# SCIENTIFIC ANALYSIS OF THE DATA RELATING TO THE RECLASSIFICATION OF CAPTAN UNDER EPA'S NEW GUIDELINES FOR CARCINOGEN RISK ASSESSMENT

**PREPARED FOR:**

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# EXECUTIVE SUMMARY

Captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) is a fungicide registered by Makhteshim-Agan and Arvesta Corporation (formerly Tomen Agro) for the control of fungal diseases in crops. In 1989, the United States Environmental Protection Agency (EPA or Agency) concluded a toxicological review of captan and classified this product as a B2 (probable human) carcinogen. (USEPA, 1985). This classification was reconfirmed in the final Reregistration Eligibility Decision (RED) document (USEPA, 1999).

The EPA has recently issued new draft final *Guidelines for Carcinogen Risk Assessment* (USEPA, 2003) that present a revised classification scheme and describe new procedures for assessing potential human carcinogenic risk. Since the current classification of captan is based on the earlier, now outdated 1986 guidelines, the EPA agreed to a request from the Captan Task Force (CTF) to have the classification of captan reevaluated in accordance with the 2003 Guidelines using an independent Third Party process. This document comprises an objective scientific evaluation of all available data on the carcinogenic potential of captan.

The following conclusions were made:

**The weight of evidence from animal bioassays indicates that captan's tumorigenic potential is restricted to one tumor type in a single animal species.** Prolonged ingestion of high dose levels of captan causes an increased incidence of crypt cell tumors (adenomas and adenocarcinomas) in the proximal portion (7 cm) of the duodenum in both sexes of mice. Tumors are observed in females only at dietary levels of at least 800 ppm (120 mg/kg/day) and in males at levels of at least 6000 ppm (900 mg/kg/day) that exceed the maximum tolerated dose. In all studies, the tumorigenic response exhibits a clear dose threshold below which no effect occurs. A careful evaluation of the results of the rat bioassays provides no evidence that captan is associated with increased incidences of either renal tumors in males or of uterine sarcomas in females. An epidemiology study of limited power involving 410 employees of a captan manufacturing plant in the U.S. provided no evidence of increased duodenal cancer or other oncogenic effects.

**The overall weight of evidence clearly indicates that captan is not genotoxic in intact animals.** Captan is mutagenic when measured in *in vitro* test systems (bacterial or eukaryotic cells) where it has ready access to the DNA. *In vivo*, however, mutagenic activity is eliminated, or substantially decreased, in the presence of protein or thiols that rapidly deactivate potentially genotoxic captan-derived species. The lack of activity *in vivo* similarly results from the rapid

deactivation of captan-derived species by reaction with a variety of nucleophilic functional groups (e.g., thiols) present in blood and tissues. Consequently, neither captan nor its breakdown products reach the duodenal stem cells and are unable to to bind to DNA or cause chromosomal aberrations in these cells. Furthermore, the rapid breakdown of captan in blood (half-life <1 second) precludes the possibility that it can be transported to other tissues in the circulation following oral or dermal administration.

**Prolonged oral ingestion of captan by mice is also associated with several non-neoplastic effects (hyperplasia, crypt cell proliferation, inflammation, cytotoxicity and erosion of the villi)** that are observed in the same proximal region (7 cm) of the duodenum where tumors are formed. These responses show clear dose thresholds similar to those observed for tumor formation and are reversible following cessation of captan exposure.

**A nongenotoxic mode of action for captan is proposed in which the tumors are a secondary consequence of a cascade of non-neoplastic events.** The proposed sequence of events is initiated by inflammation, cytotoxicity and increased loss of the epithelial cells in the villi and this is followed by increased regenerative cell proliferation and hyperplasia of the stem cells in the duodenal crypts. Over a prolonged period of time the hyperplastic state leads to neoplasia through a process whereby spontaneously initiated cells are cloned before DNA damage can be repaired. There is a strong causal association indicating that tumor formation is secondary to duodenal irritation and hyperplasia and that the latter is a key event in the sequential cascade of events leading to cancer.

- **The overall weight of evidence strongly suggests that captan induces adenomas and adenocarcinomas in the duodenum of the mouse by a non-genotoxic mode of action involving cytotoxicity and regenerative cell hyperplasia that exhibit a clear dose threshold.**

**Based on the new Guidelines for Carcinogen Risk Assessment, EPA's current B2 (probable human) carcinogen classification for captan is inappropriate.**

**Under the descriptors defined in the new guidelines, it is proposed that captan should be classified as:**

1. ***likely to carcinogenic to humans following prolonged, high-level oral exposures causing cytotoxicity and regenerative cell hyperplasia in the proximal region of the duodenum.***
2. ***not likely to be a human carcinogen at dose levels that do not cause cytotoxicity and regenerative cell hyperplasia in the intestine.***
3. ***not likely to be carcinogenic to humans in other organs/tissues or following dermal or inhalation exposure.***

## Scientific Analysis of Data Relating to the Reclassification of Captan under EPA's New Guidelines for Carcinogen Risk Assessment

## I. INTRODUCTION

Captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) is a fungicide registered by Makhteshim-Agan and Arvesta Corporation (formerly Tomen Agro) for the control of fungal diseases in crops. In 1989, the United States Environmental Protection Agency (Agency) concluded a toxicological review of captan and classified it as a B2 ("probable" human) carcinogen (USEPA, 1989). The Agency also recommended use of the linear low dose extrapolation approach (Q1*) for purposes of risk characterization. This classification, based on EPA's 1986 Guidelines for Carcinogen Risk Assessment (USEPA, 1986), was recently reconfirmed in the Reregistration Eligibility Decision (RED) on captan (USEPA, 1999) because the Agency did not reassess the classification during the RED process.

In recent years, however, the EPA has been updating its Guidelines for Carcinogen Risk Assessment and, after several iterations, has recently published (USEPA, 2003) a Draft Final version of the Guidelines. These guidelines outline new procedures for evaluating carcinogenic risk using a "weight of the evidence" approach involving in-depth analysis of mode of action data and focusing on potential *human* hazard. A new carcinogen classification scheme based on human risk has been developed. The guidelines also provide alternative approaches to dose-response assessment and cancer risk characterization that obviate EPA's long reliance on the "no dose threshold" assumption for carcinogens and default use of the linear low-dose extrapolation model.

In response to a request from the Captan Task Force (CTF), EPA has agreed to have the carcinogenic potential and classification of captan reevaluated in accordance with the 2003 Guidelines using an independent Third Party process. The purpose of this document is to provide a summary of major scientific issues relating to the potential carcinogenic risk of captan to humans. It is not intended to be a comprehensive review of the toxicology of captan.

## II. REGULATORY HISTORY

Captan was first registered in 1949 as a pesticide for the control of fungal diseases under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). A rich regulatory history has followed the initial registration:

- 1980
    - EPA Publishes the Rebuttable Presumption Against Registration or RPAR (USEPA, 1980). [The RPAR concluded that captan is a rodent carcinogen. The Agency initiated an extensive regulatory evaluation of captan].
    - Within the RPAR, the Agency included a Position Document 1 (PD1) that

addressed potential concern that captan might produce delayed toxicity following chronic usage and exposure).

- 1985
  - Captan Registrants develop Task Force -- Captan Task Force (CTF).
  - Chevron and Stauffer and, following various mergers and realignments, currently Arvesta and Makhteshim, formed a data development task force to address the concerns raised by the EPA review.
  - Agency issues public notice submission of rebuttals and other information on the presumption and affords opportunity to submit any other data on the risks and benefits of captan.
  - EPA issues captan Data Call-In (DCI) and captan Special Review Position Document 2/3 (PD 2/3) (USEPA, 1985). PD 2/3 expressed concern that captan was a human health concern for carcinogenicity. Based on cancer risks, it was proposed that all food-uses of captan be cancelled and that only seed treatment uses be retained.

- 1986
  - The Agency issued a Registration Standard for captan summarizing the data that had been submitted in support of the captan re-registration and identified data gaps (USEPA, 1986a).
  - Agency reassessed carcinogen classification for captan and maintained B2 status (USEPA, 1986b).

- 1989
  - EPA published the Position Document (PD4) to conclude the Agency's Special Review and risk/benefit analysis of captan registrations (USEPA, 1989).
  - In the PD4, EPA maintained the classification of captan as a B2 carcinogen.

- 1995
  - EPA issued the draft HED chapter for Re-registration Eligibility Decision Document (USEPA, 1995).
    - Captan classification as B2 carcinogen retained.
    - The Q1* calculated as $3.6 \times 10^{-3}$, based on the adenomas and carcinomas of duodenum and jejunum-ileum seen in the male and female ICR-derived CD1 mice (Wong *et al.*, 1981) at high doses.
    - Based on the Q1* the "...upper bound cancer risk from captan is within range of risk that the agency generally considers as negligible."
    - CTF disagreed with EPA's conclusion that captan was a rat carcinogen (Fletcher *et al.*, 1995). No formal response received.

- 1999
  - The Agency issues final RED (USEPA, 1999a).

Captan classification as B2 carcinogen retained despite having the 1996 proposed carcinogen risk assessment guidelines available.
    - The Q1* calculated as $2.4 \times 10^{-3}$, based on the intestinal tumors in male mice and a scaling factor of ¾.

- 2000
    - CTF provides Response to Agency on RED Document (Pruett, 2000).
        - CTF objected to B2 classification based upon existing mechanistic data and new blood degradation data.
        - CTF recommended that captan should be classified as "....*Not likely at low doses*."
    - CTF submitted comprehensive position paper addressing the renal and uterine tumors in rats (Foster & Elliott, 2000). No formal response received.

- 2001
    - CTF requests EPA to re-evaluate captan under new draft Guidelines for Carcinogen Risk Assessment.

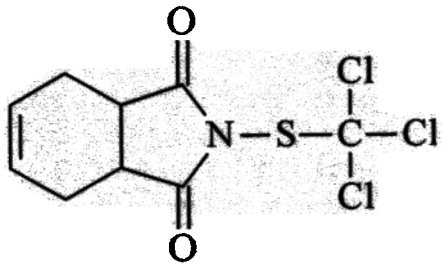
- 2002
    - EPA agrees in principal to conduct re-evaluation using an independent Third Party approach.

## III. PHYSICAL PROPERTIES AND CHEMICAL REACTIVITY

Captan (Figure 1) is a crystalline solid with low volatility and water solubility and a relatively high octanol-water partition coefficient. Selected physicochemical properties of captan are listed below (Gordon, 2001):

| | |
|---|---|
| CAS number: | 133-06-2 |
| Empirical formula: | $C_9H_8Cl_3NO_2S$ |
| Molecular weight: | 300.61 |
| Physical form: | Crystals |
| Melting point: | 178°C |
| Water solubility: | 3.3 mg/L at 25°C |
| Acetone solubility: | 3.0g/100 ml |
| Log $K_{OW}$: | 2.35 |

**Figure 1.** Structure of Captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide)



Captan is hydrolyzed in aqueous solution resulting in cleavage of the N-S bond and release of 1,2,3,6-tetrahydro-phthalimide (THPI) and thiophosgene. The rate of hydrolysis increases rapidly with increasing pH, the half-life of the reaction decreasing from 18.8 h at pH 5 to 8.3 min at pH 9 (Lee, 1989). Captan also reacts extremely rapidly with thiol-containing compounds such as cysteine and glutathione to produce THPI and thiophosgene (Figure 2). Half-lives of 18 sec at 22°C (Crossley, 1967a,b) and 0.97 sec at 38°C (Gordon *et al.*, 2001) have been reported with blood thiols. As will be discussed, this reaction is of particular importance in determining the metabolic fate and toxicological characteristics of captan *in vivo*. The thiophosgene generated from captan by either hydrolysis or reaction with thiols is a reactive molecule and has the potential to react rapidly with a number of functional groups on biological macromolecules. Thiophosgene also undergoes further hydrolysis and reaction with thiols (e.g., cysteine) as shown in Figure 3.

