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D- 8990 Propanil

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

AUG 11 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Propanil - Toxicology Chapter of the Registration Standard

FROM: William Dykstra, Ph.D. William Dykstra 8/10/87
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Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
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THRU: Robert P. Zendzian, Ph.D. 8/10/87
Registration Standard Coordinator
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

William Burnam, Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Attached is the Toxicology Chapter of the Registration Standard for Propanil.

Attachment

cc: Amy Rispin, Chief
Science Integration Staff
Hazard Evaluation Division (TS-769C)

Robert Coberly
Toxicology Branch
Hazard Evaluation Division (TS-769C)

BEST AVAILABLE COPY

9 pages

Information which may reveal inert ingredients has been removed from pages 35, 40, 44, and 83.

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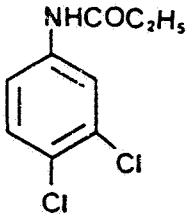
PROPANIL

Structure:



Chemical Name: 3',4'-dichlorophenylpropionanilide

Other Names: DPA, FW-734, Stam, Stampede

Chemical Properties:

Appearance - dark brown, crystalline solid
Melting point - 90.6 to 91.6 °C

A. Toxicology Summary

Propanil is a selective postemergence herbicide (contact type) with a relatively low toxicity. There are two technicals presently registered with the Agency, 98% purity and 85-88% purity technicals. The toxicology studies submitted to the Agency have been performed with one or the other technicals, but rarely both. There were no acceptable acute toxicity and irritation studies.

In rat and rabbit teratology studies, the teratogenic potentials were negative at the HDT of 100 mg/kg in each study.

A three-generation rat reproduction study had a NOEL of 300 ppm, with decreased body weights in parental animals observed at 1000 ppm (HDT).

A 90-day mouse feeding study showed an increase in hepatocytic pleomorphism and hepatocellular necrosis at 200 ppm. The NOEL was 25 ppm.

A 90-day rat feeding study showed increased relative spleen weights in females and decreased hemoglobin in males at 1000 ppm. The NOEL was 330 ppm in the study.

Similarly, a 2-year rat feeding study showed increased relative spleen weights in females at 400 ppm. The NOEL for the study was 100 ppm.

In a 2-year dog feeding study, the effects at 4000 ppm (HDT) were decreased body weights, decreased food consumption, and increased SGOT and SAP values. The NOEL for the study was 600 ppm.

The oncogenic potential of propanil could not be determined in the 2-year rat study at the MTD of 1600 ppm due to the inadequate histological examination of tissues in both sexes of rats. The oncogenic potential of propanil in mice was negative at 180 ppm (HDT). However, it is uncertain that the MTD was tested. Additionally, the 2-year mouse study employed both 98% and 85.4% technicals at the HDT of 180 ppm. NOELs for bilateral retinal degeneration in males and females and thyroiditis in females have not yet been established with the 85.4% purity technical in the mouse study at 180 ppm (only dose tested with the 85.4% technical).

Propanil was not mutagenic in gene mutation assays, chromosomal aberration assays, and in all but one (B. subtilis) direct DNA damage assays.

In a rat metabolism study using only males, approximately 90 to 92 percent of the radioactivity was recovered in urine, feces, and cage washings within 2 days. Less than 1 percent was found in rat tissues. The predominant metabolite was 3',4'-dichloroaniline.

The studies needed to complete the data base for technical propanil include acute toxicity, subchronic oral toxicity (nonrodents) and subchronic dermal toxicity, chronic (both rat and dog), oncogenic (rat and possibly mouse), and a rat metabolism.

B. Toxicology Profile

81 Series Acute Toxicity and Irritation Studies

81-1 Acute Oral

There were no acceptable studies. This creates a data gap and requires submission of an acceptable study.

81-2 Acute Dermal

There were no acceptable studies. This creates a data gap, and requires submission of an acceptable study.

81-3 Acute Inhalation

There were no acceptable studies. This creates a data gap, and requires submission of an acceptable study.

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81-4 Primary Eye Irritation

There were no acceptable studies. This creates a data gap and requires submission of an acceptable study.

81-5 Primary Dermal Irritation

There were no acceptable studies. This creates a data gap and requires submission of an acceptable study.

81-6 Dermal Sensitization

There were no acceptable studies. This creates a data gap and requires submission of an acceptable study.

81-1 Acute Delayed Neurotoxicity

No acute delayed neurotoxicity studies are required since propanil is not an organophosphate pesticide.

82 Series Subchronic Testing

82-1 Subchronic Oral

There were two studies reviewed. One was acceptable in the mouse and one was unacceptable in the rat. This creates a data gap and requires the submission of an acceptable study in the nonrodent species.

[MRID No. WD005]

Randomized groups of 10 male and 10 female CD-1 mice were fed dietary levels of 0, 25, 200, 1600, and 12,800 ppm of Stam technical (98.0% purity) for 3 months. Criteria evaluated included toxic signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, and histopathology.

The results were as follows:

NOEL = 25 ppm
LEL = 200 ppm; increase in hepatocytic pleomorphism and hepatocellular multifocal necrosis.

The study was classified as Core-Minimum.

[MRID Nos. 46259, 15419]

Randomized groups of 10 male and 10 female Wistar albino rats were fed ad libitum dietary concentrations of 0, 0.01, 0.033,

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0.10, 0.33, 1.0, and 5.0 percent of Stam technical (97% purity) for 3 months. Criteria evaluated were toxic signs, food consumption, body weight, hematology, urinalysis, organ weights, and histopathology.

The results were as follows:

NOEL = 0.033% (330 ppm)
LEL = 0.10% (1000 ppm); increased relative spleen weight in females and decreased hemoglobin in males.

The study was classified as Core-Supplementary since individual data were not provided.

82-2 Subchronic Dermal (21-Day)

No 21-day subchronic dermal studies have been submitted. This creates a data gap and requires the submission of an acceptable study.

82-3 Subchronic Dermal (90-Day)

No 90-day subchronic dermal studies have been submitted. Based on the registered use patterns this study is not required.

82-4 Subchronic Inhalation

No subchronic inhalation studies have been submitted. Based on the registered use patterns this study is not required.

82-5 Subchronic Neurotoxicity

No subchronic neurotoxicity studies have been submitted. No studies are required since the acute neurotoxicity study is not required.

83 Series Chronic and Long-Term Studies

83-1 Chronic Toxicity

Two chronic studies have been submitted for technical propanil.

Each study was unacceptable. This creates a data gap and requires the submission of both chronic rodent and nonrodent studies.

[MRID Nos. 15419, 134002, 36089]

Randomized groups of 25 male and 25 female Wistar rats were administered 0, 100, 400, and 1600 ppm of Stam technical (97% purity) in the diet ad libitum for 2 years.

Criteria evaluated included toxic signs, food consumption, body weight, hematology, urinalysis, organ weights, and histopathology.

The results were as follows:

NOEL = 100 ppm (LDT)
LEL = 400 ppm; increased relative spleen weight in females.

The oncogenic potential could not be determined at the MTD of 1600 ppm due to inadequate histological examination of tissues in both sexes of rats. The study was classified as Core-Supplementary because clinical chemistries were not performed, numerous rats were not examined histologically, and only 25 rats/sex/group were used.

[MRID Nos. 15419, 36090, 132749]

Two male and two female purebred beagle dogs were placed on each of the following dietary levels of Stam technical (97% purity) ad libitum for 2 years: 0, 100, 600, and 4000 ppm.

Criteria evaluated included toxic signs, food consumption, body weights, hematology, clinical chemistry, urinalysis, organ weights, and histopathology.

The results were as follows:

NOEL = 600 ppm
LEL = 4000 ppm; decreased body weight, decreased food consumption, and increased SGOT and SAP values.

The study was classified as Core-Supplementary because only two dogs/sex/group were used, limited clinical chemistry, and limited histopathology was performed.

83-2 Oncogenicity

One Core-Supplementary oncogenicity study in mice with technical propanil (both 98% and 85.4% purity technicals were used) has been submitted. This creates a data gap and requires the submission of an acceptable rat oncogenicity study, and possibly a repeat of the mouse oncogenicity study.

[MRID No. 155215]

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Randomized groups of CD-1 mice were used in the study. Both 98% purity and 85.4% purity propanil technicals were used in the 2 year study. The dose levels for the study were 0, 0, 5, 30, and 180 ppm for the 98% purity technical and a second dose level at 180 ppm using the 85.4% purity technical.

The oncogenic potential was negative at 180 ppm (HDT) for both the 98% purity and 85.4% purity technicals. There were no compound-related effects on survival, clinical observations, tissue masses, body weight, food consumption, hematology, and organ weights.

Dose-related histologic findings were observed in the male liver as centrilobular hepatocytic enlargement beginning by week 15 and continuing for the 104-week study.

The LEL for this effect was 180 ppm (HDT) for both the 98.0% purity and 85.4% purity Stam technical. The NOEL was 30 ppm for this lesion.

Bilateral retinal degeneration in male and female mice and thyroiditis in female mice were observed at 180 ppm (85.4% purity, Stam technical; the only dose level tested for this technical in this study). NOELs for these effects were not established in this study. The presence of these lesions may exceed Special Review Criteria. The study was classified as Core-Supplementary because the MTD was not employed and NOELs for bilateral retinal degeneration and thyroiditis with the 85.4% technical were not established. The 2-year mouse study may need to be repeated with the 85.4% technical.

83-3 Teratogenicity

Two acceptable studies were submitted. One was in rats and the second was in rabbits.

[MRID No. 58588]

Randomized groups of 25 pregnant Sprague-Dawley rats received oral doses by gavage of Stam technical (85.4% purity) at 0, 0.8, 4.0, 20, and 100 mg/kg during days 6 through 15 of gestation.

On day 20 of gestation, all animals were sacrificed. Uterine contents were examined and reproductive parameters were measured. All fetuses were evaluated externally. One-third of the fetuses were examined for soft tissue anomalies by the Wilson technique, and the remaining two-thirds of the fetuses were examined by Alizarin staining for skeletal anomalies. The teratogenic potential was negative where as developmenti toxicity was observed at 100 mg/kg/day. The study was classified as Core-Minimum.

The results were as follows:

Maternal toxic NOEL	=	20 mg/kg
Maternal toxic LEL	=	100 mg/kg (increased resorptions/dam)
Developmental toxicity NOEL	=	20 mg/kg
LEL	=	100 mg/kg (decreased pup weight, delayed ossification, absent sternebrae #5 and xiphesternum)

[MRID No. 58589]

Randomized groups of 20 NZW pregnant rabbits received Stam technical (85.4% purity) as a suspension in corn oil at dosages of 0, 4, 20, and 100 mg/kg once daily during days 6 through 18 of gestation.

On day 30 of gestation, surviving rabbits were sacrificed by CO_2 asphyxiation and reproductive parameters were measured. Fetuses were examined externally, weighed, and sexed. All fetuses were examined for soft tissue anomalies, eviscerated, stained with Alizarin, and evaluated for skeletal variations.

The teratogenic potential was negative. The study was classified as Core-Minimum.

The results were as follows:

Maternal Toxic NOEL	=	20 mg/kg
Maternal Toxic LEL	=	100 mg/kg (death, decreased body weight)
Developmental toxicity NOEL	=	20 mg/kg
LEL	=	100 mg/kg (unossified metacarpals)

83-4 Reproduction

One acceptable three-generation reproduction study in rats was submitted.

[MRID Nos. 36091, 15419]

Randomized groups of 25 male and 25 female Wistar rats were fed ad libitum dietary levels of 0, 100, 300, and 1000 ppm of Stam technical (97% purity) for three generations with each generation producing two litters.

There were no compound-related effects on fertility, gestation, pup viability, pup lactation, and sex ratios for each generation. Average litter sizes at birth and weaning were greater at all

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dietary levels of technical as compared to controls. Weaning body weights averaged less for pups at all dietary levels of technical as compared with controls, but this appears to be due to the increased litter sizes. Histopathologic examination of F3b pups did not reveal any compound-related lesions.

Reproductive and systemic	NOEL =	300 ppm
	LEL =	1000 ppm (HDT) (decreased body weights of parental animals during growth, mating, and weaning)

84 Series Mutagenicity Testing

84-2 Mutagenicity Tests

No additional mutagenicity studies are required.

Gene Mutation

There were three acceptable gene mutation studies. Two studies were bacterial and one was mammalian.

[MRID No. WD003]

Stam technical (98% purity) was evaluated both with and without S-9 in five histidine-requiring strains of S. typhimurium and one tryptophan-requiring E. coli strain at concentrations of 1, 5, 10, 50, 100, 500, 1000, and 5000 micrograms/plate. Positive controls were employed. The technical was not mutagenic under conditions of the assays. The study was acceptable.

[MRID No. WD004]

Stam technical (88% purity) was evaluated both with and without S-9 in five histidine-requiring strains of S. typhimurium and one tryptophan-requiring E. coli strain at concentrations of 10, 50, 100, 250, 500, 1000, and 5000 micrograms/plate. Positive controls were employed. The technical was not mutagenic under conditions of the assays. The study was acceptable.

[MRID No. WD002]

Stam technical (87.8% purity) was evaluated both with and without S-9 in CHO/HGPRT mammalian cells from CHO-K_1-BH_4 cell line in a gene mutation assay at concentrations of 15, 75, 125, and 150 micrograms/mL without S-9, and 100, 115, 120, 130, 140, 150, 165 and 175 micrograms/mL with S-9. Positive controls were employed. The technical was not mutagenic under the conditions of the assay. The study was acceptable.

Chromosomal Aberration

One chromosomal aberration assay study was submitted. It was an in vivo cytogenetic study in mice.

[MRID No. WD001]

Stam technical (87.8% purity) was evaluated for in vivo cytogenetic mutagenic potential in male CD-1 mice at single and multiple (5 days) dosages of 0, 26.5, 106, and 265 mg/kg/day. The positive controls received TEM in one single dose at 0.3 mg/kg.

Bone marrow was extracted from the femurs of each animal and slides of cells were prepared. The technical did not induce chromosomal aberrations in mouse bone marrow cells under conditions of the assays.

The study classification was reserved pending submission of additional data.

Direct DNA Damage

Four direct DNA damage studies were submitted. Three studies were in bacterial systems and one study was in mammalian cells.

[MRID No. WD003]

Stam technical (98% purity) was evaluated in a DNA damage and repair Rec assay in B. subtilis at dosages of 20, 100, 200, 500, 1000, and 2000 micrograms/disk. Positive and negative controls were employed. Under the conditions of the assay, the technical did not appear to induce DNA damage. The study was classified as unacceptable since minimal information was submitted.

[MRID No. WD004]

Stam technical (88% purity) was evaluated for DNA damage/repair in yeast (mitotic recombination in S. cerevisiae D3), DNA damage/repair in bacteria (B. subtilis/E. coli) and DNA damage/repair in human fibroblast cells (UDS in WI-38). Positive and negative controls were used.

The technical was not mutagenic in the mitotic recombination assay in S. cerevisiae. It also tested negatively in the relative toxicity assay in DNA repair-deficient E. coli and in the unscheduled DNA synthesis assay in WI-38 cells. It tested positively in the relative toxicity assay in DNA repair-deficient B. subtilis.

These studies were classified as acceptable. No metabolic activation was used for the differential toxicity studies.

However, the results were positive in B. subtilis without metabolic activation.

85 Series Metabolism Studies

85-1 Metabolism

One metabolism study in male rats was submitted.

[MRID No. 35686]

C^{14}-Stam (uniformly labeled in the ring) was used in the study. Groups of male rats were given C^{14}-Stam by stomach tube. Approximately 90 to 92 percent of the radioactivity was recovered in urine, feces, and cage washings during the feeding period and the following 2 days. Only small amounts (less than 1%) were found in rat tissues. Several metabolites were found in urine and feces. A major portion of the labels were derivatives of 3',4'dichloroaniline. The study was classified as Core-Supplementary because (1) individual data were not provided; (2) female rats were not studied; and (3) T 1/2 was not determined.

C. Data Gaps

Propanil is registered and tolerances are established in 40 CFR 180.274 for several raw agricultural commodities (RACs). The following Guideline Toxicology studies have been identified as data gaps for propanil and are therefore required:

81-1	Acute Oral Toxicity
81-2	Acute Dermal Toxicity
81-3	Acute Inhalation Toxicity
81-4	Primary Eye Irritation
81-5	Primary Dermal Irritation
81-6	Dermal Sensitization
82-1	Subchronic Oral (Nonrodent) waived due to requirement for chronic nonrodent study
82-2	Subchronic Dermal (21-Day)
83-1	Chronic Toxicity (Rodent and Nonrodent)
83-2	Oncogenicity (Rat)
85-1	Metabolism (Rat)

D. ADI Reassessment

In an evaluation of the ADI (July 22, 1987) by the Toxicology Branch RfD Committee, the 2-year rat feeding study was used as the critical study. The NOEL for the rat study was 100 ppm (5 mg/kg/day). The study was classified as Core-Supplementary. An uncertainty factor of 1000 was used to account for the inter- and intraspecies differences. No modifying factors were used.

The NOEL was divided by an uncertainty factor of 1000 to give the ADI (RfD).

$$\text{ADI (RfD)} = \text{NOEL} \times \frac{1}{1000}$$

$$\text{ADI (RfD)} = 5 \text{ mg/kg/day} \times \frac{1}{1000} = 0.005 \text{ mg/kg/day}$$

E. Toxicological Issues

Based on the current incomplete toxicology data base, there are three significant toxicological issues associated with propanil.

Issue 1: There are two technicals presently registered with the Agency. A 98% technical product registered in April 1985 and an 85-88% technical product registered since 1972. Additionally, a 97% purity technical was used in the 1960s.

The toxicology studies submitted to the Agency have been performed with one or the other technical, but rarely both, as shown below in Table I.

TABLE I

Summary of Toxicology Studies with Propanil

Study and Results	Percent Purity of Technicals		
	97%	85-88%	98%
90-Day Mouse			NOEL = 25 ppm LEL = 200 ppm
90-Day Rat	NOEL = 330 ppm LEL = 1000 ppm		
Rat Teratology		Negative at 100 mg/kg	
Rabbit Teratology		Negative at 100 mg/kg	
2-Year Rat	Oncogenicity invalid NOEL = 100 ppm LEL = 400 ppm		
2-Year Dog	NOEL = 600 ppm LEL = 4000 ppm		

Table I (cont'd)

Summary of Toxicology Studies with Propanil

Study and Results	Percent Purity of Technicals		
	97%	85-88%	98%
2-Year Mouse		Oncogenicity negative at 180 ppm. No NOEL for two lesions at 180 ppm; MTD issue; Only dose used was 180 ppm	Oncogenicity negative at 180 ppm; NOEL = 30 ppm; MTD issue; Doses used were 5, 30, and 180 ppm
3-Generation Reproduction	NOEL = 300 ppm LEL = 1000 ppm		
Gene Mutation		Negative for S. typhimurium and E. coli, and CHO/HGPRT mammalian cells	Negative for S. typhimurium and E. coli
Chromosomal Abberation		Negative for cytogenetic in mice	
Direct DNA Damage		Negative for S. cerevisiae, E. coli, UDS, and positive for B. subtilis	Negative for B. subtilis but unacceptable

The 2-year rat feeding study, the 2-year dog feeding study, the 3-generation rat reproduction study, the rat metabolism study, and the 90-day rat feeding study were all done in the 1960s by the Medical College of Virginia using a 97% purity technical which may be chemically different from both the 98% and 85-88% technicals used since that time.

The rat and rabbit teratology studies were performed with the 85.4% purity technical and were performed in 1980. There are no teratology studies with the 98% technical.

The mutagenicity studies were performed with either the 85-88% technical or the 98% technical.

The 2-year mouse oncogenicity study was performed with both the 98% technical (doses of 5, 30, and 180 ppm) and the 85.4% technical (dose of 180 ppm).

