ITER Peer Review on 1,3-Dichloropropene (Telone II) & Copper Meeting Summary

June 29 and 30, 1998
University of Cincinnati, College of Medicine
Cincinnati, Ohio
USA

An independent panel of expert scientists and risk assessors met on June 29 and 30, 1998 to review a hazard identification and dose-response assessment on Telone II (1,3-dichloropropene) and a protocol for a study on the acute toxicity of copper to humans through drinking water. This 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 and protocol. A comprehensive overall review of the materials was provided by the combined experience of all the reviewers.

The peer review meeting began with a discussion of conflict of interest. Prior to the meeting each reviewer certified that he or she did not have a conflict (real or apparent) with the chemical under review or sponsor, or identified the potential for such conflicts. Possible conflicts were discussed with each reviewer to determine if measures were needed to manage a potential conflict (or appearance of conflict). Options include excluding the reviewer from a particular discussion and consensus, or allowing the reviewer to participate in the discussion, but not be polled for consensus. The peer review panel discussed and agreed upon how to manage any potential conflicts. This is documented in Attachment A.

These review meetings follow a standard format beginning with a close examination of the supporting documentation and important references several weeks prior to the meeting. At the meeting, after the conflict of interest discussion and decision by the panel is made, the authors of the assessment or documentation briefly present their work. For chemical assessments, the panel then systematically discusses the assessment, starting with a discussion of the qualitative weight of evidence and a determination of whether adequate data exist on which to base a risk value, followed by a discussion of the appropriate critical endpoints and studies. Next, the quantitative aspects of the assessment are discussed, including proposed cancer risk estimates and reference doses and concentrations. For the copper protocol, discussion centered on a list of questions that had been identified by the sponsor, to help refine and strengthen the protocol.

Full discussion and participation are encouraged and agreement is reached by consensus. Consensus for the purpose of these meetings is defined as "an opinion held by all or most, or general agreement." The meeting was open to the public.

Assessment for Telone II (1,3-Dichloropropene)

Sponsor: Dow AgroSciences

Presenters:

- Dr. K.S. Rao, Dow AgroSciences
- Dr. William Stott, The Dow Chemical Company
- Ms. Linda Calhoun, The Dow Chemical Company

Chair: Dr. Michael Dourson, Toxicology Excellence for Risk Assessment (*TERA*)

Review Panel:

- Dr. Michael P. Carty, University of Cincinnati
- Dr. Joyce M. Donohue, U.S. Environmental Protection Agency, Office of Water
- Dr. Michael Dourson, Toxicology Excellence for Risk Assessment
- Dr. Marvin A. Friedman, private consulting Toxicologist
- Dr. George Leikauf, University of Cincinnati
- Dr. Peter R. McClure, Syracuse Research Corporation
- Dr. Bruce D. Naumann, Merck & Co., Inc
- Dr. Kenneth A. Poirier, The Procter & Gamble Company
- Dr. Karl H. Summer, GSF-National Research Center for Environment and Health, Germany
- Dr. Marcia van Gemert, van Gemert & Hauswirth, L.L.C.
- Dr. Vanessa T. Vu, U.S. Environmental Protection Agency, National Center for Environmental Assessment

Dr. Michael Dourson, the chairperson, opened the session with a brief overview of the meeting process. He indicated that Dow AgroSciences had used U.S. EPA risk assessment methods, including the 1996 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996). The panel was charged with determining whether Dow AgroSciences followed these methods correctly, but not necessarily to critique the EPA methods themselves.

Representatives from Dow AgroSciences and the Dow Chemical Company presented information on the Telone II (1,3-dichloropropene) assessment in three short presentations, preceding discussions on cancer hazard characterization and weight of evidence, cancer dose-response, and non-cancer dose-response. Each presentation was followed by a short period during which reviewers asked clarifying questions, followed by discussion of that portion of the assessment document.

PRESENTATIONS & CLARIFYING QUESTIONS – Cancer Assessment

Dr. William Stott of the Dow Chemical Company presented a brief summary of the toxicological basis for the hazard characterization and weight of evidence discussion. The presentation highlighted the hypothesis that the toxicity of Telone II (94% 1,3-dichloropropene) is dependent upon the saturation of substantial defense mechanisms

which protect animals and humans during normal exposures. The results of animal bioassays were summarized with emphasis on the differences between the results of the inhalation and dietary studies using the newer epoxidized soy bean oil (ESO)-stabilized formulation versus the results reported for oral gavage studies of epichlorohydrin-stabilized product. A review of the metabolism of 1,3-dichloropropene was presented. Key points regarding its rapid and nearly complete metabolism and the importance of glutathione conjugation in this process were made. The existing genotoxicity data were summarized. The lack of positive findings in *in vivo* assays and absence of evidence for the metabolic activation to an epoxide *in vivo* were presented to support the conclusion that a nongenotoxic mode of tumorigenesis accounted for the lung adenomas in mice, and liver adenomas in rats in the inhalation and dietary bioassays, respectively.

Clarifying Questions on the Presentation

Following the presentation, Dr. Stott and Dr. Gollapudi of Dow Chemical Company responded to clarifying questions from the panel.

The panel raised a number of questions regarding the specific formulation used in the toxicity testing and the potential for it to contain contaminants. A panel member asked whether the risk assessment was to be based on the consumer formulation of Telone II and whether the NTP cancer bioassay (NTP 1985) was conducted using a different formulation than was tested in the newer studies. Dr. K.S. Rao of Dow AgroSciences clarified that the current Telone II formulation is different from the Telone used for the NTP bioassay which was an epichlorohydrin-stabilized product. The newer formulation uses 2% epoxidized soybean oil, rather than epichlorohydrin. Several panel members asked questions regarding the potential for the newer ESO-stabilized formulation to contain oxidative and polar breakdown products. Dr. Stott indicated that epoxides were looked for in both the product and the headspace of the container and none were found. In addition, the newer toxicity tests were conducted using the standard formulation as available to consumers, which typically has less than 0.1% polar contaminants. Another reviewer asked about the potential for polar contaminants in the new ESO formulation as had been reported in previous studies. Dr. Stott indicated that some of the early positive mutagenic assay results attributed to Telone were subsequently shown (Talcott and King 1984) to be artifacts from the formation of polar compounds during its purification by gas chromatography. He indicated that the current ESO-stabilized formulation does not have polar metabolites due to auto-oxidation.

To address the functional implications of differences in product purity and formulation, one panelist asked whether the presence of epichlorohydrin in the old versus the new formulation was sufficient to explain all the observed target organ and tumorigenic effects. Dr. Stott indicated that the doses of epichlorohydrin present in the old formulation were not sufficient to explain all the tumors associated with that formulation based on available toxicology data for epichlorohydrin.

Dr. Stott responded to a number of questions regarding the proposed metabolic pathway for 1,3-dichloropropene. He clarified that while there is no *in vivo* evidence for formation

of an epoxide intermediate, *in vitro* evidence for oxide formation has been shown in mutagenicity testing in which the oxide is quite active. It loses activity with GSH addition and has no activity with the addition of normal cellular defenses. He commented further that these findings led to the metabolism proposal developed by the International Programme on Chemical Safety (IPCS) to include a monooxygenase oxidation that would be slow relative to very rapid glutathione conjugation. In response to panel questions regarding differential metabolism of the cis- and trans-isomers, Dr. Stott indicated both proceed through a similar pathway, but the trans-isomer has a greater tendency to be oxidized through the CO_2 pathway than the cis-isomer. A reviewer noted that in the human the amount of cis-conjugate is 75% and trans-conjugate is 23%.

In response to a question on 1,3-dichloropropene toxicokinetics, Dr. Stott indicated that the doses were the same for determination of the blood concentration curve for 1,3-dichloropropene neat versus the microencapsulated product, based on labeled carbon studies. A reviewer asked whether a two-compartment model had been fitted to the blood concentration data and that if a second compartment exists, how much of the overall metabolism of 1,3-dichloropropene would it represent. Dr. Stott acknowledged that a second pathway other than direct glutathione conjugation and oxidation to CO_2 may exist, but reiterated that no product of an epoxidation pathway has ever been measured.

A panel member asked if additional information is available on comparative metabolic differences between humans and animals, as evidenced by the existence of mouse specific metabolites. Dr. Stott provided additional detail on the metabolism of 1,3-dichloropropene indicating that the major pathway of metabolism is similar between humans, rats, and mice. However, mice have an apparent additional minor pathway that accounts for 10-15% of their 1,3-dichloropropene metabolism.