**Figure 2.** Generalized Reaction of Captan with Thiols.

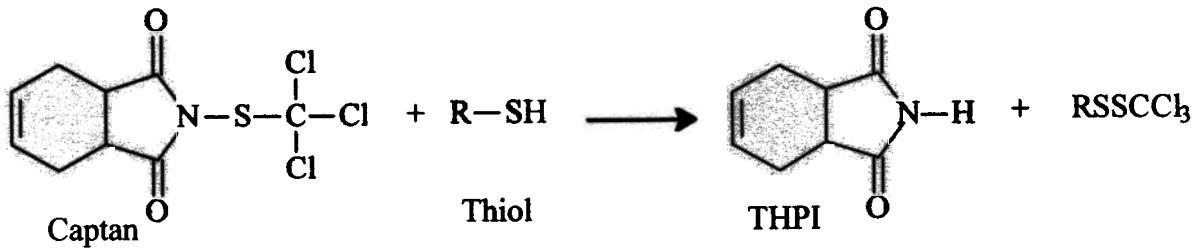
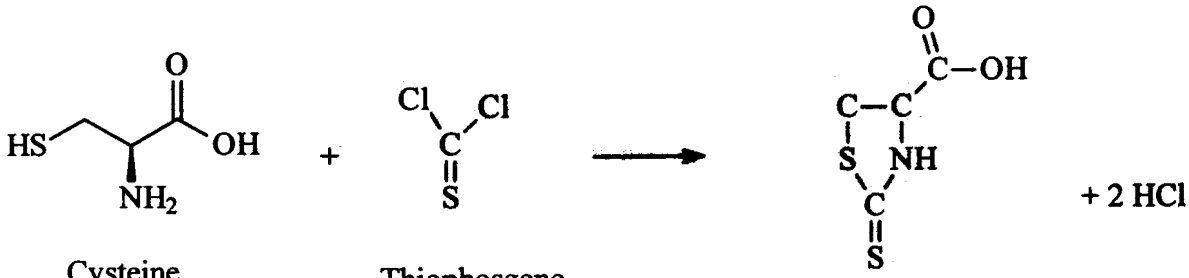



**Figure 3.** Reactions of Thiophosgene

Hydrolysis of thiophosgene in water

$$CSCl_2 + 2H_2O \longrightarrow CO_2 + 2HCl + H_2S$$ (Tilles, 1966)

Reaction of cysteine with thiophosgene




Cysteine

Thiophosgene

2-thioxothiazolidine 4-carboxylate

## IV. METABOLISM AND PHARMACOKINETICS

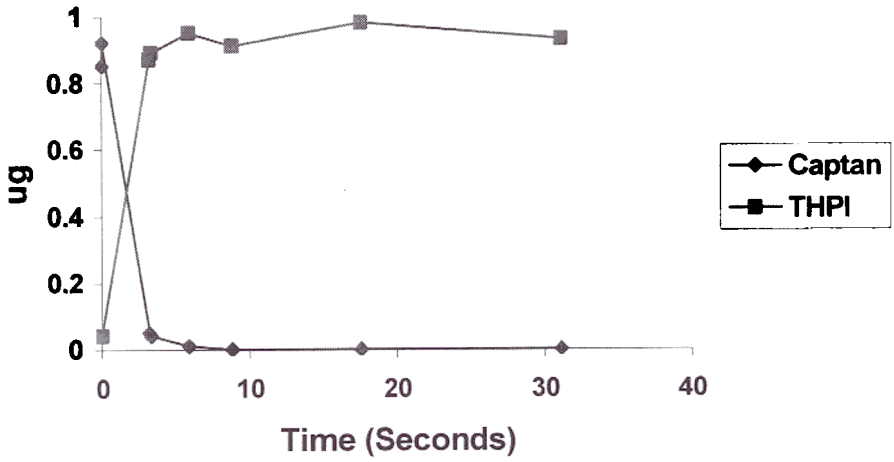
The metabolic fate of captan has been reviewed by Edwards *et al.*, (1991), Hayes (1991), Gordon *et al.*, (2001), HSDB (2001), and Trochimowicz *et al.*, (2001). When ingested, captan is relatively stable in the acidic environment of the stomach. However, it is rapidly hydrolyzed in the alkaline environment of the duodenum to THPI and thiophosgene. As discussed earlier, the latter metabolite is highly reactive and, therefore, short-lived. Alternatively, captan may react chemically with sulfhydryl-containing compounds present in the gut contents or, in the gut epithelial cells to form THPI and a thiophosgene "adduct" with glutathione (GSH). GSH concentrations in the cytoplasm of most animal tissues range from 3-10 mM so that this alone will have a significant capability of deactivating any thiophosgene produced. Furthermore, the cytoplasm of most animal cells contains high titers of GSH S-transferases that will substantially increase the rate of the non-enzymatic reaction between thiophosgene and GSH.

In the unlikely event that captan survives long enough to enter the systemic circulation, it will be broken down by thiols in the blood (half-life of less than one second) to THPI and thiophosgene-thiol adducts. *In vitro* tests with human blood have confirmed this extreme lability (Williams & Gordon, 1999; Gordon *et al.*, 2001) (Figure 4). Studies have shown that thiophosgene also reacts rapidly with thiols and other functional groups (amines, amides, imides, alcohols) in the tissues (Sharma, 1978a,b; Tilles, 1966) and EPA has concluded that thiophosgene is so labile that residues after oral ingestion of captan are not

quantifiable (USEPA, 1999).

In conclusion, from a toxicological standpoint, the rapid enzymatic or non-enzymatic reaction of captan and thiophosgene with tissue thiols results in the effective elimination of reactive species that might otherwise be transported around the body in the systemic circulation.

**Figure 4. Captan Degradation and THPI Accumulation in Human Blood**




A study by Provan and Eyton-Jones (1996) determined the distribution of captan and its metabolites from the gastrointestinal (GI) tract of mice. CD1 male mice (30 per exposure level) were administered [1,2-$^{14}$C]-cyclohexene-labeled captan in their diets at concentrations of 0, 400, or 3,000 ppm for varying lengths of time. Groups of six mice were sacrificed at 6, 12, 18, 24, and 30 hours after the start of exposure to each dose level and radioactivity was measured in the contents and the epithelial tissue of the different segments of the gastrointestinal tract. A low. Steady-state concentration of radiolabel was observed in the duodenum in both the 400 ppm and 3000 ppm groups. The radiolabel was associated with the duodenal contents rather than the epithelial tissues and did not accumulate with time. Parent captan was detectable only in the stomach of mice receiving the 3,000 ppm diets; in those receiving 400 ppm diets, only captan metabolites were found. Only THPI and its metabolites were found in duodenum, blood and urine consistent with the rapid degradation of captan observed in the stomach. Thus, following oral ingestion, captan is rapidly absorbed and distributed. Because of its rapid breakdown in the GI tract, however, captan is not absorbed as parent compound and neither captan nor its metabolites accumulate over time. The urine contained THPI-derived metabolites (seven HPLC peaks detected).

In summary, following oral ingestion, captan is rapidly absorbed, distributed,

metabolized, and excreted. It is absorbed from the gut in the form of hydrolysis products, mainly THPI. THPI may be metabolized further to other stable metabolites. Thiophosgene is a highly reactive, short-lived intermediary metabolite that rapidly reacts with all available nucleophiles, especially thiols.

## HAZARD IDENTIFICATION

Since this document is focused exclusively on evaluating the potential oncogenicity of captan, only studies relating to this particular hazard endpoint will be discussed.

### A. Mouse Bioassays

Captan has been evaluated for oncogenic potential in three carcinogenicity studies in mice (NCI, 1977; Wong *et al.*, 1981; Daly & Knezevich, 1983).

#### NCI (1977)

In the NCI study (NCI, 1977), B6C3F1 mice were fed diets containing 0, 8000 or 16000 parts per million (ppm) captan (approximately 0, 900 or 2400 mg/kg/day captan) for 80 weeks followed by an 11 week non-treatment period. The No Observed Adverse Effect Level (NOAEL) was 8000 ppm and the Lowest Observed Adverse Effect Level (LOAEL) was 16000 ppm based on decreased mean body weight. The primary effects observed in this study were histologic changes in the duodenum occurring approximately 1 cm posterior to the pylorus. Grossly, the lesions appeared either as single, well-circumscribed and slightly elevated areas or as single, thin mucosal projections. Three different types of lesions were recognized – mucosal hyperplasia, adenomatous polyps and adenocarcinomas -- with characteristics suggesting they were different stages of the same type of lesion. The incidence of duodenal adenocarcinoma in adenomatous polyps was 0/68, 1/43 and 3/46 for control, low and high dose males, respectively and 0/68, 0/49 and 3/48 for control, low and high dose females, respectively. Combination of unspecified adenomatous polyps with polyploid carcinomas yielded incidences of 0/68, 3/43 and 5/46 for control, low and high dose males, respectively. Hyperplasia of the duodenal mucosa was also noted in three high dose males.

#### Wong *et al.*, (1981)

In a second study, ICR-derived CD1 mice were initially fed diets containing 0, 2000, 6000 or 10000 ppm captan for 4 weeks (Wong *et al.*, 1981). Because of a lack of effect, the captan dietary concentrations were increased to 0, 6000, 10000, or 16000 ppm (about 900, 1500 and 2400 mg/kg/day) for the remainder of the 113-week study. A NOAEL was not established. The LOAEL for systemic toxicity was 6000 ppm based on decreased body weight gain and food consumption. Based on the effects body weight and body weight gain (>20% depression), the 16000 ppm was considered to have exceeded the Maximum Tolerated Dose. There was an increased incidence of small

intestinal (primarily duodenal) adenomas/polyps and carcinomas at all dose levels (Table 1); a positive dose-related trend for and increased incidence of these tumors was observed for both sexes. A statistically significant increase in gastric and duodenal hyperplasia was seen in males and females and an increase in jejunal hyperplasia in females. Mucosal hyperplasia of the duodenum was characterized as a progressive distortion of normal villus architecture. Intestinal crypts were irregularly dilated and lined by an increased number of cells that showed an intense basophilic staining reaction. Areas of hyperplasia of grade 2 or greater showed a flattened and distorted mucosal surface and a complete lack of normal villus architecture. The mucosal hyperplasia was inversely correlated with the relative incidence of adenocarcinoma found in each test group.

Daly & Knezevich (1983)

In another study with CD1 mice (Daly & Knezevich, 1983), the animals were fed diets containing 0, 100, 400, 800 or 6000 ppm (approximately 0, 15, 60, 120, or 900 mg/kg/day, respectively) captan for 22 months. The study was terminated at this time due to increased mortality in the high dose males. The NOAEL for systemic toxicity was 800 ppm and the LOAEL for systemic toxicity was 6000 ppm based on increased mortality in males and reduced body weight gain in males and females. The 6000 ppm dosed clearly exceeded the MTD for captan. Captan exposure resulted in an increased incidence of malignant and/or benign neoplasms of the duodenal crypt cells in male mice at 6000 ppm and female mice at 800 and 6000 ppm (Table 1). Focal hyperplasia of the duodenal mucosa was increased in both males and females from the 6000 ppm group although its occurrence is not clearly related to dose.