It is uncertain whether two complete toxicology data bases are required for the two different presently registered technicals or whether the toxicology data base can be comprised of studies using either of the two technicals which may contain significant differences in impurities. Depending upon the technical registrations that the registrant wishes to maintain and the type and quantity of impurities therein, it may be necessary to repeat certain studies, for example, the reproduction study (using 85-88% and/or 98% technicals), the teratology studies (with 98% technical), the mouse oncogenicity study (with 85-88% technicals), and possibly other studies. The registrant is required to address this issue in satisfactory detail.

Issue 2: The 2-year mouse oncogenicity study was performed at doses of 0, 5, 30, and 180 ppm (with 98% technical) and an additional dose of 180 ppm (with 85.4% technical).

At the 180 ppm dose level with 85.4% technical, two lesions occurred at statistically and toxicologically significant incidences.

One of these lesions was bilateral retinal degeneration in male mice which occurred at a grade of moderately severe in 5 out of 8 incidences at 180 ppm (85.4%). The control male mice had no lesions graded moderately severe, but each male control group had two lesions with a grade of moderate. The percentage of mice affected in the control groups was 4 and 5 percent, whereas at 180 ppm (85.4%), the affected incidence was 11 percent.

Additionally there was an increased incidence of bilateral retinal degeneration in group 6 female mice which was also considered compound-related.

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Also, at the 180 ppm dose (85.4%), an increased incidence of thyroiditis (minimal grade) was observed in female mice. A NOEL for this lesion does not appear to be established in the study.

Issue 3: The HDT in the 2-year mouse oncogenicity study may not have been the MTD. The HDT was 180 ppm and was based on the results of a 90-day range-finding study in mice. In the range-finding study, severe toxic effects were produced at 1600 and 12,800 ppm. The effects observed at 200 ppm were hepatocytic pleomorphism in 3/10 females and multifocal hepatocellular necrosis in 1/10 males. The NOEL for the range-finding study was 25 ppm. The HDT for the 2-year mouse study was chosen to be 180 ppm, which is approximately eight times lower than the dose level of 1600 ppm, where "life threatening" toxic effects were observed. Toxicology Branch considers the 180 ppm level for the MTD for the 2-year mouse study to be too low and to be based only on hepatocytic pleomorphism and multifocal hepatocellular necrosis, which may be insufficient toxic endpoints. A further explanation of the MTD by the registrant is required.

TABLE A

GENERIC DATA REQUIREMENTS FOR PROPANIL

Data Requirement	Composition[1]	Use Patterns[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?
§158.135 Toxicology					
Acute Testing					
81-1 - Acute Oral - Rat	TGAI	A	No		Yes
81-2 - Acute Dermal - Rabbit	TGAI	A	No		Yes
81-3 - Acute Inhalation - Rat	TGAI	A	No		Yes
81-4 - Eye Irritation - Rabbit	TGAI	A	No		Yes
81-5 - Dermal Irritation - Rabbit	TGAI	A	No		Yes
81-6 - Dermal Sensitization - Guinea Pig	TGAI	A	No		No
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI	A	No		No
Subchronic Testing					
82-1 - 90-Day Feeding					
- Rodent	TGAI	A	Yes	WD005	No
- Nonrodent	TGAI	A	No		No[3/]

TABLE A (cont'd)

GENERIC DATA REQUIREMENTS FOR PROPANIL

Data Requirement	Composition[1]	Use Patterns[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?
§158.135 Toxicology					
Subchronic Testing (cont'd)					
82-2 - 21-Day Dermal	TGAI	A	No		Yes
82-3 - 90-Day Dermal	TGAI	A	No		No
82-4 - 90-Day Inhalation	TGAI	A	No		No
82-5 - 90-Day Neurotoxicity	TGAI	A	No		No
Chronic Testing					
83-1 - Chronic Toxicity					
- Rodent	TGAI	A	No		Yes
- Nonrodent	TGAI	A	No		Yes
83-2 - Oncogenicity					
- Rat	TGAI	A	No		Yes
- Mouse	TGAI	A	Partial	155215	Yes[4/]

TABLE A (cont'd)

GENERIC DATA REQUIREMENTS FOR PROPANIL

Data Requirement	Composition[1]	Use Patterns[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?
§158.135 Toxicology					
Chronic Testing (cont'd)					
83-3 - Teratogenicity					
- Rat	TGAI	A	Yes	58588	No
- Rabbit	TGAI	A	Yes	58589	No
83-4 - Reproduction	TGAI	A	Yes	36091, 15419	No
Mutagenicity Testing					
84-2 - Gene Mutation	TGAI	A	Yes	WD002, WD003, WD004	No
84-2 - Chromosome Aberration	TGAI	A	Yes	WD001	No
84-2 - Other Mechanisms of Mutagenicity	TGAI	A	Yes	WD003, WD004	No
Special Testing					
85-1 - General Metabolism	PAIRA	A	No		Yes

TABLE A

GENERIC DATA REQUIREMENTS FOR PROPANIL

Footnotes

1/Composition: TGAI = Technical Grade of the Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, Radiolabeled; Choice = Choice of several test substances determined on a case-by-case basis.

2/The Use Patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic Nonfood; E = Greenhouse, Food Crop; F = Greenhouse, Nonfood; G = Forestry; H = Domestic, Outdoor; I = Indoor; IP = Industrial Preservative.

3/Data requirement waived due to requirment for a chronic nonrodent study.

4/An additional mouse oncogenicity study may be required depending on the outcome of the MTD issue.

G. Bibliography

MRID No.	Citation
46259 15419	Rohm & Haas Company (19??) Toxicologic Study on the Effect of Adding Stam F-34 (FW-734) to the Diet of Rats for Three Months. (Unpublished study received October 29, 1961 under unknown admin. no.; CDL:108773-E).
15419	Ambrose, A.M.; Larson, P.S.; Borzelleca, J.F.; et al. (1972) Toxicologic studies on 3',4'-Dichloropropion-anilide. Toxicology and Applied Pharmacology 23(?): 650-659. (Also In unpublished submission received March 22, 1976 under 5F1606; submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:094375-A).
36089 15419 134002	Rohm & Haas Company (19??) Toxicologic Study on the Effect of Adding Stam F-34 to the Diet of Rats for a Period of Two Years. (Unpublished study received June 11, 1970 under 0F0932; CDL:091587-P).
36090 15419 132749	Ambrose, A.M.; Larson, P.S. (1964) Toxicologic Study on the Effect of Adding Stam F-34 to the Diet of Beagle Dogs for a Period of Two Years. (Unpublished study received June 11, 1970 under 0F0932; prepared by Medical College of Virginia, Department of Pharmacology, submitted by Rohm & Haas Company, Philadelphia, PA; CDL:091587-Q).
36091 15419	Borzelleca, J.F.; Ambrose, A.M.; Larson, P.S. (1966) Three Generation Reproduction Study on Rats Receiving Stam F-34 in Their Diet. (Unpublished study received June 11, 1970 under 0F0932; prepared by Medical College of Virginia, Department of Pharmacology, submitted by Rohm & Haas Company, Philadelphia, PA; CDL:091587-R).
35686	Yih, R.Y.; McRae, D.H. (1965?) Studies on Metabolism of 3',4'-Dichloropropionanilide (Stam) in Rats. (Unpublished study received June 11, 1970 under 0F0932; submitted by Rohm & Haas Company, Philadelphia, PA; CDL:091588-E).
155215	Weatherholtz, W. (1983) Twenty-four Month Dietary Oncogenicity Study in Mice: Stam Technical: Final Report: Project No. 417-400. Unpublished study prepared by Hazleton Laboratories America, Inc., 6120 p.

MRID No.	Citation
- 58588	Kam, C.; Stevens, K.R.; Gallo, M.A. (1980) Teratologic Evaluation of Stam Technical in the Albino Rat: Snell Project No. 10065-008. (Unpublished study received February 11, 1981 under 707-75; prepared by Booz, Allen & Hamilton, Inc., submitted by Rohm & Haas Company, Philadelphia, PA; CDL:224328-A; 244329; 244330; 244331).
- 58589	Florek, M.C.; Christian, M.S.; Christian, G.D.; et al. (1980) Stam Technical Teratogenicity Study in Rabbits: Argus Project 018-001; Rohm & Haas Company Study 80P-113. (Unpublished study received February 12, 1981 under 707-75; prepared by Argus Research Laboratories, Inc., submitted by Rohm & Haas Company, Philadelphia, PA; CDL:244332-A).
- WD001	O'Neill, P.J.; McLeod, P.L.; McCarthy, K.L. (1983) In Vivo Cytogenetic Study in Mice with Stam(pede) Technical; Rohm & Haas Company, Toxicology Department, Spring House, PA; Accession No. 260448.
- WD002	Kruszewski, F.H.; McCarthy, K.L.; Byers, M.J. (1984) Stam® Technical CHO/HGPRT Gene Mutation Assay; Rohm & Haas Company, Toxicology Department, Spring House, PA; Accession No. 260448.
- WD003	Shirasu, Y.; Moriya, M.; Koyashiki, R. (1980) Microbial Mutagenicity Test of DCPA Propanil; DNA Damage and Repair Rec Assay (Bacillus subtilis) and Reversion Assay (E. coli and S. typhimurium); Toxicology Division, Inst. Environmental Tox. of Japan; Accession No. 260448.
- WD004	Simmon, V.F. (1979) In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides; (1) Reverse mutation in bacteria (Ames Assay/E. coli); (2) DNA damage/repair in yeast (mitotic recombination in S. cerevisiae D3); (3) DNA Damage/Repair in Bacteria (B. subtilis/E. coli; (4) DNA damage/repair in mammalian cells (UDS in WI-38); SRI International, Menlo Park, CA; Accession No. 260448.
WD005	McLaughlin, J.E. Stam (R); A Three-Month Dietary Study in Mice; Study Number 82R-065; Rohm & Haas Toxicology Department, Spring House, PA; No Accession Number.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
3-Generation Reproduction - rat; Medical College of Virginia; Project No. (unknown) MRID Nos. 36091, 15419; 1966.	Stam F-34; Technical; 97% ai.		Reproductive NOEL = 300 ppm. Reproductive LEL = 1000 ppm; decreased body weight of parental animals during growth, mating, and weaning. Levels tested: 0 (control), 100, 300, 1000 ppm (orally in feed) in Wistar rats.		Minimum
90-Day Feeding - Rat; Medical College of Virginia; Project No. (unknown); MRID Nos. 15419, 46259; 1961.	Stam F-34; Technical; 97% ai.		Systemic NOEL = 0.033% (330 ppm). Systemic LEL = 0.10%; increased relative spleen weight in females and decreased hemoglobin in males. Levels tested: 0 (control), 0.01, 0.033, 0.10, 0.33, 1.0, and 5.0% (orally in feed); 90 days in Wistar rats.		Supplementary. Individual data data not provided.
Metabolism - Rat; Medical College of Virginia; Project No. (unknown); MRID No. 35686; 1965.	C^{14}-Stam (uniformly labeled in the ring).		Approximately 90 to 92% recovered in urine, feces, and cage wash in 2 days. Only small amounts in rat tissues. Major metabolite was 3'-4'-dichloroaniline.		Supplementary. (a) T 1/2 not determined. (b) Female rats not tested (c) Individual data not provided.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2-Year Oncogenicity - Mouse; Hazelton No. 417-400; MRID No. 155215; December 2, 1983.	Stam Technical, 98.0% purity and 85.4% purity.		Oncogenic NOEL = 180 ppm (HDT) for both 98.0% and 85.4% Stam technical. HDT may not be MTD. NOEL not yet established for bilateral retinal degeneration in males and females and thyroiditis in females with 85.4% technical. Levels tested: 0, 5[a], 30[a], 180[a], 180[b] ppm (orally in feed - 2 years); CD-1 mice. a/98.0% purity technical was used. b/85.4% purity technical was used.		Supplementary. (a) MTD issue. (b) NOEL for bilateral retinal degeneration males and females and thyroidits in females not yet established with 85.4% St m technical. (c) Additional information has been requested from registrant.
90-Day Feeding - Mouse; Rohm & Haas Toxicology Department No. 82R-065; MRID No. WD005; October 6, 1983	Stam Technical 98.0% purity.		NOEL = 25 ppm. LEL = 200 ppm; increased incidence of hepatocytic pleomorphism in females and one incident of hepatocytic multifocal necrosis in males. Levels tested: 0, 25, 200, 1600, and 12,800 ppm; 10/sex/group (orally - in feed); CD-1 mice.		Minimum.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TDX Category	(DRE Grade/ Doc. No.
Teratology - Rat; Booz, Allen and Hamilton, Inc. No. 10065-008; MRID No. 58588; February 29, 1980 .	Stam Technical; 85.4% ai		Teratogenic NOEL = 100 mg/kg/day (HDT). Maternal NOEL = 20 mg/kg/day. Maternal LEL = 100 mg/kg/day; (increase in average number of resorptions per dam). Fetotoxic NOEL = 20 mg/kg/day. Fetotoxic LEL = 100 mg/kg/day; (decreased pup weight, delayed ossification in some pups, absent sternebrae #5 and xiphesternum in some pups). Levels tested: 0 (vehicle control), 0, 8. 4.0, 20, and 100 mg/kg in Sprague-Dawley rats.		Minimum.
Teratology - Rabbit; Argus No. 018-001; MRID No. 58589; December 17, 1980.	Stam Technical; 85.4% ai		Teratogenic NOEL = 100 mg/kg/day (HDT). Maternal NOEL = 20 mg/kg/day. Maternal LEL = 100 mg/kg/day (death, decreased body weight). Fetotoxic NOEL = 20 mg/kg/day. Fetotoxic LEL = 100 mg/kg/day (unossified metacarpals). Levels tested: 0 (vehicle control), 4, 20, and 100 mg/kg/day in NZW rabbits.		Minimum.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Chronic Feeding - Dog; Medical College of Virginia; Project No. (unknown); MRID Nos. 36090, 132749, and 15419; 1964.	Stam F-34, Technical; 97% ai.		NOEL = 600 ppm. LEL = 4000 ppm (HDT); decreased body weight, decreased food consumption, increased SGOT and SAP values. Levels tested: 0 (control), 100, 600, and 4000 ppm (orally in feed - 104 weeks) in beagles; 2 dogs/sex/group. Oncogenic potential could not be determined due to inadequate histopathological examination.		Supplementary. (a) Only 2 dogs/ sex/group. (b) Few clinical chemistries. (c) Limited histopathology.
Chronic Feeding - Rat; Medical College of Virginia; Project No. (unknown) MRID Nos. 15419, 134002, and 36089; 1964.	Stam F-34 Technical; 97% ai.		Systemic NOEL = 100 ppm. Systemic LEL = 400 ppm; increased relative spleen weight in females. Levels tested: 0 (control), 100, 400, 1600 ppm (orally in feed - 2 years) in Wistar rats; 25 rats/sex/group.		Supplementary. (a) No clinical chemistries. (b) Inadequate histopathology (numerous rats not examined). (c) Only 25/ sex/group.
Mutagen.-In vivo cytogenetics - Mice; Rohm & Haas; Project No. 82R-255; MRID No. WD001; November 11, 1983.	Stam(pede) Tech. 87.8% Lot No. 4-76-416.	260448	Did not induce chromosomal aberrations in mouse bone marrow cells.	N/A	Reserved.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	GDRE Grade/ Doc. No.
Mutagen.-CHO/HGPRT Gene Mutation; Rohm & Haas; Project No. 83R-142; MRID No. WD002; January 12, 1984.	Stam(pede) Tech. 87.8% Lot No. 4-76-416	260448	Was not mutagenic under conditions of study.	N/A	Acceptable.
Mutagen.-DNA Damage + Repair (rec), B. subtilis Inst. Envir. Tox. Japan; Project No. 80RC-1006; MRID No. WD003; February 14, 1980.	Propanil, 98%	260448	Did not induce DNA damage under conditions of study.	N/A	Unacceptable.
Mutagen.-Reversion (Ames + E. coli); Inst. Envir. Tox. Japan; Project No. 80-RC-1006; MRID No. WD003; February 14, 1980.	Propanil, 98%	260448	Was not mutagenic under conditions of assays.	N/A	Acceptable.
Mutagen.-Reversion (Ames + E. coli); SRI International; EPA-600/1-79-041; MRID No. WD004; October 1979.	Stam Tech. 88% Batch No. 6-2502.	260448	Was not mutagenic under conditions of assays.	N/A	Acceptable.

Tox Chem. No. 325 (Propanil) File Last Updated _____ Current Date 07/14/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagen.-DNA Damage + Repair in Yeast (mitotic recomb. S. cerevisiae); SRI International; EPA-600/1-79-041; MRID No. WD004; October 1979.	Stam Tech. 88% Batch No. 6-2502.	260448	Negative under conditions of assay.	N/A	Acceptable.
Mutagen.-DNA Damage + Repair in B. subtilis/E. Coli; SRI International; EPA-600/1-79-041; MRID No. WD004; October 1979.	Stam Tech. 88% Batch No. 6-2502.	260448	Negative in E. coli and positive in B. subtilis.	N/A	Acceptable.
Mutagen.-DNA Damage + Repair (UDS in WI-38); SRI International EPA-600/1-79-041; MRID No. WD004; October 1979.	Stam Tech. 88% Batch No. 6-2502.	260448	Tested negatively under the conditions of the assay.	N/A	Acceptable.

Reviewed By: William Dykstra
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: 82-1, Subchronic Oral TOX Chem No.: 325

Accession Number: None MRID No.: WD005

Test Material: STAM Technical, 98.0% purity

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number: 82R-065

Sponsor: Rohm & Haas Company

Testing Facility: Rohm & Haas Toxicology Department

Title of Report: STAM®; A 3-Month Dietary Study in Mice

Author: McLaughlin, J.E.

Report Issued: March 24, 1983

Conclusions:

The NOEL is 25 ppm. At the LEL of 200 ppm, there were 3/10 incidences of hepatocytic pleomorphism in females and 1/10 incidence of hepatocytic multifocal necrosis in males. At 1600 and 12,800 ppm, there were good correlations between toxic signs (cyanosis), gross necropsy (darkened blood and organs), increased absolute and relative liver and spleen weights, and increased incidences of histological lesions in the liver and spleen of both sexes. Decreased body weight gain, increased food consumption, and decreased erythrocyte counts were also observed in both sexes at 12,800 ppm. The cyanosis and darkened blood and organs were highly suggestive of methemoglobinemia.

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

A. Test Material - RH-04,767; STAM®; propanil; 98.0% purity, TD No. 78-047; Lot No. LSPP.3-0031R; black solid.

B. Test Animals - Mouse/COBS-CD1/Charles River; Wilmington, MA; age at dosing approximately 5 1/2 weeks.

C. Experimental Design

Group	No. of Animals Male	Female	Dietary Level (ppm)	Dietary Level (mg/kg/day) 1/ Males	Females
1	10	10	0	0	0
2	10	10	25	6.6	9.5
3	10	10	200	49	78
4	10	10	1600	442	566
5	10	10	12,800	5325	6467

1/ Calculated from body weight and food consumption during study.

Date of treatment initiation: September 12, 1978.
Date of treatment completion: December 18, 1978.

Randomized groups of 10 male and 10 female CD-1 mice were fed ad libitum dietary concentrations of test material at 0, 25, 200, 1600, and 12,800 ppm for 13 weeks. Mice were observed daily and given physical examinations with measurement of body weight and food consumption weekly.

Urinalysis measurements, which included color, clarity, bilirubin, glucose, acetone, occult blood, pH, and protein were made in week 11. Hematological measurements, which included hemoglobin, packed cell volume, red cell count, total white cell count, and white cell differential and clinical chemistry parameters, which included A/G ratio, albumin, alkaline phosphatase, glucose, total protein, glutamic pyruvic transaminase, and urea nitrogen were made in week 13.

