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

005571

NOV - 5 1986

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Registraton # 239-2351, Captan: Resubmission of metabolism data.
Tox. Chem. no.: 159
Accession No.: 262633

TO: H. Jacoby (PM 21)
Registration Division (TS-767C)

FROM: Marion P. Copley, D.V.M., D.A.B.T. MCopley 10/30/86
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THROUGH: Jane Harris, Ph.D., Section Head JH 10/30/86
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Chevron has resubmitted the following study comparing the metabolism of the fungicide Captan Technical in rats and mice. TITLE OF REPORT: S-2163 - The comparative metabolism of Captan in the rat and mouse (preliminary study), final report No. SOCAL 1992 dated 11/14/85.

This metabolism study was specifically designed to explain the increased incidence of duodenal tumors in mice, but not rats, following chronic administration of captan technical in the diet. This study is classified as core-supplementary and does not satisfy the metabolism regulatory requirements since its scope is limited to investivating effects in the gastro-intestinal (G.I.) tract. Although it does not supply a conclusive explaination for the species difference in captan-induced oncogenicity in the G.I. tract, it does provide supplemental information concerning the gastrointestinal distribution of captan in rats and mice.

BACKGROUND:

Captan is currently under special review (Position document 2/3 is completed) and a Registration Standard was completed in March 1986. The following data gaps still exist for captan:

- Acute dermal toxicity
- Primary dermal irritation
- 21-day dermal toxicity
- Chronic (oral) - non-rodent
- Subchronic (inhalation)
- Metabolism

Copley, Disc 8/7/1, Captan, # 159, Ln. 125/57, 10/20//30/86

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EPA: 68-02-4225
DYNAMAC No. 216A
October 29, 1986

DATA EVALUATION RECORD

CAPTAN

Metabolism in Rats and Mice

STUDY IDENTIFICATION: Wong, Z.A. and Chang, H.M. The comparative metabolism of captan in the rat and mouse. (Unpublished study No. SOCAL 1992, S-2163, by Chevron Chemical Company, Richmond, CA; dated November 14, 1985.) Accession No. 262633.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-29-86

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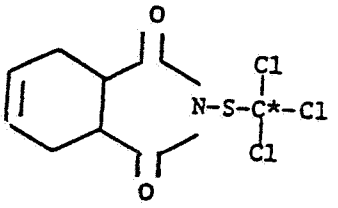
1. CHEMICAL: Captan.

2. TEST MATERIAL: [^{14}C-trichloromethylthio]captan ([^{14}C]captan) with a specific activity of 56 Ci/mole and a radiochemical purity greater than 99 percent. The structure of captan and the position of the radiolabel (*) are:



3. STUDY/ACTION TYPE: Metabolic study in rats and mice.

4. STUDY IDENTIFICATION: Wong, Z.A. and Chang, H.M. The comparative metabolism of captan in the rat and mouse. (Unpublished study No. SOCAL 1992, S-2163 by Chevron Chemical Company, Richmond, CA; dated November 14, 1985.) Accession No. 262633.

5. REVIEWED BY:

Charles E. Rothwell, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Charles Rothwell
Date: 10-29-86

Nicolas P. Hajjar, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: 10-29-86

6. APPROVED BY:

William L. McLellan, Ph.D.
Metabolism
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: 10-29-86

Marion P. Copley, D.V.M., D.A.B.T.
EPA Reviewer

Signature: Marion Copley
Date: 10/30/86

Jane Harris, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. A series of studies was conducted to help explain the increased incidence of duodenal tumors in mice, but not rats, following chronic administration of technical captan in the diet. The results indicate that a single oral dose of [^{14}C]captan at 250 mg/kg moves through the gastrointestinal (G.I.) tract of mice faster than that of rats. In addition, higher concentrations of captan and total [^{14}C] occur in the duodenum of the mouse than of the rat at 2 hours postadministration. The pH of the gastric mucosa of the mouse was also consistently higher than that of the rat, 3.5-4.0 versus 2.9-3.2 pH units, respectively. However, the species differences observed in these studies were minor in nature and do not provide a strong basis for explaining the species differences in captan-induced oncogenicity in the G.I. tract.

B. This metabolism study is inconclusive but does provide some supplemental information on the gastrointestinal distribution of captan in mice and rats.

Items 8 through 10--See footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material was [^{14}C-trichloromethylthio]captan ([^{14}C]captan) with a specific activity of 56 Ci/mole and a radiochemical purity greater than 99 percent. For dosing, the [^{14}C]captan was diluted to approximately 500 dpm/µg with unlabeled analytical grade captan and suspended in 1 percent carboxymethyl cellulose in distilled water.

