Study Title

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

Guideline Requirement

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Author

Chris F. Wilkinson, PhD
Gail Arce, PhD
Elliot B. Gordon, PhD, DABT

Sponsor

CAPTAN TASK FORCE

Arvesta Corporation
100 First Street, Suite 1700
San Francisco, CA 94105

and

Makhteshim-Agan of North America, Inc.
551 Fifth Avenue, Suite 1100
New York, NY 10176

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B) or (C).

Company: Captan Task Force

Company Agent: Elliot B. Gordon, PhD, DABT

Title: Corporate Toxicologist, Makhteshim-Agan of North America Inc.

Signature:  Date: 13 JAN 2004

GOOD LABORATORY PRACTICE STATEMENT

This study was not conducted according to the Environmental Protection Agency Good Laboratory Practices Standards (40 CFR 160).

Sponsor: _____ Date: 13 Jan 2004

Elliot B, Gordon, PhD, DABT
Makhteshim-Agan of North America Inc.

Agent For: Captan Task Force

Submitter: _____ Date: 13 Jan 2004

Elliot B. Gordon, PhD, DABT
Makhteshim-Agan of North America Inc.

TABLE OF CONTENTS

APPENDIX

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 Since the current classification of captan is based on the earlier, now outdated 1986 guidelines, the EPA agreed to a request from the Captan Task Force (CTF) to have the classification of captan reevaluated in accordance with the 2003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 reached:

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 tumors (adenomas and adenocarcinomas) in the small intestine (primarily the proximal portion 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 mortality from cancer.

2. The overall weight of evidence indicates that captan is not genotoxic in intact animals. Captan is weakly mutagenic when measured in *in vitro* test systems (bacterial or eukaryotic cells) where it has ready access to the DNA or other DNA enzymatic processes. *In vitro* mutagenic activity is eliminated, or substantially decreased, in the presence of protein or thiols that rapidly deactivate captan or its derivatives. 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 cause DNA damage or chromosomal aberrations in these cells. Furthermore, the rapid breakdown of captan in blood (half-life less than one 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 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 or cells damaged during replication are cloned before DNA damage can be repaired. There is a strong causal association (dose-response, temporality) indicating that tumor formation is secondary to cytotoxicity 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:

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

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. Literature searches were performed using Medline, Toxnet and pesticide registration databases and documents.

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 followed the initial registration, the following being points of relevance to the current discussion:

- 1980

- EPA published the Rebuttable Presumption Against Registration or RPAR (USEPA, 1980). The RPAR concluded that captan was a rodent carcinogen and the Agency initiated an extensive regulatory evaluation.

- 1985
  - Captan Registrants formed a Task Force – the 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.
  - The Agency issued a public notice submission of rebuttals and other information on the RPAR and affords the opportunity to submit any other data on the risks and benefits of captan.
  - EPA issued the captan Data Call-In (DCI) and captan Special Review Position Document 2/3 (PD 2/3) (USEPA, 1985). PD 2/3 expressed concern with respect to 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).
  - The Agency reassessed the carcinogen classification of captan and maintained the 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 the Re-registration Eligibility Decision Document (USEPA, 1995).
    - The captan classification as a B2 carcinogen was retained.
    - A Q1*, calculated as 3.6×10^{-3}, was based on the adenomas and carcinomas of the duodenum and jejunum-ileum seen in 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 a 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 has been received.

- 1999
  - The Agency issued final RED (USEPA, 1999).

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

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

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

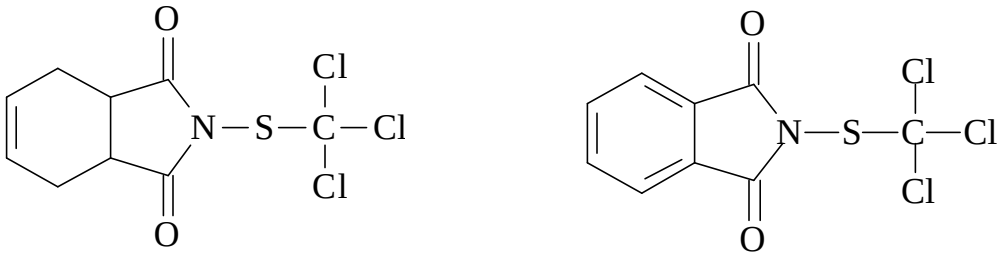
- 2002
  - EPA agreed in principal to conduct re-evaluation using an independent Third Party approach.

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:	178ºC
Water solubility:	3.3 mg/L at 25ºC
Acetone solubility:	3.0g/100 ml
Log K_{OW}:	2.35

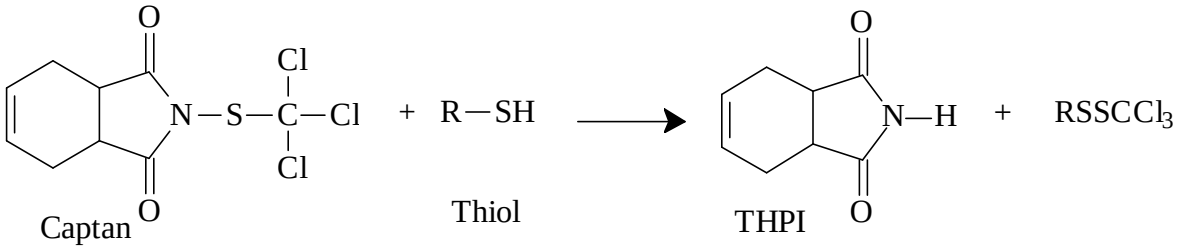
Figure 1. Structure of Captan and Folpet



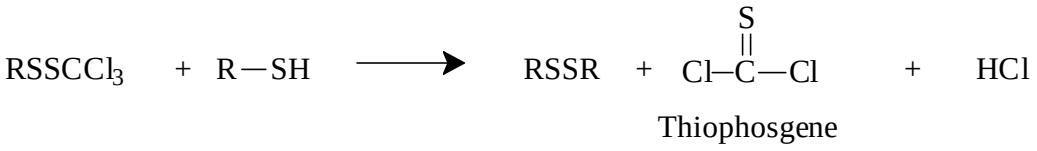
Captan Folpet

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 22ºC (Crossley, 1967a,b) and 0.97 sec at 38ºC (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.



Captan Thiol THPI



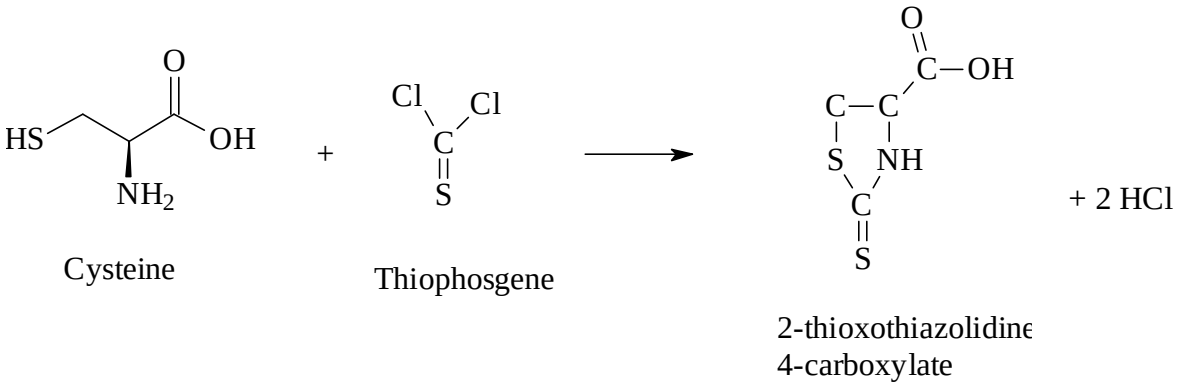
Thiophosgene

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

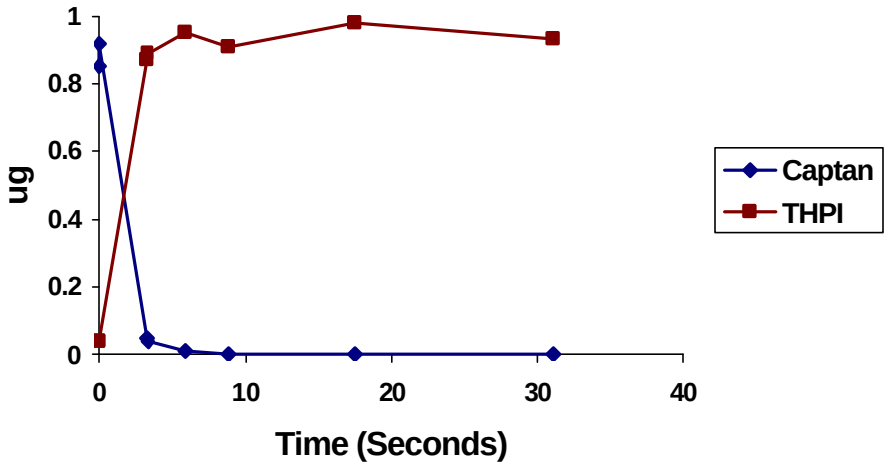
The metabolic fate of captan has been reviewed by Edward *et al.* (1991), Hayes (1991), Gordon *et al*. (2001), HSDB (2001), and Trochimowicz *et al*. (2001). When ingested, captan is relatively stable in the acidic environment of the stomach. However, it is rapidly hydrolyzed in the alkaline environment of the duodenum to THPI and thiophosgene. As discussed earlier, the latter metabolite is highly reactive and, therefore, short-lived. Captan can also 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 concentrations 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. *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) (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



Single oral bolus doses of captan result in an increase in GSH in the duodenum. This increase is seen two hours after administration and returned to normal ten days later (Katz *et al.*, 1982). Continued administration of captan in the feed results in a higher sustained GSH level (Miaullis *et al.*, 1980).

More rigorous studies have been conducted with folpet (Figure 1). Since folpet shares a common mechanism of toxicity with captan with regard to duodenal tumor induction, these data help to elucidate the effect of folpet and subsequently captan on GSH. Folpet was administered as a bolus dose at 7.6, 72, and 668 mg/kg to CD1 male mice and GSH levels were measured in the stomach, duodenum, jejunum, ileum and liver ranging from 0.5 to 24 hours post treatment. Depletion of GSH was observed 0.5 hours post-treatment in the mid- and high dosage groups in the duodenum, jejunum and ileum (Table 1). The depletion was the greatest in the duodenum and jejunum. It was still depleted in the duodenum after 1 hour post-exposure. No changes were observed in either the stomach or the liver during this time period. After 2 hours, the levels of GSH had rebounded to within control levels in all tissues and at all doses with the exception of the liver. At the mid and high dosages, the liver had statistically lower levels of GSH than controls. Six hours after exposure, the GSH levels statistically significantly increase in the duodenum

beginning at the low dose and increase in a dose responsive manner. Only the mid and high dose groups in the jejunum and ileum are significantly increased. By 24 hours, the mid-dose in all tissues except the duodenum are declining. The high dose groups in these tissues have even higher levels of GSH than at 6 hours. No effects, either increases or decreases in GSH are seen in the stomach at any dose or time period. Interestingly, the liver continues to show decreased levels of GSH at the high dose at 24 hours post-treatment (Chasseaud *et al.*, 1991). This data strongly suggests that captan GSH interaction is occurring in the small intestine. It would also imply that as the folpet or captan derivatives move through the intestinal tract, there is a longer time period for these products to be deactivated by GSH, in particular, and other thiols.

Table 1.
Folpet effect on GSH levels in various organs of the mouse

		Time after dosing (hours)				
Duodenum	Group	0.5	1	2	6	24
	Control	2.7	4.0	3.7	2.5	2.4
	7.6 mg/kg	2.5	2.6	2.5	3.2*	2.5
	72 mg/kg	2.1*	2.1*	2.2	3.9**	3.1**
	668 mg/kg	2.0**	2.1*	2.5	3.6*	4.3**
	Positive [a]	0.7**	1.2**	0.9**	1.9*	3.6*
Jejunum	Group	Time after dosing (hours)				
		0.5	1	2	6	24
	Control	3.1	2.6	2.9	2.7	2.9
	7.6 mg/kg	2.8	2.9	2.6	2.8	3.0
	72 mg/kg	2.5*	2.5	2.9	3.9**	3.6*
	668 mg/kg	2.1**	2.7	2.8	4.0**	6.0**
	Positive [a]	1.7**	2.5	2.6	3.3*	3.4
Ileum	Group	Time after dosing (hours)				
		0.5	1	2	6	24
	Control	2.6	2.4	2.5	2.4	2.5
	7.6 mg/kg	2.4	2.5	2.4	2.3	3.3
	72 mg/kg	2.2	2.2	2.2	3.1**	2.7
	668 mg/kg	2.0*	2.2	2.4	3.2**	3.9**
	Positive [a]	1.8**	2.4	2.4	2.1*	3.0
Stomach	Group	Time after dosing (hours)				
		0.5	1	2	6	24
	Control	5.0	4.4	4.1	3.9	3.8
	7.6 mg/kg	4.7	3.8	4.4	4.4	3.8
	72 mg/kg	3.8	3.6	3.6	4.0	3.8
	668 mg/kg	4.9	3.5	3.8	3.8	3.5
	Positive [a]	1.7**	1.2**	1.6**	1.1**	4.5
Liver	Group	Time after dosing (hours)				
		0.5	1	2	6	24
	Control	8.7	8.3	8.8	6.6	9.2
	7.6 mg/kg	7.8	8.5	7.8	5.9	9.7
	72 mg/kg	7.2	8.4	7.0*	5.9	8.0
	668 mg/kg	8.3	7.2	6.9**	5.7	5.3**
	Positive [a]	3.8**	3.2**	2.4**	2.0**	7.7*

Glutathione concentration is noted as mM. Data are from 5 or 6 animals and are rounded from the original three significant figures. Statistical significance is indicated by *: $p<0.05$; **: $p<0.01$. a: Positive control is diethylmaleate at 600 mg/kg (Chasseaud, Hall & McTigue, 1991).

A study by Provan and Eyton-Jones (1996) determined the distribution of captan and its metabolites from sections of 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 3000 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 (the mice had received non-radiolabeled captan diets for 8 days prior to receiving the radiolabeled material) and radioactivity was measured in the contents and the epithelial tissue of the different segments of the gastrointestinal tract; segments studied were the stomach, duodenum, 10 cm sections of the rest of the small intestine, cecum and secum to anus segment. A low, steady-state concentration of radiolabel was observed along the entire small intestine 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 3/6 mice receiving the 3,000 ppm diets; in those receiving 400 ppm diets, only captan metabolites were found. Consistent with the rapid degradation of captan observed in the stomach, only THPI and its metabolites were found in duodenum, blood and urine.

