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FINAL

DATA EVALUATION REPORT

CAPTAN

Study Type: Mutagenicity: In Vivo Cytogenetic Assay with Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Contract Number: 68D10075
Work Assignment Number: 1-05
Clement Number: 91-48
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
IN VIVO MAMMALIAN CYTOGENETICS

MUTAGENICITY STUDIES

EPA Reviewer: Paul Chin, Ph.D. Signature: Paul Chin
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Review Section II, Toxicology Branch (I)/HED Date: 11/19/93

KLD 11/19

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo cytogenetic assay with rats

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 159

MRID Number: 00062982

TEST MATERIAL: Captan

SYNONYMS: cis-N-trichloromethylthio-4-cyclohexene, 1,2-dicarboximide

SPONSOR: Makhteshim Chemical Works, Ltd, Beer-Sheeva, Israel/ICI Americas Inc., Wilmington, DE

STUDY NUMBER: 79/MAK008/292

TESTING FACILITY: Life Science Research, Essex, England

TITLE OF REPORT: Captan-Spritzpulver AA Captan: Investigation of Effects on Bone Marrow Chromosomes of the Rat After Sub-Acute Oral Administration

AUTHORS: Bootman J. and Whalley, H.E.

REPORT ISSUED: May 25, 1979

CONCLUSIONS--EXECUTIVE SUMMARY: No conclusions can be reached from the multiple dosing in vivo bone marrow cytogenetic assay conducted with captan. There was no indication of a clastogenic response in male rats (6/group) administered single daily oral gavage doses of 200, 400, or 800 mg/kg captan for five consecutive days. However, the study was seriously compromised for the following reasons:

1. No signs of compound toxicity in the animals or cytotoxic effects on bone marrow cells were seen; hence, the cumulative high dose (4000 mg/kg) did not approach the maximum tolerated dose; therefore, higher daily doses could have been used.

2. Females were not included as recommended by Guidelines.

3. No information was provided on test material purity, stability or storage conditions.

4. There was no indication that the study was conducted in conformance with good laboratory practices; a quality assurance statement was not provided.

5. Only one sampling time (5 hours) was performed. Guidelines recommend that two sampling times after the last dose (6 and 24 hours) be performed with a repeated treatment protocol unless, otherwise, justified.

Based on the above considerations it was concluded that the study does not satisfy Guideline requirements for genetic effects Category II, Structural Chromosome Aberrations.

STUDY CLASSIFICATION: The study is unacceptable.

A. MATERIALS:

1. Test Material: Captan-Spritzpulver (spray powder) AA Captan

Description: White crystalline powder
Identification number: 702/38
Purity: Not reported
Receipt date: Not provided
Stability: Not provided
Contaminants: None listed
Solvent used: 0.5% Gum tragacanth (GT)
Other provided information: Test material storage conditions were not reported. Suspensions of captan in 0.5% GT were prepared on the day of use.

2. Control Materials:

Negative/Route of Administration: None.

Vehicle/Final Concentration/Route of Administration: 0.5% GT was administered once daily for 5 consecutive days by oral gavage at a dosing volume of 10 mL/kg.

Positive/Final Dose(s)/Route of Administration: Chlorambucil (CB) was prepared in 10% ethanol and administered once daily for 2 consecutive days at a dose of 15 mg/kg/day.

3. Test Compound:

Route of administration: Oral gavage.

Volume of test substance administered: 10 mL/kg.

Dose levels used: 200, 400, and 800 mg/kg/day for 5 consecutive days.

NOTE: The high concentration was selected to yield an accumulated dose that was approximately 25-44% of the LD_{50}.

4. Test Animals:

a. Species: Rat Strain: CD Age: Not reported
Weight: At receipt 70-90 g
Sex: Males only Source: Charles River Breeding Laboratories (U.K.), Kent, England.

b. No. animals used per dose:

- Treatment groups: 6 males No females
- Positive control: 6 males No females.
- Vehicle control: 6 males No females.

c. Properly maintained? Feeding, watering, and housing conditions were reported; environmental conditions (temperature, humidity, and light cycle) were not provided.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound
Dosing: _____ once _____ twice (24 hours apart)
__x__ other (describe): Once daily for 5 consecutive days (24 hours apart).

Sampling (after last dose): _____ 6 hours _____ 12 hours
_____ 24 hours _____ 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe): 5 hours after the last dose

b. Negative and/or vehicle control
Dosing: _____ once _____ twice (24 hours apart)
__x__ other (describe): Once daily for 5 consecutive days (24 hours apart)

Sampling (after last dose): _____ 6 hours _____ 12 hours
_____ 24 hours _____ 48 hours _____ 72 (mark all that are appropriate)
__x__ other (describe): 5 hours after the last dose

c. Positive control

Dosing: _____ once __x__ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hours _____ 12 hours
_____ 24 hours _____ 48 hours _____ 72 (mark all that are appropriate)
__x__ other (describe): 5 hours after the last dose

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d. Administration of spindle inhibitor

Inhibitor used/dose: Colchicine/4 mg/kg

Administration time: 3 hours after the last dose; 2 hours prior to sacrifice.