   Additionally, diets were prepared by mixing captan technical (SX-1086, 90 percent purity) with Purina Rodent Chow to nominal concentrations of 500 and 5000 ppm. Fresh diets were prepared and offered once per week for mice and two to three times per week for rats.

2. Test animals were male and female Sprague Dawley rats and CD-1 mice from Charles River Laboratories. The animals were allowed a conditioning period of approximately 2 weeks prior to testing. Rats and mice weighed 90-160 g and 17-29 g, respectively, prior to dosing.

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[1]Only items appropriate to this DER have been included.

3. The metabolism, tissue distribution, and elimination of [^{14}C]captan was studied. Three unfasted males of each species were orally dosed with [^{14}C]captan at 250 mg/kg and placed in individual glass metabolism cages. Urine, feces, and expired air were collected at 5, 12, 24, 48, 72, and 96 hours postadministration; an additional sample of expired air was also collected at 3 hours postadministration.

   A 24-hour urine sample was taken from one rat and one mouse dosed at 250 mg/kg for metabolite analysis by TLC.

4. A preliminary study was performed to determine the approximate time when peak levels of [^{14}C] in the duodenum-jejunum were reached following an oral dose of [^{14}C]captan. Unfasted mice and rats (sex not specified) were dosed by oral intubation with [^{14}C]captan at 250 mg/kg. The animals (usually one rat and mouse per interval) were sacrificed 0.5 to 4 hours after dosing and the following sections of the G.I. tract and its contents were isolated by either ligation or applying hemostats and severing: esophagus to the pylorus, pylorus to the duodenum-jejunum junction (upper one-third of the small intestine), duodenum-jejunum junction to the ileo-cecal junction (lower two-thirds of the small intestine) and the cecum to the end of the colon.

5. Two groups of two rats and six mice of unspecified sex were orally dosed with [^{14}C]captan at 250 mg/kg and sacrificed 2 hours later. For one group, the animals were placed in individual metabolism cages to collect 0- to 2-hour urine, feces, and expired air. After sacrifice, the stomach, duodenum, distal small intestine, and large intestine were combined and radioassayed. Animals in the second group were sacrificed 2 hours postdosing, and the stomach, duodenum, and distal small intestine and their respective contents were removed and extracted first with ethyl acetate and then with methanol:water (1:1, v/v); the extracts and residues were then radioassayed. The ethyl acetate extracts were also analyzed by thin-layer chromatography (TLC) for [^{14}C]captan and metabolites.

6. Twenty rats and mice of each sex were administered diets containing technical captan at 500 or 5000 ppm for 90 or 148 days. Two rats and six mice preconditioned on the 5000-ppm captan diets were administered single oral doses of [^{14}C]-captan at 250 mg/kg after 90 days or 5 mg/kg after 148 days on the diets. The animals were sacrificed approximately 2 hours after dosing with [^{14}C]captan and the gastrointestinal sections and contents were taken for analyses of radioactivity and metabolites. Urine, feces, and expired air were also collected and radioassayed as previously described.

7. Rats and mice fed diets containing technical captan at 500 or 5000 ppm for 148 days were sacrificed, their gastrointestinal tracts were isolated, and the pH of the mucosal surfaces of the stomach and duodenum was measured using a surface pH electrode.

8. Aliquots of urine, tissue extracts, and TLC plate scrapings were radioassayed by liquid scintillation counting (LSC). All other samples were combusted and then radioassayed by LSC. Metabolites were separated by TLC in an appropriate solvent system. [^{14}C] metabolites located by autoradiography were scraped off and radioassayed by LSC; identification was performed by cochromatography with authentic standards.

B. A protocol was not included with the CBI.

12. REPORTED RESULTS:

A. Data on the homogeneity, stability, and actual concentrations of technical captan in the diet were not reported. Furthermore, data on the actual doses of [^{14}C]captan administered were not reported.

B. The mean recoveries of radioactivity in the excreta of male mice and rats administered single oral doses of [^{14}C]captan are presented in Table 1. After 96 hours, total recoveries in urine, feces, and expired air were similar for both species. However, mice excreted the administered [^{14}C] at a faster rate than rats as indicated by the higher percentage of [^{14}C] recovered in the excreta of mice at 12 hours (58.6 percent) than rats (23 percent). Very little radioactivity was recovered in the tissues and cage washes of dosed animals 96 hours postadministration (rat, 1.2 percent; mouse, 0.7 percent). Total recoveries of radioactivity 96 hours after dosing were 85.5 and 83.2 percent of the administered dose for mice and rats, respectively.