In summary, following oral ingestion, captan is rapidly degraded in the stomach and although, at high dose levels, some absorption into the epithelial cells cannot entirely be ruled out, this material undoubtedly would be rapidly degraded by reaction with GSH, other thiols or macromolecules. There is no evidence that captan *per se* is absorbed into the systemic circulation and no evidence that either captan or its metabolites accumulate in any tissues over time. Absorption from the gut is in the form of hydrolysis products, mainly THPI, that may be further metabolized to other stable products. Thiophosgene, a reactive, short-lived breakdown product of captan, rapidly reacts with available nucleophiles, especially thiols.

It should be emphasized that studies to determine the pharmacokinetic disposition of captan are confounded by methodological issues relating to high reactivity and short half-lives of captan and its breakdown products. Furthermore, it is extremely difficult to determine the relative roles of enzymatic vs. non-enzymatic (hydrolytic or nucleoplilic) reactions in captan degradation or to identify where the reactions are actually occurring (gut contents, cell surfaces or within the epithelial cells). As a consequence, attempts to correlate patterns of radiolabel binding with target site histopathology have been typically inconclusive.

V. 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). In addition, captan was included in an early NCI program to evaluate the carcinogenic potential of a large number of chemicals (Innes *et al*., 1969). Since no significant increase in tumors was observed in this study in mice receiving gavage doses of 215 mg/kg/day for 3 weeks followed by dietary exposure to 84 mg/kg/day for 18 months, this study will not be further discussed. While not a chronic biassay *per se*, a study by Pavkov (1985) will be discussed in this section, however, because it provides valuable information on the nature, location and progression of both non-neoplastic and neoplastic lesions in the mouse gastrointestinal tract following dietary exposure to captan.

i. 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 the 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. Microscopically, three different types of lesions were recognized with characteristics suggesting they were different stages of the same type of lesion:

- Mucosal hyperplasia defined as a duplication of glands and villi;
- Adenomatous polyps defined as a more accentuated proliferative lesion with glandular structures and villi aggregated and branched around supporting stalks made up of connective tissue with no evidence of malignancy; and
- Adenocarcinoma in adenomatous polyp defined as an advanced and aggressive lesion consisting of cellular polyploid structures with numerous mitotic figures, disorganized microacini and areas of neoplastic infiltration.

The incidence of duodenal adenocarcinoma in adenomatous polyps was 0/69, 1/43 and 3/46 for pooled control, low and high dose males, respectively and 0/69, 0/49 and 3/48 for pooled control, low and high dose females, respectively. The combination of unspecified adenomatous polyps with polyploid carcinomas yielded incidences of 0/69, 3/43 and 5/46 for control, low and high dose males, respectively.

Although hyperplasia of the duodenal mucosa was noted in three high dose males, the design of this study did not allow the observation of non-neoplastic effects since the 11-week recovery period between the end of captan exposure and sacrifice provided ample opportunity for the recovery of reversible effects.

ii. 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 the lack of any effect, 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 on body weight and body weight gain (>20% depression), the high dose (16000 ppm) was considered to have exceeded the Maximum Tolerated Dose (MTD). Animals at this dosage were weak, small, emaciated with distended and puffy abdomens consistent with undernutrition. There was an increased incidence of small intestinal (primarily duodenal) adenomas/polyps and carcinomas at all dose levels (Table 2); a positive dose-related trend for and increased incidence of these tumors was observed for both sexes. Jejunal neoplasms were also evident but at a much lower incidence. In males, a significantly higher incidence ($p<0.05$) of jejunal tumors was observed only in mid-dose animals (7/69, 10%) compared with controls (1%) while, in females, the incidence in treated groups (1-4%) was not significantly different from controls (3%). It should be noted that because the tumors observed in the small intestine were morphologically indistinguishable in regard to their location (duodenum, jejunum or ileum), the small intestine was grossly divided into three segments of equal length (about 10 cm) for histopathologic evaluation. The proximal and distal portions were designated duodenum and ileum, respectively, and the central segment, jejunum. The Wong *et al*. (1981) study indicated a higher number of tumors than the other chronic mouse bioassays because of its long duration (28 months) and the Swiss Roll technique used for sampling intestinal tumors. This technique allows a careful evaluation of the whole intestine rather than sectioning.

A statistically significant ($p<0.01$) increase in duodenal hyperplasia was seen in males and females with combined incidences of 48%, 49% and 38% for the low-, mid- and high-dose groups compared with 6% in the controls. An increased incidence in jejunal hyperplasia was also reported in mid-dose males and females (12%) compared to controls (males 3% and females 1%). 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.

iii. 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 dose 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 2). The incidence of focal hyperplasia of the duodenal mucosa increased compared with controls in both males and females from the 6000 ppm group (14% and 22%, respectively) although a clear dose-response relationship was not evident.

iv. Pavkov (1985)

In a long-term study, Pavkov (1985) analyzed the progression of histological changes in the small intestine of CD1 mice continuously exposed to captan by dietary administration. Male mice were exposed to 6000 ppm captan for 3, 6, 9, 12, 18 or 20 months and some animals were treated for a specified time followed by a recovery period prior to sacrifice. The recovery groups were 6/6 (months treatment/months recovery), 6/12, 12/6 and 12/8. Significant reductions in body weight gain and food consumption were seen in captan-treated mice throughout the study. The principal treatment-related effects were histopathological changes in the small intestine, particularly the initial few cm of the duodenum, at all sacrifice intervals. Diffuse epithelial hyperplasia was seen in captan-treated animals at all sacrifices but was not observed in controls at any time period. The incidence of this finding decreased with time throughout the study – 35%, 40%, 15%, 17% and 14% at 3, 6, 9, 12 and 18+ months, respectively. Focal epithelial hyperplasia was also seen in all treated animals at all sacrifice intervals. The incidence of this finding increased with exposure duration – 75%, 95%, 85%, 94% and 100% at 3, 6, 9, 12 and 18+ months, respectively. Focal hyperplasia usually, though not always, developed within areas of diffuse hyperplasia. Focal hyperplasia was also observed in control animals at 12 months (15%) and 18+ months (19%) but was more widely distributed throughout the small intestine than in the treated animals. In chronically exposed mice 95% of the focal hyperplasia occurred in the proximal 7 cm of the duodenum and 99% in the proximal 14 cm. In controls, only 28% of the focal hyperplasia occurred in the first 7 cm while 83% occurred in the proximal 14 cm. Special stains (hematoxylin and eosin H and E) confirmed that the diffuse and focal hyperplasias occurred as a result of proliferation of the crypt columnar epithelial cells. Similarly, all of the adenomas and adenocarcinomas of the duodenum developed in the proximal 7 cm of this organ and appeared to arise from the crypt epithelial cells. Incidences of neoplasms were 25%, 17%, and 36% after 9, 12, and 18+ months, respectively. In animals given a recovery period after cessation of captan exposure, the incidence of focal hyperplasia decreased considerably to that observed in concurrent controls. At least 6 months continuous exposure to captan is required for tumors to develop. If exposure is stopped prior to this time, tumors do not form.

Pavkov (1985) also noted significant pathologic changes in the stomach after 3 months of captan treatment. The stomach lesions were limited to the non-glandular forestomach and included necrotizing (ulcers and/or erosions) and proliferative (epithelial hyperplasia) changes. This type of proliferative response in rodent forestomach is characteristic of several irritant chemicals (ethyl acrylate, butylated hydroxyanisole, propionic acid, chlorothalonil) (Wilkinson & Killeen, 1996) and when prolonged can lead to neoplasia; the latter does not occur in the case of captan.

TABLE 2.

Incidence of duodenal tumors and hyperplasia 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
Focal hyperplasia	4/91	2/83	7/93	6/87	12/84				(1983)
Adenocarcinomas	0/9					1/43		3/46	NCI (1977)
Duodenal neoplasms	2/74				20/73		21/72	39/75	Wong *et al.*,
Mucosal hyperplasia	3/74				39/73		36/72	24/75	(1981)
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
Focal hyperplasia	11/85	9/82	8/83	13/81	20/91				(1983)
Adenocarcinomas	0/9					0/49		3/48	NCI (1977)
Duodenal neoplasms	2/72				24/78		19/76	29/76	Wong *et al.*,
Mucosal hyperplasia	6/72				33/78		37/76	34/76	(1981)

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

The collective results of the chronic mouse bioassays (Table 2) clearly show increased incidences of tumors (adenomas and adenocarcinomas) in the small intestine (primarily the duodenum) of 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. Two of the bioassays (Wong *et al*., 1981; Daly & Knezevich, 1983) clearly suggest focal hyperplasia of the small intestine as an important pre-neoplastic lesion associated with tumor formation and the general progression of histological changes occurring during chronic dietary exposure to captan is clearly delineated by Pavkov (1985). It is likely that the apparent localization of focal hyperplasia and tumor formation in the proximal region of the duodenum is more a function of the bioavailability of captan-derived material than any special characteristics of this portion of the small intestine. In fact there is no clear transition between different sections of the small intestine and it is not entirely clear where the duodenum ends and the jejunum begins. With increasing dose and/or duration of captan exposure more captan-derived species will be available lower down the small intestine and can be expected to result in hyperplasia and tumor formation in those sections. This is supported by the Wong et al. (1985) study where higher dose and longer duration exposures resulted in hyperplasia and tumors in the jejunum.

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.

i. 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 3). There was no increase in renal cortical/tubular cell neoplasia in females.

TABLE 3.

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**

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 correction for continuity. Both the NCI and NTP recommend that the Cochran-Armitage test be applied with correction for continuity to ensure that rare tumors are not found to be false positives. When this is done, Foster & Elliott (2000) demonstrated that there is no statistically significant trend in the incidence of kidney tumors between the groups.

It seems likely that the kidney tumors observed in this study are due to chance rather than captan exposure since Goldenthal *et al*. (1982) observed no increase in the incidence of focal pre-neoplastic lesions usually associated with chemically induced renal tubular

tumors. While the incidence of nephropathy was comparable between control and treated rats, the high dose animals had a 50% increase in blood urea nitrogen (BUN) compared to controls. This suggests that the high dose group had more rats with end-stage chronic neuropathy that puts them at increased risk for developing tumors. Some strains of rats (Fischer, SD) are so sensitive to this effect that is not of relevance to humans.

Two other modes of action are known to be involved in the development of chemically-induced male rat kidney tumors -- the nephropathies associated with alpha-2u-globulin (Swenberg *et al.,* 1989; Swenberg, 1993) and renal β-lyase (Anders *et al.,* 1992; Dekant *et al*., 1994). Each of these nephropathies results in tubular cell necrosis, regenerative hyperplasia and eventually neoplasia of the renal proximal tubules by a different mechanism. Alpha-2u nephropathy involves tubular necrosis from an accumulation of alpha-2u globulin and lysosomal overload. In contrast, tubular necrosis associated with renal β-lyase is caused by the enzymatic release of reactive thiols from cysteine S-conjugates (premercapturic acids from GSH conjugates) and the subsequent inhibition of mitochondrial respiration. There is no evidence to suggest that captan acts through either of these mechanisms that, in any case, have no relevance to humans.

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 few tumors observed are probably of spontaneous origin since :
  - There is no increase in the incidence of focal pre-neoplastic lesions usually associated with chemically induced renal tubular tumors;
  - There is no evidence that captan is acting through known mechanisms of male rat tumorigenicity (alpha-2u-globulin or renal β-lyase);
  - 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.

ii. Til (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 4.

Foster & Elliott (2000) conducted a detailed analysis of the Til study and concluded that the three types of sarcomas observed are not a homogeneous group of malignant tumors

(stromal sarcomas arise from a different tissue type) and should not have been combined in analyzing for a possible treatment-related effect. Furthermore, stromal

TABLE 4.

Incidence of uterine tumors in female Wistar rats (Til, 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**

sarcomas are known to develop from uterine polyps so that a more appropriate statistical analysis should have been conducted using combined stromal sarcomas with polyps. When this is done, the combined incidence is not statistically significant. Background incidences in the Wistar rat were reported by Eiben & Bomhard (1999) for 59 two year studies conducted at the Bayer AG Institute of Toxicology in Wuppertal Germany that were terminated between 1974 and 1994. The average incidence of benign and malignant adenomas plus carcinomas of the uterus (including the cervix) was 6.5% with a range of 0 to 24%. Benign stromal polyps including those not otherwise specified had an average background incidence of 11.9% with a range of 0 to 45%.

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.

It is concluded that the uterine tumors observed in this study are not likely to be treatment related.

For the sake of completeness, it is necessary to mention the review of Reuber (1989), that concluded 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.

It is not known why, unlike mice, rats do not develop tumors of the small intestine following chronic dietary exposure to captan. It is possible that the mouse has a higher background incidence of tumor formation than the rat because there are more pre-initiated cells in the small intestine. Alternatively, the difference may result simply from the fact that the mice are receiving a higher dose of captan per unit of body weight. Rats are more susceptible to the toxicity of captan (lower MTD) and the lower dose levels necessitated by this are insufficient for the development of intestinal tumors.

A comprehensive comparative study was conducted in which folpet, a fungicide that shares a common mechanism of toxicity with captan, was administrated to both rats and mice. An array of endpoints was evaluated. A number of endpoints varied quantitatively between the species, but there was no qualitative "smoky gun" that would unequivocally explain why mice were sensitive and rats were refractory (Chasseaud *et al.,* 1991).

C. Other Routes of Exposure

Captan-induced duodenal tumors are not only specific for the mouse but also appear to be restricted to oral exposure. There is no evidence that the tumors arise as a result of systemic effects occurring after oral, dermal, or inhalation exposures. Instead, they appear to result specifically from persistent local contact of the duodenal epithelium with captan-derived materials passing through the GI tract.

Dermal: The knowledge that captan degrades extremely rapidly in blood and tissues makes it highly unlikely that any reactive captan-derived materials will be absorbed into the systemic circulation and transported to tissues other than the skin at the point of exposure. The results of a 21-day dermal toxicity study in New Zealand rabbits (Johnson, 1987) clearly demonstrated that effects were limited to minimal irritation at all dose levels. Other than body weight and food consumption decrements in the high dose animals (1000 mg/kg), there was no evidence of any test article related systemic toxicity.

To evaluate possible differences in the ability captan to penetrate rat and human skin, an *in vitro* skin absorption study was conducted using either 0.2 g/cm^2 (neat) or 40 µg/cm^2 in acetone. In the rat skin, the rate of absorption was 0.5 µg/cm^{-2}hr^{-1}irrespective of the method of application. In contrast, in human skin, the rate of absorption was 68- to 100-fold less -- 0.005 and 0.007 µg/cm^{-2}hr^{-1} as a neat or acetone application, respectively. The results of this study indicate that, relative to rats, the absorption of captan through human epidermis is very slow. This will provide ample opportunity for degradation of captan via reaction with GSH within the epidermal cells and will prevent entry into the systemic circulation. The use of rat dermal absorption data in human risk assessment will significantly overestimate human dermal exposure (Ward *et al.*, 1989).