Clarifying questions from the review panel also addressed issues of glutathione conjugation. With regard to the glutathione assay, Dr. Stott indicated that the assays for cellular glutathione depletion measured only reduced glutathione content and that these were the only assays to evaluate sulfhydryl binding. One reviewer asked whether any consideration had been given to the potential effects of glucose-6-phopshate dehydrogenase deficiency, which might compromise glutathione conjugation in humans. Dr. Stott indicated that any effect would be dose-dependent and that one study of GST-mu deficient humans found no difference in urinary metabolites of 1,3-dichloropropene.

The panel members had additional questions on the presentation of the genotoxicity data. One reviewer commented that the *in vitro* DNA binding assay assumes that the appropriate activating enzymes are present and questioned whether the method to detect DNA adducts would be able to identify oxidized bases as well as bulky 1,3-dichloropropene adducts since the ability of the compound to decrease reduced glutathione pools may be consistent with the generation of cellular oxidative stress. Dr. Stott acknowledged this possibility, but noted that the *in vivo* assays were also negative.

Questions were asked regarding the availability of additional studies that were not discussed in the presentation or document. A panel member asked for clarification on the

results of the mechanistic cell proliferation work that had been conducted and asked if any recovery studies had been completed to determine the likelihood of a tumor promotion mechanism, particularly in light of the observation that glutathione levels decrease through out life. Dr. Stott summarized that in Fischer rats, only minor changes in cell proliferation were observed and no significant degree of non-tumor pathology was observed in tumorigenic target tissues. He also indicated that no recovery studies have been conducted in the GI tract, but that the GSH levels in the GI tract rebounded in the 4-week study. GSH levels in the mouse lung did not display a rebound effect. Regarding tumor promotion, an initiation-promotion study in which 1,3-dichloropropene was tested with a phorbol ester (tumor promoter) yielded a negative result; however, 1,3-dichloropropene has not been evaluated as a tumor promoting agent. Dr. Stott indicated that he was not aware of any cell transformation assays.

Panelists asked for additional information regarding other potential target organ effects. Dr. Stott indicated that none of the Dow studies had identified kidney effects due to 1,3-dichloropropene with the only observed renal pathology secondary to excretion of large amounts of the mercapturic metabolites. The early study reporting cloudy epithelium in the kidney has not been reproduced and was conducted in a group of rats that had endemic nephropathy. In response to another question, Dr. Stott indicated that he was not sure that bladder stones would have been detected based on the method used for tissue preparation.

DISCUSSION – Cancer Qualitative

Mode of Action

The panel agreed that there were sufficient animal data for the assessment but that the existing human data are inadequate for qualitative and quantitative assessment. A number of issues were discussed including the mode of action for the liver versus the lung, genotoxicity data, and the importance of glutathione in tumor response.

Several of the panel members indicated their agreement with the proposed mode of action, but indicated reservations about completely eliminating the possibility of a genotoxic mode of action, consistent with a linear dose-response. A reviewer indicated that the recent toxicity studies using the ESO-stabilized formulation help to rule out a genotoxic event and support a non-linear model; however, the mechanism of tumor formation is still not known and at this point a linear response could not be ruled out. This reviewer felt the proposed mechanism, although plausible, needed to be related back to humans.

Several reviewers indicated that the liver tumors were critical in terms of assessing the linearity of the dose-response. One reviewer commented that of the tumor types observed in the animal studies, the liver tumors should be the focus of the mode of action determination even though their public health significance might be minimal given that they were nonmalignant microscopic tumors with a high background incidence. The tumors in the other organs were more-concentration dependent. A second reviewer

agreed with the importance of the liver tumor response for assessing the possibility of a non-linear mode of action, but thought that one should not mix public health concern with the initial determination whether the substance is a liver carcinogen. This reviewer indicated that it might be useful to separate the mode of action for the lung tumors observed in mice and the liver tumors observed in rats. According to this reviewer, a better argument could be made for a non-linear mechanism for the lung adenomas because of irritant effects; however, the rationale for a non-linear mechanism for the liver tumors was less clear.

A number of issues also present a problem with the non-linear model, including the lack of information on start/stop studies and cell proliferation, which would address the possibility that 1,3-dichloropropene acts to promote existing initiated cells, and the fact that the metabolism data indicate that tumors are generated even when the glutathione conjugation system is not overwhelmed. Based on this, the reviewer indicated, at this point, that a threshold for the liver response has not been demonstrated and therefore the tumor response might be linear.

Another reviewer indicated that they would be uncomfortable ruling out a genotoxic effect, given the example that prolonged exposure, coupled with GSH levels decreasing later in life, may have allowed liver tumors to be initiated late and consequently not provided enough time to progress. This would be consistent with a tumor promoting effect and the lack of chronic cytotoxicity or cell proliferation. In terms of the relevance of the liver tumors, another reviewer noted that humans are likely to be exposed in short bursts which is different than the argument being made for the liver tumors. As a clarifying point, Dr. Stott indicated that humans are only exposed via inhalation and ingestion is not a factor since the environmental persistence of 1,3-dichloropropene is very low.

The panel discussed the value of the existing data to eliminate a genotoxic mode of action. One reviewer commented that the studies using the new formulation were more appropriate for an evaluation of mutagenicity and less weight should be given to studies with epichlorohydrin. Another reviewer commented that the positive results in the Ames assay might be an indication that an adduct could be formed and that the epichlorohydrin data do not explain this result. It was also noted that a number of the bacterial assays listed in the document summary table were discounted with regard to the genotoxicity discussion because they were done with the epichlorohydrin-stabilized formulation and these studies should be examined closely to determine whether the presence of epichlorohydrin can account for their positive results. In contrast, a reviewer commented that state of the art techniques for evaluation of genotoxicity were applied and resulted in negative findings. A second reviewer agreed, indicating that the amount of information available for the assessment was impressive and that a short-lived free radical in the glutathione conjugation pathway might contribute to the observed weak mutagenicity. Combined with increased cell proliferation this could explain the tumor results.

The review panel discussed the importance of the DNA adduct study on the determination of genotoxicity (Stott et al. 1997b). One reviewer noted that the cancer

might be tissue-specific and if no adducts were seen in the tissues containing tumors this would support the finding for lack of genotoxicity. Another reviewer noted that the doses used in this study were similar to the cancer bioassay (Stott et al., 1995a, b). A reviewer questioned whether the post-labeling method could detect oxidative damage of DNA. Dr. Stott indicated that this was not specifically looked at and agreed that it is theoretically possible that depletion of glutathione could lead to oxidative DNA damage. A reviewer asked about the sensitivity of the post-labeling assay (1 in 100,000,000) and how this high sensitivity relates to cancer risk. Dr. Gollapudi indicated that the technique addressed the potential for direct interactions of 1,3-dichloropropene metabolites with DNA. Another reviewer indicated that two additional studies (Ghia et al. 1993 and Kitchin and Brown 1994) reporting a NOEL for increased single strand breaks with exposure to high doses *in vivo* support the proposed non-linear mechanism.

Pertinent portions of the EPA proposed cancer guidelines (U.S. EPA 1996) were summarized by a reviewer and indicated that lack of DNA reactivity in and of itself is not sufficient to exclude a linear mode of action. This reviewer added that applying these guidelines with the fact that the epichlorohydrin data could not be totally dismissed (since the potency of epichlorohydrin itself did not explain all the observed toxicity) does not provide sufficient evidence to show a non-linear mode of action for the liver tumors. Another reviewer concurred that the liver is the key target to focus on and that the existing data are insufficient to say if the effect is linear or non-linear.

