Report of Peer Review Meeting
Cancer Assessment for Captan
September 3-4, 2003

Introduction

An independent panel of expert scientists met in Cincinnati to review a cancer weight of evidence assessment for the pesticide captan. The Sponsor for the meeting was the Captan Task Force, composed of the following member companies: Arvesta Corporation, San Francisco and Makhteshim-Agan of North America Inc., New York. The author of the document was C. Wilkinson, LLC, Burke, VA.

This peer review meeting was conducted by Toxicology Excellence for Risk Assessment (*TERA*); a non-profit organization dedicated to the best use of toxicity data in risk assessment. Expert peer reviewers donated their time and talents to provide an independent review of the assessment. The objective was a comprehensive overall review of the materials as provided by the combined experience of all the reviewers. This meeting summary represents the major discussions and conclusions of the panel as a whole.

This review meeting followed a standard *TERA* process, beginning with a close examination of the supporting documentation and important references by the panel prior to the meeting. At the meeting, the authors of the assessment briefly presented their work. The panel then systematically discussed the assessment, including a discussion of the data available to identify captan's tumorigenic potential, support alternative mode of action hypotheses, and the qualitative weight of evidence.

Full discussion and participation were encouraged and agreement was reached by consensus. Consensus for the purpose of these meetings was defined as "an opinion held by all or most, or general agreement." The meeting was open to the public, but no public observers participated.

The meeting was attended by the following parties:

Sponsor: Dr. Elliot Gordon, Makhteshim-Agan
Dr. Ephi Gur, Makhteshim-Agan
Dr. Iris Mor, Makhteshim-Agan
Dr. Scott Mobley, Arvesta Corporation
Dr. John Kinzell, Arvesta Corporation
Dr. Doina Bujor, Arvesta Corporation
Mr. Scott Rawlins, Makhteshim-Agan

Presenter: Dr. Christopher Wilkinson

Panel Advisors: Dr. Rita Schoeny, U.S. EPA
Dr. John Foster, AstraZeneca, United Kingdom

Chair: Dr. Andrew Maier, *TERA*

Review Panel:

- Dr. Matthew Bogdanffy, E.I. du Pont de Nemours and Co., Inc.
- Dr. Michael Gargas, The Sapphire Group
- Dr. Dawn Goodman, Covance Laboratories, Inc.
- Dr. Gordon Hard, Consultant
- Dr. Martha Moore, National Center for Toxicological Research, U.S. Food and Drug Administration
- Dr. Steven Robison, Procter and Gamble
- Dr. Annette Shipp, Environ Health Sciences Institute
- Dr. Lawrence Sirinek, Ohio Environmental Protection Agency

Conflict of Interest

After brief introductions, the meeting began with a discussion of conflict of interest (COI). A brief general statement explaining the COI policy was given. Each reviewer had certified in writing prior to the meeting that he or she did not have a conflict (real or apparent) with the chemical under review and he or she had no affiliation with the sponsors and authors (identified to the reviewers before the meeting). Alternatively, the reviewers identified the potential for such conflicts prior to the meeting. *TERA* staff discussed any potential conflicts with each reviewer to determine if measures were needed to manage a potential conflict (or appearance of conflict). *TERA* presented a plan for managing conflict of interest to the panel (see Appendix A).

Each panel member gave a brief introduction and added any additional statements for inclusion in their previous COI disclosure. The panel agreed to each participant's participation as documented in Attachment A.

Author Presentation

Dr. Christopher Wilkinson presented an overview of the cancer assessment for captan, assisted by Dr. Elliot Gordon of Makhteshim-Agan and Dr. Scott Mobley of Arvesta Corporation (The slides from the presentation can be found in Appendix B). The presenters noted that captan is currently classified as a B2 carcinogen following U.S. EPA's 1986 cancer risk assessment guidelines. The purpose of the September 3-4 review was to reassess the cancer classification for captan following EPA's current Draft Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2003). The presentation started with an overview of the known metabolic pathways for captan. Captan is hydrolyzed to THPI and thiophosgene, the rate of which is pH dependent. Under alkaline conditions, this occurs in a matter of minutes. Therefore, this reaction is likely to be more rapid in

the duodenum than in the acidic conditions of the stomach. Thiophosgene reacts rapidly with a variety of functional groups and is short lived. Captan and thiophosgene react rapidly with thiols (e.g., glutathione) in the gastrointestinal tract (GI tract) lumen contents or in the epithelial cells lining the GI tract. This reaction has been measured in the blood and has been shown to have a half-life of less than a second, making transportation in systemic circulation highly unlikely.

The presenters noted that duodenal tumors in mice, kidney tumors in male rats, and uterine tumors in female rats have all been observed in cancer bioassays with captan. The presenters concluded that the mouse duodenal tumors were biologically relevant and related to captan treatment. However, the kidney and uterine tumors were not considered to be related to captan treatment. Several lines of evidence for these conclusions were presented. The increased incidence of renal tumors was seen in only 1 of 4 rat studies and only in males (Goldenthal et al., 1982). In addition, the effect is limited to primarily adenomas. The increase in tumor incidence is not statistically significant by pair-wise analysis ($p>0.05$) and the dose-related trend was statistically significant only when adenomas and carcinomas were combined and only using a questionable analytical method: Cochran-Armitage trend test without correction for continuity. In its cancer assessment of captan, U.S. EPA had concluded that data "show only a borderline increase in kidney tumors." As with the kidney tumors, the increased incidence of uterine tumors in female rats was seen in only 1 of 4 studies (NOASR, 1983), even though other studies used higher dose levels. The study was unusually long (120 weeks) and no historical control data are available for a study of this duration making it difficult to judge whether the observed tumors are consistent with background rates. Also, the uterine sarcomas recorded in this study were not a homogeneous group of malignant tumors. Three separate tumor types constitute the total increased incidence recorded by U.S. EPA, and the grouping of these three tumor types into one class has dubious validity since tumors of different, or potentially different, tissue origin would not normally be treated together. There was no evidence of the usual progression of uterine sarcomas from uterine polyps. The increased incidence of sarcomas in the high dose group was statistically significant only when tumor types are inappropriately combined and a more appropriate analysis of combined sarcomas and polyps shows no treatment-related effect.

The presenters also summarized the genotoxicity data for captan. They concluded that captan is generally positive in several *in vitro* test systems for genotoxicity with bacterial (*S. typhimurium, B. subtilis, E. coli*) as well as mammalian cells (Chinese hamster ovary, mouse lymphoma). The effect is much less marked in the presence of an S9 metabolic activation system or other exogenous sources of thiols. Tests with a wide range of *in vivo* assays for mutagenicity are overwhelmingly negative. Gavage administration of high dose levels of captan (1,000 mg/kg/day for 5 days) produced no clastogenic effect in duodenal stem cells and did not increase nuclear aberration frequency in the positive control (dimethyl hydrazine). There is no evidence that ^{35}S-captan binds covalently to DNA in the duodenal stem cells following oral administration. The presenters concluded that although captan appears to be mutagenic *in vitro*, its high reactivity will prevent it from reaching the DNA in biologically relevant situations, supporting the conclusion that

captan is not genotoxic in *in vivo* assays. Therefore, the presenters concluded that captan is likely operating by a non-genotoxic mode of action.

The presenters noted that non-neoplastic effects were observed in the mouse duodenum in the bioassays in which mouse duodenal tumors were observed, including crypt cell hyperplasia, shortening of the villi, immature cells at the villus tips, and disorganization of the villi cells (Tinston, 1996). These effects occurred in the same region of the small intestine as the tumors and demonstrated similar dose-response relationships and NOAELs. The non-neoplastic effects always precede tumor formation and both non-neoplastic effects and tumors appear to be reversible following cessation of exposure to captan. Therefore, the mode of action proposed by the presenters includes irritation and inflammation of the proximal duodenal epithelial cells. This is followed by cytotoxicity and epithelial cell necrosis. Epithelial cells are rapidly lost, the villi shorten followed by regenerative hyperplasia and crypt basal cell proliferation. This cell proliferation increases the probability of "fixing" spontaneous DNA damage that would normally be repaired, resulting in neoplasia of crypt cells.

Based on this mode of action (MOA), the presenters suggested that the appropriate weight of evidence descriptors should be

- Likely to be carcinogenic to humans following prolonged, high level oral exposures causing duodenal cytotoxicity and regenerative cell hyperplasia
- Not likely to be carcinogenic to humans at dose levels that do not cause cytotoxicity and regenerative cell hyperplasia of the duodenum
- Not likely to be carcinogenic to humans following dermal or inhalation exposure

Clarifying Questions

One reviewer asked about a mouse study (Pavkov, 1985) that included two recovery phases, indicating that this study included information about non-neoplastic effects that are critical for supporting the mode-of-action conclusions. This reviewer noted that while a limited description of the study was present in the section on mechanistic studies, the description should be expanded and included in the discussion of the tumor studies. Other panel members agreed. The sponsors indicated that this study shows that at least 6 months continuous exposure to captan is needed for tumors to develop. If the exposure is stopped prior to this time, then tumors do not form.

One reviewer asked why the Wong et al. (1981) mouse study had such different results (higher incidence of tumors) than the other mouse studies. The sponsor indicated that the Wong et al. study used a different technique to sample the small intestine than the other studies; the Swiss roll technique samples more of the small intestine, resulting in a higher overall incidence of tumors identified. Another reviewer suggested that the longer duration of the Wong et al. study would result in a higher incidence of observed tumors as well.

A reviewer asked why the document described the small intestine effects as limited to the first 7 cm of the duodenum. The sponsors indicated that this was due to the pH change in the small intestine as captan passes through the GI tract. Captan breaks down more rapidly as pH increases, and lower down in the GI tract, it is unlikely that significant captan remains unreacted. However, another reviewer indicated that one study (Tinston, 1996) found hyperplasia, but no tumors, in the jejunum following captan exposure. This reviewer asked if captan/glutathione (GSH) conjugates were higher or lower in other regions of the GI tract and whether these conjugates in the GI tract could be contributing to the tumor formation. The sponsors indicated that as far as they were aware, the GSH levels were constant throughout the small intestine. In support of this, the results of a mouse study for folpet (a structurally-related compound) were cited. In this folpet study, measured GSH concentrations in the small intestine were 3.16 millimolar in the duodenum, 3.87 millimolar in the jejunum, and 3.32 millimolar in the ileum (Chasseaud and Hall, 1991). A reviewer also asked whether these GSH levels were measured in whole tissue homogenates. The sponsor indicated that they were measured in homogenates.

The sponsors also indicated that the thiol groups in dietary proteins will bind with the reactive metabolites of captan; however, at high doses, some captan may not be hydrolyzed or sequestered by GSH or proteins, and therefore, may reach lower portions of the GI tract. Another reviewer agreed, noting that as administered levels of captan increase, the biology of the small intestine changes – as the duodenum is damaged, it functions differently and may have a lower ability to sequester captan. The jejunum may take over some duodenal functions. A different reviewer asked if captan binding to GSH in the small intestine is mediated by glutathione transferases (GSTs) or by direct binding. The sponsor indicated that the reaction was likely to be direct rather than enzymatic. A different reviewer clarified, noting that while the rate for spontaneous reactivity was likely to be high, GST could be expected to contribute some to the overall binding activity.

Another reviewer asked if the sponsor had completed any GSH depletion studies. The sponsors indicated that they had for the pesticide folpet, which appears to be acting by an identical mechanism as captan in terms of duodenal effects (Bernard and Gordon, 2000; Chasseaud and Hall, 1991). The study indicated that if the compound is administered as a continuous exposure (e.g. in feed) then there are increased GSH levels, but if the compound is administered as a bolus dosed then GSH is initially depleted in a transient way. This reviewer then asked how the GSH depletion mechanism relates to the tumor mode of action. The sponsor indicated that the proposed mechanism is not based on GSH depletion being a causal event, but rather on the depletion of captan by thiols so that captan never gets down to the crypt cells. Thus, GSH prevents any genotoxic effect of captan. After reviewing the study on GSH levels following folpet dosing, the reviewer agreed that this study provides support for the proposed MOA in that it shows an initial decrease of GSH following exposure, in both the duodenum and the ileum. However, the reviewer also indicated that the study was compromised because it was conducted using whole tissue homogenates, which would have diluted the overall impact of captan on GSH concentrations in the immediate target cells. The sponsor also noted that not just

GSH is involved in this mechanism; other biological sulfhydryl groups (such as found in cysteine or other amino acids) also contribute.