The results of these studies clearly indicate that, in rats, there is no evidence of any systemic toxicity (including effects in the small intestine) following dermal administration of captan. Furthermore, since the rate of dermal absorption in humans is approximately 100-fold lower than in rats, it is highly unlikely that any systemic toxicity will occur in humans.

Inhalation: In a 90-day rat inhalation study using nose-only exposures to captan (Hext, 1989), groups of 10 male and female Alpk:ApfSD rats were exposed to concentrations of 0.13, 0.60, or 5.06 µg/l captan, and 20 males and 20 females were exposed to 12.98 µg/l captan for 6 hours per day, 5 days per week for 13 weeks. A concurrent control group consisted of 20 male and 20 female rats. Ten males and 10 females from each group were killed one week following their last exposure, and the remaining animals in the high dose and control groups were killed following a 4-week recovery phase.

No treatment-related changes were seen in ophthalmoscopy, clinical chemistry or hematological parameters in any exposed group. There was no evidence of kidney toxicity. Treatment-related effects were confined solely to the respiratory tract, specifically the larynx, and were consistent with exposure to an irritant particulate. The effects in the lung were considered responsible for five male mortalities (25% mortality) in the high dose group. Examination of the lungs from the animals allowed to recover for 4 weeks revealed they were completely normal. The NOEL for the lung was considered to be 0.6 µg/l. Effects considered to be of toxicological significance were seen at 12.98 and 5.06 µg/l. Effects considered to be an adaptive response to irritants were seen at the lower concentration. The toxicological NOEL for captan was 0.6 µg/l. No systemic effects were observed

Conclusion: All data indicate that captan induces only localized irritation following direct contact with the tissue with which it first comes into contact. Dermal toxicity studies reveal only local irritation with no systemic effects. Similarly, inhalation studies indicate irritation in the larynx leading to cytotoxicity and epidermal cell necrosis at high doses of captan. The irritation is reversible. It is concluded that both captan and its degradates (particularly thiophosgene), react extremely quickly with GSH and/or other thiols in the epidermal cells at the point of entry into the body (skin, respiratory epithelium) that the moieties responsible for the irritation and toxicity are rapidly removed before they can be absorbed into the systemic circulation. It is highly unlikely

that systemic effects leading to the tumorigenic effects observed in mouse small intestine following oral ingestion can occur following dermal or inhalation exposures of mice or other species to captan.

D. Human Exposure and Epidemiology

Captan Uses: Captan is a non-systemic fungicide used to control diseases in orchard crops, berries, seeds, turf and ornamentals. Captan is formulated into wettable powders (WP), water dispersable granules (WDG) and liquid flowable concentrates (FLC) for foliar application to food plants by aerial and ground equipment (airblast and ground boom) and to field grown ornamental and golf course turf by ground boom. These formulations may also be applied to greenhouse ornamental, fruit/nut trees in home gardens, and directly to the soil around plants in greenhouses, using ground equipment such as backpack/knapsack, low-pressure hand wand and garden hose-end sprayer. WP, FLC, and dust formulations may be applied to seeds, as a slurry or dust or directly into planter boxes.

General Population: The levels of exposure of the general population to captan residues are extremely low. For the purpose of cancer risk assessment, chronic dietary exposure and ground and surface water concentrations have been calculated by the Agency as documented in the 1999 Captan RED. The dietary exposure was estimated to be 0.00005 mg/kg/day. The estimated environmental concentrations (EECs) of captan in ground and surface water are 0.02 ppb and 4.0 ppb, respectively, for chronic exposure. Exposure via drinking water is negligible.

Agricultural**:** Agricultural occupational exposure to captan residues via dermal and inhalation routes can occur during handling, mixing, loading and application activities. Most exposure to captan in the agricultural setting is short-term (1 to 7 days) to intermediate term (1 week to several months). Exposure estimates for all captan use scenarios have been made by the Agency. For most handlers, the use of captan per season would be 7 days for strawberries and 3 days per season for the remaining fruit crops. For greenhouse use of high pressure systems, handlers were estimated to be exposed for 1 hour a day for up to 26 days a year. Golf course applications were estimated to occur up to 10 times a year. Thus, exposure is for a limited time period and is intermittent in nature.

In the Agency's 1999 RED calculation of estimated exposure, the worst-case exposure was determined by modeling to be to the mixer/loader (ML) of wettable powder for aerial application to strawberries making very conservative assumptions. This may lead to a maximal total daily combined dermal and inhalation dose of 0.24 mg/day with the inhalation exposure being a large component. The majority of other agricultural work activities led to exposure calculations that were significantly lower.

Mills (1998) reported the results of a correlation analysis between data on cancer incidence and pesticide use at a county level throughout California. A correlation coefficient (r) of 0.46 was observed between captan use and leukemia in Hispanic males

and a r value of 0.49 between captan use and prostrate cancer in black males. The results of this study should be interpreted with caution in light of its methodological limitations. Correlation does not imply causation (there are many unmeasured variables that could be involved) and there are serious questions regarding the assumption that county-wide use data provides a reasonable measure of individual pesticide exposure.

Manufacturing: 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.

E. 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).

- Dietary exposure of the U.S. population to captan is extremely low. Likewise the agricultural use pattern and exposure of workers is also very low and intermittent.

- An epidemiology study of limited power involving 410 employees of a captan manufacturing plant in the U.S. suggested no evidence of an increase in deaths by cancer or for death by duodenal tumors as reported in death certificates. Although a low correlation was observed between captan use (at a California county level) and the incidence of leukemia in Hispanic males and prostate cancer in black males, there is no evidence of any causative relationship between captan and human cancer.

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 (Bridges1975; Quest *et al.*, 1993; Edwards *et al.*, 1991; Tennekes, 1994; Bernard and Gordon, 2000; Trochimowicz *et al.*, 2001). Inevitably, when so many genetic screening assays have been performed, there are bound to be both positive and negative responses. However, a careful analysis using a weight-of-the-evidence approach shows conclusively that captan is not an *in vivo* mutagen.

A. Process

A simple three-step approach can be taken to evaluate genetic toxicological databases.

- Evaluate the quality of individual assay reports;
- Evaluate the results from multiple studies in a specific category of mutation tests; and
- Evaluate the complete database using a hierarchical evaluation of individual categories of mutation tests.

A necessary prerequisite to any weight of evidence evaluation is a careful review of the individual studies to insure that they meet minimum standards of reliability and predictivity. In order for studies of genotoxic potential to be acceptable for use in risk assessment, the following criteria must be met:

- The underlying data supporting the conclusions must be available for review, either in Agency files or in the peer-reviewed literature.
- The test system should be validated and peer-accepted, with validation studies available in the open literature.
- The methods must conform to validated methodology and performance criteria for a particular test system.
- The test sample must be adequately characterized to insure both stability and purity.

Adherence to a careful quality analysis and/or use of acceptability criteria excludes data that are inappropriate to the assessment. This is particularly important in the evaluation of studies conducted in the 1970's when the field of genetic toxicology was rapidly growing and many assay systems subsequently found to have poor specificity were prematurely reported in the literature.

The validation step is essentially the recognition that an investigator's work or assay test system should be reproducible in another laboratory. A positive result in a single assay must be judged on the basis of the entire database for any particular type of study, e.g., an *in vitro* bacterial gene mutation category or an *in vivo* cytogenetic category. Positive results cannot automatically assumed to be the only correct results, with the preponderance of negative results displaying the insensitivity of the assay system. *A*

priori, it is just as likely that a positive response is an artifact due to overly sensitive test system as it is that a negative response is the result of improper or insensitive assay systems (Ashby, 1991; Scott *et al*., 1991; Caldwell, 1993). Therefore, evaluation of the sensitivity and specificity of individual assays within a particular category of tests can help distinguish between false negative, false positive and valid test results.

The final evaluation must consist of a hierarchical evaluation of individual categories of mutagenicity tests. Conclusions for each category of test (endpoint) should be evaluated on a hierarchical basis to arrive at an overall evaluation. In a properly tiered assessment of mutagenic risk, more weight is usually placed on the results from the higher tier of *in vivo* studies. More specifically, the results of microbial testing are outweighed by the results of mammalian cell culture tests, and these, in turn, are outweighed by *in vivo* somatic cell testing and *in vivo* mammalian tests for germ cell and heritable mutations. The reasons for the weighting are embedded in the logic of the tiered testing approach. The lower tier studies are intentionally designed to optimize sensitivity at the expense of specificity, to insure that all potential mutagens are identified and evaluated for potential hazard. The higher tier studies are specifically intended to model the endpoint of direct concern, the potential for *in vivo* somatic and heritable mutations in humans, and are therefore inherently more relevant to the assessment of potential risk in humans.

B. Assessment by Genotoxicity Category

i. *In vitro* Assay systems

Bacterial Gene Mutation Assays: The results of a large number of bacterial mutation studies are listed in summary form in Table 6, although this is not inclusive of all the literature reports. Because captan was shown to be mutagenic in the early seventies, it was used in many of the early programs to evaluate the integrity and specificity of gene mutation assay systems. These screening programs evaluated large numbers of chemicals and unfortunately the data were generally presented only as positive or negative with little additional information of the sample tested, i.e. purity. Many of the tests also used only one concentration/dose of the test chemical. Although this information is useful, it does not lend itself to scrutiny with respect to any specific chemical. The report of Simmon *et al.* 1977, for example, was an EPA sponsored study. Although the study allows evaluation of data (available to the Agency), the data were not presented in the literature publication. In addition, while the studies of DeFlora *et al.*, 1984 and Moriya *et al.* 1983 used current methodology and were dose-response studies, they did not fully report the data. The results and conclusions of complete studies should be given greater weight than those from studies that are significantly deficient in data or method presentation.

Despite the paucity of individual data, nearly every bacterial study conducted with captan is positive for mutagenicity. Several conclusions can be drawn from these data. The earlier 'spot tests' or zone of inhibition tests were generally less sensitive and *S. typhimurium* tester strain TA1535 tended to be the only strain showing a positive response. As development of the 'Ames Assay' moved from the 'spot test' to a plate

incorporation method, nearly every tester strain showed mutagenic activity. In addition, several other assay systems (not all currently accepted as valid mutational assay systems) were also positive. These included the *E. coli* WP2, *S. coelicolor* and *B. subtilis* test systems.

In all the tests with various species/strains of bacteria, three major conclusions can be drawn:

- captan induces gene mutations in bacterial systems;
- strains carrying additional mutations in either DNA polymerase function or other DNA repair genes were more sensitive than their corresponding wild type strains; and
- the addition of a rat liver homogenate (S9) or other thiol-containing mixes significantly reduces and in some cases eliminates mutagenic activity (Moriya *et al.*, 1978). The significance of this with regard to the proposed mechanism of carcinogenic action of captan will be discussed later.

Metabolites: As discussed in Section II, captan breakdown results in the release of THPI and thiophosgene. THPI is of little toxicological concern and has been shown to be negative for mutagenic activity in bacterial test systems (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* (Rideg, 1982) in a 'spot test'. This was published in abstract form only and cannot be adequately reviewed. Using an *in vitro* mammalian system, Arlett (1975) generated 'thiophosgene' as a vapor by 'activating' captan, previously adsorbed to filter paper, with carbonate and produced a marginal response in a Chinese hamster cell line (unvalidated system). Overall, THPI is negative in the *S. typhimurium* test but there is little data to assess thiophosgene.

Host-Mediated Bacterial Gene Mutation Assays: In order to better mimic the metabolic activation observed *in vivo*, many investigators use host-mediated assays rather than exogenous rat liver S9. In these assays, animals are treated with the test chemical i.p. and after test chemical exposure *in situ*, the bacteria are removed, plated out and evaluated for mutation. In two separate studies (Ficsor *et al.*, 1977: Kennedy *et al.*, 1975, unacceptable) using different bacterial tester strains, no mutagenic activity with captan was observed. As discussed above, captan appears to be inactivated in the presence of thiol-containing media, in this case, the whole animal.

Mammalian *in vitro* Gene Mutation Assays: Four studies have been conducted using *in vitro* mammalian gene mutation assays. The study of Arlett *et al.* (1975) is in a system that was not validated. The data cannot be interpreted and will not be discussed further. The Oberly (1984) study had control mutation frequencies that were too low for an acceptable study and is also excluded from the assessment. A summary of all reports can be found in Table 6.

The study by O'Neill *et al.* (1981) evaluated captan in a CHO/HGPRT assay, pioneered in this laboratory. A positive but weak response was observed in three independent trials. Although the purity of the captan was not reported, the source was the manufacturer, Chevron. No trials were done in the presence of liver homogenate.

Two mouse lymphoma $TK^{+/-}$ (ML) assays were conducted. Oberly *et al.* (1984) tested captan from 0.01 to 0.3 mg/ml and obtained marginally positive responses (2- to 3-fold background levels) at 0.06 mg/ml; these induced frequencies were more analogous to those expected as background levels. It is concluded that the background mutation frequency was too low and that the study does not meet current acceptability criteria. Data were not presented to allow a full assessment of the number of mutants or the cytotoxicty. For all these reasons, this study was excluded from the current assessment of captan mutagenicity.

The study by Edgar *et al.* (1985) was conducted both with and without liver homogenate. Positive responses, approximately four-fold background levels, were seen at 0.2 and 0.3 mg/ml. However, the criteria for assessing positive and negative responses in the ML assay have evolved since this study was performed. The response observed at 0.3 mg/ml in trial 1 was associated with excessive cytotoxicity (less than 6% survival) and is not useful for assessing mutation frequency; likewise, the 0.2 mg/ml dose showed survival of less than 5%. The effects at these doses should be excluded from the mutation frequency calculation. Additional acceptance criteria require a positive response to show an induced mutation frequency of at least 100×10^{-6} and a negative response to be less than 50×10^{-6}. The results of this study meet the currently acceptable criteria for a negative assessment. This study was also negative in the presence of S9 activation.

Overall, the data from the *in vitro* mammalian gene mutation tests range from weakly positive to negative. In the *in vitro* mammalian assays, captan displays a significantly lower mutation activity than in the bacterial test systems.

Mammalian *in vitro* Chromosomal Assays: Tezuka *et al.* (1978) evaluated the potential of captan to induce chromosomal aberrations in diploid human fibroblast cultures. EPA deemed this study acceptable for regulatory purposes. Doses of 0 through 4 µg/ml captan were tested. Significant mitotic inhibition was observed but no chromosomal aberrations were induced at cytotoxic dosages of 3 and 4 µg/ml captan.