At study termination, all mice were sacrificed and necropsied, and brains, gonads, hearts, livers, kidneys, and spleens were weighed.

Liver fractions were assayed for p-nitroanisole demethylation activity.

The following tissues were examined microscopically from all mice in groups 1 and 5 only:

adrenals (2)		lymph gland (mesenteric)
bone marrow and smear		mammary gland
brain		masses
esophagus		muscle, skeletal
eyes (2)		nerve, peripheral
gonads (2)		pancreas
gross lesions (to include a border of apparently normal tissue)		pituitary
		prostate
		salivary gland
heart (with coronary vessels)		seminal vesicles
		skin
intestine		spinal cord (cervical)
colon	rectum	spleen
duodenum	cecum	stomach
jejunum	ileum	trachea
kidneys (2)		thymus
larynx		thyroid/parathyroid
liver		urinary bladder
lung		uterus

To detect internal lesions, brain, kidney, liver, and lung tissue was serially sliced, either pre- or postfixation, as appropriate.

Although all tissues were examined grossly and saved, only the heart, kidneys (2), liver, any observed gross lesion or masses, and any suspected target organs based on the clinical or gross findings were examined from middle-dose groups 2, 3, and 4.

The Student's t-test was used for statistical analysis with $p < 0.05$ being significant.

Results:

No mice died, but one male control and one male from group 5 escaped and were eliminated from the study. Cyanosis was observed in all group 5 males, all group 4 males, and three of 10 group 5 males. It appeared as a bluish-grey discoloration of the ears and skin.

Food consumption was significantly increased in both sexes at 12,800 ppm and averaged 44.1 percent greater for males and 19.3 percent greater for females.

Body weight was significantly reduced for males (-16.2%) and females (-13.6%) of the 12,800 ppm (group 5) during the study.

Clinical chemistry and urinalysis values did not show any treatment-related effects. RBC was significantly decreased in both sexes at 12,800 ppm. At 1600 ppm, absolute and relative spleen weight of both sexes and relative liver weight of females were increased significantly. At 12,800 ppm, absolute and relative spleen weight of both sexes and absolute and relative liver weight of females and relative liver weight of males were increased significantly.

Relative heart and brain weight at 12,800 ppm in females was also increased significantly whereas absolute and relative gonad weight was decreased in females at 12,800 ppm.

MFO activity (p-nitroanisole-o-demethylase assay) was significantly increased in both sexes at 1600 and 12,800 ppm. At gross necropsy observations, both sexes at 1600 and 12,800 ppm had darkened blood, spleen, liver, heart, lungs and kidneys.

Microscopic evaluation showed lesions in the spleen and liver which could be correlated with organ weights and gross necropsy findings.

Histologically, the liver showed hepatocytic pleomorphism, focal or multifocal necrosis, and pigment in von Kupffer cell cytoplasm. The incidences of these lesions are shown below.

Group	1		2		3		4		5	
Dosage (ppm)	0		25		200		1600		12,800	
	M	F	M	F	M	F	M	F	M	F
No. Examined	9	10	10	10	10	10	10	10	9	10
Liver										
Hepatocytic pleomorphism	0	1	1	1	1	3	5	7	6	6
Multifocal necrosis	0	0	0	0	1	0	1	1	2	0
Focal necrosis	0	0	0	0	0	0	0	0	0	1
Nuclear variation	2	0	1	0	0	0	0	2	3	2
Pigment, Kupffer Cells	0	0	0	0	0	0	2	0	6	5

In the spleen, there was an increase in the severity of the grade of the lesions in males and females of groups 4 and 5 (average scores 3.0-3.5) in comparison to control (0.8-1.6). The lesions observed in the spleen were hemosiderin and extramedullary hematopoiesis.

Discussion:

The NOEL is 25 ppm for both sexes in the study.

At 200 ppm, there was an incidence (3/10) of hepatocytic pleomorphism in females and one incidence of multifocal hepatocytic necrosis in males. The LEL is 200 ppm. At 1600 and 12,800 ppm, there were good correlations between toxic signs (cyanosis), gross necropsy observations (darkened blood and organs), increased liver and spleen weights, and increased incidences of histological lesions in the liver (hepatocytic pleomorphism, focal and multifocal necrosis, nuclear variation, and pigmented Kupffer cells) and the spleen (extramedullary hematopoiesis and hemosiderin). Additionally, body weight was decreased, food consumption was increased, and red blood cell counts were decreased at 12,800 ppm. The cyanosis and darkened blood and organs were highly suggestive of methemoglobinemia.

Classification: Core-Minimum.

William Dykstra

William Dykstra
Toxicology Branch
Hazardous Evaluation Division (TS-769C)

Edwin Budd 7/27/87

Edwin Budd, Section Head
Toxicology Branch
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Reviewed By: William Dykstra
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin Budd
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DATA EVALUATION REPORT

Study Type: 82-1, Subchronic Oral TOX Chem. No.: 325

Accession Number: N/A MRID No.: 46259 1549

Test Material: STAM F-34 (97% technical)

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): Not reported

Sponsor: Rohm & Haas Company

Testing Facility: Medical College of Virginia

Title of Report: Toxicological Study on the Effect of Adding Stam F-34 to the Diet of Rats for Three Months

Author(s) Not reported

Report Issued: Unpublished study received October 29, 1961

Conclusions:

All rats at the 5.0 percent dietary level died. Decreased body weight and food consumption was observed at the 0.33 and 1.0 percent level in females and the 1.0 percent level in males. At all dietary levels in females and at the 0.10 and 1.0 percent level in males, there were increases in neutrophils. Hemoglobin was decreased at 0.33 and 1.0 percent in females and 0.10, 0.33, and 1.0 percent in males. Increased relative spleen weight was observed in females at 0.10, 0.33, and 1.0 percent levels and in males at 0.33 and 1.0 percent. There were no compound/related histological findings. The NOEL is 0.033 percent (330 ppm) dietary level. The LEL is 0.10 percent (1000 ppm) dietary level and the effects are increased relative spleen weight in females and decreased hemoglobin in males.

Classification:

Core-Supplementary. Individual data not provided.

Special Review Criteria (40 CFR 154.7): N/A

Review:

Test Material

STAM F-34 technical containing 97% 3',4'-dichloropropionanilide, [redacted] It is a dark brown crystalline material with a slight aromatic odor.

Randomized groups of 10 young male and 10 young female Wistar albino rats were fed ad libitum dietary concentrations of 0, 0.01, 0.033, 0.10, 0.33, 1.0, and 5.0 percent of test material for 3 months. The rats were individually caged and were weighed once a week. Food consumption was measured over a 3-day period during the thirteenth week. Blood studies were made on five rats/sex/group at the end of the third month. Urine samples, pooled from five rats/sex/group, were collected toward the end of the third month and tested semiquantitatively for sugar (Morris Anthrone Method) and protein (Pro-Teen, Sulfosalicylic Acid, and Shevky and Stafford Methods). Organ to body weight ratios for liver, kidney, heart, spleen, and testes were determined at sacrifice of 3-month survivors. Histopathologic studies were made on the following tissues: heart, lung, liver, kidney, spleen, gastroenteric, bladder, bone marrow (rib, femur, and vertebrae), muscle, skin, brain, thyroid, adrenal, and pancreas. Animals examined included all survivors from 0, 0.033, 0.10, 0.33, and 1.0 percent diets and a few from the 5.0 percent diets.

Results:

All rats died at the 5.0 percent dietary level during the first 3 weeks. These deaths are considered compound-related. Three other rats that died were not considered due to treatment. These rats were the following: one female at the 0.33 percent level in the twelfth week, one male at the 0.01 percent level in the sixth week, and one male at the 1.0 percent level in the eleventh week.

Body weight was decreased for females at 0.33 and 1.0 percent and for males at 1.0 percent during the study. Food consumption data from the thirteenth week showed similar decreases in females at 0.33 and 1.0 percent and males at 1.0 percent. These decreased findings in body weight and food consumption are considered compound-related.

At all dietary levels in females and at 0.10 and 1.0 percent in males there were increases in neutrophils (polychromatocytes). Hemoglobin was decreased at 0.33 and 1.0 percent in females and 0.10, 0.33, and 1.0 percent in males. The report states that the peripheral blood showed appearance of immature forms of red cells. The following table from the study report shows the hematology results.

Average of Hematologic Data Obtained on Rats
Receiving STAM F-34 in Their Diet for 3 Months[1]

Sex	Diet Conc. (%)	Hemato-crit	Hb g/100 mL	WBC x 10^3	Differential WC Count (%)				
					Poly	Lymph	Mono	Eos	Baso
Female	0	49.6	15.5	14.5	12	83	3	2	0
	0.01	54.0	15.5	18.5	16	77	3	4	0
	0.033	47.8	13.9	18.1	18	74	3	5	0
	0.1	52.2	14.3	12.7	14	81	1	4	0
	0.33	50.4	13.5	19.4	15	80	2	3	0
	1.0	45.2	12.7	18.5	15	80	4	1	0
Male	0	52.0	15.6	16.0	19	76	2	3	0
	0.01	49.6	15.3	16.2	18	78	2	2	0
	0.033	51.2	14.3	21.1	19	76	4	1	0
	0.1	51.2	12.6	17.7	23	72	2	3	0
	0.33	47.2	13.3	21.8	19	76	2	3	0
	1.0	46.8	12.7	24.0	20	72	7	1	0

[1]Table III (page 6) of study report.

Urinalyses did not show any treatment-related effects.

Evaluation of relative organ weights showed significantly compound-related increased spleen weight in females at 0.10, 0.33, and 1.0 percent and in males at 0.33 and 1.0 percent. These increased relative spleen weights correlated with the hemolytic anemia observations in the hematology data.

Additionally, the following relative organ weights were significantly increased: The heart in males and females at 1.0 percent, the liver in females at 1.0 percent, and the testes in males at 1.0 percent.

The following table from the study report shows relative organ weights.

Organ to Body Weight Ratio Data for Rats Receiving STAM F-34 in Their Diet for 3 Months[1]

Sex	Diet Conc. (%)	No. Rats	Organ/Body Weight x 10^{-3}				
			Heart	Spleen	Kidney	Liver	Testes
Female	0	10	2.77 ± 0.31	1.67 ± 0.38	6.32 ± 0.65	30.7 ± 4.5	
	0.01	10	2.80 ± 0.41	1.77 ± 0.34	6.78 ± 0.56	34.9 ± 4.3	
	0.033	10	2.77 ± 0.24	1.91 ± 0.20	7.05 ± 0.66	35.6 ± 3.4	
	0.1	10	2.94 ± 0.27	2.05 ± 0.27	6.49 ± 0.65	33.3 ± 5.1	
	0.33	9	2.95 ± 0.42	3.16 ± 0.46	7.26 ± 0.57	36.2 ± 5.2	
	1.0	10	3.18 ± 0.48	4.10 ± 0.94	6.79 ± 0.61	46.5 ± 4.8	
Male	0	10	2.49 ± 0.22	1.53 ± 0.85	6.61 ± 0.72	35.7 ± 4.8	8.26 ± 1.20
	0.01	9	2.43 ± 0.23	1.31 ± 0.22	6.33 ± 0.36	31.0 ± 3.2	7.88 ± 0.92
	0.033	10	2.52 ± 0.16	1.39 ± 0.24	7.10 ± 0.30	36.8 ± 2.6	7.72 ± 0.94
	0.1	10	2.67 ± 0.34	1.58 ± 0.22	6.56 ± 0.52	36.5 ± 6.2	8.79 ± 1.31
	0.33	10	2.59 ± 0.42	2.64 ± 0.60	6.65 ± 0.54	37.1 ± 5.7	9.84 ± 0.63
	1.0	9	2.88 ± 0.47	4.04 ± 0.77	7.27 ± 0.77	39.3 ± 4.9	14.01 ± 2.33

[1]Table IV (page 7) from study report.

There were no compound-related histological findings.

Conclusion:

The NOEL is 0.033 percent (330 ppm). The LEL is 0.10 percent (1000 ppm), and the effects are increased relative spleen weight in females and decreased hemoglobin in males.

Classification:

Core-Supplementary. Individual data were not provided.

William Dykstra
Section II, Reviewer
Toxicology Branch

Edwin Budd 7/21/87
Edwin Budd, Section Head
Review Section II
Toxicology Branch

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Reviewed By: William Dykstra
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: 83-1, Chronic Toxicity, Nonrodent

TOX Chem. No.: 325

MRID No.: 36090, 132749, 15419

Accession Number: N/A

Test Material: STAM F-34; technical, 97% ai

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): Not Reported

Sponsor: Rohm & Haas Company

Testing Facility: Medical College of Virginia

Title of Report: Toxicologic Study of the Effect of Adding Stam F-34 to the Diet of Beagle Dogs for a Period of Two Years

Authors: Ambrose, A.M.; P.S. Larson

Report Issued: (1964); Unpublished study received June 11, 1970 under 0F0932

Conclusions:

There were no deaths. Compound-related decreases in body weight and food consumption were observed at 4000 ppm. No compound-related effects were observed in hematology, urinalyses, organ weights, organ-to-body weight ratios, and histopathology. The NOEL is 600 ppm (mid-dose). The LEL is 4000 ppm (HDT) and the effects are decreased body weight, decreased food consumption, and increased SGOT and SAP values.

Classification:

Core-Supplementary.

a. Only 2 dogs/sex/group were used.
b. Few clinical chemistries.
c. Limited histopathology.

Special Review Criteria (40 CFR 154.7): N/A.

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INERT INGREDIENTS IS NOT INCLUDED.

Review:

1. Test Material: STAM F-34; technical grade; 97% 3'4'-dichloropropionanilide, [redacted] It is a dark brown crystalline material with a slight aromatic odor.

2. Experimental: [Copied from study report (pages 1-2).]

"Two male and two female purebred beagle dogs of about six months of age were placed on each of the following dietary levels of Stam F-34: 0, 100, 600, and 3000 ppm; the 3000 ppm diet was raised to 4000 ppm at the start of the fifth week. The stock diet used consisted of 87% Foundation Diet (Hill Packing Company), 12% Mazola Oil and 1% Cod Liver Oil, mixed fresh daily. To the Foundation Diet was added, with thorough mixing, an amount of Stam F-34 calculated to result in the 3000 ppm concentration (raised to 4000 ppm at start of fifth week) in the completed formulation and the lower concentrations were made by serial dilution with Foundation Diet. At feeding, the food was moistened with an equal weight of water."

"Prior to being placed on these diets, the dogs were immunized against distemper, hepatitis and leptospirosis and treated for intestinal parasites."

"Food consumption was measured daily and the dogs were weighed once a week."

"Hematologic values (hematocrit, hemoglobin, total white cell and differential white cell) were obtained prior to placing the dogs on diet and at three-month intervals while on diet. Urine tests for concentrations of reducing substances (Benedict and Uristix methods) were made at the same time intervals."

"Liver function tests (bromsulphalein, serum glutamate-oxalacetate transaminase and serum alkaline phosphatase) were made during the twenty-fourth month of the study."

"Organ to body weight ratio data were determined at sacrifice for heart, liver, kidney, spleen, and testes. Tissues preserved and studied microscopically were: heart, lung, liver, kidney, spleen, gastroenteric, urinary bladder, skin, skeletal muscle, mesenteric lymph nodes, bone marrow, brain, pituitary, thyroid, pancreas, adrenal and gonad." [End of quotation.]

Results:

There were no deaths.

Body weight and food consumption were decreased at 4000 ppm during the study. The following tables present body weight and food consumption data at representative intervals.

Average Body Weight (kg)
Weeks

Dose (ppm)	No. of Dogs	0	1	3	6	13	26	52	78	104
0	4	7.7	7.4	7.8	8.5	10.1	11.4	13.5	14.2	15.5
100	4	7.8	7.6	8.5	9.0	10.5	11.9	13.2	14.6	15.0
600	4	7.8	7.6	8.0	9.2	10.7	11.6	12.7	13.9	14.5
4000	4	7.9	7.6	7.7	8.0	7.8	9.6	9.9	11.0	11.5

Cumulative Food Consumption (kg)
Weeks (*Averages)

Dose (ppm)	No. of Dogs	13	26	39	52	65	78	91	104
0	4	31.8*	67.8	104.2	140.6	176.9	213.4	249.8	286.2
100	4	32.9	69.0	105.4	141.8	178.2	214.6	251.0	287.4
600	4	33.2	69.1	105.5	141.9	178.3	214.7	251.1	287.5
4000	4	25.6	58.7	95.1	131.5	167.9	204.3	240.7	277.0

Hematologic values were comparable between control and treated groups at each sampling interval. Liver function tests (SGOT and SAP) were increased at 4000 ppm at 24 months. At termination slightly decreased absolute heart and liver weights were found in dogs at the 4000 ppm dosage level, although these effects were not dose-related. Organ-to-body weight ratios indicated slight dose-related increased relative kidney weights.

In light of the hematological, clinical data, and histologic findings these slight organ weight changes are not considered toxicologically significant. Histopathological findings did not reveal any compound-related effects.

Conclusion:

There were no deaths. Compound-related decreases in body weight and food consumption were observed at 4000 ppm. No compound-related effects were observed in hematology, organ weights, organ-to-body weight ratios, and histopathology. The NOEL is 600 ppm (the mid-dose). The LEL is 4000 ppm (HDT) and the effects are decreased body weight, decreased food consumption, and increased SGOT and SAP values.

Classification:

Core-Supplementary; only 2 dogs/sex/group were used; few clinical chemistries were done; there was limited histopathology.

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DATA EVALUATION REPORT

Study Type: 83-1 - Chronic Toxicity, Rodent TOX Chem. No.: 325

Accession Number: N/A MRID Nos.: 15419, 134002, 36089

Test Material: STAM F-34 (Technical, 97% ai)

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): Not reported

Sponsor: Rohm & Haas Company

Testing Facility: Medical College of Virginia

Title of Report: Toxicologic Study on the Effect of Adding STAM F-34 to the Diet of Rats for a Period of Two Years

Authors: Ambrose, A.M.; Larson, P.S.; Borzelleca, J.F.; Hennigar, G.R.

Report Issued: Unpublished study received June 11, 1970 under 0F0932 and published study, Toxicology and Applied Pharmacology 23, 650-659 (1972).

Conclusions:

The oncogenic potential could not be determined at the MTD of 1600 ppm due to inadequate histological examination of tissues in both sexes of rats. Increased mortality in male rats at 1600 ppm was observed only after 20 months. Both male and female rats at 1600 ppm had significantly reduced body weight during the 2-year period. Food consumption was comparable between groups for both sexes. Hemoglobin and hematocrit values were decreased in female rats at 1600 ppm at 3, 6, 12, and 24 months. There was increased relative spleen weight in both sexes at 1600 ppm and in females at 400 ppm, liver weight in females, and testes weight in males at 1600 ppm. There were no compound-related histologic effects. The NOEL is considered to be 100 ppm (the low dose). The LEL is 400 ppm and the effect is increased relative spleen weight in females.

Classification:

Core-Supplementary, because:

1. Clinical chemistries not performed;
2. Inadequate histopathological examination (numerous rats not examined); and
3. Only 25 rats/sex/group were used.

Special Review Criteria (40 CFR 154.7): N/A.

Review:

Test Material

STAM F-34, technical grade; 97%, 3',4'-dichloropropionanilide, [redacted]

Randomized groups of 25 Wistar-derived young albino male and female rats were administered 0, 100, 400, or 1600 ppm of test material in the diet ad libitum for 2 years. Finely ground Purina Laboratory Chow served as the basic diet. The rats were individually caged and were weighed once a week. Food consumption data were obtained over a 3-day period at the end of 1, 3, 6, 12, and 24 months. Hematologic determinations (hematocrit, hemoglobin, total white, and differential white cell counts) and tests for urine concentrations of reducing substances (Morris-Anthrone or Benedict's method) and protein (Shevky and Stafford method) were performed at 3-month intervals.