A reviewer asked whether the proposed MOA was that neither captan nor the metabolites reached the basal cells in the small intestine. The sponsors indicated that that was correct. This reviewer also asked whether THPI, the primary metabolite of captan is mutagenic. The sponsor indicated that it is not. However, another reviewer pointed out that the Pritchard and Lappin (1991) study (discussed in the document on page 19) suggested that some of the captan appeared to be reaching the stem cells in the small intestine, since following administration of ^{35}S labeled captan, ^{35}S-radioactivity was associated with DNA in all tissues tested. A different reviewer indicted, however, that the Pritchard and Lappin (1991) study was conducted using tissue homogenates, so it does not address what compounds are specifically getting into different cell populations.

A reviewer asked a question regarding the data presented on the kinetic curve of captan in blood (see slide 6 of presentation, Appendix B). Specifically, this reviewer wanted to know if the binding to sulfhydryl groups overwhelms the role of pH in degrading captan. If so, the reviewer questioned how this observation applies to the kinetics in the duodenum. The sponsors replied that even at pH 7, captan hydrolysis still takes about 2 hours, suggesting that the chemical reaction between sulfhydryl groups (or other nucleophilic centers) and captan would likely overshadow the loss through hydrolysis.

Discussion of Charge Questions

Following clarifying questions, the panel discussed the charge questions that had been submitted to them for consideration. The charge questions were loosely organized following the analytical approach presented in U.S. EPA's 2003 Draft Guidelines for Carcinogen Risk Assessment and included questions regarding data availability, analysis of tumor data, analysis of other key data, the mode of action, and the weight of evidence narrative. The entire charge document is available in Appendix C.

> **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?**

A reviewer asked how the sponsors had conducted the literature search and how they had decided which studies to include in the report. The sponsors replied that they continually search all relevant toxicity databases to identify new studies regarding captan and that all relevant studies that were located were included in the document. They also noted that many of the relevant studies on captan are not published in the open literature. Another reviewer suggested that the document would be improved by including a description of the literature search strategy used to identify studies, as well as criteria that would be used for excluding any studies from discussion in the document.

Another reviewer noted that an epidemiology study relevant to captan (Mills, 1998) was located that had not been included in the document. The study identified a correlation between leukemia and one county in California where captan was used. The sponsors noted that they were aware of this study. However, the sponsors felt that the study has limited overall use for a captan assessment because it was of an ecological study design, was of limited geographical scope, and included exposure to several pesticides. The impact of this study on the document was discussed in more detail during the discussion of human data. Another reviewer suggested evaluating the utility of available studies for agricultural workers who may be exposed to captan.

Two reviewers commented that the document should include more data from the genotoxicity studies than just whether the studies were positive or negative. Additional information on the methods and the study quality would help with study interpretation and is useful when genotoxicity is the key issue in a cancer assessment. These reviewers gave a more detailed summary of the types of data that are important to include during the discussion of genotoxicity.

One reviewer indicated that the document was generally well done for an overview document. However, this reviewer suggested that the document should include more data on the general molecular pathology of GI tumors since summarizing this data would have given good support to the proposed MOA. In addition, the document should include descriptions of the available data on studies by the inhalation and dermal routes of exposure, since currently the document does not provide any data to support the statements made in the weight of evidence narrative on those pathways. The sponsors replied that there are limited data by the inhalation and dermal pathways. Only limited human studies by the inhalation route are available. In one study of workers, air levels of captan were not measured although captan made up 40% of the dust. No increase of deaths from cancers were observed. One dermal study (Antony et al., 1994) shows that captan will act as an irritant and promote tumors in mouse skin. One reviewer asked if, based on these studies, one would also expect that captan would act as a lung irritant if inhaled. The sponsors replied that this would be a reasonable conclusion. The reviewer then indicated that given this conclusion, it is not reasonable to state that captan will not cause lung tumors without specific data to support the statement.

Other reviewers commented that the document should include better data on historical control values for comparison with the bioassays on captan. Two reviewers had identified sources of historical control data and agreed to provide it to the sponsors (Handbook of Toxicology, 2002).

Conclusions for Charge Question #1. 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.

- The document should include a description of the literature search and the criteria used to determine which studies would be included in the analysis.
- The document should include more detail in the study summaries.

- 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.
- 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 statements regarding those routes of exposure.
- Include data on GI tract pathogenesis for other agents (as well as data on GI tract pathogenesis and physiology in general).
- Use updated references for tumor incidence for historical controls.
- For mechanistic data, add discussion of studies that support the MOA such as GSH depletion studies, and rat kinetic studies.

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

One reviewer opened discussion by noting that the major problem with the epidemiology study of Wong and Harris (2000) is lack of statistical power to conclude that captan exposure is not associated with increased cancer deaths in humans. In addition, dosimetery might be another issue. The study only looked at cancer deaths instead of cancer incidence. If duodenum cancer is a reasonable endpoint, it is extremely difficult to draw the conclusion that captan is not carcinogen from this study because, based on some NCI data, the small intestinal cancer rate is very low (about 2 per100,000 in general population). It is very hard to test any increase in a population of 400 for a change from such low background incidence. The sponsor noted that the population of 410 in this study included both exposed and control populations.

One reviewer disagreed with the conclusions regarding the epidemiology study that are presented on page 14 of the document. This reviewer felt that it was inappropriate to conclude that the study offers no evidence of increased duodenal cancer because the study did not measure cancer incidence in the cohort, rather cancer mortality was evaluated. Several other reviewers agreed. One reviewer suggested that information on the background incidence of duodenal cancer in humans should be added. Reviewers suggested that this study description be revised to indicate that the study shows no evidence of increased cancer mortality or no evidence of increased deaths from duodenal cancer.

Another panel member advised that U.S. EPA's (2003) cancer guidelines do not require tumor site concordance between humans and animals for studies to be considered in the overall weight of evidence. Therefore, this panelist suggested that a revised document should not focus only on the fact that no increased deaths from duodenal cancers were observed in humans, since absence of cancer deaths attributed to a single site are not required in making overall weight of evidence conclusions.

Another reviewer asked whether any epidemiology studies of captan applicators had been conducted. Any negative results from these types of studies would provide

additional support for the document. One reviewer noted that NCI had done a study of pesticide applicators, and the sponsor indicated that one study of captan applicators is still ongoing. The sponsor noted, however, that multiple exposures confounded the study. One reviewer asked if there were any air monitoring data in manufacturing facilities that could provide an upper bound on exposure for the epidemiology study. The sponsor replied that levels of 2 mg/m^3 dust containing captan have been measured in production facilities. Another reviewer asked if captan was applied by being dissolved in water and sprayed. Based on this line of questions, the reviewer suggested that the document should include a discussion of how captan is used and the potential exposure routes in humans.

Another reviewer asked what other compounds could be found in captan formulations. Since some of the genotoxicity assays were conducted with technical grade captan, some of the materials in the formulation other than the active ingredients could be contributing to the assay results. The sponsor noted that "pure" captan is about 92% captan and 8% inert ingredients, but that applied formulations only contain about 75% captan. While the inert ingredients vary significantly with the formulation, a significant portion of the total inert ingredient content is talc.

One reviewer suggested that literature on the acute effects of captan in humans should be discussed in the document. Since the irritation-based duodenal effects in mice are essentially acute effects, acute data in humans may provide support for the proposed MOA. Other reviewers agreed with this suggestion.

Conclusions for Charge Question # 2. The panel reached unanimous consensus that the Wong and Harris (2000) epidemiological study is insufficient to support definitive 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 in the document 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 the Mills (1998) study, information from studies of pesticide applicators, and information on the acute effects of captan in humans should be included in the discussion of the proposed MOA.

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 discussed issues surrounding the data on rat kidney tumors, rat uterine tumors, and mouse small intestine tumors.

Rat Kidney Tumors. An increase in kidney tumors was observed in one of the four bioassays conducted in rats (Goldenthal et al., 1982). One reviewer noted that the tumors observed in this study were not statistically significant by a pairwise test, but that they

were found to be statistically significant by a Cochran-Armitage test run without continuity, as reported in an earlier assessment by U.S. EPA (1989). However, this reviewer also noted that the National Toxicology Program (NTP) recommends that this statistical test be run with continuity to ensure that rare tumors are not found to be false positives. When the Cochran-Armitage test is run with continuity, the kidney tumors are not statistically significant.

This reviewer also noted that when a chemical induces renal tubule tumors, there is usually an associated increase in the incidence of focal pre-neoplastic lesions. However, these lesions were not observed in the Goldenthal et al. (1982) study, suggesting that the kidney tumors are most likely to be due to chance, rather than captan exposure. Also, this reviewer observed that while the incidence of nephropathy was comparable between the control and treated animals; the high dose animals had a 50% increase of blood urea nitrogen (BUN) compared to controls. This might suggest that the high-dose group had more rats with end-stage chronic nephropathy that puts them at an increased risk for developing tumors, which would suggest the tumors are irrelevant to humans. This reviewer also mentioned that there is a specific morphological phenotype for some rat kidney tumors that form spontaneously. Although the study did not identify the phenotypes of the tumors, the reviewer noted that information on the phenotype would have helped to determine if the kidney tumors had formed spontaneously.

One reviewer noted that chronic progressive nephropathy is a proliferative and regenerative disease that can be enhanced by some chemicals, but that this is not relevant to humans. Another reviewer indicated that the key point in considering the kidney tumors is that no corresponding non-neoplastic renal toxicity was observed. A different reviewer asked how the incidence of kidney tumors compared to historical controls. The sponsors replied that they were essentially the same as historical controls.

Other reviewers mentioned two other modes of action that are known to contribute to the development of male rat kidney tumors for some chemicals: alpha-2-microglobulin accumulation and beta-lyase activation of conjugated metabolites. The sponsor noted that some unpublished studies had been conducted using anti-alpha-2-microglobulin antibodies and these studies indicated that this MOA was not involved in the formation of kidney tumors following captan exposure. The panel suggested that the document should include a brief discussion presenting the evidence for or against a role of these or other common MOA hypothesis for kidney tumors.

The sponsors asked whether the document should include any discussion of structure-activity relationship analysis, such as using data for related compounds such as folpet and captafol. Several reviewers agreed that this type of information should be included, noting that information of this type can provide support for an overall proposed MOA. One reviewer mentioned a 28-day study for THPI, which is a metabolite of captan, (BIBRA, 1997) in which spontaneous nephropathy was observed. The reviewer suggested that this study be included in the document because it will give support to the issue of spontaneously forming kidney tumors.

One reviewer asked whether in a statistical analysis of tumor incidence data the correction for continuity is commonly used. Another reviewer answered that the kidney tumors did appear to be spontaneous tumors, but that test for continuity should be done to confirm this, and that NTP recommends that this correction be used. Another reviewer asked whether the animals that died early were included in the statistical analysis. The sponsor replied that they were not. Two reviewers indicated that current policy is to include them, and noted that when animals are censored (e.g., excluded from the analysis) statistical significance would occur that would not occur if the animals had not been censored. Another reviewer suggested that the statistical analysis should be redone including all animals. One reviewer noted that it is policy to conduct a pairwise test first, then a trend test.

