Peer Reviewer Comments on Revised 2004 Captan Report

The revised report (dated 1/13/2004) was reviewed by all panel members who attended the September 3-4, 2003 peer review.[1]

Dr. Matthew Bogdanffy, E.I. du Pont de Nemours and Co., Inc.
Dr. Dawn Goodman, Covance Laboratories, Inc.
Dr. Gordon Hard, Consultant
Dr. Andrew Maier, Toxicology Excellence for Risk Assessment (*TERA*)
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 (now with the West Virginia Department of Environmental Protection)

Each panel member was sent the revised report, a CD containing new studies cited in the revised report, and a copy of the final report of the September 2003 peer review meeting. In addition, each panel member was asked to certify whether he or she agreed with the revised document's conclusions and if they had additional comments, or recommendations for minor or major revisions. A compilation of all panel members' comment and individual certification forms are attached.

All panel members indicated that they agreed with the statements and approved the report. Two reviewers indicated no changes were needed, four reviewers required minor revisions to the text, and two reviewers indicated that major revisions were needed to improve the document. Many of the revisions included editorial suggestions to make the meaning of statements more correct or clearer. Many comments were also provided to highlight corrections of typographical errors.

Although all the reviewers agreed that the document provides adequate support for the proposed mode of action and weight of evidence conclusions, several substantive comments were made. Two comments of particular note were made regarding the weight of evidence conclusions. Reviewers commented that the statement regarding the carcinogenic potential of dermal and inhalation exposure should be revised to better reflect the limited data for these routes of exposure. Alternative wording of the statements regarding the level of certainty in the conclusion regarding *in vivo* mutagenic potential of captan was also suggested. In addition, there were a number of requests to add more information or clarify the meaning of some statements or to better develop arguments presented in the document. For example, reviewers noted that sections related to toxicokinetics of captan in the duodenal microenvironment, structure activity relationships (particularly for THPI), and susceptibility of children could be enhanced.

[1] Note that Dr. Mike Gargas provided written comments for the September 2003 review, although he was not able to participate in the meeting due to a last minute schedule conflict. Since the September meeting, Dr. Gargas has developed a conflict of interest that prevented him from participating in the follow-up review.

Individual reviewer's comments are presented below

Reviewer 1

I agree with these statements and approve the report with no additional comments or recommendations.

Reviewer 2

I agree with these statements and approve the report with minor revisions as indicated in the attachment.

Reviewer 2 provided the following comments on an annotated copy of the report.

p. 18

- Last paragraph on Daly & Knezevich (1983) study. See the pathology report for the study (not the summary) for discussion of stomach lesions and jejunum ileum lesions.
- Last line – reference to duodenal crypt cells is incorrect as crypt cells are not mentioned in this report. Replace with "duodenum" or "duodenal mucosa."

p. 20

- First paragraph, last sentence. This is also support by Daly and Knezevich to a lesser extent.

p. 23

- Third bullet near bottom of page. It should be noted that the incidences of uterine tumors in this study were within the historical range for studies o shorter duration (104 vs. 120 wks).

p. 46

- First bullet. Two strains of mice with the same response - B6C3F1 from NCI. Add "B6C3F1 and" before "CD1 mice…" in first line.

p. 47

- First paragraph after bullets, last line. Delete "proximal" from sentence.
- Second to last line, add "primarily in the" just before "proximal region of the small intestine…"

p. 48

- First full paragraph, line 5. Goblet cells are specialized cells within the epithelium. The epithelial cells rest upon (line, cover) the lamina propria. Revise sentence to delete goblet cells. "Within each villus is a core of lamina propria that include smooth muscle, blood vessels, and lymphatic vessels."

p. 51

- Second paragraph, last sentence. "chronically treated controls" is an oxymoron. One can't have "controls" and "chemically treated" together. Controls are of same age as recoverably animals.

p. 53

- First paragraph, eighth line. Reference to "pathologists' interpretations" is misleading. These two studies don't discuss pathogenesis. Pathologist reviewing multiple studies conclude the mechanism is irritation followed by tumor formation.