Organ weights for heart, liver, kidney, spleen, and testes were obtained at sacrifice of the 2-year survivors. Tissues preserved for histopathologic study on animals sacrificed, and those dying in which little autolysis had set in were: heart, lung, liver, spleen, gastroenteric, kidney, bladder, thyroid, adrenal, pancreas, gonad, pituitary, skeletal muscle, skin, bone marrow, and brain.

Results:

Body weight decreases were significant during the entire study for male and female rats at 1600 ppm. Increased mortality occurred after 20 months in males of the 1600 ppm group. The following table obtained from the study shows these data:

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BODY WEIGHT AND SURVIVAL DATA ON RATS RECEIVING 3',4'-DICHLOROPROPIONANILIDE IN THEIR DIET FOR 2 YEARS[1]

Sex	Diet Conc. (ppm)	Average Body Weight (g ± SD)									No. of Survivors
		0 Wk	1 Wk	3 Wk	6 Wk	13 Wk	16 Wk	52 Wk	78 Wk	104 Wk	
Female	0	78	120 ± 10	158 ± 8	199 ± 16	238 ± 17	284 ± 29	306 ± 40	344 ± 41	356 ± 58	15
	100	78	118	156	200	240	280	300	323	351	10
	400	78	118	153	195	231	271	292	320	329	12
	1600	78	107 ± 12[a]	141 ± 9[a]	173 ± 15[a]	207 ± 14[a]	241 ± 11[a]	261 ± 13[a]	274 ± 37[a]	292 ± 24[a]	12
Male	0	88	143 ± 12	221 ± 17	317 ± 26	422 ± 40	486 ± 51	539 ± 64	578 ± 71	597 ± 72	17
	100	88	145	222	324	429	493	530	566	576	15
	400	88	141	219	318	427	491	520	520	542[a]	11
	1600	88	124 ± 13[a]	197 ± 17[a]	278 ± 11[a]	365 ± 55[a]	427 ± 51[a]	464 ± 54[a]	470 ± 68[a]	441 ± 87[a]	5[b]

[1]Table I (page 4) of study report (with minor modifications).
[a]Value differs significantly from control, $p < 0.05$.
[b]Increased mortality did not appear until after 20 months.

Food consumption data were comparable between treated and control groups of both sexes.

Decreased hematocrit and hemoglobin values were observed for female rats at 1600 ppm in comparison to controls at 3, 6, 12, and 24 months. Urinalyses evaluation for protein and reducing substances showed no effects peculiar to treatment.

Organ-to-body weight ratio data showed dose-related increases which were significant for spleen weight in both sexes at 1600 ppm and in females at 400 ppm. Relative liver weight in females and testes weight in males at 1600 ppm were also increased. These data are shown below in a table obtained from the study.

AVERAGES OF ORGAN-TO-BODY WEIGHT RATIO DATA OF RATS RECEIVING 3',4'-DICHLOROPROPIONANILIDE IN THEIR DIET FOR 2 YEARS[1]

Sex	Diet Conc. (ppm)	No. of Rats	Ratio of Organ-to-Body Weight (g/kg + SD)				
			Heart	Spleen	Kidney	Liver	Testes
Female	0	15	3.2 ± 0.4	1.8 ± 0.3	8.1 ± 1.6	32.2 ± 4.5	
	100	10	3.2 ± 0.4	2.2 ± 0.6	8.0 ± 1.4	34.1 ± 4.3	
	400	12	3.4 ± 0.5	2.3 ± 0.7[a]	7.5 ± 1.5	32.1 ± 6.5	
	1600	12	3.4 ± 0.5	3.3 ± 0.8[a]	8.3 ± 1.3	40.5 ± 8.6[a]	
Male	0	17	2.6 ± 0.3	1.7 ± 0.5	6.9 ± 1.3	30.9 ± 6.8	6.1 ± 1.4
	100	15	2.7 ± 0.3	1.9 ± 0.7	7.2 ± 0.9	33.0 ± 7.5	6.2 ± 0.9
	400	11	2.6 ± 0.3	2.0 ± 0.6	7.5 ± 0.9	34.7 ± 5.7	6.5 ± 1.2
	1600	5	3.1 ± 0.6	2.4 ± 0.3[a]	7.5 ± 0.9	32.1 ± 5.0	8.5 ± 1.4[a]

[1]Table IV (page 81) of study report (with minor modifications).
[a]Value differs significantly from control, $p < 0.05$.

Histologic evaluation of tissues showed no compound-related lesions.

However, the following data were noted: (1) at the control level (0 ppm), only 15 female rats that were sacrificed at 104 weeks and 1 female rat that died at 92 weeks were examined (out of 25 rats); therefore 9 female control rats were not examined, and (2) at 400 ppm, only 13 female rats that were sacrificed at 104 weeks and 2 female rats that died at 96 weeks (animal 20) or 102 weeks (animal 25) were examined (out of 25 rats). Therefore, 10 of the female rats at 400 ppm were not examined.

No explanation for the unexamined rats is presented in the report. These unexamined rats may be due to excessive autolysis, since the report states that "those dying in which little autolysis had set in" were examined.

The following table illustrates the numbers of unexamined rats in the study:

ppm	No. Rats Initiated	No. Rats Examined for Entire Study	No. Rats Not Examined
		Females	
0	25	16	9
400	25	15	10
1600	25	12	13
		Males	
0	25	18	7
400	25	12	13
1600	25	8	17

Rats at 100 ppm were not examined histologically.

Conclusion:

The oncogenic potential could not be determined at the MTD of 1600 ppm due to inadequate histological examination of tissues in both sexes of rats. Increased mortality in male rats at 1600 ppm was observed only after 20 months. Both male and female rats at 1600 ppm had significantly reduced body weights during the 2-year period. Food consumption was comparable between control and treated groups of both sexes. Hemoglobin and hematocrit values were decreased in female rats at 1600 ppm at 3, 6, 12, and 24 months. Organ-to-body weight ratio data showed dose-related increased spleen weights which were significant at 1600 ppm for both sexes and in females at 400 ppm. Relative liver weight in females and testes weight in males at 1600 ppm were increased. There were no compound-related histologic effects. The NOEL is considered to be 100 ppm (the low dose). The LEL is 400 ppm and the effect is increased relative spleen weight in females.

Classification:

Core-Supplementary, because:

1. Clinical chemistries were not performed.
2. Only 25 rats/sex/group were used.
3. Inadequate histopathological examination (numerous rats were not examined).

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DATA EVALUATION REPORT

Study Type: 83-2--Oncogenicity TOX Chem. No.: 325

Accession No.: N/A MRID No.: 155215

Test Material: STAM Technical, 98.0% and 85.4% purity

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study No.(s): 417-400

Sponsor: Rohm & Haas Company

Testing Facility: Hazleton Laboratories America, Inc.

Title of Report: 24-Month Dietary Oncogenicity Study in Mice

Author(s): Weatherholtz, W.

Report Issued: December 2, 1983

Conclusions:

The oncogenic potential was negative at 180 ppm (HDT), but Toxicology Branch has serious reservations as to whether or not a maximum tolerated dose (MTD) was used in this study.

There were no compound-related effects on survival, clinical observations, tissue masses, body weight, food consumption, hematology, and organ weights.

Dose-related histologic findings were observed in the male liver as centrilobular hepatocytic enlargement beginning by week 15 and continuing for the 104-week study. Similar lesions were not observed in the female liver.

The LEL for this effect in males was 180 ppm (HDT) for both the 98.0% purity STAM Technical and the 85.4% STAM Technical. Although 85.4% STAM Technical was not tested below 180 ppm, the results produced for both technicals are toxicologically comparable and lower levels of the 85.4% purity STAM Technical are not required to be evaluated for this lesion. The NOEL was 30 ppm for this lesion.

Bilateral retinal degeneration in male and female mice and thyroiditis in female mice were observed at 180 ppm (85.4% purity, STAM Technical). NOELs for these effects were not established in this study for the 85.4% technical.

Classification: Core-Supplementary.

a. The registrant is required to justify the highest dose tested (180 ppm) as the MTD.

b. NOEL not established for bilateral retinal degeneration in males and females and thyroiditis in females (for the 85.4% technical).

Special Review Criteria (40 CFR 154.7)

The presence of thyroiditis in female mice and bilateral retinal degeneration in male and female mice mice at 180 ppm ai (85.4% purity STAM Technical) may exceed Special Review Criteria. NOELs for these lesions were not established (since lower dose levels of 85.4% STAM Technical were not tested in this study). We have requested historical control data from the registrant for these lesions. We have also requested from the registrant a detailed explanation of the chemical differences between the 98.0 % and 85.4% technicals.

Review:

A. Materials:

1. Test Compound

a. STAM Technical; 98.0% purity; Lot No. LSPP3-0031R, TD Nos. 78-47 and 81-536A; crystalline black solid.

b. STAM Technical; 85.4% purity; Lot No. 9287, TD Nos. 79-333 and 81-519; crystalline black solid.

2. Test Animals

a. Species and strain: CD-1 mice.

b. Age: Approximately 6 weeks old at initiation of study.

c. Weights: Body weight ranged from 17.2 to 35.5 g for the males and from 14.3 to 24.9 g for the females.

d. Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

1. Randomized CD-1 mice were assigned to the following groups:

Group	No. of Animals Male	Female	Dietary Level (ppm)[a/]
1. Vehicle control[b/]	66	66	0
2. Vehicle control[b/]	66	66	0
3. STAM Technical 98.0%	80	80	5
4. STAM Technical 98.0%	80	80	30
5. STAM Technical 98.0%	80	80	180
6. STAM Technical 85.4%	80	80	180

[a/]Concentration of active ingredient (ai).
[b/]Acetone (vehicle) was added to the diet in amount equal to the high-dose level.

Ten mice per sex per group were sacrificed at 14 weeks and at 53 weeks (scheduled interim sacrifices).

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weekly intervals.

Results - Residues of propanil in all analyzed feed samples were comparable to intended levels.

3. Animals received food (Purina Rodent Laboratory Chow®) and water ad libitum for the duration of the study (104 weeks).

4. Statistics - The following procedures were utilized in analyzing the numerical data:

"Absolute body weights, growth rates (Rao's growth parameters), a total food consumption, clinical pathology data (excepy leukocyte differentials and erythrocyte morphology) and organ weight data of the control groups (separate and combined) were compared statistically against the da.a of the compound-treated groups of the same sex with $p < 0.05$ being significant.

"Cumulative survival through week 104 was analyzed using the National Cancer Institute Package.

"Trend analysis of survival through week 104 was evaluated at $p < 0.05$ by one-tailed probability level, while control versus compound-related group comparisions of survival were evaluated at $p < 0.05$ by two-tailed level.

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"Trend analysis using Cockran-Armitage test for linear trend and Fisher's exact test were performed on selected histopathological findings at $p < 0.05$ by one-tailed level.

"Statistically significant differences at $p < 0.05$ are designated as follows:

S+ = Significantly higher than group 1 control.
s+ = Significantly higher than group 2 control.
S+s+ = Significantly higher than combined controls.
S- = Significantly lower than group 1 control.
s- = Significantly lower than group 2 control.
S-s- = Significantly lower than combined controls."

5. Quality assurance inspections were performed.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality.

Results

a. Toxicity - There were no compound-related clinical signs observed in any group of either sex. Hunched posture, thinness, urine stains, alopecia, rough hair coat, sores, squinted eyes, lacrimation, and swellings were noted with the most frequency in male and female mice in all groups.

b. Mortality - There were no compound-related effects in mortality between control and treated groups for either sex.

Summaries of survival for males and females as presented in the report are shown below:

	Males					
Group	1	2	3	4	5	6
ppm ai	0	0	5	30	180	180
Purity (%)	--	--	98.0	98.0	98.0	85.4
No. on test	66	66	80	80	80	80
Interim sac. week 14	10	10	10	10	10	10
Interim sac. week 53	10	10	10	10	10	10
No. found dead or sacrificed moribund	29	28	33	34	34	31
Terminal sac. week 105	15	16	22	21	25	28
Percent adjusted survival to terminal sacrifice (%)	34	36	40	38	42	47

	Females					
Group	1	2	3	4	5	6
ppm ai	0	0	5	30	180	180
Purity (%)	--	--	98.0	98.0	98.0	85.4
No. on test	66	66	80	80	80	80
Interm sac. week 14	10	10	10	10	10	10
Interim sac. week 53	10	10	10	10	10	10
No. found dead or sacrificed moribund	24	30	35	33	31	37
Terminal sac. week 105	20	16	24	26	28	23
Percent adjusted survival to terminal sacrifice (%)	45	35	40	44	47	38

2. Body Weight - Animals were weighed at study initiation, weekly through week 12, and biweekly for the remainder of the study.

   Results - There were no significant differences in mean body weight between male and female control and treated groups.

3. Food Consumption and Compound Intake - Food consumption values were recorded at study initiation, weekly through week 12, and biweekly for the remainer of the study. Compound intake was calculated from the consumption and body weight data.

   Results - Food consumption was slightly increased for treated males in comparison to controls. However, the increases did not exceed 6 percent. Food consumption in group 4 females also slightly exceeded control values during the first 52 weeks of the study.

   Mean compound consumption values for the study as presented in the report are shown below:

	Males			
Group	3	4	5	6
ppm ai	5	30	180	180
Purity (%)	98.0	98.0	98.0	85.4
Mean (mg/kg/day)	0.71	4.39	26.06	26.15
S.D.	0.082	0.434	2.667	2.387

	Females			
Group	3	4	5	6
ppm ai	5	30	180	180
Purity (%)	98.0	98.0	98.0	85.4
Mean (mg/kg/day)	0.88	5.35	32.41	31.50
S.D.	0.137	0.764	4.293	0.767

4. Ophchalmological examinations were not performed on the animals.

5. Blood was collected on 10 mice/sex/group selected for sacrifice at weeks 14, 53, and 105. The following parameters were examined:

   - Hematocrit (HCT), hemoglobin (HGB), erythrocyte count (RBC), leukocyte count (WBC), platelet count, leukocyte differential, erythrocyte morphology, and methemoglobin (MET HGB). All animals were fasted prior to sample collection.

   Results - Mean hematology and methemoglobin values were comparable between control and treated groups of both sexes. Although a trend of lowered segmented neutrophils and increased lymphocytes was observed for treated male groups at weeks 14, 53, and 105, these differences were not statistically significant or dose-related and were not considered compound-related.

6. Clinical chemistry analyses and urinalyses were not performed.

   However, mean liver p-nitroanisole demethylase activity values at weeks 53 and 105 showed no compound-related findings.

7. Sacrifice and Pathology - Ten mice/sex/group were randomly selected for sacrifice during weeks 14 and 53, and all surviving animals were sacrificed after 104 weeks of treatment (during week 105). Gross necropsies were performed and findings recorded on all mice at the scheduled sacrifice intervals, and on any animals which died or were sacrificed moribund during the study.

   The following organs from each sacrificed mouse were weighed and organ/body weight ratios were determined prior to fixation: brain, heart, kidneys, liver, spleen, and testes with epididymides; postfixation: ovaries.

   The following tissues from each necropsied mouse were preserved in 10% neutral buffered formalin:

   Adrenals (2)
   Bone with marrow and bone marrow smear
   Brain (3 sections to include frontal cortex and basal ganglia, parietal cortex and thalamus,
   Gross lesions (to include a border of apparently normal tissue)
   Heart (with coronary vessels)
   Intestine
   Colon; rectum;

(cont'd)

- cerebellum, and pons)
- Esophagus
- Eyes (2)
- Gallbladder
- Gonads
- Liver (2 lobes, median and left lateral; 1 section each)
- Lung (representative sections of right and left lung; 1 section each)
- Lymph gland (mesenteric)
- Mammary gland
- Masses
- Muscle, skeletal
- Nerve, peripheral
- Pancreas
- Pituitary
- cecum
- Duodenum
- Ileum
- Jejunum
- Kidneys (2)
- Larynx
- Prostate
- Salivary gland
- Seminal vesicles
- Skin
- Spinal cord (mid-cervical and thoracolumbar area)
- Spleen
- Stomach (body, pylorus)
- Trachea
- Thymus
- Thyroid/parathyroid
- Urinary bladder
- Uterus

The carcass of each animal was also preserved.

Histopathology - All of the preserved tissues from the groups 1 (0 ppm ai), 2 (0 ppm ai), 5 (180 ppm ai, 98.0% purity), and 6 (180 ppm ai, 85.4% purity) animals sacrificed at week 53 and at study termination (week 105) were embedded in paraplast, sectioned, stained with hematoxylin and eosin, and examined microscopically.

The tissues listed below were processed and examined microscopically from animals in both control groups, and groups 5 and 6 that were found dead or sacrificed moribund during the study, and from all animals in groups 3 (5 ppm ai) and 4 (30 ppm ai) (excluding animals sacrificed during week 14).

- Adrenals (2)
- Bone with marrow
- Gallbladder
- Brain (3 sections to include frontal cortex and basal ganglia, parietal cortex and the thalamus, cerebellum and pons)
- Gonads (2)
- Kidneys (2)
- Liver (2 lobes, median and left lateral; 1 section each)
- Lymph gland (mesenteric)
- Pancreas
- Pituitary
- Spleen
- Thyroid/parathyroid

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(cont'd)

Gross lesions (to include a border of apparently normal tissue)
Heart

Tissue masses
Lungs (representative sections of right and left lung; 1 section each)

Only the liver and spleen from all mice sacrificed at week 14 were processed and examined microscopically.

Results

a. Organ Weights - Statistical analysis of the week 14 organ weight data showed significantly lower absolute liver weights in male control group 2 and in treated male groups 3, 4, 5, and 6 when compared to control group 1. In addition, significantly lower liver weights were observed in treated male groups 4 and 6 when compared to combined controls. Mean relative liver weights were significantly lower in male groups 3, 4, 5, and 6 when compared to control group 1, and significantly lower in male group 6 when compared to combined controls. The mean absolute spleen weights of male groups 4 and 6 were significantly lower than those for control group 1, and the relative weight of the group 4 males was significantly lower than the control group 2 males. Mean absolute and relative spleen weights of male group 4 were significantly lower when compared to combined controls.

The terminal body weight and organ weight values between the two male control groups are shown below:

Males (g) Week 14

Control Groups	Body	Brain	Heart	Liver	Spleen	Kidney	Testes
1	38.53	.4580	.227	2.180	.106	.692	.388
2	35.83	.4570	.195	1.849	.113	.637	.396

Due to the variability in body and organ weight values between the two male control groups, compound-related effects in absolute and relative organ weights of the treated male groups could not be established.

Evaluation of the female organ weight data at week 14 revealed no compound-related findings.

Evaluation of the male and female organ weight data at weeks 53 and 105 revealed no compound-related findings.

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b. Gross Pathology - Gross pathology examinations of 10 mice/sex/group at weeks 14 and 53 did not reveal any compound-related findings. Gross pathology examination of mice sacrificed at termination (week 105) showed no compound-related findings in males or females. However, the occurrence of liver masses at 105 weeks in both control and treated mice of both sexes showed an unusually high incidence as shown below:

	Males					
Group	1	2	3	4	5	6
No. examined	15	16	22	21	25	28
Liver masses	13	7	4	9	7	8
Percent (%)	87	44	18	43	28	29

	Females					
Group	1	2	3	4	5	6
No. examined	20	16	24	26	28	23
Liver masses	8	4	2	5	7	5
Percent (%)	40	25	8	19	25	22

D. Microscopic Pathology:

1. Non-neoplastic - Histopathological examination of liver and spleen in males and females sacrificed at week 14 did not reveal any compound-related lesions.

