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

DEC 1 1989

007632

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyproconazole-*In Vitro* Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

TO: Lewis/Gable PM 21
Registration Division (H7509C)

FROM: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II (HFAS)
HED (7509C)

K. Clark Swentzel 11/21/89

THRU: Marcia van Gemert, Ph.D.
Chief, Toxicology Branch II (HFAS)
HED (7509C)

M van Gemert 11/22/89

EPA ID No. 55947-RGG
Acc. No. 411587-01
Project No. 9-1791
Caswell No. 272E
Registrant: Sandoz Corp.

Requested Action

Review subject study.

Response

The primary review of this study was performed by Dynamac Corp. and the secondary review was done by Dr. John Chen, Section I/Tox Branch II; the DER is attached. Cyproconazole was evaluated in a multiple-harvest cytogenetic assay for the potential to induce chromosome aberrations in Chinese hamster ovary cells. Concentrations of test material were 100, 150 or 200 ug/ml without S9 activation and 100, 150, 200 or 250 ug/ml with S9 activation; cultures were harvested at 4, 9 and 19 hours after treatment. A positive response was observed in both the S9 activated and nonactivated assays at different dose levels and at different harvest times. Therefore, it was concluded that Cyproconazole was clastogenic in this mammalian cell test system and that metabolic activation was not required to demonstrate the effect.

Classification: acceptable

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EPA No.: 68D80056
DYNAMAC No.: 242-A
TASK No.: 2-42A
November 15, 1989

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

CYPROCONAZOLE

Mutagenicity--*In vitro* Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 11/15/89

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DATA EVALUATION RECORD

CYPROCONAZOLE

Mutagenicity--*In vitro* Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll

Date: 11-15-1989

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Ira Cecil Felkner

Date: 11-15-89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: [illegible] for Roman J. Pienta

Date: 11/15/89

K. Clark Swentzel
EPA Reviewer and
Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel

Date: 11/20/89

007632

DATA EVALUATION RECORD

CHEMICAL: Cyproconazole.

STUDY TYPE: *In vitro* chromosome assay with Chinese hamster ovary (CHO) cells.

MRID NUMBER: 411587-01.

TEST MATERIAL: Cyproconazole.

SYNONYM(S): 2-(4-Chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol; SAN 619F/94361-06-5, 94361-07-6.

SPONSOR: Sandoz Crop Protection Corporation, Basle, Switzerland.

TESTING FACILITY: Research and Consulting Company B.V., Hertogenbasch, the Netherlands.

TITLE OF REPORT: Evaluation of the Ability of Cyproconazole to Induce Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO) Cells.

AUTHOR(S): Enninga, I.C.

STUDY NUMBER(S): 0883/ECC 153.

REPORT ISSUED: June 28, 1988.

CONCLUSIONS/EXECUTIVE SUMMARY:

Cyproconazole was evaluated in a multiple-harvest cytogenetic assay for the potential to induce chromosome aberrations in Chinese hamster ovary cells. Cultures exposed to 100, 150, or 200 µg/mL without S9 activation and cultures exposed to 100, 150, 200, or 250 µg/mL with S9 activation were harvested 4, 9, and 19 hours after treatment. Results indicated that significant increases in cells with aberrations were scored in both the nonactivated and S9-activated assays at different dose levels and at different harvest times. The effect was not dose or time dependent. Regardless of the condition, dose, or harvest interval, the frequency and type (chromatid breaks and acentric fragments) of aberrations that were induced were comparable. This finding, in conjunction with the narrow dose range, suggests that the clastogenic activity of cyproconazole occurs within a limited dose range. We conclude, from the positive response observed in the nonactivated and S9 activated assays, that cyproconazole was clastogenic in this mammalian cell test system and that metabolic activation was not required to demonstrate the effect.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material: Cyproconazole.
   Description: Light brown powder.
   Batch No.: 8507.
   Purity: 95.6 ± 1%.
   Contaminants: Not listed.
   Solubility: In water, 140 ± 4 ppm at 22°C; in ethanol and dimethylsulfoxide (DMSO) >23% at 25°C.
   Solvent Used: DMSO.
   Stability: Decomposition <5%/2 years at 20°C.
   Other Comments: The test material was stored at room temperature in the dark. Solutions of the test material were prepared immediately prior to use and were protected from light.

2. Cell Line: The Chinese hamster ovary (CHO) cells (subline CHO-K1) used in this assay were obtained from Dr. A. T. Natarajan, State University of Leiden, the Netherlands. Prior to use, the CHO cells were grown in Ham's F-10 medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics.

