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FINAL

DATA EVALUATION REPORT

CAPTAN

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 4/24/92

Independent Reviewer Sanjay Diwan, Ph.D. Date 4/24/92

QA/QC Manager Sharon Segal, Ph.D. Date 4/27/92

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Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
SALMONELLA

MUTAGENICITY STUDIES

EPA Reviewer: Paul Chin, Ph.D. Signature: Paul Chin
Review Section II, Toxicology Branch (I)/HED Date: 12/14/93
EPA Section Head: Joycelyn Stewart, Ph.D. Signature: JS KLD
Review Section II, Toxicology Branch (I)/HED Date: 12/14/93 11/07/

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 159

MRID Number: 00149489

TEST MATERIAL: Captan

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

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: SOCAL 2042

TESTING FACILITY: Chevron Environmental Health Center, Richmond, CA

TITLE OF REPORT: Microbial/Mammalian Microsome Mutagenicity Plate Incorporation Assay: Comparison of Captan Technical (SX-1086), Chevron Folpet Technical (SX-1388), and Chevron Captafol Technical (SX-945)

AUTHORS: Machado, M.L., Carver, J.H., and Kodama, J.K

REPORT ISSUED: December 18, 1984

CONCLUSIONS--EXECUTIVE SUMMARY: The direct-acting mutagen, captan[1,2] at 7.5 and 10 µg/plate (0.025 and 0.033 µmoles/plate, respectively) was assayed in the absence and presence of two sulfhydryl amino acids (cysteine or glutathione) and five non-thiol analogs (alanine, glycine, methionine, serine, and threonine) to determine the efficacy of the various amino acids to inhibit captan induced mutagenesis in a modified Salmonella typhimurium mutagenicity assay using S. typhimurium strain TA100. Results indicate that the five non-thiol amino acids had no effect on the mutagenic response provoked by

[1]Bridges, B.A. The mutagenicity of captan and related fungicides. Mutat. Res. (1975) 32:3-34.

[2]McCann, J., Choi, E., Yamasaki, E., Ames, B.N. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci, USA 72 (1975):5135-5139.

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captan. By contrast, both cysteine and glutathione effectively inhibited the mutagenic activity of captan at molar ratios ≥5 (the ratio of inhibitor to test substance). We conclude that the study was properly conducted and the modifications to the standard S. typhimurium/mammalian microsome assay (i.e., no S9-activation, no positive controls, and the use of strain TA100 only) were justifiable and based on sound scientific judgement. Although the study does not fully comply with Guideline requirements for genetic effects Category I, Gene Mutation, the results clearly demonstrated that captan is a mutagen; therefore, full compliance with Guideline requirements is not necessary for this special modified Salmonella study.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Captan technical

Description: White solid
Identification number: SX-1086
Purity: 92.4%
Receipt date: December 12-16, 1982
Stability: Not provided
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was reported to be soluble in DMSO. The initial stock solution was sonicated for 3 minutes prior to further dilution.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO; final concentration was not reported.

Positive: None. Positive controls were not used because the test material is a known mutagen (see Appendix A, Protocol Amendment).

Activation: The test was not performed under S9-activated conditions.

3. Activation: Not used

4. Test Organism Used: S. typhimurium strains

_____ TA97 _____ TA98 __x__ TA100 _____ TA102 _____ TA104
_____ TA1535 _____ TA1537 _____ TA1538
list any others:

Test organism were properly maintained: Yes
Checked for appropriate genetic markers (rfa mutation, R factor): Yes

5. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: Only one concentration (10 µg/plate) was assayed with S. typhimurium TA100.

   (b) Mutation assay: Two concentratins (7.5 and 10.0 µg/plate) were evaluated using S. typhimurium TA100 in the presence and absence of two sulfhydryl amino acids (cysteine and glutathione) and five non-thiol analogs (alanine, glycine, methionine, serine, and threonine).

