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DATA EVALUATION REPORT

CAPTAN

STUDY TYPE: TIME-COURSE ORAL - MOUSE

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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CAPTAN **Time Course Study (no guidelines)**

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DATA EVALUATION RECORD

STUDY TYPE: Time course- oral
(No guidelines)

DP BARCODE: D225683
P.C. CODE: 081301

SUBMISSION CODE: SSE04312
TOX. CHEM. NO.: MRID 43982201

TEST MATERIAL (PURITY): Captan (89.4% w/w)

SYNONYMS: 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thiol]-1H-isoindole-1,3(2H)-dione; N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide; N-trichloromethyl-thio-3a,4,7,7a-tetrahydrophthalimide; Merpan, Orthocide-406

CITATION: Tinston, D. (1996) Captan: A time course study of induced changes in the small intestine and stomach of the male CD1 mouse. Central Toxicology Laboratory, Aldery Park, Macclesfield, Cheshire, UK SK10 4TJ. Study No. PM/005, Report No. CTL/P/4893, January 24, 1996. MRID 43982201. Unpublished.

SPONSOR: Zeneca Agricultural Products, P.O. Box 15458, Wilmington, DE 19850-5458.

EXECUTIVE SUMMARY: In a 28-day feeding study, groups of 25 male CD1 mice were given 3000 ppm dietary Captan (89.4% w/w; batch no. WRC14264-13-1) or untreated diet. A third group of 25 served as pair-fed controls. Five mice of each group were sacrificed on days 1, 3, 7, 14, and 28 to assess the time course of gastrointestinal effects.

Although two mice of the treatment group and one mouse in the pair-fed control group were terminated due to excessive body weight loss, no deaths could be attributed to the direct effects of the test material and clinical findings were limited to reduced body weight gain. The reduced body weight gain in the Captan-treated group was consistent with that observed in the pair-fed controls and could be attributed to food avoidance (i.e., reduced palatability) and consequent inanition. Based upon mean body weights and feed consumption, the 3000 ppm dietary exposure was equivalent to ≈621 mg Captan/kg body weight. Twenty-eight day dietary exposure to 3000 ppm Captan resulted in

duodenal distention on days 3-14 and histopathological findings characterized by crypt cell hyperplasia, villous shortening, and disorganization of villus enterocytes. Histopathological findings were observed by day 3 of treatment and persisted throughout the treatment period but were limited to the duodenal region of the intestine. Only a few incidences of minor findings were noted for the stomach and there were no findings in the ileum or jejunum. The results of this study demonstrated the exposure duration-dependent irritant effect of dietary Captan on duodenal epithelium of mouse.

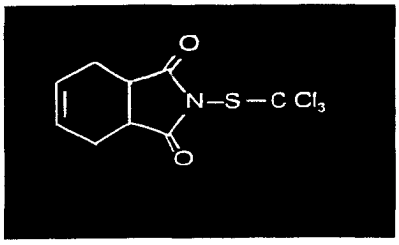
COMPLIANCE: This study is considered acceptable (nonguideline) for the purpose of describing the time course of intestinal lesions in mice during a 28-day dietary exposure to Captan. Signed and dated GLP (04/15/96), quality assurance (01/24/96) and flagging criteria statements (01/24/96) were present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Captan

Description: off-white/beige solid
Lot/Batch #: WRC14264-13-1
Purity: 89.4% w/w
Stability of compound: not stated
CAS #: 133-06-2
Structure:



Captan

2. Vehicle and/or positive control

Powdered rodent chow (CT1 diet) served as the vehicle; no positive controls were used

3. Test animals

Species: Mouse
Strain: CD-1
Age and weight at study initiation: 29-33 days on delivery (approximately 41-52 days at study initiation); 31.1 g (control and treated groups); 32.9 g (pair-fed controls)
Source: Charles River (no address provided)
Housing: Housed singly after assignment to experimental group.