p. 54

- Paragraph completed at top of page. These crypt cells probably have increased proliferation secondary to the increased loss of more superficial cells. They are likely more susceptible to spontaneous mutations as compared to controls for this reason alone, therefore the increased tumor formation.
- Third full paragraph, Concur!

p. 56

- Fourth paragraph. Once tumors are formed, they are irreversible. This indicates that the hyperplasia is reversible, removing the stimulus (mechanism) for tumor formation. Therefore, the tumors don't develop.

p. 58

- Great!

p. 63

- Third full paragraph under "i. Neoplastic Effects." Revise first sentence – "Based on the results….limited to the formation of small intestinal (primarily duodenal) adenomas and adenocarcinomas in mice."

p. 64

- First paragraph under "iii. Non-Neoplastic Effects" fifth line. Delete the word "same" from "the same crypt cells."

Reviewer 3

I agree with these statements and approve the report with minor revisions as indicated in the attachment.

Reviewer 3 provided the following comments by telephone to Dr. Maier.

p. 16

- Line 10, "secum" should be "cecum"

p. 17

- Lower half of text, the term "polyploid" should by "polypoid" (2 occurences)

p. 18

- Line 9, insert "and" after "small,"
- Line 12, third word should be "an"
- First paragraph, last sentence, revise to read "This technique allows for a careful evaluation of the whole intestine compared to conventional sectioning."

p. 22

- Line 4, “neuropathy” should be “nephropathy”
- Line 4, Regarding statement “Some strains of rats (Fischer, SD) are so sensitive to this effect that it [sic] is not of relevance to humans.” The rationale for this statement is not clear, in that differences in sensitivity are not the same as an underlying biological mechanism that has no counterpart in humans.

p. 23

- Line 2, statement beginning “Furthermore, stromal sarcomas are known….” The combined incidence of stromal sarcomas with polyps should be an additional test, not the only test.

p. 24

- First bullet, is not exactly correct. The statistical text of combined polyups and and sarcomas is an appropriate *secondary or additional* test.
- 2 lines before “C. Other Routes of Exposure”, “smoky gun” should be “smoking gun”?, or preferably reword to a more formal phrase

p. 25

- Line 1, insert “of” between “ability” and “captan”

p. 27

- Line 1, second word, change “a” to “an”

p. 28

- Last full sentence, “Positive results ….” Add “be” between “automatically” and “assumed”. This sentence is awkward.

p. 29

- First line, add “an” between “to” and “overly”

p. 35

- 5 lines from end, plase clarify what is meant by “c-“

p. 37

- Line 4, Should Table 5 be Table 7?

p. 38

- 2 lines above “C. Overall….”, “as” should be “an”

p. 39

- Last bullet, first line, remove apostrophe from“its’”

p. 40

- First bullet, add the word “in” before “*in vitro*”

p. 47

- Line 15, histopathological is misspelled

p. 48

- Line 7, please clarify reference to goblet cells location - lining not lamina.
- Third full paragraph, 2 reference citation – “Allison” should be “Alison”

p.51

- Third paragraph, second to last sentence, suggest rewording” The inability to detect a more significant increase in BrdU labeling may be due to the fact that there is a high normal background rate of cell ….”

p. 53

- Line 6, delete second “proximal”. In same sentence add commas after duodenum and bioassays.

- Second paragraph, “Firth” should be “Frith”
- Third line from bottom, add “the” between “near” and “bottom”

p. 54

- Second full paragraph, last line, add “and” between “females” and “considerably”

p. 56

- First paragraph, last sentence is awkward.

p. 58

- last sentence, add comma after “Figure 6”

p. 70

- Epstein et al., 1972 reference is missing the title.

Reviewer 4

I would agree with these statements and approve the report only after major revisions, as indicated in the attachment, are made to the report.