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
   - __x__ Standard plate test
   - _____ Pre-incubation (_____) minutes
   - _____ "Prival" modification
   - _____ Spot test
   - __x__ Other (describe)

The purpose of this study was to determine the potential of the identified amino acids to inhibit the mutagenic activity of captan. A detailed protocol for microbial mutagenicity assays (see Appendix B) and a general description of the test procedure used in this study were provided. However, there was no information on the modifications to the standard procedure (i.e., incorporation of the amino acids into the test system) that were undertaken in the reported assay. We assume, therefore, that increasing molar concentrations of the various inhibitors were incorporated into the top agar-organism-test material reaction mixture prior to overlaying on minimal bottom agar. In this manner, the specified molar ratios (0 to 30) of inhibitor to test material were probably achieved. Plates were incubated for 48 hours at 37°C; and the means and standard deviations of the colony counts from three replicate plates/test dose/amino acid concentration were counted. A sterility test of the highest captan concentration and the solvent (DMSO) was performed.

2. Protocol: See Appendix B.

C. REPORTED RESULTS: The study authors stated that 10 µg/plate was not cytotoxic in the preliminary cytotoxicity assay. Accordingly, two concentrations 7.5 and 10 µg/plate (0.025 and 0.033 µmoles/plate, respectively) were evaluated in the presence and absence of the seven inhibitory agents using strain S. typhimurium TA100. The study authors further stated that the non-thiol analogs (alanine, glycine, methionine, serine, and threonine) did not reduce the mutagenic activity of captan at any molar ratio; no data were presented to support this statement. However, representative results from the assays with glutathione and cysteine indicated that both sulfhydryl amino acids effectively inhibited the mutagenesis of 7.5 and 10.0 µg/plate captan (Table 1). As the data further show, the mutagenic activity of captan was markedly reduced in the presence of increasing

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TABLE 1. Representative Results of the Inhibitor Effects of Amino Acids on the Mutagenic Action of Nonactivated Captan in the Modified Salmonella typhimurium Mutation Assay

		Response of S. typhimurium TA100				
		Solvent Control	Test Material (Captan)			
		Dimethyl sulfoxide	7.5 µg/plate (0.025 µmoles/plate)		10 µg/plate (0.033 µmoles/plate)	
Inhibitor	Molar[a] Ratio	Colony Count[b]	Colony Count[b]	Mutagenic Activity[c]	Colony Count[b]	Mutagenic Activity[c]
Glutathione	+[d]	100±18	--	--	--	--
	0.0	116±21	499±11	5.0	601±30	6.0
	1.0[e]	--	473±42	4.7	474±24	4.7
	3.0	--	262±28	2.6	153±24	1.5
	5.0	--	158±22	1.6	--	--
	6.0	--	--	--	159±26	1.6
	9.0	--	--	--	128±13	1.3
Cysteine	+[d]	100±18	--	--	--	--
	0.0	116±21	499±11	5.0	601±26	6.0
	2.5[f]	--	265±20	2.7	309±25	3.1
	5.0	--	111±9	1.1	142±9	1.4
	10.0	--	101±23	1.0	108±14	1.1

[a]Molar ratio = ratio of inhibitor to test material.

[b]Means and standard deviations from the counts of triplicate plates.

[c]Mutagenic Activity = $\frac{\text{No. of Mutant Colonies (Test Dose)}}{\text{No. of Mutant Colonies (Solvent + Inhibitor)}}$; calculated by our reviewers.

[d]Concentration not specified.

[e]Results for other molar ratios (2, 4, or 9) generally showed a similar pattern of decreased mutagenesis with increasing glutathione concentrations.

[f]Results for other molar ratios (15, 20, or 30) generally showed a similar pattern of decreased mutagenesis with increasing cysteine concentrations.

APPENDIX A

PROTOCOL AMENDMENT

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_____Captan review_____

Page _____ is not included in this copy.

Pages __7__ through __20__ are not included in this copy.

The material not included contains the following type of information:

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