Diet: CT1 diet provided ad libitum (Special Diet Services, Ltd, Witham, Essex, UK) for controls and treatment group.
Water: tap water provided ad libitum
Environmental conditions:
Temperature: 22±3°C
Humidity: 30-70%
Air changes: at least 1.5.hr; positive pressure
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 12-19 days

B. STUDY DESIGN

1. In life dates

Not specfically provided. Estimated as 03/21-28/95 to 04/18-25/95 due to staggered starting dates as per food intake (Appendix 3, MRID43982201).

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: Study design

Treatment group	Captan	No. of	Comments
Group 1 (untreated	0	25 males	Five mice were terminated at each
Group 2 (Captan	3000	25 males	Five mice were terminated at each
Group 3 (pair-fed	0	25 males	Five mice were terminated at each

Data taken from p.17 of MRID 43982201

3. Diet preparation and analysis

Experimental diet was made as a 10 kg batch which contained 9.5 kg of diet thoroughly mixed with a 0.5 kg premix that contained the appropriate amount of the test material and untreated diet. Samples of the experimental diet and control diet were analyzed quantitatively for Captan by gas chromatography.

Homogeneity analysis: Homogeneity analyses indicated that concentrations of the test material in the diet were within 0.1% of the overall mean.

Stability analysis: The test material appeared to be stable in the diet over a period of 42 days exhibiting only a 4.3% reduction relative to initial concentration.

Concentration analysis: Captan concentration was 101.0% of nominal. No Captan was detected in the control diets

4. Statistics

Statistical analyses were limited to simple descriptive statistics (mean and standard deviation) and application of two-sided Student's t-test for group comparisons (0.05 significance level).

C. METHODS

1. Observations

Prior to the start of the study, all mice were examined to assure health status and normal activity. Animals were inspected once daily for clinical signs of toxicity and for mortality.

2. Body weight

Individual body weights were recorded prior to the experiment and daily on days 2-8, 15, 22, and 29.

3. Food consumption and compound intake

Animals in Groups 1 and 2 were fed and watered *ad libitum*. Food consumption was recorded daily for the first week and weekly for the remaining three weeks. Mean feed consumption was provided for these periods. The food consumption of the Captan experimental group (Group 2) was used to determine the food provided to the Group 3 pair-fed controls.

4. Sacrifice, tissue and sample analysis

a. Sacrifice and tissue preparation

All of the surviving mice were anesthetized with halothane and exsanguinated. Two mice of the Captan-treatment group were sacrificed on day 4 due to excessive body weight loss, and one mouse of the pair-fed control group that exhibited adverse clinical signs and excessive body weight loss was also sacrificed on day 4. Blood samples (0.5 ml) were collected in lithium/heparinized tubes and retained at -20°C. All terminal and interim sacrifice animals were examined for gross pathology. The small intestine and stomach were prepared and fixed in Bouin's fixative for 24 hours. Duodenum, ileum, jejunum and stomach were embedded in paraffin, sectioned at 5 μm,

and stained with hematoxylin and eosin. Histopathologic examinations were conducted using light microscopy.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

With the exception of what appeared to be food avoidance-induced inanition, there were no treatment related signs of toxicity reported.

2. Mortality

Two mice were terminated on day 4. One mouse in the 3000 ppm group exhibited excessive body weight loss and one mouse in the pair-fed control group exhibited adverse clinical findings (piloerection, tremors, labored breathing, hypothermia) and excessive body weight loss. No adverse findings were observed in any of the remaining mice.

B. BODY WEIGHT AND WEIGHT GAIN

Mean body weight values are shown in Table 2. Relative to untreated controls, the Captan-treated mice exhibited significant ($p<0.01$ or 0.5) decreases in body weight at all time points measured beyond day 1. The final mean body weight of the Captan-treated mice at day 28 was approximately 11% less than that of the untreated controls. The reduced body weight gains corresponded to a concurrent decrease in feed consumption and were consistent with the reduced body weight gains observed for the pair-fed controls. The mean body weight for mice in Groups 1, 2, and 3 were 32.0 ± 1.1 g, 28.4 ± 1.6 g, and 29.2 ± 2.3 g, respectively.