Reviewer 4 provided the following comments as annotations to the report:

General statement – The document is much improved. Changes are generally responsive to the panel’s recommendations. I agree with the basic conclusion regarding the proposed mode of action, although would suggest some rewording of the weight of evidence statements. In several places the document could be further enhanced to more fully document the rationale and basis for conclusions that are presented. Detailed as well as general comments are noted below as they occur in the document - they are not sorted by relative importance.

p. 6

- Statement 2 at bottom of page – consider rewording to emphasize that the weight of evidence suggests that captan *is unlikely to be* genotoxic in vivo and a genotoxic mode of action is *unlikely to contribute significantly* to the tumorigenic potential of captan.
- Fifth line from bottom, please clarify what is meant by the phrase “DNA enzymatic processes.”

p. 7

- Line 1, Regarding the statement “Consequently, neither captan nor its …..” It was not directly shown that captan or its reactive breakdown products do not reach these cells, although toward the end of the document a good case is made based on indirect lines of evidence. This point should be clarified. Therefore I do not agree with this statement. The final sentence in this paragraph should note that this is true for captan and reactive products. THPI can be transported systemically, and the genotoxicity of this compound or its further downstream metabolites is not well studied.
- First bullet under number 6, clarify that this is by the oral route – insert “by the oral route” between “carcinogen” and “at”
- Second bullet, the conclusion regarding inhalation and dermal exposure is too strong, based on the very limited database. This statement may be supported for systemic

tumors, but portal of entry tumors to the skin or respiratory tract would not be inconsistent with the proposed mode of action.

- Third bullet, rather than "likely" to be carcinogenic, it may be appropriate to characterize the risk as "suggestive" rather than likely since the finding is observed in a single species.

p. 8

- Last sentence of Introduction Section indicate how the literature search was done. This is an enhancement over the prior version, but could be improved. In particular, dates of the search (month and year) should be stated. If a decision was made not to include any available studies, the decision logic should be presented here. In addition, you should note that the assessment included unpublished company-sponsored studies.

p. 12

- Section IV should be retitled to "Metabolism and Toxicokinetics." This section needs to more clearly paint a picture of the overall disposition of captan, thiophosgene and THPI following an oral dose. In particular I found the absence of a clear discussion of the disposition of captan in duodenal lumen surprising. Secondly since THPI is relatively stable and likely to be absorbed, its downstream metabolism should be discussed in greater detail. If no reactive metabolites are expected this finding would strengthen the case for the absence of internal tumors.
- Line 4, replace "alkaline" with "neutral"
- Line 8 and 9 – This argument does not always hold, since most potent electrophiles that are mutagenic can react with GSH, yet they can have significant DNA reactivity even at less than millimolar concentrations. It is a point worth raising, but not one to rely too heavily on.
- Line 9, first word replace "tissues" with "cells.
- Last line of first paragraph, is it "non-enzymatic" or just "enzymatic"?

p. 13

- Fourth line from top, replace "tissue" with "blood"
- First line below figure 4, do single oral bolus doses result in an increase, only after an initial decrease?
- Last paragraph, first line, add "likely" to- "Since folpet likely shares a…."
- Last paragraph, fourth line, "7.6, 72, OR 668"
- Last paragraph, fifth line, add "at time periods" between "liver" and "ranging"
- Last paragraph, seventh line, and second to last line - replace "dosage" with "dose"
- Last line, revise to read "the GSH levels WERE statistically significantly increased in the duodenum beginning at the low dose and increased in a…."

p. 14

- Line 2, replace "are" with "were"
- Line 3, "the duodenum declined"
- Line 7, replace "captan" with "folpet", but note that captan would presumably exhibit similar behavior

- Why is the liver affected if captan is not absorbed systemically? Due to gavage dosing and overload of the capacity of duodenal contents to degrade captan or is this an effect of THPI?

p. 16

- Line 3, insert ", which reflects the disposition of THPI or unreacted captan" after "cyclohexene-labeled"
- First paragraph, please indicate what percent of the administered radiolabel was recovered.
- Second paragraph, first line. Change to "…degraded in the small intestines"
- Second paragraph, fourth line. While "there is no evidence that captan per se is absorbed into the systemic circulation.." There is no evidence it is NOT either.
- Third paragraph, last sentence, delete "typically"

p. 17

- Line 7, bioassay is misspelled

p. 18

- Line 9, "small AND emaciated"
- First paragraph, last line, "rather than ISOLATED sections"
- Second paragraph, present an explanation as to why the incidence might be lower in the high dose group.
- Last line on page, "incidence of malignant and benign…." Delete OR.