In a subsequent study, Tezuka *et al.* (1980) investigated the ability of captan to induce chromosomal aberrations in Chinese hamster V79 cells *in vitro*. Chromosomal aberrations were induced beginning at 450 mM captan (13.5 µg/ml). No cytotoxicity was observed even at the highest dose level in the V79 cell culture. These data were not presented and could not be evaluated. This is a significantly higher concentration of captan than used in the diploid human fibroblast cell culture test. The difference in the results is probably due mainly to the different sensitivities of the two test systems. Human cells in particular are more karyotypically rugged than the V79 culture system, known for its chromosomal and karyotypic instability.

Overall, captan can induce a weak positive response in some tissue culture systems in the absence of activation. No studies were performed using exogenous activation systems. In the more relevant study to assess human risk, captan did not induce chromosomal damage to the human cell culture system.

ii. Ancillary Genetic Toxicity Endpoints

Other Genotoxicity Assays: Other assays have been developed to assess the ability of a compound to cause DNA or chromosomal damage and are often useful in elucidating the mode of action of a particular mutagen. They tend to be sentinel assays that indicate the potential for mutation but do not demonstrate the compound is actually a mutagen. For example, Unscheduled DNA Synthesis (UDS) detects DNA damage that is repaired by the excision repair system. The fact that the damage is repaired is in and of itself evidence that, although the cell sustained damage, it has the ability to recover before further damage occurs. Likewise, Sister Chromatid Exchange (SCE) assays monitor the exchange of sister chromatids within a chromosome. This recombinational event is a natural process to repair damaged DNA and is increased following damage to DNA. DNA binding or adduct formation provides a more direct indicator of DNA damage. Because DNA is continually being damaged and repaired, DNA binding or adduct formation alone is not evidence that a chemical is a mutagen. It simply provides evidence that a chemical can react with DNA.

***In vivo/in vitro* Unscheduled DNA Synthesis (UDS) assay**: In an OECD guideline study, Kennely (1990) evaluated the ability of captan to induce UDS *in vivo*. Male Alpk:ApfSD rats were administered captan by gavage at 500, 1000 and 2000 mg/kg body weight and UDS was evaluated at 4 and 12 hours post-treatment in cultures of excised liver cells. Captan did not cause a significant increase in net nuclear grain counts or the percentage of cells in repair. It is concluded that captan is negative in this assay.

***In vitro* SCE:** One *in vitro* SCE study by Tezuka et al. (1980) was performed in Chinese hamster V79 cultures. This study was positive, as was the Chinese hamster V79 chromosomal aberration study performed in the same laboratory.

DNA strand break and binding studies: Many different laboratories have looked at captan-induced DNA damage and captan-DNA binding. Unfortunately, conflicting results have been obtained. Bridges (1975) reported (as a personal communication from Andersen and Rosenkranz) that captan has the ability to bind to nucleic acid to produce a 7-(trichloromethylsulphenyl) guanine adduct. This was subsequently reported in a paper published in Henry Ford Hospital Medical Journal by Andersen and Rosenkranz in 1974 suggesting that captan may bind to DNA and quoted by Elder (1989). However, because of the obscurity of this journal, it was not possible to evaluate or validate the method of analysis employed.

Swenberg *et al.* (1976) reported that captan causes chromosomal breaks in Chinese hamster V79 cells as evidenced by alkaline elution of the DNA extracted from these cells; the effect was obviated in the presence of a rat S9 activation system. These data are

consistent with the induction of SCE and chromosomal breaks in the same cell culture system as reported by Tezuka *et al*. (1980). Although the alkaline elution assay is sometimes considered evidence of DNA 'binding', it measures the degree of chromosomal/chromatid breakage (already shown by Tezuka *et al*. (1980)).

Selsky (1981) investigated the ability of captan to interact with DNA. Osborne-Mendel rats and CD1 mice were fed 300 and 1600 mg/kg ^{14}C captan (methyl label). Although they found an association of the captan with the DNA, they concluded the majority of the detected radioactivity was either non-covalently associated or that some captan-DNA products were covalently associated but they were lost during hydrolysis at pH 7.0. This study was inconclusive with regard to captan-DNA binding.

Snyder (1992) evaluated the effects of captan on DNA and DNA metabolic processes in *in vitro* cultured human diploid fibroblasts. Captan is very cytotoxic to this cell line and consistent with the results of Tezuka *et al*. (1978), exposure for 30 minutes to as little as 1.65 uM (5 µg/ml) captan resulted an 80% reduction in cloning ability. Snyder showed that captan was able to induce DNA strand breaks in this culture at 99.0 uM. Addition of the excision repair inhibitor 1-B-D-arabinofuranosyl- cytosine (20uM) and hydroxyurea (2mM) led to an increased number of strand breaks at lower doses of captan (45.9uM). Strand breaks were only observed in closed systems leading to the conclusion the volatile thiophosgene degradate was responsible for the effect. Snyder was also able to detect a 3- to 4-fold increase in protein/DNA cross-linking at excessively high doses of 200 uM captan. Protein/DNA crosslinking can result in distortion of chromatin can lead to DNA breaks. However, because the concentrations of captan required to induce the effects are so much higher than those causing cell death, their biological relevance is questioned.

Snyder (1992) also reported the ability of captan to form DNA adducts when treated in cell free systems. Thus, when 3 mg of ^{14}C-captan (methyl labeled) was reacted with 4 or 4.6 mg herring sperm DNA for 1 hour at 37^{o}C in 7.5 ml of buffer, DNA adducts were detected. Initial binding calculations revealed one adduct in 130 and 190 base pairs, respectively, at pH values of 6.0 and 7.5. This unusually high level of binding is confounded by the high level of captan in the thermal (100^{o}C)/acid (0.1N HCl) hydrolyzed sample as evidenced in the HPLC trace. If this level of captan (nearly equal to the reported number of adducts) is present, it is likely that heat and acid degradates are also present. Because no standards were used in the HPLC analysis, confirmation of 'real' adducts is difficult. No attempt was made to identify the radiolabelled peaks. Clearly, the samples were not cleaned of unreacted captan prior to the analysis. Snyder also reported binding to DNA from treated cultures of diploid human fibroblasts although no data were provided and the level of binding was very low; this could easily be explained by a similar contamination as that described above. It is concluded that the DNA-binding reported in this report is not convincing due to the high level of captan contamination in the purified DNA and inadequate analytical methodology employed.

In an effort to understand the mode of action of captan in the duodenum of mice exposed to captan, a series of DNA binding studies were performed by Pritchard and Lappin (1991), Provan *et al*. (1995), and Provan and Eyton-Jones (1996).

Pritchard and Lappin (1991) evaluated the ability of captan to bind to DNA in the stomach, duodenum, jejunum, liver and bone marrow of mice exposed to either ^{35}S- or ^{14}C- (methyl position) labelled captan. CD1 mice were given a single oral dose of captan (900 mg/kg), appropriate tissue was excised 6 hours after exposure, and DNA was extracted and analyzed for radioactivity. Binding was calculated to be 2.8, 38, 38, 91, and 46 x 10^{-6} adducts per nucleotide in the bone marrow, liver, stomach, jejunum and duodenum, respectively. Since the binding appeared higher in the jejunum than in the duodenum (the presumed target tissue), the results were somewhat puzzling. Subsequent purification of the DNA and additional purification of the DNA by cesium chloride gradient separation showed that not all the radioactivity was associated with the DNA. No attempt was made to digest the DNA to confirm the presence of captan- DNA adducts. However, it was clear from the lack of co-elution that the 'binding' was not to DNA but to a material co-purified with the DNA.

To better understand the nature of this 'bound' co-eluting material, Provan *et al.* (1995) compared the elution patterns of DNA from CD mice fed ^{35}S captan to ^{35}S-N-acetylcysteine and two thiazolidine derivatives. Hepatic DNA was analyzed for DNA binding products using the methodology of Prichard and Lappin (1991). The cesium chloride elution patterns from DNA isolated from the animals fed acetylcysteine and thiazolidine derivatives were analogous to the material observed following treatment with radiolabeled captan. Again, the radiolabelled peaks did not co-elute with the DNA. These experiments demonstrated that the 'DNA binding' observed in the GI tract after exposure to captan was due to the incorporation of free sulfur into protein that was contaminating the DNA preparations. Thus, there was no evidence of captan DNA binding.

Provan and Eyton-Jones (1996) subsequently studied the disposition of ^{14}C (cyclohexene labeled) captan through the digestive tract. This study showed that the radiolabel was associated mainly with the contents of the GI tract and not the tissue. Because of the location of the radiolabel (on the ring structure), the presence or absence of DNA binding cannot be determined from this study since thiophosgene or its degradates are the presumptive DNA reactive moieties.

DNA Polymerase Studies: A chemical does not need to bind to DNA to induce gene mutation. DNA damage is a constant event in the life of a cell and DNA polymerases and other DNA repair enzymes are continually repairing this damage. If these enzymes are functionally impaired, spontaneous DNA damage may go unrepaired.

In a series of studies spanning over 15 years, Lewis and colleagues have investigated the role of captan in inhibiting DNA and RNA polymerases. These kinetic studies help to elucidate a potential mechanism by which captan exerts its mutagenic activity *in vitro*. Lewis and Brown (1977) postulated that captan irreversibly inhibits DNA synthesis by a mechanism located within the nucleus. Subsequent studies showed that captan inhibited DNA polymerase by competing for the same site as the DNA while not interferring with the fidelity of the DNA polymerase I copy of the DNA template (Dillwith and Lewis, 1982). Using captan as an inhibitor of viral reverse transcriptase, Freeman-Wittig *et al.*

(1986) studied differential effects of captan on DNA polymerase and RNase H activity. RNase H activity was 10-fold more sensitive to captan than either DNA-dependent or RNA-dependent DNA polymerase. Captan inhibition of the RNA-dependent polymerase activity could be prevented by dTTP (deoxythymidinetriphosphate). The calculated KdTTP of the uninhibited reaction was 5.6 uM. Additional kinetic studies indicated that captan was interacting with the polymerase at the active site of the molecule. Since dithiothreitol prevented captan inhibition, it was concluded the (trichloromethyl)thio moiety of captan was involved in the inhibitory action. Luo and Lewis (1992) went on to show that captan was acting at the nucleoside triphosphate (NTP) binding site and the DNA binding site of the RNA polymerase.

GSH and Other Thio Compounds: As discussed in Section IV. Metabolism and Pharmacokinetics, GSH and other thiol-containing molecules play an essential role in the inactivation of captan and other electrophilic compounds in the cell. Moriya *et al.* (1978) recognized this in their reports of captan genotoxicity in an *E. coli* repair deficient strain. The elimination of captan mutagenicity by these agents is shown in Table 5. The presence of an activation system (S9) significantly reduced or eliminated all genotoxicity. They found that captan mutagenic activity was very sensitive not just to the liver homogenates used as chemical activation systems, but to cysteine and blood, which also abolished all mutagenic activity. Their results suggested that captan is rapidly destroyed by compounds containing sulfhydryl groups. Fiscor *et al.* (1977) using the *S. typhimurium* tester strains, also demonstrated captan was inactivated by rat or human blood during a 45-minute incubation. Rat plasma inactivated captan but not as readily as whole blood. This observation correlated with the presence of GSH associated with erythrocytes but not plasma. Dalvi and Ashley (1979) showed that the addition of glutathione prior to addition of captan afforded complete protection of the cytochrome P-450 system *in vitro*. Rahden-Staron *et al.* (1988) reported that the captan induction of c-mitosis (15% over control) in V79 cells was accompanied by a 77% decrease in non-protein sylfhydryl groups, mainly GSH. It can be concluded from these studies that captan is inactivated and subsequent mutagenic activity *in vitro* is diminished or eliminated in the presence of thiol-containing systems.

TABLE 5.

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

Test System	Component Added	Revertants per Plate
E.coli WP2 hcr	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

[1] Data from Moriya *et al*. (1978)

iii. In vivo Assay Systems

A large number of captan *in vivo* mutagenicity studies, both gene mutation and clastogenicity endpoints, have been reported. Table 7 summarizes the results of these studies.

Gene Mutation Somatic Cell Assays: Considered by many to be a higher tier heritable gene mutation test, the mouse specific locus test measures gene mutation in heterozygous alleles for coat color in mouse embryos exposed *in utero*. Captan was tested in this assay at dietary concentrations of 100, 1000, and 5000 ppm (equivalent to 11, 105, 419 mg/kg/day) along with positive and negative controls (Nguyen, 1981). Pregnant dams were fed captan for five days beginning on day 8 and ending on day 12 of gestation. Pups were evaluated for 'spots' on day 12 and at weaning. The results show that captan did not induce somatic mutations in mice at the three concentrations tested. Integrity of the assay was demonstrated.

In another mouse specific locus study, Imanishi (1987) reported captan induced a frequency of recessive color spots of 2.2% after an i.p. injection of 15 mg/kg. Unfortunately, since this report was an abstract, no data could be evaluated and no background or positive control data were given. The background level of recessive color spots in the Nguyen (1981) study was 2.9%, well above the reported 'positive' response reported in the Imanishi (1987) abstract. For these reasons, this study is excluded from the present genotoxicity assessment of captan.

Overall, captan does not induce *in vivo* somatic cell mutation.

Chromosomal Somatic Cell Assays: There has been a wide range of studies (literature as well as company sponsored) to assess the ability of captan to induce chromosomal aberrations *in vivo*. With the exception of one poorly reported study, all are negative. The details of these studies can be found in Table 5.

Bootman and Whalley (1979) showed captan to be negative in a CD rat bone marrow chromosomal aberration study. Tezuka *et al*. (1978) also confirmed captan to be negative when evaluated in Wistar rat bone marrow. Fry and Fiscor (1978) exposed Swiss albino mice to a 50% captan formulation and were unable to induce any chromosomal aberrations.

Micronucleus assays confirm these results. Jacoby (1985), in an OECD guideline study, determined that captan did not induce micronuclei (MN) in the bone marrow of male or female CD-1 mice. In more sophisticated studies to evaluate MN in duodenal crypt cells of C57Bl/6J mice, Chidiac and Goldberg (1986) showed that captan did not induce MN at the tumor target site. This important study will be discussed in greater detail in relationship to the proposed mode of tumorigenic action of captan.

In a study of highly questionable quality, Feng and Lin (1987) reported that in mice (unspecified strain) captan induced both chromosomal aberration in spermatogonia and MN in bone marrow. The source of the chemical was a local Chinese producer with a stated purity of 96.5%, although the LD_{50} (2850 mg/kg) reported was lower than that usually seen. The report of this study contains so many mathematical errors, some by orders of magnitude, the data are difficult to interpret and are not credible. Furthermore, the results are inconsistent with all other published reports of better quality. This study is excluded from this assessment of captan genotoxicity.

Overall, captan does not induce *in vivo* chromosomal damage.