Rat Uterine Tumors. The panel discussed Table 3 on page 13 of the document, which showed that one rat study (NOASR, 1983) out of four studies reported uterine stromal sarcomas, poorly differentiated sarcomas, and unclassified sarcomas, as well as fibromatous and multiple fibrous polyps. Two reviewers noted that the stromal sarcomas arise from a different tissue type than the other sarcomas, and should be statistically analyzed alone rather than by combining them with other sarcoma types. In addition, these sarcomas are known to develop from uterine polyps, so that stromal sarcomas should also be combined with polyps for statistical analysis. These reviewers expressed the opinion that the uterine tumors were not likely to be treatment related. Other panel members agreed. One panel member noted that background data on uterine tumors in Wistar rats is available (Handbook of Toxicology, 2002) and should be included in the document.

One reviewer asked if it was possible to determine that the "unclassified" sarcomas were not actually stromal. Another reviewer replied that it is difficult to determine without the slides, but that stromal sarcomas are very characteristic tumors, and they generally appear with other characteristic tumor types. Therefore, it is likely that stromal sarcomas would be clearly identified and that the "unclassified" sarcomas likely arose from other tissues. Therefore, this reviewer would not combine the unclassified sarcomas with the stromal sarcomas. A different reviewer noted that when the stromal sarcomas are combined with the stromal polyps, the combined incidence was not statistically significant. In addition, if a risk assessment would take the conservative approach that all sarcomas be combined, when all sarcomas are combined with polyps, which is an appropriate additional approach, then the combined incidence is not statistically significant.

One reviewer suggested that the document should add a more detailed description of each of the rat studies, including those that did not observe either kidney or uterine tumors. In addition to giving the strains of rat in these studies, the document should discuss how these studies contribute to the overall weight of evidence that the rat tumors are not biologically relevant or treatment related.

Mouse Small Intestine Tumors. The panel first discussed the differences between rats and mice, and the possible reasons for the lack of small intestine tumors observed in rats.

Although the panel acknowledged that there were no data to conclusively support a specific conclusion, it did discuss several theories. First, it is possible that the mouse has a higher background rate of tumor formation because there are more pre-initiated cells in the mouse small intestine. Second, it was noted that that the mouse actually consumed higher doses of captan per body weight than rats. Finally, it was noted that the maximally-tolerated dose (MTD) is lower in rats, so rats may be dying before they have the opportunity to develop small intestine tumors. The panel suggested that the document should include a discussion of the potential explanations for the differences between rats and mice.

The panel then discussed the studies that observed small intestine tumors in mice (Daly and Knezevich, 1983; NCI, 1977; Wong et al., 1981). It was noted that at the time these studies were conducted, the focus of the study was on the proliferative lesions, not on the accompanying non-neoplastic effects. One reviewer noted that there were small numbers of animals in the control groups and that the historical control data shows very small numbers of tumors, indicating that this tumor type is rare. The Daly and Knezevich study also observed some tumors in the jejunum and ileum in addition to the duodenum, and that some of these tumors were observed in animals that also had duodenal tumors.

A reviewer opened discussion on this topic by noting that the Wong et al. (1981) study might have had higher numbers of tumors for two reasons – first was the long duration of the study (28 months) which gave the opportunity for more tumors to develop. Second, the study used the Swiss Roll technique for sampling intestinal tumors. This technique samples the entire length of the small intestine and is more likely to identify small tumors and lesions. In this study, survival was decreased after 22 weeks, and the document should address the effect of decreased survival. There were hyperplastic lesions in the stomach and jejunum, and this reviewer suggested that the incidence of hyperplasia should be included in the document. This study also observed tumors in the jejunum of female mice and the incidence of these tumors should be included in the document. It was not clear if these tumors would be statistically significant, but they are biologically significant, based on the overall proposed mode of action.

This reviewer noted that the NCI (1977) study observed adenomatous polyps that were listed as tumors. These lesions should be described in the document. Also, from the NCI study, the incidence of duodenal adenomas and carcinomas should be added together. This reviewer considered that captan is carcinogenic in the small intestine of mice and that the tumors observed are both treatment related and relevant to humans. This reviewer also suggested that the document should discuss the significance of lesions observed in the stomach and jejunum. In addition, this reviewer commented that the statement in the document that captan is primarily carcinogenic in the "proximal 7 cm portion of the duodenum" is not supported by the mouse bioassays, but rather is information that was gathered from the mechanistic studies.

Another reviewer suggested that the tumor nomenclature used in the report is inaccurate – the bioassays do not identify the small intestine tumors as "crypt cell adenomas." Rather these tumors should be identified as adenomas of the duodenum. In addition, this

panel member indicted that there is not a clear transition between the different sections of the small intestine – there are gray areas and some of the tumors could have actually been located in the areas in between the distinct sections. For this reason, this reviewer suggested that the MOA could be considered to apply to the entire small intestine. With increasing dose and duration of captan exposure, more captan would be available lower down in the small intestine, resulting in hyperplasia and tumors in those sections. This reviewer felt that the document should discuss and explain this possibility.

Other reviewers agreed with this idea, noting that it is supported by the observation of hyperplasia in the stomach and jejunum. It also supported by the Wong et al. (1981) study in which both higher doses and longer durations resulted in tumors in the jejunum.

The panel then discussed the proper nomenclature for the tumors in the document. One reviewer suggested that the tables in the document should reflect the actual tumor nomenclature used by the study authors. Then the discussion can mention where the tumors arise, if known, and discuss the mechanism of tumor formation. A different reviewer suggested that the Pavkov study would help with this discussion because it used specials stains to identify the cells of tumor origin, and suggested that the discussion of the Pavkov (1985) study be expanded and included in the section on tumor studies in the document.

One reviewer mentioned that dilation of the duodenum was observed in the bioassays, and suggested that the document could discuss the significance of this finding. If this is related to stasis in the small intestine, this could help explain why tumors are found primarily in the upper GI tract (since the lumen contents, including captan, would move more slowly down the GI tract under stasis conditions). Another reviewer indicated that there are several reasons for a finding of dilation, and that it might be speculative to place too much weight on this finding.

The panel also discussed the inflammatory response generated by the irritant nature of captan. It was noted that based on the histology slides, it appeared that altered structure in the lamina propria had occurred, which would be followed by macrophage infiltration into the lamina propria and inflammatory infiltrate. This inflammatory response could be contributing to the reaction observed in the small intestine, and one panel member also noted that chronic inflammatory response from crypt cells could also be producing free radicals. Another panel member commented that this change in histology might make the lumen leaky, which could impact the systemic absorption of captan.

The panel continued to discuss the contribution of non-neoplastic lesions to the overall conclusions regarding carcinogenicity. One panel member noted that in the NCI (1977) study, the animals were off captan exposure for 10 weeks prior to sacrifice, so it is possible that any non-neoplastic effects that might have occurred were reversible. However, in the Wong et al. (1981) and Daly and Knezevich (1983) studies, the incidence of hyperplasia does support the MOA, even if the inflammatory component observed in the shorter-term studies was not observed in these studies. One reviewer asked how this conclusion could be drawn when tumors were observed in the Daly and

Knezevich (1983) study at lower doses than the hyperplasia. Another reviewer replied that, although the incidence of hyperplasia was small at the lower doses and not statistically significant, it does support the conclusion that hyperplasia occurs before tumor formation. Another reviewer suggested giving the Pavkov (1985) study more consideration in the totality of the data because it does give good support for the MOA since it gives information on what is happening along the GI tract, and adds data on the temporal relationship. This reviewer suggested that the MOA analysis should give less focus to the duodenum, and more focus to discussing the events that occur along the length of the small intestine. Other reviewers agreed, noting that the document needs to include an interpretive analysis of how the MOA occurs, building the story sequentially.

Conclusions for Charge Question #3. 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. The panel also agreed that the rationale for these conclusions needs to be strengthened in the document to reflect the balance and totality of the data.

- For rat 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-2-microglogulin accumulation or beta-lyase activation of thiol conjugates. Use structure activity relationship information to strengthen arguments.
- For uterine tumors, it is appropriate to evaluate stromal sarcomas separately from other undifferentiated or unclassified sarcomas. This is consistent with NTP guidelines. In addition, it is appropriate to evaluate stromal sarcomas combined with uterine polyps as a secondary analysis.
- The explanation for the species differences between rats and mice in the development of small intestine tumors is not known, but should be discussed in the document. The panel speculated on two explanations that should be explored: 1) mouse has a higher background rate and so may have more pre-initiated cells which give rise to more spontaneous lesions; 2) for a given feed concentration, since rats eat more food they reach the MTD earlier than mice, and develop systemic toxicity prior to doses that induce tumors.
- 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, nor 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.

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?

One reviewer opened the discussion of the kinetics data by noting that these data are fairly straightforward, although, the document needs to strengthen the discussion of the interactions of captan with GSH and other macromolecules. Generally, the metabolism data are not sufficient to add significantly to the data in support of the proposed MOA, but the data do not detract from it either. This reviewer indicted that the bioavailability study (Provan and Eyton-Jones, 1996) shows distribution of radioactivity along the entire small intestine. However, the study was conducting by analyzing both the tissue and the intestine contents together, so it is not possible to tell if the radioactivity is associated with intestinal tissue or food.

This reviewer noted that Provan et al. (1995) measured covalent binding of captan to DNA in the stomach, then jejunum, then duodenum; however, the differences in binding among the tissues are not large and may not be statistically significant. In addition, the Pritchard and Lappin (1991) study, which measured ^{35}S-captan DNA binding, was also not informative because it looked at whole tissue homogenate which could not distinguish binding in the different cell types. Also, the study design was limited because it could not distinguish DNA binding from binding with other macromolecules. The kinetics data are consistent with the toxicity data, but none of the studies show definitively that captan does not reach the crypt cells.

One reviewer asked about statements in the document (page 8) that appear contradictory. The document indicates "Only THPI and its metabolites were found in the duodenum, blood and urine consistent with rapid degradation of captan in the stomach" and "following oral ingestion, captan is rapidly absorbed and distributed." The sponsors indicated that captan comes in contact with and may enter epithelial cells of the stomach and small intestine, but that it is not absorbed systematically. Another reviewer indicated that, the studies do not distinguish between radiolabeled parent compound versus metabolites, so they do not clearly indicate the form of the radioactivity found throughout the body.

One reviewer asked for clarification on which stable metabolites could be formed from captan, and on the degree of certainty that thiophosgene is short-lived. The sponsors indicated that the 3- and 4-hydroxy groups of THPI could form epoxides. In addition, there could be open ring metabolites. The sponsors indicated that studies are currently

ongoing to measure the half-life of thiophosgene. The studies are not yet complete because the half-life is so fast that it is difficult to measure using the current analytical technique. One reviewer asked about the possibility of using a single cell assay that tells if the chemical reaches the target. The sponsor replied that they could not separate cells in the GI tract and that they were trying to develop a method for scraping the villi off GI tract sections to allow the analysis of only the crypt cells.

One reviewer asked about the availability of toxicity data for captan metabolites. Another reviewer indicated that THPI has a negative genotoxicity profile. The sponsor replied that there are 2 mutagenicity studies on THPI that are negative and some negative aquatic toxicity tests. A reviewer indicated that there is a BIBRA (1997) study of THPI that the sponsors should locate and include in the document.

One reviewer asked if there were any kinetic data for THPI in rats. The sponsor indicated that there are some metabolism studies and that THPI appears to have the same ring metabolites as captan itself. A reviewer asked if captan itself is absorbed, and the sponsor replied that THPI is absorbed systematically. Another reviewer asked if captan reacted with the thiol groups on the cell surface or inside the cell. The sponsor replied that the answer is uncertain. It is clear that thiols are a sink for captan, so it is reasonable that thiols on the cell surface will degrade captan. However, it also reasonable to conclude that with high doses of captan, some may get into the cells. The sponsor also indicated that following a bolus dose of captan, GSH decreases temporarily, but then increases. A panel member suggested that these data on GSH need to be discussed in the document.

A reviewer suggested that hydrolysis may be playing a smaller role in the degradation of captan in the duodenum than indicated by the document. This reviewer noted that while the duodenum is less acidic than the stomach, it is not alkaline as discussed in the document. This reviewer also suggested that the document should add a discussion of the lumen contents and their role in modifying the bioavailability of captan.

