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Chemical:	**Pyrethrins**
PC Code:	**069001**
HED File Code	**13000 Tox Reviews**
Memo Date:	**06/11/1997**
File ID:	**TX012258**
Accession Number:	**412-01-0083**

HED Records Reference Center
01/11/2001

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

JUN 11 1997

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum:

Subject: EPA Id No.: 069001. Pyrethrins: Review of a chromosome aberration study in CHO cells.

PC Number: 069001
Tox Chem No.: 715
DP Barcode: D227458
Submission No.: S507244

From: John Doherty 6/11/97
Toxicology Branch II
Health Effects Division 7509C

To: Karen Whitby
Risk Characteriation and Analysis Branch
Health Effects Division 7509C

Through: K. Clark Swentzel 6/11/97
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Toxicology Branch II
Health Effects Division 7509C

I. Conclusions.

A series 84-2 chromosome aberration study (MRID No.: 43987001) in Chinese Hamster Ovary (CHO) cells with Pyrethrum Extract FEK-99 was reviewed and it was concluded that pyrethrins did not induce chromosome aberrations up to and including dose levels that cause excessive cytotoxicity. The study was classified as ACCEPTABLE. The requirement for additional series 84-2 mutagenicity/genetic toxicity studies will be determined at a later time when pyrethrins are due for reregistration.

II. Action Requested

The Chemical Specialties Manufacturing Association on behalf of the Pyrethrin Joint Venture (refer to letter from Ralph Engel dated April 22, 1996) has submitted a series 84-2 chromosome aberration study (MRID No.: 43987001) in order to

fulfil the mutagenicity/genetic toxicity data requirements for pyrethrins.

III. Toxicology Branch Comments

The study was reviewed and a copy of the DER is attached.

IV. Study Reviewed

Study Identification	Executive Summary
84-2. Chromosome aberration in CHO cells. Microbiological Associates, Study No.: 8004, March 26, 1996, MRID No.: 43987001.	In two independent mammalian cell cytogenetics chromosome aberration assays (MRID No.: 43987001), CHO cell cultures were exposed to Pyrethrum Extract Blend FEK-99 (55.98% purity) in DMSO at concentrations initally over the range of 6.25, 12.5, 25, 50, 100 or 150 µg/mL (adjusted to 100% active ingredient) with and/or without metabolic activation (rat liver S9 derived from Aroclor treated rats) and repeat levels of 10, 25, 40, 55, 70, 85 or 100 µg/mL. Dose levels of 50 µg/mL and above were cytotoxic in both nonactivated and activated assays. 100 cells were scored for each replicate. There was no indication of increases in percentage of structural aberrations or severely damaged cells or average aberrations per cell for either assay in the presence or absence of met-abolic activation at dose levels up to and including excessive cytotoxicity. The positive controls mitomycin C and cyclo-phosphamide induced the appropriate responses. **There was no evidence of induction of chromosome aberrations in CHO cells induced over background by Pyrethrum Extract Blend FEK-99.** This study is classified as ACCEPTABLE and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic chromosome aberration data.

EPA Reviewer: John Doherty
Toxicology Branch II (7509C)
EPA Secondary Reviewer: Irving Mauer, Ph.D., Geneticist
Toxicology Branch II (7509C)

6/11/97

06-11-97

DATA EVALUATION RECORD

012258

STUDY TYPE: *In vitro* mammalian chromosome aberration in Chinese Hamster Ovary Cells OPPTS 870.5375 [§84-2]

DP BARCODE: D227458 SUBMISSION CODE: S507244
P.C. CODE: 069001 TOX. CHEM. NO.: 715

TEST MATERIAL (PURITY): Pyrethrum Extract; Blend Batch FEK-99, purity 55.98%.

CITATION: P.T. Curry, 1996, "Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells", Microbiological Associates, Inc., Study No.: G96AC14.330001, March 26, 1996. MRID No.: 43987001. Unpublished.

SPONSOR: Pyrethrum Joint Venture (PJV) /Chemical Specialties Manufacturers Association (CSMA).

EXECUTIVE SUMMARY:

In two independent mammalian cell cytogenetics chromosome aberration assays (MRID No.: 43987001), CHO cell cultures were exposed to Pyrethrum Extract Blend FEK-99 (55.98% purity) in DMSO at concentrations initially over the range of 6.25, 12.5, 25, 50, 100 or 150 µg/mL (adjusted to 100% active ingredient) with and/or without metabolic activation (rat liver S9 derived from Aroclor treated rats) and repeat levels of 10, 25, 40, 55, 70, 85 or 100 µg/mL. Dose levels of 50 µg/mL and above were cytotoxic in both nonactivated and activated assays. 100 cells were scored for each replicate.

