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

008308

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 690021. Pyrethrins: Review of an unscheduled DNA synthesis study (Genotoxicity Category III) with rat hepatocytes.

TOX CHEM No.: 715
TOX PROJECT No.: 0-0642
Record No.: 259162

FROM: John Doherty 5/31/90
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The Chemical Specialty Manufacturers Association on behalf of the Pyrethrin Joint Venture has submitted an unscheduled DNA synthesis genotoxicity assay with pyrethrum extract in order to partially fulfill the requirements for mutagenicity/genotoxicity testing. This study was reviewed and determined to be ACCEPTABLE. Refer to DER attached.

Additional mutagenicity/genotoxicity studies with pyrethrum extract have also been submitted under separate cover and these will be reviewed and returned to Special Review and Reregistration at a later time. When all studies thus far submitted have been reviewed, an overview of the mutagenicity/genotoxicity studies will be prepared and needs for additional testing (if any) will be determined.

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Reviewed by: John Doherty 3/19/91
Section I, Toxicology Branch I, 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 III)

MRID NO.: 413445-01 TOX. CHEM. NO.: 715

TEST MATERIAL: Pyrethrum Extract, Blend FEK-99 obtained from the Fairfield American Co.

TEST SYSTEM: Rat primary hepatocytes derived from the livers of normal adult Fisher 344 rats obtained from the Charles River Co..

STUDY NUMBER(S): Lab # T8729.380009

SPONSOR: Pyrethrum Joint Venture/Chemical Specialties Manufacturers Association.

TESTING FACILITY: Microbiological Associates, Rockville, Maryland.

TITLE OF REPORT: "Unscheduled DNA synthesis assay in rat primary hepatocytes with a confirmatory assay".

AUTHOR(S): Rodger D. Curren, Ph.D.

REPORT ISSUED: December 22, 1989

CONCLUSIONS:

Not demonstrated to increase net nuclear grain counts over the dose range of 0.03 to 1.0 ul/ml.

Classification: ACCEPTABLE.

Quality Assurance Statement: A statement signed by Joan McGowan attested that four inspections were made. No deficiencies in the conduct of the study or reporting of the data were indicated by the QAS.

REVIEW

In this study pyrethrum extract was evaluated for its potential to induce unscheduled DNA synthesis by using a method as described by G.M. Williams (Cancer Research 37:1845-1851, 1977). The indicator cells were obtained from rat liver from rats sacrificed with metofane. The liver was perfused with 0.5 mM EGTA solution and than with a collagenase solution. The liver

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was removed from the animal and cells dissociated, counted and seeded into 35 mm dishes (approximately 500,000 viable cells per dish in a 2 ml volume). The cultures were incubated at 37 degrees for 90 to 180 minutes, washed with medium and refed with serum free medium and used for the test.

Preliminary Cytotoxicity Testing.

Before the main test, preliminary cytotoxicity tests were run. In these studies, the test material was dissolved in acetone to produce concentrations of 1000, 300, 100, 30, 10, 3.0, 1.0, 0.3, 0.03 and 0.01 ul/ml. 20 ul of each solution were added to the culture dishes to produce a 1:100 dilution. Eighteen to 20 hours later the cultures were assessed for lactic dehydrogenase (LD) activity. The relative toxicities of the test solutions were obtained by comparing the LD activity in the treated cultures with the LD activity of the solvent treated cultures.

The preliminary test indicated that the test material was immiscible in the culture medium at 1 ul/ml and above. The pyrethrum extract was also determined to interfere with the LD assay and the LD data had to be normalized by including a test condition consisting of test article plus 1% triton which resulted in 100% lysis. Based on these studies it was determined that the highest practical concentration of pyrethrum extract for testing would be 3.0 ul/ml, a condition that may result in about 80% relative toxicity.

Definitive Testing.

Twenty ul of pyrethrum extract solution in acetone were placed with the 2 ml of cells and ^{3}H thymidine (final concentration 10 uCi/ml) were also added and 18-20 hours were allowed for reactions. Separate preparations of the positive control (7,12-diimethylbenz(a)-anthracene, DMBA) dissolved in DMSO were also prepared. Three cultures of each condition were prepared. After the incubation period, samples (2) were taken for parallel cytotoxicity testing. The cells were washed in serum free medium, swelled in 1% sodium citrate and fixed in ethanol-acetic acid fixative. The cover slips were prepared for radioassay and coated with Kodak NTB emulsion and stored in a refrigerator for eight days.

The slides were read blind on an Artek Colony Counter. Nuclear grains were counted in 50 cells at random on each of three cover slips per treatment. The net nuclear counts were determined by counting three nucleus-sized areas adjacent to each nucleus and subtracting the average cytoplasmic count form the nuclear count. Nuclei exhibiting replicative synthesis and from cells exhibiting toxic effects were not counted.