One panel member asked how much data addresses the issue of whether captan gets into the crypt cell. Another reviewer answered that there are no direct data to answer this question. The panel discussed that a study that evaluates the concentration gradient from the lumen to the crypts would be useful. In addition, it might be useful to build a PBPK model for the duodenal microenvironment using estimates to parameterize the model. It was also suggested that kinetic data for related compounds may be helpful for assessing captan kinetics in the GI tract.

Conclusion for Charge Question # 4. 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 from it either. The mechanistic studies are confounded by methodological issues related to the high reactivity of the metabolites, and because the studies are of inadequate design to demonstrate a pattern of localization that matches with captan-induced histopathology.

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?

One panel member opened the discussion of the mechanistic studies by commenting that overall the mechanistic studies were adequate and helpful for understanding the proposed MOA. The histopathological (morphometry) studies (Foster, 1994; Tinston, 1995; Tinston, 1996; Allen, 1994) were compelling, and the Pavkov study, particularly, provides strong support for the proposal that the villi and crypts are the primary target of captan. The cell proliferation studies (Foster, 1994; Tinston, 1995; Allen, 1994) are supportive, but not remarkable. The studies with BrDU labeling only demonstrated no increases or a minimal increase above background. It was noted that, typically, much larger increases are seen for proliferative agents, but since crypt cells are rapidly proliferating naturally, there may be little additional effect of captan if it acts by inducing cell proliferation. Therefore, these studies are not inconsistent with the MOA, even if they are not strongly positive. The BrDU studies (Foster, 1994; Tinston, 1995) did demonstrate localization of the increased labeling to the duodenum. The study by Chidiac and Goldberg (1987) is generally supportive of the MOA, since it shows that captan does not induce nuclear aberrations in duodenal crypts even in under conditions of thiol depletion. This study rules out an effect of captan on DNA at the level of structural and numerical aberrations

Other reviewers agreed with the above conclusions regarding the cell proliferation studies noting that there was too much normal proliferative response to see any increase in proliferation using BrDU or PCNA labeling. A panel member suggested that a study which investigates a change in the rate of movement up the crypt rather than a basic proliferation study may help support the proposed MOA.

A reviewer commented that the proposed hypothesis is biologically plausible, and this reviewer summarized some common modes of action for general classes of GI toxicants. This reviewer suggested that the document add some text comparing captan to other compounds that are known to act on the GI tract in a similar manner.

Another reviewer suggested that the document, for example on page 26 in table 8, should integrate the short-term mechanistic studies with the bioassays and other longer-term studies. It is critical to address temporality – to demonstrate that the precursors happen before the tumors develop. Another panel member added that it is also critical to address the issue of dose, discussing the effects observed in the short-term studies that occur within the dose range of the chronic studies. A third reviewer pointed to the jejunal lesions in the oncogenicity studies noting that the Pavkov (1985) study also mentions these lesions. The late appearing nature of these lesions was suggested by the reviewer as supporting the MOA and correlating well with the mechanistic studies. Other reviewers agreed, noting that the Pavkov (1985) study is a good bridge between the short-term and the chronic studies.

Conclusions for Charge Question # 5. The panel reached unanimous consensus that the overall body of data are very supportive of the MOA; in particular they concluded that the histopathology data were strongly supportive. They noted that the cell proliferation data are not robust, but that they are not inconsistent given the limited sensitivity of this measure in tissues with a high background proliferation rate. Other mechanistic data for captan, including the ^{35}S 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 to support the general MOA and to include a discussion of the THPI toxicity data.

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 evidence, can it be concluded that captan genotoxicity does not contribute significantly to human carcinogenic potential at environmentally relevant doses?

Two reviewers indicated that, based on the available genotoxicity data, captan is not likely to be a genotoxic carcinogen. However they also noted that some data are missing in order to be certain about this conclusion. These reviewers also indicated that the document should do a much better job of completely discussing the genotoxicity data so that these conclusions are more apparent. The reviewers noted that it is not sufficient to just describe which studies were positive and which were negative. Rather, each study should be thoroughly evaluated for its quality, as any other toxicity study would be. These reviewers gave the following suggestions on how the genotoxicity data for the captan database should be discussed in the report.

These reviewers suggested that data that are only available in abstract form should be noted, however given the limited amount information presented in an abstract these studies cannot be adequately evaluated or considered as part of the weight of evidence. They indicated that the following studies were reported as abstracts: Imanishi et al. (1987); Rideg (1982); Jorgenson et al. (1976); and Simmon et al. (1977).

Each assay should be evaluated for appropriate dose selection, and acceptable negative and positive controls. These reviewers also suggested that regulatory accepted guidelines for conducting genotoxicity testing (FDA, 2003) provide good criteria for evaluating studies and a good rationale for eliminating poor quality studies as a part of the weight of evidence analysis. A weight of the evidence evaluation should be made once the individual studies are evaluated. A complete discussion of the reviewers' recommended approach to the weight of the evidence analysis can be found in Moore and Harrington-Brock (2000).

These reviewers then discussed their analysis of the available genotoxicity data for captan. They suggested that the document should be revised to improve the presentation of the data. The data for the genetic toxicology assays should be summarized in tabular form, which will permit easy evaluation of the results.

The *in vitro* data was analyzed first. Of the available bacterial mutation assays, the strongest response for captan was observed in the Moriya et al. (1978) paper. There were a number of *in vitro* mammalian genetic toxicology studies available for review. The results of the Arlett et al. (1975) study cannot be interpreted because the study had an unusually high response rate relative to the reviewers' experience with the same endpoint. The paper by Swenberg et al. (1976) gives some indication of DNA single strand breaks, but this effect is most likely the result of high dose toxicity. The study by Oberly et al. (1984) does not meet the currently accepted criteria for mouse lymphoma assay data and should not be included in the genotoxicity evaluation. The study by O'Neill et al. (1981), which showed a weak positive result for captan, is of questionable use because the positive response was only observed when there was unacceptably high cytotoxicity. The reviewers also discussed some unpublished data in the mouse lymphoma assay that showed that captan was only marginally positive, and gave negative results when S9 was added to the assay. The reviewers concluded that together data support the proposal that adding protein with thiol groups decreases the potential for mutagenicity. The reviewers also discussed a study by Tezuka et al. (1978) that should be discussed in the document. This study was considered of good quality, and reported that captan does not induce chromosome aberrations in human fibroblasts *in vitro*.

These reviewers then discussed their conclusions regarding the *in vivo* studies. They suggested reorganizing the discussion of the *in vivo* studies in the document, first describing the micronucleus and chromosome aberration studies, followed by the dominant lethal and other less commonly used study types.

The Tezuka et al. (1978) study mentioned above also conducted several good quality *in vivo* assays that should be added to the document. For example, Tezuka et al. (1978) conducted a good quality dominant lethal study of captan that was negative. This study was very similar to the dominant lethal study by Collins (1972), which was weakly positive, except that Tezuka used analytical grade captan while Collins used technical grade captan. Therefore, these reviewers suggested that the results in Collins could be due to contaminants. Tezuka et al. (1978) also conducted a good quality, negative chromosome aberration study. These reviewers indicated that the Feng and Lin (1987) study is of questionable quality, and that it appears to have mathematical errors (values appeared off by one or more orders of magnitude) and there was limited data presentation. It was noted that the Chidiac and Goldberg (1987) study is not a traditional chromosome aberration study because it did not evaluate the typical target tissue, bone marrow but assessed chromosome effects in duodenal cells, a not well validated approach.

These reviewers noted that both THPI and its methyl analog are negative in salmonella assays and *in vitro* and *in vivo* cytogenetics assays. They also noted that there are limited data to conclude that thiophosgene is positive in genotoxicity assays.

Overall, these reviewers concluded that there is evidence that captan is mutagenic in bacteria. However, they were not convinced that captan is mutagenic in mammalian cells, and this difference could be due to a difference protein and thiol content in the growth medium used in individual assays. A different panel member suggested that the positive

results in some bacterial assays but not in mammalian cells, could be due to the differences in DNA folding (lack of histone protein), and the fact that bacteria are genetically engineered to be more porous. Thus, this could explain the difference between bacteria and mammalian cells.

In addition, these reviewers concluded that the weight of evidence supports that captan is not genotoxic *in vivo*. A different reviewer asked if the *in vivo* studies actually measured mutagenicity *in vivo*. The first reviewer replied that most of the *in vivo* studies are cytogenetic studies (e.g., measure chromosome effects), rather than mutagenicity studies. This panel member indicated that ideally to strengthen the *in vivo* database, one would conduct a point mutation study *in vivo* – either in Big Blue or MutaMouse, taking care in dose selection to avoid doses that are irritant and cause the release of free radicals. Another reviewer agreed with his conclusion.

One reviewer stated that it is important to recognize that just because a chemical demonstrates genotoxicity does not mean that the tumors arise from a genotoxic event. Chloroform was cited as a relevant example for comparison to captan. Another reviewer agreed, but noted that in order to satisfy U.S. EPA's cancer risk assessment guidelines, the burden of proof is to show with data that genotoxic potential is not connected to the tumor response. A reviewer then noted that the default assumption in cancer risk assessment is that genotoxicity is equivalent to a linear dose response assessment, and there the question was asked whether there are any genotoxic compounds that are analyzed by a non-linear dose response assessment. A different panel member answered that for EPA cancer risk assessment purposes arsenic is an example of a compound that may not be genotoxic but for which the MOA has not been adequately characterized to use a non-linear assessment approach. Chloroform was given as an example of a compound that has some genotoxic potential, but for which an adequate argument has been made to move from the default linear approach.

One reviewer believed that the overall weight of evidence is that captan is unlikely to be causing cancer by a genotoxic mechanism, but acknowledged that there are some missing pieces of information. For example, the data on thiophosgene is limited. The sponsor indicated that there is one study that measured mutagenicity with direct contact with captan, which suggested that thiophosgene was responsible for this mutagenicity. Rideg (1982) tested thiophosgene directly, however, this is an abstract only and cannot be cited. The study used captan treated filter paper; captan did not directly interact with the test cells. The filter paper was made alkaline, presumably releasing thiophosgene. This study was positive, suggesting thiophosgene via vapor is mutagenic. The panel suggested expanding the discussion of that study.

However, the panel also acknowledged that based on the existing data set for captan, it may be hard to convince EPA that captan is not a genotoxic carcinogen. Suggestions from the panel to make the arguments in the document more convincing include:

- Compare the magnitude of captan mutagenicity to other mutagens
- Compare captan mutagenicity to the literature on chloroform and phosgene

- Expand the discussion of the effect of adding S9 and other thiol group donators to the *in vitro* assays and their relevance to the *in vivo* findings, since this provides support for the MOA

Conclusions for Charge Question # 6. 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* mammalian cell studies. Captan is negative in *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.

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?

One reviewer opened discussion of the MOA by summarizing what the data demonstrate about the steps that occur between the contact of captan with a cell and the formation of tumors. This reviewer commented that it is known that tumors form in the mouse small intestine, increasing in incidence with increasing dose and duration of exposure. In addition, tumors are observed further down the small intestine with increasing dose and duration. There is a correlation between tumors and localized hyperplasia that increases in severity and moves down the small intestine with dose and duration of captan exposure. Histopathological evidence shows damage to the small intestine with increasing exposure duration. There is evidence of crypt cell hyperplasia and immature cells at the tip of the villi. This effect shows a dose and temporal response at a given dose.

The reviewer then summarized what is not known about the formation of tumors following captan exposure noting that it is not known exactly how captan is acting to cause damage to the epithelium of the small intestine. It is likely that captan acts by interacting with sulfhydryl groups on cysteine and GSH, but it is not known if this interaction occurs on the surface of the villi, or inside the cells. The suggestion that a captan/thiol interaction occurs is supported by the evidence of GSH depletion. Also, there is much general knowledge about the ways that GI damage can occur. NSAIDS interfere with prostaglandins; alcohol increases membrane permeability; radiation causes crypt cell necrosis; ethyl acrylate binds to thiol groups.

