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

MAY 18 1990

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

**SUBJECT:** EPA ID 0120: Captan: 90-day rat inhalation study and request for waiver on the 90-day rat dermal inhalation study.

**TO:** L. Schnaubelt/K. Samek (PM74)  
Special Review & Reregistration Division (H-7508C)

**FROM:** Marion P. Copley, D.V.M., Section Head  
Section 2, Toxicology Branch I  
Health Effects Division (H-7509C) *Marion Copley*  
*5/8/90*

**THRU:** Karl Baetcke, Ph.D., Branch Chief  
Section 2, Toxicology Branch I  
Health Effects Division (H-7509C) *Karl P. Baetcke*  
*5/15/90*

Tox. Chem. No.:159  
Proj. No.:9-2216  
Record No.:252887

- 1) A new 90-day inhalation study has been submitted to the Agency in response to the Registration Standard for Captan.
- 2) In addition the Agency has been requested to waive the data requirement for a 90-day dermal rat study based on the results of this study.

CONCLUSIONS:

As discussed in a previous memorandum from M. Copley to L. Schnaubelt of RD, the following conclusions can be made:

- 1) The 90-day inhalation study satisfies the guideline requirements for a 90-day inhalation study.
- 2) There are no indications of the renal effect that was observed in a previous study. Therefore, a subchronic dermal toxicity study should not be required.
- 3) Toxicology Branch 1 (TB1) has no objections to the waiver request for the 90-day dermal rat study with Captan.

**BACKGROUND:**

The Agency has currently completed a Special Review on Captan. Position Document (PD) 2/3 was completed (June, 1985). The PD4 was published in the Federal Register on 2/24/89. The Health Effects Division Peer Review Committee determined that Captan is a B<sub>2</sub> oncogen (12/29/86 and 4/13/88). The following data gaps identified in the Registration Standard for Captan (March 1986) still remain:

- ° Chronic (oral) - non-rodent
  - ° Metabolism
- (either supplementary or not submitted)

A 90-day inhalation study (#86-7951) was submitted using doses between 5 and 50 mg/m<sup>3</sup>. This study was determined to be supplementary since signs of renal and upper respiratory tract alterations occurred at all doses. A 90-day dermal study was required by DCI in order to determine if dermal and inhalation exposures should be combined. If combined, there were inadequate margins-of-exposure (MOE) when compared to an estimated NOEL of 0.5 mg/m<sup>3</sup> (this was 0.1 of the LEL from the flawed study). Following this determination, ICI reevaluated the exposure methods of the earlier inhalation study and determined (TBI agreed) that they were seriously flawed (see memorandum from M. Copley to R. Mountford dated 10/20/88) and should not be used to regulate captan. As a result, a second 90-day inhalation study was conducted using doses between 0.13 and 12.98 mg/m<sup>3</sup>. Preliminary data indicated that the renal lesion was not present in the new study. Therefore an extension was granted to the registrant for the 90-day dermal study until the new 90-day study could be submitted and evaluated by the Agency.

**CURRENT ACTION**

ICI has submitted the final 90-day inhalation study (MRID 412344-02, study # PR0735) and a 3 week range-finding study (MRID 412344-01). Results of this well conducted study confirm the preliminary data submitted previously.

- 1) Exposure data indicates that rats received up to 13 ug/l captan. These concentrations were numerically greater than those supposedly obtained at the lowest dose in the flawed study. Particle sizes were within the required range.
- 2) Histologic data indicates that renal lesions, observed in the flawed study at all doses, were not present at any dose. This suggests that the earlier study may have markedly under-estimated the actual exposure.

Special stains were used to confirm the absence of the previously observed renal tubular lesions.

- 3) In addition, a 21-day range finding study (MR0113), testing to 25 ug/l did not result in renal lesions at any dose. The type of lesion observed in the flawed study would be expected to be present during this time frame.

Based on the above studies, it is unlikely that exposure to Captan, by the inhalation route, would result in inadequate MOE for renal changes. The NOEL for renal effects is at least 25 ug/l or 3.365 mg/kg/day. MOE for the combined exposure, when dermal exposure and protective clothing is considered are greater than 100 (worst case applicator exposure for mangos, nectarines, peaches = 0.154 mg/kg/day, times 0.2 for protective clothing). This diminishes significantly, our concern for dermally induced renal toxicity.

#### SUMMARIES OF STUDIES

- Subchronic (90 day) Inhalation Toxicity Study - Rats/ICI Central Toxicology Lab., UK/ Study # CTL/P/2543/Aug. 1, 1989.  
Core-Minimum . MRID 412344-02

Wistar-derived rats were exposed (nose-only) to captan at levels of 0.13, 0.60, 5.06 or 12.98 ug/l for 13 weeks (6 hr/day, 5 days/wk).

NOEL for systemic effects  $\geq$  12.98 ug/l

LEL for systemic effects > 12.98 ug/l

NOEL for local irritation < 0.13 ug/l

LEL for local irritation  $\leq$  0.13 ug/l based on squamous hyperplasia in the larynx in females.

In addition at 0.60 ug/l squamous hyperplasia was observed in males. At 5.06 ug/l there was necrosis or attenuation of bronchial epithelium and mild epithelial necrosis of bronchi/bronchioles, metaplasia and vacuolar degeneration of squamous epithelium. At 12.98 ug/l there was rhinitis and degeneration of the olfactory epithelium and deaths due to respiratory lesions characterized by marked bronchial necrosis, alveolar changes, congestion and edema of the lungs, degenerative and proliferative changes of nasal cavity and larynx.

Following a 4 week recovery period, all but the laryngeal lesions were reversed in the 12.98 ug/l survivors.

- Subchronic (21 day) Preliminary Inhalation Toxicity Study - Rats/ICI Central Toxicology Lab., UK/ Study # CTL/P/2534/Aug. 1, 1989.

Captan

4 90-day dermal waiver request

Acceptable

MRID 412344-01

Wistar-derived rats were exposed (nose-only) to captan at levels of 0.8, 5.3, or 24.8 ug/l for 3 weeks (6 hr/day, 5 days/wk).

NOEL for systemic effects  $\geq$  24.8 ug/l

LEL for systemic effects > 24.8 ug/l

NOEL for local irritation = 5.3 ug/l

LEL for local irritation = 24.8 ug/l based on nasal histopathology and laryngeal ulceration (females); alveolar edema, congestion and hemorrhage in lungs along with bronchilar necrosis in 2 decedent males.

COPLEY, PC6\CAPTAN\MEMO2.272, #9-2216, 5/9/90

007938

EPA No.: 68D80056  
DYNAMAC No.: 260-B  
TASK No.: 2-60B  
March 16, 1990

DATA EVALUATION RECORD

CAPTAN

Subchronic Inhalation Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.  
Program Manager  
Dynamac Corporation

Signature: *Roman Penta for*

Date: 3-16-90

EPA No.: 68D80056  
DYNAMAC No.: 260-B  
TASK No.: 2-60B  
March 16, 1990

DATA EVALUATION RECORD

CAPTAN

Subchronic Inhalation Toxicity Study in Rats

REVIEWED BY:

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Marion P. Copley, D.V.M.,  
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Toxicology Branch I  
(H-7509C)

Signature: Marion Copley  
Date: 5/9/90

DATA EVALUATION RECORD

GUIDELINE § 82-4

STUDY TYPE: Subchronic Inhalation Toxicity Study in Rats.

MRID NUMBER: 412344-02.

TEST MATERIAL: Captan.

SYNONYM: N-Trichloromethylthio-4-cyclohexene 1,2-dicarboxamide.

STUDY NUMBER: CTL/P/2543.

SPONSOR: Captan Task Force: Chevron Chemical Co., Makhteshim Agan, ICI Americas, Inc.

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom.

TITLE OF REPORT: Captan: 90-Day Inhalation Toxicity Study in the Rat.

AUTHOR: P. M. Hext.

REPORT ISSUED: August 1, 1989.

CONCLUSIONS: Wistar-derived rats (10/sex/group) were exposed (nose-only) to captan at levels of 0.13, 0.60, 5.06, or 12.98  $\mu\text{g}/\text{l}$  for 13 weeks (6 hours/day, 5 days/week). Additional groups were exposed at the highest level and allowed a 4-week recovery period. No systemic toxicity was observed. Histologic changes were confined to the respiratory system and were consistent with effects of exposure to an irritant particle.