Two tests were run, a first assay and a confirmatory assay. The first assay tested dose levels of 3.0, 1.0, 0.6, 0.3,

0.1, and 0.03. The critical endpoint for evaluation of an effect of the test material was "average net grains per nucleus". The results of both test are summarized in the following table.

Dose level	Grand Mean for average net grains/nucleus[1] Initial Study	Confirmatory Study
Solvent Control	-1.4 ± 2.5	-0.1 ± 1.8
BMBA (3ug/ml)	20.7 ± 6.7*	6.8 ± 4.2*
0.03 ul/ml	-0.3 ± 1.9	-0.3 ± 1.7
0.10 ul/ml	0.2 ± 1.9	0.0 ± 2.1
0.30 ul/ml	0.5 ± 1.8	0.0 ± 1.7
0.60 ul/ml	-0.4 ± 2.8	0.0 ± 1.6
1.0 ul/ml	1.9 ± 3.2	-0.3 ± 2.2
3.0 ul/ml	Too Toxic	

*Meets test criteria for being significant: i.e the mean count is at least 5 counts over the control.
[1] Based on the average from three slides from three separate preparations which counted 50 nulcei.

The above table indicates that the test material pyrethrum extract did not demonstrate a positive response.

There were some possible indications of a test chemical effect when the cells with 5 or more net nuclear grains are examined. For example in the initial study at 0.6 ul/ml (4%) and at 1.0 ul/ml (17% cells) vs 0% in the controls. This was not reproduced in the confirmatory assay (see table attached). In both the initial and confirmatory assays the positive control substance DMBA produced the expected positive result.

CONCLUSION. This study is ACCEPTABLE. The data generated indicate that the pyrethrum extract did not induce unscheduled DNA synthesis under the conditions of this assay.

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TABLE 5
SUMMARY OF THE REPEAT UDS ASSAY
PYRETHRUM EXTRACT; BLEND FEK-99

TREATMENT	RELATIVE SURVIVAL	SLIDE DESIGNATION	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS	S.D.	GRAND MEAN	S.D.	PERCENT CELLS WITH 5 OR MORE NET NUCLEAR GRAINS
Pyrethrum Extract; Blend FEK-99								
3.0 ul/ml	14%			Too Toxic to be Evaluated for UDS				
1.0 ul/ml	51%	35A	50	-1.3	+/- 2.3	-0.3	+/- 2.2	0%
		35C	50	0.2	+/- 2.0			
		35C	50	0.1	+/- 1.9			
0.6 ul/ml	92%	34A	50	-0.3	+/- 1.8	0.0	+/- 1.6	0%
		34B	50	-0.2	+/- 1.6			
		34C	50	0.2	+/- 1.3			
0.3 ul/ml	99%	32A	50	-0.4	+/- 1.6	0.0	+/- 1.7	0%
		32B	50	-0.2	+/- 1.6			
		32C	50	0.7	+/- 1.7			
0.1 ul/ml	100%	36A	50	-0.7	+/- 2.0	0.0	+/- 2.1	1%
		36B	50	-0.1	+/- 1.8			
		36C	50	-0.9	+/- 2.1			
0.03 ul/ml	101%	33A	50	0.0	+/- 1.7	-0.3	+/- 1.7	0%
		33B	50	-0.4	+/- 1.5			
		33C	50	-0.4	+/- 1.8			
DMBA								
10 ug/ml	81%	12A	50	17.6	+/- 6.5	16.9*	+/- 6.4	98%
		12B	50	17.3	+/- 6.4			
		12A	50	15.8	+/- 6.3			
3.0 ug/ml	90%	10A	50	5.1	+/- 2.8	6.8*	+/- 4.2	67%
		10B	50	8.5	+/- 5.1			
		10B	50	6.9	+/- 3.5			
DMSO (Solvent Control for DMBA)								
10 ul/ml	100%	17A	50	-0.5	+/- 1.0	-1.1	+/- 1.5	0%
		17B	50	-0.8	+/- 1.1			
		17C	50	-2.1	+/- 1.7			
Acetone (Solvent Control for the Test Article)								
10 ul/ml	100%	14A	50	0.4	+/- 1.6	-0.1	+/- 1.8	0%
		14B	50	-0.7	+/- 1.8			
		14C	50	0.6	+/- 1.6			
MME (Media Control)								
	102%	11A	50	-1.3	+/- 1.4	-1.4	+/- 1.4	0%
		11B	50	-1.3	+/- 1.2			
		11C	50	-1.5	+/- 1.6			

Relative survival = 100% - relative toxicity
S.D. Standard Deviation
* Significant (See Protocol: Section 8.0, Evaluation of Test Results)

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END