TABLE 2. Mean body weight (g) of mice

Day	0 ppm	3000 ppm	0 ppm
1	31.1±1.7	31.1±1.6	32.9±1.9**
2	31.4±1.6	28.6±1.9**	29.4±1.8**
3	31.2±1.3	26.6±1.5**	29.4±1.8**
4	31.3±1.2	26.2±2.1**	28.0±1.8**
5	31.3±1.4	26.7±2.4**	27.1±1.4**
6	31.2±1.2	27.4±1.9**	27.1±1.7**
7	31.8±1.4	28.2±1.7**	26.1±1.9**
8	32.0±1.4	28.6±1.6**	28.3±2.3**
Mean days 1-8	31.4±0.3	27.9±1.6	28.5±2.1
15	32.9±1.4	29.3±1.8**	29.0±2.0**
22	33.3±2.0	29.3±2.2*	32.1±1.5
29	34.5±1.7	30.6±2.2*	32.0±1.7*
Mean[a]	33.0±1.3	29.3±1.0	30.4±1.7

Data taken from Table 5, p. 32, MRID 43982201.
[a] Calculated by reviewer; mean of days 1-8 and weeks 2-4.
* Statistically significant at $p<0.05$ (two-tailed Student's t-test).
** Statistically significant at $p<0.01$ (two-tailed Student's t-test).

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Mean food consumption values for all three groups are presented in Table 3.

TABLE 3. Food consumption (g/mouse/day) in mice given dietary captan for 28 days

Captan Day	0 ppm (untreated control)	3000 ppm	0 ppm (pair-fed control)
One	7.0±1.0	4.4±1.4**	4.4±0.5**
Two	6.6±1.2	4.2±1.9**	4.2±0.4**
3	6.8±1.1	4.7±1.5**	4.6±1.1**
4	6.7±0.6	5.3±1.1**	4.7±0.6**
5	8.0±2.5	5.9±1.6*	5.5±0.5**
6	6.6±1.1	5.4±0.6**	5.4±0.5**
7	7.5±1.0	7.4±1.7	7.6±1.0
Mean days	7.0±0.5	5.3±1.2	5.2±1.2
15	6.7±0.5	6.3±0.8	6.3±0.3*
22	8.5±1.4	7.5±1.3	7.3±0.3
29	7.5±0.5	6.9±0.8	6.8±0.3*
Mean[a]	7.4±0.8	6.5±0.9	6.4±0.9

Data taken from Table 6, pp. 33-34, MRID 43982201

[a] Calculated by reviewer; mean of days 1-7 and weeks 2-4.

* Statistically significant at $p<0.05$ (two-tailed Student's t-test).

** Statistically significant at $p<0.01$ (two-tailed Student's t-test).

2. Compound consumption

Compound intake was not provided in the study report but was estimated by the reviewer based upon food intake (5.8 g/mouse = 207.1 g/kg), body weight (mean body weight = 0.028 kg), and the test article concentration in the food (3000 ppm = 3 mg/g):

Captan intake = 207.1 g diet/kg b.w.x3 mg Captan/g diet = 621 mg Captan/kg b.w.

3. Food efficiency

Food efficiency ({body weight gain [g]/food consumption [g per unit time]} x 100) was not calculated by the study authors. Food efficiency, however, may be estimated from the data provided:

Group 1: = (3.4 g [b.w. gain]/208.1 g feed) x 100 = 1.63
Group 2: = (-0.5 g [b.w. gain]/182.2 g feed) x 100 = -0.27
Group 3: = (-0.9 g [b.w. gain]/179.2 g feed) x 100 = -0.50

Food efficiency for the Captan-treated mice was similar to that of the pair-fed controls suggesting that the reduced body weight gain of the Captan-treated group was likely the result of reduced feed intake and inanition.