The collective results of these studies (Table 1) clearly show increased incidences of duodenal tumors in mice following prolonged exposure to high dietary levels of captan. The effect is observed in females only at dietary levels of at least 800 ppm (although no clear dose-response relationship is evident) and in males at levels of at least 6000 ppm. As will be discussed (Section VI.B), genetic toxicity studies have shown that captan is not genotoxic *in vivo*. The lack of genotoxicity *in vivo*, coupled with the clear dose threshold for tumor induction, strongly suggests an epigenetic mechanism of tumor production. In addition to the studies described above, captan was also included in an early NCI program to evaluate the carcinogenic potential of a large number of chemicals (Innes *et al.*, 1969). No significant increase in tumors was seen in mice initially receiving gavage doses of 215 mg/kg/day for 3 weeks followed by dietary exposure to 560 ppm for 18 months.

## TABLE 1.

Incidence of duodenal tumors in mice

| Dose (ppm) | 0 | 100 | 400 | 800 | 6000 | 8000 | 10000 | 16000 | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Males | | | | | | | | | |
| Adenomas | 2/91 | 3/83 | 0/93 | 1/87 | 4/84 | | | | Daly &* |
| Carcinomas | 0/91 | 0/83 | 0/93 | 0/87 | 2/84 | | | | Knezevich |
| Adenocarcinomas | 0/9 | | | | | 1/43 | | 3/46 | NCI |
| Duodenal neoplasms | 2/74 | | | | 20/73 | | 21/72 | 39/75 | Wong |
| Females | | | | | | | | | |
| Adenomas | 3/85 | 1/82 | 1/83 | 7/81 | 3/91 | | | | Daly &* |
| Carcinomas | 0/85 | 0/83 | 0/83 | 0/81 | 1/91 | | | | Knezevich |
| Adenocarcinomas | 0/9 | | | | | 0/49 | | 3/48 | NCI |
| Duodenal neoplasms | 2/72 | | | | 24/78 | | 19/76 | 29/76 | Wong |

*Incidence reported in pathology re-evaluation (Robinson, 1993)

### B. Rat Bioassays

Four acceptable carcinogenicity studies have been conducted with captan in rats. The first two of these (Hazleton, 1956; NCI, 1977) showed no increased incidence of tumors up to and including dietary levels of 10000 and 6050 ppm (about 500 and 300 mg/kg/day), respectively. EPA's current position on captan's oncogenic potential is based, in part, on two more recent studies (Goldenthal *et al.*, 1982; NOASR, 1983). Consequently, the two former studies will not be discussed here although certainly the existence of two negative studies should be taken into account in a total weight of evidence analysis.

#### Goldenthal *et al.*, (1982)

In the first of the two studies reviewed by EPA (Goldenthal *et al.*, 1982), Charles River CD rats were fed diets containing 0, 500, 2000 or 5000 ppm captan (approximately 0, 25, 100 or 250 mg/kg/day) for two years. The NOAEL was 500 ppm and the LOAEL was 2000 ppm based on hepatocellular hypertrophy, increased relative organ weights (kidney in male and female and heart, brain, liver and thyroid in male) and decreased bodyweight. EPA concluded that there was a significant increasing trend in males only for combined adenoma and carcinoma of the kidney (Table 2). There was no increase in renal cortical/tubular cell neoplasia in females.

EPA concluded (USEPA, 1989) that *"...rat data show only a borderline increase in kidney tumors"* and has noted that there was no statistically significant increase in renal tumors when the data were analyzed by the pair-wise procedure ($p>0.05$). The Agency was only able to show a dose-related trend for combined adenomas and carcinomas.

However, as pointed out by Foster & Elliott (2000), the statistical methodology employed by the Agency to conduct the trend analysis was a Cochran-Armitage test without

**TABLE 2.**

Incidence of renal tumors in male Charles River CD rats (Goldenthal *et al.*, 1982)

| Dose (ppm) | 0 | 500 | 2000 | 5000 |
|---|---|---|---|---|
| Number examined | 69 | 69 | 67 | 66 |
| Liposarcomas | 1 | 0 | 1 | 0 |
| Adenomas | 1 | 0 | 2 | 3 |
| Adenocarcinomas | 0 | 0 | 0 | 1 |
| Unilateral renal cell carcinomas | 0 | 1 | 1 | 0 |
| Total (Adenoma + carcinoma) | 1 | 1 | 3 | 4 |

correction for continuity. Both the NCI and NTP recommend that the Cochran-Armitage test be applied with correction for continuity. When this is done, Foster & Elliott (2000) demonstrated that there is no statistically significant trend in the incidence of kidney tumors between the groups.

In conclusion, EPA's position with respect to the association of captan with the rat renal tumors must be considered questionable in light of the following:

The finding of increased kidney adenomas has been seen in only one of four rat carcinogenicity studies;
The increase is seen only in males;

- The small numerical increase in tumors is not statistically significant when analyzed by the correct methodology;
The increase in kidney adenomas in the high dose group is a single animal outside the historical control database for this tumor type; and
The increase is only in benign tumors – there is no increase in malignant renal tumors (carcinomas) between treated and control groups.

NOASR (1983)

In this study, Wistar rats were fed diets containing captan at 0, 125, 500 or 2000 ppm (approximately 0, 6.25, 24 or 98 mg/kg/day) for 120 weeks. The NOAEL and LOAEL for systemic toxicity were at least 2000 ppm. The only observation made was a *"slight but statistically significant increase in uterine sarcomas in the high dose group"* (USEPA, 1999). The incidence of uterine tumors in this study is shown in Table 3.

Foster & Elliott (2000) conducted a detailed analysis of the NOASR study and concluded that the three types of sarcomas observed are not a homogeneous group of malignant tumors and should not have been combined in analyzing for a possible treatment-related effect.

**TABLE 3.**

Incidence of uterine tumors in female Wistar rats (NOASR, 1983)

| Dose (ppm) | 0 | 125 | 500 | 2000 |
|---|---|---|---|---|
| Number examined | 40 | 49 | 50 | 50 |
| Fibromatous polyps | 7 | 11 | 12 | 10 |
| Multiple fibrous polyps | 1 | 1 | 1 | 2 |
| Adenocarcinomas | 6 | 4 | 5 | 7 |
| Papilloma | 0 | 0 | 1 | 0 |
| Carcinoma in situ | 1 | 0 | 0 | 1 |
| Stromal sarcoma | 0 | 0 | 0 | 2 |
| Poorly differentiated sarcoma | 0 | 0 | 0 | 1 |
| Unclassified sarcoma | 0 | 0 | 0 | 1 |
| **Total sarcoma** | **0** | **0** | **0** | **4** |

In summary, Foster & Elliott (2000) pointed out that:

- The small numerical increase in uterine sarcomas in the high dose group is only significant when the different tumor types are inappropriately combined;
- An increase in uterine tumors of this type were seen only in one out of the four studies conducted even though the other studies used considerably higher dose levels;
  The study was unusually long (120 weeks) and there are no historical control data against which to compare the tumor incidence;
- There is no evidence for usual progression of uterine sarcomas from uterine polyps; and
  A more appropriate analysis of combined sarcomas and polyps shows no treatment-related effect on tumor incidence.

For the sake of completeness, it is necessary to mention the review of Reuber (1989), which concluded that captan *"is highly carcinogenic in rats and mice."* Reuber apparently based this conclusion on a personal interpretation of the histological sections combined with a highly unorthodox and flawed analysis in which tumors of distinct types and in different tissues/organs are combined. The review is considered unscientific. It was not cited by EPA and should not be used in the weight of evidence evaluation.

C. Human Epidemiology

An epidemiological cohort study was conducted of 410 employees who had worked for at least one day at a captan manufacturing plant in Ohio between January 1, 1954 and December 31, 1997 (Wong & Harris, 2000). The mortality experience of the cohort within this time period was determined through a number of sources including company records, the Social Security Administration's Death Master File, and the National Center for Health Statistics' National Death Index. Cause-specific standardized mortality ratios (SMRs) were calculated for the entire cohort by length of employment, by latency, and by job category.

Although of very limited size, the results of the study suggest that the employees at the captan plant were not at increased risk of cancer-related deaths. Of the deaths recorded during the study period, none were diagnosed as due to duodenal cancer. This study indicates that captan is not a human carcinogen under conditions of routine daily occupational exposure by those actually engaged in the manufacture of this chemical.

### D. Conclusions

The weight of evidence from animal bioassays indicates that prolonged ingestion of high dose levels of captan causes an increased incidence of tumors in the proximal portion of the duodenum in both sexes of mice. Careful evaluation of the results of the rat bioassays provides no evidence that captan is associated with increased incidences of either renal tumors in males or of uterine sarcomas in females as concluded by EPA. Consequently, captan is associated with the formation of tumors in only one tissue (duodenum) in only one test animal species (mice).

An epidemiology study of limited power involving 410 employees of a captan manufacturing plant in the U.S. suggested no evidence of increased duodenal cancer or other oncogenic effects.

## VI. GENETIC TOXICOLOGY

The genetic toxicology of captan remains a controversial area and, over the years, an extensive database comprising all types of studies has been compiled. This has been the subject of several comprehensive reviews (Bridges, 1975, Quest *et al.*, 1993; Edwards *et al.*, 1991; Tennekes, 1994; Bernard & Gordon, 2000; Trochimowicz *et al.*, 2001) and no attempt is made here to conduct another all-inclusive evaluation. Instead, an objective overall assessment and interpretation of the available data is made and, where possible, an attempt made to identify consistent patterns of activity.

In interpreting the results of the many genotoxicity studies conducted, it is important to consider the conditions of each test in relation to rapid nonenzymatic and/or enzymatic breakdown of captan in living organisms. As discussed (Section II), this breakdown results in the release of THPI and thiophosgene. THPI is of little toxicological concern

and has been shown to be consistently negative for mutagenic activity in bacterial assays (*S. typhimurium* TA1535, TA1537, TA98, TA100, TA102; and *E. Coli*, WP2 uvrA) with and without the addition of rat liver S9 preparation (Carver, 1985). Thiophosgene, however, is a reactive molecule and has an inherent reactivity and ability to bind avidly to many biological molecules. It has been shown to be a mutagen when applied directly to *S. typhimurium* assays (Rideg, 1982) or when administered as a vapor produced after by "activating" captan, previously absorbed onto filter paper, with carbonate (Arlett, 1975). Consequently under test conditions in which thiophosgene is able to reach DNA it has the potential to cause a mutagenic effect.

A *In Vitro* Mutagenicity Tests

The total weight of evidence clearly indicates that, *in vitro*, captan is a mutagen in both bacterial (Table 4) and eukaryotic cell (Table 5) assays. Captan induces treatment and dose-related increases in the incidence of mutations including frame-shift, base-substitution and other point mutations. The mutagenic potency of captan *in vitro*, however, is highly dependent on the presence (or absence) of thiols or other molecules capable of reacting with captan itself or the thiophosgene released. The results in Table 4, for example, show that the presence of the rat liver S9 microsomal fraction that contains substantial amounts of proteins and other macromolecules decreases mutagenic activity substantially. This is also true for the *in vitro* eukaryotic cell assay systems where positive results are only observed in serum-free incubation mixtures (Table 5).

The effect of exogenous proteins and thiols (cysteine) on decreasing the mutagenic activity of captan in bacterial assay systems is dramatic (Table 6). Clearly, these materials provide alternative sites that rapidly bind any thiophosgene released from captan and prevent it from reaching and potentially interacting with DNA (Trochimowicz *et al.*, 2001).

