 0.468 mg/L were 2.8 and 21.1% lower than the pre-exposure values.

D. Necropsy: Gross pathological examination of animals which died during the study revealed pale livers with lobular patterns; reddened and/or ulcer-like foci of the glandular stomach; abnormal contents of the gastrointestinal tract; pale spleens; distended, hepatoid foci, and/or edematous lungs; pale kidneys; and reddened renal pelvis. At terminal sacrifice, with the exception of distended lungs in animals dosed at 0.293 mg/L and higher, no other treatment-related gross pathological changes were observed.

E. At the lowest dose tested (0.068 mg/L), plasma and RBC cholinesterase activities were markedly inhibited at 30 to 40 min and 20 hours post-dosing (Table 3)..

Table 3: Percent Inhibition (Compared to Pre-exposure Activity) of Plasma and RBC ChE Activities at 0.068 mg/L [a]

Time Post-Dosing	Plasma ChE		RBC ChE	
	Male	Female	Male	Female

30-40 min	81	94	67	75
20 hr	58	84	49	84

[a] Data summarized from table on page 30 of the study

E. Deficiencies: The aerodynamic particle size should have been determined hourly during exposure. In this study, it was apparently determined only once per exposure, at an unspecified time. However, upon examination of the data provided, it is evident that the aerosol generation system produced particles within the optimum respirable range (1-4 µm) and this deficiency is considered minor.

Clinical observations were not conducted on a daily basis, and individual data were not provided. However, the summarized data provided were adequate in establishing the general nature of effects observed as well as their onset and duration. As a result, this deficiency is considered minor.

The study author reported that the mean chamber humidity was 15%, which is below the 40-60% limits outlined in Subdivision F guidelines. This deficiency should have no significant effect on the results of the study and is considered minor.

DATA EVALUATION RECORD

Isofenphos

Study Type: Primary Eye Irritation (§81-4)

Work Assignment No. 3-15C (MRID 41609911)

Prepared for

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

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 7-22-97

Quality Assurance:
William J. Spangler, Ph.D.

Signature: William J. Spangler
Date: 7/23/97

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 07/24/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch II (7509C)

Robert F. Fricke 26 Aug 97

EPA Work Assignment Manager: Jess Rowland, M.S.
Science Analysis Branch (7509C)

Jess Rowland 8/26/97

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit

OPPTS Number: 870.2400 | OPP Guideline Number: §81-4

DP BARCODE: None | SUBMISSION CODE: None
P.C. CODE: 109401 | TOX. CHEM. NO.: 447AB

TEST MATERIAL (PURITY): Isofenphos,Technical (90.8%)

SYNONYMS: 1-Methylethyl 2-[[ethoxy[(1-methylethyl)amino] phosphinothioyl]oxy]benzoate

CITATION: Sheets, L. (1990) Primary eye irritation study with technical grade Isofenphos in rabbits. Mobay Corporation, Stilwell, KS. Laboratory Study Nos. 100269 and 90-335-EP. July 30, 1990. MRID 41609911. Unpublished.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, MO.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 41609911), 0.1 mL of technical-grade Isofenphos (90.8%) was instilled into the conjunctival sac of the left eye of three adult New Zealand White rabbits/sex. The treated eyes were not rinsed. The animals were observed for up to 72 hours following treatment, and eye irritation was scored using a modified Draize scheme.

Ocular irritation was greatest in the treated eyes 1 hour following instillation, and included slight conjunctival redness, very slight conjunctival chemosis, and severe conjunctival discharge in 6/6 treated eyes. Two male animals died from acute cholinergic toxicity prior to the 24-hour observation interval. In the remaining four animals, slight conjunctival redness persisted in 4/4 eyes at 24 hours and 2/4 eyes at 48 hours. No corneal or iridial changes were observed during the study, and conjunctival redness completely subsided by 72 hours.

TOXICITY CATEGORY III

This study is classified **acceptable (§81-4)** and satisfies the guideline requirement for a primary eye irritation study in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Technical-grade Isofenphos
   Description: Clear, colorless liquid
   Lot/Batch #: 8-00-5258 and 8-03-0052
   Composition: 90.8% Purity
   CAS #: 25311-71-1

2. Vehicle and/or positive control: None employed

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age: Young adult (approximately 10 weeks)
   Weight: Not provided
   Source: Small Stock Industries, Pea Ridge, Arkansas
   Acclimation period: ≥6 Days
   Diet: Agway Prolab Rabbit Diet, approximately 125 g/animal/day
   Water: Tap water, *ad libitum*

B. STUDY DESIGN and METHODS

1. In-life dates: January 9-12, 1990

2. Animal assignment and treatment: A 0.1-mL aliquot of technical-grade Isofenphos was instilled into the conjunctival sac of the left eye of three young adult rabbits/sex. The upper and lower lids were held together for approximately 1 second before releasing to prevent loss of the material. The treated eyes were not rinsed, and the right eye of each animal served as an untreated control. The animals were observed for ocular irritation and signs of toxicity at 1, 24, 48, and 72 hours following instillation; eye irritation was scored by a modified Draize method[1].