P. 19

- The description of the tumor data for this important study needs to be clearer - consider adding a table. The current text does not present the tumor findings for groups given a recovery period. Change the last sentence in the paragraph to past tense.
- Last paragraph, regarding the stomach lesion findings – This is consistent with irritant response in tissues that have neutral pH.

p. 20

- Table 2 – add Innes et al, 1969 and Pavkov 1985.
- Last line, NOASR, 1983 - indicate that this is the same study as Til et al.

p. 21

- First sentence indicates that 2 studies will not be discussed here. I disagree, these additional studies should be discussed to determine if the negative findings are credible. Furthermore, describing these studies is helpful to support the contention that remaining studies identified all sensitive strains, used appropriate doses, etc.

p. 22

- Line 4 - replace neuropathy with nephropathy?
- First full paragraph, last sentence. Verify that humans have no capacity for activation of GSH conjugates via a beta-lyase mechanism. I'm not sure of this point.

- Fourth bullet – extra space between since and the colon
- Awkward wording in description of NOAEL and LOAEL. In other words, non-tumor effects were not seen at any dose? If so, why not just say this?

p. 23

- End of first sentence add a conclusion that, the observations were within historical background.
- Fourth bullet – …from uterine polyps, *based on a statistical analysis of combined sarcomas and polyps?*

p. 24

- First full paragraph – the description of the Reuber et al. analysis should state clearly and specifically and errors in the analysis. Reuber evaluated slides from which bioassays? This text should be reworded to be less passionate.
- Paragraph 2 also should discuss how the GI tract response in rats versus mice differs in non-neoplastic responses as well. More detailed evaluation of the reason for differences in responsiveness could be presented.
- Paragraph 3 – "smoky gun" – reword
- Paragraph 4 - …or inhalation exposures, *although standard chronic bioassays have not been conducted for these alternative routes of exposure*.
- Paragraph 5 – Why is the study by Antony et al. (1994) not mentioned here? Decreased body weight can be an indicator of systemic toxicity, particularly for a dermal study. If it is discounted as due to dermal irritation that would need further justification.

p. 25

- Line 1 – ability *of* captan…using *captan doses* of either 0.2…
- Line 7 reword – These results suggest that dermal exposure will provide ample…
- Paragraph 2 – "These" studies – only one was presented. What happened to Antony et al. 1994?
- Paragraph 4 – describe more fully the specific respiratory tract effects that were observed
- Paragraph 5 – I do not agree that decreased body weight seen in the dermal study does not represent a systemic effect. This point needs further analysis in the document.
- Paragraph 5 – I agree with the general conclusion regarding GI tract tumors, but these data are not sufficient to conclude that portal of entry tumors would not occur following dermal or inhalation exposures.

p.26

- Paragraph 3 - change to "Dietary exposure was…"
- Paragraph 4- Do handlers experience irritation under typical conditions of use?
- Add recent agricultural worker study [Lebailly P, Devaux A, Pottier D, De Meo M, Andre V, Baldi I, Severin F, Bernaud J, Durand B, Henry-Amar M, Gauduchon P Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan.1: Occup Environ Med. 2003 Dec; 60(12): 910-7].

p.27

- First bullet- add to the end, "The reason for this differential sensitivity among species is not known, and it is unclear whether humans would respond most like rats or mice."
- Second bullet – At the end of the first sentence add "low, xx-fold below tumorigenic doses." This is needed to put the doses in perspective.
- Third bullet – replace "there is no evidence of any" with "These studies were inadequate to identify a .."

p.28

- Line 3 – comma needed in Bridges citation.
- Line 7 – soften this statement – *shows that captan is unlikely to be an in vivo mutagen*.

p.29

- Paragraph 2 – add a statement that in vivo studies that measure the same endpoint as an invitro study are given geater weight. For example, it is not clear that an in vivo study of chromosome damage would out weigh a strong in vitro mutagenicity finding.

p.30

- Paragraph 2 – the conclusions regarding the inactivity of THPI may be correct, but are not adequately supported by the very minimal data presented. I believe the panel recommended supplemting the limited database on this point with data for structural analogs of THPI that have been more extensively tested. This is one of the major weak points in the analysis.
- Paragraph 3 – replace colon with semicolon in citation. The word "unacceptable appears in the parenthesis – what is this?