***In vivo* Germ Cell Gene Mutation:** Although not a mammalian test system, the *Drosophila* Sex Linked Recessive Lethal (SLRL) test is considered a test for germ cell gene mutation *in vivo*. Several studies have been performed and all show captan to be negative. In an EPA sponsored study undertaken to validate the assay system, Valencia *et al*. (1981) reported that captan exhibited weak activity at 2000 ppm. However, when multiples or clusters were removed from the statistical assessment, both the chromosomal and dominant lethal endpoints were not statistically significant from the controls. Studies by Mollet (1973) and Mollet and Wurgler (1974) demonstrated that captan did not induce SLRLs up to toxic concentrations nor did it induce somatic recombination and mutation utilizing a mosaic eye test (unvalidated test). Kramers and Knaap (1973) tested an 83% formulation of captan and found it negative for SLRLs.

Overall captan is considered negative for the endpoint of *in vivo* germ cell gene mutation.

***In vivo* Germ Cell Chromosomal Mutation:** Several studies of varying quality have been conducted to evaluate the ability of captan to induce dominant lethality in mice and

rats. This study endpoint assesses major chromosomal defects. Male animals are exposed, mated with untreated females and progeny, and pre- and post-implantation sites are evaluated in the dams. These studies utilize a variety of dosing regimens. Some expose the animals to the test compound by i.p. injection (1-5 injections) while others expose the animals via the feed for 5 days, 7 weeks or 8 weeks. Tezuka *et al*. (1978) obtained negative results with analytical grade captan in C3H mice. Doses of 200 or 600 mg/kg bw of captan for five days did not affect fertility. The mean number of corpora lutea, implants and live embryos were within the normal range for both groups treated with captan. No dose-dependent increases in the frequency of induced lethal mutations and of litters with early deaths were observed. The positive control substance elicited a distinct effect on all parameters.

Salaman and Smith (1977) found 89% technical captan negative in CD mice. However, since this study was conducted at IBT, subsequent GLP issues at this laboratory necessitate eliminating this study from the assessment. Epstein *et al*. (1972) reported Orthocide (Captan, purity not provided) as negative in Swiss mice following both i.p and dietary administration. Individual data were not provided. Since the report is of suspect quality, it should not be given much weight in the assessment.

Collins (1972) evaluated a technical grade captan administered in five daily i.p. injections (2.5, 5.0,or 10.0 mg/kg/day) or by gavage (50, 100, or 200 mg/kg/day). No fertility effects were observed after captan treatment. When dominant lethal mutants are expressed as decreased total implants per pregnant female, no mutagenic effects were observed after i.p. or oral administration. However, when both mice and rats were treated orally, there was a statistically significant increase in the mean number of early pup deaths at the highest dose at week 1 and with the two highest doses at week 2 (100 and 200 mg/kg/day). No increase in early deaths was observed in females mated with males receiving captan by i.p. injection. Despite the statistical significance of these data, their biological meaning and relevance to captan's mutagenicity are unclear. Genetically damaged zygotes result mainly in pre-implantation losses, with a consequent reduction of the total number of implants per pregnancy. In this study no reduction in the mean number of implants was seen after captan exposure.

In an EPA sponsored study, Jorgenson *et al*. (1976) found captan negative with respect to its ability to induce heritable translocations in mice. Unfortunately, the study is reported only as an abstract and since only the Agency has access to the data, it was not possible to evaluate the quality of the study. Consequently, this study has been excluded from this assessment.

Overall captan was not shown to induce any dominant lethal mutation as is considered negative for *in vivo* chromosomal germ cell mutation.

C. Overall Role of Captan in Genotoxicity Induction

Although captan is able to induce gene mutation in bacterial cells, it is only weakly active in the *in vitro* eukaryotic Chinese hamster V79 cell line. *In vivo* results are clearly

negative, both for gene mutation and chromosomal endpoints. Heritable gene mutation assays are also negative.

The DNA binding, DNA polymerase and GSH studies provide insight into the pattern of genotoxicity observed. In very rare and relatively unsubstantiated studies, some DNA binding was reported. However, the overwhelming evidence indicates that neither captan nor its thiophosgene metabolite bind to DNA under normal cellular circumstances. Thiophosgene does bind to the active site of some of the DNA/RNA polymerases and this may affect the fidelity of replication. In bacterial cells with deficient repair capabilities, mutations are detected with much higher frequencies. This implies that, as would be anticipated, captan binding to DNA polymerase is more detrimental in cells with deficient repair capabilities. The concurrent stimulation of the exonuclease activity combined with the inhibition of the polymerase activity of the DNA polymerases may result in breaks and gaps in the chromatin following naturally occurring DNA damage. Mutations are generally not detected when full repair function is present *in vitro* and were not present *in vivo*.

In order for captan to disrupt cellular function, it must reach the cell. Glutathione and other electrophile scavengers inactivate captan as well as the 'active' metabolite, thiophosgene, by binding and making it unavailable. The gene mutation endpoint, like the cancer endpoint, is governed by the basic processes of distribution, activation, and inactivation. Thiophosgene is capable of reaching bacterial genetic material and causing mutation only in repair deficient cells. In the presence of S9 or other thiol-containing systems, it is inactivated and no gene mutation is observed. In the *in vitro* mammalian gene mutation and chromosomal assays, the presence of S9 eliminates any captan activity. In the whole animal, captan and its reactive metabolites are rapidly inactivated. GSH depletion studies described in Section IV provide additional evidence that captan will be inactivated before reaching target cells *in vivo*. The tissue relevant studies of Chidiac and Goldberg (1987) underscore the lack of genetic activity of captan in the crypt cells of the duodenum of mice.

Although most gene mutation is the result of DNA binding, specific binding to the active sites of DNA polymerases can also result in gene mutation. Captan is an established inhibitor of DNA polymerase. However, this effect is governed like any other enzymatic process by a threshold and it is highly unlikely that, in intact animals, cellular levels of captan will ever approach those required to cause inhibition of DNA polymerase. The mode of action of captan in inducing duodenal tumors in the mice cannot be due to gene mutation.

D. Conclusions

- The overall weight of the 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 some eukaryotic cells. Although the precise mechanism is not known, it is suspected to involve the inactivation of DNA and RNA polymerases by binding in the active sites of these enzymes.

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

- 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.

Table 6. Captan *In Vitro* Genotoxicity Studies

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
In vitro Bacterial Gene Mutation Assays						
Salmonella typhimurium test – Plate Incorporation						
Salmonella reversion assay E. coli	*S. typhimurium* *TA1535,TA1537,* *TA1538, TA100* *WP2*	0, 1, 2, 10, 15, 25 µg/plate -S9 + S9	Positive Reduced activity Positive	Chevron	EPA Acceptable Pre-1991 guideline	Simmon *et al.*, (1977)
Salmonella reversion assay	*S. typhimurium* TA1535, TA1537 TA1538 TA98, TA100, TA97	2.5-250 µg/plate -S9 + rat S9	Positive (all strains) TA1538 weak Reduced activity	Reagent grade Serva Feinbio-chemica	Acceptable methodology Dose-response study, no concentrations nor revertants reported	DeFlora *et al.* (1984)
Salmonella reversion assay E. coli	*S. typhimurium* TA1535,TA1537 TA1538, TA98 TA100 WP2 *hcr*	-S9 + S9	Positive (all strains) Reduced activity with S9 Positive	Purity and source not reported	Acceptable methodology Only data for TA100 in graph form. All other data presented as + or -	Moriya *et al.* (1983)
Salmonella reversion assay E. coli	*S. typhimurium* TA1535,TA1537 TA1538, TA98 TA100, TA102 WP2 *uvrA*	0.1 to 10 mg/plate-S9 0.1 to 10 mg/plate + S9 0.1 to 10 mg/plate+ S9	Negative Negative Negative	99.9% THPI Metabolite	Acceptable, guideline/GLP study	Carver (1986)
Bacterial Spot Tests						
Salmonella Spot test	*S. typhimurium* TA1535, TA1536 TA1537, TA1538 TA98, TA100	50, 100, 300 µg/plate -S9 +S9	Weak Positive (all but negative in TA1536) Negative to Equivocal	Not reported Chevron	Good methodology for spot test Revertants not reported	Shiau *et al.* (1981)
Salmonella Spot test	*S. typhimurium* TA1535, TA1536 TA1537, TA1538	50 µg/plate	Positive (TA1535 only)	Not reported	Acceptable spot test	Shirasu *et al.* (1976)

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
Salmonella Spot test S. coelicolor	*S. typhimurium* TA1535, TA1536 TA1537, TA1538 *A3*	20 µg/plate + rat S9 2000 µg (spot) 200 µg (incorp)	Positive TA1535 Reduced Negative Weak Pos	93% Bayer	Spot test, old tester strains	Carere *et al.* (1978)
E coli DNA repair test Liquid micro method	*E. coli* WP2 (wt) WP67(*uvrA⁻polA⁻*) CM871(*uvrA⁻recA-lexA⁻*)	2.5 to 250 µg/plate +/- S9	Negative Positive Positive S9 reduced activity	Reagent grade Serva Feinbio-chemica	Unusual methodology No concentrations provided, dose-response study No data provided, only calculated responses	DeFlora *et al.* (1984)
E. coli Spot Test	WP2*hcr*+ WP2*hcr*-	50 µg/plate	Positive	Not reported	More positive in repair deficient strain	Shirasu *et al.* (1976)
E. coli Spot Test	WP2*hcr*+ WP2*hcr*-	1 to 3 mg crystal or 20-25µl unknown concentration	Positive Positive	50% WP formulation/ Chevron	Inadequate description of dose: results in conflict with Shirazu *et al* (1975) and DeFlora *et al* (1984)	Nagy *et al.* (1975)
B subtilis Zone of Inhibition	H17 *rec*+ M45 *rec*-	1 mg/ml (20µg)	Positive Positive	Not reported	More positive in M45*rec*-	Shirasu *et al.* (1976)
B subtilis Zone of Inhibition B subtilis Plate incorporation Pre-incubation	TKJ5211*(uvrA10)* TKJ6321*(uvrA10 polA151spp-1)* TKJ5211 TKJ6321	50, 100, 300 µg/plate + rat S9 1, 5, 10, 25 µg/plate + rat S9	Positive Positive Positive Reduced	Not reported Chevron	Acceptable methodology Individual data not presented Much greater activity in repair deficient strain	Shiau *et al.* (1981)
Host Mediated Bacterial Assays						
Host Mediated *S.typhimurium* gene mutation	Mouse *S. typh* G46 *S. typh* G46 Rat *S. typh* TA1950 (oral)	*500, mg/kg x 3 (sc)* *1g/kg (oral)* 2g/kg	Negative Negative Negative	Captan 50WP (50%) (Expressed as technical) Stauffer Chemical Co	EPA Acceptable Pre-1991 guideline	Ficsor *et al.* (1977)
Host Mediated *S.typhimurium* gene mutation	*S. typhimurium* G46 -rat *S. typhimurium* TA1530	125, 250 mg/kg 0.5-5 mg/kg	Negative 88% ai	Purity not reported Chevron	Excluded IBT Laboratory	Kennedy *et al.* (1975)

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
In Vitro Mammalian Cell Gene Mutation Assays						
In vitro Mammalian gene mutation assay	Chinese hamster ovary (CHO) hgprt	0, 0.1, 0.25, 0.5, 1.0, 2.0, and 4.0 µg/ml; in DMSO Non-activated only	Positive above 0.25 ug/ml Dose responsive 3 replicate trials	Purity not reported Chevron	Acceptable methodology	O'Neill *et al.* (1981)
In vitro Mammalian gene mutation assay	Mouse Lymphoma $TK^{+/-}$	0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 µg/ml Non-activated only	Extremely weak positive above 0.06ug/ml 1 trial	Purity not reported Chemical Services, Inc	Excluded: Suspect quality Not currently accepted methodology: Negative control values abnormally low.	Oberly *et al.* (1984)
In vitro Mammalian gene mutation assay	Mouse Lymphoma TK+/-	0, 0.05, 0.075, 0.1, 0.2, 0.3 mg/ml 0, 12.5, 15, 20, 25, 30 35, 50 with rat liver S9	Positive above 0.2ug/ml Reassessed as Negative Negative	Purity not reported	Acceptable methodology	Edgar *et al.* (1986)
In vitro Mammalian gene mutation assay	Chinese Hamster V79 (8-AG and Oubain)	0, 0.01, 0.05, 1 mg/pad - vapor 0, 5, 10 µg/ml (suspension)	Negative 8-AG; Equivocal Oubain	Purity not reported Orthocide, Murphy Chemical	Excluded Unvalidated methodology: Results cannot be interpreted	Arlett *et* al. (1975)
In vitro Mammalian Cell Chromosomal Damage						
Chromosomal aberrationChromo-somal aberrations	Human diploid fibroblastChinese hamster ovary (CHO) cells	0, 0.5, 0.15, 3.0, and 4.0 µg/ml2-10 µg/ml (± rat S9) in DMSO	Negative 89.1% Sanachem 800 WP product	>98% Wako 99.9% Nishio Positive	EPA Acceptable	Tezuka *et al.* (1978)
Chromosomal aberrationChromo-somal aberrations	Chinese hamster V79	0, 6 to 60 µM	Positive at 45 uM: positive doses show 'cell stickiness'	99.9% Nishio	EPA Acceptable No cytotoxicity data reported	Tezuka *et al.* (1980)
DNA Damage						
In vivo /in vitro Unscheduled DNA synthesis (UDS)	Primary rat hepatocytes from male Alpk:APfSD rats	0, 500, 1000, 2000 mg/kg	Negative (also for S phase induction)	91.2% Western Research Center	EPA Guideline/GLP	Kennely (1990)

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
Sister chromatid exchange (SCE)	Chinese hamster V79	0, 6 to 60 µM (serum free media)	Positive at 30 uM	99.9% Nishio	EPA acceptable No cytotoxicity data reported	Tezuka *et al.* (1980)
Alkaline Elution/DNA strand breaks	Chinese hamster V79	0.01 to 3.0 mM -S9 +S9	Positive Reduced activity	Not reported	Excluded: Only +/- reported. No data provided. Suspect quality	Swenberg *et al.* (1976)
DNA Binding						
Radiolabelled Captan, methyl	CD1 mice Osborne-Mendel rats	300, 1600 mg/kg additional 156 mg/kg in mice	Non-covalent binding: lost during hydrolysis	98% Amersham	Non-GLP research study: acceptable methodology	Selsky (1981)
Radiolabelled Captan, methyl	Mouse stomach, s. intestine, liver, bone marrow	900 mg/kg oral.	Negative: Radioactivity did not co-elute with DNA	98%, SpA 888MBq/mM Cambridge Research Biochem.	GLP research study	Pritchard & Lappin (1991)
Radiolabelled N-acetylcysteine, thiazolidine derivatives	CD-1 mice	3 mmole/kg ^{35}S-captan; N-acetyl-^{35}S]cysteine; 2-thio-oxo[^{35}S]thiazolidine-4-carb-oxylate; 2-oxo[^{35}S]thiazol-idine-4-carboxylate	Labelled material similar to cesium gradient of DNA sample (Pritchard)	99% captan 98%^{35}S captan 96%^{35}Scys-teine 96% 2-oxo 93% 2-thiooxo	Research study acceptable methodology	Provan *et al.* (1995)
Radiolabeled Captan, cyclohexene	Mouse GI tract, blood	400, 3000 ppm diet	No conclusion	99.1%, SpA 1.265 GBq/mM Zeneca	GLP research study Inadequate design to assess DNA binding	Provan & Jones (1996)
Radiolabeled Captan, methyl	Herring sperm	3mg captan/4 mg DNA	Binding	Tech 98% Stauffer	HPLC chromatography with no identification – *in vitro*, cell free	Snyder (1992)

