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

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

JUN 21 1990

PC 069001

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id #69001. Pyrethrin Extract: Review of mutagenicity (Ames Test) and chromosome aberration study and overview of mutagenicity/genotoxicity testing.

TOX CHEM No.: 715
TOX PROJECT No.: 0-0582
Record No.: 258653

FROM: John Doherty John Doherty 6/6/90
Section I, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda DeLuise
Product Manager Team #50
Special Review and Reregistration Division
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THROUGH: Roger Gardner Roger Gardner 6-15-90
Acting Section Head
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Health Effects Division (H7509C)

The Chemical Specialty Manufacturers Association (CSMA) on behalf of the Pyrethrin Joint Venture has submitted a mutagenicity study with Salmonella typhimurium strains (an Ames Test) and a chromosome aberration study with Chinese Hamster Ovary Cells in order to partially fulfil the Agency's requirements to provide a study from each of the three categories of mutagenicity testing. The two studies were reviewed and the DERs are attached. An unscheduled DNA synthesis study was previously submitted and reviewed by Toxicology Branch I (TB-I).

All three studies were determined to be ACCEPTABLE by current standards and are listed in the following table. Since the registrant has provided at least one study from each of the three categories of mutagenicity/genotoxicity testing, no additional testing is required at this time as per discussion with Dr. Irving Mauer, geneticist, HED.

Study	Results/Classification
Category I	
Bacterial mutagenicity study in Salmonella typhimurium (Ames Test). MIcrobiological Associates #T8729.501014, 12/28/89. MRID #413447-01	No evidence of mutagenicity strains TA98, TA100, TA1535, TA 1537 and TA 1538 with and without metabolic activation at dose levels of 292, 585, 877, 2924, 5848 and 8772 ug/plate. ACCEPTABLE (DER attached).
Category II	
Chromosome aberration study in Chinese hamster ovary cells. Microbiological Associates #T8729.337, 12/28/89. MRID #413466-01.	No evidence of chromosome aberrations in either the presence (dose levels 0.04, 0.08, 0.16 and 0.32 ul/ml) or absence (0.01, 0.02, 0.04 and 0.08 ul/ml) of metabolic activation. ACCEPTABLE (DER attached).
Category III	
Unscheduled DNA synthesis in rat liver hepatocytes. Microbiological Associates #T8729.380009, 12/22/89.	Not demonstrated to increase net nuclear grain counts over the dose range of 0.03 to 1.0 ul/ml. ACCEPTABLE (DER with Tox Project No.: 0-0642).

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Reviewed by: John Doherty 5/31/90
Section I, Toxicology Branch, Health Effects Division (H7509C)
Secondary reviewer: Irving Mauer, Ph.D.
Geneticist, Health Effects Division (H7509C) 05 31-90

DATA EVALUATION REPORT

STUDY TYPE: 84-2. Mutagenicity (Category 1)

MRID NO.: 413447-01 TOX. CHEM. NO.: 715

TEST MATERIAL: Pyrethrum Extract: Blend PTK-99 obtained from Pyrethrum Joint Venture, Chemical Specialties Manufacturers Association.

TEST SYSTEM: Tester strains of Salmonella typhimurium histidine auxotrophs TA98, TA100, TA1535, TA1537 and TA1538 were supplied (originally from Dr. Bruce Ames of the University of California, Berkeley). Microsomal enzymes were prepared from rat liver from Aroclor treated rats by a method described in the study report.

STUDY NUMBER(S): Lab Study # T8729.501014

SPONSOR: Pyrethrum Joint Venture and Chemical Specialty Manufacturers Association

TESTING FACILITY: Microbiological Associates, Inc. Rockville, Md.

TITLE OF REPORT: "Salmonella/Mammalian Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay"

AUTHOR(S): R.H.C. San and K.A. Springfield

REPORT ISSUED: 12/28/89

CONCLUSIONS:

No evidence of mutagenicity in strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation at dose levels of 292, 585, 877, 2924, 5848 and 8772 ug/plate.

Classification: ACCEPTABLE.

Quality Assurance Statement: A statement signed by Paula E. Gentry of the Quality assurance Department attested that four inspections of the study were made. No deficiencies in the conduct of the study were indicated by the Quality Assurance Statement.