The mechanism by which captan induces these mutations is not clear, although a similarity with alkylating agents is noted (Bridges *et al.*, 1972). Direct evidence for interaction between captan and DNA is weak, the ability of captan to induce unscheduled DNA synthesis (UDS) either *in vitro* or *in vivo* is lacking, and except for one report (Bridges, 1975), alkylated DNA products *in vivo* are not evident. The one-carbon thiophosgene is too small for DNA-DNA cross-linking, but would allow DNA-protein cross-linking. Regardless of the mechanism involved, it is clear that captan has the potential to induce mutations when sensitive targets are exposed.

**TABLE 4.**

*In vitro* mutagenic activity of captan in bacterial assay systems

| Test System | | -S9 | +S9 | Reference |
|---|---|---|---|---|
| *S. typhimurium* | TA1535 | +[2] | +/- | Carere *et al.* (1978)<br>Shirasu *et al.* (1976) |
| *S. typhimurium* | TA1536 | | | Shiau *et al.* (1981) |
| *S. typhimurium* | TA1537 | + | +/- | Carere *et al.* (1978)<br>Shirasu *et al.* (1976) |
| *S. typhimurium* | TA1538 | + | +/- | Carere *et al.* (1978)<br>Shirasu *et al.* (1976) |
| *S. typhimurium* | TA98 | + | +/- | Shiau *et al.* (1981) |
| *S. typhimurium* | TA100 | ++ | + | Moriya *et al.* (1983)<br>DeFlora *et al.* (1984) |
| *B.subtilis* | TKJ5211 | +++ | +++ | Shiau *et al.* (1981) |
| *B.subtilis* | TKJ6321 | +++ | +++ | Shiau *et al.* (1981) |
| *E. coli* | WP2 $try^{-}$ $her^{+}$ | +++ | | Nagy *et al.* (1975)<br>Shirasu *et al.* (1976) |

[1] Data from Shiau *et al.*, (1981) with other references as shown.

[2] Spot Test scoring reported for 50 μg (167 nm)/plate.

- \- Equal to or less than the spontaneous rate.
- +/- Almost identical with the background, but with an inhibition zone.
- \+ Has an inhibition zone and a few more revertants than the background.
- ++ Has an inhibition zone and more revertants than the background.
- +++ Has an inhibition zone and too numerous to count revertants.

## TABLE 5.

*In vitro* mutagenic activity in mammalian cell systems

| Assay System | Results | Reference |
|---|---|---|
| Chinese Hamster V79/Hgprt | Positive in absence of S9 fraction | Arlett *et al.* (1975) |
| Chinese Hamster V79 Alkaline elution | 0.03-0.1 mM. Positive for single strand DNA in absence of S9<br>3.0 nM. Negative for single strand DNA in presence of S9 fraction | Swenberg *et al.* (1976) |
| Chinese Hamster CHO/Hgprt | Approx. 35 mutants/106 cells per μg captan/ml medium (-S9). Cultures maintained in serum-free medium. | O'Neill *et al.* (1981) |
| Mouse lymphoma L5178Y/TK assay | Positive forward mutation index in absence of S9 fraction. | Oberly *et al.* (1984) |

Abbreviations: Hgprt = Hypoxanthine guanine phosphoribosyl transferase; TK = Thymidine kinase;

## TABLE 6.

Effect of exogenous proteins and thiols on captan mutagenicity[1]

| Test System | Component Added | Revertants per Plate |
|---|---|---|
| *E.coli* WP2 bcr | None | 3200 |
| with 0.15 μM (45 μg) | S-9 | 30 |
| captan per plate | 20 mM cysteine | 19 |
| | rat blood | 32 |
| | 0 μM Cysteine/μM captan | 2900 |
| | 0.5 " | 2580 |
| | 1.0 " | 1660 |
| | 2.5 " | 183 |
| | 5.0 " | 10 |

Data from Moriya *et al.*, (1978)

B *In Vivo* Mutagenicity Assays

In contrast to the positive mutagenic activity exhibited by captan in a variety of *in vitro* test systems, the results of several *in vivo* assays are overwhelmingly negative (Table 7). One weakly positive effect has been reported in a *Drosophila* sex-linked recessive lethal assay (Valencia, 1981) and "slight" increase in early litter deaths has been reported in a mouse dominant lethal assay (Collins, 1972). Three other *Drosophila* tests and four other mouse dominant lethal assays were negative (Table 7). In a single oral study by Feng & Lin (1987), captan was reportedly positive in one micronucleus study and chromosomal aberration assays in bone marrow and testis. This paper contains numerous errors and in

**TABLE 7.**

*In vivo* mutagenic activity of captan

| Assay System | Result | Reference |
|---|---|---|
| Somatic cell mutation (mouse spot test) | Negative (oral) | Litton (1980) |
| | | Moore (1981) |
| | 2.2% frequency after 15 mg/kg i.p. | Imanishi *et al.* (1987) |
| Somatic mutation & recombination (*Drosophila* SMART) | Negative | Mollet & Wurgler (1974) |
| Mouse heritable translocation | Negative | Simmon *et al.* (1977) |
| Drosophila sex-linked recessive lethal | Negative | Kramers & Knaap (1973) |
| | | Mollet (1973) |
| | Weakly positive | Valencia (1981) |
| Mouse dominant | Negative | Jorgenson *et al.* (1976) |
| Lethal | Negative | Kennedy *et al.* (1975) |
| | Negative | Rideg (1982) |
| | Negative | Simmon *et al.* (1977) |
| | *Positive* | Collins (1972) |
| Micronucleus | Negative | Jakoby (1985) |
| | *Positive* | Feng & Lin (1987) |
| Chromosomal aberration | Negative | Tezuka *et al.* (1978) |
| | Negative | Fry & Fiscor (1978) |
| | Negative | Chidiac & Goldberg (1987) |
| | *Positive* | Feng & Lin (1987) |
| Heritable translocation | Negative | Jorgenson *et al.* (1976) |

Data from Bernard & Gordon (2000)

light of several other negative studies of this type the data it contains must be considered questionable. In view of the rapid biodegradation of captan, it is highly unlikely whether any reactive species derived from orally administered material would survive to reach remote tissues such as the bone marrow or testis.

Because captan induces duodenal tumors in mice, a critical study was undertaken by Chidiac & Goldberg (1987) to determine whether, at oncogenic dose levels, orally administered captan was genotoxic *in vivo* to the cells believed to be the origin of the tumors (i.e., the stem cells within the crypts). Following administration of captan by gavage at doses of 0 or 1,000 mg/kg/day for 5 consecutive days no evidence of clastogenic activity was seen and it was concluded that there was no evidence of nuclear interaction in the stem cells of the mouse duodenum. Furthermore, captan did not potentiate the nuclear aberration frequency induced by a positive control, dimethyl hydrazine, in the duodenal stem cells. The study strongly suggests that neither captan nor its degradates reach the stem cells deep within the crypts of the villi of the duodenum or, if they do reach the stem cells, they are not clastogenic. Consequently, despite the established ability of captan to interact with DNA *in vitro*, it is not clastogenic to the cells that give rise to the duodenal tumors seen in mice when administered orally *in vivo*.

C. DNA Binding

Although when $^{35}$S-captan is mixed directly with calf thymus DNA a low level of radioactivity becomes associated with the DNA, the investigators (Couch & Siegel, 1977) were unable to conclude it involved covalent bonding. Captan does react with DNA, however, as evidenced by the formation of N-7-(trichloromethylsulfenyl) guanine (Elder, 1989). It is also able to inhibit *in vitro* DNA polymerase activity in calf thymus nuclei although this is thought to occur through a thiol-related mechanism (Lewis & Brown, 1978).

Several studies have focused on the question of whether, following oral administration, captan or its metabolites might covalently bind to DNA in mouse duodenal stem cells. In a study by Pritchard & Lappin (1991), a single dose of either 0 or 900 mg/kg of $^{35}$S-labeled captan was administered by gavage to individual groups of 100 CD-1 mice and DNA was extracted from stomach, duodenum, jejunum, liver, and bone marrow six hours after dosing. Low levels of $^{35}$S-radioactivity were found to be associated with DNA in all tissues evaluated. Cesium chloride gradient ultracentrifugation showed that radioactivity from captan-treated mice did not overlap precisely with the DNA peaks measured by UV absorption. The authors concluded that, while radioactivity was associated with DNA, it may not represent covalently bound captan adducts. A subsequent study (Provan *et al.*, 1995) supported this conclusion. These investigators showed similar low level residues of $^{35}$S following administration of $^{35}$S-radiolabeled N-acetylcysteine and two thiol metabolites of captan. It is likely, therefore, that the low levels of $^{35}$S radiolabel observed in DNA by Pritchard & Lappin (1991) reflect an exchange between the sulfur atom in captan and the endogenous thiol-containing amino acid pool.

Based on the results of these two studies, it can be concluded that captan-derived

radioactivity was associated with DNA but that the low activity observed in the duodenum (the target organ for tumor development) was no higher than that in other non-target tissues. Importantly, the activity may not represent covalently bound captan-DNA adducts since ultracentrifugation of samples in a cesium chloride gradient revealed that the $^{35}S$-radioactivity did not correspond to DNA peaks measured by UV absorption.

### D. Conclusions

- The overall weight of evidence indicates that captan is not an *in vivo* mutagen when administered to intact animals.

- If provided with ready access to DNA, as in *in vitro* test systems, captan and/or its degradates, particularly thiophosgene, have the ability to induce mutagenic effects in prokaryotic and eukaryotic cells. The precise mechanism(s) through which these mutagenic effects occur are not clear.

- The mutagenic potency of captan in *in vitro* test systems is markedly reduced in the presence of proteins (e.g., S9 microsomal fractions) and other exogenous materials, such as thiols. These materials rapidly react with the reactive captan-derived products and serve to prevent interactions with the DNA.

- With very few exceptions, captan is negative for mutagenicity in *in vivo* assay systems.

  Neither captan nor its breakdown products reach the stem cells deep within the crypts of the villi of the duodenum or, if they do reach the stem cells, they are not clastogenic. There is no evidence that captan or its breakdown products bind to DNA or cause chromosomal aberrations in the duodenal stem cells following oral administration.

## VII. PROPOSED MODE OF ACTION

### A. Summary and Key Precursor Events

Based on the discussion so far, it can be concluded that:

- Prolonged dietary exposure of male and female CD1 mice to high dose levels of captan is associated with an increased incidence of crypt cell adenomas and adenocarcinomas in the proximal region of the duodenum after the pylorus of the stomach. These were the only tumor types related to captan treatment in mice and rat bioassays.

- The mouse duodenal tumors exhibit clear dose thresholds of 800 and 6000 ppm (about 120 and 900 mg/kg/day) in female and male mice, respectively.

The total weight of evidence indicates that, although captan is mutagenic in *in vitro* tests, it is not mutagenic or clastogenic *in vivo* because its high reactivity with thiols and other functional groups serves to protect the DNA.

The lack of genotoxicity *in vivo* combined with the clear dose threshold for tumor induction strongly suggests that captan exerts its effect through a non-genotoxic mechanism. Furthermore, pathology evaluation of the mouse bioassays indicates that the tumors are closely associated with mucosal hyperplasia and adenomatous polyps and that these lesions precede formation of tumors in the same proximal region of the duodenum.