Table 7. Captan *In Vivo* Genotoxicity Studies

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
In vivo Chromosomal Alteration Somatic Cell						
Bone marrow cytogenetics	Male CD rats	0, 200, 400, 800 mg/kg oral	Negative	Lot number provided Makhteshim	Proprietary study, pre-GLP	Bootman & Whalley (1979)
Bone marrow cytogenetics	Male Wistar rats	0, 500, 1000, 2000 mg/kg, oral 0, 200, 400, 800 mg/kg x5 days oral	Negative Negative	98 % Wako, 99.9% Nichio	EPA acceptable Currently non-guideline	Tezuka *et al* (1978)
Bone marrow cytogenetics	Swiss albino mice	250 mg/kg ip	Negative	Captan 50WP: 50% tech Stauffer	Dose resulted in 68% mortality at 54 hours post treatment	Fry, & Fiscor (1978)
Bone marrow cytogenetics	Mice (species not specified)	0, 100, 400, 600, 800, 1000 mg/kg 5 days oral	Positive at 400 mg/kg/day	96.5% Danyan Chemical, China	Excluded Questionable quality: Many mathematical errors: No cytotox	Feng & Lin (1987)
Micronucleus	Mice (species not specified)	0, 10, 50, 100, 400, 800 mg/kg 2 days oral	Positive at 100 mg/kg/day	96.5% Danyan Chemical, China	Excluded Questionable quality: Many mathematical errors: One sampling time: no cytotox	Feng & Lin (1987)
Micronucleus assay	Male and female CD-1 mice: bone marrow	0, 40, 200, 1000 mg/kg b.w.	Negative	94% Makhteshim	OECD 474: GLP	Jacoby (1985)
Micronucleus assay	C57B1/6J mice CD1 mice Evaluation of duodenal crypt cells	0, 8000, 16000 ppm diet for 1 week (tech) 0, 20, 200, 1000 mg/kg (tech) diet for 5 days 0, 200, 2000 mg/kg single dose (tech, anal, and form)	Negative Negative Negative	99% Analytical 92.4% technical 50% formulation	Research study: good data but system not validated for genetic testing programs	Chidiac & Goldberg (1986)
In vivo Gene Mutation Germ Cell						
Drosophila SLRL	*Drosophila melanogaster*	0, 2, 3, 2000 ppm	Weak positive 2000 ppm only Negative if clusters excluded	Not reported	Presence of multiples (clusters). When removed, statistical significance reduced: Chromosomal aberration and dominant lethal tests negative	Valencia, Warf Ins. (1981)
Drosophila SLRL	*Drosophila melanogaster* injection technique	0.15% Non-toxic, 3% high concentration;	Negative	99.5% Chevron	Only abstract in English	Mollet (1973)
Drosophila SLRL	*Drosophila melanogaster* injection technique	0.3, 1% in media pure 0, 0.5% formulation	Negative	Pure, 83% formulation Ligtermoet Chemical Co	Typical injection methodology	Kramers & Knaap (1973)
Drosophila somatic recombination	*Drosophila melanogaster* Mosaic eye	0.78, 1.45% (feeding)	Negative	99.5% Chevron	Unvalidated methodology	Mollet and Wurgler (1974)

Test System	Test Object	Concentration Dose	Assay Results	Purity Source	Acceptability/ Quality Comments	Reference
In vivo chromosomal alteration germ cell						
Dominant Lethal	Male C3H mice	0, 200, 600 mg/kg 5 days	Negative	>98% Wako, 99.9% Nishio	EPA acceptable	Tezuka *et al* (1978)
Dominant Lethal	Male CBA-J mice Male Osborne-Mendel rats	0, 2.5, 5, 20 mg/kg ip 5 days 0, 20, 100, 200 mg/kg oral 5 days	Statistical significance without biological significance	% not reported, lot number 36423, Chevron	Results inconsistent Suspect quality	Collins (1972)
Dominant Lethal	Male CD mice	0, 500, 3000, 7000 ppm 8 weeks	Negative	89%	Excluded: IBT study, suspect quality	Salaman & Smith (1977)
Dominant Lethal	Male Swiss mice	0, 9, 12, 15, 30 mg/kg ip 0, 500, 800 mg/kg oral 5 days	Negative	Orthocide (not reported)	Individual data not reported Suspect quality	Epstein *et al* (1972)
Dominant Lethal	Male mice	0, 1250, 2500, 5000 mg/kg diet 7 weeks	Negative	Not reported	EPA Sponsored	Jorgenson (1976) Abstract Also reported by Simmon *et al* (1977) full data sets
Heritable Gene Mutation						
Mouse specific locus test	T-strain male, C57Bl/6J female mice	0, 100, 1000, 5000 ppm diet 5 days (days 8-12)	Negative	92.2% Technical Chevron	EPA acceptable study	Nguyen (1981) Litton
Mouse specific locus test	PW male, C57Bl/6J female mice	15 mg/kg ip (day 10)	Positive	Not reported	Excluded Abstract with no data 2.2% induced frequency a typical control frequency. Suspect quality	Imanishi *et al* (1987)

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 adenomas and adenocarcinomas in the small intestine, primarily 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 incidence and severity of the tumors increase with increasing dose and duration of exposure. 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. There is evidence that tumors are observed further down the small intestine (jejunum) with increasing dose and duration of exposure.

- There is a correlation between tumor formation and localized hyperplasia that increases in severity and moves down the small intestine with dose and duration of exposure.

- Cessation of captan exposure results in a marked reduction in the incidence of hyperplasia and malignant tumors with the incidence reverting toward control levels.

- The total weight of evidence indicates that, although captan can induce weak mutagenic activity 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, the progression of non-neoplastic histopatholgical changes indicates that the tumors are closely associated with mucosal hyperplasia and adenomatous polyps and that these lesions always precede formation of tumors in the same proximal region of the small intestine.

It is proposed, therefore, 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 mucosal epithelium of the small intestine. The precise nature of the mechanism causing cytotoxicity is unknown. It probably involves the reaction of captan-derived species with SH groups on cysteine or GSH and may occur on the surface of the villi or inside the cell. Based on the folpet studies, the initial depletion of GSH in the intestine coupled with the increased levels of GSH with concomitant decreases of GSH in the liver might suggest that the reaction with GSH is occurring on the surface of the villi. Captan and its breakdown products are known chemical irritants and clearly have the ability to cause inflammation and cytotoxicity in the mucosal epithelium of the duodenum. Following sustained 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, inflammation and necrosis of the mucosal epithelial cells in the proximal region of the small intestine 2) regenerative hyperplasia and crypt cell proliferation and 3) neoplasia. Importantly, hyperplasia and cell proliferation are

threshold-based effects with clear NOAELs and, 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.

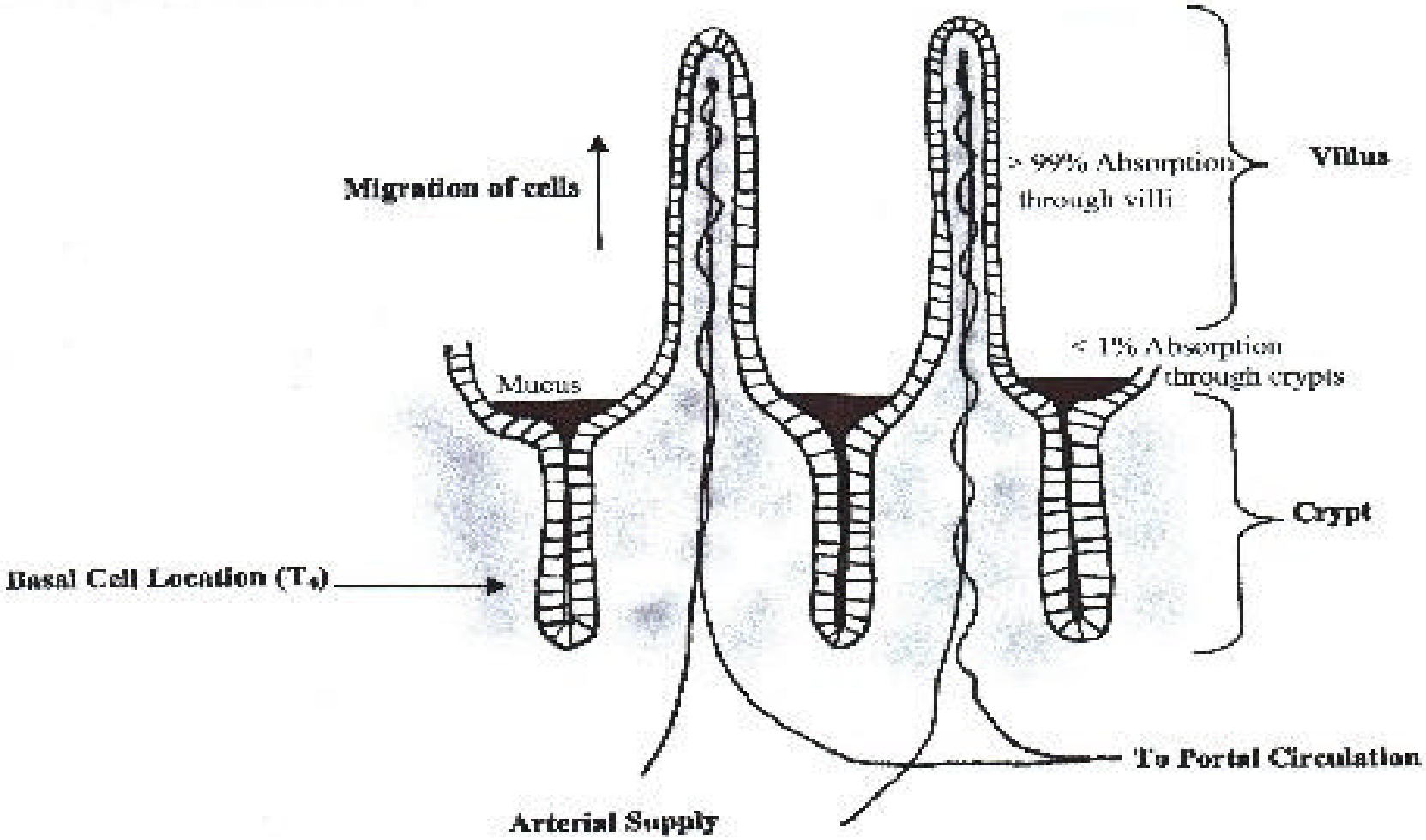
Within the crypt, approximately two-thirds of the cells (150-160 per crypt) are in a proliferative state, rapidly passing through the cell cycle. The proliferating cells are derived from a population of stem cells. Several investigators have concluded that the actual stem cells comprise a ring of about 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). A number of reviews regarding cell kinetics and the organization of the intestinal crypts have been published (Potten *et al*., 1983; Wright & Allison, 1984; Potten & Morris, 1987; Potten & Hendry, 1983).

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 the only obvious cytotoxicity observed following captan exposure is restricted to the villi.

A key finding in support of this conclusion is that subchronic and chronic feeding studies show that only 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).

Figure 5. Schematic of Duodenum Villi and Crypts.



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.

The long-term study by Pavkov (1985), discussed earlier, provides important information on the location and progression of non-neoplastic histological changes occurring in the small intestine during sustained exposure of male mice to captan (6000 ppm). Pavkov (1985) clearly showed that the histological changes were restricted primarily to the proximal region of the duodenum. 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 the duodenum 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 studies (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, a modest 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. The inability to detect a more significant increase in BrdU labeling may be due to the fact that there is a significant normal background rate of cell proliferation in the crypt cells. Based on this study, the NOAEL for non-neoplastic histopathological changes in the duodenum 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 1	Day 3	Day 7	Day 14
Crypt cell hyperplasia	0/5	4/5	5/5	5/5
Shortening of villi	0/5	3/5	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

i. 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 proximal region 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, the adenomas and adenocarcinomas occur in the same proximal proximal region of the duodenum and although the cells of origin are not identified in the bioassays. Pavkov (1985) reports that they originate 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 more 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 could ultimately lead to tumor formation. This is supported by the observation that a low incidence of duodenal tumors is seen in concurrent and historical controls (Bomhard & Mohr, 1989; Bomhard, 1992; Chandra & Firth, 1992). In the case of control mice, however, focal hyperplasias (and possibly tumors) are more widely distributed throughout the small intestine and not restricted to the proximal portion of the duodenum as with those induced by captan (Pavkov, 1985).

Histological changes in intestinal crypts resulting in mutagenic and/or neoplastic effects could potentially occur in the proliferating transit cells as a result of errors in DNA replication (Lijtha, 1979). It is highly unlikely, however, 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 must be considered at very low risk for mutagenic transformations that would result in a carcinogenic response, because the rapid transit time and eventual 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 length of time. 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). In this position, the cells are protected from 1) the turbulence of extensive cell movement 2) factors that control differentiation

and maturation and 3) the potentially harmful environment of the gut lumen. The stem cells are also likely to be protected by their relatively slow rate of turnover (several months in the mouse and several decades in humans) and the fact that they spend less time in the error susceptible stages of DNA synthesis and mitosis than transit cells. They may also benefit from periods of apparent inactivity to reorganize themselves and repair any deficiencies (Potten & Loeffler, 1990; Lijtha, 1979). The Brunner's gland, specific to duodenal tissue, affords further protection for the intestinal crypts. This gland secretes a thick mucous that protects the epithelial tissue from bile salts, digestive enzymes and presumably reactive products in the lumen.

ii. 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 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 2).

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.

iii. Temporal

Several independent studies have demonstrated that the key non-neoplastic event on which tumor formation is proposed to depend, hyperplasia of the proximal region of the small intestine, occurs shortly after the start of exposure and always precedes tumor formation in long-term bioassays.