REVIEW

The experimental techniques and procedures were based on the methods originally published by Ames. In this study there were two phases: a dose range finding study and the main study which also included a confirmatory assay.

In the dose range finding study, ten dose levels of the test material in acetone were plated at one plate per dose with an overnight culture of TA100 in both the presence and absence of microsomal enzymes. The dose levels tested ranged from 8.8 to 8772 ug of test material per plate and was administered in 50 ul of acetone. There was no appreciable toxicity due to the pyrethrum extract at any dose level tested. Dose levels of 585 ug/plate resulted in precipitate.

In the main study, pyrethrum extract was tested at dose levels of 292, 585, 877, 2924, 5848 and 8772 ug/plate in each of the five tester strains with and without metabolic activation. After plating the mixture of agar, tester strain, test substance or positive control, the plates were incubated for 48 hours at 37 $\pm$ 2° C. Following incubation the samples were counted by either hand or by an automatic colony counter. Each test condition was assessed in triplicate and the results of each of the three replicates were reported.

The following positive control substances were used:

2-aminoanthracene: TA98, TA100, TA1535, TA1537 and TA1538 with metabolic activation.

2-nitrofluorene: TA98, and TA1538 without metabolic activation.

sodium azide: TA100 and TA 1535 without metabolic activation.

ICR-191: TA1537 without metabolic activation.

The summary of the results from both the initial and the confirmatory studies are shown in the accompanying tables. The positive controls produced the expected positive results for each condition. There was no evidence that the pyrethrum extract produced a positive response for any strain in either the presence or absence of metabolic activation. Some apparent increases were present such as a doubling of the revertants per plate but no dose response was evident and the observation was not confirmed in the confirmatory assay. The tester strain TA100 does have the appearance of dose related increases for both the initial and confirmatory tests especially in the presence of the microsomal activation system. The criteria that the test substance must double the solvent control response was, however, not met and the readings for the pyrethrum treated plates were

within the range for spontaneous revertants.

Inspection of the individual tables reporting the study results indicates that for the initial study there was either a slight or moderate precipitate formed at dose levels of 877 ug/plate and above. In the confirmatory study there was a slight or moderate precipitate at 292 ug/plate (the lowest dose tested) and above. The testing laboratory did not provide an explanation for this discrepancy and does not make any issue that the presence of the precipitate compromises the study results.

CONCLUSION. This study is ACCEPTABLE. The study demonstrated that pyrethrum extract does not induce revertants in any of the tester strains in either the presence or absence of metabolic activation.

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article Id : Pyrethrum Extract; Blend FEK-99
Study Number : T8729.501014 Experiment No : B2

Average Revertants Per Plate* ± Standard Deviation

Liver Microsomes: None

Dose (ug)	TA98	TA100	TA1535	TA1537	TA1538
0.0**	10 ± 1	109 ± 6	10 ± 5	4 ± 3	5 ± 1
292	12 ± 2	99 ± 7	7 ± 4	4 ± 2	5 ± 2
585	15 ± 1	137 ± 24	10 ± 4	5 ± 2	5 ± 2
877	11 ± 4	124 ± 16	12 ± 6	3 ± 1	6 ± 3
2924	12 ± 2	125 ± 6	9 ± 1	8 ± 2	5 ± 2
5848	16 ± 3	148 ± 12	12 ± 4	3 ± 2	6 ± 3
8772	20 ± 4	152 ± 9	10 ± 1	5 ± 3	5 ± 2
Pos***	221 ± 9	394 ± 50	271 ± 2	185 ± 23	413 ± 16

Liver Microsomes: Rat liver S-9

Dose (ug)	TA98	TA100	TA1535	TA1537	TA1538
0.0**	20 ± 6	136 ± 12	13 ± 7	5 ± 2	15 ± 2
292	23 ± 7	133 ± 8	12 ± 4	4 ± 2	9 ± 4
585	20 ± 2	141 ± 20	14 ± 3	7 ± 1	11 ± 2
877	23 ± 5	133 ± 6	12 ± 1	4 ± 2	10 ± 2
2924	23 ± 4	146 ± 10	12 ± 1	3 ± 1	11 ± 7
5848	21 ± 1	150 ± 8	9 ± 2	5 ± 1	13 ± 4
8772	21 ± 5	155 ± 5	10 ± 1	5 ± 4	11 ± 5
Pos***	363 ± 28	523 ± 18	61 ± 11	58 ± 3	489 ± 26