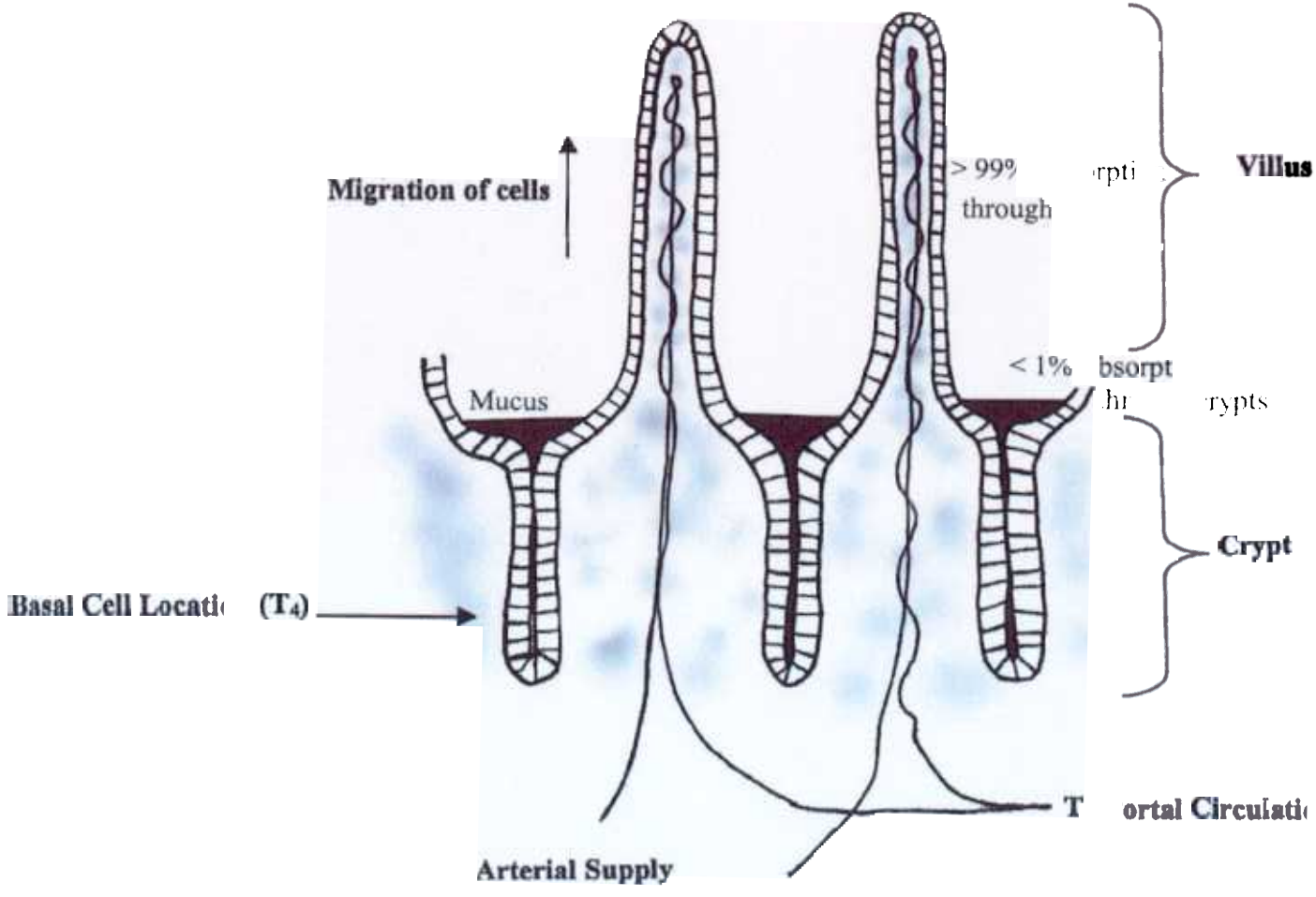
It is proposed that the hyperplasia and the adenomatous polyps are key precursor events in the carcinogenic process and that these lesions are part of a proliferative response to inflammation and cytotoxicity in the duodenal epithelia. Captan and its breakdown products are known chemical irritants and clearly have the ability to cause inflammation and cytotoxicity to the duodenal epithelial cells. Over a long period of exposure, these effects lead to regenerative cell proliferation which, in turn, has been associated with increased tumor incidence in certain tissues (Pitot *et al.*, 1991). When, through whatever mechanism, a chemical causes an increase in cell proliferation, the capacity of the cell to repair damaged DNA is decreased and the probability of converting spontaneous DNA damage to heritable change is increased. Since all tissues normally contain some initiated cells, cell proliferation can result in "fixing" a spontaneous initiated event that would normally be repaired (Cohen & Ellwein, 1990).

In summary, the proposed sequence of events in the carcinogenic action of captan in the mouse duodenum is 1) irritation and inflammation of the proximal duodenal epithelial cells 2) cytotoxicity and cell necrosis 3) regenerative hyperplasia and crypt cell proliferation and 4) neoplasia. Importantly, hyperplasia and cell proliferation are threshold-based effects with clear NOAELs and that, except in extreme cases, are likely to be fully reversible on cessation of exposure (Ghanayem *et al.*, 1991).

B Morphology of Intestinal Epithelium

The intestinal mucous membrane is composed of epithelium, lamina propria, and muscularis mucosa. The epithelial cells extend upward into the lumen in cylindrical finger-like projections (0.3-1 mm long) called villi, which line the entire length of the intestine. Within each villus is a core of lamina propria that include smooth muscle, blood vessels, lymphatic vessels and goblet cells. The epithelial cells also extend downward from the lumen to form the crypts of Lieberkuhn. The crypts of the small intestine are small, flask-shaped epithelial structures containing about 250 cells comprised of columnar enterocytes (an intestinal epithelial cell), mucus-secreting goblet cells (which secrete a protective mucous layer), Paneth cells which secrete digestive enzymes and infrequent enteroendocrine cells. The morphology of the duodenal villi and crypts as well as the direction of migration of the cells proliferating from the stem cells within the crypts to the villi tips are depicted in Figure 5.

F re Sch f D od V Cry



Within the crypt, approximately two-thirds of the cells (150-160 per crypt), the stem cells, are in a constant proliferative state. Several investigators have concluded that the actual stem cells comprise a ring of about 16 cells near the bottom of the crypt but above the Paneth cells (about the 4$^{th}$ position from the bottom)(Leblond & Cheng, 1976; Cheng & Leblond, 1974; Potten *et.al.*, 1983; Potten & Hendry, 1983; Wright & Alison, 1984; Potten & Morris, 1987).

Cell proliferation is restricted to a centrally located band of about 10 cell layers deep within the crypts (Potten *et al.*, 1990). Within this band, the cells rapidly divide and move upward, out of the crypt onto the villus, migrating to the villi tips where they are rapidly sloughed off the into the lumen (Potten *et al.*, 1983; Potten & Loeffler, 1990; Wright & Allison, 1984; Potten & Morris, 1987; Potten & Hendry, 1983). This proliferative process is driven by a high rate of mitotic activity, resulting in a normal cell replacement time for the villi of approximately 2 days in rats, 3 days in mice and 3 to 4 days in humans (Ham, 1965).

The proliferative nature of most of the cells comprising the crypts and villi indicate that the stem cells are the only epithelial cells present for a sufficient period of time to sustain a carcinogenic insult. The architectural features of the crypts and villi and the well established instability of captan *in vivo*, strongly support the conclusion that neither captan nor its reactive degradates are likely to reach the stem cells deep within the crypts.

A key finding in support of this conclusion is that subchronic and chronic feeding studies show that only the tips of the villi sustain damage from captan exposure. No damage occurs to cells further down the villi or to the stem cells within the crypts and there is no indication that captan or its breakdown products bind to DNA or cause chromosomal aberrations in the stem cells (Chidiac & Goldberg, 1987).

### C. Experimental Support for Proposed Mode of Action

Numerous studies have been conducted in attempts to elucidate the mode of action through which captan induces tumor formation in mice and to clarify the chain of key causal events involved.

In a long-term study (Pavkov, 1985), male mice were exposed to 6000 ppm captan via the diet for periods of from 3-20 months the principal treatment-related effects were histopathological changes in the first few cm of the duodenum at all sacrifice intervals. Diffuse and focal epithelial hyperplasia was seen in captan-treated animals at all sacrifice intervals, the focal hyperplasia increasing with animal age and exposure duration. Focal epithelial hyperplasia was also seen in control animals (15-20% incidence) but was more widely distributed throughout the small intestine. Special stains confirmed that the diffuse and focal hyperplasias were a result of proliferation of the crypt columnar epithelial cells. All of the adenomas and adenocarcinomas of the duodenum also occurred in the proximal region (7 cm) of this organ and appeared to arise from the crypt epithelial cells. In animals given a period of recovery (6-12 months) after treatment, the

incidence of focal epithelial hyperplasia significantly decreased until it was comparable to that seen in concurrent chronically treated controls.

In two essentially identical 56-day study (Foster, 1994; Tinston, 1995), male and female CD1 mice were exposed to captan at dietary levels of 0, 400, 800, 3000 or 6000 ppm. On the day of sacrifice, the mice were given a single intraperitoneal dose of bromodeoxyuridine (BrdU) as a marker for cell proliferation in the intestine. The duodenum was evaluated for histological changes, for the average number of cells in the crypt cell population, for BrdU labeling index (% cells labeled with BrdU as a ratio to the total number of cells in the crypts) and for the ratio of villus height to crypt depth. Additionally, the stomach, jejunum and ileum were evaluated for histopathological changes only. The results of each study were essentially the same. Exposure to 3000 ppm and 6000 ppm captan resulted in moderate to marked crypt cell hyperplasia, an increase in the crypt cell BrdU labeling index (a measure of mitotic activity), and concomitant shortening of the associated villi (decreased villus-crypt height ratio). These findings were limited to the 7 cm of the duodenum proximal to the pylorus of the stomach. Increased inflammatory cell infiltrate (primarily mononuclear cells) was present in the lamina propria within the same defined area as the hyperplasia. Diffuse crypt cell hyperplasia (females only) and an increased number of crypt cells (both sexes) was also seen at 800 ppm but no increase in BrdU labeling was not noted for this group. Based on this study, the NOAEL for duodenal effects was at least 400 ppm for male and female mice.

Allen (1994) fed male CD1 mice diets containing 0 or 6000 ppm captan for 28, 56 or 90 days. Histologic changes seen in the first 7 cm of the duodenum at all sacrifice intervals consisted of marked crypt cell hyperplasia (initially diffuse and later focal) with atrophy of the villi. A marked increase in the number of mitotic figures was noted in the hyperplastic crypts. PCNA labeling index was increased in the crypt cell population of the proximal duodenum and the average number of cells per duodenal crypt was increased in mice at all sacrifice intervals. Villus-to-crypt height ratio showed biologically significant reductions in mice at all time periods. Inflammatory cell infiltrate, consisting mostly of mononuclear cells, was present in the lamina propria in the region of the duodenum showing diffuse crypt cell hyperplasia. The effect was most prominent at day 29.

To better define the time course for the development of histopathologic changes in the intestine, 25 male CD1 mice were fed diet containing 3000 ppm captan for 28 days (Tinston, 1996). Five mice from each group were sacrificed after 1, 3, 7, 14 and 28 days of the study. Duodenal changes consisting of crypt cell hyperplasia (4/5 mice), shortening of the villi (3/5 mice) and general disorganization of the villus enterocytes (2/5 mice) were detected in captan-treated mice after only three days; mice sacrificed after 7 days showed immature cells at the villus tips (5/5 mice). All of the duodenal findings were also seen in the mice sacrificed after 14 and 28 days of exposure. Table 8 clearly shows the rapid onset of these non-neoplastic effects, with crypt cell hyperplasia occurring in most animals within three days of feeding followed almost immediately by a shortening of the villi, disorganization of the villus enterocytes and the appearance of

immature cells at the villus tips. In addition to these effects, increased inflammatory cell infiltrate, was commonly noted in the lamina propria within the region of crypt cell hyperplasia.