3. S9 Fraction: The S9 fraction was derived from the livers of young adult male Wistar or Sprague Dawley rats induced with Aroclor 1254. Prior to use, the concentration of cytochrome P-450 in the S9 fraction was determined and found to be 173.6 nM cytochrome P-450/g wet liver. The S9-cofactor mix contained 0.5 mL S9/mL of the reaction mixture.

4. Control Compounds: DMSO served as the negative control; mitomycin C (MMC) at 0.075 μg/mL was used as the nonactivated positive control and cyclophosphamide (CP) at 10 μg/mL was used as the S9-activated positive control.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Prepared cultures (four replicates), seeded at ≈5 x 10^5 cells/dish, were exposed for 2 hours to eight concentrations of the test material (1.0 to 2000 μg/mL) or the solvent control (DMSO) both in the presence and absence of S9 activation. Following exposure, cells were washed; cells from two of the four replicate cultures were counted to determine survival immediately following treatment. The remaining cultures were refed fresh medium and reincubated for 18 to 20 hours; cells were collected and counted, and the relative percent survival was determined.

2. Cytogenetic Assay:

a. Treatment: Based on the preliminary cytotoxicity assay results, doses were selected for the nonactivated and S9-activated cytogenetic assays. Duplicate cultures containing ≈4 x 10^6, ≈2 x 10^6, or ≈1 x 10^6 cells/flask were exposed for 2 hours to the selected doses of the test material, solvent (DMSO), or positive controls (2 x 10^6 cells/flask dosed with 0.075 μg/mL MMC/-S9 or 10 μg/mL CP/+S9).

Cultures were washed, refed with fresh medium, and re-incubated for 4 hours (cultures containing ≈4 x 10^6 cells), 9 hours (cultures containing ≈2 x 10^6 cells), or 19 hours (cultures containing ≈1 x 10^6 cells). Colchicine (2 μg/mL) was added to cultures exposed to the highest test material dose or the positive controls 2 to 2.5 hours prior to cell harvest.

Metaphase cells were collected by mitotic shake-off, treated with hypotonic 1% sodium citrate, and fixed with methanol:acetic acid (3:1). Slides were stained with 5% Giemsa and coded.

b. Metaphase Analysis: Two hundred metaphase cells per treatment group (100/culture) were scored for structural chromosome aberrations. Numerical variations were noted but not included as aberrations.

3. Statistical Analysis: The data were evaluated for statistical significance at p values of 0.05, 0.01, and 0.001 by the Chi-square test.

4. Evaluation Criteria:

   a. Assay Validity: The assay was considered valid if 1) the number of chromosome aberrations in the solvent control cultures fell within the historical range of data recorded by the reporting laboratory and 2) the positive control chemicals induced a significant ($p<0.05$) increase in the number of cells with aberrations.

   b. Positive Response: The test material was considered positive if it induced a significant ($p<0.05$) and dose-related increase in the number of cells with chromosome aberrations. In the absence of a dose-response relationship, the test material was considered positive if it induced a significant effect that was reproducible.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The eight nonactivated and S9-activated doses of the test material evaluated in the preliminary cytotoxicity assay ranged from 1 to 2000 µg/mL. Compound precipitation was observed in cultures containing the three highest doses (333, 1000, and 2000 µg/mL) with and without S9 activation. In the nonactivated test, no cells survived exposure to the three highest doses of the test material. At nonactivated doses $\leq$ 100 µg/mL, $\geq$ 84% of the cells survived. Under S9-activated conditions, the two highest doses were completely cytotoxic and less than 5% of the cells survived exposure to 333 µg/mL/+/-S9. Lower S9-activated doses (1 to 100 µg/mL) were relatively noncytotoxic. Based on these results, the cytotoxic assay was repeated using a narrower range of test material doses (100, 150, 200, and 250 µg/mL/+/-S9). In the absence of S9 activation, 8% of the cells exposed to 250 µg/mL were viable 18 to 20 hours posttreatment; at 200 µg/mL, 55% of the cells were recovered 18 to 20 hours posttreatment. The inclusion of S9 activation reduced cytoxicity as indicated by the 18- to 20-hour posttreatment recovery of 64% of the cells exposed to 250 µg/mL. The doses selected for the cytogenetic assay were 50, 100, 150, and 200 µg/mL/-S9 and 100, 150, 200, and 250 µg/mL/+S9.

