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FINAL

DATA EVALUATION REPORT

CAPTAN

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

Prepared for:

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Office of Pesticide Programs
Environmental Protection Agency
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GUIDELINE SERIES 84: MUTAGENICITY
SALMONELLA

MUTAGENICITY STUDIES

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DATA EVALUATION REPORT

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

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 159

MRID Number: 00073207

TEST MATERIAL: Captan

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

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: Not applicable

TESTING FACILITY: Pesticide Research Laboratory, Pennsylvania State University, University Park, PA

TITLE OF REPORT: Excerpts from Mutagenesis Induced in Mutant Strain of Salmonella typhimurium by Pesticides. Presented at the 174th American Chemical Society National Meeting, Division of Pesticide Chemistry

AUTHORS: Ercegovich, C.D., and Rashid, K.A.

REPORT ISSUED: August 30, 1977

CONCLUSIONS--EXECUTIVE SUMMARY: As part of a screening program of 70 pesticides and related degradation products, captan was evaluated for the potential to induce gene mutations in the Salmonella typhimurium/mammalian microsome mutagenicity assay. Qualitative results indicated that nonactivated and S9-activated captan was mutagenic in S. typhimurium strains TA1535 and TA100. Captan was negative in strain TA98. The nonactivated test material was also not mutagenic in strains TA1537 or TA1538; however, in the presence of S9 activation, mutagenic responses were induced in these strains. Based on the evaluation criteria of the investigators, captan was classified as a mutagen. However, the lack of quantitative results renders the study unacceptable as an individual data source. The study, therefore, does not satisfy Guideline requirements for genetic effects, Category I, Gene Mutations.

STUDY CLASSIFICATION: The study is unacceptable but can be used to support the findings of other microbial mutation assays.

A. MATERIALS:

1. Test Material: Captan

Description: Not provided
Identification number: Not provided
Purity: 99%
Receipt date: Not provided
Stability: Not provided
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was supplied by Chevron. The frequency of test material preparation was not reported. All solutions were sterilized by passage through 0.045 μ Millipore membrane filters.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/Final concentration was not reported

Positive:

Nonactivation:

Nitrosoguanidine	1 and 5 μg/plate TA100, TA1535
2-Nitrofluorene	1 μg/plate TA98, TA1538
9-Aminoacridine	100 μg/plate TA1537

Other:

Activation:

Aflatoxin-B_1 :	Neither the concentration nor the strains were reported
Benzo(a)pyrene:	Neither the concentration nor the strains were reported

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 liver homogenate was prepared by the performing laboratory.

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S9 mix composition:

Component	Concentration/mL of S9 mix
Sodium phosphate buffer	100 µmoles
Glucose 6-phosphate	5 µmoles
NADP	4 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
S9	0.08 mL

4. Test Organism Used: S. typhimurium strains
____ TA97 _x_ TA98 _x_ TA100 ____ TA102 ____ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538
list any others:

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

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: The spot test was used to evaluate the cytotoxic activity of 50, 250, 500, and 1000 µg of each test material in the absence of S9 activation. The strain(s) that were used were not reported.

(b) Mutation assay: Five doses (1, 5, 25, 125, and 325 µg/plate) were assayed without S9 activation; concentrations were doubled for the S9-activated test. Five replicated plates were prepared for each dose.

B. TEST PERFORMANCE:

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

2. Protocol:

(a) Preliminary cytotoxicity assay: Cytotoxicity was assessed by impregnating sterile filter discs (6 mm), positioned on preinoculated minimal Vogel-Bonner medium E plates containing 12.6 µg/plate L-histidine and 14.7 µg/plate biotin, with solutions of the test material ranging from 50 to 1000 µg. The concentration of biotin and histidine was 3 times higher than the levels used in the mutation assay. Zones of inhibition were measured 24 hours following incubation at 37°C.

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(b) Mutation assay:

(1) Plating procedures: Immediately prior to use, biotin and histidine (final concentration 4.2 and 4.9 µg/plate, respectively) were added to 8-hour broth cultures (1.0-2.0 x 10^8 cells/mL) of each tester strain. Approximately 0.2 mL of the test dose or the solvent and 0.1 mL of the prepared culture of the appropriate strain were added to 2 mL molten top agar. For the S9-activated phase of testing, 0.5 mL of the S9 mix was also added. The contents of the tubes were mixed, poured over minimal medium, and incubated at 37°C for 3 days.

(2) Sterility test: A sterility test was performed on the top agar, the histidine-biotin solution, and the test solutions.

(3) Statistical methods: The data were evaluated by an analysis of variance and Duncan's multiple range test.

(4) Evaluation criteria: Test chemicals that caused a ≥2-fold increase in revertant colonies over the spontaneous reversion frequency were considered positive.

C. REPORTED RESULTS: Captan was one of 70 pesticides and related degradation products that were evaluated in this S. typhimurium mammalian/microsome mutagenicity testing program. Although primary data were not reported for any of the examined test substances, qualitative results indicated that nonactivated and S9-activated captan was strongly mutagenic in S. typhimurium strains TA1535 and TA100 (Table 1). Captan was negative with and without S9 activation in strain TA98 and negative without S9 activation in strains TA1537 and TA1538. Strong to questionable responses were recorded for strains TA1537 and TA1538, respectively, under S9-activated conditions. The doses that induced a mutagenic response were not reported. Based on these findings, the study authors concluded that captan was mutagenic with and without S9 activation.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the lack of quantitative data precludes the use of these results as valid evidence of a mutagenic effect. The study, therefore, is unacceptable as an individual data source, but the positive findings in this study can be used to support the results from other S. typhimurium mammalian/microsome mutation assays conducted with captan.

TABLE 1. Qualitative Results from the Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay Conducted with Captan[a]

Test Substance	S9 Activation	Response of Bacterial Tester Strain[b]				
		TA1535	TA1537	TA1538	TA98	TA100
Captan	-	S	N	N	N	S
	+	S	S	D	N	S

[a]Information extracted from CBI, Table 2, p. 40.

[b]Response categories:

N = Negative (i.e., 1.0 to 1.5-fold increase over the spontaneous reversion frequency).

D = Doubtful (i.e., ≤2-fold increase over the spontaneous reversion frequency).

S = Strong (i.e., >5-fold increase over the spontaneous reversion frequency).

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? NO The study was conducted as part of a research program and was not intended for submission to a regulatory agency.

F. ___ APPENDIX: Appendix A, Materials and Methods, pp. 11-18.

APPENDIX A

MATERIALS AND METHODS
pp. 11-18