II. RESULTS AND DISCUSSION:

A. Clinical observations: Ocular irritation was greatest in the treated eyes 1 hour following instillation, and included slight conjunctival redness (score of 1), very slight conjunctival chemosis (score of 1), and severe conjunctival discharge (score of 3) in 6/6 treated eyes. Two male animals died prior to the 24-hour observation interval. In the remaining four animals, slight conjunctival redness (score of 1) persisted in 4/4 eyes at 24 hours and 2/4 eyes at 48 hours. No corneal or iridial changes were observed during the study, and conjunctival redness completely subsided by 72 hours. In this study, technical-grade Isofenphos is a mild ocular irritant.

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[1]The area of corneal opacity was not graded.

During the 3-day study, perianal staining was observed in the single surviving male, and perianal and urine staining were observed in a single female animal. Gross necropsy of the two decedent males revealed signs of diarrhea and salivation. The study author concluded that the deaths and clinical signs were a result of acute cholinergic toxicity.

B. Deficiencies: Although only four treated eyes were evaluated between 24 and 72 hours, Subdivision F guideline §81-4 offers no option to reduce the dose of 0.1 mL; therefore, this deficiency does not jeopardize the acceptability of the study [refer to page 17 of the MRID].

DATA EVALUATION RECORD

Isofenphos

Study Type: Primary Dermal Irritation (§81-5)

Work Assignment No. 3-15D (MRID 41609904)

Prepared for

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

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
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Signature: Christie E. Padova
Date: 7-22-97

Quality Assurance:
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Signature: William J. Spangler
Date: 7/23/97

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 07/24/97

Disclaimer

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EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch II (7509C)

Robert F. Fricke 26 May 97

EPA Work Assignment Manager: Jess Rowland, M.S.
Science Analysis Branch (7509C)

Jess Rowland 8/26/97

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS Number: 870.2500 OPP Guideline Number: §81-5

DP BARCODE: None SUBMISSION CODE:
P.C. CODE: 109401 TOX. CHEM. NO.: 447AB

TEST MATERIAL (PURITY): Technical-grade Isofenphos (90.8% purity)

SYNONYMS: 1-Methylethyl 2-[[ethoxy[(1-methylethyl)amino] phosphinothioyl]oxy]benzoate

CITATION: Sheets, L. (1990) Primary dermal irritation study with technical grade Isofenphos in rabbits. Mobay Corporation, Stilwell, KS. Laboratory Study Nos. 100185 and 90-325-EM. May 29, 1990. MRID 41609904. Unpublished.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, MO.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 41609904), New Zealand White rabbits (3/sex) were dermally exposed (6 cm^2 site/animal) to 0.5 mL of Isofenphos (90.8%) for 4 hours. Animals were observed for dermal irritation for up to 72 hours following application, and irritation was scored by the Draize scale.

Very slight to well-defined erythema was observed at up to 5/6 sites between 1 and 24 hours following patch removal. No other dermal irritation was observed during the study. The primary dermal irritation index was 0.42.

TOXICITY CATEGORY IV

This study is classified **acceptable (§81-5)** and satisfies guideline requirements for a primary dermal irritation study in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Technical-grade Isofenphos
   Description: Clear, colorless liquid
   Lot/Batch #: 8-00-5258 and 8-03-0052
   Purity: 90.8%
   CAS #: 25311-71-1

2. Vehicle and/or positive control: None employed

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age: Young adult (approximately 10 weeks)
   Weight: Not provided
   Source: Small Stock Industries, Pea Ridge, Arkansas
   Acclimation period: 6 Days
   Diet: Agway Prolab Rabbit Diet, approximately 125 g/animal/day
   Water: Tap water, *ad libitum*

B. STUDY DESIGN and METHODS:

1. In-life dates: January 9-12, 1990

2. Animal assignment and treatment: Fur from the dorsal trunk areas of three young adult animals/sex was clipped 24 hours prior to dermal administration with 0.5 mL of technical-grade Isofenphos. The test substance was applied as received to a single intact 6-cm^2 application site/animal and covered with a gauze patch secured with hypoallergenic tape. The patch was then covered with a piece of plastic secured with Vetrap adhesive bandage. Following a 4-hour exposure period, the coverings were removed, and the test sites were gently washed with water-moistened paper towels. The rabbits were observed for dermal irritation and signs of toxicity 30-60 minutes and 24, 48, and 72 hours following patch removal. Erythema and edema were scored separately using the Draize scale.

II. RESULTS AND DISCUSSION:

A. Clinical observations: Very slight to well-defined erythema (scores of 1-2) was observed at up to 5/6 sites between 1 and 24 hours following patch removal. No other dermal irritation was observed during the study. The primary dermal irritation index was 0.42. Based on the results of this study, technical-grade Isofenphos is not a significant dermal irritant.

B. Deficiencies: There were no deficiencies that affected the validity of the study results.