2. Cytogenetic Assay:

a. Nonactivated Test: Under nonactivated conditions, statistically significant ($p<0.05$) increases in the number of cells with aberrations were observed in cultures harvested 4 and 9 hours postexposure to 200 µg/mL. A significant increase ($p<0.05$) in chromosome aberrations was also noted in the 100 µg/mL test group harvested 4 hours posttreatment. No significant effects were seen in cultures harvested at the 19-hour interval. Representative nonactivated results were selected from the 9-hour cell harvest and are presented in Table 1; reported results from the 4- and 19-hour fixation times are presented in Appendix A; study author's Tables Nos. 2 and 6, respectively.

b. S9-Activated Test: Metaphases were not available from the high-dose group (250 µg/mL) at the 4- and 9-hour harvest times. Significant increases in cells with aberrations were seen in the following test groups: 100 µg/mL (9- and 19-hour harvests), 150 µg/mL (4-hour harvest), 200 µg/mL (9-hour harvest), and 250 µg/mL (19-hour harvest). No dose- nor time-related trend was apparent. It was noted, however, that under both non-activated and S9-activated conditions, the predominant types of aberrations that were scored at doses inducing a significant effect were generally chromatid breaks and acentric fragments. S9-activated results from the 9-hour cell harvest were selected as representative and appear in Table 1; reported results from the 4- and 19-hour fixation times are presented in Appendix B; study author's Table Nos. 3 and 7, respectively.

Based on the combined results of the multiple-harvest nonactivated and S9-activated assays, the study author concluded that cyproconazole is "weakly clastogenic" in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study author interpreted the data correctly. Although cyproconazole did not induce a dose- or time-dependent clastogenic effect, significant increases were consistently seen under nonactivated and S9-activated conditions. Similarly, the type and frequency of aberrations were generally comparable regardless of the condition, dose, or harvest interval. It would, therefore, appear that the clastogenic activity of cyproconazole is confined to an extremely narrow range of reactive doses and that S9 activation was not required to demonstrate this effect.

TABLE 1. Representative Results of the Multiple-Harvest CHO Cell *in vitro* Cytogenetic Assay with Cyproconazole

Substance	Dose (µg/mL)	S9 acti- vation	Harvest[a] Time (Hours)	No. of Cells Scored	No. Cells with Aberra- tions[b]	% Cells with Aberra- tions	Biologically Significant Aberrations[c] (No./Type)
Solvent control							
Dimethylsulfoxide	--	-	9	200	7	3.5	4TB; 1SB; 2AF
	--	+	9	200	5	2.5	1TB; 1SB; 4AF
Positive control							
Mitomycin C	0.075	-	9	200	18*	9.0	7TB; 2SB; 7AF; 3E
Cyclophosphamide	10	+	9	200	56**	28.0	22TB; 7SB; 24AF; 15E
Test Material							
Cyproconazole	100[d]	-	9	200	5	2.5	2TB; 4AF
	150	-	9	200	7	3.5	1SB; 5AF; 1D
	200	-	9	200	17*	8.5	10TB; 1SB; 7AF; 1E
	100[e]	+	9	200	12*	6.0	5TB; 2SB; 5AF; 1MA
	150	+	9	200	5	2.5	5TB
	200	+	9	200	12*	6.0	5TB; 1SB; 6AF; 2D

[a]Time after compound administration.

[b]Gaps not included.

[c]Abbreviations used:

TB - Chromatid break
SB - Chromosome break
D - Dicentric
AF - Acentric fragment
E - Exchange figure
MA - Multiple aberrations

[d]Significant increases in cells with aberrations were also noted in the 100- and 200-µg/mL nonactivated test groups harvested 4 hours postexposure.

[e]Significant increases in cells with aberrations were also noted in the following S9-activated test groups: 150 µg/mL at 4 hours and 100 and 250 µg/mL at 19 hours.

*Significantly different than the solvent control ($p<0.05$) by the X^2 test.

**Significantly different than the solvent control ($p<0.001$) by the X^2 test.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated June 28, 1988.

F. CBI APPENDIX: Appendix A, Results from the 4- and 19-hour Nonactivated Fixative Times, CBI pp. 13 and 17; Appendix B, Results from the 4- and 19-hours S9-activated Fixative Times, CBI pp. 14 and 18; and Appendix C, Materials and Methods, CBI pp. 8-10.

APPENDIX A

Results from the 4- and 19-Hour Nonactivated Fixative Times

CBI pp. 13-17

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__X__ A draft product label.

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APPENDIX B

Results from the 4- and 19-Hour S9-Activated Fixative Times

CBI pp. 14-18

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APPENDIX C

Methods and Materials

CBI pp. 8-10

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