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	

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

iv. 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 treatment with 6 months recovery or 6/12 = 6 months treatment 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, 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 further support 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 sustained cytotoxicity and chemical-induced regenerative cell proliferation is plausible and consistent with our current understanding of the biology of cancer (Correa, 1996). It is also plausible with regard to our knowledge of the histology and morphometrics of the gastrointestinal tract.

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). While a high rate of cell proliferation is not always necessarily associated with a high rate of cancer, regenerative cell proliferation linked to persistent cytotoxicity appears to be a potentially important risk factor.

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; USEPA, 2001); 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; Swenberg *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 or 1992; 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.

Perhaps the best-known example of an accepted tumorigenic mechanism that comes closest to that being proposed for captan is chloroform (USEPA, 2001). Thus, the increased incidence of rodent liver and kidney tumors following high level exposure to chloroform are considered secondary to sustained cytotoxicity and secondary regenerative hyperplasia. As with captan, a major metabolite of chloroform (phosgene) is a highly reactive species potentially capable of having mutagenic effects on DNA. The genetic toxicity profile of chloroform, however, is mainly negative and it is generally considered that phosgene is so unstable that, once formed, it is likely to react with cytoplasmic molecules before reaching nuclear DNA (USEPA, 2001). This suggestion is precisely analogous to that being proposed here for the thiophosgene and other reactive species released during the degradation of captan.

In addition to promoting the clonal expansion of nascent tumor cells *in situ*, abnormally high proliferation may: 1) increase fixation and expression of pre-mutagenic 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.

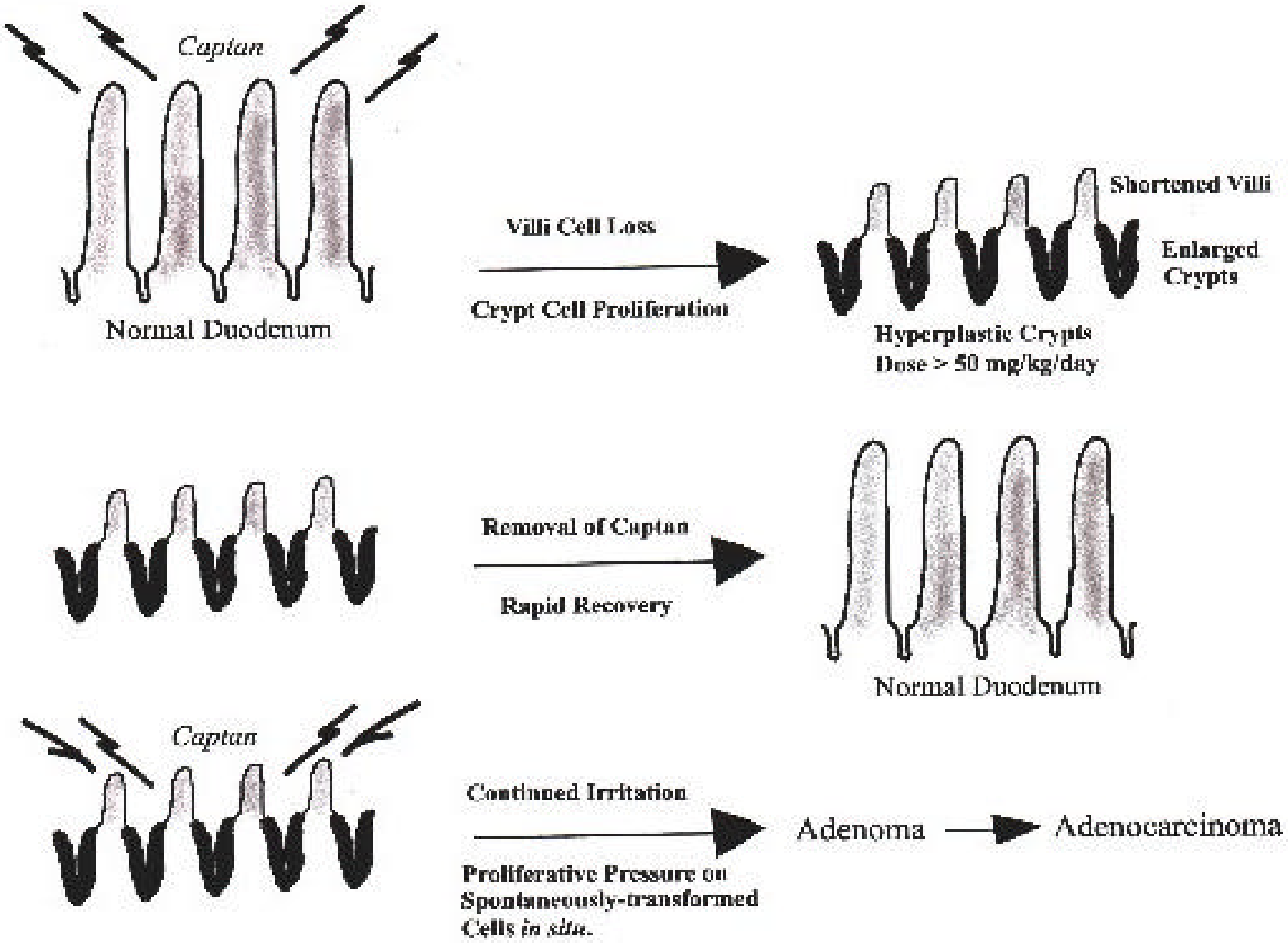
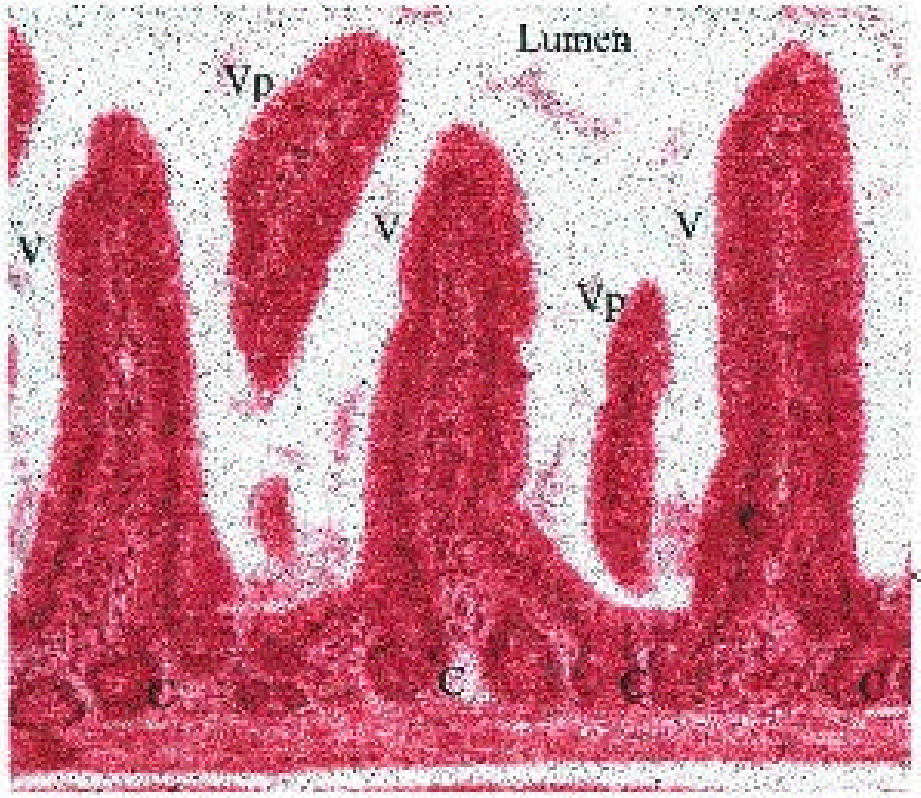


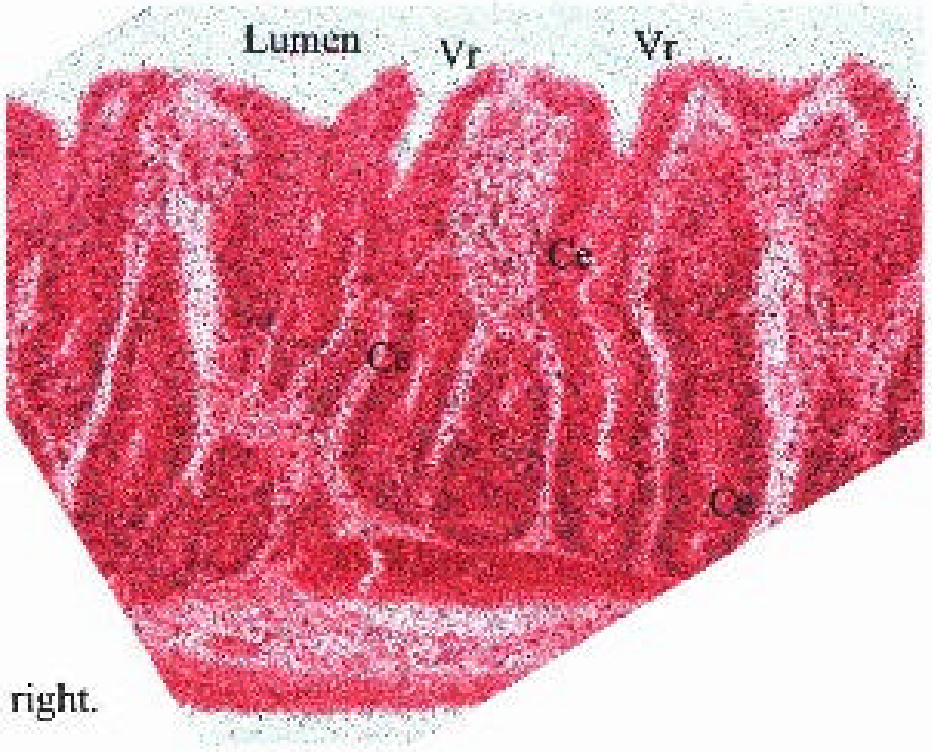
Figure 7. Micrographs of Normal and Captan Treated Mouse Duodenum.

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. As discussed earlier, the reasons for these differences are not clear. In view of the general similarities in the morphology and physiology of the mammalian intestinal tract, however, there is no reason why the proposed mode of action and response to captan in humans should be inherently different from that in other mammals. If a worst-case 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 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. It is, of course, equally possible that the human response to captan may be more similar to rats than mice. In this case, captan would not be considered to be of any significant cancer risk to humans.

Despite the paucity of data available to assess susceptibility of children to captan, what little exists implies children are no different than adults with regard to the mode of action of captan. Reports of Pavkov (1985) indicate that animals exposed at earlier life stages did not have increased susceptibility to duodenal tumors. Even assuming adults could be as sensitive to captan as mice, the exposure levels that would be required to cause any effect would be so large that any differences in child exposure (for a given exposure level children often have a somewhat greater intake per unit of body weight) would be of little or no consequence.

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 pH of the lumen and the breakdown products will come into 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 less than one 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. The histopathological effects of captan are of local rather than systemic origin.

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.

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

Based on the new Guidelines for Carcinogen Risk Assessment, it is proposed that captan should be classified as:

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

A. Human Carcinogenic Potential

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. Based on U.S. EPA calculations human dietary exposure, both children and adult and all modeled sub-populations, is minimal. Occupational exposure, via dermal and inhalation routes, is also minimal and intermittent. Furthermore, because of its extreme lability in blood (half-life less than one second), captan is not likely to be carcinogenic to humans in other organs/tissues following dermal or inhalation exposures at any dose level/concentration. Although no long-term oncogenicity studies were conducted via the dermal or inhalation route of exposure, there is no reason to believe that captan exposure via these routes will result in small intestine tumors. Captan does not exert its tumorigenic mode of action systemically as demonstrated by 90 day inhalation studies or 21 day dermal toxicity studies. Captan exposure, however, results in irritation at the site of contact. Conversely,

captan is likely to be carcinogenic to humans only following prolonged, high-level oral exposures causing cytotoxicity and regenerative hyperplasia in the proximal region of the small intestine.

B. Summary of Key Evidence

i. 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 proximal region of the small intestine. 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 death from duodenal cancer or an increase in deaths from other cancers.

ii. Genotoxicity

Although captan induces mutagenic effects *in vitro* in bacterial and weak effects in some 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 or key DNA processes.

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 similar evidence that captan-derived species do not bind to DNA or cause chromosomal aberrations in the duodenal stem cells following oral administration.

iii. 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. At least one study has shown that the tumors occur in the same area of the proximal duodenum as the hyperplasia 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 mucosal epithelium, 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 links between non-neoplastic and neoplastic effects (dose-response, thresholds, temporal association, reversibility).

C. Overall Process of Captan Tumorigenicity

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

1. Following oral ingestion, captan is rapidly degraded to THPI, thiophosgene and other reactive species in the stomach and proximal part of the small intestine – breakdown occurs by either hydrolysis or reaction with GSH and other thiols;
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 damaged DNA and increases the probability of cloning a transformed cell; and
6. The increased cloning of cells containing naturally occurring DNA damage leads to an increased incidence of duodenal adenomas and adenocarcinomas.

D. 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 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 less than one second), *captan is not likely to be carcinogenic to humans in other organs/tissues following dermal or inhalation exposures at any dose level/concentration*. Conversely, *captan is likely to be carcinogenic to humans only following prolonged, high-level oral exposures causing cytotoxicity and regenerative hyperplasia in the proximal region of the small intestine.*

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 duodenum of mice; and 2) strong evidence that, although captan is mutagenic in some *in vitro* tests ameliorated by exogenous thiols, 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 tumors (adenomas and adenocarcinomas) in the small intestine (primarily the proximal portion 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 mortality from cancer.

2. The overall weight of evidence indicates that captan is not genotoxic in intact animals. Captan is weakly mutagenic when measured in *in vitro* test systems (bacterial or eukaryotic cells) where it has ready access to the DNA or other DNA enzymatic processes. *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 less than one 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 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 or cells damaged during replication are cloned before DNA damage can be repaired. There is a strong causal association (dose-response, temporality) indicating that tumor formation is secondary to cytotoxicity 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:

- ***not likely to be a human carcinogen at dose levels that do not cause cytotoxicity and regenerative cell hyperplasia in the proximal region of the small intestine*;**

- ***not likely to be carcinogenic to humans in other organs/tissues or following dermal or inhalation exposure, and***
- ***likely to be carcinogenic to humans following prolonged, high-level oral exposures causing cytotoxicity and regenerative cell hyperplasia in the proximal region of the small intestine.***

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Appendix

Response to the Toxicology Excellence for Risk Assessment (TERA) Report of Peer Review Meeting: Cancer Assessment for Captan, September 3-4, 2003

Response to the Toxicology Excellence for Risk Assessment (TERA) Report of Peer Review Meeting: Cancer Assessment for Captan, September 3-4, 2003

The Captan Task Force is very grateful for the careful and comprehensive review of the overview document "Scientific Analysis of Data Relating to the Reclassification of Captan under EPA's New Guidelines for Carcinogenic Risk Assessment (Overview Document). This review was helpful in strengthening and clarifying the scientific presentation of data supporting the cancer risk reclassification of captan. We have revised the document to reflect the Panel's comments and suggestions.