D. TISSUE AND SAMPLE ANALYSIS

All of the surviving mice were anesthetized with halothane and exsanguinated. Two mice of the Captan-treatment group were sacrificed on day 4 due to excessive body weight loss, and one mouse of the pair-fed control group that exhibited adverse clinical signs and excessive body weight loss was also sacrificed on day 4. Blood samples (0.5 ml) were collected in lithium/heparinized tubes and retained at -20°C. All terminal and interim sacrifice animals were examined for gross pathology. The small intestine and stomach were prepared and fixed in Bouin's fixative for 24 hours. Duodenum, ileum, jejunum and stomach were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Histopathologic examinations were conducted using light microscopy.

1. Gross pathology

Distension of the duodenal lumen was observed in 4 of 5 mice at day 1, 2 of 5 mice at days 3 and 7, and 5 of 5 mice at day 14. The duodenal distention was no longer observed at day 28. There were no gross findings in other sections of the small intestine or the stomach at any time periods throughout the study, and there were no gross findings in the two mice killed on day 4.

2. Microscopic pathology

There were no histopathologic findings in mice following one day of exposure to dietary the Captan. However, at later time periods, mice given dietary Captan (3000 ppm) exhibited histopathologic changes in the duodenum and stomach that were not observed in the untreated controls or the pair-fed controls.

1) Non-neoplastic - At three days, mice given Captan exhibited duodenal crypt cell hyperplasia (4 of 5 mice

examined), shortening of the villi (3/5), and general disorganization of the villus enterocytes (2/5). At day 7, immature cells were observed at the villus tips in all mice examined. Examination upon interim sacrifice revealed that the incidences and severity of these findings in the duodenum were maintained throughout the treatment. Other findings of less frequent incidences included inflammation and edema of the lamina propria and focal erosion/enteritis, none of which were maintained throughout the treatment period. There were no histopathologic findings in the ileum or jejunum sections of the small intestine. Findings in the stomach were limited to glandular dilatation (1/5), focal gastritis (1/5), and luminal cell exudate (1/5) on day 3, and focal paraketosis (1/4) on day 28. The findings in the stomach were not maintained throughout the treatment and some were also observed in untreated controls and, therefore, were not definitively treatment related. Nonneoplastic findings in individual animals are shown in Table 4.

2) Neoplastic - Neoplastic changes were not observed in any of the mice in the study.

TABLE 4. Individual pathologic findings in the duodenum of male mice following dietary exposure to 3,000 ppm Captan

Animal No. (On study)	Edema/ Inflammation	Villous shortening	Immature villous enterocytes	Enterocyte disorganziation	Erosion/ enteritis	Hyperplasia	Metaplasia
26 (2 d/1 wk)	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-
28	+ +	-	-	-	-	-	-
29	-	-	-	-	-	-	-
30	+	-	-	-	-	-	-
31 (4 d/1 wk)	-	+ +	-	-	-	+[a]	-
32	-	+	-	+	-	+[a]/+ +[b]	-
33	+	-	-	+	-	+[a]	-
34	+ + +	-	-	-	-	-	-
35	-	+	-	-	+	+[a]	-
36 (8 d/2 wks)	-	+ + +	+ +	+ +	-	+ + +[a]	+
37	-	+ + +	+ +	+ +	-	+ + +[a]	-
38	-	+ + +	+ +	+ +	-	+ + +[a]	-
39	-	+ +	+ + +	+ +	-	+ + +[a]	-
40	-	+ + +	+ +	+ +	-	+ + +[a]	-
41	-	+ + +	+ + +	+ +	-	+ + +[a]	-
42 (15 d/3 wks)	-	+ + +	+ + +	+ +	-	+ + +[a]	-
43	-	+ + +	+ + +	+ +	-	+ + +[a]	-
44	-	+ + +	+ + +	+ +	-	+ + +[a]	-
44	-	+ + +	+ + +	+	-	+ + +[a]	-
45	-	+ + +	+ + +	+ + +	-	+ + +[a]	+ +
46 (29 d/5 wks)	-	+ + +	+ + +	+ + +	-	+ + +[a]	-
47	-	+	+ + +	+	-	+ +[a]	-
48	-	+ +	+ + +	+ +	-	+ + +[a]	-
49	-	+ + +	+ + +	+ + +	-	+ +[a]	-
50	-	+ + +	+ + +	+ + +	-	+ + +[a]	-