This reviewer commented that the origin of the pre-initiated cell that gives rise to the tumors is also uncertain from the data, as is the specific mechanism involved. Several hypotheses were considered by the reviewer. GI tumors are rare in the mouse, making questionable the hypothesis that captan-induced proliferation targets a waiting pre-initiated cell population. It is possible that binding of captan to DNA associated proteins interferes with DNA replication. However, this theory is not supported by the genotoxicity data. Another mechanistic hypothesis is that GSH depletion and lack of protection from oxidative damage may be involved. Although the specific mechanisms remain unknown, the reviewer stated that it is clear, that tumors will not be expressed without sustained injury to the GI tract.

This reviewer then summarized the key events in the carcinogenicity for captan. These include disruption of the villi in the small intestine that shows a dose response and duration relationship. There is a temporal relationship between this disruption and tumor formation. The effects on the small intestine are reversible, as demonstrated by the Pavkov (1985) study. There is growing acceptance of the biological plausibility of the theory of cell damage leading to cell proliferation leading to tumors as an underlying MOA. This MOA is relevant to humans with the caveat that both dose and exposure need to reach a certain level before tumors will form.

Another reviewer noted that the biological plausibility of the proposed MOA is supported by general pathophysiological response of the GI tract, and data for other site of contact tumorigens. This information would be a more appropriate introduction to the discussion of plausibility than the current paragraph (page 30, first paragraph).

Another panel member noted that chloroform is a good analogy for captan, since chloroform's reactive metabolite, phosgene, likely interacts with nucleophiles in the cytoplasm and does not reach DNA. Since this is an accepted MOA hypothesis, a similar argument could be made for captan's reactive degradation product, thiophospgene. This panel member also noted that the general MOA of sustained injury leading to hyperplasia followed by neoplasms is a well-accepted concept. The reviewers agreed that it was not critical to know the precise cellular origin of tumors for this MOA to be accepted. One reviewer commented that in the kidney tumor literature for other compounds, it is often not known exactly which specific cell population gives rise to the observed tumors, but that does not invalidate the general MOA.

Another reviewer acknowledged the general acceptance of the proposed MOA, but also suggested that the document should focus on the divergence of captan from chloroform and other similar chemicals, and incorporate more of the data on what is known about the chemistry of captan in the GI tract, mapping captan through the GI tract. This reviewer also suggested that the document should expand the discussion of the potential for special risk to children. Specific considerations or data sources to discuss include: 1) differences in the GI tract physiology and captan metabolism between children and adults, 2) available genotoxicity assays that looked at effects in the F1 generation, 3) the non-enzymatic nature of the MOA reduces the likelihood of common considerations such as

age-related differences in gene expression, 4) results of the Pavkov (1985) study, which reported that animals exposed at earlier life stages did not have increased susceptibility.

Another panel member noted that there is no plausible explanation for why no small intestine tumors develop in the rat, other than some genetic predisposition in the mouse. This reviewer noted that since there is an unexplained species difference, there is no data to indicate whether humans will respond to captan more like mice or rats. It is equally possible that the mouse tumors are not relevant to humans because humans respond to captan more like rats. The discussion of human relevance in the document should be revised to consider the alternative possibilities.

Conclusions for Charge Question # 7. The panel reached unanimous consensus that the proposed MOA was adequately supported by the weight of 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 the explanations for the species specificity of the small intestine tumors are not known, nor are there data to demonstrate if humans would respond more like rats (non-responsive) or mice (responsive) to this tumor type. The panel suggested that the document should 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.

Charge Question # 8: Does the weight of evidence narrative in the document adequately explain captan's human carcinogenic potential and the conditions (e.g., route, 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?

One reviewer opened the discussion of the weight of evidence statement by indicating that the narrative should include all the data that are supportive of the MOA and should address the causality criteria (e.g., the modified Bradford-Hill criteria presented in U.S. EPA's cancer risk assessment guidelines) for the proposed MOA as well as why or why not other MOAs are applicable.

Several reviewers disagreed with the narrative conclusions regarding the dermal and inhalation pathways of exposure, because these statements are not supported by data or by the analysis presented in the sponsor's document.

Conclusions for Charge Question # 8. 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 U.S. EPA's 2003 Draft Guidelines for Carcinogen Risk Assessment and current U.S. EPA cancer assessments (e.g., chloroform, atrazine) when revising the WOE narrative for captan.

Panel Conclusions

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.

- Include a description of the literature search and the criteria used to determine which studies would be included in the analysis.
- Include more detail in the study summaries.
- 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.
- 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 statements regarding those routes of exposure.
- Include data on GI tract pathogenesis for other agents, as well as data on GI tract pathogenesis and physiology in general.
- Use updated references for tumor incidence for historical controls.
- For mechanistic data, add a discussion of studies that support the MOA such as GSH depletion studies and rat kinetic studies.

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 the Mills study and information on the acute effects of captan in humans should be included in the discussion of the proposed MOA.

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 rat 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-2-microglogulin 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 sarcomas. This is consistent with NTP guidelines. In addition, it is appropriate to also evaluate stromal sarcomas combined with uterine polyps as a secondary measure.

There appears to be no apparent explanation for the species differences between rats and mice in the development of small intestine tumors. The panel speculated on two explanations that should be explored: mouse has a higher background rate and so may have more pre-initiated cells which give rise to more spontaneous lesions; for a given concentration in feed, rats which eat more food, reach the MTD earlier than mice, and develop systemic toxicity prior to doses that induce tumors.

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, nor 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.

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 from 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.

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 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.

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 *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.

The panel reached unanimous consensus that the proposed MOA was adequately supported by the weight of 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.

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.

Specific Suggestions by Individual Panel Members

Several individual reviewers made specific suggestions for revisions to the document; these suggestions are listed below.

- Include a description of the literature search strategy used to identify studies.
- Include more data on the general molecular pathology of GI tumors since summarizing this data would have given good support to the proposed MOA.
- Include descriptions of the available data on studies by the inhalation and dermal routes of exposure, since currently the document does not provide any data to support the statements made in the weight of evidence narrative on those pathways.
- Include better data on historical control values for comparison with the bioassays on captan.
- A revised document should not focus on the fact that no increased deaths from duodenal cancers were observed in humans, since this finding does not contribute significantly to the overall weight of evidence conclusions.
- Include a discussion of how captan is used and the potential exposure routes in humans.
- Include a discussion on the acute effects of captan in humans. Since the duodenal effects in mice are essentially acute effects, acute data in humans may provide support for the proposed MOA.
- Include a brief discussion ruling out other common MOAs for kidney tumors.
- Include a discussion of structure-activity relationship analysis, including use of data for related compounds such as folpet and captafol. This type of information would provide support for the overall proposed MOA
- Since the animals that died early from kidney tumors were not included in the statistical analysis, it is possible that statistical significance occurred that would not occur if the animals had not been censored. Consider re-doing the statistical analysis including all animals.
- Include a description of a 28-day study of THPI in which spontaneous nephropathy was observed (BIBRA, 1997). This study may give support to the issue of spontaneously forming kidney tumors.
- Add a description of the rat studies that did not observe either kidney or uterine tumors. In addition to giving the strains of rat in these studies, the document should use these studies to contribute to the overall weight of evidence that the rat tumors are not biologically relevant or treatment related.
- Include a discussion of the potential explanations for the differences between rat and mouse in the development of intestinal tumors. There are no data to indicate whether humans will respond to captan more like mice or rats. It is equally possible that the mouse tumors are not relevant to humans because humans respond to captan more like rats. The discussion of human relevance in the document should be revised to consider both possibilities.
- Address the effect of decreased survival in the Wong et al. (1981) study. Include the incidence of hyperplasia in the stomach and jejunum and incidence of tumors in the jejunum of female mice. It was not clear if these tumors would be statistically significant, but they are biologically significant.
- Describe the lesions listed in the NCI study as adenomatous polyps. Add the incidence of duodenal adenomas and carcinomas from the NCI study together.
- Discuss the significance of lesions observed in the stomach and jejunum.

- Check the accuracy of the tumor nomenclature used in the report – the bioassays do not identify the small intestine tumors as "crypt cell adenomas." Rather these tumors should be identified as adenomas of the duodenum. Revise the tables in the document to reflect the actual tumor nomenclature used by the study authors. Then the discussion can mention where the tumors arise, if known, and discuss the mechanism of tumor formation.
- Revise discussion of the MOA so that it applies to the entire small intestine. With increasing dose and duration of captan exposure, more captan would be available lower down in the small intestine, resulting in hyperplasia and tumors in those sections. The document should discuss and explain this possibility.
- Expand the discussion of the Pavkov (1985) study. Include this study in the section on tumorigenicity in the document, because this study provides significant information on tumor formation in the small intestine and specifically mentions the crypt cells. This study gives good support for the MOA because it gives information on what is happening along the GI tract.
- Discuss the significance of dilation of the duodenum that was observed in the bioassays.
- Strengthen the discussion of the interactions of captan with GSH and other macromolecules.
- Hydrolysis may be playing a smaller role in the degradation of captan in the duodenum than indicated by the document. While the duodenum is less acidic than the stomach, it is not alkaline as discussed in the document. Add a discussion of the lumen contents and their role in the bioavailability of captan.
- Add some text comparing captan to other compounds that are known to act on the GI tract in a similar manner. Examples of several mechanism of GI tract effects were noted.
- Integrate the short-term mechanistic studies with the bioassays and other longer-term studies (e.g. on page 26 in table 8). It is critical to address temporality – to demonstrate that the precursors happen before the tumors develop and to address the issue of dose, discussing the effects observed in the short-term studies that occur within the dose range of the chronic studies.
- Improve discussion of the genotoxicity data so that these conclusions are more apparent. It is not sufficient to just describe which studies were positive and negative. Rather, the studies should be thoroughly evaluated for their quality, as any other toxicity study would be. See the extensive specific suggestions in the genotoxicity section.
- Eliminate several genotoxicity studies from consideration in the document because they are from abstracts: Imanishi et al. (1987); Rideg (1982); Jorgenson et al. (1976); Simmon et al. (1977). Reorganize the discussion of the *in vivo* studies in the document, first describing the micronucleus and chromosome aberration studies, followed by the dominant lethal and other less well know study types.
- Add a discussion of the Tezuka et al. (1978) study because it conducted several good quality *in vivo* assays.
- Investigate the possibility that results in Collins genotoxicity study are due to contaminants.

- Expand the discussion of the Rideg (1982) study that measured mutagenicity without direct contact with captan, which suggests that thiophosgene responsible for this mutagenicity.
- Compare the magnitude of captan mutagenicity to other mutagens.
- Compare captan mutagenicity to the literature on chloroform and phosgene.
- Expand the discussion of the effect of adding S9 and other thiol group donators to the *in vitro* assays, since this provides support for the MOA.
- Support discussion of the biological plausibility of the propose MOA by adding information about general GI tract knowledge. Add this information to the introduction of plausibility in place of the current paragraph (page 30, first paragraph).
- Focus document discussion on the divergence of captan from chloroform and other similar chemicals, and incorporate more of the data on what is known about the chemistry of captan in the GI tract, mapping captan through the GI tract.
- Expand the discussion of the potential for special risk to children.

The panel did not conclude that any studies were required before they could reach conclusions regarding the mode of action for captan carcinogenicity. However, several individual reviewers mentioned the following studies that would give additional support to the proposed mode of action, if conducted:

- a study which investigates a change in the rate of cell movement up the crypt rather than a basic proliferation study,
- a study that evaluates the concentration of captan gradient from the lumen to the crypts. For example a PBPK model that evaluates captan kinetics in the duodenal environment, using estimates to parameterize the model,
- a study evaluating point mutation *in vivo* – either in Big Blue or MutaMouse, taking care in dose selection to avoid doses that are irritant and cause the release of free radicals.