**TABLE 8.**

Appearance of Non-Neoplastic Microscopic Effects in Mice Fed Diets Containing 3000 ppm Captan[1]

| Effect | Day | Day 3 | Day 7 | Day 14 |
|---|---|---|---|---|
| Crypt cell hyperplasia | 0/5 | | 5/5 | 5/5 |
| Shortening of villi | 0/5 | | 5/5 | 5/5 |
| Disorganization of Villus enterocytes | 0/5 | 2/5 | 5/5 | 5/5 |
| Immature cells at villus Tips | 0/5 | 0/5 | 5/5 | 5/5 |

[1] Data from Tinston (1996)

D. Causality Between Key Events and Tumor Formation

Tissue Localization:

All key non-neoplastic precursor effects – hyperplasia, increased number of crypt cells, shortening of the villi (indicative of inflammation and cytotoxicity) – are only observed in the same section (proximal 7 cm) of the duodenum. Special stains confirmed that the diffuse and focal hyperplasias are a result of proliferation of the crypt columnar epithelial cells (Pavkov, 1985). Similarly, all of the adenomas and adenocarcinomas occur in the same proximal 7 cm of the duodenum and appear to arise from the crypt epithelial cells. This observation is consistent with the pathologists' interpretations that the tumors seen in both the NCI (1977) and the Daly & Knezevich (1983) studies are formed by an irritation mechanism and that the characteristics of the lesions represent a continuous spectrum of change exacerbated by captan administration. It is probable that this specific location reflects the increasing pH of the intestinal lumen (relative to the stomach) that will cause the rapid breakdown of captan to inflammatory cytotoxic products.

The literature indicates that there are a small number of spontaneously-transformed cells in the duodenum that ultimately lead to tumor formation. This is supported by the observation that a low incidence of duodenal tumors is seen in bioassay and historical control mice (Bomhard & Mohr, 1989; Chandra & Firth, 1992). In the case of control mice, however, focal hyperplasias (and possibly tumors) are more widely distributed and not restricted to the proximal portion of the duodenum as with those induced by captan (Pavkov, 1985).

It is highly unlikely that the tumors originate from the newly formed epidermal cells migrating up the villi since these are shed into the intestinal lumen within 3 to 5 days of initial cell division within the crypt. These migrating cells are at very low risk from mutagenic transformations that would result in a carcinogenic response because the rapid transit time and elimination of "damaged" cells along with "normal" cells naturally precludes the amplification of cells containing DNA errors. Potten (1984) compared this natural turnover of epidermal cells to a rapidly moving escalator that would not permit either normal or abnormal cells to stop their rate of turnover. Thus, even if these cells sustained a spontaneous "initiating" event during transit, they are too short-lived to be considered as viable targets for carcinogenesis.

The only cells that remain within the crypt for any extended time are the stem cells. These are the only cells for which genetic changes could persist for any duration. As noted previously, the stem cells are located near bottom of the crypt above the Paneth cells (about the $4^{th}$ position from the bottom).

Dose-Response and Threshold

Table 9 summarizes data from several subchronic feeding studies on the non-neoplastic lesions observed in the proximal duodenum of mice. These results clearly demonstrate that that NOAEL for crypt cell hyperplasia and increased inflammatory cell infiltrate is 400 ppm for females. Both male and female mice also showed a slight, but statistically significant, increase in the number of cells per crypt at 800 ppm (Foster, 1994, Tinston, 1995) although this increase was not accompanied by an increase in crypt cell labeling with BrdU. Increases in crypt cell labeling and decreased size of the villi (along with all other non-neoplastic lesions) were observed in mice fed diets containing 3000 ppm captan.

The dose-response data for the neoplastic effects of captan in mice are quite consistent with the occurrence of the non-neoplastic lesions, especially hyperplasia. Thus, NOAEL values for adenomas and adenocarcinomas in mice following chronic dietary exposure to captan are 400 ppm in females considerably higher in males (Table 1).

The non-neoplastic effects of captan in the duodenum of male and female mice strongly suggest a probable role for crypt cell hyperplasia as a key precursor to the observed tumorigenic response. No treatment-related duodenal tumors are seen in male mice chronically exposed to 800 ppm, a dose that does not induce crypt cell hyperplasia. Conversely, an increased incidence of duodenal tumors is seen in the female mice at 800 ppm, a dose that consistently induces crypt cell hyperplasia. These results strongly support a conclusion that the tumorigenic response is directly related to crypt cell hyperplasia. Another important conclusion that can be reached with respect to both the non-neoplastic and neoplastic lesions induced by captan is that both are clearly threshold-based effects.

## TABLE 9

### Dose-Response for Occurrence of Captan-Induced Non-Neoplastic Lesions In Mouse Duodenum

**DOSE LEVELS AT WHICH INDICATED NON-NEOPLASTIC LESIONS OCCUR[1]**

| Non-Neoplastic Lesion | 400 ppm | 800 ppm | 3000 ppm | 6000 ppm | Ref. |
|---|---|---|---|---|---|
| Focal Epithelial Hyperplasia | | | | Positive | Pavkov (1985) |
| Crypt Cell Hyperplasia | Negative | Positive, females only | Positive | Positive | Foster (1994) |
| Increased Crypt Cell Labelling | Negative | Negative | Positive | Positive | |
| Increased # Cells/Crypt | Negative | Positive | Positive | Positive | |
| Decreased Crypt:Villus Ratio | Negative | Negative | Positive | Positive | |
| Increased Inflammatory Cell Infiltrate in the Lamina Propria | Negative | Positive, females only | Positive | Positive | |
| Crypt Cell Hyperplasia | | | | Positive | Allen (1994) |
| Atrophy of Villi | | | | Positive | |
| Increased Crypt Cell Labelling | | | | Positive | |
| Increased # Cells/Crypt | | | | Positive | |
| Decreased Crypt:Villus Ratio | | | | Positive | |
| Increased Inflammatory Cell Infiltrate in the Lamina Propria | | | | Positive | |
| Crypt Cell Hyperplasia | Negative | Positive, females only | Positive | Positive | Tinston (1995) |
| Increased Crypt Cell Labelling | Negative | Negative | Positive | Positive | |
| Increased # Cells/Crypt | Negative | Positive | Positive | Positive | |
| Decreased Crypt:Villus Ratio | Negative | Negative | Positive | Positive | |
| Increased Inflammatory Cell Infiltrate in the Lamina Propria | Negative | Positive, females only | Positive | Positive | |
| Crypt Cell Hyperplasia | | | | Positive | Tinston (1996) |
| Shortened Villi | | | | Positive | |
| Disorganization of the Villus Enterocytes | | | | Positive | |
| Immature Cells at the Villus Tips | | | | Positive | |

Blank space indicates no observations were made at dose level shown.

Temporal

Several independent studies have demonstrated that the key non-neoplastic event on which tumor formation is proposed to depend, hyperplasia of the proximal duodenum, occurs shortly after the start of exposure and consistently precedes tumor formation.

Reversibility

As indicated earlier, one of the characteristics of modes of neoplasia involving hyperplasia and cell proliferation is that, except in extreme cases, the effects observed should show evidence of reversibility. The extent of the reversibility of captan-induced non-neoplastic and neoplastic lesions in the duodenum was examined by Pavkov (1985) in a study in which male mice were exposed to 6000 ppm captan via the diet for varying periods (e.g., 6, 12, 18 months) after which they were allowed periods of recovery on non-captan-treated diets (e.g., 6/6 = 6 months nontreatment with 6 months recovery or 6/12 = 6 months nontreatment with 12 months recovery).

The results of the study clearly indicated that the incidence of focal epithelial hyperplasia significantly decreased during recovery periods, such that it became comparable to the incidence seen in concurrent controls. The histological appearance of the epithelium in some of the recovery animals, however, differed from the concurrent controls in that, while having much less epithelial hyperplasia, it exhibited muscular hyperplasia and submucosal fibrosis and ectasia of submucosal vessels. These lesions appeared to be resolving foci of focal hyperplasia in which the extent of epithelial repair exceeded the repair of mesenchymal tissues.

When comparable exposure durations were considered, the incidence of benign adenomas in the 6/6 and 12/6 recovery groups exceeded that of the controls sacrificed after 6 or 12 months of test. It is noteworthy that the incidence of malignant adenocarcinomas in the 6/6 recovery group was not different from that of the concurrent control mice (both 0 at 12 months). Since the tumorigenic responses are age-related, however, it is more appropriate to compare the tumor incidence in recovery and continuously treated animals with controls of similar age. When age-matched evaluations were performed, the numbers of mice with nonneoplastic and neoplastic findings were comparable in the 6/12 recovery group and the 18+ month controls. Two malignant adenocarcinomas occurred in the 6/12 recovery animals while one occurred in the untreated controls. This difference is of questionable biological significance.

In summary, the results of the Pavkov (1985) study indicate that cessation of captan exposure results in a marked reduction in the incidence of hyperplasia and malignant tumors with the incidence reverting towards control levels. These results provide further support for the linkage between hyperplasia and tumorigenicity and indicate recovery towards control levels when treatment is discontinued.

## E Biological Plausibility

The proposed mode of action of captan through a mechanism involving prolonged chemical-induced cell proliferation is consistent with our current understanding of the biology of cancer. Indeed, it is completely consonant with the classical two-stage model for skin carcinogenesis that emphasizes the importance of sustained proliferation in the promotion of initiated cells into tumors (Berenblum & Armuth, 1977).

The basis for the proposed mode of action is the increasing body of experimental evidence demonstrating that, while not carcinogenic *per se*, abnormally high cell proliferation, can play a central role in tumor development (i.e., the clonal expansion of spontaneously- or chemically-initiated cells) (Butterworth *et al.*, 1992; Pitot *et al.*, 1991

There are now several examples of chemicals known to induce cancer in various organs by the mechanism of cell proliferation. These include the effects of chloroform and several other compounds on liver (Larson *et al.*, 1994; Solt *et al.*, 1977); butylated hydroxyanisole (BHT), ethyl acrylate, propionic acid and chlorothalonil on rodent forestomach (Nera *et al.*, 1988; Ghanayem *et al.*, 1986; Kroes & Wester, 1986; Wilkinson & Killeen, 1996); sodium saccharin on the bladder (Cohen & Ellwein, 1990) and the induction of α2u-globulin-mediated nephropathy by unleaded gasoline or 2,2,4-trimethylpentane (Short *et al.*, 1989; Swenburg *et al.*, 1989). Another example is the induction of thyroid follicular cell hyperplasia and neoplasia by compounds such as ethylene thiourea that decrease circulating levels of thyroid hormones and stimulate the thyroid to synthesize more hormone (Chhabra *et al.*, 1989; Hill *et al.*, 1989). All of these chemicals cause marked biochemical and/or pathological changes that occur soon after the start of exposure and eventually lead to cell hyperplasia and cell proliferation. Cell proliferation may result from cell regeneration following prolonged irritation, inflammation and cytotoxicity or, in the case of the thyroid follicular cells, from the continuous over stimulation of endocrine tissues.

In addition to promoting the clonal expansion of nascent tumor cells *in situ*, abnormally high proliferation may: 1) increase fixation and expression of premutagenic DNA lesions; 2) increase the number of spontaneously initiated cells during replication; 3) perturb checkpoints in the cell cycle, leading to mutagenic events; and 4) increase the number of spontaneously initiated cells by blocking cell death/elimination. Thus, there are two avenues for duodenal tumors to develop; promotion of the nascent tumor cells and initiation of normal basal cells through disruptions in normal DNA replication.