There was no indication of increases in percentage of structural aberrations or severely damaged cells or average aberrations per cell for either assay in the presence or absence of metabolic activation at dose levels up to and including excessive cytotoxicity. The positive controls mitomycin C and cyclophosphamide induced the appropriate responses. **There was no evidence of induction of chromosome aberrations in CHO cells induced over background by Pyrethrum Extract Blend FEK-99.**

This study is classified as ACCEPTABLE and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic chromosome aberration data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Pyrethrum Extract Blend FEK-99
   Description: Yellow liquid
   Lot/Batch #: FEK-99
   Purity: 55.98% a.i.
   Stability of compound: Considered stable in proper storage.
   CAS #: 8003-34-7
   Structure: Not in HED's computer file system and not provided in study report. Pyrethrum extract is a mixture of plant alkaloids.
   Solvent used: DMSO (dimethylsulfoxide)

2. Control Materials:
   Negative: Solvent
   Solvent/final concentration: 50 µL/5 mL
   Positive:
   Nonactivation: Mitomycin C (MMC) at 0.08 µg/mL in distilled water. Obtained from Sigma Chem. Co.
   Activation: Cyclophosphamide (CP) at 10 µg/mL in distilled water. Obtained from Sigma Chem. Co.

3. Activation: S9 derived from

   x Aroclor 1254 x induced x rat x liver

4. Test compound concentrations used.

   Preliminary toxicity study: 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 or 300 µg/mL.

   Initial chromosome aberration study: 6.25, 12.5, 25, 50, 100 or 150 µg/mL.

   Repeat Chromosome aberration study: 10, 25, 40, 55, 70, 85 or 100 µg/mL.

   The same dose levels were used in both the presence or absence of metabolic activation.

5. Test cells: Chinese Hamster Ovary (CHO) cells obtained from the American Type Culture Collection, Rockville, Md.

   Properly maintained? Y (assumed).

   Cell line or strain periodically checked for Mycoplasma contamination? Y (by the Hoechst staining procedure).

   Cell line or strain periodically checked for karyotype stability? Y (not used beyond passage 20).

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay. The method and procedure were described in detail in the report. The cells (5 x 10^5 per 25 cm^2 flask) were treated with 50 µl of test material in DMSO in a 5 mL suspension of the cells in McCoy's 5A medium (with or without added S-9). The cells were stated as being treated for 6 hours with or without the activation medium. Two hours after initiation of treatment, bromo-2'-deoxyuridine (BrdU) was added and the incubation continued. After the exposure period, the medium was removed and washed with calcium and

magnesium free buffer, then refed with 5 mL of complete medium containing BrdU and reincubated for 24 additional hours. Two hours prior to cell harvest, Colcemid^R was added to arrest the cell division. The cells were harvested by trypsinization and counted using a Coulter counter. Cell viability was determined by trypan blue dye exclusion. Metaphase preparations were prepared and stained for sister chromatid differentiation using a modified fluoresence-plus-Giesma technique. Slides were evaluated for the percentage of first, second and third-plus-subsequent-division metaphase cells for estimation of the test article effect on cell cycle kinetics. The average generation time (AGT) was calculated for each treatment condition.

2. Cytogenetic Assay: Based on the method of Evans (1974) exposing duplicate cultures of CHO cells to test article as well as positive and negative controls.

Cell treatment: The cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask for harvests of 24 hours or less and at 2.5 x 10^5 cells/flask for harvests greater than 24 hours and were incubated at 37°±1°C (5% CO_2 in humidified air). Treatment was carried out by refeeding duplicate flasks with 5 mL of complete medium (McCoy's 5A, composition described) with or without the activation (S9) preparation. 50 µl of dosing solution in DMSO were added and six hours were allowed for interaction. After the exposure period, that medium was removed and the cells were washed with medium (calcium and magnesium free) and then refed with the McCoy's medium.

The dividing cells were harvested two hours after addition of Colcemid^R at three approximate time points from the initiation of treatment: 24 hours (the estimated AGT at the highest dose tested), 48 hours (the estimated AGT plus 24 hours) and 12 hours (no adjustment for cell cycle delay). After harvesting, an aliquot of the cell suspension was assessed for cytotoxicity.