Much of the panel's discussion was regarding the importance of glutathione in the tumorigenic response generated by Telone II. A reviewer noted that the mode of action discussion in the Dow AgroSciences document was not particularly strong, but was presented more clearly in the presentation. This reviewer indicated that the existing data set was lacking critical information, including the effects of 1,3-dichlropropene on glutathione-deficient animals, metabolic pathways in addition to the central pathway, protein adduct evaluations, and information on human metabolites. Several reviewers agreed that a study in GSH deficient animals would be useful. A reviewer added that if the proposed mechanism was correct, then treatment under conditions that cause glutathione depletion, for example following inhalation of 20 ppm, should result in post-labeled bases which would give information on the secondary metabolism pathways that may be taking place. Dr. Stott commented that at doses of 60 ppm no mutagenic activity adducts were identified. Another reviewer noted that glutathione deficiency could itself generate toxicity. Another reviewer suggested that since there may be more than one metabolic pathway for 1,3-dichloropropene such that under conditions in which the central pathway is minimized other metabolic pathways may be highlighted. In response to a question from another reviewer, it was indicated that arguments could be made for either linear or non-linear response from this idea, for example if the diminution in GSH could be linear. Another reviewer noted that the kinetics are important, what is going on in one pathway could affect the other metabolic pathways. A reviewer disagreed indicating that in a physiological environment there must be a dose that does not deplete glutathione, which argues for a threshold or non-linear response. Another reviewer concurred that the data strongly support the use of non-linear methods based on the weight of the evidence.

One reviewer commented that the trans-isomer also forms a GSH conjugate in humans (Creedy et al., 1984) and that differences in cis- and trans-isomer must be taken into account. Dr. Stott indicated that there is more rapid conjugation with the cis- than the trans-isomer. In animal studies the trans-isomer is also conjugated, but is also metabolized by the oxidative pathway, but the *in vivo* mutagenesis assays did not detect direct interactions of potential oxidative metabolites and DNA.

One reviewer questioned whether there is a point at which no tumors are formed or just that the power of the animal studies was insufficient to see the linear tumor response. Another reviewer proposed plotting the numbers of tumors and GSH depletion with dose to provide evidence for linearity or non-linearity. Several members of the panel indicated that this analysis would be problematic in that the GSH depletion was seen at 3 days but compensatory levels were seen at 26 days, the tumor data do not increase linearly so one probably would not see a clear correlation, and that this analysis could only provide qualitative support since it would be difficult to interpret the importance of the early events to tumors which occur late. A reviewer suggested that existing literature on other substances that act by depleting glutathione might be useful to review.

The panel discussed the correlation between glutathione depletion and the results of the genotoxicity assays and whether these results support a linear or non-linear response. A reviewer noted that the *in vitro* data did show that mutations were blocked in the presence of glutathione protection. Dr. Stott added that under certain conditions, a positive result *in vitro* mutagenicity testing can be obtained, but if natural defenses are added back, the positive effect is removed. Another panelist asked whether the *in vitro* assays were all or none responses, which would bear on the issue of partial protection. Regarding the dose-response effect of glutathione levels, a reviewer noted that a 50% decrease in glutathione should lead to a significant decrease in protection and mutation frequency should increase. Another reviewer confirmed that no increase in DNA adducts was observed with increasing glutathione depletion and commented that a good attempt was made to study metabolism at tumorigenic doses and one would expect to see damage, yet no damage was observed. A reviewer clarified that this assay did not measure oxidative damage. One reviewer indicated that an *in vivo* mutagenicity assay using a transgenic rat model would be useful to parallel the negative findings in the Big Blue mouse study. The chair indicated that if the conclusion is that that there is mutagenicity and tumors are observed, then this does not argue for a non-linear response. A panel member indicated, however, that if depletion of glutathione causes these observed effects then there is a threshold.

The panel addressed several other issues regarding utility of glutathione depletion to predict the tumor incidence in animal studies. A reviewer commented that one question is whether high doses predict the effect of low doses, or are the results of the animal studies similar only at doses that saturate natural defenses. Another panel member agreed and added that the question also remains regarding the compensation for glutathione depletion over time as seen in the liver versus the lung. According to this reviewer, these organ specific effects are important and thus one cannot assume non-linearity on the basis that increased dose will generate a predictable decrease in glutathione. One panel

member asked if partial reduction in glutathione levels would generate excess risk. Another panelist noted that at doses that caused decreased glutathione, tumors were observed in animal studies. A reviewer indicated that the question is where the dose-response curve intersects the response axis, at some point with a significant decrease in GSH one may still see tumors, but the low tumor incidence may not be detectable with the power of the animal study. Other reviewers again raised the issue of geriatric animals and that a study with older rats may be useful since adequate GSH decreased as the animal ages, which may account for the late developing tumors.

Reviewers discussed that the toxicity profile is much different in the dog with the occurrence of hematopoietic effects. Dr. Stott indicated that dogs get anemia, but nothing in the bladder and there is no evidence of bone marrow toxicity.

The chairperson asked the panel to comment on additional data that might be useful to further support the proposed mode of action. Additional studies were suggested to determine the effect of GSH deficiency on toxicity, toxicokinetics of the GSH conjugation pathway and non-GSH metabolism, and additional *in vivo* genotoxicity assays. The additional study in GSH-deficient animals was suggested by several panel members to assess the physiological relevance of glutathione conjugation capacity on Telone II toxicity. Several panel members also suggested the need for additional data on the metabolism pathways for Telone II. One reviewer indicated that additional studies to quantify non-glutathione metabolism would be desirable. Dr. Stott commented that based on mass balance studies, the oxidative pathway is not extensive. This reviewer noted however, that under certain circumstances, one should see this difference, for example, if under normal conditions less than 5% of the non-glutathione pathway could be utilized, but under conditions of glutathione depletion this may increase to 10%. Assuming this scenario, Telone II might be weakly carcinogenic, but under rare conditions it might be a tumor promoter and this activity could be modeled with the kinetics of the metabolism pathway.

Other reviewers suggested that the kinetics of the reaction rates with GSH and the toxicokinetics of metabolite formation would be useful to know. The chairperson suggested that with these kinetic assay data, one could tie the metabolism with GSH depletion, DNA adduct formation, and tumor incidence. Dr. Stott indicated that even under depleted conditions the data have not shown any large increase in unknown metabolism and that *in vitro* kinetic data and GSH conjugation kinetics have also been determined. A reviewer noted that in the study (Stott et al., 1997b) the GSH levels were depleted at 3 days but had rebounded at two weeks. If the same metabolites were observed this could answer questions about the importance of the alternative metabolism pathways. Dr. Stott commented that it is doubtful that GSH would be saturated fully. Dr. Rao commented that even assuming no oxidative pathway was evident based on metabolite analysis on day 3 this would still not identify the mechanism.

Some of the panel members also indicated that additional *in vivo* genotoxicity assays would be useful to clarify remaining questions. A reviewer commented that a next step might be evaluation of 8-OH-guanine to correlate oxidative DNA damage with increased

tumors under conditions of depleted glutathione. Dr. Stott noted that this mechanism does not explain the negative results in the *in vivo* genotoxicity assays.

The panel had mixed opinions on whether the data on Telone II were sufficient to recommend the use of a non-linear model for the dose-response assessment. Most of the panel members indicated that a non-linear approach appears to be the best in this case, but some could not absolutely rule out a genotoxic mechanism and thought that additional investigation into oxidative damage, the relative contributions of metabolites of the alternate pathways, differences in metabolism across species, and additional studies at lower doses could assist in this determination.

The chairperson asked the panel to consider whether the mode of action for the lung and liver tumors should be separated. The panel did not concur with this suggestion, citing that there is not conclusive evidence that the lung tumors are the result of an irritation mechanism, in light of the lack of cell proliferation and the lack of lower airway irritation.

The chair also asked the panel to consider whether testing for oxidative damage would contribute to the weight of evidence for a nongenotoxic mechanism. The panel agreed that the document could include a more comprehensive synthesis of the kinetic data for GSH across species, which might lead to identification of additional studies, e.g. role of oxidative damage.

The panel discussed a number of consensus statements that capture the mode of action discussion.

1. We do not know the underlying mechanism for tumors
2. Mutations cannot be ruled out as a mechanism.
3. Tumorigenicity is only seen at high doses.
4. Time-dependent glutathione depletion seems to be associated with increase in tumors.
5. There is limited evidence of carcinogenicity in animal studies with the new ESO-stabilized formulations.

Weight of Evidence Narrative

The panel discussed the weight of evidence narrative in some detail and recommended revisions to reflect a number of key points.