Females, but not males, exposed to 0.13  $\mu\text{g}/\text{l}$  showed an increased incidence of squamous hyperplasia in the larynx; there were no histologic changes in the lungs, bronchi, or nasal cavity. Squamous hyperplasia was increased in both sexes exposed at 0.60  $\mu\text{g}/\text{l}$ .

At 5.06  $\mu\text{g}/\text{l}$ , necrosis or attenuation of the bronchial epithelium and mild epithelial necrosis of the bronchi/bronchioles were observed in both sexes and hyperplasia of the bronchial epithelium was observed in females; changes in the larynx of both sexes were metaplasia, hyperplasia, and vacuolar degeneration of the squamous epithelium.

At 12.98  $\mu\text{g}/\text{l}$ , similar changes in the respiratory tract were observed at terminal sacrifice; in addition, the nasal cavity showed rhinitis and degeneration of the olfactory epithelium. Four deaths occurred in males at 12.98  $\mu\text{g}/\text{l}$  (and one in a satellite male at 12.98  $\mu\text{g}/\text{l}$ ) which were related to respiratory lesions rather than to systemic toxicity. In addition to marked necrosis of the epithelium of the bronchi and large bronchioles (all five), there were alveolar changes, congestion, and edema of the lungs, and degenerative and proliferative changes in the nasal cavity and larynx. In the surviving rats (12.98  $\mu\text{g}/\text{l}$ ) allowed to recover for 4 weeks, all changes except those of the larynx were reversed.

There were no effects of captan exposure on clinical signs of toxicity, food consumption, clinical laboratory parameters, or organ weights. Weight gains were decreased in exposed males, but this was not clearly related to dosing.

The systemic NOEL is greater than 12.98  $\mu\text{g}/\text{l}$  (HDT), and the systemic LOEL is greater than 12.98  $\mu\text{g}/\text{l}$  (HDT). The respiratory LEL (local irritation) is  $\leq 0.13 \mu\text{g}/\text{l}$  in females, based on squamous hyperplasia in the larynx, and a NOEL was not established. In males, the respiratory LEL is 0.60  $\mu\text{g}/\text{l}$ , and the NOEL is 0.13  $\mu\text{g}/\text{l}$ , based on hyperplasia in the larynx.

Classification: CORE Minimum since there was no NOEL for local effect in females.



A. MATERIALS:

1. Test Compound: Captan (technical); description: off-white powder; batch No.: 11240-37-1; purity: 88.7%.
2. Test Animals: Species: rat; strain: Alpk:APfSD (Wistar-derived albino); age: 5-6 weeks; weight: males--154 to 162 g, females--140 to 145 g; source: Animal Breeding Unit (BABU), Alderley Park, Cheshire, United Kingdom.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimatized for at least 1 week, and those free of unhealthy signs were randomly assigned to cages among the following test groups:

| Test Group | Target Concentration of Captan ( $\mu\text{g/l}$ ) | Main Study (13 weeks) |         | Satellite Groups <sup>a</sup> (17 weeks) |         |
|------------|--|-----------------------|---------|--|---------|
|            |  | Males                 | Females | Males                                    | Females |
| 1          | 0  | 10                    | 10      | 10                                       | 10      |
| 2          | 0.1  | 10                    | 10      | --                                       | --      |
| 3          | 0.5  | 10                    | 10      | --                                       | --      |
| 4          | 5.0  | 10                    | 10      | --                                       | --      |
| 5          | 15.0   | 10                    | 10      | 10                                       | 10      |

<sup>a</sup>Satellite groups were exposed for 13 weeks and then allowed a 4-week recovery period.

All animals were acclimated to restraint tubes for 4 days prior to exposure. Rats were exposed for 6 hours/day, 5 days/week. The rats were housed five to a cage in a room with an overall temperature range of 15-25°C and an overall humidity range of 25-80%. There was a 12-hour light/dark cycle, and the airflow through the holding chambers was 400 l/min.

2. Inhalation Exposure Conditions: Animals were exposed in nose-only restraining tubes (Battelle) inserted in a 46-l Perspex exposure chamber. Temperature and humidity ( $22 \pm 3^\circ\text{C}$  and  $50 \pm 15\%$ ) were measured at 30-minute intervals. Atmospheres were generated into a reservoir chamber with a Wright's dust feed mechanism, and a concentric glass jet atomizer pulled test atmosphere into the exposure chamber. Dried filtered air was used to supply the atomizer and diluting air. Airflow rates supplied to the atomizer and

directly to the chamber (dilution air) were measured with variable area flowmeters. The airflow rates for the atomizer and diluting air were determined in preliminary trial generation studies. Atmospheres were sampled close to the breathing zone at a sampling rate of 2 l/minute, and dust was collected on 25-mm diameter Gelman VM-1 filters. Test samples were collected at least three times during each exposure.

Samples at 5 and 15  $\mu\text{g}/\text{l}$  were monitored gravimetrically. For target concentrations of 0.1 and 0.5  $\mu\text{g}/\text{l}$ , a Scattered Light Instrument was used with continual recording during exposure. The aerodynamic particle size of the test atmosphere was measured with a Marple Cascade Impactor using predetermined size ranges. These measurements were made daily for the first week and once a week for the remainder of the study. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated with a microcalculator. Captan was determined analytically after extracting filters with ethyl acetate using gas chromatography with an electron capture detector.

Results: Table 1 summarizes data for analyzed atmospheric concentrations and the aerodynamic particle size distribution of captan. The mean concentrations were acceptably close to target levels. The particle sizes were well within the respiratory range. The increase in MMAD at 5 and 15  $\mu\text{g}/\text{l}$  indicated some particle aggregation with increasing concentration.

3. Food and Water Consumption: Animals received food (Porton CT-1 diet, Batch Number 3658) and water ad libitum except during exposure periods.
4. Statistics: The following procedures were utilized in analyzing the numerical data:

Data for main study animals were analyzed separately from data for animals in satellite groups.

The body weights, food consumption and utilization, and organ weights were analyzed by analysis of variance separately for males and females. Organ weights were also considered by analysis of covariance on final body weights.

Biochemical and hematological data were analyzed by analysis of variance; however, with the exception of urine protein, male and female data were analyzed together. All data presented in the report, however, were separated by sex for analysis.

TABLE 1. Mean Atmospheric Concentrations of Aerodynamic Particle Size Distributions in the 90-Day Inhalation Study of Captan in Rats<sup>a</sup>

| Treatment Group | Target Concentration $\mu\text{g/l}$ | Mean Analyzed Concentration $\mu\text{g/l}$ (range) <sup>b</sup> | Mass Median Aerodynamic Diameter $\mu\text{m} \pm \text{S.D.}$ | Geometric Standard Deviation GSD $\pm \text{S.D.}$ |
|-----------------|--------------------------------------|--|--|--|
| 2               | 0.10                                 | 0.13 (0.050-0.389)   | $0.95 \pm 0.2$   | $1.82 \pm 0.6$                                     |
| 3               | 0.50                                 | 0.60 (0.272-1.424)   | $1.23 \pm 0.3$   | $1.80 \pm 0.2$                                     |
| 4               | 5.00                                 | 5.06 (3.356-6.853)   | $1.57 \pm 0.3$   | $1.84 \pm 0.1$                                     |
| 5               | 15.00                                | 12.98 (9.020-16.870)   | $1.60 \pm 0.4$   | $2.00 \pm 0.2$                                     |

<sup>a</sup>Data were extracted from study No. CTL/P/2534, pp. 46-48.

<sup>b</sup>The values in parentheses are the ranges of individual daily means recorded from 10/19/88 to 01/19/89.

Each treatment group mean was compared with the control group mean using a two-sided Student's t-test, based on the error mean square in the analysis.

5. Quality Assurance: A quality assurance statement was signed and dated July 24, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least once daily for signs of morbidity and mortality. In addition, all animals were observed frequently during each exposure for signs of toxicity or abnormal behavior.

Results: Four males exposed at 12.98  $\mu\text{g/ml}$  were found dead (weeks 5, 7, 8, and 13), and one satellite male at the same exposure level was sacrificed moribund at week 11. A female in the group exposed to 0.13  $\mu\text{g/l}$  and one exposed to 5.06  $\mu\text{g/l}$  were sacrificed moribund at weeks 6 and 10; these mortalities were not considered compound related. A female in the highest exposure group was accidentally returned to the wrong home cage after exposure, became pregnant and was removed from the study at week 6.