* Average and standard deviation from replicate platings

** 0.0 = Vehicle control

*** Pos = Positive Control concentrations as specified in Materials and Methods section.

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article Id : Pyrethrum Extract; Blend FEK-99
Study Number : T8729.501014 Experiment No : B3

Average Revertants Per Plate* ± Standard Deviation

Liver Microsomes: None

Dose (ug)	TA98	TA100	TA1535	TA1537	TA1538
0.0**	41 ± 7	94 ± 6	6 ± 2	4 ± 2	5 ± 2
292	27 ± 2	87 ± 10	7 ± 3	5 ± 3	3 ± 2
585	42 ± 7	94 ± 1	6 ± 1	5 ± 3	5 ± 4
877	42 ± 11	98 ± 3	4 ± 2	4 ± 2	4 ± 4
2924	48 ± 9	118 ± 18	8 ± 3	2 ± 3	3 ± 1
5848	48 ± 14	127 ± 8	7 ± 2	4 ± 2	3 ± 1
8772	25 ± 7	157 ± 10	6 ± 4	3 ± 1	2 ± 2
Pos***	274 ± 77	337 ± 60	285 ± 24	96 ± 13	296 ± 11

Liver Microsomes: Rat liver S-9

Dose (ug)	TA98	TA100	TA1535	TA1537	TA1538
0.0**	45 ± 9	115 ± 14	10 ± 4	6 ± 2	8 ± 4
292	61 ± 6	122 ± 9	8 ± 4	2 ± 2	10 ± 1
585	59 ± 10	136 ± 4	8 ± 2	6 ± 2	6 ± 2
877	53 ± 4	123 ± 6	8 ± 3	3 ± 2	8 ± 1
2924	48 ± 11	155 ± 7	10 ± 1	5 ± 4	10 ± 1
5848	65 ± 7	167 ± 9	8 ± 1	6 ± 2	8 ± 2
8772	48 ± 22	182 ± 20	9 ± 2	4 ± 2	6 ± 2
Pos***	660 ± 34	731 ± 53	68 ± 6	73 ± 6	486 ± 19

* Average and standard deviation from replicate platings

** 0.0 = Vehicle control

*** Pos = Positive Control concentrations as specified in Materials and Methods section.

Reviewed by: John Doherty 6/6/90
Section I, Toxicology Branch I, Health Effects Division (H7509C)
Secondary reviewer: Irving Mauer, Ph.D.
Geneticist, Health Effects Division (H7509C) 6-6-90

DATA EVALUATION REPORT

STUDY TYPE: 84-2. Mutagenicity (Category II)

MRID NO.: 413446-01 TOX. CHEM. NO.: 715

TEST MATERIAL: Pyrethrum Extract, blend FEK-99 received from the Fairfield American Corporation.

TEST SYSTEM: Chinese Hamster Ovary (CHO-K_1) cells were obtained from American Type Culture Collection, Rockville, Md. Metabolic activation system obtained from the liver from Aroclor induced rats and preparation of the S-9 supernatant.

STUDY NUMBER(S): Laboratory Study # T8729.337

SPONSOR: Pyrethrum Joint Venture and Chemical Specialties Manufacturers Association, Washington, D.C.

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD.

TITLE OF REPORT: "Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells".

AUTHOR(S): Donald L. Putnam and Marcia J. Morris

REPORT ISSUED: 12/28/89

CONCLUSIONS:

No evidence of increased chromosome aberrations at dose levels of 0.01, 0.02, 0.04, and 0.08 ul/ml in the absence of activation and 0.04, 0.08, 0.16, and 0.32 ul/ml in the presence of metabolic activation.

Classification: ACCEPTABLE.

Quality Assurance Statement: A statement signed by Joan C. McGown attested that three inspections were made. No discrepancies in the conduct or reporting of the study were indicated by the QAS.