In its most simplistic form, the proposed mode of action of captan involves two major sequential events (Allen, 1994; Foster, 1994). First, epithelial cells that comprise the villi in the proximal duodenum are damaged by exposure to captan or its breakdown products and are sloughed off into the intestinal lumen at an increased rate. As a result of this event, the height of the villi is reduced. Second, basal stem cells in the crypt compartment that normally divide at a rate commensurate with their need to replace cells normally lost from the tips of the villi, increase their rate of proliferation to a

hyperphysiological level and become hyperplastic. Over a prolonged period of time, the basal cell hyperplasia will lead to errors in replication or will decrease the capacity of the stem cells to repair spontaneous DNA damage. The proposed sequence of events described above are illustrated diagrammatically in Figure 6 and Figure 7 is a photomicrograph of the duodenal tissues from normal and captan treated CD1 mice.

**Figure 6. Mode of Action for Captan in the Mouse Duodenum.**

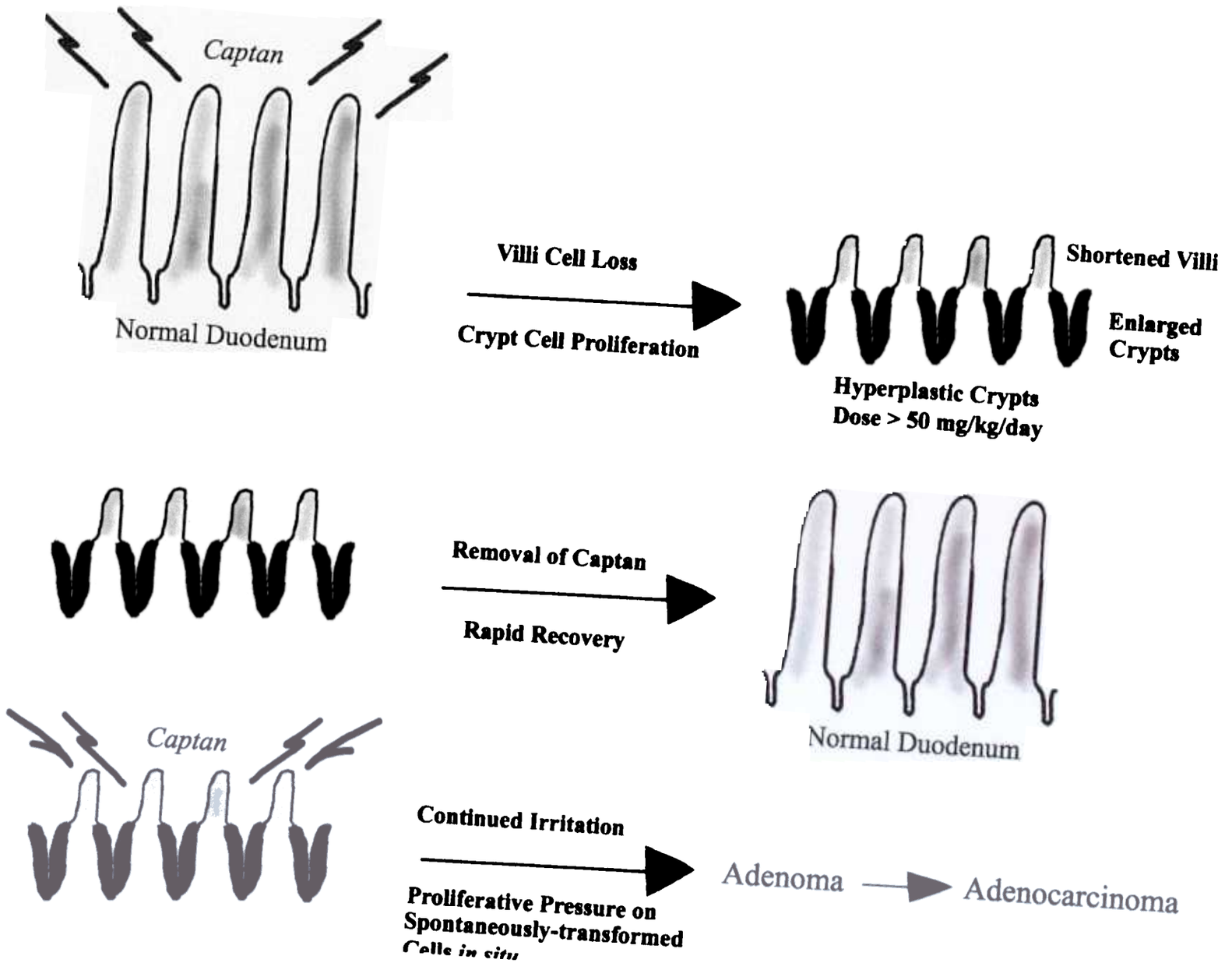
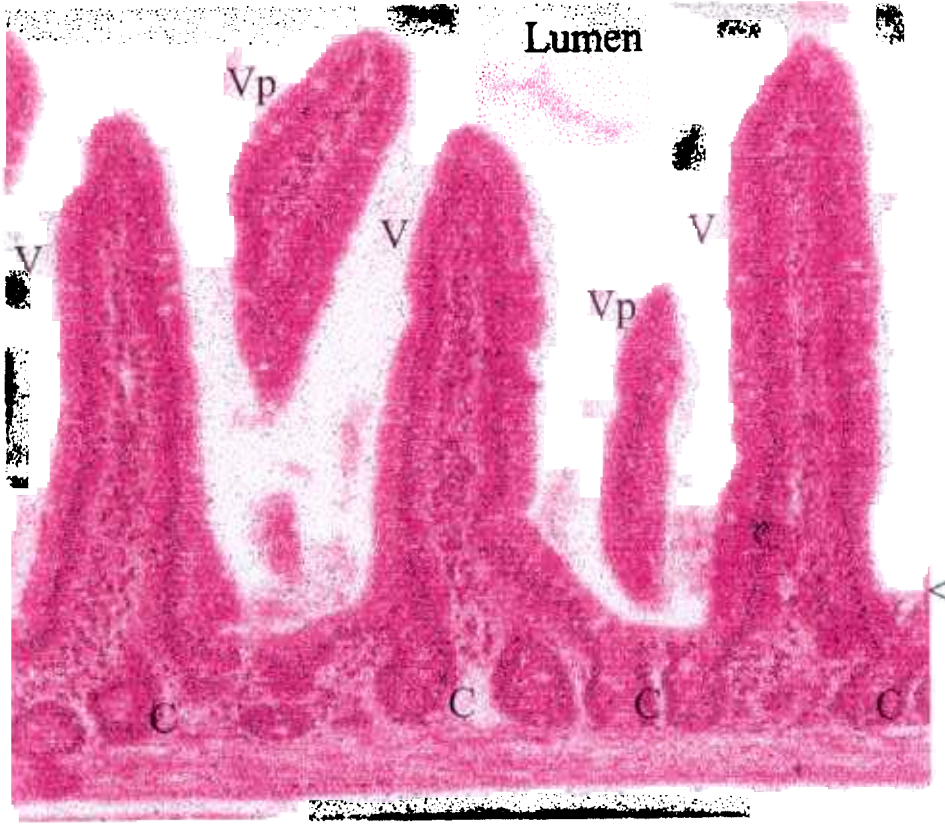


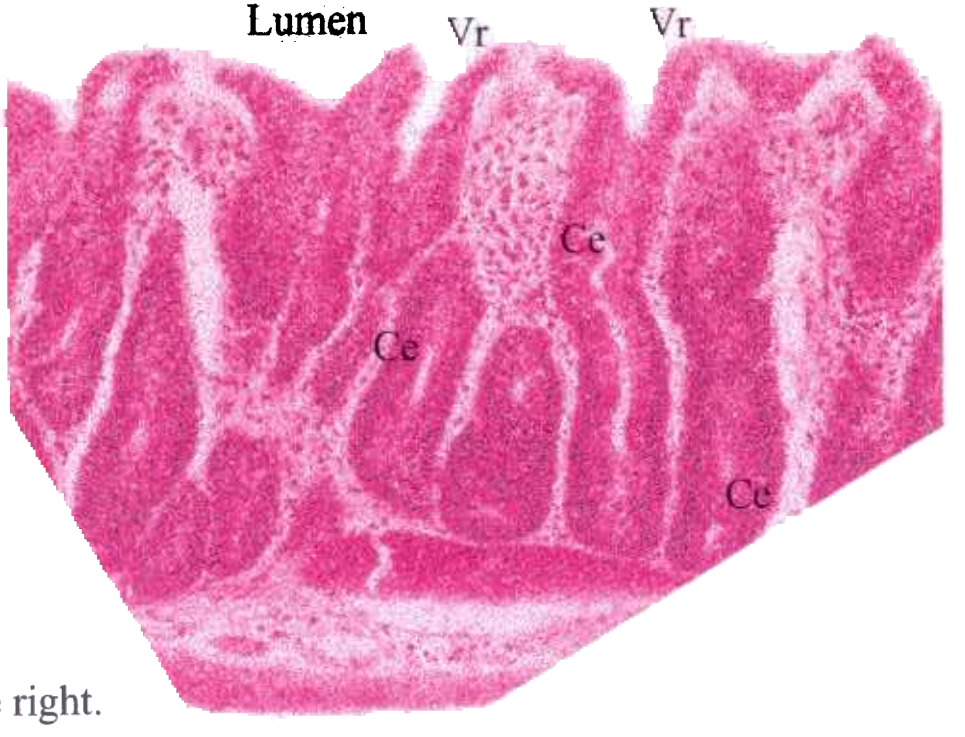
**Figure 7. Micrographs of Normal and Captan Treated Mouse Duodenum.**

Control Duodenum.




Captan Treated Duodenum.
6000 ppm Captan for 28 Days.
Hematoxylin and Eosin Stain.

Note tall villi of control duodenum at left are severely shortened in the captan treated duodenum below.




Vb

V = Villus
Vp = Villus (partial view)
Vr = Villus Remnant
Vb = Base of Villi

C = Crypt
Ce = Crypt Enlarged

Note small crypts of control duodenum above are greatly enlarged in captan treated duodenum to the right.

## F. Relevance of Proposed Mode of Action to Humans

Since apparently mice are susceptible to the duodenal effects of captan and rats are not, different mammalian species can be expected to show considerable variation with regard to their responses to captan. The reasons for these differences are not clear. In view of the general similarities in the morphology and physiology of the intestinal tract, however, there is no reason why the proposed mode of action and human response to captan should be inherently different from that of other mammals. If the assumption is made that humans are as susceptible as mice, and if humans were to be subject to a similar level of exposure to captan as the mice in the chronic bioassay, it is possible that induction of tumors would result. Based on the bioassay results with the mice, however, dose levels of at least 900 mg/kg/day and 120 mg/kg/day for a lifetime would be required to elicit an effect an effect in males and females, respectively. These equate to the highly unlikely *lifetime daily intakes* for a 70 kg male and a 60 kg female of about 63 g/day and 7 g/day, respectively.

Finally, there is no evidence to suggest children or any other human population subgroup would be any more susceptible than healthy adults.

## G. Other Possible Modes of Action

The only viable alternative to the mode of action proposed here is that captan or its reactive breakdown products, especially thiophosgene, could induce tumors through direct genotoxic action on the stem cells in the duodenal crypts.