During exposure and for a period after exposure, clinical observations normally associated with restraint were observed in all groups including controls. These signs were mucus excretion from the nose and chromodacryorrhea during or after exposure, in addition to piloerection and postural changes (as hunching) after exposure. Abnormal respiratory noise was observed in all rats including controls. This finding was not consistent in animals in that it occurred, there was recovery, and there was also relapse. It also was observed in some rats during the recovery period. In the high-exposure group it was seen in one female at weeks 9, 12, and 13 but in two to five females during the recovery period. It appeared to be slightly more frequent at the high exposure level in males (four to six rats) than in controls (one to three rats) during the exposure period and also slightly more frequent in high-exposure females; one to four females during weeks 1 to 13 compared with no control females in the same period. Other clinical findings were considered normal for all groups.

2. Body Weight: Body weights of rats were recorded prior to the first exposure and weekly thereafter.

Results: Table 2 presents representative data for mean body weights and body weight gains. There were no effects on body weights or body weight gains in females exposed to captan. Mean weight gains in all male groups exposed to

TABLE 2. Mean Body Weights and Weight Gains at Selected Intervals in Rats Exposed to Captan for 13 Weeks (Main Groups)

| Exposure Level<br>( $\mu\text{g/l}$ ) | Mean Weight (g $\pm$ S.D.) at Week <sup>a</sup> |              |              | Mean Weight Gains (g) Between Weeks <sup>b</sup> |       |       |  |  |  |
|---------------------------------------|---|--------------|--------------|--|-------|-------|--|--|--|
|                                       | Initiation                                      | 6            | 13           |  |       |       |  |  |  |
|                                       | 0   |              |              |  |       |       |  |  |  |
|                                       |   |              |              | <u>Males</u>                                     |       |       |  |  |  |
| 0                                     | 157 $\pm$ 13                                    | 346 $\pm$ 23 | 407 $\pm$ 52 | 124  | 189   | 264   |  |  |  |
| 0.13                                  | 154 $\pm$ 12                                    | 327 $\pm$ 11 | 390 $\pm$ 15 | 114*   | 173*  | 235** |  |  |  |
| 0.60                                  | 155 $\pm$ 11                                    | 337 $\pm$ 23 | 419 $\pm$ 23 | 117  | 182   | 265   |  |  |  |
| 5.06                                  | 162 $\pm$ 14                                    | 329 $\pm$ 17 | 390 $\pm$ 26 | 109**  | 167** | 228** |  |  |  |
| 12.98                                 | 159 $\pm$ 14                                    | 327 $\pm$ 15 | 386 $\pm$ 24 | 105**  | 167** | 230** |  |  |  |
|                                       |   |              |              | <u>Females</u>                                   |       |       |  |  |  |
| 0                                     | 142 $\pm$ 8.6                                   | 230 $\pm$ 15 | 260 $\pm$ 29 | 61   | 88    | 118   |  |  |  |
| 0.13                                  | 142 $\pm$ 7.1                                   | 227 $\pm$ 14 | 255 $\pm$ 14 | 61   | 85    | 113   |  |  |  |
| 0.60                                  | 140 $\pm$ 7.3                                   | 229 $\pm$ 13 | 263 $\pm$ 16 | 60   | 89    | 123   |  |  |  |
| 5.06                                  | 141 $\pm$ 9.9                                   | 225 $\pm$ 13 | 255 $\pm$ 12 | 56   | 84    | 112   |  |  |  |
| 12.98                                 | 142 $\pm$ 8.1                                   | 228 $\pm$ 14 | 255 $\pm$ 15 | 58   | 87    | 114   |  |  |  |

$\bar{a}$  means and standard deviation calculated by the reviewers. Initial weights are recorded as week 1 in the report.

<sup>b</sup>Extracted from study pp. 64 and 66; standard deviations were not provided.

\*Significantly different from the control value,  $p < 0.05$ .

**\*\*Significantly different from the control value,  $p < 0.01$ .**

captan were slightly lower than control gains. The decreases were consistently significantly ( $p < 0.05$ ) lower in groups exposed at 5.06 and 12.98  $\mu\text{g/l}$ , being decreased approximately 13% by week 13. Decreases were not significant at 0.60  $\mu\text{g/l}$ . The study authors did not consider the effects on body weight to be related to exposure and considered the effect to be caused, in part, by group caging. The mean body weight gain in the satellite group of males exposed at 12.98  $\mu\text{g/l}$  was 236 g compared to 274 g for controls (weeks 1-13), but gain during the recovery period (49 g) was similar to the control gain (55 g).

3. Food Consumption and Compound Intake: Food consumption was recorded for each cage of rats weekly throughout the study, and mean daily diet consumption was calculated. Food utilization per cage was calculated weekly and expressed as the weight gained (g) by the animals in the cage per 100 g of food consumed.

Results: Food consumption (total) was decreased about 10% in males exposed at 5.06 or 12.98  $\mu\text{g/l}$ ; the decrease was significant at sporadic intervals and there was no apparent dose-related trend. Food consumption was consistently higher in exposed females than in controls. There was no dose trend and significance was sporadic. Food efficiency was not affected (Table 3).

4. Ophthalmology: Ophthalmologic examinations were performed on all animals prior to the start of the study, following application of a mydriate, using a binocular indirect ophthalmoscope. Rats in the control (0  $\mu\text{g/l}$ ) and high-exposure (12.98  $\mu\text{g/l}$ ) groups were similarly examined in week 13, and animals in the satellite groups were examined in week 18.

Results: There were no treatment-related effects on ophthalmic findings.

TABLE 3. Food Consumption and Utilization in Rats Exposed to Captan for 13 Weeks

| Exposure Level<br>( $\mu$ g/l) | Total Consumption (g) |         | g Gain/100 g Food |         |
|--------------------------------|-----------------------|---------|-------------------|---------|
|                                | Males                 | Females | Males             | Females |
| 0                              | 2414                  | 1660    | 10.93             | 7.08    |
| 0.13                           | 2325                  | 1708    | 10.10             | 6.61    |
| 0.60                           | 2412                  | 1731    | 10.97             | 7.08    |
| 5.06                           | 2208*                 | 1747    | 10.31             | 6.43    |
| 12.98                          | 2258                  | 1735    | 10.19             | 6.55    |

\*Significantly different from control value,  $p < 0.05$ .

5. Hematology and Clinical Chemistry: Blood was collected from all main study and satellite animals at termination. The samples were obtained by cardiac puncture. The CHECKED (X) parameters were examined:

a. Hematology:

|                             |   |
|-----------------------------|---|
| X Hematocrit (HCT) †        | X Leukocyte differential count              |
| X Hemoglobin (HGB) †        | X Mean corpuscular HGB (MCH)                |
| X Leukocyte count (WBC) †   | X Mean corpuscular HGB concentration (MCHC) |
| X Erythrocyte count (RBC) † | X Mean corpuscular volume (MCV)             |
| X Platelet count †          | X Kaolin-cephalin time (KCT)                |
| Reticulocyte count (RETIC)  | X Prothrombin time (PT)                     |
| Red cell morphology         |   |

Results: No treatment-related effects on hematology parameters were observed. There were no effects of biological importance in the satellite groups. At the end of the recovery period, the red cell count was slightly increased and the MCV and MCH were slightly decreased in females previously exposed to captan when compared to controls, but the values for controls were slightly different from those normally expected.

b. Clinical Chemistry:

| <u>Electrolytes</u>                       | <u>Other</u>           |
|---|------------------------|
| X Calcium†                                | X Albumin†             |
| Chloride†                                 | Albumin/globulin ratio |
| Magnesium†                                | X Blood creatinine†    |
| X Phosphorus†                             | Blood urea nitrogen†   |
| X Potassium†                              | X Cholesterol†         |
| X Sodium†                                 | Globulins              |
|   | X Glucose†             |
| <u>Enzymes</u>                            | X Total bilirubin†     |
| X Alkaline phosphatase (ALP)              | Direct bilirubin       |
| Cholinesterase                            | X Total protein†       |
| X Creatine phosphokinase (CK)†            | X Triglycerides        |
| X Lactic acid dehydrogenase               | X Urea                 |
| X Serum alanine aminotransferase (ALT)†   |                        |
| X Serum aspartate aminotransferase (AST)† |                        |
| Gamma glutamyltransferase (GGT)           |                        |

†Recommended by Subdivision F (November 1984) Guidelines.