   Histopathologic evaluation of tissues of males and females sacrificed at week 53 showed centrilobular hepatocytic enlargement in male mice from each group but was slightly increased in incidence and severity in group 5 (180 ppm ai, 98.0% purity) animals.

   The summary incidence of this finding in male mice is shown below.

	Liver (Males) Week 53					
Group	1	2	3	4	5	6
No. examined	10	10	10	10	10	10
Centrilobular hepatocytic enlargement	5	5	6	3	8	5

The hepatic enlargement had a grade of moderate in three of eight group 5 males and in one control male. The remaining control animals had a minimal to slight grade for this lesion.

These findings suggest a marginal compound-related effect. Other non-neoplastic lesions observed in both sexes of mice at week 53 were unrelated to treatment.

There was good correlation between individual gross observations and corresponding microscopic diagnoses for animals sacrificed at termination, found dead, or sacrificed moribund.

There were three non-neoplastic histopathological lesions with a relationship to treatment. These were centrilobular hepatocytic enlargement in males, bilateral retinal degeneration in males, and thyroiditis in females.

For centrilobular hepatocytic enlargement the following distribution was observed as reported:

Centrilobular Hepatocytic Enlargement
(Animals Sacrificed at Weeks 53 and 105;
Deaths and Moribund Sacrifices Weeks 15-105)

	Males					
Group	1	2	3	4	5	6
Total examined	53	52	64	65	68	68
Not affected	41	43	49	49	41	41
Slight	8	3	10	8	8	16
Moderate	4	5	4	7	9	11
Moderately severe	0	1	1	1	4	0
Total affected	12	9	15	16	21	27 S+, s+, S+s+
Total percent affected	23	17	23	25	31	40

A dose-related increase in this lesion in males with a significant pairwise-comparison for group 6 is present. It can be seen from the total percent affected that a NOEL appears to be established in group 4 (30 ppm). Although the 85.4% purity STAM Technical was not tested below 180 ppm, the results produced in groups 5 and 6 are comparable and lower levels of the 85.4% purity technical are not required to be evaluated for this lesion.

With respect to bilateral retinal degeneration, the effect in group 6 males is considered compound-related.

For animals sacrificed at weeks 14, 53, and 105, together with deaths and moribund sacrifices, the following incidences were reported in the addendum to the final report (February 12, 1985).

	Males					
Group:	1	2	3	4	5	6
Slight	0	1	0	0	1	1
Moderate	2	2	5	1	3	2
Moderately severe	0	0	1	0	0	5 S+,S+s+
Severe	0	0	0	0	0	0
Total affected	2	3	6	1	4	8
Number examined	64	63	75	75	79	79
Retina: cannot be examined	9	5	7	10	5	8
Adjusted total examined	55	58	68	65	74	71
% Affected	4%	5%	9%	2%	5%	11%

	Females					
Group:	1	2	3	4	5	6
Slight	0	1	0	1	0	0
Moderate	0	1	3	1	1	4
Moderately severe	0	0	4 S+s+	1	0	3
Severe	0	0	0	1	0	0
Total affected	0	2	7 S+,S+s+	4	1	7 S+,S+s+
Number examined	63	66	80	78	79	79
Retina: cannot be examined	6	6	4	4	3	7
Other adjustments*	0	0	0	0	0	1
Adjusted total examined	57	60	76	74	76	71
% Affected	0%	3%	9%	5%	1%	10%

*Animal A87975.

The incidences and grades of these lesions in males and females of group 6 (85.4% purity, 180 ppm) may be compound-related. NOELs for these lesions using 85.4% purity technical have not been established in this study.

Unilateral retinal degeneration was unaffected by treatment.

With respect to the effects of STAM Technical on the thyroid, the following incidences and grades were reported in female mice:

	Females					
Group	1	2	3	4	5	6
Number Examined	54	54	68	67	68	67
Cystic Follicles						
Not affected	47	49	55	57	61	59
Minimal	4	3	9	7	4	6
Slight	1	1	3	3	1	1
Moderate	2	1	1	0	2	1
Total Affected	7	5	13	10	7	8
Thyroiditis						
Not affected	52	52	65	61	66	58
Minimal	1	2	2	3	1	8 S+,S+s+
Slight	1	0	1	3	0	0
Moderate	0	0	0	0	0	1
Moderately severe	0	0	0	0	1	0
Total Affected	2	2	3	6	2	9 S+s+

Statistically significant increases in group 6 females (180 ppm, 85.4% purity) for minimal thyroiditis and total number of animals affected with thyroiditis were observed. A NOEL for thyroiditis in female mice due to STAM Technical (85.4% purity) was not established in this study.

In males, the incidences and grades of cystic follicles and thyroiditis are without a clear relationship to treatment as shown below:

	Males					
Group	1	2	3	4	5	6
Number Examined	54	53	63	64	68	66
Cystic Follicles						
Not affected	48	48	50	56	56	55
Minimal	2	3	9 S+,S+s+	4	9 S+s+	3
Slight	3	2	3	3	3	4
Moderate	1	0	1	1	0	4
Total Affected	6	5	13	8	12	11

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	Group	Males (cont'd)					
		1	2	3	4	5	6
Number Examined		54	53	63	64	68	66
Thyroiditis							
Not affected		54	52	63	63	68	66
Minimal		0	2	0	0	0	0
Slight		0	0	0	1	0	0
Moderate		0	1	0	0	0	0
Moderately severe		0	0	0	0	0	0
Total Affected		0	1	0	1	0	0

Other non-neoplastic lesions, considered at one time to be possibly related to treatment but subsequently dismissed upon further inspection, included dilated mucosal glands of the stomach in males, regenerative epithelium of the kidneys in males and females, myocarditis in males, hemosiderin pigment in the spleen of males and females, and hepatitis in males.

2. Neoplasms

a. Week 14 Sacrifice - No neoplasms were observed in the mice/sex/group at week 14 in the liver and spleen.

b. Week 53 Sacrifice - A few neoplasms were identified in the 10 mice/sex/group at week 53. These included a β-cortical adenoma of the adrenal in a group 6 male; a thyroid follicular adenoma in one group 3 male and one group 4 female; a thyroid follicular carcinoma in one group 6 male; a lung alveolar/bronchiolar adenoma in one group 1 male, one group 5 male, and one each in females of groups 1, 2, 3, and 6; a lung alveolar/bronchiolar carcinoma in one each in males of group 1, 4, and 5; and liver hepatocellular adenoma in two males of group 2, one male of group 3, and two males of group 4. The incidences of lung and liver tumors in male and female mice observed at the 53 week interim sacrifice are presented in Tables I and II.

There were no compound-related tumors at week 53.

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Table I

Incidence of Lung and Liver Tumors in Male CD-1 Mice Administered STAM Technical in the Diet; 53-Week Sacrifice

Group ppm	1 0	2 0	3 5	4 30	5 180	6 180
Number examined	10	10	10	10	10	10
Lung						
A/B[a]/ Adenomas	1 (10)	0	0	0	1 (10)	0
A/B Carcinomas	1 (10)	0	0	1 (10)	1 (10)	0
Total tumor-bearing animals	2 (20)	0	0	1 (10)	2 (20)	0
Liver						
HC[b]/ Adenomas	0	2 (20)	1 (10)	2 (20)	0	0
HC Carcinomas	0	0	0	0	0	0
Total tumor-bearing animals	0	2 (20)	1 (10)	2 (20)	0	0

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a/Alveolar/Bronchiolar.
b/Hepatocellular.
() Figures in parentheses indicate percentage response.

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Table II

Incidence of Lung and Liver Tumors in Female CD-1 Mice Administered STAM Technical in the Diet; 53-Week Sacrifice

Group	1	2	3	4	5	6
ppm	0	0	5	30	180	180
Number examined	10	10	10	10	10	10
Lung						
A/B[a] Adenoma	1 (10)	1 (10)	1 (10)	0	0	1 (10)
A/B Carcinoma	0	0	0	0	0	0
Total tumor-bearing animals	1 (10)	1 (10)	1 (10)	0	0	1 (10)
Liver						
HC[b] Adenoma	0	0	0	0	0	0
HC Carcinoma	0	0	0	0	0	0
Total tumor-bearing animals	0	0	0	0	0	0

[a] Alveolar/Bronchiolar.
[b] Hepatocellular
( ) Figures in parentheses indicate percentage response.

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c. Moribund Deaths and Terminal Sacrifice - There were no compound-related increases in any tumors in animals which died moribund during the study or were sacrificed at study termination or any effect on latency. The most frequently observed neoplasms were lung and liver tumors and malignant lymphomas.

1. Male Mice - In male mice, the percentage response of total liver tumor-bearing animals was 36, 40, 24, 24, 28, and 28 for groups 1, 2, 3, 4, 5, and 6, respectively. These results are presented in Table III.

With respect to lung tumors in male mice, the percentage response of total lung tumor-bearing animals was 34, 20, 24, 18, 17, and 29 for groups 1, 2, 3, 4, 5, and 6, respectively. These data are presented in Table IV.

Additionally, there was no indication of a decrease in latency of lung or liver tumors in male mice found dead or sacrificed moribund during the study.

For male mice bearing malignant lymphomas, the percent response was 29, 18, 13, 14, 19, and 20 for groups 1, 2, 3, 4, 5, and 6, respectively. These data are presented in Table V. Additionally, there was no decrease in latency for male mice as shown in the statistical analyses of the data in Table IX.

Table III

Incidence of Liver Tumors in Male CD-1 Mice Administered STAM Technical in the Diet; Moribund Deaths and Terminal Sacrifice

Group	1	2	3	4	5	6
ppm	0	0	5	30	180	180
Number examined	44[a]	43[a]	55[a]	55	58[a]	58[a]
HC[b] adenomas	11 (25)	8 (19)	9 (16)	1 (2)	7 (12)	7 (12)
HC carcinomas	9 (20)	13 (30)	8 (15)	12 (22)	10 (17)	10 (17)
Total tumor-bearing animals	16 (36)	17 (40)	13 (24)	13 (24)	16 (28)	16 (28)

a/ Four group 1 males, four group 2 males, four group 3 males, one group 5 male, and one group 6 male exibited both adenoma and carcinoma and were counted only once for calculating the total number of tumor-bearing animals.

b/ Hepatocellular.

( ) Figures in parentheses indicate percentage response.

Table IV
Incidence of Lung Tumors in Male CD-1
Mice Administered STAM Technical in the Diet;
Moribund Deaths and Terminal Sacrifice

Group	1	2	3	4	5	6
ppm	0	0	5	30	180	180
Number examined	44[a]	44[b]	55	55	59	59
A/B[c] Adenomas	6 (14)	4 (9)	3 (5)	4 (7)	4 (7)	6 (10)
A/B carcinomas	10 (23)	7 (16)	10 (18)	6 (11)	6 (10)	11 (19)
Total tumor bearing animals	15 (34)	9 (20)	13 (24)	10 (18)	10 (17)	17 (29)

---

a/One group 1 male exhibited both adenoma and carcinoma and was counted only once for calculating the total number of tumor-bearing animals.
b/Two group 2 males exhibited both adenoma and carcinoma and were counted only once for calculating the total number of tumor-bearing animals.
c/Alveolar/Bronchiolar.
()Figures in parentheses indicate percentage response.

Table V
Malignant Lymphomas

Animal Numbers of Tumor-Bearing Male Mice

Group 1: 148, 152, 155, 156, 157, 169, 170, 172, 175, 182, 184, 187, 189, 192, 193, 195.

Group 2: 281, 285, 289, 291, 295, 296, 311, 331, 337, 342.

Group 3: 412, 425, 426, 430, 435, 436, 443, 458, 486.

Group 4: 589, 592, 605, 607, 614, 618, 627, 634, 640, 641.

Group 5: 734, 735, 740, 742, 758, 762, 768, 786, 787, 793, 800, 804, 805.

Group 6: 891, 894, 904, 906, 907, 909, 925, 934, 938, 937, 943, 956, 960.

Incidence of Tumor-Bearing Male Mice

Group / ppm	1 / 0	2 / 0	3 / 5	4 / 30	5 / 180	6 / 180
No. examined	56	56	70	70	70	70
T-B mice	16	10	9	10	13	14
Percent Response	29	18	13	14	19	20

2) Female Mice - In female mice, the percentage response of total liver tumor-bearing animals was 23, 9, 14, 23, 10, and 16 for groups 1, 2, 3, 4, 5, and 6, respectively. These results are presented in Table VI.

With respect to lung tumors in female mice, the percentage response of total lung tumor-bearing animals was 25, 13, 11, 28, 25, and 17 for groups 1, 2, 3, 4, 5, and 6, respectively. These data are presented in Table VII.

Additionally, there was no indication of a decrease in latency of lung or liver tumors in female mice found dead or sacrificed moribund during the study.

For female mice bearing malignant lymphomas, the percentage response was 21, 30, 23, 27, 31, and 31 for groups 1, 2, 3, 4, 5, and 6, respectively. These data are presented in Table VIII.

Additionally, there was no decrease in latency for female mice with malignant lymphoma as shown by statistical analyses of the data in Table IX.

Table VI
Incidence of Liver Tumors in Female CD-1 Mice Administered STAM Technical in the Diet; Moribund Deaths and Terminal Sacrifice

Group	1	2	3	4	5	6
ppm	0	0	5	30	180	180
Number examined	44[a/]	46[a/]	58[a/]	57	59[a/]	58[a/]
HC[b/] adenoma	5 (11)	2 (4)	5 (9)	3 (5)	3 (5)	5 (9)
HC carcinoma	6 (14)	3 (7)	4 (7)	10 (18)	5 (8)	6 (10)
Total tumor-bearing animals	10 (23)	4 (9)	8 (14)	13 (23)	6 (10)	9 (16)

a/ One group 1 female, one group 2 female, one group 3 female, two group 5 females, and two group 6 females exhibited both adenoma and carcinoma and were counted only once for calculating the total number of tumor-bearing animals.
b/ Hepatocellular.
( ) Figures in parentheses indicate percentage response.

Table VII

Incidence of Lung Tumors in Female CD-1 Mice Administered STAM Technical in the Diet; Moribund Deaths and Terminal Sacrifice

Group ppm	1 0	2 0	3 5	4 30	5 180	6 180
Number examined	44	46	59	60	59	60
A/B[a] adenomas	3 (7)	4 (9)	5 (8)	8 (13)	6 (10)	7 (12)
A/B carcinoma	8 (18)	2 (4)	2 (3)	9 (15)	9 (15)	3 (5)
Total tumor-bearing animals	11 (25)	6 (13)	7 (11)	17 (28)	15 (25)	10 (17)

a/Alveolar/Bronchiolar.
()Figures in parentheses indicate percentage response.

Table VIII
Malignant Lymphomas

Animal Numbers of Tumor-Bearing Female Mice

Group 1: 215, 216, 221, 222, 256, 232, 246, 255, 265, 266, 273, 278.

Group 2: 347, 351, 359, 362, 366, 375, 371, 376, 379, 386, 387, 388, 392, 393, 395, 402, 410.

Group 3: 492, 495, 491, 507, 517, 521, 532, 525, 534, 539, 542, 545, 549, 552, 561, 565.

Group 4: 653, 658, 662, 664, 683, 689, 690, 695, 698, 686, 697, 703, 707, 719, 730, 718, 724, 725, 706.

Group 5: 811, 816, 817, 825, 838, 854, 856, 858, 860, 861, 869, 874, 883, 880, 881.

Group 6: 976, 982, 985, 993, 994, 992, 996, 997, 998, 011, 016, 015, 017, 009, 013, 035, 026, 024, 043, 050, 047, 049.

Incidence of Tumor-Bearing Female Mice

Group ppm	1 0	2 0	3 5	4 30	5 180	6 180
No. examined	56	56	70	70	70	70
T-B mice	12	17	16	19	15	22
Percent response	21	30	23	27	21	31

70 70

Table IX

Malignant Lymphomas

Week of Death for Tumor-Bearing Male Mice

Group 1: 88, 66, 53, 96, 58, 72, 84, 73, 105, 98, 83, 73, 53, 53, 97, 84.

Group 2: 70, 74, 96, 105, 89, 90, 105, 39, 90, 92.

Group 3: 80, 1, 58, 53, 60, 105, 100, 105, 77.

Group 4: 105, 48, 75, 75, 100, 77, 60, 60, 84, 42.

Group 5: 24, 56, 77, 28, 105, 79, 94, 50, 91, 87, 42, 53, 105.

Group 6: 48, 49, 58, 14, 47, 43, 92, 53, 82, 105, 40, 88, 49, 94.

Week of Death for Tumor-Bearing Female Mice

Group 1: 89, 53, 105, 66, 98, 66, 52, 79, 85, 101, 74, 86.

Group 2: 68, 105, 53, 65, 97, 35, 92, 44, 93, 54, 102, 90, 88, 103, 77, 101, 72.

Group 3: 77, 73, 21, 105, 96, 69, 46, 89, 84, 52, 65, 100, 103, 54, 92, 76.

Group 4: 55, 76, 95, 102, 63, 68, 85, 73, 105, 79, 43, 76, 105, 33, 27, 95, 74, 105, 89.

Group 5: 65, 27, 105, 100, 81, 86, 105, 47, 47, 70, 43, 48, 101, 81, 45.

Group 6: 41, 88, 83, 95, 80, 70, 79, 76, 81, 86, 19, 99, 43, 90, 53, 44, 21, 85, 82, 94, 99, 99.

D. Discussion

There were no compound-related effects on survival, clinical observations, tissue masses, and body weight. For treated males during the study, a slight increase (6%) in food consumption was observed. However, this slight increase was not considered toxicologally significant. Food consumption in group 4 females also slightly exceeded control values during the first 52 weeks of the study. This finding was not considered toxicologically significant.

The dose-related decrease in liver weight at week 14 in males was not considered toxicologically significant since it was not observed at weeks 53 and 105.

There was a dose-related increase in centrilobular hepatocytic enlargement in male mice which is considered compound-related. The percentage response was 23, 17, 23, 25, 31, and 40 for groups 1, 2, 3, 4, 5, and 6, respectively. The grade of the lesion was comparable between groups. The LEL for this effect was 180 ppm (HDT) for both the 98.0% purity and the 85.4% purity STAM technical. Although 85.4% technical was not tested below 180 ppm, the results produced for both technicals are toxicologically comparable at 180 ppm and lower levels of the 85.4% purity technical are not required to be evaluated at lower dosages for this lesion. The NOEL for this lesion is 30 ppm.

This hepatic lesion was the only minimum evidence that an MTD was employed. The effect only occurred in male mice. This finding is not considered as adequate evidence that an MTD was used in the study. The registrant is required to justify the highest dose tested (180 ppm) as the MTD.

Additional information relating to the MTD was found in the 90-day range-finding study in mice (Protocol Number 78P-079; Report Number 82R-065; March 24, 1983). The dosages in that study were 0, 25, 200, 1600, and 12,800 ppm (98.0% purity, STAM technical). The NOEL in the range-finding study was 25 ppm. At 200 ppm, hepatocytic pleomorphism was observed in 3/10 females and 1/10 males. Also at 200 ppm, multifocal

hepatocytic necrosis was observed in 1/10 males. The lesions observed in the liver in the range-finding study were as follows:

Group	1		2		3		4		5	
Dosage (ppm)	0		25		200		1600		12,800	
No. examined	F 10	M 9	F 10	M 10	F 10	M 10	F 10	M 10	F 10	M 9
Liver										
Hepatocytic pleomorphism	1	0	1	1	3	1	7	5	6	6
Multifocal necrosis	0	0	0	0	0	1	1	1	0	2
Focal necrosis	0	0	0	0	0	0	0	0	1	0
Nuclear variation	0	2	0	1	0	0	2	0	2	3
Pigment Kupffer cells	0	0	0	0	0	0	0	2	5	6

Additional effects observed in the range-finding study included decreased body weight gains and food consumption increases in both sexes at 12,800 ppm. Cyanosis was observed in males and females at 12,800 ppm and males at 1600 ppm. RBC values were decreased in both sexes at 12,800 ppm. Clinical chemistry and urinalysis evaluations did not show any treatment effects. At 1600 ppm, absolute and relative spleen weights were increased in males and females. At 12,800 ppm, absolute and relative spleen and liver weights were increased in both sexes, as well as increased relative heart and brain weight in females. Increased mixed function oxidase activity, as measured by the p-nitroanisole-o-demethylase assay, in the liver of both sexes at 1600 and 12,800 ppm was observed.

