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

005305

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUL 15 1986

MEMORANDUM

SUBJECT: 707-75. Propanil. Review of Mutagenicity Studies for Registration of Stampede™ 3E Herbicide on Barley and Oats.

Tox. Chem. No. 325
Project No. 1231

TO: Robert Taylor (PM Team #25)
Registration Division (TS-767c)

FROM: Pamela M. Hurley, Toxicologist Pamela M Hurley
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Record No. 164402

Rohm and Haas Company submitted a package of mutagenicity studies requested by the Agency as a condition for registration of Stampede™ 3E herbicide on barley and oats. The Toxicology Branch (TB) was requested to review the submitted studies. The attached table summarizes the studies and the results of the TB evaluation.

Table I

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Study	Results	Classification
In vivo cytogenetic study in mice	Stam(pede) did not induce chromosomal aberrations in mouse bone marrow cells.	Reserved pending submission of additional data[1]
CHO/HGPRT gene mutation assay	Stam was not mutagenic under the conditions of the assay.	Acceptable
DNA damage and repair rec assay B. subtilis	Propanil did not appear to induce DNA damage under the conditions of the assay.	Unacceptable[2]
Reverse mutation assay (Ames) and E. coli	Propanil was not mutagenic under the conditions of the assays.	Minimally Acceptable
Reverse mutation assay (Ames) and E. coli	Propanil was not mutagenic under the conditions of the assays.	Acceptable
DNA damage/repair in yeast (mitotic recombination in S. cerevisiae D3)	Propanil was not mutagenic under conditions of the assay.	Acceptable
DNA damage/repair in bacteria (B. subtilis/E. coli)	Propanil tested negatively under the conditions of the assay for E. coli and positively for B. subtilis.	Acceptable
DNA damage/repair in mammalian cells (UDS in WI-38)	Propanil tested negatively under the conditions of the assay.	Acceptable

1. In this study, 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 hour 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.

2. Minimal information was submitted on this assay. The methods section was too brief. 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 once. Finally, no means of metabolic activation was used in the assay.

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Reviewed by: Pamela Hurley
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Secondary Reviewer: Edwin Budd
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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 RJ, 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)

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 (1 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.

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.

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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: 87.8%
Source: Not given (assumed Rohm and Haas)
Vehicle (if applicable): Dimethyl sulfoxide (DMSO)
Positive Control(s) (if applicable): Ethyl methanesulfonate (EMS) for unactivated 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_4 Cell line

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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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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-nitro-fluorene (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.

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-Aminoanthracene 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.

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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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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 mammaliam 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 St dies 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)

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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

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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 x 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 1.8 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.