REVIEW

Preliminary Cytotoxicity Assay

In this study nine concentrations of the pyrethrum extract (not corrected for purity) ranging from 0.0005 to 0.5 ul/ml were incubated with CHO cells (approximately 5 x 10^5 cells/25 cm^2 flask) at 37 $\pm$ 1° C in a humidified atmosphere for 16-20 hours. Separate experiments were run to include the presence and absence of the metabolic activation system. The test volumes were 5 ml of medium (with or without the S-9 preparation) to which was added 50 ul of test material in DMSO. Two hours after initiation of treatment a 50 ul aliquot of 1 mM BrdU was added to each flask and incubation was continued. After two or six hours (the exposure/treatment period for the activated and nonactivated systems respectively), the medium was removed, the cells washed with PBS and refed with 5 ml of complete medium containing 0.01 mM BrdU and reincubated for a total of 24 hours from BrdU treatment. Two hours prior to harvest by trypsinization, Colcemid was added to each flask to make a final concentration of 0.1 ug/ml. Metaphase preparations were prepared and stained for sister chromatid differentiation using a modified Geisma technique. The slides were evaluated for the percentage of first, second and third division metaphase cells for estimation of the test article effect on cell cycle kinetics. The mitotic index was determined for each treatment condition as the percentage of mitotic cells in a population of 500 cells scored.

The preliminary cytotoxicity study indicated that the test material was partially insoluble at dose levels of 0.5, 1.5 and 5 ul/ml. The dose levels (uncorrected for purity) of 0.005, 0.01, 0.02, 0.04, 0.08 ul/ml were selected for the non-activated study based on the 35% at 0.05 ul/ml and 92% change at 0.15 ul/ml in the mitotic index. The dose levels of 0.02, 0.04, 0.08, 0.16 and 0.32 ul/ml were selected for the study with metabolic activation based on 32% and 97% change in the mitotic index at 0.15 and 0.5 ul/ml respectively.

Chromosome Aberration Study

Duplicate preparations of 5 x 10^5 cells/25 cm flask were preincubated and treated by refeeding the cells with either 5 ml medium or medium containing the S-9 supernatant to which was added 50 ul of stock test solution in DMSO for a 1:100 dilution. In the non-activation assay the cells were exposed for 18 hours at 37 $\pm$ 1° C in a humidified atmosphere of 5 $\pm$ 1% CO_2 . Two hours prior to scheduled harvest, the treatment medium was removed in order to avoid interferences of the test article with cell collection and fixatation and the cells were washed with PBS and refed with medium containing 0.1 ug/ml of Colcemid. The S-9

activated cells were treated for only two hours with the test material before the media was removed and the cells washed and returned to the incubator for an additional 16 hours. After 16 hours, Colcemid was added to the duplicate samples at a final concentration of 0.1 ug/ml.

After a total of 20 hours of incubation (2 hours with Colcemid), the cells were harvested by trypsinization and centrifugation (800 rpm for 5 min). The cell pellet was resuspended in 2-4 ml of 0.075 M KCl and allowed to stand at room temperature for 5 minutes. The cells were collected by centrifugation and the supernatant aspirated and the cells fixed with two washes of methanol:glacial acetic acid fixative and stored overnight at approximately 4°C. The fixed cells were then prepared on slides, stained with 5% Giesma, dried and mounted.

Metaphase cells with 20 $\pm$ 2 centromeres were examined under oil immersion. The protocol called for reading a minimum of 100 metaphase spreads (50 per duplicate flask) to be read and scored for chromatid type and chromosome type aberrations.

The positive controls used were triethylenemelamine (TEM, 0.5 ug/ml) for the non-activation system and cyclophosphamide (CP, 5 mg/ml) for the S-9 activation study.

Results

Table 3 (non-activation system) and Table 4 (S-9 activation system) xeroxed from the study report and attached illustrate the results of this study. Both of the positive controls produced the expected positive results as indicated by the increase in aberrant cells, gaps, breaks and exchanges.

None of the test article concentrations exceeded the criteria for a positive response. In addition to the four dose levels of pyrethrum extract shown in these tables, dose levels of 0.005 for the non-activated system and 0.02 for the activated system were also tested and did not result in evidence of chromosome aberrations.

CONCLUSION. This study is ACCEPTABLE. Sufficient data were generated to demonstrate that pyrethrum extract does not induce chromosome aberrations under the conditions of this assay.