p.32

- Top of page – to assess human risk *(i.e., the human diploid fibroblast study of Tezuka et al. 1978)*, captan…"
- Paragraph 3 – 1000 *or* 2000 mg/kg…
- Paragraph 4 – et al. needs to be italics.

p.33

- Paragraph 2 – 300 *or* 1600 mg/kg
- Paragraph 2 – change the u in micromolar to µM, here and in later sections
- Paragraph 2 – chromatin *and* lead to…
- Bottom of page – delete phrase "exposed to captan"

p.35

- Line 5 – KdTTP is awkward – suggest describing in words

p.36

- Last Line – this is true. However, no in vivo assay to detect *mutations* are available in the target tissue. This should be noted.

P.38

- Paragraph 2 – eve though studies were of suspect quality – doses should be described.

P.39

- Paragraph 1- If DNA polymerase inhibition is a driving mechanism – then need to explain a little about why this impacts fidelity of DNA repair, but not overall ability of cells to proliferate.
- Paragraph 2, Line 5 – the ability of thiophosgene to reach bacterial DNA is independent of repair status. This sentence mixes two ideas.
- First bullet – captan is *unlikely to be* an in vivo mutagen.

p.40

- Second bullet – add comma after "e.g."
- Last bullet – I agree that it is unlikely that captan or its reactive degradation products reach the stem cells, but the text to this point in the document has not presented a cohesive argument for this conclusion, although one can be built on several lines of available indirect evidence. Either build the argument or simplify the conclusion here that captan is not clastogenic or DNA reactive in this population of cells.

p.41

- This table was an excellent addition to the document. Overall it is very informative, but needs a little technical editing help. For example, in some rows the concentration data do not line up with the corresponding assay results.

p.47

- Third bullet – the conclusion that it is not mutagenic in vivo due to thiol protection is still speculative and this should be softened.
- Paragraph 1 – experimental dose thresholds for tumors are not a compelling argument for a non-genotoxic mechanism. This statement is found in several places in the document. The apparent threshold may simply reflect lack of sensitivity in animal bioassays.
- Paragraph 2 – I agree that hyperplasia is a finding in the causal chain of events, but I think measures of cell cytotoxicity or inflammation are a better choice as the "key precursor event." This is because the damage to the villi is the hypothesized underlying cause of the hyperplasia and can be argued to be a clear threshold based phenomenon. Hyperplasia itself can be observed following events triggered by either linear or threshold responses.
- Paragraph 2 – In the discussion of GSH levels following folpet administration, the importance of liver involvement is unclear. This confuses the discussion of the lack of systemic distribution of captan. Please clarify.

p.48

- Paragraph 3 - "sloughed off into the …" - remove "the"
- Paragraph 4 - I think this is a critical point in the analysis that needs further elaboration. This is the first mention in the document of the mucus layer and the potential kinetics in the microenvironment of the duodenum. I would suggest the scientific underpinnings for this argument be described in the toxicokinetics section under the category of intralumenal microenvironment kinetics.

p.53

- Line 8 - delete extra “proximal”

p.54

- Paragraph 2, line 3 - “that *the* NOAEL…”

p.61

- Paragraph 1 - I would recommend that you calculate the daily intakes as mg/kg-day and compare to the tumorigenic dose. This comparison which shows that exposures are 4 or more orders of magnitude below tumorigenic doses is useful to put the results into perspective.
- Paragraph 2 - the presentation on potential children’s risk is very minimal and should be enhanced. There are many additional considerations that could be raised in this section that would support the conclusion that children are not likely to be more susceptible. To name a few: captan reactivity is not highly dependent on enzyme-dependent metabolism, eliminating this as a possible difference in susceptibility with age. The effect occurs in a highly proliferative tissue in adults (animals), so the rationale for greater childhood sensitivity based on greater basal proliferation is lee important. There is no effect in germ cell mutation assays and distribution of reactive products in vivo is unlikely limiting *in utero* exposure. There are others that could be addressed as well.