- "1,3-Dichloropropene is unlikely to present a carcinogenic hazard to humans under normal agricultural use conditions (i.e. low level airborne exposure). 1,3-D is likely to be carcinogenic to humans only under conditions of high exposure (e.g., ingestion, or inhalation leading to metabolic saturation). The apparent lack of mutagenicity of 1,3-D in mammalian systems coupled with its rapid detoxification at nonsaturating doses as well as the general growth-promoting effect on high background tumor rates suggest a nonmutagenic mode of action for

this materials. Based upon this cancer hazard characterization, a nonlinear or threshold dose-response extrapolation is appropriate for 1,3-D." (page 41 draft document)

One reviewer noted that as proposed, the statement indicated a lack of mutagenicity in mammalian systems yet in some studies mutagenicity was observed. Several reviewers expressed reservations about the low dose effects, noting that the panel did not decide definitively on a linear or non-linear mechanism. One noted that when relying on bioassays with 50 animals per group, it might be difficult to see an effect in a susceptible subgroup.

The panel evaluated the statement regarding the likelihood of Telone II causing cancer at high exposures, with some panel members expressing doubts about the potential of Telone II to generate human cancer, citing that only benign tumors were observed in the animal studies and the bulk of the genotoxicity data were negative, if one considers data on the new formulation (without epichlorohydrin) only. Other reviewers held a different view indicating that the weight of evidence must reflect whether the animal data are sufficient to decide on human carcinogenic potential based on the principle that the data do not suggest humans would behave differently than animals. Another reviewer indicated that EPA guidelines assume benign tumors constitute a potential for cancer unless the data are sufficient to show otherwise. A second reviewer agreed, indicating that two different tumor types were observed in two different species and that at much higher doses there is potential for progression as in the NTP bioassay. A reviewer commented that the overall animal data are weak, but still support a potential for human carcinogenicity, but additional language on conditions that would describe the exposure that may lead to carcinogenicity risk, such as route or degree of exposure, could be included. The wording "likely to be carcinogenic" was discussed and the panel agreed with revised wording that Telone II "may be carcinogenic to humans."

The ambiguity of the low and high dose terminology was discussed. Referring to the relative level of the doses associated with tumor formation in the animal studies, Ms. Calhoun indicated that a dose level of 60 ppm as used in the animal study is equivalent to 300,000 ug/cu.m. and that peak and average concentrations of Telone II following field application are 1600 ug/cu.m. and 20 ug/cu.m, respectively. One reviewer indicated that the dose should be expressed in terms that provide a link to a biological event that implies the mechanism of action. Another reviewer suggested that there are only sufficient data to support a statement on physiological mechanisms regarding the GSH levels. Based on the consensus statements agreed upon for mode of action, reviewers suggested adding statements indicating that the tumorigenic mechanism in animals is unknown and that the effects occur at doses that deplete glutathione. Other suggestions regarding the effect of GSH included making a statement to indicate the level depletion of glutathione that would be considered relevant and to reflect the effect of a compromise on GSH levels.

One reviewer commented that the initial statement should state that a carcinogenic hazard may be expressed after long term exposure to levels associated with decreased glutathione and add a second statement that cancer hazard is unlikely under conditions of

intact physiological defense mechanisms. Ms. Calhoun indicated that the original narrative placed the unlikely statement first, based on the greater weight of evidence for this statement. A reviewer suggested reversing the order of these statements, noting that typically in EPA risk documents what is the concern is typically placed first, followed by what is not a concern. Dow AgroSciences expressed reservations that individuals may read only the first statement and would be mislead. While noting the concern, the panel agreed with the suggestion of placing the "may be carcinogenic" statement first.

Other comments from the panel included one reviewer's question if there are any data on irritation thresholds for the formulation in humans -- based on the idea that if a worker cannot smell it, is that below the level of concern. Dr. Stott indicated that in his experience the detectable level for humans is 1 to 2 ppm. Another reviewer suggested that the narrative indicates why a margin of exposure approach was recommended. The panel also recommended showing both MOE and linear extrapolations because a linear response could not be ruled out. One panel member asked what classification Telone II would be given under EPA's 1986 cancer guidelines (U.S. EPA, 1986). Two panel members responded that based on the data for the ESO-stabilized formulation, a classification of C would probably be assigned, but that this would not obviate the derivation of a quantitative risk value.

Based on these discussions and subsequent post-meeting comments and review, the panel agreed with the following Weight of Evidence Narrative statement. It was noted that the phrase "may be carcinogenic" is not consistent with the EPA proposed cancer guidelines (U.S. EPA 1996).

- **Telone II may be carcinogenic to humans after long-term exposure to high concentrations. This conclusion is based on limited evidence of carcinogenicity in animal studies using the current ESO-stabilized formulation (lacking epichlorohydrin). These newer studies with the current ESO-stabilized formulation, show an increase in benign tumors in animals, observed at doses which were also associated with depletion of reduced glutathione. Telone II is unlikely to present a carcinogenic hazard to humans when protective mechanisms are present, such as sufficient GSH to completely conjugate and detoxify the administered dose. While the mechanism of tumor formation is not completely understood, a genotoxic mode of action cannot be ruled out.**

The majority of the panel agreed that the weight of the evidence supported a non-linear mechanism and therefore a MOE approach was appropriate for regulatory purposes; however, some panel members noted that a linear mode of action could not be ruled out. The panel agreed that the risk document should include both approaches.

PRESENTATION AND CLARIFYING QUESTIONS - Cancer Dose-Response Assessment

Ms. Linda Calhoun of Dow Chemical Company briefly presented the dose-response assessment used to derive the quantitative estimates for inhalation and oral cancer risk values. Tables of results using a number of different models were presented for female mouse nasal lesions, male mouse lung tumors, male and female rat stomach lesions, and male rat liver tumors. Uncertainty factors and factors for margin of exposure were presented. Ms. Calhoun indicated that they selected the Weibull model because it is flexible and widely used. Many of the models used provided similar results and the data set fit the model.

For the inhalation route of exposure, a lower bound on the effective concentration (LEC) was proposed based on application of a Weibull model to lung adenomas in male mice (Lomax et al. 1989). The modeling procedure resulted in a proposed LEC_{10} of 8.6 mg/cu.m. Proposed factors for the margin of exposure included a value of 3 for human to animal sensitivity, a value of 10 for human variability, 1 for persistence, and 3 for mode of action / slope of the response curve, resulting in a combined factor of 100. The resulting proposed margin of exposure value was 0.2 mg/cu.m.

For the oral route of exposure, a lower bound on the effective dose (LED) was proposed based on application of a Weibull model to liver adenomas in male rats (Stott et al. 1995). The modeling procedure resulted in a proposed (LED_{10}) of 8.9 mg/kg-day. Proposed factors for the margin of exposure included a value of 10 for human to animal sensitivity, a value of 10 for human variability, 1 for persistence, and 3 for mode of action / slope, resulting in a total factor of 300. The resulting proposed margin of exposure value was 0.03 mg/kg-day.

Clarifying Questions on the Presentation:

Following the presentation, the review panel asked a number of clarifying questions regarding the calculations that were performed to arrive at the point of departure estimates. In response to these questions, Ms. Calhoun indicated that for the inhalation estimate the data were first converted to mg/cu.m (a conversion factor from the IRIS file 1 ppm = 4.54 mg/cu.m was used), were adjusted for the percentage of 1,3-dichloropropene in the formulation, and doses were adjusted to a continuous concentration (5/7 days and 6/24 hours). Several reviewers commented on the choice of models. One reviewer asked whether an attempt had been made to evaluate how well the predicted values matched the observed values. This reviewer pointed out that the data appear to indicate an EC_{10} of approximately 20 mg/cu.m, while the model predicted an EC_{10} of 15.2 mg/cu.m for lung adenomas. A second reviewer commented that the EPA software used for the modeling has not been validated for all the models that were presented and because the goodness of fit statistic is best with the greatest value, the linear model may actually have given the best result. Ms. Calhoun reiterated that a number of different models had been run and that the resulting LEC_{10} value is similar for many of these alternative models. A reviewer raised a question regarding the adjustments to the LEC_{10} for lung adenomas questioning why both a LEC_{HEC} was derived and body weight scaling was done.

A reviewer asked if any consideration was given to the response for the MOE since the effect that was modeled was a tumor endpoint. Ms. Calhoun indicated that no factor had been added since this effect was significantly increased at only one dose. Ms. Calhoun also indicated that a body weight scaling adjustment (3/4 body weight) had been done for the liver tumor modeling for the oral cancer MOE determination.

DISCUSSION -- Cancer Inhalation Dose-Response

Choice of Dose: Dow AgroSciences proposed a margin of exposure value base on application of a Weibull model to lung adenomas in male mice (Lomax et al. 1989). This modeling procedure resulted in a proposed LEC_{10} of 8.6 mg/cu.m.