TLC analyses of 24-hour urine and feces from a male rat and mouse dosed with [^{14}C]captan at 250 mg/kg indicated similar metabolite distribution for both species. Unmetabolized captan comprised 96.3 and 93.0 of the recovered fecal radioactivity from the rat and mouse, respectively. Thiazolidine-2-thione-4-carboxylic acid (TTCA) and dithiobis (methane sulfonic acid) derivatives comprised 2.1 and 1.6 percent of the fecal [^{14}C] from the rat, respectively, and 3.2 and 3.8 percent from the mouse, respectively. For the urine, dithiobis (methane sulfonic acid) derivatives comprised 77.5 and 68.4 percent of the sample radioactivity from the rat and mouse, respectively, and TTCA accounted for 19.1 and 29.7 percent from the rat and mouse, respectively. Unmetabolized captan comprised only 0.2 percent of the urinary [^{14}C] for both species and less than 3.3 percent was unidentified.

TABLE 1. Excretion of [^{14}C] by Male Mice and Rats after Single Oral Dose of [^{14}C]Captan at 250 mg/kg[a].

Hours After Dosing	Mean percent of administered dose in[b]					
	Urine		Feces		Expired Air	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
12[c]	31.1	12.0	9.8	0.0	17.7	11.0
24	8.0	19.6	8.7	7.5	0.4	10.0
48	3.3	9.4	0.9	4.5	0.3	2.6
72	1.3	0.9	0.2	3.0	0.2	0.5
96	0.4	0.3	2.3	0.6	0.1	0.2
Total	44.0	42.2	22.0	15.6	18.8	24.2

[a]These animals were not given diets containing technical captan.

[b]Values are the means of three animals.

[c]Combined 3-, 5-, and 12-hour collections.

C. Results of the preliminary study indicated that no obvious peak distribution of [^{14}C] occurred in the duodenum of rats or mice 0.5 to 4 hours after an oral dose of [^{14}C]captan at 250 mg/kg. The mouse consistently had a higher percentage of radioactivity in the duodenum, 1.5 to 8 percent of the dose for the mouse versus 0 to 2 percent for the rat.

D. The distribution of radioactivity in expired air, excreta, and various sections of the G.I. tracts of mice and rats 2 hours after dosing with [^{14}C]captan is presented in Table 2. [^{14}C] elimination by nonpretreated animals dosed at 250 mg/kg was 8.9 and 2.2 percent of the dose for mice and and rats, respectively. However, total recoveries of [^{14}C] were not reported. Also, the mouse showed higher levels of [^{14}C] in the duodenum (6.8 percent, 397 ppm) and large intestine (28 percent, 1004 ppm) than the rat (0.6 percent, 109 ppm and 0.1 percent 3 ppm, respectively. In contrast, the rat had higher levels of [^{14}C] in the distal small intestine than the mouse (39.5 versus 7.1 percent of the dose, or 2850 and 364 ppm, respectively).

Two hours after oral administration of [^{14}C]captan at 250 mg/kg to rats fed diets containing technical captan at 5000 ppm for 90 days, 5.3 percent of the dose (410 ppm) and 0.7 percent of the dose (150 ppm) were found in the duodena of mice and rats, respectively. In contrast, levels of [^{14}C] in the duodena of mice and rats dosed with [^{14}C]captan at 5 mg/kg after 148 days on diets containing technical captan at 5000 ppm were not remarkably different from each other; 1.1 percent (2 ppm) and 1.7 percent (8 ppm), respectively. Similarly, higher levels of [^{14}C] were found in the large intestine of the mouse compared to the rat, but [^{14}C] levels were higher in the distal small intestine of the rat than mouse, for both pretreated dose groups. The authors did not make comparisons between pretreated and nonpretreated animals.

E. Ethyl acetate extraction of the various sections of the G.I. tracts of rats and mice removed 66.6 to 97.7 percent of the sample radioactivity (Table 3). An additional 0.5 to 21.6 percent of the sample [^{14}C] was extracted with methanol:water (1:1, v/v), and 1.8 to 11.7 remained bound and was unextractable. TLC analysis of the gastric ethyl acetate extracts showed that greater than 98 percent of the [^{14}C] in this fraction for both rats and mice was unmetabolized [^{14}C]captan (Table 4). In the mouse duodena, only 65.7 percent of the ethyl acetate extractable [^{14}C] was captan; 32.1 and 2.2 percent were unidentified polar and nonpolar material, respectively. The ethyl acetate extracts of the rat duodenums contained 33.0 and 58.2 percent of the [^{14}C] as captan and polar material, respectively. In the distal small intestine, a larger proportion of captan was found in the rat than in the mouse.