Route of administration __x__ i.p. _____ other (describe)

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):

No. of cells per animal per treatment group examined: 100.

No. of cells per animal per control group examined: 100.

3. Details of Cell Harvest and Slide Preparation:

Animals in all groups were sacrificed by cervical dislocation 5 hours after the last treatment with the selected doses of the test material, vehicle, or positive control. Bone marrow cells were collected from both femurs by aspiration into Hanks' solution. Cells were centrifuged, treated with 0.56% KCl containing 1 IU/mL heparin, and fixed in methanol:glacial acetic acid (3:1). Slides were stained with 10% Giemsa and coded.

4. Statistical Evaluation: The data were analyzed for statistical significance at p values of 0.05, 0.01, and 0.001 by a modified chi-square test.

5. Evaluation Criteria: No criteria were provided to establish the validity of the assay or the biological significance of the results.

C. REPORTED RESULTS:

1. Animal Observations: Body weights and toxic signs were recorded daily. No signs of clinical toxicity were reported and no effects on body weight were observed over the 5-day administration period of 200, 400, or 800 mg/kg captan.

2. Cytogenetic Assay: The study authors stated that the reported results were from a repeat test with captan. The initial assay, conducted with comparable captan doses, was aborted due to the poor performance of colchicine. Representative results from the repeat cytogenetic study are presented in Table 1. No significant increase in the percentage of cells with structural chromosome aberrations was seen at any dose. Metaphase plates from two males in the low-dose group and one male in the mid-dose group contained single chromosome exchanges. However, in the absence of a dose response and/or an appreciable increase in chromatid breaks, this finding does not constitute

evidence of a clastogenic effect. By contrast, the positive control (2x15 mg/kg CB) induced a powerful clastogenic response. Based on the overall results, the study authors concluded that the oral administration of five daily doses of captan did not produce adverse effects on chromosome structures.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the findings of this study do not support a valid negative conclusion for the following reasons:

1. The lack of overt toxicity in the animals or cytotoxic effects on the target organ indicated that the cumulative high dose (4000 mg/kg) did not approach the MTD; therefore, higher daily dosing could have been used.

2. Females were not included as recommended by Guideline.

3. Test material purity was not provided.

4. Only one sampling time (5 hours) was performed. Guidelines recommend that two sampling times after the last dose (6 and 24 hours) be performed with a repeated treatment protocol unless, otherwise, justified.

Based on the above considerations, it was concluded that the study is unacceptable.

E. QUALITY ASSURANCE MEASURES: There was no quality assurance statement or indication that the study was conducted in compliance with good laboratory practices.

F. APPENDIX: Appendix A, Materials and Methods, pp. 8-13.

TABLE 1. Representative Results of the In Vivo Cytogenetic Assay in Male Rats Receiving Five Daily Oral Gavage Doses of Captan

Substance	Dose	Number of Animals Examined per Group	Number of Metaphases Examined	Total Number of Cells with Aberrations[a]	Percent Cells with Aberrations[a]	Total Number of Aberrations[a]	Aberrations per cell[a]	Biologically Significant Aberrations No./Type
Vehicle Control								
Gum Tragacanth	0.5%	6	600	6	1.0±0.63	6	0.010	4TB; 2F
Positive Control								
Chlorambucil	2x15 mg/kg	6	464	165	36.33±33.24[b]	334	0.729	116TB; 5SB; 68E; 74F 63M; 2D; 6P
Test Material								
Captan	5x800 mg/kg[c]	6	600	7	1.17±1.17	9	0.015	2TB; 6F; 1A

[a]Gaps excluded
[b]Significantly higher than the vehicle control ($p<0.001$) by a modified chi-square test.
[c]Results for lower doses (5x200 and 5x400 mg/kg) did not suggest a clastogenic effect.

Abbreviations used:

TB = Chromatid break
SB = Chromosome break
F = Fragment
E = Exchange
M = Multiple aberrations (≥8 aberrations/cell); counted as one aberration
D = Dicentric
P = Pulverized cell
A = Abnormal chromosome (not otherwise specified)

APPENDIX A

MATERIALS AND METHODS
pp. 8-13

_____Captan review_____

Page _____ is not included in this copy.

Pages __9_ through __14_ are not included in this copy.

The material not included contains the following type of information:

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

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_____ Information about a pending registration action.

__x__ FIFRA registration data.

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