p.62

- As indicated earlier the weight of evidence statement regarding dermal and inhalation exposure needs modified to more accurately reflect the limitations in the data.

p.64

- Paragraph 2 - “following oral administration, *or cause cytotoxicity in these cells*”
- Paragraph 3 - “strong causal *link* between”
- Bullets - This is a very nice summary. Consider moving it to the front of the MOA discussion to guide the reader to arguments that will be made.

p.65

- Line 4 - “position *at the last evaluation* is that”
- Paragraph 2 - “other *systemic* organs/tissues” this change needed to address possibility of skin and lung portal of entry effects.

p.68

- References need some technical editing to ensure consistency in format. For example, using and between author names, periods after journal abbreviations, etc.

Reviewer 5

I would agree with these statements and approve the report only after major revisions, as indicated in the attachment, are made to the report.

Reviewer 5 provided the following comments as annotations to the report:

p. 6

- Second to last line. Replace "similarly" with "likely also". While what is stated here is likely true, it has not been demonstrated.

p. 7

- Item number 4, line 7, the word "cloned." This is not the correct word to use, not sure what is intended.

p. 28

- Line 5, "Inevitably, when so many genetic screening assays have been performed, there are bound to be both positive and negative responses." This is not necessarily true and suggest you replace with something like "The available literature shows some positive and some negative responses for captan."
- Line 7, delete the word "conclusively."
- The third bullet under "A. Process", I don't agree with the hierarchical evaluation as described on the next page.
- Seventh bullet – I'm not sure this last criteria is met by any of the "older" studies.
- Second paragraph from bottom, last sentence. None of the assays have "good specificity" for cancer. Revise the sentence to read "This is particularly important in the evaluation of studies conducted in the 1970's when the field of genetic toxicology was rapidly growing and many assay systems subsequently gained little use."
- Last paragraph, first sentence, reword- "The validation step is essentially the proof that an investigator's work or assay test system can be reproduced in another laboratory." Revise third sentence to read "Positive results cannot automatically be assumed to be the only correct result, when there are also a large number of negative results, and vice-versa."

p. 28 and 29

- Last paragraph on page 28 finished on page 29. Cannot agree with this paragraph's concluding sentence because none of the genetox assays have good sensitivity or specificity (for cancer).

p. 29

- First full paragraph, as noted above I cannot agree with the hierarchical evaluation. Please re-read the Moore and Harrington-Brock paper.

p. 31

- Lines 1 and 2, end sentence after "assay" and delete "pioneered in this laboratory."
- Third paragraph, fifth sentence. Need a reference for this sentence, "additional acceptance criteria…."

p. 36

- Gene Mutation Somatic Cell Assay paragraph discusses the mouse specific locus test. This is an insensitive test.

p. 41

- Table 6 is very nicely done.

p. 47

- First paragraph after the bullets. Revise beginning of sentence to read “The weight of the evidence that captan is not likely to be genotoxic *in vivo*, combined with the clear….”

Reviewer 6

I agree with these statements and approve the report with no additional comments or recommendations.

Reviewer 7

I agree with these statements and approve the report with minor revisions as indicated in the attachment.

Reviewer 7 had the following comments:

p.19:

- I suggest including a table with the Pavkov data for hyperplasia and neoplasia incidence at for animals sacrificed at the end of dosing and those sacrificed at the end of the recovery period. Visual inspection of these data will reinforce the finding relevant to the MOA. Recommended action: Insert Table
- The last paragraph on the page that refers to changes in the forestomach (epithelial hyperplasia) but unlike other chemicals that have an irritant effect on the upper GI, captan dose not produce neoplasms in that region. Why do you think that's the case? Recommended action: none -just curious.

p.21

- Last sentence in paragraph immediately below Table 3. The sentence, The Agency was only able to show..." reads as if evaluating adenomas and carcinomas combined is unusual, e.g., use of the word "only". Rather, combined analyses are standard procedure for EPA. Recommended action: Suggested rewording. A dose-response trend was noted for adenomas and adenocarcinomas combined but not for either type alone.