Results: The authors reported that there were several statistically significant differences between control and test groups, but these were not considered compound related since the differences were small and not dose related. Table 4 presents data for ALT, AST, CK, and triglycerides. Means and standard deviations were recalculated by the reviewers with and without outlying values (see Section E, Reviewers' Discussion and Interpretation of Results). Other parameters (not tabulated) had a few sporadic changes in exposed groups when compared to controls.

6. Urinalysis: Urine was collected from fasted animals at week 13 (males) and week 14 (females). The CHECKED (X) parameters were examined:

|   |                                     |   |                        |
|---|-------------------------------------|---|------------------------|
|   | Appearance <sup>†</sup>             | X | Glucose <sup>†</sup>   |
| X | Volume <sup>†</sup>                 | X | Ketones                |
| X | Specific gravity <sup>†</sup>       |   | Bilirubin <sup>†</sup> |
| X | pH                                  | X | Blood <sup>†</sup>     |
|   | Sediment (microscopic) <sup>†</sup> |   | Nitrate                |
| X | Protein <sup>†</sup>                | X | Urobilinogen           |

Results: No effects of biological importance on urinary parameters were present. Significant increases in specific gravity and decreases in pH were seen in dosed males, but values were within the normal range and there were no corresponding changes in females.

<sup>†</sup>Recommended by Subdivision F (November 1984) Guidelines.

TABLE 4. Representative Clinical Chemistry Data ( $\pm$  S.D.) in Rats Exposed to Captan for 3 Months<sup>A</sup>

| Exposure Level ( $\mu$ g/l) | Alanine Amino-transferase (U/l) | Aspartic Amino-transferase (U/l) | Creatinine Kinase (U/l)      | Triglycerides (mg/dl) |
|-----------------------------|---------------------------------|----------------------------------|------------------------------|-----------------------|
| <u>Main group</u>           |                                 |                                  |                              |                       |
| 0                           | 44.2 $\pm$ 10.2                 | 59.0 $\pm$ 15.6                  | 30.3 $\pm$ 17.5 <sup>f</sup> | 128 $\pm$ 17.5        |
| 0.13                        | 39.5 $\pm$ 6.8                  | 50.1 $\pm$ 4.7                   | 28.7 $\pm$ 12.9              | 106 $\pm$ 10.8*       |
| 0.60                        | 46.3 $\pm$ 9.3 <sup>a</sup>     | 58.6 $\pm$ 8.0 <sup>c</sup>      | 48.9 $\pm$ 27.3 <sup>g</sup> | 117 $\pm$ 27.3        |
| 5.06                        | 41.1 $\pm$ 5.5 <sup>b</sup>     | 54.8 $\pm$ 4.8 <sup>d</sup>      | 41.2 $\pm$ 22.4 <sup>h</sup> | 105 $\pm$ 28.2*       |
| 12.98                       | 45.2 $\pm$ 9.4                  | 57.0 $\pm$ 11.3                  | 29.2 $\pm$ 4.6               | 108 $\pm$ 9.4         |
| <u>Recovery group</u>       |                                 |                                  |                              |                       |
| 0                           | 43.8 $\pm$ 8.1                  | 54.9 $\pm$ 7.7                   | 48.6 $\pm$ 14.7 <sup>i</sup> | 126 $\pm$ 13.2        |
| 12.98                       | 42.6 $\pm$ 16.0                 | 47.0 $\pm$ 4.4 <sup>e</sup>      | 42.0 $\pm$ 19.1 <sup>j</sup> | 117 $\pm$ 24.0        |
| <u>Main group</u>           |                                 |                                  |                              |                       |
| 0                           | 36.8 $\pm$ 8.8                  | 52.1 $\pm$ 14.6                  | 27.0 $\pm$ 5.8               | 81 $\pm$ 22.5         |
| 0.13                        | 39.6 $\pm$ 9.5                  | 49.8 $\pm$ 7.9                   | 22.8 $\pm$ 6.2               | 107 $\pm$ 40.5*       |
| 0.60                        | 47.3 $\pm$ 14.3                 | 59.7 $\pm$ 7.5                   | 30.9 $\pm$ 11.3              | 95 $\pm$ 13.1         |
| 5.06                        | 36.7 $\pm$ 6.6                  | 49.9 $\pm$ 7.5                   | 30.9 $\pm$ 17.5              | 108 $\pm$ 31.8*       |
| 12.98                       | 33.8 $\pm$ 3.2                  | 51.1 $\pm$ 7.5                   | 30.6 $\pm$ 12.2              | 95 $\pm$ 19.8         |
| <u>Recovery group</u>       |                                 |                                  |                              |                       |
| 0                           | 35.3 $\pm$ 6.9                  | 50.6 $\pm$ 9.2                   | 30.1 $\pm$ 16.6              | 114 $\pm$ 20.7        |
| 12.98                       | 39.8 $\pm$ 18.6                 | 61.5 $\pm$ 38.5                  | 32.0 $\pm$ 20.4              | 121 $\pm$ 29.7        |

\*Significantly different from control value,  $p < 0.05$ .

<sup>A</sup> Means and standard deviations calculated by reviewers. The values with a superscript were recalculated omitting the following outlier values:

| <u>Reported</u>                | <u>Outliers</u> | <u>Reported</u>              | <u>Outliers</u> |
|--------------------------------|-----------------|------------------------------|-----------------|
| <sup>a</sup> 89.2 $\pm$ 112.3* | 401, 121        | <sup>f</sup> 43.4 $\pm$ 44.5 | 127             |
| <sup>b</sup> 50.7 $\pm$ 30.3   | 137             | <sup>g</sup> 165.5 $\pm$ 357 | 1175            |
| <sup>c</sup> 195.8 $\pm$ 186.8 | 564, 425        | <sup>h</sup> 55.4 $\pm$ 49.6 | 183             |
| <sup>d</sup> 132.4 $\pm$ 245.5 | 831             | <sup>i</sup> 51.9 $\pm$ 29.8 | 127             |
| <sup>e</sup> 106.9 $\pm$ 167.4 | 521             | <sup>j</sup> 139.4 $\pm$ 276 | 821             |

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

| <u>Digestive System</u>        | <u>Cardiovasc./Hemat.</u>      | <u>Neurologic</u>                               |
|--------------------------------|--------------------------------|---|
| X Tongue                       | X Aorta <sup>†</sup>           | XX Brain  |
| X Salivary glands <sup>†</sup> | XX Heart <sup>†</sup>          | X Peripheral nerve (sciatic nerve) <sup>†</sup> |
| X Esophagus <sup>†</sup>       | X Bone marrow <sup>†</sup>     | X Spinal cord (3 levels) <sup>b</sup>           |
| X Stomach <sup>†</sup>         | X Lymph nodes <sup>†</sup>     | X Pituitary <sup>†</sup>                        |
| X Duodenum <sup>†</sup>        | X Spleen <sup>a</sup>          | X Eyes (optic nerve) <sup>†b</sup>              |
| X Jejunum <sup>†</sup>         | X Thymus                       |   |
| X Ileum <sup>†</sup>           |                                |   |
| X Cecum <sup>†</sup>           |                                |   |
| X Colon <sup>†</sup>           |                                |   |
| X Rectum                       |                                |   |
| XX Liver <sup>†</sup>          | <u>Urogenital</u>              | <u>Glandular</u>                                |
| X Gallbladder <sup>†</sup>     | XX Kidneys <sup>†a</sup>       | XX Adrenals <sup>†</sup>                        |
| X Pancreas <sup>†</sup>        | X Urinary bladder <sup>†</sup> | Lacrimal gland                                  |
|                                | XX Testes <sup>†</sup>         | X Mammary gland <sup>†b</sup>                   |
|                                | X Epididymides                 | X Thyroids <sup>†</sup>                         |
|                                | X Prostate                     | X Parathyroids <sup>†</sup>                     |
|                                | X Seminal vesicle              | X Harderian glands <sup>b</sup>                 |
|                                | X Ovaries                      |   |
|                                | X Uterus                       |   |
|                                | X Cervix                       |   |
| <u>Respiratory</u>             |                                |   |
| X Trachea <sup>†</sup>         |                                |   |
| XX Lung <sup>†a</sup>          |                                |   |
| X Larynx <sup>a</sup>          |                                |   |
| X Nasal passages <sup>a</sup>  |                                |   |
|                                |                                | <u>Other</u>                                    |
|                                |                                | X Bone (sternum and femur) <sup>†</sup>         |
|                                |                                | X Skeletal muscle <sup>†b</sup>                 |
|                                |                                | X Skin <sup>b</sup>                             |
|                                |                                | X All gross lesions and masses <sup>a</sup>     |
|                                |                                | X Bone (femur)                                  |

<sup>†</sup>Recommended by Subdivision F (November 1984) Guidelines.