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APPENDIX A

Panel Member Biographies and Conflict of Interest Disclosures

Conflict of Interest Disclosure

Peer Review of a Cancer Assessment for Captan
September 3-4, 2003

An essential part of panel selection is the identification and disclosure of conflicts of interest to ensure credible results and confidence in the panel's recommendations. Prior to selecting the panel, *TERA* determined that a conflict of interest that would prevent a person from being considered for the panel would include authorship or previous review of this document; anyone employed by the Sponsor or Author organizations; anyone currently receiving financial support, e.g., thru contracts or grants, from the Sponsor or Author; and those with direct personal financial interests in the outcome of the review. Each panel member was asked to complete a questionnaire to determine whether their involvement in certain activities could pose a conflict of interest or could create the appearance that the peer review lacks impartiality. An answer of "yes" to any of these questions does not necessarily mean that the individual has a conflict of interest, but that additional information needed to be gathered. *TERA* staff carefully reviewed these forms and discussed the answers with the panel members to ascertain whether conflicts of interest might exist. *TERA* determined that none of the panel members has a conflict of interest as defined above. However, some of the panel members have past experience with either captan or the Sponsor or Author that may be perceived as a conflict. Information from each panel member relevant to these activities is summarized below to make sure the other panel members and the public are fully aware of these activities.

While these activities are not conflicts of interest, they are disclosed here as they may create an appearance that a panel member lacks impartiality because they have previously reached conclusions on similar issues or questions. The panel members are asked to objectively evaluate the materials for this review, and use this information, along with their personal knowledge and expertise, to independently reach conclusions on this document. In addition, if any panel member feels at any time that another member is trying to influence the outcome of the review in an inappropriate way, he or she should bring this to the attention of the Chair so that it may be addressed. These disclosures will be discussed by the panel at the beginning of the meeting.

The peer reviewers have donated their time and talents to this effort. They have been selected based upon their expertise and qualifications and are employed by many types of organizations. *TERA* strives to create a balance of expertise and affiliations for each meeting. However, individual peer reviewers represent their own expertise and views, not those of their employer, of any group who may have nominated them, or any group with which they may be associated. This peer review panel is a distinguished group, with many years experience in a wide range of disciplines.

Matt Bogdanffy, PhD. Dr. Bogdanffy is Director, Biochemical and Molecular Toxicology at the DuPont Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Co., Inc. He is a Diplomate of the American Board of

Toxicology, with research interests in inhalation carcinogenesis and dosimetry. Dr. Bogdanffy was selected for the panel due to his expertise in mode of action analysis, kinetics, and mutagenesis. He has no conflicts.

Michael Gargas, PhD. Dr Gargas, Managing Principal of *The Sapphire Group*™, is a toxicologist with over 25 years of related environmental experience. Dr. Gargas was selected for the panel due to his expertise in the area of human health risk assessment and biochemical toxicology research. His research emphasizes chemical metabolism, physiologically based pharmacokinetic (PBPK) modeling, and chemical dosimetry, with specific application of these approaches to risk assessments. Dr. Gargas has no conflicts.

Dawn Goodman, VMD. Dr. Goodman is the Director of Pathology North America, Covance Laboratories, Inc. She is a Diplomate of the American College of Veterinary Pathology and has over 30 years experience in the design, conduct, and pathology evaluation of animal bioassays, with emphasis on chemical carcinogenesis. Dr. Goodman has served on cancer peer review panels for U.S. EPA, the National Cancer Institute (NCI), the International Agency for Research on Cancer (IARC), and the National Toxicology Program (NTP). Dr. Goodman was selected for the panel due to her expertise in veterinary pathology and cancer bioassays. Hazelton Laboratory, the predecessor of Covance, did conduct some early (1956) studies on captan. In addition, Dr. Goodman was employed at NCI when the captan bioassay was conducted, but she was not involved with the study. *TERA* has determined that these activities are not conflicts because they are not ongoing and did not involve the sponsors of this review. Dr. Goodman has no conflicts.

Gordon Hard, PhD. Dr. Hard has been the Director of Administration and Senior Pathologist/Toxicologist for the American Health Foundation. His research has involved studying the pathogenesis of renal carcinogenesis and toxicity by developing and using animal models. This research has produced over 150 publications, including chapters on aspects of rodent kidney that were commissioned by IARC (International Agency for Research on Cancer, WHO), ILSI (International Life Sciences Institute), and U.S. EPA (US Environmental Protection Agency). For U.S. EPA, Dr. Hard has reviewed the science pertinent to chemically-induced alpha-2u-globulin nephropathy, resulting in publication of EPA's purple booklet on the subject. His microscopic re-evaluation of the rat carcinogenicity bioassays on chloroform produced new findings that were instrumental in bringing EPA to a conclusion on the risk assessment of this by-product of drinking water chlorination, applying the Agency's newly drafted risk assessment guidelines. Dr. Hard has also served in an *ad-hoc* capacity on sub-committees for IARC (monograph series), ILSI (special scientific panels), JECFA (Europe), and FIFRA (EPA). Dr. Hard was selected for the panel due to his expertise in kidney pathology and peer review experience. He has no conflicts.

Andy Maier, PhD, Chair. Dr. Maier is the Verifiable Estimates for Risk Assessment Program Manager for *TERA*. In this capacity he oversees the program for derivation of cancer and noncancer risk assessment values, and thus, has detailed knowledge of EPA risk assessment methods. He has participated in numerous *TERA* peer reviews. He is

also certified by the American Board of Industrial Hygiene. Dr. Maier was selected for participation on the panel based on his expertise in developing and evaluating cancer mode of action analyses, and his research interests in molecular mechanisms of toxicity. He has no conflicts.

Martha Moore, PhD. Dr. Moore is the Director of the Division of Genetic and Reproductive Toxicology at the National Center for Toxicological Research, Department of Health and Human Services, U.S. Food and Drug Administration. She is an international expert in genetic toxicology and until recently was Chief of the Genetic Toxicology Branch in the Environmental Carcinogenesis Division of U.S. EPA's National Health and Environmental Effects Research Laboratory. Dr. Moore was selected for the panel due to her expertise in using short-term assays to detect genotoxicity of environmental contaminants. She has no conflicts.

Steve Robison, PhD. Dr. Robison is a Senior Scientist in the Chemical Product Safety and Regulatory Affairs Department at Proctor and Gamble. He has more than 14 years of corporate toxicology experience. Prior to his work in the industrial sector, he served as an Assistant Professor in the Department of Neurology at the University of Vermont, where he conducted research on cellular mechanisms of genotoxicity. Dr. Robison has an extensive publication history in this area. He is a member of the Environmental Mutagen Society as well as the Society of Toxicology. He was selected for the panel due to his expertise in evaluating genotoxicity data and its implications for human safety assessment. He has no conflicts.

Annette Shipp, PhD. Dr. Shipp is the Managing Principal of the Environ Health Sciences Institute. Dr. Shipp is a toxicologist with experience in quantitative risk assessment including evaluations of chemicals in environmental or occupational settings, as well as investigations of cancer risk assessment methodology. Her recent research has been in the area of complex cancer hazard identification and dose response assessments for chemicals such as acrylamide, PFOA, and coal-tar containing shampoos. Dr. Shipp was selected for the panel due to her expertise in cancer risk assessment methodology. She has no conflicts.

Lawrence Sirinek, PhD. Dr. Sirinek is the Risk Assessment Coordinator for the Ohio Environmental Protection Agency. In this capacity, Dr. Sirinek is responsible for developing and reviewing site-specific risk assessment documentation as well as developing state risk assessment policies. Prior to his appointment with OEPA, Dr. Sirinek was an assistant professor and senior research scientist at Ohio State University's Childrens Hospital Research Foundation where his research focused on organ transplantation and cell-mediated immunology. Dr. Sirinek has served on previous *TERA* peer review panels and is an *ad hoc* member of the FIFRA Scientific Advisory Panel. He was selected for the panel due to his expertise in risk assessment issues and peer review experience. Dr. Sirinek has no conflicts.

APPENDIX B

Sponsor Presentation

Overview Analysis of Carcinogenic Potential of Captan

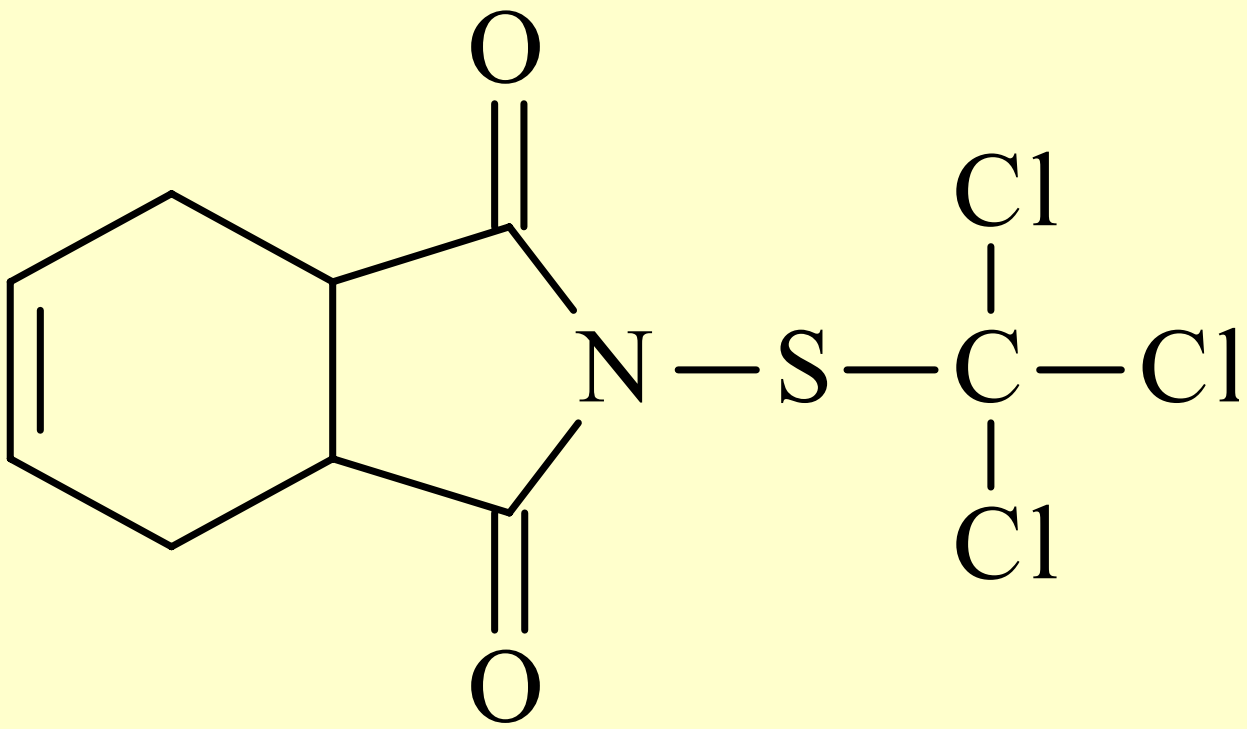
Chris F. Wilkinson, Ph.D.