A panel member began the discussion on the modeling for the inhalation cancer risk. This reviewer indicated that the choice of lung adenomas was the appropriate effect to model and indicated that similar results were obtained for this data set when run on other modeling software. The reviewer questioned the methodology for the HEC calculation and upon reevaluation by this reviewer and Ms. Calhoun, it was determined that HEC presented in the document was based on EPA 1991 methods for dosimetric adjustment. Recalculation of these data using the 1994 methodology resulted in a change in the proposed LEC_{10HEC} to 75 mg/cu.m. The panel recommended that the document be revised to use the current EPA dosimetric adjustment method. In addition, the panel recommended that the document should discuss and justify the selection for the choice of gas category used.

The panel discussed at length the choice of lung adenomas as the effect to model, with much of the discussion centered on the issue of the severity of lung adenomas. Several reviewers were concerned about this endpoint, noting that typically an earlier effect such as hyperplasia is modeled. Another reviewer agreed with this comment, but noted that lung adenomas were the only effect with sufficient dose-response data to model. Another reviewer noted that nasal lesions occur at lower concentrations than the lung adenomas, but that there were no other intermediate responses. Other panel members did not have the same reservations regarding the severity of the adenomas, noting that the adenomas were microscopic and not life threatening, although another reviewer responded that the microscopic nature of the tumors could be an artifact of the tissue staining technique. The chair summarized that some reviewers were uncomfortable with adenomas as the response to model, and commented that carcinogenic testing is crude and many precursor events may have been missed and without these, it may be appropriate to use an additional uncertainty factor. In spite of these issues, the panel agreed that the lung adenomas were the best choice of an effect to model.

The panel also discussed the appropriateness of the dosimetric adjustment methodology given the different responses in the nasal and pulmonary regions. One reviewer commented that the dosimetric adjustment is based on effects in the pulmonary region. Another reviewer noted that the study did not clearly indicate if the adenomas were derived from Type II or Clara cells which occur differently in the bronchioles versus the

alveolar regions of the lung. This is important information to determine if the dosimetric adjustment was appropriate.

The panel agreed with the point of departure estimate as revised to reflect the EPA (1994) dosimetric adjustments.

Margin of Exposure: Dow AgroSciences proposed factors for the margin of exposure which included a value of 3 for human to animal sensitivity, a value of 10 for human variability, a value of 1 for persistence, and a value of 3 for mode of action / slope of the response curve. This results in a combined factor of 100.

The panel raised questions on the issue of the nature of the response with regard to the modeling of the benign tumors. Two of the reviewers commented that normally for a margin of exposure approach more complete mode of action data would be desired before selecting tumors as an endpoint and that mode of action decisions were being made without sufficient data. In contrast, another reviewer noted that the lack of mutagenicity observed in the Big Blue mouse ruled out mutations. A reviewer responded that this negative finding did not rule out the adenomas and that the test has limits since it represents effects in only one strain of mice. It was suggested that since mutations could not be ruled out, it would be appropriate to present both MOE and linear approaches. Several reviewers agreed and commented that it is not clear if there are enough mode of action data to exclusively use the MOE approach and that it would be preferable to present both an MOE and linear approach. The panel agreed that both approaches should be presented in the document.

Human to Animal Sensitivity: Dow AgroSciences proposed a value of 3. The panel agreed on a value of 3 for human to animal sensitivity since a dosimetric adjustment had been made.

Human Variability: Dow AgroSciences proposed a value of 10. The panel agreed with the default value of 10 for human variability but recommended that text be added to justify the use of the default value, particularly with regard to potential children's risk issues. One reviewer added that justification of the default with regard to the aged should also be included and commented that the default factor of ten was not sufficiently justified or discussed in the document.

The panel also discussed the possibility of using a lower value for this factor based on the lack of difference in Telone II urinary metabolites in a group of Glutathione-s-transferase mu deficient workers. Several reviewers raised concern about relying on this study as sufficient evidence to modify the default value as indicated in the mode of action discussion.

Persistence: Dow AgroSciences proposed a value of 1. The panel all agreed on the value of 1 for persistence.

Mode of Action / Slope: Dow AgroSciences proposed a value of 3. The panel agreed with the proposed value of 3 for mode of action / slope. Several panel members noted that the slope of the dose-response curve was shallow, with another reviewer noting that in terms of the children's risk issue, a shallow slope generally argues for an increase in the uncertainty factor.

Severity (Nature of Response): Dow AgroSciences did not propose a value for this area. The panel discussed the need for an additional factor to account for the severity or nature of the response to better follow the EPA proposed cancer guidelines. Some reviewers advocated an additional factor of 10 to account for the severity of the effect based on reliance on tumors as an endpoint and the incomplete data on the mode of action. Other reviewers; however, indicated that since the response was a benign tumor, a factor of 3 would be more appropriate. One reviewer suggested that the data on glutathione levels in the lung would provide a precursor effect to model that would address severity. Although some reviewers indicated modeling this effect would reduce the value of the factor for severity, other panelists expressed concern with this approach. There were concerns with the reliance on the time of GSH measurement post-exposure since levels can rebound, and the problem with the effect of multiple isozymes with overlapping function. In addition to the panel members supporting factors of 3 and 10, other reviewers believed that the adenomas were a precursor lesion and supported a factor of 1. One reviewer added that if the effects were reversible then they would not be severe enough for an additional factor. The panel did not reach consensus on the appropriate value of the factor for severity with panelists supporting values of 1, 3, and 10.

Risk Value: Dow AgroSciences proposed a margin of exposure value of 0.2 mg/cu.m based on an MOE factor of 100. The panel agreed to a final MOE value ranging from 0.6 to 0.06 mg/cu.m based on a point of departure of 55 mg/cu.m and a total MOE factor of 100 to 1000. With the revised dosimetric adjustment, the 10^{-5} risk specific concentration from the linear extrapolation is changed to 0.006 mg/cu.m.

DISCUSSION – Cancer Oral Dose-Response

Choice of Dose: Dow AgroSciences proposed a Margin of Exposure value based on application of a Weibull model to liver adenomas in male rats (Stott et al. 1995). The modeling procedure resulted in a proposed LED_{10} of 8.9 mg/kg-day.

The panel discussed the choice of liver adenomas as the effect to model. One reviewer raised the possibility of modeling the observed liver foci; however, another reviewer indicated that the only dose-dependent trends were observed within certain severity grades and also that the dose-response is similar to adenomas. Dr. Stott commented that the liver foci data may present an apparent shallow slope due to the fact that at high doses less foci were noted because some foci merged. The panel agreed that liver adenomas are the appropriate effect to model.

Margin of Exposure: Dow AgroSciences proposed factors for the margin of exposure including a value of 10 for human to animal sensitivity, a value of 10 for human

variability, a value of 1 for persistence, and a value of 3 for mode of action / slope. This results in a combined factor of 300.

Human to Animal Sensitivity: Dow AgroSciences proposed a factor of 10. The panel agreed that a value of 3, instead of the proposed value of 10, would be appropriate for the LED_{10} of 8.9 mg/kg-day after adjustment by body weight scaling (3/4 power) to a value of 2.5 mg/kg-day.

Human Variability: Dow AgroSciences proposed a value of 10. The review panel agreed with the proposed value of 10 for intraspecies variability, although the panel noted that the text needed additional justification for the use of the default value.

Persistence: Dow AgroSciences proposed a value of 1. The panel agreed with the proposed value of 1 for persistence.

Mode of Action/ Slope: Dow AgroSciences proposed a value of 3. The panel agreed with the proposed value of 3 for mode of action / slope.

Severity (Nature of Response): Dow AgroSciences did not propose a value for this area. The panel discussed the issue of severity of the response due to the reliance on modeling of benign tumors. The majority of the panel supported a factor 3 for a number of reasons. One panel member noted that the lack of an *in vivo* liver genotoxicity assay justified applying a factor of 3 instead of 1. Another reviewer favored a 3 instead of 10, noting the late onset of the adenomas. A factor of 3 was also recommended because liver toxicity, but not tumors, was present in the inhalation study, reflecting a threshold response. Some of the panel members preferred a factor of 10, indicating that if the liver foci could be modeled as a precursor then a lower value for this factor could be applied. One reviewer noted that the proper choice of a factor was not clear. The consensus of the panel was that a value of 3 would be appropriate. Some of the panel members who preferred 3 indicated that they could be persuaded to choose 10, and a minority of the panel felt that a value of 10 should be assigned.