Darkened blood, spleen, liver, lungs, kidneys, and heart in both sexes at 1600 and 12,800 ppm were considered compound-related. The grade of extramedullary hemopoiesis and hemosiderin in the spleen was increased in comparison to controls at 1600 and 12,800 ppm in both sexes.

The toxic effects at 1600 and 12,800 ppm are indicative of an MTD. The effects observed at 200 ppm (essentially hepatocytic

pleomorphism in 3/10 females) is considered by Toxicology Branch to be an insufficient basis to establish an MTD. The HDT in the 2-year mouse study was 180 ppm. This HDT is approximately eight times lower than the dose level of 1600 ppm, where "life-threatening" toxic effects were observed. A further explanation by the registrant of the MTD in the 2-year mouse study is needed.

Bilateral retinal degeneration may be compound-related in male and female mice at 180 ppm with the 85.4% purity technical.

NOELs for these lesions using 85.4% purity technical have not been established in this study.

Unilateral retinal degeneration was unaffected by treatment.

Statistically significant increases in group 6 females for minimal thyroiditis and total number of animals affected with thyroiditis were observed. A NOEL for thyroiditis in female mice due to STAM Technical (85.4% purity) was not established in this study.

William Dykstra

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74 74

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DATA EVALUATION REPORT

Study Type: Teratology; 83-3 TOX Chem. No.: 325

Accession Number: N/A MRID No.: 58589

Test Material: Stam Technical (85.4% ai)

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): 018-001

Sponsor: Rohm & Haas Company

Testing Facility: Argus Research Laboratories, Inc.

Title of Report: Stam Technical Teratogenicity Study in Rabbits

Authors: Floret, M.C.; Christian, M.S., et al.

Report Issued: December 17, 1980

Conclusions:

Teratogenic potential: negative up to 100 mg/kg/day (HDT); maternal toxicity LEL = 100 mg/kg/day (death, decreased body weight); maternal toxicity NOEL = 20 mg/kg/day; fetotoxic LEL = 100 mg/kg/day, (unossified metacarpals); fetotoxic NOEL = 20 mg/kg/day.

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

3-75

Review:

1. Test Material - Stam Technical (85.4% ai); Lot No. 9287; Sample No. 80-70; black, lumpy solid.

2. Test Animals - Ninety-four female DLI:NZW rabbits; received from Dutchland Laboratories, Inc., Swampbridge Road, Box 139A, Denver, PA. Rabbits were 167 to 179 days old at receipt (birth dates October 1 to October 15, 1979).

   Randomized groups of 20 NZW rabbits were placed into each treatment group. Stam technical was given to each animal orally as a suspension in corn oil at dosages of 0 (vehicle), 4, 20, and 100 mg/kg/day once daily during days 6 through 18 of gestation at volumes of 1 mL/kg/day. Doses were adjusted daily on the basis of maternal body weight.

   On day 30 of gestation, surviving female rabbits were killed with CO_2 and uterine contents were examined. Reproductive parameters were measured. Fetuses were examined externally, weighed, and sexed. All fetuses were examined for soft tissue anomalies, eviscerated, stained with Alizarin Red-S, and evaluated for skeletal variations.

Results:

Pregnancy occurred in 18, 19, 15, and 17 rabbits given 0, 4, 20, and 100 mg/kg/day, respectively. Five rabbits at the high dose died during days 13 through 20 of gestation. These deaths are considered treatment-related. There were no other deaths in either the control or treatment groups.

Abortion occurred in 1, 3, 0, and 4 rabbits of the control, low-, mid-, and high-dose groups. These abortions are not considered compound-related, since there was no relationship to dose.

Two low-, one mid-, and one high-dose group rabbits delivered naturally on day 29 or 30 of gestation prior to sacrifice.

Pregnant rabbits treated with 100 mg/kg/day showed significantly reduced body weight ($p < 0.01$) in comparison to controls during days 6 to 12 of gestation. Changes in body weight during days 6 to 12 were -0.01, +0.02, 0.00, and -0.19 kg for the control-, low-, mid-, and high-dose groups, respectively. Body weight gain of these rabbits was comparable to control by day 30 of gestation.

As a result of death, abortion, or early delivery, the following rabbits were sacrificed at day 30:

control: 17;
low: 14;
mid: 14; and
high: 7.

There were no compound-related effects in the number of corpora lutea, implantations, resorptions, or live and dead fetuses. Fetal sex ratio and body weights of fetuses were comparable between groups.

Totals of 113, 106, 65, and 49 fetuses in the control-, low-mid-, and high-dose groups were examined for visceral and skeletal anomalies.

There were no compound-related visceral abnormalities.

Significantly fewer ($p < 0.01$) metacarpals were ossified in live fetuses of the high-dose group (due to 8 of 10 fetuses in one litter). Other skeletal data were comparable between control and treated groups.

Conclusion:

Teratogenic potential: negative up to 100 mg/kg/day (HDT); maternal toxicity LEL = 100 mg/kg/day (death, decreased body weight); maternal toxicity NOEL = 20 mg/kg/day; fetotoxic LEL = 100 mg/kg/day (unossified metacarpals); fetotoxic NOEL = 20 mg/kg/day.

Classification: Core-Minimum.

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DATA EVALUATION REPORT

Study Type: Teratology; 83-3 TOX Chem. No.: 325

Accession Number: N/A MRID No.: 58588

Test Material: Stam Technical (85.4% ai)

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): 10065-008

Sponsor: Rohm & Haas Company

Testing Facility: Booz, Allen and Hamilton, Inc.

Title of Report: Teratogenic Evaluation of Stam Technical in the Albino Rat

Authors: Kam, C.; Stevens, K.R.; Gallo, M.A.

Report Issued: February 29, 1980

Conclusions:

Teratogenic potential: negative up to 100 mg/kg/day (HDT); maternal toxicity LEL = 100 mg/kg/day (increase in average number of resorptions/dam); maternal toxic NOEL = 20 mg/kg/day; fetotoxic LEL = 100 mg/kg/day (decreased pup weight, delayed ossification in some pups, absent sternebrae (No. 5) and xiphesternum in some pups); fetoxic NOEL = 20 mg/kg/day.

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

Review:

1. Test Material - Stam Technical, Lot 09287 (85.4% ai, ID No. 79-314; FDS Lot No. 4649).

2 Test Animals - Sexually mature BLU:(SD)BR female albino rats, approximately 13 weeks of age (weighing an average of 250 g) were mated 3:1 with sexually mature BLU:(SD)BR male albino rats. The matings were managed by Blue Spruce Farms, Inc., Altamont, NY. The dams were then delivered to the Booz, Allen and Hamilton, Inc. Laboratory on December 21, 1979 by Taconic Farms, Inc., Germantown, NY.

Randomized groups of 25 pregnant Sprague-Dawley rats received doses of 0, 0.8 mg/kg, 4.0 mg/kg, 20 mg/kg, and 100 mg/kg of test material in corn oil at 10 mL/kg during days 6 to 15 of gestation. The dosage formula was prepared daily and administered by gavage.

Each animal was observed daily for toxic signs. The body weight of each animal was recorded on the day of receipt and on days 6, 10, 15, and 20 of gestation. Food consumption was recorded for each animal on days 6, 10, 15, and 20 of gestation.

On day 20 of gestation, all animals were sacrificed by exposure to CO_2 vapor. The uterine contents were examined and the following recorded for each dam:

a. Number of corpora lutea per ovary.
b. Number of implantation sites.
c. Number of early and late resorption sites.
d. Number of live and dead fetuses.
e. Body weight of each live fetus.
f. Sex of each fetus.

At the time of uterine examination, all fetuses were examined grossly for the presence of external abnormalities.

One-third of the fetuses were examined for soft tissue anomalies by the Wilson technique.

The remaining two-thirds of the fetuses were examined by Alizarin staining for skeletal anomalies.

Results:

A few scattered incidences of rales, diarrhea, red nasal discharge, and alopecia were observed. These observations were not dose-related. One of the animals died during the course of the study: Dam #5119 of the high-dose group. Upon necropsy, the

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dam was apparently not pregnant. In addition, a red stomach lining and red fluid filling the intestines were observed.

Body weights and food consumption were similar among all groups with the exception of the dams of the 4 mg/kg group. These dams were lighter ($p < .05$) than dams of all other groups. This effect is considered by this reviewer to be of no toxicologic significance.

The litter reproduction data showed an increase in the average number of resorptions/dam of the high dose:

Observation	I (Vehicle Control)	II (0.8 mg/kg)	III (4 mg/kg)	IV (20 mg/kg)	V (100 mg/kg)
Number of pregnant rats	20	23	23	21	22
Number of live litters	20	23	22	21	22
Average number of live fetuses/dam	11.5	11.1	11.3	9.7	11.6
Average number of resorptions/dam	0.5	0.5	0.3	0.5	0.9
Average weights of gravid uteri	69.8	66.5	68.7	61.1	67.9

No gross findings attributable to the test material were recorded for the pups. There were no significant dose-related soft tissue abnormalities detected in the pups. The most frequently observed abnormalities were slightly dilated brain ventricles, hydroureter, and hydronephrosis, but these occurred at similar frequencies in control and treated animals and are not considered by this reviewer to be related to the test material.

Smaller pups were seen in the high-dose group (average fetus weight per dam was 4.00, 3.80, 3.89, 3.92, and 3.63 grams in groups I, II, III, IV, and V, respectively). At 100 mg/kg, there were increased incidences of (1) delayed ossification in the manubrium and sternebrae No. 3 and No. 4, (2) partial ossification of cervical and thoracic vertebrae, and (3) absence of sternebrae No. 5 and xiphisternum.

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Conclusions:

Teratogenic potential: negative up to 100 mg/kg/day (HDT); maternal toxic LEL = 100 mg/kg/day (increase in average number of resorptions/dam); maternal toxic NOEL = 20 mg/kg/day; fetotoxic LEL = 100 mg/kg/day (decreased pup weight, delayed ossification in some pups, absent sternebrae (No. 5), and xiphisternum in some pups); fetotoxic NOEL = 20 mg/kg/day.

Classification: Core-Minimum.

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Section II, Reviewer
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Edwin Budd 7/21/81
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81

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Secondary Reviewer: Edwin Budd
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DATA EVALUATION REPORT

Study Type: 83-4, Reproduction

TOX Chem. No.: 325

Accession Number: N/A

MRID Nos.: 36091
15419

Test Material: STAM F-34; technical, 97% ai

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): Not reported

Sponsor: Rohm & Haas Company

Testing Facility: Medical College of Virginia

Title of Report: Three Generation Reproduction Study on Rats Receiving STAM F-34 in Their Diets

Authors: Borzelleca, J.F.; Ambrose, A.M.; Larson, P.S.

Report Issued: (1966) Unpublished study received June 11, 1970 under 0F0932

Conclusions:

Average body weights of parental animals were less (up to 10%) than controls at 1000 ppm during growth, mating, and weaning. There were no compound-related effects in fertility indices, gestation indices, viability indices, lactation indices, and sex ratios for each generation. Average litter sizes at birth and weaning were greater at all dietary levels of STAM F-34 as compared with controls. Weaning body weights averaged less for pups at all dietary levels of STAM F-34 as compared with controls, but this appears to be due to the increased litter sizes. Histopathological examination of 10 pups/sex/dose of the F/3b litters did not show any compound-related lesions. The NOEL for the study is 300 ppm. The LEL is 1000 ppm and the effects are decreased body weights of parent animals.

Classification:

Core-Minimum. Individual animal data were not provided.

Special Review Criteria (40 CFR 154.7): N/A.

Review:

1. Test Material: STAM F-34; Lot No. 9315; technical; 97% 3',4'-dichloropropionanilide, [redacted] It is a dark brown crystalline material with a slight aromatic odor.

2. Experimental: [Copied from study report (pages 1-2).]

"Albino rats of Wistar strain were used. At 28 days of age, litter mate rats, within but not between sexes, were separated into 4 groups of 25 rats of each sex and individually caged to constitute the F/0 generation. One group was placed on each of the following dietary concentrations of STAM F-34: 0, 100, 300, and 1000 ppm. Finely ground Purina Laboratory Chow served as the basic diet into which the STAM F-34 was incorporated with thorough mixing by means of a rotary-type blender. Diets were prepared in 6 kg batches. A weighed amount (6 g) of STAM F-34 in 10 mL of diethylether was added, followed by two 10 mL vessel rinses, to the basic diet to prepare the 1000 ppm concentration. Lower concentrations were prepared from the 1000 ppm diet by suitable dilution with the basic diet."

"After 11 weeks on the above dietary regimes, 20 females on each diet were transferred to individual breeding cages and each was mated with a male rat on the same dietary level of STAM F-34 for the F/1a generation. Male rats within each group were rotated to a different female on each of three successive 7-day periods, if found necessary. Records were kept of mating, number of pregnancies, number of litters cast, young in the litter at 1, 5, and 21 days, and the weight of the litter at 21 days (weaning). Litters containing more than 10 were reduced to this number on day 5. Indices calculated were:

1. Fertility Index - (pregnancies/matings) x 100

2. Gestation Index - (total litters cast/pregnancies) x 100

3. Viability Index - (live pups at 5 days/live pups born) x 100

4. Lactation Index - (weaned pups/live pups minus discards at day 5) x 100"

"F/1a rats were sacrificed and autopsied following weaning. F/0 rats were remated as above to produce second (F/1b) litters. Following weaning, F/0 generation rats were sacrificed and autopsied."

"Twenty-five male and twenty-five female F/1b rats from each diet level were continued on their respective parents' diet until about 105 days of age, at which time 20 of each sex within each group were mated and the same procedure followed as with the F/0 generation through production and weaning of two litters (F/2a and F/2b). F/1b rats were then sacrificed and autopsied."

"F/2b rats were continued through the same procedure as with the F/1b generation through production and weaning of two litters (F/3a and F/3b)."

"Histopathologic studies were performed on 10 male and 10 female F/3b offspring from each diet level, tissues examined being heart, lung, liver, kidney, urinary bladder, spleen, gastroenteric (stomach, small and large intestine), bone marrow, skeletal muscle, skin, brain, pituitary, thyroid, adrenal, pancreas, and gonad." [End of quotation.]

Results:

Body weights of male and female parental animals were less than controls (up to 10%) at 1000 ppm during growth, mating, and weaning. The following table from the study report shows parental body weight at mating and weaning:

Body Weights at Mating and Weaning in Percent of Control of Rats Receiving STAM F-34 in Their Diets[1/]

Diet Conc. (ppm)	F/0 to F/1a				F/0 to F/1b				F/1b to F/2a			
	Mating		Weaning		Mating		Weaning		Mating		Weaning	
	F	M	F	M	F	M	F	M	F	M	F	M
0	100	100	100	100	100	100	100	100	100	100	100	100
100	98	101	102	103	102	103	98	103	106	105	107	101
300	96	99	98	103	98	103	96	103	97	98	97	95
1000	93	90	95	95	95	95	92	93	96	96	98	95

Diet Conc. (ppm)	F/1b to F/2b				F/2b to F/3a				F/2b to F/3b			
	Mating		Weaning		Mating		Weaning		Mating		Weaning	
	F	M	F	M	F	M	F	M	F	M	F	M
0	100	100	100	100	100	100	100	100	100	100	100	100
100	107	101	106	102	100	105	103	103	103	103	102	103
300	97	95	100	95	94	105	95	101	95	101	95	103
1000	98	95	95	94	92	98	93	100	93	100	94	99

1/ Table 13 (page 28) of study report.

There were no compound-related effects in fertility indices, gestation indices, viability indices, lactation indices, and sex ratios for each generation. The following table shows the values for the reproductive indices:

Diet Level	Litter	First Gener.	Second Gener.	Third Gener.	First Gener.	Second Gener.	Third Gener.
		FERTILITY			GESTATION		
		1st	2nd	3rd	1st	2nd	3rd
0	a	100	85	100	95	100	100
0	b	78	63	75	100	100	93
100	a	95	90	95	100	94	100
100	b	67	84	100	100	94	95
300	a	100	95	95	100	100	100
300	b	63	85	84	92	88	100
1000	a	95	90	100	100	100	100
1000	b	83	70	85	100	100	100
		VIABILITY			LACTATION		
0	a	91	90	96	73	82	75
0	b	86	86	93	94	98	92
100	a	99	97	99	86	89	82
100	b	97	99	95	98	91	85
300	a	88	98	98	81	80	89
300	b	90	96	99	98	98	93
1000	a	90	98	98	85	83	93
1000	b	98	96	90	97	96	90

Compared with control rats, average litter sizes at birth and weaning were usually greater at all dietary levels of STAM F-34. This increased litter size resulted in body weights for pups at all dietary levels of STAM F 34, which were less than controls. The following table from the study report shows the results of each generation:

Average Number of Pups Born per Litter, Weaned per Litter, and Weaning Weights for Rats Receiving STAM F-34 in Their Diet[1/]

Diet Conc. (ppm)	F/0 to F/1a: Average No. Pups Born/Litter*	F/0 to F/1a: Average No. Pups Weaned/Litter	F/0 to F/1a: Av. Weaning Wt. (g)	F/0 to F/1b: Average No. Pups Born/Litter	F/0 to F/1b: Average No. Pups Weaned/Litter	F/0 to F/1b: Av. Weaning Wt. (g)
0	9.9	5.8	36.9	8.5	5.6	38.7
100	10.8	8.1	33.7	9.3	7.1	37.9
300	11.1	7.2	33.6	9.1	5.9	40.3
1000	9.7	7.1	34.1	8.6	7.4	37.9
	F/1b to F/2a			F/1b to F/2b		
0	9.5	6.3	40.2	8.8	6.6	46.4
100	10.1*	7.6	36.6	10.3	7.4	41.6
300	10.5	7.5	35.7	9.1	7.8	45.6
1000	11.0	8.0	34.2	10.0	7.8	36.9
	F/2b to F/3a			F/2b to F/3b		
0	12.2	7.3	33.7	8.6	6.4	40.1
100	10.8	7.3	35.4	10.2	7.2	40.1
300	11.2	7.9	34.9	8.0	6.3	36.8
1000	10.5	8.4	31.6	11.8	7.9	34.1

*Including stillborn.

[1/] Table 16 (page 31) from study report (with minor modifications).

Histopathological examination of 10 pups/sex/dose of the F/3b litters did not show any spontaneous or compound-related lesions.

Conclusion:

Average body weights of parental animals were less (up to 10%) than controls at 1000 ppm during growth, mating, and weaning. There were no compound-related effects in fertility indices, gestation indices, viability indices, lactation indices, and sex ratio for each generation. Average litter sizes at birth and weaning were greater at all dietary levels of STAM F-34 as compared with controls. As a result of increased litter sizes, weaning body weights averaged less for pups at all dietary levels of STAM F-34 as compared with controls. Histopathological evaluation of 10 pups/sex/dose of the F/3b litters did not show any spontaneous or compound-related lesions. The NOEL for the study is 300 ppm, the LEL is 1000 ppm, and the effects are decreased body weight of parental animals.

Classification:

Core-Minimum. Individual animal data were not provided.