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TABLE 2. Distribution of Radioactivity in the Excreta and G.I. Tracts of Rats and Mice 2 Hours After Administration of a Single Oral Dose of [^{14}C]Captan

Sample	Percent of Recovered Dose [a]					
	250 mg/kg		250 mg/kg[b]		5 mg/kg[c]	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Expired air	6.2	2.1	7.1	1.6	12.9	6.6
Cage wash	0.1	0.1	2.8	0.1	0.3	0.6
Feces	0.1	0.1	0.2	0.0	0.0	0.0
Urine	2.7	-[d]	11.4	1.2	23.1	7.6
Carcass	N.D.[e]	N.D.	4.1	1.9	13.8	15.7
Stomach	48.9(2928)[f]	57.6(5051)	10.5(1313)	66.6(9109)	6.3(14)	21.1(74)
Duodenum	6.8(397)	0.6(109)	5.3(410)	0.7(150)	1.1(2)	1.7(8)
Distal small intestine	7.1(364)	39.5(2850)	13.0(688)	27.8(1853)	8.5(7)	46.4(55)
Large intestine	28.2(1004)	0.1(3)	45.4(1620)	0.1(3)	33.9(23)	0.3(1)

[a]Results are from combined samples of two rats and six mice (sex not reported).

[b]Animals were placed on diets containing 5000 ppm technical captan for 90 days prior to dosing with [^{14}C]captan.

[c]Animals were placed on diets containing 5000 ppm technical captan for 148 days prior to dosing with [^{14}C]captan.

[d]No sample.

[e]N.D., not detected.

[f]Values in parentheses are concentrations of [^{14}C] as captan equivalents in ppm. The authors do not clearly indicate whether these concentrations were measured in the tissues, their contents, or both.

TABLE 3. Extractable and Nonextractable Radioactivity in the G.I. Tracts of Mice and Rats 2 Hours After Dosing with [^{14}C]Captan at 250 mg/kg

Fraction	Percent of Sample [^{14}C][a]					
	Stomach		Duodenum		Distal small intestine	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Ethyl acetate extract	97.0	97.7	77.5	71.6	66.6	90.2
Methanol:water (1:1, v/v) extract	0.8	0.5	17.1	18.1	21.6	5.7
Unextractable	2.2	1.8	5.4	10.3	11.7	4.0

[a]Results were from combined samples of two rats and six mice.

TABLE 4. TLC Analysis of Ethyl Acetate Extracts of Various Sections of the G.I. Tracts of Mice and Rats Dosed with [^{14}C]Captan at 250 mg/kg

Metabolic Fraction	Percent of [^{14}C] in Ethyl Acetate Extracts					
	Stomach		Duodenum		Distal small intestine	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Nonpolar[a]	0.3	0.1	2.2	8.7	8.2	0.4
Captan	98.2	99.0	65.7	33.0	55.3	92.8
Origin (polar)[a]	1.5	0.8	32.1	58.2	36.4	6.8

[a]These fractions were not further defined.

F. Ether and methanol extracts of the duodenum, stomach, and distal small intestine of rats and mice dosed with [^{14}C]captan at 5 mg/kg after 148 days on diets containing technical captan at 5000 mg/kg were analyzed by TLC. The amount of extractable and unextractable [^{14}C] in these samples were not reported. Levels of [^{14}C]captan, TTCA, and unidentified polar metabolites in the three sections of the G.I. tracts were similar for both rats and mice.

G. The mean gastric and duodenal mucosal pH in rats and mice fed diets containing technical captan at 0, 500, or 5000 ppm for 148 days are presented in Table 5. The pH values for the gastric mucosa of mice were generally 0.5-0.7 pH units higher than for rats; the ranges were 3.5-4.0 and 2.9-3.2, respectively. No compound-related effects on the gastric mucosal pH were observed.