Peer Review Meeting

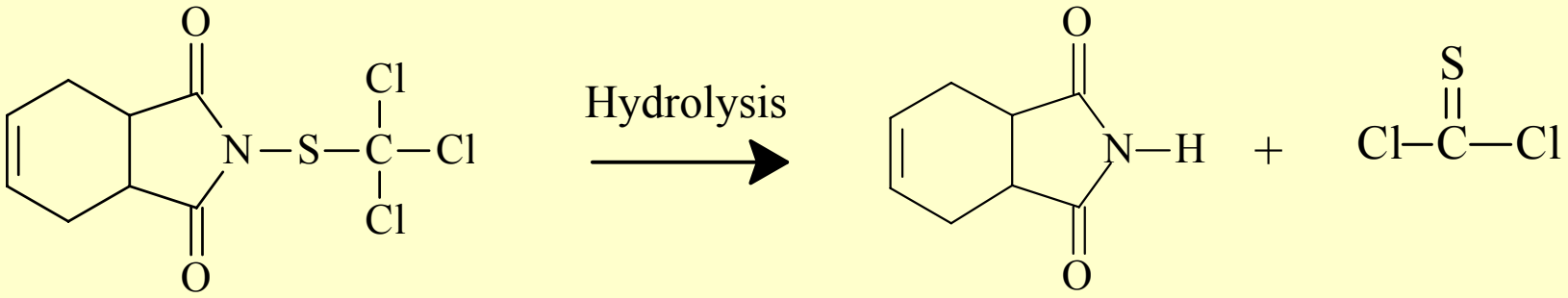
Cincinnati, September 3-4, 2003

Captan

(N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide)



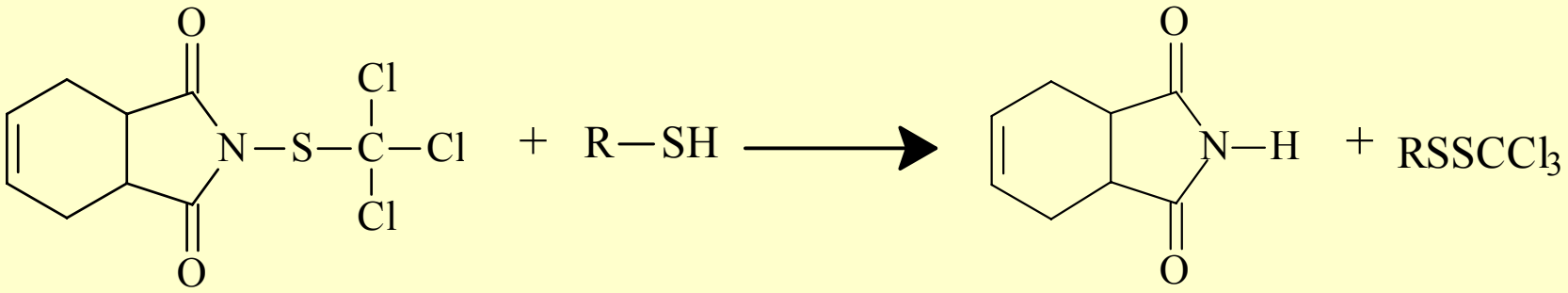
Hydrolysis of Captan



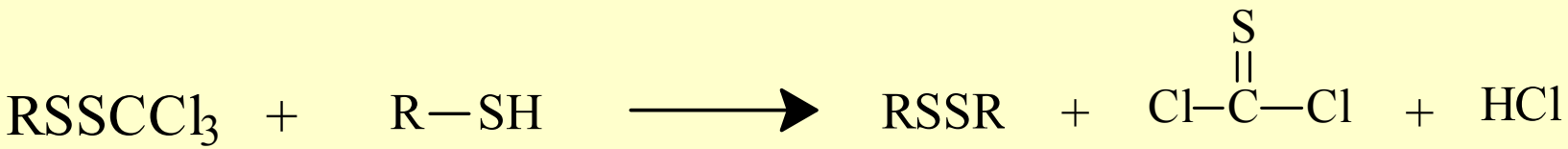
Captan THPI Thiophosgene

	Half-Life
pH 5.0	18.8 h
pH 7.0	4.9 h
pH 9.0	8.3 min.

Generalized Reactions of Captan with Thiols



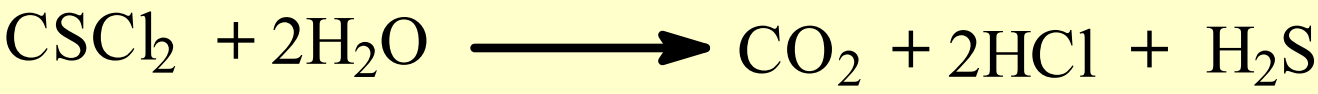
Captan Thiol THPI



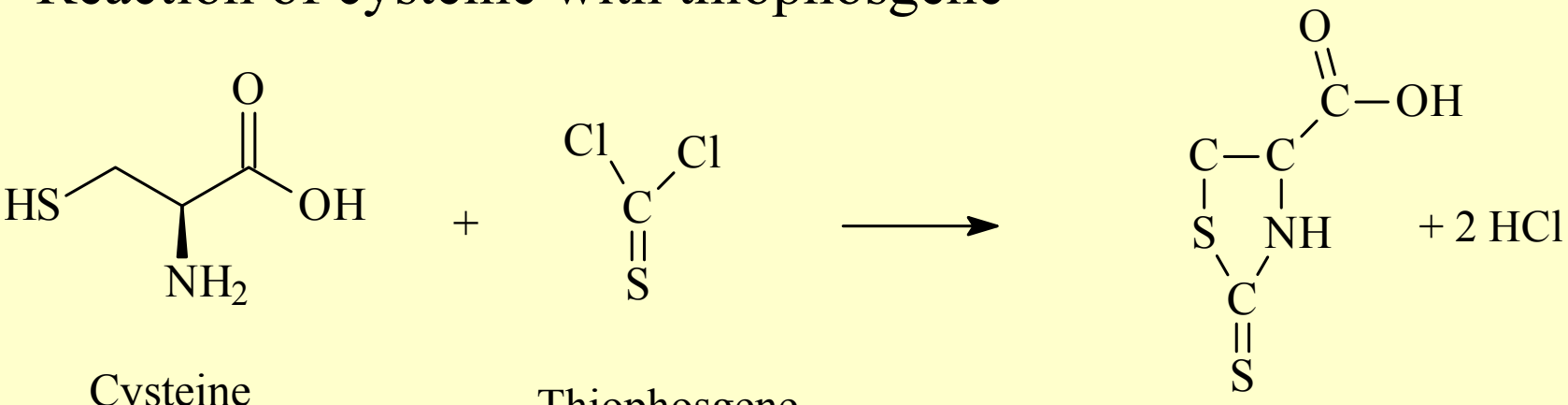
Thiophosgene

Reactions of Thiophosgene

Hydrolysis of thiophosgene in water



Reaction of cysteine with thiophosgene

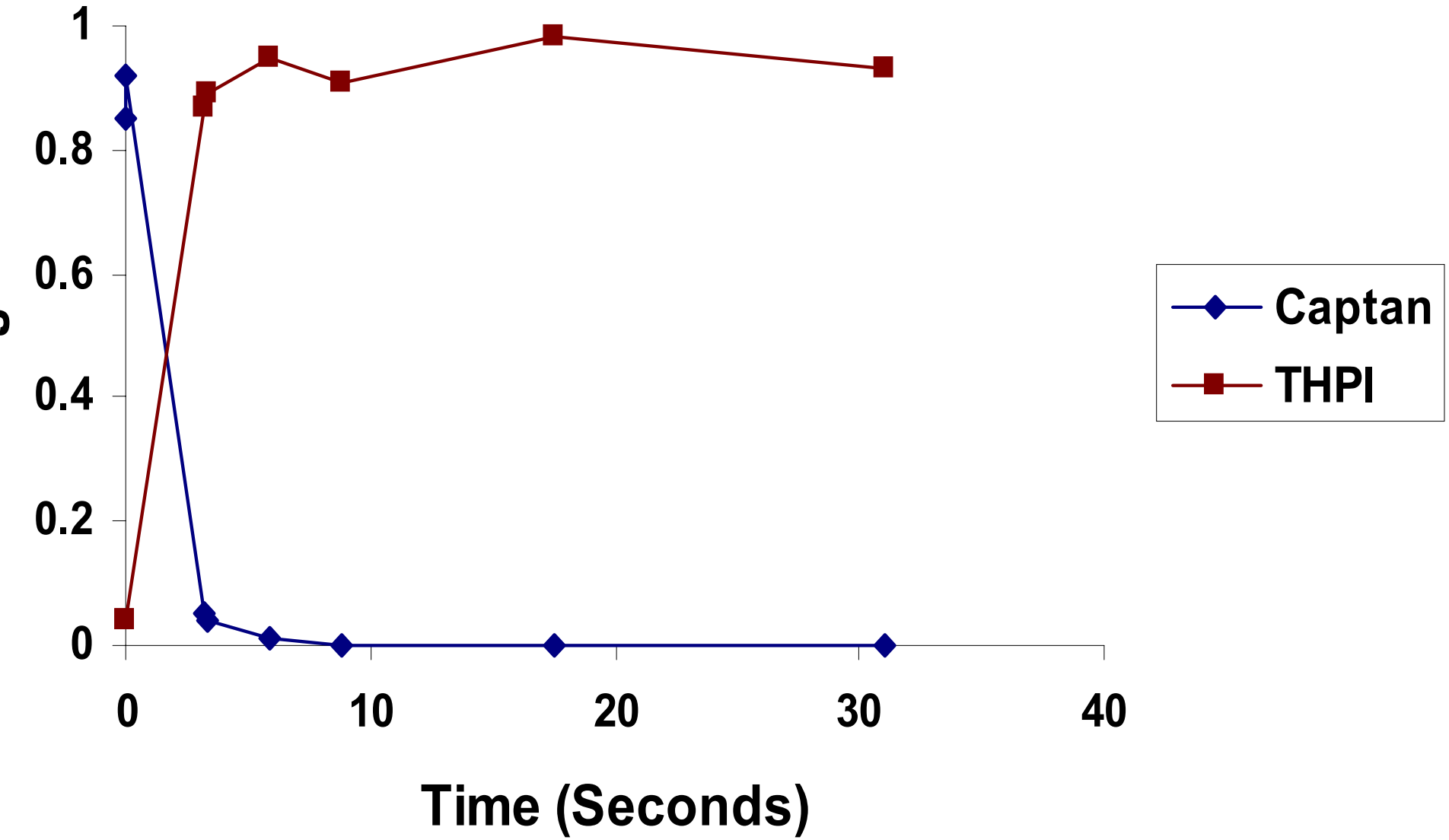


Cysteine

Thiophosgene

2-thioxothiazolidine 4-carboxylate

Figure 4. Captan Degradation and THPI Accumulation in Human Blood



Extreme Reactivity of Captan

- **Captan (and thiophosgene) rapidly hydrolyzed in the GI tract at a rate dictated by pH (duodenum > stomach)**
- **Captan (and thiophosgene) rapidly react with GSH and other nucleophiles (amides, alcohols) in lumen of gut or epithelial cells**
- **Captan (and thiophosgene) rapidly react with thiols in blood – transportation in systemic circulation highly unlikely**
- **EPA has concluded that residues of captan or thiophosgene after ingestion are not quantifiable**

Factors considered in analysis

- Animal tumor data (chronic bioassays)
- Human epidemiology
- Genotoxicity
- Mechanistic information
- Relevance to humans

Incidence of Duodenal Tumors in Mice

DOSE (ppm) (mg/kg/day)	0 0	100 15	400 60	800 120	6000 900	8000 1200	10000 1500	16000 2400
Males								
Adenomas[1]	2/91	3/83	0/93	1/87	**4/84**			
Carcinomas[1]	0/91	0/83	0/93	0/87	**2/84**			
Adenocarcinomas[2]	0/68					1/43		3/46
Duodenal neopl.[3]	2/74				20/73		21/72	39/75
Females								
Adenomas[1]	3/85	1/82	1/83	**7/81**	3/91			
Carcinomas[1]	0/85	0/83	0/83	0/81	1/91			
Adenocarcinomas[2]	0/68					0/49		3/48
Duodenal neopl.[3]	2/72				24/78		19/76	29/76

[1] Daly and Knezevich (1983) [2] NCI (1977) [3] Wong *et al*. (1981)

Incidence of Renal Tumors in Male Charles River CD Rats[1]

DOSE (ppm) **(mg/kg/day)**	**0** **0**	**500** **25**	**2000** **100**	**5000** **250**
Number examined	69	69	67	66
Liposarcomas	1	0	1	0
Adenomas	1	0	2	3
Adenocarcinomas	0	0	0	1
Unlilateral renal cell carcinomas	0	1	1	0
Total (adenoma + carcinoma)	**1**	**1**	**3**	**4**