The metaphase cells were harvested following trypsinization and centrifugation at about 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL of KCl (0.075 M) and allowed to stand at room temperature for 4-8 min, recentrifuged and the supernatant aspirated and the cells fixed with washes of Carnoy's fixative (methanol:glacial acetic 3:1 v/v). The cells were stored overnight or "longer" in fixative at approximately 2-6°C.

The slides were cells in fixative were recentrifuged and the pelletted cells were resuspended "to opalescence" in fresh fixative. Drops of the suspension were allowed to air dry overnight on a glass slide and the dried slides were stained with 5% Giemsa and redried and mounted.

Metaphase analysis was assessed for 200 metaphase cells; 100 from each flask whenever possible.

Scored for structural defects or aberrations: Y
(Chromatid type aberrations: chromatid and isochromatid breaks and exchange figures such as quadriradials

(symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations: chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric), pulverized chromosomes and cells and severely damaged cells (≥10 aberrations. Chromatid and isochromatid gaps were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage.

Scored for numerical chromosomes: N (at least there were no specific indications for diploidy, triploid or polyploidy)

Coded prior to analysis: Y

Evaluation criteria was based on the number and types of aberrations found, the percentage of structurally damaged cells and the frequency of structural aberrations per cell (mean aberrations per cell).

Statistical analysis consisted of analysis by pair wise comparison of the percent aberrant cells using Fisher's Exact test. If the Fisher's test was positive, the Cochran-Armitage test was used to measure dose responsiveness.

II. REPORTED RESULTS

A. Analytical Chemistry report. The analytical chemistry report was prepared by the McLaughlin Gormley King Company (a member of the PJV) and was presented in Appendix III of the study report. This report indicated that samples of Pyrethrum Extract prepared at nominal concentrations of 0.003 to 30 mg/mL were analytically determined be reasonably close (-3% to 19%) to stated concentration. The lower the dose solution the higher the percentage difference. For example the 0.003 mg/mL dose was 0.00357 or 10% higher.

B. Cytotoxicity and chromosome aberrations assays. The results of this study were presented by the author in 17 tables. Table 17 which summaries the results of this study is attached (photocopied from the study report).

I. Preliminary cytotoxicity assay. There was a large increase in cytotoxicity between 30 μg/mL (29% cell growth inhibition) and 100 μg/mL (82% cell growth inhibition) in the absence of metabolic activation. At 300 μg/mL cell growth inhibition was complete. At doses of 0.03 to 10 μg/mL there was a range of cell growth inhibition from -10% to +17%. In the presence of metabolic activation, the dose level of 100 μg/mL resulted in 53% and 300 μg/mL resulted in 100% cell growth inhibition. The preliminary test also indicated that the Average Generation Time (AGT) for 100 μg/mL was 24 hours in both the presence and absence of metabolic activation. The AGT was from about 12.2 to 15.5 hours for the lower dose levels.

II. Chromosome aberration assays. Table 17 (photocopied from the study report) illustrates the results of the assessment of the chromosomes at 12, 24 and 48 hours after harvest. Both assessments indicated significant cytotoxicity as indicated by the reduction in the mitotic index (MI) at 100 µg/mL in the absence of metabolic activation (68% MI at 24 hours) and presence (81% MI also at 24 hours). Cell growth inhibition (CGI) was also evident as indicated in several other tables (not attached) at 100 µg/mL in both the absence (78% CGI at 24 hours) and presence of metabolic activation (66% CGI at 24 hours). The author described that some samples, particularly for higher dose levels, the Pyrethrum Extract resulted in a cloudy solution (apparently due to insolubility) and that excessive toxicity or insufficient toxicity confounded the interpretation of the results. Thus, repeat experiments were run using dose concentrations of 10, 25, 40, 55, 70, 85 or 100 µg/mL in order to obtain the proper level of cytotoxicity (i.e. sufficient but not excessive). The results of these studies are also included in Table 17 (appended).

There were no increases in aberrant cells, chromatid type or chromosome type aberrations of severely damaged cells in the pyrethrin treated samples as indicated in Table 17. The positive controls MMC and CP produced their expected positive results.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is classified as ACCEPTABLE and satisfies the requirement for a series 84-2 chromosome aberration study in vitro.

B. Study deficiencies - There was no specific reference to cells being scored for numerical chromosome aberrations. No study deficiencies were noted that were considered to compromise the integrity of the data or study interpretation.

ATTACHMENTS

**THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
SEE THE FILE COPY.**

Table 17. "Summary" (two pages).