p.22

- Last sentence of second paragraph. The sentence says that there is no evidence that captan acts by these other 2 modes but provides no citations for that evidence. Recommended action: add citations to sentence

p. 21& 22

- Bottom of 21 and top of 22. The idea presented on page 21 is that these lesions are likely spontaneous because of no increase in incidence of focal pre-neoplastic lesions. Then the rest of the paragraph refers to increased BUN levels and the possibility that high dose group animals were at greater risk of developing tumors due to chronic "neuropathy" (should that be nephropathy?). The sentence implies that tumors were secondary to nephropathy, which would be treatment-related (only in the high dose group). Aren't these two ideas contradictory? Recommended action: Authors re-read this paragraph.

p.24

- Why are animals in the high dose group losing weight? Why is food consumption decreased? Were animals off feed because of irritation? Recommended action: provide a simple explanation because text says that it's unlikely that reactive captan materials are absorbed into systemic circulation.

p. 27, p. 53

- On p. 27, in the last sentence in the first bullet under Conclusions, text states that "...tumors in only one tissue (duodenum) ..." ; On p. 53 in the first sentence, first paragraph states, ". ...are only observed..." and in the last sentence, second paragraph states, "….restricted to the proximal portion of the duodenum. ..". Recommended action: replace only with primarily; and refer to small intestines rather than duodenum in the sentence with the word "restricted".

p. 46, p. 63

- The second sentence states, "…exhibit clear dose thresholds…." This is more a matter of philosophy. I don't think you can experimental identify a threshold and attribute anyone dose as a threshold due to experimental limitations. I think you can say something like. ..the data consistently demonstrated that no tumors developed below xxx ppm, strongly suggestive of a threshold.

p.47

- In the second full bullet, it states that the incidence of malignant tumors reverted. Should this be benign tumors?

p.53

- In the first paragraph, fifth sentence, it states, "This observation is consistent with the pathologists' interpretations...". Which pathologists? Recommended action: insert citation or refer reader back to section where this is explained.

p.61

- In the first paragraph it states that assuming humans are as susceptible as mice is a "worst case" assumption. Why is that the case? What evidence is there that the human GI would not be more susceptible. Recommended action: remove words - worst case-
- In the first paragraph, it states that exposure at certain levels for a lifetime would be required. Did tumors develop in mice in the Pavkov study develop with less than lifetime exposure? The text on p. 19 states that, " At least 6 months continuous exposure to captan is required. ..." Again a table of the Pavkov data would be helpful. Recommended action: If correct that tumors developed in mice with less than lifetime exposure, then change the text on page 61 to emphasize high dose, long-term exposure or chronic being somewhat ambiguous about lifetime.

p.62

- Last paragraph. Sentence refers to the inhalation and dermal studies as evidence that captan does not act systemically. First, both studies were of too short a duration to support that conclusion. I assume that the two-year studies examined other tissues and therefore provide stronger evidence.

Reviewer 8

I agree with these statements and approve the report with minor revisions as indicated in the attachment.

Reviewer 8 had the following comments:

Additional Comments to Revised Captan Reclassification Report:

I found the revised document to be completely responsive to previous comments provided during the initial evaluation provided by the TERA peer review group. The Captan Task Force is to be commended for the completeness of the revised report. My only concern is related to wording in the proposed classification regarding other exposure routes. Clearly no evidence provided in the report demonstrates carcinogenesis in other tissues following inhalation and dermal exposure, however a very strong case is made for a mechanism of action in the small intestine that includes chronic irritation of the exposed epithelial surface, cytotoxicity of epithelial components and increased cell proliferation.

Because similar irritation is also reported following short-term inhalation and dermal exposures and chronic data are apparently not available, a similar proliferative response to chronic epithelial irritation leading to neoplasia is not implausible. For this reason I would prefer to have some qualifying language which focuses on the expectation that captan is not likely to be carcinogenic at sites remote to areas of exposure, or alternatively, that it is not likely to be carcinogenic following dermal or inhalation exposure at concentrations and duration unlikely to cause cytotoxicity and hyperplasia in exposed tissues. Because there is a lack of data from studies of duration similar to that used in the intestinal studies, it is not clear that continued administration by either pathway would not result in tumor formation. Thus either or both of the qualified conclusions presented above seem to be better supported by data presented in the report.