Risk Value: The resulting proposed margin of exposure value, without the body weight scaling, is 0.03 mg/kg-day. After body weight scaling (3/4 power), the MOE value is 0.008 mg/kg-day. These values are based on a point of departure of 2.5 mg/kg-day and a total MOE factor of 300. The linear extrapolation generated a risk specific dose of 0.00025 mg/kg-day at the 1 in 100,000 risk level.

The chair asked if there were any additional recommendations from the panel. Several panel members indicated that greater detail about the studies should be in the document and additional discussion about mechanism should be included.

PRESENTATION AND CLARIFYING QUESTIONS - Noncancer Assessment

Ms. Linda Calhoun of Dow Chemical Company presented information on the hazard identification and dose-response assessment for derivation of a proposed reference

concentration (RfC) and reference dose (RfD) using U.S. EPA methods. A RfC was derived based on benchmark concentration (BMC) modeling of the nasal epithelial hyperplasia in female mice from the Lomax et al (1989). In that study, the lowest concentration at which effects were seen was 20 ppm in females. The resulting BMC_{10} (mouse) was 3.1 mg/cu.m, using a Weibull model. (The BMC_{10} equals the 95% lower confidence limit on the estimated duration-adjusted concentration producing a 10% quantal response in mice.) Dosimetric adjustments were made for a Category 1 gas to determine a BMC_{10HEC} of 0.57 mg/cu.m. An uncertainty factor of 30 (10 for intraspecies variability and 3 for animal to human extrapolation) was applied to derive the proposed RfC of 0.02 mg/cu.m.

A RfD was derived based on benchmark dose (BMD) modeling of the basal cell hyperplasia in male rats in a chronic dietary study (Stott et al., 1995). Male rats exhibited epithelial changes in the forestomach at a dose of 12.5 mg/kg-day. The resulting BMD_{10} was 2.2 mg/kg-day using a Weibull model. An uncertainty factor of 100 (10 for intrahuman variability and 10 for animal to human extrapolation) was applied to derive the RfD of 0.022 mg/kg-day.

Ms. Calhoun presented a table comparing results from a number of models and noted that they selected the Weibull model as most appropriate for derivation of these values.

Clarifying Questions on the Presentation:

The panel had no clarifying questions.

DISCUSSION – Noncancer Inhalation

Hazard Identification:

The panel agreed that the existing database was sufficient for development of a reference concentration (RfC) and that selection of nasal lesions as the critical effect for inhalation was appropriate. It was noted that this response is consistent across studies and other effects such as urinary bladder and forestomach lesions occurred only at higher doses. In addition there was not a clear difference in the severity of the nasal effects in the chronic versus subchronic study, and the effects were observed late in the study and were not progressive. Others noted that the response was at 24 months. Dr. Rao added that progression in the nasal epithelial hyperplasia was not seen; however, a reviewer noted that the NOAELs in the subchronic versus the chronic study were different, potentially indicating progression. Another reviewer added that there was no evidence for these lesions in humans.

The panel discussed the apparent difference between toxicity observed in mice and rats following inhalation exposure. The panel discussed possible efficiency as related to differences in gas deposition across species. A panel member commented that mice are more efficient at filtering due to surface area and convolution of the nasal turbinates and the upper respiratory tract gets 20-30% of the dose. Dr. Stott indicated that the actual

dose was 2-3 times greater in the mouse than in the rat. A reviewer questioned why no systemic effects were observed if the dose to the upper respiratory was 20-30% of the total dose. Another reviewer indicated that with irritation of the respiratory system, changes in respiration could generate systemic pH problems. This reviewer suggested that a respiratory irritation study would be useful to answer these questions and may have a bearing on the issue of humans' ability to detect odors of Telone II.

Dose-Response Assessment

Choice of Dose: Dow AgroSciences proposed a BMC_{10HEC} of 3.1 mg/cu.m, using the same dosimetric adjustments that EPA used for the IRIS RfC. The panel discussed the selection of concentration and calculation of the BMC briefly. One reviewer noted that the dose-response modeling was not model dependent and that he reproduced the same results using different programs than Dow AgroSciences. This reviewer worked with the Dow AgroSciences scientists to revise the dosimetric adjustments using methods from EPA's 1994 RfC methodology (U.S. EPA, 1994) for Category 1 gases. The revised RGDR was 0.395, which when multiplied by the BMC_{10} for the mouse, results in a BMC_{10HEC} for humans of 1.22 mg/cu.m. The panel agreed with the revised $BMC_{10HEC.}$

Uncertainty Factor: Dow AgroSciences proposed an overall uncertainty factor of 30, with 10 for intraspecies variation and 3 interspecies variation.

Intraspecies Variability: Dow AgroSciences proposed a factor of 10 for this area. A reviewer noted that this is the same as was used by EPA for the RfC on IRIS (U.S. EPA 1998). This value for intraspecies variability may be conservative, but there are not sufficient data to move from the default value of 10. Dr. Stott mentioned a study conducted in the Netherlands (Vos et al., 1991). where no difference in metabolism was determined in a group of GST-mu deficient workers whose urinary metabolites had been measured. Several reviewers speculated on why these results were seen, including that this may indicate that GSH conjugation is not the critical step in the metabolic pathway or that it could be that the chemical reaction versus enzymatic reaction was compensating in that group. Dr Stott thought that alternatively this result probably reflects human reserve capacity at the occupational exposure level. One reviewer commented that the coefficient of variation observed in this study on plasma and excretion data of 30% would translate to a ratio of the upper confidence limit to the mean of 1 to 1.5 instead of three for pharmacokinetics. This would support moving the UF for intrahuman variability closer to a value of 3 to 6. Another reviewer; however, indicated that the actual range varies from 300 to 2000 which is almost a factor of 6 for pharmacokinetics alone.

A reviewer noted a study (Vos et al., 1991) that compared variability in enzyme activity of various GST isozymes which indicated as much as a three-fold difference. Another reviewer pointed out that the data from an occupationally exposed group could reflect healthy worker effects and may not represent effects on children or aged individuals. The panel agreed that the data were insufficient to move away from the default value of 10 for intraspecies variability. It was suggested that the document include a discussion of the

above studies and indicate what additional data might help move away from the default of 10.

Interspecies Variation: Dow AgroSciences proposed using a factor of 3 for this area of uncertainty because dosimetric adjustments in the methodology have addressed the pharmacokinetic component of the factor. One reviewer noted that the observed hyperplasia is probably secondary to irritation, which is a well-defined threshold effect, and therefore, the same set of default uncertainty factors would not necessarily be required since the dosimetric adjustment accounts for some of the interspecies differences. Another reviewer commented that this might be true if the nasal tissue were the only tissue of concern, however, EPA's method is designed to protect against all noncancer toxicity and the other effects of inhalation exposure are not precluded. A reviewer suggested applying the methodology of upper respiratory tract deposition of vapors and use of these specific models to determine the uncertainty factor for pharmacokinetics across species. This would provide a biologically based adjustment.

The panel agreed that a factor of 3 was appropriate.

Database Deficiencies: Dow AgroSciences proposed a factor of 1. The panel agreed.

The panel briefly discussed confidence in the RfC. Panel members however, were unclear on what a confidence rating was measuring, whether it is a rating relative to other RfCs or perhaps confidence reflects the confidence in predicting the correct RfC. Most reviewers thought confidence was high, however one reviewer indicated that the lack of irritation studies suggested a lower confidence.

Risk Value: Dow AgroSciences proposed a RfC of 0.02 mg/cu.m. The panel agreed to a RfC of 0.04 mg/cu.m, based on the same study and uncertainty factor, but with revised dosimetric adjustments.

DISCUSSION – Noncancer Oral

Hazard Identification

The panel agreed that the database is sufficient to derive a risk value.

A reviewer summarized the data, indicating that there is an array of data identifying the same NOAEL for the mouse, rat, and dog. Because of differences in dosing, the lowest LOAEL was from the study in rats and with epithelial changes in the rat stomach identified as the critical effect. This reviewer thought the document should evaluate the hematopoietic effects in dogs due to the high background incidence of anemia in young children in the U.S. which merits a thorough discussion of the significance of the dog finding, a possible mechanism for the effect, and the possibility of anemic children as a sensitive population. One reviewer questioned whether dogs might be the more relevant species. A reviewer commented that the discussion only talks about high dose groups but does not adequately discuss the effects observed in the middle dose groups. This reviewer

also mentioned that the risk document and the original study are in disagreement regarding the 13-week rat study in which the support document indicates there were differences at the 5 mg/kg-day dose but the original study does not show this.