TABLE 3

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH PYRETHRUM EXTRACT; BLEND FEK-99 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Flask	Mitotic Index[2] (%)	Cells Scored	Aberrant Cells[3] (%)	Total Number of Structural Aberrations						Severely Damaged Cells[6]	Average Aberrations Per Cell[3,7]
					Gaps	Chromatid-type[4] Breaks	Chromatid-type[4] Exch	Chromosome-type[5] Breaks	Chromosome-type[5] Dic	Chromosome-type[5] Ring		
20 Hour Harvest												
Untreated cells	A	4.2	50	0	0	0	0	0	0	0	0	0.000
	B	5.2	50	2	0	0	0	0	1	0	0	0.020
DMSO	A	2.2	50	0	0	0	0	0	0	0	0	0.000
	B	4.6	50	0	1	0	0	0	0	0	0	0.000
Pyrethrum Extract; Blend FEK-99												
0.01 ul/ml	A	4.6	50	0	2	0	0	0	0	0	0	0.000
	B	2.8	50	0	2	0	0	0	0	0	0	0.000
0.02 ul/ml	A	1.8	50	0	0	0	0	0	0	0	0	0.000
	B	1.8	50	4	0	0	0	0	2	0	0	0.040
0.04 ul/ml	A	4.2	50	0	0	0	0	0	0	0	0	0.000
	B	4.8	50	0	0	0	0	0	0	0	0	0.000
0.08 ul/ml	A	0.8	50	0	0	0	0	0	0	0	0	0.000
	B	0.6	50	4	2	1	0	0	0	1	0	0.040
TEM,	A	1.8	50	40	7	15	9	4	0	0	0	0.560
0.5 ug/ml	B	1.6	50	44	10	12	15	3	0	0	0	0.600

[1] CHO cells were treated for 18 hours at 37±1°C in the absence of an exogenous source of metabolic activation.

[2] Mitotic index = number mitotic figures x 100/500 cells counted.

[3] Excluding cells with only gaps.

[4] Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures include quadriradials, triradials and complex rearrangements.

[5] Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.

[6] Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

[7] Severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 4

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH PYRETHRUM EXTRACT; BLEND-99 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Flask	Mitotic Index[2] (%)	Cells Scored	Aberrant Cells[3] (%)	Total Number of Structural Aberrations							Average Aberrations Per Cell[3,7]
					Gaps	Chromatid-type[4] Breaks	Chromatid-type[4] Exch	Chromosome-type[5] Breaks	Chromosome-type[5] Dic	Chromosome-type[5] Ring	Severely Damaged Cells[6]	
20 Hour Harvest												
Untreated cells	A	6.6	50	0	0	0	0	0	0	0	0	0.000
	B	6.6	50	2	0	1	0	0	0	0	0	0.020
DMSO	A	5.8	50	0	0	0	0	0	0	0	0	0.000
	B	7.6	50	2	0	1	0	0	0	0	0	0.020
Pyrethrum Extract; Blend FEK-99												
0.04 ul/ml	A	9.2	50	0	0	0	0	0	0	0	0	0.000
	B	6.4	50	2	0	0	0	0	1	0	0	0.020
0.08 ul/ml	A	5.0	50	2	0	3	0	0	0	0	0	0.060
	B	5.8	50	0	0	0	0	0	0	0	0	0.000
0.16 ul/ml	A	3.2	50	4	0	0	0	0	2	0	0	0.040
	B	3.8	50	2	1	0	0	0	1	0	0	0.020
0.32 ul/ml	A	0.2	26	4	0	0	1	0	0	0	0	0.038
	B	0.4	40	5	0	0	1	1	0	0	0	0.050
CP, 50ug/ml	A	5.0	50	22	3	5	5	1	0	1	0	0.240
	B	7.6	50	22	0	6	4	2	0	0	0	0.240

[1]CHO cells were treated for 2 hours at 37±1°C in the presence of an exogenous source of metabolic activation.

[2]Mitotic index = number mitotic figures x 100/500 cells counted.

[3]Excluding cells with only gaps.

[4]Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures include quadriradials, triradials and complex rearrangements.

[5]Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.

[6]Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

[7]Severely damaged cells and pulverizations were counted as 10 aberrations.