This summary of our revisions should assist the independent panel of experts in reviewing the changes made to the Overview Document. The Panel was asked to consider eight 'charge questions' loosely organized following the approach presented in the U.S. EPA's 2003 Draft Guidelines for Carcinogen Risk assessment and provided their comments and suggestions for revision of the Overview Document. The report provides comments to each charge question and reached several conclusions in which there was either consensus or unanimous consensus with regard to changes in the presentation of the data or conclusions drawn from the data. The response will document what was changed and direct the panel to the location of the changes in the Overview document in relationship to the charge questions.

Charge Question 1. Was the literature search/document review complete enough to locate all studies pertinent to developing a cancer assessment for captan? Can you recommend any additional studies or data that should be included in this assessment?

Overall, the document was fairly complete, including most of the available studies. However, the panel did suggest several additional studies and sources of information that should be considered.

a) *Include a description of the literature search and the criteria used to determine which studies would be included in the analysis.*
b) *Include more detail in the study summaries.*
c) *For human data, add a description of the Mills (1998) study. Also consider the available human data on acute toxicity and data available from health studies of pesticide applicators.*
d) *For tumor data, add a discussion of Antony et al (1994), as well as describing any other data for inhalation or dermal routes, to support WOE statement regarding those routes of exposure.*
e) *Include data on GI tract pathogenesis for other agents, as well as data on GI tract pathogenesis and physiology in general.*
f) *Use updated references for tumor incidence for historical controls.*
g) *For mechanistic data, add a discussion of studies that support the MOA such as GSH depletion studies and rat kinetic studies.*

Revision: The Panel's suggestions were incorporated into the Overview Document as outlined below:

a) A sentence was added to the Introduction indicating that Medline, Toxnet and regulatory databases were used. Since the toxicology of captan has been extensively reviewed by the U.S. EPA, only the more recent studies were located by literature search.
b) Relevant detail was added to the study summaries in V. Hazard Identification.
c) A discussion of the Mills (1998) paper was added. It can be found in V. Hazard Identification, D. Human Exposure and Epidemiology, Agricultural.
d) The Antony (1994) study was very poor. Since it would only tend to add ambiguity, it was not discussed. A few of the many problems include high animal mortality, insufficient controls groups, improper methodology, unusual mouse strain (for this assay), no purity information on the commercial captan test material, etc. Only papillomas/group are reported and only for a subset of the groups tested with little discussion of the pathology of the papillomas. Because it is of little value, it was decided to not discuss this paper in the Overview Document.

In the section V. Hazard Identification, section C. Other Routes of Exposure was added that discussed relevant studies conducted by either dermal or inhalation exposure.

e) GI tract pathogenesis was addressed for other agents. This can be found in section VII. Proposed Mode of Action, E. Biological Plausibility.

f) Additional references for historical control incidences were added in the V. Hazard Identification section for the A. Mouse Bioassays and B. Rat Bioassays.

g) A discussion of the GSH depletions studies with folpet (a fungicide that shares a common mechanism of action with captan) was added to section IV. Metabolism and Pharmacokinetics.

Charge Question 2. What conclusions can be drawn from the human data regarding the potential human carcinogenicity of captan?

The panel reached unanimous consensus that the only available epidemiological study is insufficient to contribute to conclusions regarding the carcinogenicity of captan. In general, the panel agreed with the conclusions of the document regarding this study. However, the panel unanimously agreed that the summary statement should be clarified to read "Based on this limited study, there is no evidence of an increase in deaths by cancer or for death by duodenal tumors as reported in death certificates." As above, the panel suggested that Mills study and information on the acute effects of captan in humans should be included in the discussion of the proposed MOA.

Revision: This statement 'Based on this limited study…' was inserted in the summary statement of the section V Hazard Identification, E. Conclusions, final bullet point. The Mills study was added to V. Hazard Identification, D. Human Exposure and Epidemiology, Agricultural. Because there were so few incident reports of acute human exposures and most resulted from exposures to other pesticides in addition to captan, it was decided that the addition of this information would add little insight and was not included.

Charge Question #3. Are the available long-term bioassays adequate to evaluate the potential human carcinogenicity of captan? Based on the weight of evidence, what tumor types are biologically relevant and related to treatment with captan?

The panel reached unanimous consensus that the kidney and uterine tumors observed in rats are not biologically relevant or treatment related and that the mouse small intestine tumors are biologically relevant and treatment related. However, the rationale for these conclusions needs to be strengthened in the document to reflect the balance and totality of the data.

For the kidney tumors, enhance the argument that tumors are spontaneous based on the observation of a lack of increased atypical hyperplasia, and possible observation of chronic renal nephropathy as indicated by increased BUN levels. Address the contribution of, or rule out, other possible MOAs such as Alpha-2μ-globulin or beta-lyase activation of thiols. Use structure activity relationship information to strengthen arguments.

For uterine tumors, it is appropriate to evaluate stromal sarcomas separately from other undifferentiated or unclassified carcomas. This is consistent with NTP guidelines. In addition, it is appropriate to also evaluate stromal sarcomas combined with uterine polyps as a secondary measure.

For mouse data, acknowledge and discuss the observation of effects (both non-neoplastic and tumors) in the stomach and jejunum and the contribution of these effects to the overall proposed MOA. The document should change focus from "duodenal tumors" to "small intestinal tumors, primarily of the duodenum." The higher incidence of small intestine tumors in the Wong et al. (1981) study can be attributed to the sensitive sectioning technique and the long study duration. The document should show the incidence of hyperplasia in the tables in addition to tumor incidence and also clarify the underlying cell type and region of the small intestine of tumors, if presented in the study. (However, the panel noted that the tumor studies themselves do not identify which region of the small intestine is the location of the tumors, not do they identify the tumors as crypt cell adenomas). The panel recommended adding discussion of the Pavkov (1985) study to the discussion of tumor studies as well as the Antony et al. (1994) study of tumor promotion following dermal treatment.

Revision: Rat Kidney: A full discussion of the spontaneous nature of the kidney tumors was inserted in Goldenthal *et al.* (1982) found in section V. Hazard Identification, B. Rat. Also, lack of contributions of Alpha-2µ-globulin and beta-lyase activation of thiols was also inserted in the same section.

Species Difference: The two possible explanations for the species differences was inserted at the end of NOASR (1983) in V. Hazard Identification, B. Rat. Note: the designation of this study was changed from NOASR (1983) to Til (1983) to be more accurate.

Mouse Data: To the extent feasible, the focus was shifted to the small intestine, keeping in mind that the overwhelming majority of the tumors were confined to the duodenum. References to the proximal 7 cm portion of the duodenum were de-emphasized. In the discussion of the Wong *et al.* (1981) study, the higher incidence was discussed with regard to the more sensitive 'Swiss roll' sectioning technique. More discussion was added with regard to the non-neoplastic lesions in the mouse. Also, Table 2 now documents the incidence of hyperplasia. The underlying cell type was discussed in the text as it was not practical to include this in Table 2. A full discussion of the Pavkov (1985) study was included which does address the underlying cell types. Based on the reasons listed in item d) of the Charge Question 1 response, the Antony *et al.* (1994) study was not discussed. The study was unacceptable.

Charge Question #4: Are the available data on physical and chemical properties adequate, and do they contribute to an understanding of the potential human carcinogenicity of captan? What conclusions can be drawn regarding the absorption, active metabolites, half-life, and elimination of captan? Would you expect that metabolism and kinetics of captan would be significantly different by different routes of exposure? How do these data contribute to the understanding of captan's cancer mode of action?

> *The panel noted that the kinetic data are not well developed. They reached unanimous consensus that the kinetic data do not completely support the MOA, but they do not detract form it either. The mechanistic studies are confounded by methodological issues related to the high reactivity of the metabolites, and because the studies do not show the pattern of localization that matches with the histopathology.*

Revision: With the addition of the folpet GSH depletion studies that measure GSH levels in specific regions of the intestinal tract as a function of time, this section was strengthened.

Charge Question #5: Are the available mechanistic data adequate and relevant to identify the chain of key causal events leading to tumor formation by captan? How do the data from the mechanistic studies contribute to an understanding of captan's cancer mode of action?

The panel reached unanimous consensus that the histopathology data are very supportive of the MOA. They noted that the cell proliferation data are not robust, but that they are not inconsistent given the limited sensitivity of this measure in the tissue with a high background proliferation rate. Other mechanistic data for captan, including the S^{35} binding studies, have limited interpretation due to methodological issues. Other suggestions by the panel are to include information on the pathogenic mechanisms for other gastrointestinal toxicants and including a discussion of the THPI toxicity data.

Revision: Although there may be methodology issues with one of the S^{35} binding studies, the totality of the DNA binding studies indicates that captan does not covalently bind to DNA. The section discussing these studies was redrafted, presenting these issues more clearly. It is located in section VI. Genotoxicity, B. Assessment by Genotoxicity Category, ii. Ancillary Genetic Toxicity Endpoints, DNA strand breaks and binding studies. Information on the pathogenic mechanisms for other gastrointestinal toxicants was added to section VII. Proposed Mode of Action, E. Biological Plausibility. Unfortunately the THPI toxicity data is not sufficient to address in the discussion on pathogenic mechanisms.

Additionally, a section was added to address temporality specifically in VII Proposed Mode of Action, D Causality Between Key Events and Tumor Formation. Temporal. The incidence and severity of tumors with increasing dose and duration of exposure was brought to the top of the introduction VII Proposed Mode of Action, A Summary of Key Events.

Charge Question #6. Are the available genotoxicity data adequate to evaluate the role of genotoxicity in captan's mode of action? Based on the weight of the evidence, can it be concluded that captan genotoxicity does not contribute significantly to human carcinogenic potential at environmentally relevant doses?

The panel reached unanimous consensus that, based on the weight of evidence, captan genotoxicity does not contribute significantly to human carcinogenic potential at environmentally relevant doses (note that a formal exposure assessment was not reviewed by the panel). Overall, the panel concluded that captan is probably not a genotoxic carcinogen; although there are some limitations in the existing data regarding thiophosgene. Based on the weight of evidence, captan is a weak mutagen in the in vitro *bacterial studies and a very weak mutagen in the* in vitro *eukaryotic cell studies. Captan is negative in the* in vivo *assays. The panel recommended that the document be revised to include a detailed evaluation of the genotoxicity studies using standardized study quality criteria to aid in weighing conflicting results and to explain questionable studies. Also, the document should expand discussion of the genotoxicity data on THPI and its close analogues. If additional data were to be generated, an* in vivo *mutagenicity study, for example Big Blue or MutaMouse, would be useful.*

Revision: Section V. Genotoxicity was completely redrafted per the Panel's suggestions. It contains the process for evaluation of studies as well as the process for weighting studies. As requested, Tables were added to better display the results. Studies, previously not discussed were documented and discussed. The section was properly organized to reflect the types of studies and their hierarchy in the genotoxicity evaluation process. Studies that did not meet the criteria for acceptability were excluded from the data re-evaluation. After a critical review of all the DNA binding studies and biochemical/kinetic studies on captan and DNA polymerases, it became apparent that the *in vitro* mutation probably is not be the result of direct interaction with DNA. With the two mouse specific locus studies, there should be no need to consider any additional *in vivo* gene mutation. Since the GSH and blood studies also support the lack of activity *in vivo*, additional testing adds little information to the genetic toxicology database. There were little to no data on THPI (one Ames test) or analogues, or thiophosgene (bacterial spot test) to warrant a full discussion.

Charge Question 7: Is the body of data adequate to describe a mode of action for captan and can a list of events be identified that are key to the carcinogenic process? The proposed mode of action involves irritation and inflammation, followed by regenerative proliferation of duodenal epithelial cells, leading to neoplasia. Do the data support this mode of action under EPA's draft cancer guidelines?

> *The panel reached unanimous consensus that the proposed MOA was adequately supported by the weight of the evidence and that the proposed MOA was relevant to humans at environmentally relevant doses. (However, the panel noted that they did not conduct a thorough exposure analysis). However, there are some remaining uncertainties regarding the cellular mechanisms and the cell of origin involved. There was a suggestion that the document draw comparisons to U.S. EPA's chloroform assessment for similarities in arguments regarding the rapidity of the reaction of thiophosgene. The panel noted that there are no known explanations for the species specificity of the small intestine tumors, nor are there data to demonstrate if humans would respond more like rats (non-responsive) or mice (responsive) for this tumor type. The panel suggested that the document include a discussion of the susceptibility of children under this proposed MOA, and noted several lines of evidence that could be considered in this type of evaluation.*

Revision: Anticipated human exposures were added to Section V Hazard Identification, D. Human Exposure and Epidemiology. The U.S. EPA has calculated the human dietary exposure and agricultural worker exposure. A discussion of chloroform was included in section VII. Proposed Mode of Action, E. Biological Plausibility. A multifaceted comparative study in rats and mice has been performed with folpet, the fungicide that shares a common mechanism of toxicity with captan, many of the endpoints evaluated showed quantitative differences between these two species; however, there were no qualitative differences that would unequivocally explain why mice are susceptible to tumors and rats are not. We assume the worst case; that is, humans will respond as mice. Although there is little direct data, a discussion of the susceptibility of children was

added in section VII Proposed Mode of Action, F. Relevance of proposed mode of Action to Humans.

Charge Question #8: Does the weight of evidence narrative in the document adequately explain captan's human carcinogenic potential and the conditions (e.g. routes, magnitude and duration of exposure) that characterize its expression? Does it adequately summarize the key evidence supporting these conclusions? What scientific uncertainties remain with respect to captan's mode of action, and what data are needed to resolve these issues?

> *The panel did not vote on consensus regarding the weight of evidence narrative because the panel felt that the document would need to be revised to incorporate the suggestions and scientific points summarized throughout the course of the meeting before an accurate WOE narrative could be prepared. Therefore, the panel did not provide specific wording changes for the WOE narrative, but did agree that the science supports the statement that captan is "likely to be carcinogenic only following prolonged oral exposures at doses causing cytotoxicity and regenerative hyperplasia in the gastrointestinal tract (primarily duodenum)" and that captan is "not likely to be carcinogenic at doses that do not result in cytotoxicity and regenerative hyperplasia." The panel suggested that the authors follow examples from the EPA's (2003) Draft Cancer Guidelines and current EPA cancer assessments, e.g., chloroform and atrazine) when revising the WOE narrative for captan.*

Revision: The WOE narrative was reorganized to better reflect the EPA's (2003) Draft Cancer Guidelines. We listed the "Not likely" scenarios first as they most relevantly reflect captan's carcinogenic potential to humans.