<sup>a</sup>Only tissues marked with this symbol were processed to paraffin blocks for rats exposed at 0.13, 0.60, and 5.06 µg/l.

<sup>b</sup>These tissues were collected, but not processed to paraffin blocks in any group.

## Results:

- a. Organ Weights: No toxicologically important changes in any organ weights were apparent. The variation of mean weights and mean organ-to-body weight ratios (calculated by the reviewers) between groups was as normally expected. Statistically significant changes in organ weights occurred but they were neither consistent between absolute and relative values nor dose-related. In the recovery groups, the mean liver weights were significantly lower in the previously exposed group than in control ( $p < 0.01$ ). Liver-to-body weight ratios were approximately 7 and 11% lower in previously exposed males and females, respectively, than in the controls (calculated by reviewer).
- b. Gross Pathology: No gross findings were considered related to dosing. Red discoloration and partial deflation of the lung in animals that died were considered to be agonal changes.
- c. Microscopic Pathology: No histologic findings that were compound-related were found in any tissues or organs with the exception of the respiratory system. In the proximal tubules of the kidney, proteinaceous globules that stained red with Martians Scarlet blue were seen in all groups including controls; the incidence was greater in males (80 to 100%) than in females (ca. 10%), but was not increased in exposed groups of either sex. Alpha-2-u-globulin was measured in kidneys of 6 to 10 rats/sex in control and high-exposure groups using an immunocytochemical detection method. Moderate levels of this marker of hyaline droplets was observed in 8 of 10 control males and 5 of 6 exposed at 12.98  $\mu\text{g/l}$ . Levels were low in 2 of 9 control females and 1 of 7 females exposed at 12.98  $\mu\text{g/l}$ . The severity and incidence were not increased by exposure.

Table 5 summarizes histologic findings in the lungs, larynx, and nasal passages at the terminal sacrifice.

Changes in the nasal cavity were confined primarily to rats exposed at 12.98  $\mu\text{g/l}$ ; they were generally not severe and were nearly completely reversed after the recovery period. For the five males that died after exposure to 12.98  $\mu\text{g/l}$ , findings were similar to those found at terminal sacrifice: rhinitis (2), degeneration of olfactory epithelium (2), necrosis of squamous epithelium (1), and goblet cell hyperplasia (1). In addition, squamous metaplasia of the lacrimal gland was seen in two decedents, and vacuolar

TABLE 5. Histologic Findings in the Nasal Cavity, Larynx, Lung, and Bronchi of Rats Exposed to Captan for 3 Months

| Organ/Finding   | Exposure Levels ( $\mu\text{g/l}$ ) |      |      |      |       |         |      |      |      |       |
|---|-------------------------------------|------|------|------|-------|---------|------|------|------|-------|
|   | Males                               |      |      |      |       | Females |      |      |      |       |
|   | 0                                   | 0.13 | 0.60 | 5.06 | 12.98 | 0       | 0.13 | 0.60 | 5.06 | 12.98 |
| <u>Nasal cavity</u>                                   | (10) <sup>a</sup>                   | (10) | (10) | (10) | (6)   | (10)    | (9)  | (10) | (9)  | (9)   |
| Rhinitis  | 0                                   | 0    | 1    | 1    | 3     | 0       | 0    | 0    | 0    | 2     |
| Degeneration of olfactory epithelium in dorsal meatus | 0                                   | 0    | 0    | 0    | 3     | 0       | 1    | 1    | 0    | 4     |
| Necrosis of squamous epithelium                       | 0                                   | 0    | 0    | 1    | 0     | 0       | 0    | 0    | 0    | 0     |
| Goblet cell hyperplasia                               | 0                                   | 0    | 1    | 0    | 1     | 0       | 0    | 0    | 0    | 2     |
| <u>Lungs and bronchi</u>                              | (10)                                | (10) | (10) | (10) | (6)   | (10)    | (9)  | (10) | (9)  | (9)   |
| Attenuated bronchial epithelium                       | 0                                   | 0    | 0    | 8    | 6     | 0       | 0    | 0    | 9    | 9     |
| Subepithelial necrosis, bronchial                     | 0                                   | 0    | 0    | 6    | 2     | 0       | 0    | 0    | 2    | 1     |
| Exfoliation of bronchial epithelium                   | 0                                   | 0    | 0    | 0    | 2     | 0       | 0    | 0    | 0    | 0     |
| Hyperplasia of bronchial epithelium                   | 0                                   | 0    | 0    | 2    | 0     | 0       | 0    | 0    | 0    | 0     |
| Alveolitis  | 3                                   | 2    | 1    | 0    | 1     | 2       | 0    | 2    | 1    | 1     |
| Alveolar macrophage infiltration                      | 0                                   | 0    | 1    | 0    | 0     | 1       | 0    | 0    | 0    | 1     |
| Perivascular cuffing                                  | 3                                   | 1    | 0    | 0    | 1     | 1       | 0    | 3    | 0    | 2     |
| <u>Larynx</u>   | (10)                                | (10) | (10) | (9)  | (6)   | (10)    | (9)  | (10) | (9)  | (9)   |
| Squamous metaplasia                                   | 0                                   | 0    | 0    | 9    | 6     | 0       | 0    | 0    | 7    | 9     |
| Squamous hyperplasia                                  | 0                                   | 1    | 5    | 9    | 6     | 0       | 4    | 7    | 8    | 9     |
| Vacuolar degeneration, squamous                       | 0                                   | 0    | 0    | 3    | 4     | 0       | 0    | 0    | 4    | 7     |
| Glandular dilation                                    | 8                                   | 3    | 4    | 2    | 5     | 3       | 3    | 1    | 3    | 5     |
| Ulceration  | 0                                   | 0    | 0    | 0    | 1     | 0       | 0    | 0    | 3    | 1     |

<sup>a</sup>The numbers in parentheses denote the number of animals examined histologically.

degeneration of the sensory cells in the vomeronasal gland was seen in all animals that died or were sacrificed during the study. One male and the one female that died had focal ulceration in the anterior ventral meatus. These latter changes were not seen at termination.

According to the authors, the primary cause of death in the males was necrosis of the epithelial lining of the bronchi and large bronchioles (all five); one male had subepithelial necrosis in a bronchus. Alveolar changes, alveolitis, alveolar macrophage infiltration, and perivascular cuffing, congestion, and edema were frequent in exposed males that died.

The bronchial epithelia were "attenuated" with loss of cilia at terminal sacrifice in all males and females exposed at 12.98  $\mu\text{g}/\text{l}$  and most exposed at 5.06  $\mu\text{g}/\text{l}$ . Subepithelial cellular necrosis of the bronchi was observed in several males and females exposed at the two highest levels at terminal sacrifice (Table 5). Other changes in conducting airways occurring at a low incidence at these exposure levels were hyperplasia of the bronchial epithelia and squamous metaplasia. Alveolar changes were found at a low incidence in exposed rats of both sexes at termination. All changes in the lungs and bronchi were reversed during the recovery period.

Changes in the larynx seen at termination in males and females exposed at 5.06 and 12.98  $\mu\text{g}/\text{l}$  were moderate squamous metaplasia, minimal squamous epithelial cell hyperplasia and vacuolation, and a slight increase in parakeratosis; laryngitis and squamous metaplasia in the larynx were also seen in male rats that died. Minimal squamous hyperplasia unaccompanied by other changes was observed at 0.60 and 0.13  $\mu\text{g}$  captan/ $\text{l}$  in both sexes; the authors considered these to be an adaptive effect and not of toxicologic importance. After the recovery period, the squamous metaplasia and hyperplasia persisted in the trachea of the males and females that had been exposed at 12.98  $\mu\text{g}/\text{l}$ . One male that died had slight hyperplasia in the trachea; this finding was not seen in either sex at the 13-week sacrifice.