[1] Goldenthal *et al*. (1982)

Comment

- **Increased renal tumors seen only in 1 of 4 rat studies and only in males**
- **Effect is limited to benign tumors**
- **Small increase in tumors is not statistically significant by pair-size analysis ($p>0.05$)**
- **EPA showed dose-related trend only for combined adenomas and carcinomas and only using a questionable analytical method**
- **EPA concluded that data *"show only a borderline increase in kidney tumors"***

Incidence of Uterine Tumors in Female Wistar Rats[1]

DOSE (ppm) (mg/kg/day)	0 0	125 6.25	500 24	2000 98
Number examined	40	49	50	50
Fibromatous polyps	7	11	12	10
Multiple fibrous polyps	1	1	1	2
Adenocarcinomas	6	4	5	7
Papillomas	0	1	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 sarcomas	**0**	**0**	**0**	**4**

[1] NOASR (1983)

Comment

- **Increased uterine tumors seen only in 1 of 4 rat studies even though other studies used higher dose levels**
- **Small numerical increase in sarcomas in high dose group is statistically significant only when tumor types are inappropriately combined**
- **The study was unusually long (120 weeks) – no historical control data available**
- **No evidence of usual progression of uterine sarcomas from uterine polyps**
- **A more appropriate analysis of combined sarcomas and polyps shows no treatment-related effect**

Conclusions: Animal Tumorigenicity

- **Prolonged ingestion of high dose levels of captan causes an increased incidence of tumors in the proximal region (7 cm) of the duodenum in both sexes of mice**

- **The tumorigenic response exhibits a clear threshold [females 800 ppm (120 mg/kg/day) and males 6000 ppm (900 mg/kg/day)]**

- **There is no evidence that ingestion of captan is associated with an increased incidence of renal, uterine or other tumors in rats**

<u>Overall Conclusion</u>: Captan is associated with tumor formation in only one tissue (duodenum) in one test animal species (mice)

Human Epidemiology

- **A limited-power epidemiology study of 410 workers in a captan manufacturing plant suggested no evidence of increased duodenal or other cancers.**

Genotoxicity

In Vitro

- **Captan is a generally positive in several *in vitro* test systems with bacterial (*S. typhimurium*, *B. subtilis*, *E. coli*) as well as mammalian cells (Chinese hamster ovary, mouse lymphoma)**

- **The effect is much less marked in the presence of an S9 metabolic activation system**

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

Test System	Component Added	Revertants per Plate
E.coli WP2 bcr with 0.15 μM (45 μg) captan per plate	None	3200
	S-9	30
	20 mM cysteine	19
	rat blood	32
	0 0.0 μM Cysteine/μM captan	2900
	0.5 "	2580
	1.0 "	1660
	2.5 "	183
	5.0 "	10

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

Genotoxicity

In Vivo

- **Tests with a wide range of *in vivo* assays for mutagenicity are overwhelmingly negative**

- **Gavage administration of high dose levels of captan (1,000 mg/kg/day for 5 days) produced no clastogenic effect in duodenal stem cells and did not increase nuclear aberration frequency of positive control (dimethyl hydrazine)**

- **There is no evidence that ^{35}S-captan binds covalently to DNA in the duodenal stem cells following oral administration**

Interim Conclusions: Tumorigenicity

- **Captan increases the incidence of crypt cell adenomas and adenocarcinomas in the proximal duodenum of mice**
- **The effect exhibits a clear dose threshold**
- **Captan is not genotoxic *in vivo***
- **The data strongly suggest that captan is acting via a non-genotoxic mechanism**
- **Pathology evaluation indicates that tumor formation is preceded by crypt cell hyperplasia in same area of proximal duodenum**

Proposed Mechanism of Action

SEQUENCE OF TUMORIGENIC EVENTS

1. **Irritation and inflammation of the proximal duodenal epithelial cells**
2. **Cytotoxicity and epithelial cell necrosis**
3. **Epithelial cells rapidly lost and villi shorten**
4. **Regenerative hyperplasia and crypt basal cell proliferation**
5. **Increase in probability of "fixing" spontaneous DNA damage that would normally be repaired**
4. **Neoplasia of crypt cells**

Appearance of Non-Neoplastic Duodenal 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)**

Causality Between Key Non-Neoplastic Events and Tumor Formation

- **Tissue Localization** All non-neoplastic precursor events occur in same section (proximal 7 cm) of duodenum as tumors and involve same crypt cells

- **Dose-Response and Threshold** NOAELs for crypt cell hyperplasia, increased inflammatory cell infiltrate, number of cells per crypt, etc. similar to those for tumor formation and indicate clear threshold-based effects

Causality Between Key Non-Neoplastic Events and Tumor Formation (Cont.)

- **Temporal** Non-neoplastic events always precede tumor formation

- **Reversibility** Reversibility of hyperplasia and reduced incidence of malignant tumors demonstrated on cessation of captan exposure

- **Biological Plausibility of Proposed Mechanism** Mechanism involving cell proliferation is consistent with current understanding of cancer biology

Relevance to Humans

- **Mechanism proposed may be relevant to humans exposed to captan under similar conditions (dose and duration) – lifetime dose levels of 900 mg/kg/day and 120 mg/kg/day for males and females respectively.**

- **Lifetime daily intakes at this level are highly unlikely.**

Alternative Mode of Action

Direct genotoxic action on stem cells in duodenal crypts.

This is unlikely because:

- **Crypt cells are well protected from reactive captan species in lumen of duodenum**
- **No evidence of any crypt cell DNA effects**
- **Captan cannot reach stem cells via systemic circulation because of extremely rapid breakdown in blood (half-life <1 second)**

Weight of Evidence Characterization

- **Captan currently classified as a Group B2 "*probable human carcinogen*" according to EPA's 1986 Guidelines**
- **Overall weight of evidence strongly suggests that captan exerts its effect through a non-genotoxic mechanism involving cytotoxicity and regenerative cell hyperplasia**
- **Under EPA's new Guidelines the B2 classification for captan is inappropriate**

CONCLUSION

Under the new Guideline descriptors captan should be classified as:

- **Likely to be carcinogenic to humans following prolonged, high level oral exposures causing duodenal cytotoxicity and regenerative cell hyperplasia**
- **Not likely to be carcinogenic to humans at dose levels that do not cause cytotoxicity and regenerative cell hyperplasia of the duodenum**
- **Not likely to be carcinogenic to humans following dermal or inhalation exposure**

APPENDIX C

Panel Charge Questions

Peer Review of Cancer Assessment for Captan
Charge to the Reviewers

Introduction

Captan has been in use as a nonselective fungicide for over fifty years. U.S. EPA currently classifies captan as a "probable human carcinogen" using their 1986 Guidelines for Cancer Risk Assessment; the weight of evidence characterization has not been updated using U.S. EPA's more recent draft guidelines. Registrants as well as other investigators have developed additional data that can be used to describe a mode of action for captan. In 2001, the Captan Task Force (CTF) requested that U.S. EPA (OPP) re-evaluate captan under its current draft cancer risk assessment guidelines. The Agency was not able to allocate resources to this task, reflecting budgetary constraints and higher priorities. The Agency, however, saw value in addressing this issue, particularly with the pending tolerance reassessments for other B2 compounds, and agreed in principle with the proposal to reevaluate captan by using an independent Third Party review. This alternative approach is an option U.S. EPA is *making available* to the Registrants; that is, while it is noted as a viable approach, the Agency is *not directing* that a Third Party review be undertaken.

The document to be reviewed presents a cancer hazard assessment and weight of evidence narrative for captan following U.S. EPA's 2003 Draft Final Guidelines for Cancer Risk Assessment. The objective of this peer review is to review the document for the validity of the arguments and conclusions regarding the characterization of captan. The panel will consider all relevant data; resolve all questions posed; or, specify where insufficient data are available for resolution of specific questions. Principles of sound science will be used throughout this review process.

To help the Panel discuss the sponsor's submission and address whether captan has been adequately characterized, *TERA* has prepared these charge questions. The charge questions are loosely organized following the analytical approach presented in EPA's 2003 Draft Guidelines for Cancer Risk Assessment. Full discussion and participation of all panel members is encouraged and the Panel is encouraged to reach consensus on each of the charge questions as well as on a weight of evidence narrative for captan. Consensus for the purpose of these meetings is defined as "an opinion held by all or most, or general agreement."

Availability of Data

- 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?

Analysis of Tumor Data

Human Data

- What conclusions can be drawn from the human data regarding the potential human carcinogenicity of captan?

Animal Data

- 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?

Some issues to consider:

One (Warner et al., 1982) of four rat bioassays demonstrated increased incidence of some renal tumors following captan exposure. Which tumor types, if any, are appropriate to combine in order to test statistical significance? What statistical tests are appropriate for evaluating significance of renal tumors? Is the increased incidence of renal tumors statistically and biologically significant? Are there other issues related to the renal tumors that should be discussed and resolved?

One rat bioassay (NOASR, 1983), demonstrated increased incidence of some uterine sarcomas. Which tumor types, if any, are appropriate to combine in order to test statistical significance? What is the significance of the length of the study (120 weeks) on the analysis of the uterine tumors? Is the increased incidence of uterine sarcomas statistically and biologically significant? Are there other issues related to the uterine sarcomas that should be discussed and resolved?

Three carcinogenicity studies in mice (NCI, 1977; Wong et al., 1981; Daly and Knezevich, 1983) demonstrated increased incidence of duodenal tumors. Is the increased incidence of these tumors statistically and biologically significant? Are there other issues related to the duodenal tumors that should be discussed and resolved?

Comment on the other treatment-related, toxicological effects identified by the long-term bioassays. What do these effects contribute to the understanding of the cancer mode of action?

Analysis of Other Key Data

Physical/Chemical Properties

- Are the available data on physical and chemical properties adequate, and do they contribute to an understanding of the potential human carcinogenicity of captan?

Some issues to consider:

Do the data on physical and chemical properties identify degradation pathways and reactive intermediates that are relevant to the cancer mode of action? Are these pathways and intermediates influenced by route of exposure?

How do the physical/chemical data contribute to the understanding of the cancer mode of action? What conclusions can be drawn from these data?

Metabolism and Kinetics

- Are the available data adequate to describe the absorption, distribution, metabolism and excretion 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?

Genotoxicity

- Are the available genotoxicity data adequate to evaluate the role of genotoxicity in captan's mode of action?

- Based on the weight of evidence, can it be concluded that captan genotoxicity does not contribute significantly to human carcinogenic potential at environmentally relevant doses?

Some issues to consider:

Captan is mutagenic when assayed in a variety of *in vitro* tests. However, some data suggest that the *in vitro* mutagenicity is dependent on the absence of thiols or other molecules that react with captan intermediates from the test system. Are these data reliable? What conclusions can be drawn from these data regarding the potential genotoxicity of captan?

Captan is generally not mutagenic when assayed in a variety of *in vivo* tests. However, some studies have shown positive results with *in vivo*

assays. Are these data reliable? What conclusions about the potential genotoxicity of captan can be drawn from the *in vivo* studies?

Can the apparent paradox between *in vitro* and *in vivo* assay results be explained by the metabolism and kinetic data for captan? Do the metabolism and kinetics data support the conclusion that captan or its metabolites will not reach the DNA in duodenal crypt cells to cause mutations?

Mechanistic

- 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?

Mode of Action

- 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? Does this mode of action support the conclusion that a nonlinear dose-response assessment is appropriate for captan?

Some issues to consider:

Do the data support the biological plausibility and coherence; strength, consistency, specificity of association; dose-response concordance; and temporal relationship for key events?

Is the proposed mode of action relevant to humans at environmental levels of exposure?

Can the database on captan equally support other possible modes of action?

Weight of Evidence Narrative

- Does the weight of evidence narrative in the document adequately explain captan's human carcinogenic potential and the conditions (e.g., route, 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?