After some discussion, the panel agreed that the choice of the critical effect was appropriate, however, one reviewer noted a concern about using forestomach basal cell hyperplasia as the critical effect because gastric irritation is a concentration dependent, not a dose-dependent effect. The panel agreed that the supporting document should discuss in greater detail the human data and provide a more in-depth evaluation of the other effects observed in the animal studies (e.g., as noted above with the dog). The panel also indicated that it might be worthwhile to model the hematopoietic effect in the dog study (specifically modeling the mean corpuscular volume and mean corpuscular hemoglobin). Another reviewer indicated that this modeling may not work well due to the small number of animals. In addition, individual reviewers suggested that the document discuss that the application of other models that were fitted gave similar results, and that the default to extra risk should be justified in the text.

A reviewer indicated that the document could still present a RfD based on the NOAEL observed for the hematopoietic effects observed in the dog and that this would generate a similar value.

Dose-Response Assessment

Choice of Dose: Dow AgroSciences used the rat forestomach basal cell hyperplasia data from Stott et al. (1995) to determine a BMC_{10} of 2.2 mg/kg-day using the Weibull model. The panel agreed.

Uncertainty Factor: Dow AgroSciences proposed an uncertainty factor of 100, 10 each for intra- and inter-species variability.

Intraspecies Variability: Dow AgroSciences proposed a factor of 10. A reviewer asked if an additional factor was warranted (as indicated by the Food Quality Protection Act [FQPA]) due to children's high background incidence of anemia and the hematopoietic effects observed in the dog study. Others indicated that this is more appropriately placed in a risk characterization or management discussion. Another reviewer indicated that the default value be applied unless there are specific data to indicate that children are more susceptible, and in this case there are not enough data to change the default. The panel discussed the dog study, noting that both the decrease in hematocrit and erythrocytes were reversible. Another reviewer commented that the effects on bone marrow in an evaluation of micronuclei and sister chromatid exchange (SCE) observed in rats, is consistent with some effect on bone marrow (Kevekordes et al., 1996). Dr. Gollapudi of Dow Chemical Company added that two other studies failed to show this effect, and Dr. Stott commented that the micronuclei test is a test for genotoxicity not bone marrow toxicity. A reviewer clarified that the FQPA question only relates to hematopoietic effects, and it is not clear if this is a species-specific effect with only the stomach irritation consistent across species. Another reviewer indicated discomfort with saying

any more than the data are suggestive of a hematopoietic effect. The panel agreed that a factor of 10 was appropriate and there were not enough data to move away from the default, but that the document should address the FQPA issue and the results of the dog study.

Interspecies Variability: Dow AgroSciences proposed a factor of 10 for this area of uncertainty. The panel agreed.

Database Deficiencies: Dow AgroSciences proposed a factor of 1 for database deficiencies. The panel agreed, with one reviewer indicating support for a value of 1, but preferring an oral reproductive and developmental study.

Risk Value: Dow AgroSciences proposed a RfD of 0.02 mg/kg-day. The panel agreed with this proposed value.

The chairperson asked the panel their thoughts on the confidence in the value. The chairperson indicated that a confidence of medium to high would be appropriate, due to the reliance on the inhalation reproductive and developmental study to complete the database for these oral endpoints. Dr. Rao commented that this should not present a problem because the systemic dose would be higher for inhalation than oral exposure due to the first pass effect in the liver. A reviewer pointed out that this might not be the case if hepatic activation is occurring, but also agreed with the confidence of medium to high. Another reviewer indicated that a confidence of medium was appropriate because of the hematopoietic effects and the lack of an oral reproductive and developmental study. The panel agreed that confidence of medium to high was appropriate.

The panel reached unanimous consensus on the RfD proposed by Dow AgroSciences.

Other Issues – Noncancer

The chairperson asked if there were any additional issues from the panel. One reviewer asked whether potential dermal exposure should be addressed even if in a qualitative manner. Mr. Roby of Dow AgroSciences indicated that dermal exposure has never been a concern for Telone II due to its subsurface application and the use of protective equipment by the applicators. Dr. Rao indicated that the EPA OPP (U.S. EPA, 1997a) supported the conclusion that due to its use pattern, 1,3-dichloropropene does not represent a hazard through the dermal route. A panel member indicated that one human study indicates dermal absorption of 2 to 5%. Another reviewer indicated that it would be useful to capture this data in the kinetics section so readers could conduct risk assessment for dermal if they needed.

RECOMMENDATIONS

- The panel recommended that the document and assessment be identified as for Telone II, the formulation, rather than 1,3-dichloropropene, a component, and that the conclusions be based on the Telone II data.

- The panel suggested the risk assessment document include additional information and discussion on:

- better discussion of human studies and add quantitative information, including the dermal study in Dutch flower growers (Kezic et al., 1996),
- a more in-depth discussion of the animal toxicity studies with better synthesis of the effects,
- more information on the hematopoietic effects observed in dogs,
- the different BMD models that were used and justification for use of extra risk, and
- FQPA concerns for both cancer and noncancer.

- Several reviewers found the document somewhat difficult to follow and suggested reorganizing the text in a number of ways. The panel did not recommend a particular format for the document, but the format of the IRIS Toxicological Reviews was suggested as a model to consider. The panel asked to review the revised document and International Toxicity Estimates for Risk (*ITER*) summaries before the assessment is released and loaded on *ITER*.

- Additional studies were suggested to determine the effect of GSH deficiency on toxicity, toxicokinetics of the GSH conjugation pathway and non-GSH metabolism, and additional *in vivo* genotoxicity assays.

- Additional investigation was suggested into oxidative damage, the relative contributions of metabolites of the alternate pathways, differences in metabolism across species, and additional studies at lower doses that could assist in making a determination for a non-linear mode of action.

- A respiratory irritation study was suggested to help answer questions about possible human ability to detect Telone II odors.

- The panel agreed that the document could include a more comprehensive synthesis of the kinetic data for GSH across species, which might lead to identification of additional studies, e.g. role of oxidative damage.

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PROTOCOL FOR A STUDY ON THE ACUTE THRESHOLD FOR HUMANS FROM COPPER IN DRINKING WATER

Sponsor: International Copper Association

Presenters:

- Dr. Scott Baker, International Copper Association
- Dr. Magdalena Araya, University of Chile

Chair: Dr. Kenneth Poirier, The Procter & Gamble Company

Review Panel:

- Dr. Pamela H. Dalton*, Monell Chemical Senses Center
- Dr. Robert Devlin, U.S. EPA National Health and Environmental Effects Research Laboratory.
- Dr. Joyce M. Donohue, U.S. EPA Office of Water
- Dr. Ernest C. Foulkes, Department of Environmental Health, University of Cincinnati
- Dr. Marvin A. Friedman, private consulting toxicologist
- Dr. Heidi J. Kalkwarf, Children's Hospital Medical Center, Cincinnati, Ohio
- Dr. Peter R. McClure, Syracuse Research Corporation
- Dr. David W. Morry*, California Environmental Protection Agency
- Dr. Bruce D. Naumann, Merck & Co., Inc
- Dr. Kenneth A. Poirier, The Procter & Gamble Company
- Dr. Bonny L. Specker, South Dakota State University
- Dr. Karl H. Summer –GSF-National Research Center for Environment and Health, Neuherberg, Germany

* Provided written comments for the panel's consideration. Additional comments were received from Dr. Judith Turnlund of the USDA Western Human Nutrition Research Center.

A representative from the International Copper Association (ICA) and the principal investigator presented information on the proposed protocol. This was followed by a short period during which reviewers asked clarifying questions. The panel then discussed the list of questions which made up their charge.

PRESENTATIONS & CLARIFYING QUESTIONS

Dr. Scott Baker, ICA

Dr. Baker provided background on the ICA, its environmental research program and its goals for the proposed acute study of copper in drinking water. The ICA has about 35 member companies with a focus on promoting copper products and their manufacture, but not mining or the processing of copper ore. One of the major issues for ICA is the environment, with 10-12% of its budget devoted to its ecological and human health research program. A wide range of research projects are funded, with most conducted by researchers at academic institutions. ICA seeks the best researchers around the world and insists on independence and peer-reviewed publication of findings. The research agenda is based on filling scientific data gaps and specific needs to reduce scientific uncertainty. In the area of human health, research is being conducted in many areas including acute toxicity and excess dietary supplementation of copper.