D. STUDY AUTHOR'S CONCLUSIONS:

Exposure of rats for 90 days to captan at atmospheric concentrations of 0.13, 0.6, 5.06, and 12.98  $\mu\text{g}/\text{l}$  resulted in five treatment-related deaths in males exposed at the highest concentration. Other mortalities were considered incidental. No toxicologically important effects on body weight, food consumption, clinical laboratory findings, ophthalmological, or organ weight findings were seen. Treatment-related effects were confined to the respiratory system and were consistent with the effects of exposure to an irritant particle. Effects in the lungs were seen only at 5.06 and 12.98  $\mu\text{g}/\text{l}$  in both sexes. A small treatment-related incidence of minimal changes was seen in the nasal passages at 12.98  $\mu\text{g}/\text{l}$ . Effects in the larynx, which were considered to be an adaptive response to irritation, were observed at 5.06 and 12.98  $\mu\text{g}/\text{l}$ . All changes in surviving rats except those in the larynx were completely reversed after a 4-week recovery period. Based on the histologic effects on the lungs, the NOEL was considered to be 0.60  $\mu\text{g}/\text{l}$ .

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The design and conduct of the study were acceptable. The exposure levels were adequately high based on the results in the range-finding study and the mortality in males in the present study. The mean analyzed concentrations were reasonably close to target, and the range of aerodynamic particle size of 0.55 to 1.6  $\mu\text{m}$  indicate that the dust should have been highly respirable.

Decreased weight gains in exposed males did not show an apparent dose trend, although gains were reduced in all exposed groups. We tend to agree with the study author that this is not related to treatment with captan. The author attributed the weight gain effect to group housing. It would have been preferable to cage the rats separately, but no injuries related to fighting were apparent.

The hematology and clinical chemistry data were present for individual animals, and mean values were reported without standard deviations (approximate 95% confidence limits for all four groups were presented). Since outlier values were present for clinical chemistry parameters and were apparently included in the means, our reviewers recalculated selected data with and without outliers (Table 4). Outlier values were confined to a few animals. In males exposed at 0.60  $\mu\text{g}/\text{l}$ , No. 61 had extremely high values for AST, ALT, and CK, and No. 67 had high values for ALT and AST. In males No. 86 (5.06  $\mu\text{g}/\text{l}$ ) and No. 126 (12.98  $\mu\text{g}/\text{l}$ ) values were high for both AST and CK. There

were no correlating histologic liver changes and, with the exception of male No. 67, the liver-to-body weight ratios were not increased from the means. We assess that none of the changes were related to exposure.

We agree with the study author's assessment that the only treatment-related effects were confined to the respiratory system. It is reasonable to attribute the deaths to respiratory effects on both the lungs (edema and congestion) and bronchi (degenerative and necrotic changes). The histologic changes in the respiratory system in this 90-day study are consistent with those found at 24.8  $\mu\text{g}/\text{l}$  in the previous 3-week inhalation study. Since no histologic changes were seen at 3 weeks with an exposure level of 5  $\mu\text{g}/\text{l}$ , whereas there were changes at 5.06  $\mu\text{g}/\text{l}$  in the present study, it is apparent that an extended period of exposure is required to cause changes at the lower exposure levels.

The study author considered the histologic changes in the larynx to be an adaptive response and not toxicologically important and set a NOEL of 0.6  $\mu\text{g}/\text{l}$  for respiratory (local) effects. However, it is our assessment that the effect levels should be based on the increased incidence of squamous hyperplasia in the larynx. On this basis, the LEL in males is 0.60  $\mu\text{g}/\text{l}$  and in females is 0.13  $\mu\text{g}/\text{l}$ ; the NOEL in males is 0.13  $\mu\text{g}/\text{l}$ , and a NOEL for local effects in females was not established. A LOEL for systemic toxicity was greater than 12.98  $\mu\text{g}/\text{l}$  (HDT), and the NOEL is equal to or greater than 12.98  $\mu\text{g}/\text{l}$ .



007938

EPA No.: 68D80056  
DYNAMAC No.: 260-A  
TASK No.: 2-60A  
March 19, 1990

DATA EVALUATION RECORD

CAPTAN

Preliminary Inhalation Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.  
Program Manager  
Dynamac Corporation

Signature:

*Roman J. Penta for*

Date:

3-16-90

EPA No.: 68D80056  
DYNAMAC No.: 260-A  
TASK No.: 2-60A  
March 19, 1990

DATA EVALUATION RECORD

CAPTAN

Preliminary Inhalation Toxicity Study in Rats

REVIEWED BY:

William L. McLellan, Ph.D.  
Principal Reviewer  
Dynamac Corporation

Signature: William L. McLellan

Date: March 16, 1990

Margaret E. Brower, Ph.D.  
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Date: March 16, 1990

APPROVED BY:

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Signature: Roman J. Pienta

Date: March 16, 1990

Marion P. Copley, D.V.M.,  
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Section II, Toxicology  
Branch I (TS-7509C)

Signature: Marion P. Copley

Date: 5/8/90

DATA EVALUATION RECORD

GUIDELINE § N.A.

STUDY TYPE: 3-Week inhalation toxicity study in rats.

MRID NUMBER: 412344-01.

TEST MATERIAL: Captan.

SYNONYM: N-Trichloromethylthio-4-cyclohexene 1,2-dicarboxamide

STUDY NUMBER: CTL/P/2534.

SPONSOR: Captan Task Force: Chevron Chemical Company,  
Makhteshim Agan, ICI Americas, Inc.

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley  
Park, Macclesfield, Cheshire, United Kingdom.

TITLE OF REPORT: Captan: 3-Week Preliminary Inhalation Toxicity  
Study in the Rat.

AUTHOR: Hext, P. M.

REPORT ISSUED: August 1, 1989.

**CONCLUSIONS:** Wistar-derived rats (five/sex/group) were exposed (nose only) to captan at mean atmospheric levels of 0.8, 5.3, or 24.8  $\mu\text{g/L}$  for 3 weeks (6 hours/day, 5 days/week). Two treatment-related mortalities occurred in males exposed at 24.8  $\mu\text{g/L}$ . There was little effect of exposure on body weights and no clinical chemistry or hematology changes of importance. No organ weight changes were observed. Treatment-related histopathologic findings were consistent with the direct-irritant effect of captan on the upper respiratory tract and were confined to the males and females exposed at 24.98  $\mu\text{g/L}$ . Changes in the lungs (alveolar edema, congestion, and hemorrhage) and bronchioles (necrosis) were observed in the two dead males. The male that was sacrificed moribund also had changes in the nasal cavity (rhinitis, loss of respiratory epithelium, and necrosis of the nasopharyngeal duct) and slight changes in the trachea and larynx. Changes at the terminal sacrifice were observed in the nasal cavity and larynx of females exposed at 24.8  $\mu\text{g/L}$ . The respiratory LEL (local irritation) is 24.8  $\mu\text{g/L}$  based on histologic changes in the nasal cavity and ulceration of the larynx in females and alveolar edema and bronchilar necrosis in the two decedent males; the NOEL for both sexes is 5.6  $\mu\text{g/L}$ . The systemic LOEL is greater than 24.8  $\mu\text{g/L}$  (HDT) and the NOEL is equal to or greater than 24.8  $\mu\text{g/L}$ .

**Classification:** Acceptable--Not CORE classified.

**A. MATERIALS:**

1. **Test Compound:** Captan (Technical); description: off-white powder; batch No.: 11240-37-1; purity: 88.7% (active ingredient).
2. **Test Animals:** Species: rat; strain: Alpk:APfSD (Wistar-derived) albino; age: approximately 8 weeks; weight: (range) males--242 to 278 g, females--200 to 238 g; source: Alderley Park, Cheshire, United Kingdom.

**B. STUDY DESIGN:**

1. **Animal Assignment:** Animals were acclimated for a minimum of 8 days prior to exposure, and were assigned to the following test groups using a randomized block design:

| Test Group | Target Concentration of Captan ( $\mu\text{g/L}$ ) | Main Study (21 days) |         |
|------------|--|----------------------|---------|
|            |  | Males                | Females |
| 1          | 0  | 5                    | 5       |
| 2          | 1  | 5                    | 5       |
| 3          | 5  | 5                    | 5       |
| 4          | 25   | 5                    | 5       |

The rats were housed by sex, five to a cage in a room with a temperature between 15°C and 24°C and a relative humidity of 50 ± 15%. The air-exchange system was designed to give 20-30 air changes per hour, and there was a 12-hour light/dark cycle.