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87 85

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Secondary Reviewer: Edwin Budd
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00E305

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2) - In Vivo Cytogenetic Study in Mice

TOX. CHEM. NO.: 325

ACCESSION NUMBER: 260448

TEST MATERIAL: Stam(pede) Technical

SYNONYMS: Propanil

REPORT NUMBER: 82R-255

SPONSOR: Rohm and Haas Co., Philadelphia, Pa.

TESTING FACILITY: Rohm and Haas Co., Toxicology Dept., Spring House, Pa. 19477

TITLE OF REPORT: Stam(pede) Cytogenetic Study in Mice

AUTHOR(S): O'Neill PJ, McLeod PL, and KL McCarthy

REPORT ISSUED: November 11, 1983

IDENTIFYING VOLUME: Volume 1 of 1, Tab 2a

CONCLUSION: Under the conditions of the study, Stam(pede) did not induce chromosomal aberrations in mouse bone marrow cells.

Classification: Reserved pending submission of additional data (see discussion)

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: 3,4-dichloropropionanilide, TD 82-148
Description: black solid
Batch #(s), Other #(s): lot # 4-76-416
Purity: 87.8%
Source: Agricultural Chemicals Discovery & Development Dept., Rohm & Haas
Vehicle (if applicable): Corn oil
Positive Control(s) (if applicable): Triethylene melamine (TEM)

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): CD-1 mice (male)
Age: Not given
Weight(s): 8-14 g; 20.0-29.9 g on Day 1 of study
Source(s): Charles River Kingston Breeding Farms (Stone Ridge, NY)

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3. Procedure:

a. Range-Finding Study for the Cytogenetic Assay

Male CD-1 mice were tested in an acute oral LD_{50} study in order to determine the dosage levels to be used in the cytogenetics assay. Ten animals were used per dose level: 0, 1.00, 1.19, 1.41, 2.00 and 2.80 g/kg. Clinical signs of toxicity, onset of signs, recovery, body weights, time of death and gross pathological changes were reported. The LD_{50} was reported to be 1.21 (1.10-1.43) g/kg.

b. Main Study

Stam(pede) was dissolved in corn oil and administered po at a volume of 10 ml/kg. The doses selected were approximately 1/4, 1/10, and 1/40 of the acute oral LD_{50} determined from the range-finding study. The concentration of the chemical administered was based on the active ingredient content of the technical material. The positive control, TEM, was administered ip at a dose of 0.3 mg/kg in a volume of 16 ml/kg, using distilled water as the vehicle. Two dosing schedules were used for the test compound and vehicle controls, one in which the animals were given a single dose and one in which the animals were given one dose per day for 5 days. The following dosages were administered: 0, 26.5, 106 and 265 mg/kg/day. The positive controls received TEM in one single dose.

In the single dose groups, animals were killed at 6, 24 and 48 hours after the administered dose. The positive controls were killed only at 24 hours. In the multiple dose groups, animals were killed only at 6 hours after the last dose. Eight animals were killed per dose per time period. All animals received colchicine (2 mg/kg, ip, at 10 ml/kg in distilled water) 3 hours prior to being killed by cervical dislocation. Bone marrow was extracted from the femurs of each animal and the cells were isolated, fixed in an absolute methanol:glacial acetic acid mixture, 3:1, pipetted onto microscope slides and stained with Giemsa stain. Three slides were prepared per animal. Up to 50 metaphase spreads were read per animal. If less than 50 were available, then the maximum number of acceptable spreads were read. Positive and negative controls and the high dose groups were read first. The low and mid dose groups were read only if an effect was detected in the high dose group. Only groups showing more than 50% survival of the animals were considered acceptable for evaluation.

Six criteria were used for selection of metaphase spreads for scoring: proper staining, observable centromeric region, well-defined chromatids, little or no overlap of individual chromosomes, spreads existing singly in a well-defined area and lack of a wide separation at the centromeric region. The following items were scored: breaks, gaps, fragments (or pulverized), translocations and rearrangements and inversions.

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B. RESULTS:

All animals survived until scheduled termination. Toxicologic signs were noted in the high and mid-dose animals in both dosing regimens. Decreased spontaneous motor activity, lethargy and piloerection were the major signs observed. Stam(pede) did not induce an increase in chromosomal aberrations in bone marrow cells at either 106 or 265 mg/kg at any of the time periods after exposure in either of the dosing regimens. A significant adverse effect on chromosomes occurred in mice treated with TEM (positive control).

C. DISCUSSION:

No mitotic indices were reported. However, the report stated that a decreased number of metaphases was observed in the slides from the high dose 6 and 48 hr. animals from the acute regimen. This could be an indication that the chemical was reaching the target tissue. The specific data on the number of metaphases was not reported (including any statistical analyses). The acceptability of the study is pending submission of these data. In addition, if any data on chromosome counts were collected, these data should be submitted as well.

90

viewed by: Pamela Hurley
ction 3 , Tox. Branch (TS-769C)
condary Reviewer: Diwin Budd
ction 3 , Tox. Branch (TS-769C)

005305

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2) - CHO/HGPRT Gene Mutation Assay

TOX. CHEM. NO.: 325

ACCESSION NUMBER: 260448

TEST MATERIAL: STAM Technical

SYNONYMS: Stampede, Propanil

STUDY NUMBER(S): Not given

REPORT NUMBER: 83R-142

SPONSOR: Rohm and Haas Company, Philadelphia, PA

TESTING FACILITY: Rohm and Haas Company, Toxicology Dept., Spring House, PA

TITLE OF REPORT: STAM^R Technical CHO/HGPRT Gene Mutation Assay

AUTHOR(S): Kruszewski FH, McCarthy KL, Byers MJ

REPORT ISSUED: 1/12/84

IDENTIFYING VOLUME: Vol. 1 of 1, Tab 3

CONCLUSION: Stam was not mutagenic under the conditions of the study.

Classification: Acceptable

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: 3,4-dichloropropionanilide
Description: Solid
Batch #(s), Other #(s): TD 82-149, Lot # 4-76-416
Purity: 97.8%
Source: Not given (assumed Rohm and Haas)
Vehicle (if applicable): Dimethyl sulfoxide (DMSO)
Positive Control(s) (if applicable): Ethyl methanesulfonate (EMS) for inactivated system; 7,12-Dimethylbenzanthracene (DMBA) for activated system

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): CHO/HGPRT mammalian cells from CHO-K_1-BH_1 Cell line

3. Experimental Procedures

a. Range Finding Test

STAM was tested for toxicity in range finding tests both with and without metabolic activation. The concentrations of the test material ranged from 0.05 to 1000 micrograms/ml (0.044 to 880 micrograms/ml ai). As part of the range finding test, some cells were exposed to either the positive or the negative control agents instead of the test material. Exposure to the chemicals was either for 18 to 20 hours without metabolic activation or for 5 hours with activation (S-9 mix, consisting of the S-9 liver homogenate fraction from Aroclor 1254 induced male Sprague-Dawley rats plus co-factor). Toxicity was determined by assessment of either suspension cell growth or plating efficiency. The results of these toxicity assays are given in the results section of this report.

b. CHO/HGPRT Gene Mutation Assay

The test concentrations were selected to span the toxicity range of 10-90% survival. The concentrations for repeat trials were selected on the basis of the results of the initial trials. For the nonactivated test, 15, 75, 125 and 150 micrograms/ml of the test compound were tested and for the activated test, 100, 115, 130 and 140 micrograms/ml of the test compound were tested, followed by a separate additional test with treatment concentrations of 120, 150, 165 and 175 micrograms/ml.

Cells were obtained from a frozen stock culture and grown in Ham's nutrient medium F-12 supplemented with fetal calf serum. Upon initiation of each test, cells from logarithmic phase cultures were plated at 5×10^5 cells/plate and incubated at 37°C. Approximately 24 hours after the cells were placed in the plates, they were exposed to the test material, with or without accompaniment of the metabolic activation S-9 mix (described above in range finding tests). Without activation the treatment was for 18 to 20 hours at 37°C, and with activation the treatment was for 5 hours at 37°C. At the end of the exposure period, the cells were washed with a buffered saline solution.

In the test without metabolic activation, the cells were immediately subcultured and in the test with activation, the cells were incubated overnight before subculturing. The cells were suspended with the aid of Trypsin. Suspensions were prepared at a concentration of 1×10^5 cells/ml, seeded onto a culture plate at a concentration of 1×10^6 cells and carried through 2 additional subcultures to allow for an 8-day mutation expression period.

The cells in each treatment group (prior to the additional 2 subcultures were tested for toxicity by either the plating efficiency method (seeded onto plates, incubated for 7 days, stained and counted to determine survival) or the suspension growth method (seeded into a 100 mm non-tissue culture plate in which cells did not attach but instead grow in suspension, and counted 2 or 3 days later to determine growth relative to controls).

After the 8-day mutation expression period, cells in each treatment group were resuspended and counted. They were then suspended in medium without hypoxanthine. Some of the suspension was used for plating efficiency determinations and to some, 6-thioguanine (6TG) was added. The latter group were tested for 6TG resistant mutants (selection plates).

B. RESULTS:

1. Without Metabolic Activation

Range-Finding Test

The toxicity results as assessed by plating efficiency ranged from 0% survival at 1000 micrograms/ml to 96% survival at 0.05 micrograms/ml. No surviving cells were observed at either 500 or 1000 micrograms/ml. In addition, a visible precipitate was observed in the latter culture dishes. Similar results were observed with the suspension growth toxicity determination. Based on these results, the following concentrations were selected for the mutation test: 15, 75, 125 and 150 micrograms/ml with expected relative survival rates estimated from the range finding test of 75-90, 50-75, 20-50 and 10-20% respectively.

Mutation Assay

The toxicities were observed to be similar to those predicted by the range-finding test. The % survival of the two replicates relative to solvent control for each of the dose levels were as follows: 101 and 82, 93 and 68, 54 and 47, and 6 and 14% for 15, 75, 125 and 150 micrograms/ml respectively. The DMSO solvent control mutant frequency assessment plates averaged 2.5 6-TG resistant mutants/10^6 survivors (only one replicate was available because the other was lost due to an accident in handling), and the mutant frequency assessment plates for the positive control (EMS) averaged 586.5 6-TG resistant mutants/10^6 survivors, a clearly significant response. Treatment with Stam resulted in mutant frequencies from 0-9 mutants/10^6 survivors. These results indicate that without metabolic activation, Stam is not a mutagen under the conditions of this assay.

2. With Metabolic Activation

Range-Finding Test

In the presence of a metabolic activating system, approximately 90% or more cell survival occurred at concentrations less than or equal to 100 micrograms/ml. There were no surviving cells following treatment with either 500 or 1000 micrograms/plate. A visible precipitate was also noted at these concentrations. Similar results were observed with the suspension growth method. An additional range finding test was conducted using only the suspension growth method. Concentrations of 100 to 500 micrograms/ml were tested in this test. Survival at concentrations equal to or greater than 200 micrograms/ml was less than 3% (a precipitate was also noted at these concentrations). Eighteen percent survival was noted at 150 micrograms/ml and 83% survival was observed at 100 micrograms/ml.

In response to a much higher survival rate in the first assay for mutation in the presence of metabolic activation, a third range-finding test was conducted. The range of concentrations tested were 120-200 micrograms/ml. Survival ranged from 85% (120 micrograms/ml) to 0.1% at 200 micrograms/ml.

Mutation Assay

Based upon the results of the range finding test, Stam was first tested at 100, 115, 130 and 140 micrograms/ml. The relative cell survival ranged from 80-98%, substantially different from the range-finding test. The mutant frequency of the DMSO solvent control averaged 28.1 mutants/10^6 survivors (duplicate treatment groups were 8.6 and 49.5; mean was 10x the value for the solvent control in the nonactivated system) and the mutation frequency of the positive control (DMBA) averaged 381.2 mutants/10^6 survivors. In one replicate at 115 micrograms Stam/ml, 23.4 mutants/10^6 survivors were noted as opposed to 8.6 mutants/10^6 survivors in the concurrent control. Although the p value was 0.051 relative to the control value in this case, it was not significant relative to the combined frequencies of the replicate controls, or to historical control values. Unfortunately, the other 115 micrograms/ml replicate was lost due to bacterial contamination. None of the frequencies at any of the other dose levels were significantly elevated over solvent controls.

In a second activated test using concentrations of 120, 150, 165 and 175 micrograms/ml, the toxicity assessment plates showed a dose response that ranged from 18 to 86% survival. No mutant colonies were detected in the solvent control plates and the DMBA positive control mutant frequency was 220.4 mutants/10^6 survivors. The mutant frequencies in the Stam treated cells ranged from 0 to 16.7 mutants/10^6 survivors. The 16.7 value was statistically significant (observed only at the LDT, 120 micrograms/ml), however, the replicate value was 0. In addition, the 16.7 value was within the range of historical control values from the testing laboratory (range 0 to 63.9 mutants/10^6 survivors, mean = 9.2, standard deviation = 11.8, median = 6.0; N= 594 plates for both activated and non-activated systems, including the test results from this report). Therefore, the results indicate that with metabolic activation, Stam was not mutagenic under the conditions of the study.

C. DISCUSSION/CONCLUSIONS:

This study is ACCEPTABLE, indicating that Stam is not mutagenic for the HGPRT locus +/- S-9 to CHO cells.

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007553

005305

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity - (84-2) - DNA Damage + Repair Rec Assay (Bacillus subtilis) and Reversion Assay (E. coli and S. typhimurium)

TOX. CHEM. NO.: 325

ACCESSION NUMBER: 260448

TEST MATERIAL: Propanil

SYNONYMS: Stampede

STUDY NUMBER(S): 80RC-1006

REPORT NUMBER: Not given

SPONSOR: Rohm and Haas Co., Philadelphia, PA

TESTING FACILITY: Toxicology Division, Inst. Environmental Tox. of Japan

TITLE OF REPORT: Microbial Mutagenicity Test of DCPA Propanil

AUTHOR(S): Shirasu Y., Moriya M., and Koyashiki R.

REPORT ISSUED: February 14, 1980

IDENTIFYING VOLUME: Volume 1 of 1, Tab 4

CONCLUSION: Under the conditions of the tests, propanil did not appear to induce DNA damage nor did it induce mutations under the conditions of the tests.

Classification: The DNA damage and repair Rec assay is UNACCEPTABLE and the Reversion Assay is MINIMALLY ACCEPTABLE.

A. MATERIALS AND METHODS:

1. Test Compound(s)- the following criteria apply for both studies unless specified otherwise:

Chemical Name: 3',4' - dichloropropionanilide
Description: Not given
Batch #(s), Other #(s): Not given
Purity: 98%
Source: Not given
Vehicle (if applicable): Dimethylsulfoxide (DMSO)
Negative Control (Rec Assay): Kanamycin
Positive Control(s) (if applicable): Mitomycin C (Rec Assay), 2-aminoanthracene; AF-2, [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide]; beta-propiolactone; 9-aminoacridine; and 2-nitrofluorene (reversion assay)

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2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Rec Assay - B. subtilis (H17 and M45) Reversion Assay - S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100; E. coli WP2 hcr

3. Test Protocol:

a. Rec Assay

Two strains of B. subtilis were used in the assay, one having the recombination repair mechanism intact (H17) and one deficient in the repair mechanism (M45). Each strain was streaked on B-11 agar medium in such a way that the starting points did not contact. The test chemical was dissolved in DMSO and spotted on paper disks (0.02 ml solution on a 10 mm diameter disk) which were in turn placed on the streaked agar culture, each at the starting point of each streak. The culture was incubated overnight at 37°C, and the length of the inhibition zones were measured. The following concentrations of test chemical were used: 20, 100, 200, 500, 1000 and 2000 micrograms/disk. Negative and positive controls were also run. Kanamycin was tested at a concentration of 10 micrograms/disk and mitomycin C was tested at a concentration of 0.1 micrograms/disk.

b. Reversion Assay

Five histidine requiring strains of S. typhimurium and one tryptophan requiring E. coli strain was used for this assay. Soft agar solutions were prepared, containing either histidine or tryptophan for the appropriate strains. The bacterial strains were suspended in a buffered solution and added to the agar solution along with the test chemical, either with or without metabolic activation (S-9 mix prepared from livers of Aroclor 1254 induced male SD rats). The mixture was then spread on minimal agar medium and incubated for two days at 37°C. Revertant colonies were counted. The following concentrations of test chemical were used: 1, 5, 10, 50, 100, 500, 1000 and 5000 micrograms/plate. DMSO was tested with S-9 mix alone and 2-aminoanthracene was tested both with and without metabolic activation in all the strains tested. AF-2 was tested without activation in WP2 hcr, TA 100 and TA 98 at 0.25, 0.05 and 0.1 micrograms/plate respectively. 2-Nitrofluorene, beta-propiolactone and 9-aminoacridine were tested at 50, 50 and 200 micrograms/plate in TA 1538, TA 1535 and TA 1537 respectively.

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B. RESULTS:

1. Rec Assay

Propanil induced growth inhibition zones ranging from 0 to 1.5 mm in both strains up to 2000 micrograms/disk. The difference between the results from the two strains was a consistent 0. The negative control, Kanamycin, induced inhibition zones of 4.5 and 4 mm for the M45 and H17 strains respectively, creating a difference of 0.5 mm between the two strains. The positive control, Mitomycin C, induced inhibition zones of 8.5 and 2 mm for the M45 and H17 strains respectively, creating a difference of 6.5 mm. The results were considered by the author to be negative.

2. Reversion Assay

In the tests without metabolic activation, growth inhibition of all the bacterial strains was observed at dose levels of 1000 micrograms propanil/plate and above. In the tests with metabolic activation, growth inhibition was observed in TA 100 and in TA 1537 at a dose level of 1000 micrograms/plate and in all strains at 5000 micrograms/plate. Propanil did not significantly increase the number of reversions/plate under the conditions of the assay at any dose level either with or without metabolic activation. 2-Amino-anthracene increased the number of reversion colonies/plate by 5 to >200 times the negative control with metabolic activation, depending upon the strain tested. Without metabolic activation, the number of reversion colonies/plate was similar to the negative controls. AF-2 increased the number by approximately 100 times in WP2 hcr, 8 times in TA 100 and 23 times in TA 98 without activation. Beta-propiolactone increased the number by 100 times in TA 1535, 2-nitrofluorene increased the number by >180 times in 1538 and 9-aminoacridine increased the number by >1666 times in TA 1537 without metabolic activation.

C. DISCUSSION:

1. Rec Assay

Minimal information was submitted on this assay. The methods section was too brief. There was little discussion on the form of the B-11 agar medium (i.e. what was it in - plates?). The distance between the streaks should have been more specific other than the fact that the starting points did not contact one another. In addition, there was insufficient information to determine whether or not the dose levels were high enough and none of the dose levels were tested more than one time. Finally, no means of metabolic activation was used in the assay. Due to lack of sufficient detail in either the methods section or the results section, and due to inadequate conduct and design, this study is UNACCEPTABLE as it stands.

2. Reversion Assay

Again, minimal information was submitted on this assay. No information was submitted on the bacterial strains as to whether or not they had been recently tested for the desired genotype characteristics (i.e. histidine requirement, deep-rough character, ultraviolet sensitivity, etc.). The cell density of the suspended cells was not reported. Only one (2 plates/strain) test was done. At least two independent tests should have been done. In the data reporting section, no statistical analysis was done and no means and standard deviations were reported. This study is MINIMALLY ACCEPTABLE in light of the comments noted above.