For the duodenum, the surface mucosa in male mice fed 500 and 5000 ppm diets had significantly lower ($p \leq 0.05$) pH values than the controls. No compound-related effects were seen in female mice or male and female rats.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The levels of captan and total [^{14}C] were higher in the duodenum of the mouse than the rat at 2 hours after oral administration of 250 mg/kg [^{14}C]captan; however, levels for both species were similar at 5 mg/kg. The elimination and gastromobility rates of captan and the gastric pH of the mouse and rat were different. The pH at the surface of the duodenal mucosa in male mice appeared to be lower as a result of subchronic dietary intake of captan. These species differences in gastrointestinal pH and duodenal concentration of captan and total [^{14}C] may be related to the different oncogenic response observed in the two species.

B. A quality assurance statement was not included with the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The results and conclusions presented in this report do not provide an explanation for the observation that dietary intake of captan produces duodenal tumors in mice and not in rats. The authors suggest that the differences in the pH of the gastric mucosa in mice when compared to rats may alter the degradation of captan leading to the observed oncogenicity in chronic studies. However, their own data show that the distribution of [^{14}C] metabolites/degradates in the stomachs of both mice and rats are almost identical with regards to extractable versus nonextractable [^{14}C] (Table 3) and TLC profile of the ethyl acetate extracts (Table 4). The reported differences in mean duodenal mucosal pH (Table 5), although statistically significant, are so minor (<0.3 pH units) as to be biologically

TABLE 5. Mean pH Values for Gastric and Duodenal Mucosa of Rats and Mice Placed on Diets Containing 0, 500, or 5000 ppm Technical Captan for 148 Days[a]

	Stomach		Duodenum	
	Mouse	Rat	Mouse	Rat
Males				
Control	3.58±0.74	3.17±0.85	6.33±0.18*[a]	6.10±0.29*[a]
500 ppm	4.01±0.56*[b]	3.02±0.73*[b]	6.09±0.19*[c]	6.18±0.28
5000 ppm	3.96±0.53*[b]	2.98±0.87*[b]	6.08±0.21*[c]	6.19±0.28
Females				
Control	3.53±0.79	3.09±0.82	6.15±0.29	6.07±0.22
500 ppm	3.50±1.03	2.91±0.61	6.11±0.14	6.11±0.22
5000 ppm	3.58±0.75	2.96±0.59	6.13±0.24	6.27±0.34

[a]Mean pH values of 5-15 animals per group.

*[a]Mean duodenal pH of control rats is significantly lower ($p \leq 0.05$) than that of the mouse (Student's t-test).

*[b]Mean gastric pH of the rat is significantly lower ($p < 0.05$) than that of the mouse (Student's t-test).

*[c]Mean duodenal pH is significantly lower ($p \leq 0.05$) than the control (two-tail analysis of variance).

insignificant. Additionally, the authors suggest that the higher concentrations of [^{14}C] in the duodenum of mice dosed at 250 mg/kg, as compared to rats may be related to the oncogenicity differences observed between the two species. However, this is also contradicted somewhat by the reported results. In mice dosed at 250 mg/kg, [^{14}C] levels in the distal small intestine (364 and 688 ppm) were similar to those in the duodenum (397 and 410 ppm), and [^{14}C] levels in the large intestine (1004 and 1620 ppm) were much higher (Table 2). In rats similarly dosed, [^{14}C] levels in the distal small intestine (2850 and 1853 ppm) were also much higher than those in the duodenum of the mouse, indicating that concentration is not the most important factor in determining the oncogenicity of captan. Also, the authors did not clearly indicate whether these comcentrations were determined on the tissues or contents of the G.I. tracts.

Finally, the results obtained are inadequate to explain the species differences in captan-induced intestinal oncogenicity because of the study design and incomplete reporting. The authors utilized captam radiolabeled at the trichloromethylthio moiety; therefore, the fate of the cyclic moiety of the molecule is unknown. It is umlikely, however, that the ring structure is responsible for these tumors because: 1) captafol, which has the same ring but a different side chain, does not cause these tumors and 2) folpet, which has a trichloromethylthio side chain but a different ring structure tham captan and captafol, is associated with an increased incidence of duodenal tumors. The authors also combined samples from animals im some studies; thus, individual animal data and variance for those results are not available. Additionally, the report was poorly organized and written, which made a full understanding of exactly what was done impossible.

In summary, this study provides some interesting informatiom on the gastroimtestinal fate of [^{14}C]captan, but does not explain the differences in captan oncogenicity between mice and rats.

Item 15--See footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-7.

APPENDIX A

Materials and Methods

(CBI pp. 3-7)

Captan Science Reviews

Page _____ is not included in this copy.

Pages _16_ through _20_ are not included in this copy.

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