Although, as discussed, captan is mutagenic in *in vitro* test systems, the total weight of evidence clearly shows it to be nongenotoxic in *in vivo* assays. The primary reason for the lack of genotoxicity *in vivo* is that captan and its products are extremely labile and rapidly bind to a variety of nucleophiles in the blood or tissues before they can reach the DNA. When captan reaches the duodenum following oral ingestion, it will rapidly break down in the alkaline environment of the lumen and the breakdown products will come intro contact with the epithelial cells of the villi. Whether or not the reactive species penetrate the epithelial cells or even whether they reach the DNA is of little importance because these cells are continuously sloughed off into the lumen and are not targets of concern for tumor formation. The cellular replacement time for epithelial cells in the villi is approximately 2 days in rats, 3 days in mice and 3 to 4 days in humans.
The stem cells located deep within the duodenal crypt are the only type of cell that could undergo a mutagenic event and be transformed to a carcinogenic state, since they are the only cell type with sufficient longevity for neoplasia to develop. Because of their location near the bottom of the crypt, however, stem cells are well protected from materials in the lumen of the duodenum and from the active species released during captan breakdown. It is highly unlikely that any of these active species ever reach the stem cells. In view of the rapid breakdown of captan in blood (half-life <1 second), it is equally unlikely that any genotoxic captan-derived products will be transported to the stem cells in the circulation following absorption from the GI tract.

As discussed earlier (Section VI.B and C), there is no evidence that oral administration of captan induces nuclear aberrations in duodenal stem cells in mice and no evidence that $^{35}S$-labeled captan binds to duodenal DNA. A low level of tissue binding was observed but this was interpreted as $^{35}S$ exchange with the endogenous thiol pool. This was supported by the fact that cesium chloride gradient ultracentifugation did not clearly establish that binding was associated with the DNA.

Based on the experimental evidence available, it is concluded that neither captan nor its breakdown products bind to or cause genetic aberrations with the DNA of the duodenal stem cells.

## VIII. WEIGHT OF EVIDENCE SUMMARY

### A Neoplastic Effects

Captan was evaluated for oncogenic potential in three chronic dietary studies in mice. These studies demonstrated that chronic dietary exposure of mice to captan results in an increased incidence of adenomas and adenocarcinomas in the duodenum. These tumors are consistently observed in both sexes at dietary levels of 6000 ppm (900 mg/kg/day) and greater and, in one study, a statistically significant increase in duodenal adenomas was seen in females exposed to a dietary level of 800 ppm (120 mg/kg/day). Chronic dietary exposure of male mice at dose levels up to and including 120 mg/kg/day and female mice up to and including 60 mg/kg/day did not result in an increase in duodenal tumors. These data indicated a clear threshold for the tumorigenic response in mice.

Four chronic bioassays have also been conducted with rats. Two of these showed no increased incidence of tumors up to and including dietary levels of 10000 and 6050 ppm (about 500 and 300 mg/kg/day), respectively. The two remaining studies, one with Charles River CD rats and the other with the Wistar strain, were equivocal with respect to slightly increased incidences of renal tumors (males only) and uterine sarcomas (females), respectively.

The increased kidney adenomas were seen only in males in only one of the four rat studies. Furthermore, the small increase in renal tumors (all benign) was not statistically significant when analyzed by the correct methodology and was only marginally different from historical control values. The small numerical increase in uterine sarcomas seen in another study (again in only one of the four conducted) was only significant when different tumor types were inappropriately combined. A more appropriate analysis of combined sarcomas and polyps shows no treatment-related effect on tumor incidence.

Based on the results of three mouse and four rat chronic bioassays, it is concluded that the oncogenicity of captan is limited to the formation of duodenal adenomas and adenocarcinomas in mice. There is no evidence that captan is oncogenic in the rat. A limited power epidemiology study involving 410 employees of a captan manufacturing

plant in the U.S. showed no evidence of increased duodenal cancer or other oncogenic effects.

### B Genotoxicity

Although captan induces mutagenic effects *in vitro* in bacterial and eukaryotic cell test systems where it has ready access to DNA, the overwhelming weight of evidence indicates that it is not genotoxic when administered to intact animals *in vivo*. The primary reason for the lack of genotoxic activity *in vivo* is that captan is rapidly degraded by hydrolysis or reaction with tissue nucleophiles, especially thiols such as cysteine or glutathione, and any reactive species formed are quickly inactivated before they can reach DNA.

Consequently, there is no evidence that captan or its breakdown products reach the stem cells deep within the crypts of the villi of the duodenum. There is a similar lack of evidence that any captan-derived species bind to DNA or cause chromosomal aberrations in the duodenal stem cells following oral administration.

### C Non-Neoplastic Effects

Pathology evaluations of the duodenal tissues from the mouse bioassays clearly show a number of non-neoplastic lesions (inflammation, cytotoxicity, hyperplasia, increased cell proliferation) in the duodenal crypts. All of the tumors are found in the same area of the proximal duodenum in which hyperplasia occurs and appear to arise from the same crypt cells. The close association observed between tumorigenicity and hyperplasia leads directly to the proposal (Section VII.D ) that the tumor response is secondary to inflammation and cytotoxicity of the duodenal mucosa, which in turn leads to increased hyperplasia and cell proliferation of the stem cells in the duodenal crypts. Inflammation and cytotoxicity, the initial stages of the process, are clearly indicated by a shortening of the villi, a general disorganization of the villus enterocytes and increased inflammatory cell infiltrate in the lamina propria. The proposed mode of action is supported by mechanistic studies that establish a strong causal linkage (dose-response, thresholds, temporal association, reversibility).

### D Overall Process of Captan Tumorigenicity

The proposed sequence of events in the carcinogenic process is as follows

Following oral ingestion, captan is rapidly hydrolyzed in the alkaline environment of the proximal duodenum forming primarily tetrahydrophthalimide (THPI) and thiophosgene;

2 Captan and thiophosgene, both strong chemical irritants, cause inflammation, cytotoxicity and necrosis of the epithelial cells of the villi in the proximal portion of the duodenum;

3. Cytotoxicity causes the cells to be sloughed off the tips of the villi at a faster rate than normal, resulting in a shortening of the height of the villi;
4. The enhanced cell loss in the villi causes an increase in crypt cell proliferation and regenerative hyperplasia in the stem cells from which the epithelial cells are derived;
5 Prolonged hyperplasia in the stem cells overwhelms their capacity to repair spontaneously damaged DNA and increases the probability of cloning a transformed cell; and
6. The increased cloning of cells containing damaged DNA leads to an increased incidence of duodenal adenomas and adenocarcinomas.

## E Weight of Evidence Characterization

Under the 1986 EPA Guidelines for Carcinogen Risk Assessment (USEPA, 1986), captan was classified as a Group B2 *probable human carcinogen* based primarily on evidence available from animal bioassays. The EPA's position is that (USEPA, 1999):

> *In chronic studies, captan causes cancer in mice and rats. The Agency has classified captan as a B2 (probable human) carcinogen based on an increased incidence of intestinal tumors in mice. It also caused an increased incidence of renal neoplasms in male Charles River CD rats and an increased incidence of uterine sarcomas in Wistar rats. A Q1* approach is used for cancer risk assessment.*

Under the new Guidelines for Carcinogen Risk Assessment (USEPA, 2003), captan is *likely to carcinogenic to humans only following prolonged, high-level oral exposures causing inflammation and regenerative hyperplasia in the proximal region of the duodenum*. Captan is *not likely to be a duodenal carcinogen in humans at dose levels that do not cause cytotoxicity and cell regeneration in the intestine*. Furthermore, because of its extreme lability in blood (half-life <1 second), *captan is not likely to be carcinogenic to humans in other organs/tissues following dermal or inhalation exposures at any dose level/concentration.*

This weight of evidence conclusion is based on: 1) observations that *sustained cytotoxicity and regenerative hyperplasia always precede, and are a pre-requisite for, the tumorigenic action of captan* in the proximal duodenum of mice; and 2) strong evidence that, although captan is mutagenic in *in vitro* tests, it is not genotoxic *in vivo* because of its extremely rapid inactivation by thiols (e.g., glutathione, cysteine) and other nucleophiles. Consequently, *it is highly unlikely that captan exerts its tumorigenic activity through a genotoxic mode of action*. Any tumorigenic effects of captan following absorption and transport in the circulation are highly unlikely because of the almost instantaneous inactivation of captan in blood.

## IX. CONCLUSIONS

**1. The weight of evidence from animal bioassays indicates that captan's tumorigenic potential is restricted to one tumor type in a single animal species.** Prolonged ingestion of high dose levels of captan causes an increased incidence of crypt cell tumors (adenomas and adenocarcinomas) in the proximal portion (7 cm) of the duodenum in both sexes of mice. Tumors are observed in females only at dietary levels of at least 800 ppm (120 mg/kg/day) and in males at levels of at least 6000 ppm (900 mg/kg/day) that exceed the maximum tolerated dose. In all studies, the tumorigenic response exhibits a clear dose threshold below which no effect occurs. A careful evaluation of the results of the rat bioassays provides no evidence that captan is associated with increased incidences of either renal tumors in males or of uterine sarcomas in females. An epidemiology study of limited power involving 410 employees of a captan manufacturing plant in the U.S. provided no evidence of increased duodenal cancer or other oncogenic effects.

**2. The overall weight of evidence clearly indicates that captan is not genotoxic in intact animals.** Captan is mutagenic when measured in *in vitro* test systems (bacterial or eukaryotic cells) where it has ready access to the DNA. *In vitro*, however, mutagenic activity is eliminated, or substantially decreased, in the presence of protein or thiols that rapidly deactivate potentially genotoxic captan-derived species. The lack of activity *in vivo* similarly results from the rapid deactivation of captan-derived species by reaction with a variety of nucleophilic functional groups (e.g., thiols) present in blood and tissues. Consequently, neither captan nor its breakdown products reach the duodenal stem cells and are unable to bind to DNA or cause chromosomal aberrations in these cells. Furthermore, the rapid breakdown of captan in blood (half-life <1 second) precludes the possibility that it can be transported to other tissues in the circulation following oral or dermal administration.

**3. Prolonged oral ingestion of captan by mice is also associated with several non-neoplastic effects (hyperplasia, crypt cell proliferation, inflammation, cytotoxicity and erosion of the villi)** that are observed in the same proximal region (7 cm) of the duodenum where tumors are formed. These responses show clear dose thresholds similar to those observed for tumor formation and are reversible following cessation of captan exposure.

**4. A nongenotoxic mode of action for captan is proposed in which the tumors are a secondary consequence of a cascade of non-neoplastic events.** The proposed sequence of events is initiated by inflammation, cytotoxicity and increased loss of the epithelial cells in the villi and this is followed by increased regenerative cell proliferation and hyperplasia of the stem cells in the duodenal crypts. Over a prolonged period of time the hyperplastic state leads to neoplasia through a process whereby spontaneously initiated cells are cloned before DNA damage can be repaired. There is a strong causal association indicating that tumor formation is secondary to duodenal irritation and hyperplasia and that the latter is a key event in the sequential cascade of events leading to cancer.

**5. The overall weight of evidence strongly suggests that captan induces adenomas and adenocarcinomas in the duodenum of the mouse by a non-genotoxic mode of action involving cytotoxicity and regenerative cell hyperplasia that exhibit a clear dose threshold.**

**6. Based on the new Guidelines for Carcinogen Risk Assessment, EPA's current B2 (probable human) carcinogen classification for captan is inappropriate. Under the descriptors defined in the new guidelines, it is proposed that captan should be classified as:**

- ***likely to carcinogenic to humans following prolonged, high-level oral exposures causing cytotoxicity and regenerative cell hyperplasia in the proximal region of the duodenum*;**
  ***not likely to be a human carcinogen at dose levels that do not cause cytotoxicity and regenerative cell hyperplasia in the intestine*; *and***
  ***not likely to be carcinogenic to humans in other organs/tissues or following dermal or inhalation exposure.***

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