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007559

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2): (1) Reverse mutation in bacteria (Ames Assay/E. coli
(2) DNA damage/repair in yeast (mitotic recombination in S. cerevisiae D3)
(3) DNA damage/repair in bacteria (B. subtilis/E. coli)
(4) DNA damage/repair in mammalian cells (UDS in WI-38)

TOX. CHEM. NO.: 325

ACCESSION NUMBER: 260448

TEST MATERIAL: Propanil

SYNONYMS: Stam Tech

STUDY NUMBER(S): EPA-600/1-79-041

REPORT NUMBER: Contract # 68-01-2458

SPONSOR: Health Effects Research Lab., ORD, USEPA, RTP, No. Carolina 27711

TESTING FACILITY: SRI International, Menlo Park, CA 94025

TITLE OF REPORT: In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides

AUTHOR(S): Vincent F. Simmon

REPORT ISSUED: October, 1979

IDENTIFYING VOLUME: Volume 1 of 1, Tab 5

CONCLUSION: Propanil was not mutagenic in the Salmonella typhimurium and E. coli reverse mutation assays or in the mitotic recombination assay in Saccharomyces cerevisiae. It also tested negatively in the relative toxicity assay in DNA repair-deficient E. coli and in the unscheduled DNA synthesis assay in WI-38 cells. It tested positively in the relative toxicity assay in DNA repair-deficient B. subtilis.

Classification: ACCEPTABLE

A. MATERIALS AND METHODS:

1. Test Material(s)- the following criteria apply for all 5 studies (listed below as letters under #2) unless specified:

Chemical Name: 3',4'-dichloropropionanilide
Description: Not given
Batch #(s), Other #(s): 6-2502
Purity: 88.0%
Source: EPA
Vehicle (if applicable): DMSO
Positive Control(s) (if applicable): beta-propiolactone, 2-anthramine (a); 1,2,3,4-diepoxybutane (b); 1-phenyl-3,3-dimethyltriazine (c); 4-nitroquinoline-N-oxide (4NQO), dimethylnitrosamine (d).
Negative Control(s): chloramphenicol (c)

S-9 metabolic activation mix: Prepared from Aroclor 1254-induced male Sprague-Dawley rats (a), (b); Prepared from liver homogenate of adult Swiss-Webster mice (d); Not used (c).

2. Test Animals and/or Other Test System (if applicable):

Five test systems were described in this study. They are:

(a) Reverse mutation in Salmonella typhimurium (Ames) assay and reverse mutation in E. coli WP2 (obtained from D. McCalla). Salmonella strains TA 1535, TA 1537, TA 1538, TA98 and TA 100 were used (obtained from Dr. Bruce Ames).

(b) Induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3.

(c) Relative toxicity assays in DNA repair-proficient and -deficient strains of E. coli (strains W3110 and p3478, obtained from H. Rosenkranz) and of Bacillus subtilis (strains H17 and M45, obtained from T. Kada).

(d) Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

3. Test Protocols:

For all the microbial assays, propanil was tested at least twice on separate days, using one plate per dose. The first experiment was a test over a wide range of doses to look for toxicity or mutagenicity. If no toxicity or mutagenicity was observed, the second experiment was conducted at higher concentrations. An assay that gave a mutagenic response was always repeated to confirm that the results were reproducible.

a. Salmonella and E. coli reverse mutation assays:

Salmonella - new stock cultures plates were made every 4 to 6 weeks from single colony reisolates that have been checked for their genotypic characteristics and for the presence of plasmid pKM101. For each test, an inoculum from the stock culture was grown up overnight at 37°C. Propanil was then tested in the presence of the indicator organisms according to the Ames assay either with or without metabolic activation (S-9 mix prepared from Aroclor 1254-induced male Sprague-Dawley rats). The following dose levels were tested: 10, 50, 100, 250, 500, 1000 and 5000 micrograms compound/plate. The number of his^+ revertant colonies were counted. A positive response was indicated by a reproducible, dose-related increase in the number of revertants in one or more tester strains. 50 micrograms beta-propiolactone/plate (without activation) and 10 micrograms 2-anthramine/plate (both with and without activation) were used as positive controls. In a third separate test, 2-anthramine was tested at 2.5 micrograms/plate (with activation) as the positive control. A negative control was also used.

E. coli WP2 (uvrA) - the procedure was similar to the Ames procedure. However, the minimal agar was supplemented with a trace of tryptophan required for enhancement of any mutagenic effect of the test chemical. The following dose levels were tested: 1, 10, 50, 100, 500 and 1000 micrograms compound/plate. 2.5 micrograms 2-anthramine/plate with metabolic activation was used as the positive control. A negative control was also used.

b. Saccharomyces cerevisiae mitotic recombination assay:

The tester strain was stored at -80°C. For each assay, the tester strain was inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30°C with aeration. The overnight culture was centifuged, resuspended and added to a test tube containing the test chemical dissolved in DMSO and either buffer or the metabolic activation mixture (same as above). The following dose levels of propanil were tested: 0.1, 0.5, 1.0 and 5.0 % concentration (w/v or v/v) (Experiment 1); and 0.010, 0.025, 0.050 and 0.10 % concentration (Experiment 2). The positive control was 1,2,3,4-diepoxybutane (0.04% w/v or v/v). A negative control was also used. The suspension mixture was incubated at 30°C for 4 hours on a roller drum. Several dilutions were made and spread on tryptone-yeast agar plates, which were in turn incubated for 2 days at 30°C, followed by an additional 2 days at 4°C. The plates were then counted for the number of red colonies (mitotic recombinants). A positive response was indicated by a dose-related increase in the absolute number of mitotic recombinants/ml as well as in the number of mitotic recombinants per 10^5 survivors.

c. E. coli W3110/p3478 and Bacillus subtilis H17/M45 Differential Toxicity of Repair-Proficient and Deficient Microorganisms Assay:

An inoculum from frozen stock cultures of each strain was grown overnight at 37°C in nutrient broth containing 1% tryptone and 0.5% yeast extract. The cultures were then mixed with nutrient broth and agar and poured onto plates. When the plates had solidified, filter discs impregnated with the test substance were placed in the center of the plates. The plates were incubated at 37°C for 16 hours; then the width (diameter) of the zone of inhibition of growth was measured. DMSO was used as a diluent and solvent. The following dose levels of propanil were used: 0.01, 0.10, 1.0 and 5.0 mg in 10 microliters DMSO applied to the disc. 20 micrograms chloramphenicol was used as a negative control and 2 mg 1-phenyl-3,3-dimethyltriazine was used as the positive control. No metabolic activation was used for this assay.

d. Unscheduled DNA Synthesis Assay:

WI-38 cells were used for this assay. Replicate cultures of these cells were initiated, grown to confluency and maintained for 5 or 6 days preceding the assay. The cultures were preincubated for 1 hour with 10^{-2} M hydroxyurea before each assay to reduce the possibility of incorporation of ^{3}H-Tdr by an occasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements

of UDS. The hydroxyurea was also added during each subsequent step of the assay. The cells were incubated at 37°C with dilutions of propanil and with 1 microcurie/ml ^{3}H-TdR. In the absence of metabolic activation, the cells were exposed to the test chemical for 3 hours. For testing with metabolic activation, the cells were exposed to the test chemical, the ^{3}H-TdR and the metabolic activation system for 1 hour. In both cases, the cells were then incubated with ^{3}H-TdR and hydroxyurea without the test chemical for an additional 3 hours. The metabolic activation system consisted of a preparation of the 9000 x g supernatant of a liver homogenate from adult Swiss-Webster mice plus cofactors. DNA was extracted from the cells, the DNA content was measured, and incorporation of the ^{3}H-TdR into the DNA was measured by scintillation counting. The following dose levels of propanil were used in the assay: 0.1, 1.0, 10, 100 and 1000 micrograms/ml, both with and without metabolic activation. 0.5% DMSO was used as the negative control. 10^{-5} M 4NQO was used as the positive control without metabolic activation and 5×10^{-2} M DMN was used as the positive control with metabolic activation.

B. RESULTS:

1. Salmonella typhimurium reversion assay:

Three separate experiments were run with propanil, both with and without metabolic activation. The first two involved all five of the tester strains. The third tested propanil only in TA100. In experiment 1 without metabolic activation, the chemical was toxic in all strains except TA 100 at 1000 micrograms/plate and in all strains at 5000 micrograms/plate (HDT). With metabolic activation, the chemical was toxic in all strains except TA 98 and TA 100 at 1000 micrograms/plate and in all strains at 5000 micrograms/plate (HDT). There was no indication of any mutagenic response at any of the dose levels tested. The positive control, beta-propiolactone induced an increase in histidine revertants/plate at a range of 3 to 62 times the negative controls (without metabolic activation in TA 1535, TA 1537 and TA 100). The positive control, 2-anthramine did not induce an increase in revertants/plate in TA 1538 and TA 98 without metabolic activation, but did induce an increase in revertants/plate at a range of 50 to 77 times in the same two tester strains with metabolic activation.

In experiment 2, propanil was toxic to all strains except TA 1535 at a dose level of 1000 micrograms/plate without metabolic activation (HDT). With metabolic activation, the chemical was toxic to all strains at 1000 micrograms/plate (HDT). There was no mutagenic response at any of the dose levels tested, either with or without metabolic activation. Beta-propiolactone, the positive control, induced a response of 1.7 to 17 times the negative control value without activation in TA 1535 and TA 100. 2-Anthramine, the other positive control did not induce an increase in revertants over the control values without metabolic activation and induced an increase of 11 to 51% the control values with metabolic activation in TA 1537, TA 1538 and TA 98.

In experiment 3, propanil was not toxic at any dose level up to 1000 micrograms/plate in TA 100. In addition, there was no indication of any mutagenic response at any dose level. 2-Anthramine, the positive control induced a response of 1.3 times the negative control with metabolic activation.

2. E. coli WP2 reverse mutation assay:

There was no indication of either toxicity or mutagenicity at any of the dose levels tested, either with or without metabolic activation. The positive control, 2-anthramine with metabolic activation, induced a response of 15 times the negative control.

3. Repair -deficient and -proficient strains of B. subtilis and E. coli - differential toxicity assay:

The authors of the report stated that propanil gave a positive response in this assay. In two separate plates, chloramphenicol, the negative control gave a toxic response in both strains of bacteria (repair-deficient and wild type), B. subtilis and E. coli. The positive control, 1-phenyl-3,3-dimethyl-triazine induced a zone of inhibition in the repair-deficient strain that was greater than the one in the wild type strain in both B. subtilis and E. coli. Propanil induced a greater zone of inhibition in the repair-deficient strain than in the wild type strain for B. subtilis only. The results were response-related, but were not nearly as positive as the positive control. The following table gives the results of the test:

Compound	mg of Compound in 10 microliters of DMSO Applied to Disc	Diameter of Zone of Inhibition (mm)* B. subtilis H17	B. subtilis M45	E. coli W3110	E. coli p3478
Negative control	20 micrograms	24	23	26	27
Chloramphenicol	20 micrograms	38	40	40	40
Positive control 1-phenyl-3,3-dimethyl-triazene	2	12	20	13	20
	2	34	64	36	65
Propanil	0.01	6	6	6	6
	0.10	7	12	6	6
	1.0	8	15	8	8
	5.0	8	16	9	9

* The diameter of the disc was 6 mm

4. Mitotic recombination assay in Saccharomyces cerevisiae:

Two separate experiments were conducted on propanil. In one experiment, propanil was tested at levels of 0.1 to 5.0 % (w/v or v/v) and in the other it was tested at levels of 0.01 to 0.10 %, both with and without metabolic activation. Propanil was toxic at at the 1.0% level and above,

both with and without metabolic activation. The % survival increased from 0 to over 100% (compared to controls) over the dose ranges of both the experiments (0.01 to 5.0%). In the first experiment, the highest number of recombinants/10^5 survivors was at 0.1 % (LDT), both with and without activation. The values were 22 versus 9.6 for controls (with activation) and 30 versus 9.8 (without activation). The authors stated that the results were negative. The positive controls were 2111 and 2063 with and without activation respectively.
In the second experiment, the highest number of mitotic recombinants with activation was at 0.01% (LDT, 9.8 versus 5.6 for controls), and without activation was at 0.1% (HDT, 13.0 versus 5.7 for controls). The positive control gave values of 2505 and 3181 recombinants/10^5 survivors, with and without metabolic activation respectively. Again, the authors stated that the results were negative.

5. Unscheduled DNA synthesis assay:

Five concentrations of propanil were tested both with and without metabolic activation, ranging from 0 to 1000 micrograms/ml. The test sample precipitated at 1000 micrograms/ml. There was no indication of increased unscheduled DNA synthesis at any of the dose levels tested, either with or without metabolic activation. Without activation, the positive control, 4NQO induced a mean of 2458 dpm/microgram DNA whereas the negative control (0.5% DMSO) induced a mean of 141 dpm/microgram DNA. With metabolic activation, the means were 593 for DMN (positive control) versus 136 for DMSO.

C. DISCUSSION:

To summarize the results of the 5 studies on Propanil: Propanil was not mutagenic in the Salmonella typhimurium and E. coli reverse mutation assays or in the mitotic recombination assay in Saccharomyces cerevisiae. It also tested negatively in the relative toxicity assay in DNA repair-deficient E. coli and in the unscheduled DNA synthesis assay in WI-38 cells. It tested positively in the relative toxicity assay in DNA repair-deficient B. subtilis. These studies are ACCEPTABLE. No metabolic activation was used for the differential toxicity studies. However, the results were positive without metabolic activation.

007559

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DATA EVALUATION RECORD

Study Type: 85-1, Metabolism Tox. Chem. No.: 325

Accession Number: N/A MRID No.: 35686

Test Material: C^{14}-Stam (uniformly labeled in the ring)

Synonyms: Propanil, 3',4'-dichloropropionanilide

Study Number(s): Not reported

Sponsor: Rohm & Haas Company

Testing Facility: Medical College of Virginia

Title of Report: Studies on Metabolism of 3',4'-dichloropropionanilide in Rats

Authors(s): Yih, R.Y.; McRae, D.H.

Report Issued: 1965 (Unpublished study received June 11, 1970)

Conclusions:

Approximately 90 to 92 percent of the radioactivity was recovered in urine, feces, and cage washings during the feeding period and the following 2 days. Only small amounts (less than 1%) were found in rat tissues. Several metabolites were found in urine and feces. A major portion of the label were derivatives of 3',4'-dichloroaniline.

Classification:

Core-Supplementary, because:

a. Individual data were not provided;
b. Female rats were not studied; and
c. T 1/2 not determined.

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Special Review Criteria (40 CFR 154.7): N/A

"Radioactive Stam uniformly labeled with C^{14} in the ring was prepared in corn oil. The solution contained 20 mg Stam per 0.4 mL and 5.48 microcurie C^{14} per 0.4 mL or 0.274 millicuries C^{14} per gram of Stam. Six adult male albino rats, averaging 243 g (217 to 265 g), were placed on a control diet for a period of 7 weeks prior to the onset of the metabolism study. Another group of six adult male albino rats, averaging 263 g (250 to 280 g), were placed on a diet containing 400 ppm Stam for a period of 7 weeks prior to the onset of the metabolism study. Twenty-four hours prior to the administration of the C^{14}-Stam, the rats were placed in individual metabolism cages and both groups received a control diet. All rats continued to receive the control diet for the remainder of the experimental period. C^{14}-Stam was administered via stomach tube between 12:00 and 12:45 p.m. each day. Each rat received 0.4 mL of the C^{14}-Stam solution per day for 7 consecutive days. The rats were held for 2 additional days on the control diet after they stopped receiving the C^{14}-Stam. Urine and feces from each rat were collected for each 24-hour period. Total samples from each group were pooled daily and frozen. At the end of the experiment (9 days after the administration of the first dose of C^{14}-Stam), two rats from each group were sacrificed, the intestinal tracts were washed out, and the individual washings frozen. These rats were placed in individual plastic bags and frozen. The remaining four rats from each group were sacrificed. Blood was drawn from the heart and pooled, and livers, kidneys, spleens, fat, muscle, hearts, and brains removed and frozen. Intestinal washings from these rats were also collected and frozen. The cages were washed down with a small quantity of water on the fourth and ninth day after the administration of the first dose of C^{14}-Stam. The washings from the individual cages of each group were pooled and frozen." (End of quote.)

The wastes and organs were radioassayed to obtain a material balance and studies were carried out to determine the number, amount, and identity of metabolites present.

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Results:

The distribution and recovery of C^{14} from the two series of rats are shown below as presented in the report:

Material Balance of C^{14}-Stam-Fed Rats

C^{14} Recovered

Fractions	C^{14}-Stam-Fed Only Short Period Feeding	Previously Fed Cold Stam, Then C^{14}-Stam (Long Period Feeding)
	%	%
Urine	57.84	48.77
Feces	20.94	17.59
Cage washings	10.89	24.20
Intestinal washings	0.17	0.42
Rat body	0.57	1.34
Total Recovered	90.41	92.32

It can be seen that total radioactivity recovered ranged from 90 to 92 percent. Additionally, there was no significant difference in the amount of C^{14} recovered between a long and a short period of feeding.

Although C^{14} was distributed in all of the organs, the percent of C^{14} and the amount in micrograms varied in different organs, as presented in the report:

The Distribution of Radioactivity in Organs of C^{14}-Stam-Fed Rats

Organs	C^{14}-Stam Treated (%)	C^{14}-Stam Treated (ug)	Previously Treated Cold Stam, Then C^{14}-Stam (%)	Previously Treated Cold Stam, Then C^{14}-Stam (ug)
Heart	0.0009	1.26	0.0010	1.41
Spleen	0.0028	4.09	0.0021	2.96
Liver	0.0841	117.87	0.0886	124.02
Skeletal muscle	0.0055	7.79	0.0052	7.23
Fat	0.0018	2.57	0.0019	2.73
Brain	0.0004	0.49	0.0004	0.57
Kidney	0.0108	15.22	0.0061	8.46
Blood	0.0135	18.95	0.0105	14.85
Total	0.1198	168.24	0.1158	162.23

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It can be seen that the liver contained the major amount of radioactivity. The concentrations of C^{14} varied in different organs as follows:

Liver > blood > kidney > skeletal muscle > spleen > fat > heart > brain.

"Six metabolites were found in the urine from Stam-fed rats, in addition to low concentrations of free Stam and free 3,4-dichloroaniline. About 40 percent of the C^{14} was recovered in one metabolite with lesser amounts, down to a low of 3 percent, in the other metabolites. Approximately 5 percent of the total radioactivity found in the urine was present as Stam and 3,4-dichloroanaline. Hydrolysis of urine showed that four of the six metabolites were derivatives of 3,4-dichloroaniline and two of the metabolites were derivatives of 2-hydroxy-4,5-dichloroaniline. The latter aniline was unstable and was found difficult to work with. A major metabolite was tentatively identified as 2-hydroxy-4,5-dichloroaniline N-glucuronide but the other hydroxyaniline derivative was not characterized. One of the 3,4-dichloroaniline derivatives was shown to be 3,4-dichloroactanilide. The other three 3,4-dichloroaniline derivatives were not identified with certainty.

"Four metabolites were found in the feces. The major metabolite was shown to be 3',4'-dichloroactanilide. Two other major metabolites were not completely characterized. One was chromatographically comparable to 2-hydroxy-4,5-dichloroaniline N-glucuronide found in the urine. The other appeared to be an aniline but was not chromatographically comparable to 3,4-dichloroaniline or a hydroxyaniline. The fourth metabolite was present at a very low level and has not yet been obtained in sufficient quantity to determine its nature.

"Two organic solvent soluble compounds were recovered from the rat liver. One was identified as 3,4-dichloroaniline and the other was chromatographically comparable to the aniline-like compound found in the feces." (End of quote.)

Conclusion:

Approximately 90 to 92 percent of the radioactivity was recovered in urine, feces, and cage washings during the feeding period and the following 2 days. Only small amounts (less than 1%) were found in rat tissues. Several metabolites were found in urine and feces. A major portion of the label were derivatives of 3',4'-dichloroaniline.

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Classification:

Core-Supplementary, because:

a. Individual data were not provided,
b. Female rats were not studied; and
c. T 1/2 not determined.

William Dykstra
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