TABLE 17

SUMMARY

Treatment	S9 Activation	Harvest Time (hrs)	Mitotic Index	Cells Scored	Aberrations Per Cell[1] (Mean ± SD)	Cells With Aberrations[2] (%)
Untreated	-	12	7.1	200	0.010 ± 0.100	1.0
DMSO	-	12	6.4	200	0.010 ± 0.100	1.0
Pyrethrum Extract; Blend FEK-99						
40 µg/ml	-	12	5.1	200	0.000 ± 0.000	0.0
55 µg/ml	-	12	4.1	200	0.005 ± 0.071	0.5
70 µg/ml	-	12	3.8	200	0.015 ± 0.122	1.5
85 µg/ml	-	12	1.2	200	0.005 ± 0.071	0.5
MMC, 0.08 µg/ml	-	12	4.9	200	0.130 ± 0.379	11.5**
Untreated	+	12	8.3	200	0.015 ± 0.158	1.5
DMSO	+	12	5.7	200	0.015 ± 0.158	1.0
Pyrethrum Extract; Blend FEK-99						
25 µg/ml	+	12	7.0	200	0.020 ± 0.140	2.0
40 µg/ml	+	12	6.0	200	0.010 ± 0.100	1.0
55 µg/ml	+	12	3.6	200	0.025 ± 0.157	2.5
70 µg/ml	+	12	1.4	106	0.028 ± 0.216	1.9
CP, 10 µ/ml	+	12	1.8	200	0.280 ± 0.931	18.5**
Untreated	-	24	6.5	200	0.050 ± 0.329	3.5
DMSO	-	24	6.7	200	0.020 ± 0.140	2.0
Pyrethrum Extract; Blend FEK-99						
12.5 µg/ml	-	24	5.6	200	0.000 ± 0.000	0.0
25 µg/ml	-	24	9.0	200	0.025 ± 0.186	2.0
50 µg/ml	-	24	3.1	200	0.010 ± 0.100	1.0
100 µg/ml	-	24	2.1	200	0.020 ± 0.140	2.0
MMC, 0.08 µg/ml	-	24	6.9	200	0.165 ± 0.423	14.5**
Untreated	+	24	10.2	200	0.000 ± 0.000	0.0
DMSO	+	24	9.5	200	0.015 ± 0.122	1.5
Pyrethrum Extract; Blend FEK-99						
12.5 µg/ml	+	24	9.6	200	0.010 ± 0.100	1.0
25 µg/ml	+	24	12.0	200	0.030 ± 0.264	1.5
50 µg/ml	+	24	6.6	200	0.020 ± 0.140	2.0
100 µg/ml	+	24	1.9	200	0.030 ± 0.171	3.0
CP, 10 µg/ml	+	24	2.3	200	0.530 ± 1.075	32.0**
Untreated	-	48	10.5	200	0.010 ± 0.100	1.0
DMSO	-	48	7.4	200	0.005 ± 0.071	0.5
Pyrethrum Extract; Blend FEK-99						
6.25 µg/ml	-	48	7.6	200	0.000 ± 0.000	0.0
12.5 µg/ml	-	48	7.6	200	0.005 ± 0.071	0.5
25 µg/ml	-	48	6.3	200	0.005 ± 0.071	0.5
50 µg/ml	-	48	1.3	200	0.015 ± 0.122	1.5
MMC, 0.08 µg/ml	-	48	5.2	200	0.200 ± 1.037	10.5**

TABLE 17 (continued)

SUMMARY

012258

Treatment	S9 Activation	Harvest Time (hrs)	Mitotic Index	Cells Scored	Aberrations Per Cell[1] (Mean ± SD)	Cells With Aberrations[2] (%)
Untreated	+	48	7.9	200	0.020 ± 0.140	2.0
DMSO	+	48	11.2	200	0.035 ± 0.210	2.5
Pyrethrum Extract; Blend FEK-99						
40 µg/ml	+	48	9.6	200	0.000 ± 0.000	0.0
55 µg/ml	+	48	6.7	200	0.020 ± 0.140	2.0
70 µg/ml	+	48	8.7	200	0.015 ± 0.122	1.5
85 µg/ml	+	48	4.8	200	0.090 ± 0.472	4.5
CP, 10 µg/ml	+	48	7.3	157	2.847 ± 3.488	63.7**

[1]Severely damaged cells were counted as 10 aberrations.
[2]*, p≤0.05, **, p≤0.01; Fisher's exact test.