2. Inhalation Exposure Conditions: Animals were exposed in nose-only restraining tubes (Battelle) inserted into four 9.5-L Perspex exposure chambers. Temperature and humidity were measured at 30-minute intervals. Atmospheres were generated into a reservoir chamber with a Wright's dust feed mechanism, and a concentric glass jet atomizer pulled test atmosphere into the exposure chamber. Dried, filtered air was used to supply the atomizer and diluting air. Air flow rates supplied to the atomizer and directly to the chamber (dilution air) were measured with variable area flowmeters. The airflow rates for the atomizer and diluting air were determined in preliminary trial generation studies. Atmospheres were sampled close to the breathing zone at a sampling rate of 2 L/minute, and dust was collected on 25-mm diameter Gelman VM-1 filters. Test samples were collected at least three times during each exposure.

Samples were monitored gravimetrically. Captan was also determined analytically after extracting filters with ethyl acetate and using gas chromatography with an electron-capture detector. The aerodynamic particle size of the test atmosphere was measured with a Marple Cascade Impactor using predetermined size ranges. These measurements were made daily for the first week and once a week for the remainder of the study. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated with a microcalculator.

Results: Table 1 summarizes data on atmospheric concentrations of captan and aerodynamic particle size distribution. There was a fairly good correlation between the Mean Particulate Concentration (measured gravimetrically) and the Mean Analyzed Concentrations. Both were close to the target. The MMAD was only slightly increased at the highest exposure concentration indicating no problem with particle aggregation, and the sizes indicated that the dust was well within the respirable range.

3. Food and Water Consumption: Animals received Porton Combined Diet (Special Services, Ltd., Essex, U.K.) and water ad libitum except during exposure.
4. Statistics: The following procedures were utilized in analyzing the numerical data: Body and organ weights were

TABLE 1. Mean Atmospheric Concentrations of Aerodynamic Particle Size Distributions in the Preliminary Inhalation Study of Captan in Rats

| Target<br>Concentration<br>$\mu\text{g/L}$ | Mean<br>Particulate<br>Concentration<br>$\mu\text{g/L}$ | Mean<br>Analyzed<br>Concentration<br>$\mu\text{g/L} \pm \text{S.D.}$ | Mass<br>Median<br>Aerodynamic<br>Diameter<br>$\mu\text{m} \pm \text{S.D.}$ | Geometric<br>Standard<br>Deviation<br>$\text{GSD} \pm \text{S.D.}$ |
|--|---|--|--|--|
| 1.0  | 0.9   | $0.83 \pm 0.15$  | $1.87 \pm 0.28$  | $2.18 \pm 0.37$  |
| 5.0  | 5.2   | $5.25 \pm 1.00$  | $1.86 \pm 0.20$  | $1.98 \pm 0.11$  |
| 25.0                                       | 27  | $24.78 \pm 4.03$   | $2.48 \pm 0.24$  | $2.21 \pm 0.07$  |

analyzed by analysis of variance, separately for males and females. Organ weights were also considered by analysis of covariance on final body weights. Biochemical and hematological data were analyzed by analysis of variance. Each treatment group mean was compared with the control group mean using a two-sided Student's t-test, based on the error mean square in the analysis.

5. Quality Assurance: A quality assurance statement was signed and dated July 31, 1989.

#### C. METHODS AND RESULTS:

1. Observations: Animals were inspected for changes in clinical condition and behavior before and after exposure and approximately every 30 minutes during exposure. They were also examined daily on nonexposure days for morbidity and mortality. Detailed clinical examinations were conducted on days 1, 4, 11, 18, and 22.

Results: One male exposed to 24.8  $\mu\text{g/L}$  died, and one was sacrificed in extremis, both on day 14. The rat that was sacrificed had labored breathing, increased breathing depth, and lung collapse. All other rats survived to termination.

Abnormal signs normally associated with restraint such as stains around the nose and chromodacryorrhea were seen in both controls and treated rats during exposure and piloerection and hunched posture were seen after exposure. Treatment-related signs during exposure were mucoid discharge from the nose and reduced response to noise. Abnormal respiratory noise was observed in some rats exposed at 24.8  $\mu\text{g/L}$  following exposure. Throughout the study, abnormal respiratory noise was seen in all treated groups but not in controls. It was more frequent in males than in females at the lower exposure levels (0.8 and 5.3  $\mu\text{g/L}$ ) occurring only after day 18, and was noted in most males and females exposed to 24.8  $\mu\text{g/L}$  by day 4.

2. Body Weight: Individual body weights were recorded prior to exposure (day 1) and on days 4, 11, 18, and 22.

Results: There was an initial variation in body weight gain with losses seen in some groups at day 3. The losses were considered to be unrelated to test compound but to be related to the stress of restraint. For the total study, weight gains were similar in control and test groups (Table 2).

TABLE 2. Mean Body Weight Gains in Rats Exposed to Captan for 28 Days

| Exposure<br>Level<br>( $\mu\text{g/L}$ ) | Mean Weight Change (g) at Day: |      |       |      |
|--|--------------------------------|------|-------|------|
|  | 3                              | 10   | 17    | 21   |
| <u>Males</u>                             |                                |      |       |      |
| 0  | 3.4                            | 23.2 | 37.0  | 47.6 |
| 0.8                                      | -0.4                           | 25.0 | 50.4  | 59.2 |
| 5.3                                      | 0.4                            | 24.4 | 49.4  | 60.6 |
| 24.8                                     | -4.8*                          | 15.6 | 35.0  | 48.0 |
| <u>Females</u>                           |                                |      |       |      |
| 0  | -4.2                           | 2.6  | 8.4   | 14.0 |
| 0.8                                      | -2.0                           | 5.8  | 14.4  | 18.8 |
| 5.3                                      | -6.4                           | 1.4  | 12.4  | 17.2 |
| 24.8                                     | 0.6                            | 9.8  | 16.4* | 19.6 |

\*Significantly different from control value,  $p < 0.05$ .



3. Food Consumption: Food consumption was recorded weekly for each cage of rats, and mean daily diet consumption was calculated from measurements made on days 1, 4, 11, 18, and 22.

Results: Food consumption was similar in exposed and control groups of males and females. It was slightly depressed in control males during week 3.

4. Ophthalmological Examinations: Ophthalmological examination were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from all animals by cardiac puncture during postmortem examination for hematology and for clinical analysis. The CHECKED (X) parameters were examined:

a. Hematology:

|   |                            |   |   |
|---|----------------------------|---|---|
| X | Hematocrit (HCT)           | X | Leukocyte differential count              |
| X | Hemoglobin (HGB)           | X | Mean corpuscular HGB (MCH)                |
| X | Leukocyte count (WBC)      | X | Mean corpuscular HGB concentration (MCHC) |
| X | Erythrocyte count (RBC)    | X | Mean corpuscular volume (MCV)             |
| X | Platelet count             | X | Coagulation:thromboplastin time (PT)      |
|   | Reticulocyte count (RETIC) |   |   |
|   | Red cell morphology        |   |   |

Results: No hematologic effects of concern were observed. All values were within the normal range.

b. Clinical Chemistry:

| <u>Electrolytes</u> |  | <u>Other</u> |                        |
|---------------------|--|--------------|------------------------|
| X                   | Calcium                                | X            | Albumin                |
|                     | Chloride                               |              | Albumin/globulin ratio |
|                     | Magnesium                              | X            | Blood creatinine       |
| X                   | Phosphorus                             |              | Blood urea nitrogen    |
| X                   | Potassium                              | X            | Cholesterol            |
| X                   | Sodium                                 |              | Globulins              |
|                     |  | X            | Glucose                |
|                     |  | X            | Total bilirubin        |
|                     |  |              | Direct bilirubin       |
| X                   | Alkaline phosphatase (ALP)             | X            | Total protein          |
| X                   | Creatine phosphokinase                 | X            | Triglycerides          |
|                     | Lactic acid dehydrogenase              | X            | Urea                   |
| X                   | Serum alanine aminotransferase (ALT)   |              |                        |
| X                   | Serum aspartate aminotransferase (AST) |              |                        |
|                     | Gamma glutamyltransferase (GGT)        |              |                        |

Results: Minor changes, sometimes of statistical significance, were observed for clinical chemistry parameters, but these were of a small magnitude and were not considered of toxicologic importance. ALP was increased in males and females exposed at 24.8  $\mu\text{g/L}$ , and the increase was significant ( $p < 0.01$ ) compared to controls in males. AST was decreased in females at the two highest exposures, but not in males; triglycerides were decreased ( $p < 0.05$ ) in females exposed at 24.8  $\mu\text{g/L}$ . Table 3 summarizes data for these parameters. Total protein and albumin were decreased in females at all exposure levels, but the changes were small and probably related to increased levels in controls. Other differences between test and control groups were sporadic.

6. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination. The following tissues were examined histologically: trachea, lungs, nasal cavity, larynx, liver, kidney, esophagus, stomach, and other abnormal tissues from intercurrent animals. The liver, kidneys, and lungs were weighed at the terminal sacrifice. The kidneys were not examined histochemically for alpha-2u globulin.

Results:

- a. Organ Weights: There were no significant increases in the weights of lung, liver, or kidney in rats exposed to captan. The authors indicated that kidney weights were slightly increased in females at the 24.8- $\mu\text{g/L}$  exposure level but that it was debatable whether this was treatment related. Table 4 summarizes mean kidney weights and kidney-to-body weight ratios in females.
- b. Gross Pathology: Dark red areas on the lung were seen on the two males that died; one had a partially collapsed lung. One male also had an enlarged thyroid.
- c. Microscopic Pathology: In both of the males that died, alveolar edema, congestion, and hemorrhage in the lungs with alveolar macrophage infiltration were observed, and both had marked bronchiolar necrosis. Rhinitis, moderate loss of respiratory epithelium in the nasal cavity, and moderate goblet cell hyperplasia in the nasal septum were seen in the rat killed moribund. The one male that died had slight tracheitis and moderate loss of epithelium of the larynx.

TABLE 3. Selected Clinical Chemistry Parameters in Rats Exposed to Captan for 3 Weeks<sup>a</sup>

| Exposure Level | Alkaline Phosphatase (U/L) |          | Aspartic aminotransferase (U/L) |         | Triglycerides (mg/kL) |          |
|----------------|----------------------------|----------|---------------------------------|---------|-----------------------|----------|
|                | Males                      | Females  | Males                           | Females | Males                 | Females  |
| 0              | 204 ± 3                    | 138 ± 20 | 65 ± 6                          | 76 ± 11 | 122 ± 18              | 109 ± 17 |
| 0.8            | 214 ± 18                   | 123 ± 23 | 65 ± 8                          | 69 ± 4  | 114 ± 17              | 113 ± 19 |
| 5.3            | 214 ± 24                   | 160 ± 34 | 58 ± 3                          | 56 ± 4* | 106 ± 13              | 88 ± 18  |
| 24.8           | 266 ± 27**                 | 170 ± 30 | 65 ± 6                          | 64 ± 5* | 110 ± 33              | 77 ± 12* |

<sup>a</sup>Mean ± S.D.

\*Significantly different from control value, p <0.05.

\*\*Significantly different from control value, p <0.01.

TABLE 4. Mean Kidney Weights and Kidney-to-Body Weight Ratios ( $\pm$  S.D.) in Female Rats Exposed to Captan

| Exposure Level<br>( $\mu\text{g/L}$ ) | Kidney Weight $\pm$ S.D.: <sup>a</sup> |                               |
|---------------------------------------|--|-------------------------------|
|                                       | g                                      | %                             |
| 0                                     | 1.73 $\pm$ 0.035 <sup>b</sup>          | 7.19 $\pm$ 0.212 <sup>c</sup> |
| 0.8                                   | 1.76 $\pm$ 0.082                       | 7.45 $\pm$ 0.308              |
| 5.3                                   | 1.82 $\pm$ 0.098                       | 7.66 $\pm$ 0.437              |
| 24.8                                  | 1.88 $\pm$ 0.197                       | 7.93 $\pm$ 0.403              |

<sup>a</sup>Values calculated by the reviewer.

<sup>b</sup>The value is 1.82  $\pm$  0.216 if an outlier of 2.205 g is included.

<sup>c</sup>The value is 7.51  $\pm$  0.733 with the outlier included.

Table 5 summarizes histologic findings at the terminal sacrifice. Lesions were confined to the respiratory system and were most frequent in the nasal cavity of females exposed at 24.8  $\mu\text{g/L}$ ; minimum rhinitis was observed in four females, minimum/slight goblet cell hyperplasia in the nasal epithelia of two, and mucopurulent exudate in three. Laryngitis was seen in several females in all exposed groups, and focal ulceration of the epithelium overlying the arytenoid cartilage of the larynx was seen in two females exposed at 24.8  $\mu\text{g/L}$ . One male exposed at 24.8  $\mu\text{g/L}$  was observed with ulceration of the squamous epithelium of the nasal cavity.

All other findings were incidental and not of toxicologic importance.

D. STUDY AUTHOR'S CONCLUSIONS:

Exposure of rats to aerosols of captan at concentrations of 0.8, 5.3, and 24.8  $\mu\text{g/L}$  resulted in two mortalities at the highest concentration. Signs of toxicity resulting from exposure to captan were consistent with those produced in rodents exposed to respiratory tract irritants. No treatment-related effects were observed on body weight gain, food consumption, or clinical laboratory parameters. Minor changes in clinical chemistry parameters were not considered to be of toxicologic importance but may reflect minor disturbances of liver metabolism. No organ weight effects were seen and none of the organs examined histologically except the respiratory tract showed any treatment-related changes. Histologic changes in exposed rats surviving to termination were restricted to the nasal cavity and larynx, which are particularly sensitive to particulate irritants. Findings in the lungs of the two males that died (alveolar edema, congestion, and hemorrhage) also were related to irritation. There was no systemic toxicity. The NOEL was 5.3  $\mu\text{g}$  captan/L.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study was adequate for a range-finding study. The exposure atmospheres were close to target, acceptably stable, and highly respirable. The test aerosols should reach the alveolar regions. Reporting of data was complete, and individual animal data were present and supported the means presented. No systemic toxicity was seen. We agree with the study author's conclusions that the effects seen at 24  $\mu\text{g/L}$ , death in two males, and histologic changes in the upper respiratory tract were treatment related and typical of changes seen with particulate irritants. The respiratory LEL (local irritation) for captan is 24.8  $\mu\text{g/L}$ , and the NOEL 5.6  $\mu\text{g/L}$ . The systemic LEL was greater than 24.8  $\mu\text{g/L}$  (HDT) and the systemic NOEL was  $\geq 24.8$   $\mu\text{g/L}$ .

TABLE 5. Histologic Findings of the Respiratory Tract of Rats Exposed to Captan for 3 Weeks<sup>a</sup>

| Organ/Finding                   | Exposure Level ( $\mu\text{g/L}$ ) |     |     |      |         |     |     |      |
|---------------------------------|------------------------------------|-----|-----|------|---------|-----|-----|------|
|                                 | Males                              |     |     |      | Females |     |     |      |
|                                 | 0                                  | 0.8 | 5.3 | 24.8 | 0       | 0.8 | 5.3 | 24.8 |
| <u>Lung</u>                     | (5) <sup>b</sup>                   | (5) | (5) | (3)  | (5)     | (5) | (5) | (3)  |
| Bronchiolitis                   | 0                                  | 1   | 1   | 1    | 0       | 0   | 1   | 0    |
| Pneumonitis                     | 1                                  | 2   | 1   | 1    | 0       | 0   | 1   | 0    |
| <u>Nasal Cavity</u>             | (5)                                | (5) | (5) | (3)  | (5)     | (5) | (5) | (5)  |
| Ulceration, squamous epithelium | 0                                  | 0   | 0   | 1    | 0       | 0   | 0   | 2    |
| Rhinitis, minimum               | 0                                  | 0   | 0   | 0    | 0       | 0   | 0   | 4    |
| Goblet cell hyperplasia         | 0                                  | 0   | 0   | 0    | 0       | 0   | 0   | 2    |
| Mucopurulent exudate            | 0                                  | 0   | 0   | 0    | 0       | 0   | 0   | 3    |
| <u>Larynx</u>                   | (5)                                | (5) | (5) | (3)  | (5)     | (5) | (5) | (5)  |
| Laryngitis, minimum/slight      | 2                                  | 3   | 5   | 1    | 1       | 2   | 4   | 3    |
| Ulceration                      | 0                                  | 0   | 0   | 0    | 0       | 0   | 0   | 2    |
| <u>Trachea</u>                  |                                    |     |     |      |         |     |     |      |
| Tracheitis, minimum             | 2                                  | 0   | 1   | 0    | 1       | 0   | 0   | 1    |

<sup>a</sup>Does not include findings for two males that died or were sacrificed at study termination.

<sup>b</sup>The numbers in parentheses are the number of tissues examined.