+ minimum; + + slight; + + + moderate; + + + + marked

[a] crypt cell hyperplasia

[b] goblet cell hyperplasia

Data taken from *Appendix 5*, pp. 113-146, MRID 43982201.

III. DISCUSSION

A. DISCUSSION

This study was designed to assess the time-course of intestinal and gastric changes in mice following 28-day dietary exposure to 3000 ppm Captan. In this 28-day feeding study, groups of 25 male CD1 mice were given dietary Captan (89.4% w/w; 3000 ppm) or untreated diet. A third group of 25 served as pair-fed controls. Five mice of each group were sacrificed on days 1, 3, 7, 14, and 28 to assess the time course of gastrointestinal effects. The study appeared to be well conducted, used adequate numbers of animals, and diet analysis indicated acceptable test article concentration, homogeneity and stability.

There were no deaths during the study that could be attributed directly to the test material. Three mice were sacrificed on day 4 (two mice in the 3000 ppm Captan group and one in the pair-fed control group) due to excessive body weight loss and, for the mouse in the pair-fed control group, adverse clinical findings. Based upon mean body weights and feed consumption, the 3000 ppm dietary exposure was equivalent to ≈621 mg Captan/kg body weight. Because of the reduced feed intake and resulting inanition, it is difficult to determine if this dose was approaching a maximally tolerated dose. Food efficiency for the Captan-treated mice and that of the pair-fed controls were similar, and both were lower than that of the untreated controls. This implies that the reduced body weight gain of the Captan-treated group was likely the result of reduced feed intake (i.e., feed avoidance) and inanition.

The irritant mode of Captan on specific regions of the gastrointestinal tract was confirmed by gross and histopathological findings in the treated mice and the absence of such effects in the pair-fed controls. Histopathological findings including duodenal crypt cell hyperplasia, villous shortening, and disorganization of villus enterocytes were observed by day 3 of treatment indicating that onset of Captan-induced irritation is relatively rapid. The histopathological changes appeared to be limited to the duodenal region of the intestine; only a few incidences of minor findings were noted for the stomach and there were no findings in the ileum or jejunum. The severity and incidence of the histopathologic responses increased or was at least maintained with duration of treatment.

The results of this study demonstrated the irritant effect of dietary Captan on duodenal epithelium. Various indices of tissue irritation in the absence of cellular destruction were concurrent with observed hyperplasia. The study author contended that the observed irritation results in a hyperplastic response indicative of a nongenotoxic mechanism for the formation of adenomas and adenocarcinomas following long-term oral exposure of mice to Captan. Although such a conclusion may be tenable, data from genotoxicity studies and cancer bioassays would be neeeded to confirm such a contention. Considering the duration and scope of this study, the study author's conclusion based solely on the results of this study may be premature.

B. STUDY DEFICIENCIES

Although the response of only male mice was examined, the irritant effects of ingested Captan would not likely be different in female mice. There were no deficiencies noted that would compromise the validity of the study.

13544

R180699

Chemical Name: Captan

PC Code: 081301
HED File Code: 61400 SRRD DERs
Memo Date: 1/30/1998
File ID: DPD225683
Accession #: 000-00-8016

HED Records Reference Center
11/17/2010