The acute study of copper in drinking water is needed because there is too little known about:

- The benefits of copper in drinking water to human health and copper's contribution to daily intakes;
- The bottom of the U-shaped dose-response curve, where adverse effects are found with both copper deficiency and copper excess;
- The health effects of copper in elevated, pulse-dose concentrations, especially in "sensitive populations;" and,
- The science behind current regulations and the threat of potential future restrictive regulation based on this weak science.

ICA has selected three research centers, well known for their work in copper, to conduct this study. They are the University of Ulster, Northern Ireland; the Institute of Nutrition and Food Technology of the University of Chile, Santiago, Chile; and the Grand Forks Human Nutrition Research Center of the U.S. Department of Agriculture, Grand Forks, North Dakota, USA. ICA asked Toxicology Excellence for Risk Assessment (*TERA*) to provide independent oversight and coordination of the project to ensure independent results. *TERA* will also review the results.

An acute study is desired because nausea and other gastrointestinal (GI) symptoms are the first symptomatic, clinically adverse effects observed upon exposure to copper. These GI symptoms appear to be the critical effect. Prevention of nausea is also the basis for many regulations. Other gastrointestinal effects occur from copper in drinking water that are episodic and reversible acute exposure. However, some of these effects, such as those on gastric motility can be attributed to other factors (e.g., chewing gum, hunger), and whether it is adverse is questionable. Chronic systemic effects are being pursued in a separate research program.

The evolving approach to this investigation is to initially do a univariate study with the dose varying, but all else held constant. Multivariate studies will follow and will consider other factors including:

- Copper speciation
- Taste confounder
- Water characteristics
- Water pipe characteristics
- Population characteristics
- Population differences
- Sensitive receptors
- Diet

This acute toxicity program for copper will use fixed parameters including distilled deionized water, one copper species, one consolidated age group, and taste control. Subsequent studies will include defined and natural waters with variable characteristics, variable copper species, variable volumes, no taste control and consideration of dietary factors.

Dr. Baker posed an additional question for the review panel to consider: "Will the current univariate study provide sufficient information to supplant weaker existing studies, and will it provide the necessary preliminary information to enable the subsequent multivariate study?"

Dr. Magdalena Araya, Institute of Nutrition and Food Technology of the University of Chile

Dr. Araya is the Principal Investigator for this study. She presented background on and details of the proposed study. She indicated many variables were considered to determine what should be included in this first study including copper concentration, speciation, water hardness, pH, and bioavailability. Subject variables included age, physiology (whether copper is taken with meals, water or fasting) and psychological aspects.

For this initial study the investigators propose to minimize these variables by using only healthy adults as subjects, deionized water and an overnight fast. Investigators will carefully design the questionnaires used to record symptoms so that there is equivalent understanding by subjects in all three locations

Defining the threshold for nausea is an outstanding issue. It could be defined in a number of ways, including:

- the first person to respond;
- the level at which nausea has been detected in a certain population (e.g., 5%, 10%, 50%?);
- existence of a sensitive subpopulation, significant differences in responses to different doses; or,

- a no-observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), or upper safe limit (USL).

Some difficulties in determining a threshold are that gastrointestinal symptoms of nausea are nonspecific, the baseline for nausea in the early morning is unknown, and the influence of fasting on the nausea threshold is not known.

The proposed study uses a latin square design for random dosing of 0, 2, 4, 6, 8 and 10 mg copper/L in deionized water. A total of 60 subjects is proposed for each location. Subjects will include healthy males and females, but not extreme ages. There will be no stratification by age. Subjects will be asked to fast overnight and remain for one hour of observation after taking the copper. For the first 15 minutes the subjects will be alone with a researcher, for the last 45 minutes they will be in a common lounge. Subjects will be sent home with a log type questionnaire to recall symptoms for 24 hours. Subjects will return once a week for a total of 6 weeks and 6 different doses.

If a threshold is identified, then additional studies at lower doses are planned. If the threshold is not identified, higher doses will subsequently be used.

The hypothesis is that nausea and other GI symptoms appear when individuals drink water containing between 2 and 10 mg copper per liter of water. The null hypothesis is that concentrations between 2 and 10 mg Cu/L drinking water do not induce nausea and other GI symptoms in adults. To calculate the sample size investigators considered a number of approaches and Dr. Araya presented the results of Chi-square for comparison of categorized results and paired differences between proportions. The critical question is "how many individuals are going to present symptoms at the baseline (zero exposure)?"

Using a Chi-square approach, investigators assumed a 5% response at baseline and reviewed a number of scenarios for assumptions of responses at higher dose levels. Using best scientific judgement, they selected possible responses of 5, 5, 20, 40, 60 and 60% at doses of 0, 2, 4, 6, 8, and 10 mg , respectively, as the most plausible case. The resulting sample size is 52 subjects.

Using the paired differences between proportions method, they estimated the baseline frequency at 5%, determined there would be a 20% difference between groups with alpha at 5% and statistical power of 90%. This results in a sample size of 56 subjects.

The investigators proposed using a latin square design for determining order of doses, with 60 subjects each receiving each concentration in random order.

Clarifying Questions on the Presentations

In response to a number of clarifying questions, Dr. Baker indicated that the purpose of this study is to examine effects of excess copper in drinking water. Also, the issue of excess copper in nutritional supplements and how their use affects an individual’s

response to copper present in the drinking water will be addressed by this protocol and subsequently in later research being proposed in this program.

One reviewer asked whether there are enough data to identify nausea as the most sensitive indicator of copper toxicity. Dr. Araya indicated that nausea is the most sensitive symptom and previous studies have shown that nausea precedes other symptoms.

Another question involved the use of single doses when long-term consumption of low levels in drinking water is a concern. While the concern is copper in drinking water consumed over a longer period of time, the research team thinks the study design is conservative using a pulse dose to test the worst case situation.

A reviewer asked whether the investigators are concerned about the possibility of the subjects acquiring a tolerance to copper. The researchers recognize the issue, but they do not think that 200 ml once a week for 6 weeks would be sufficient to develop a tolerance.

Reviewers had a number of questions about the design and administration of the questionnaire. These discussions are reflected under questions number six and seven below.

Question Posed to the Peer Review Panel

The sponsor requested that the reviewers evaluate and respond to a number of questions to focus their review and comments. Dr. Baker also posed an additional question (#12) at the meeting.

1. Is the n of 60 for this study sufficiently powerful to find the expected no effect level?
2. Does the study need six doses?
3. An alternate study design would be to randomly subdivide 60 subjects into at six different exposures groups (~5 subjects per sex per exposure). This study design would not be confounded with the multiple individual exposures of the current design, but would suffer because of a fewer number of subjects per group. Also the control groups would be different. In the current study design, everyone serves as his/her control. In the alternate design, one group serves as control. Is the alternative study design superior for the purposes of the stated objectives?
4. This study specifically excludes age stratification, based in part on the results of recent work which indicates that children may not be as sensitive to the effects of copper in drinking water as perhaps previous work indicates, and in part on the expected similarities among young and old adults on gastric physiology. Is this exclusion reasonable?
5. Does chronic exposure to copper at concentration just below the gastric response level lead to elevated positive copper balance in healthy individuals? In healthy infants?

6. Is the one hour observation period sufficient time for the development of potential gastrointestinal problems?
7. Are the questionnaires sufficient to address the potential effect? Are the approaches to questioning needed in this protocol?
8. Should subjects receive the same copper solutions described in protocols 1 and 2, plus the addition of aspartame to each solution in a sufficient quantity in order to mask the taste of copper?
9. Does the presence of solid foods in the GI tract influence the response to copper in drinking water? How should this potential confounder be addressed?
10. Does a person become acclimatized to elevated copper concentrations in his/her drinking water? Will a person respond on first exposure to a lower concentration than would cause a problem with repeated exposures.
11. The results of this study have potential use in two ways: as a way of judging the appropriate amount of copper in drinking water and as an initial study to support future development of guidance on copper supplementation. Is this dual purpose evident? Will the results be meaningful for both purposes?
12. Will the current univariate study provide sufficient information to supplant weaker existing studies, and will it provide the necessary preliminary information to enable the subsequent multivariate study?

DISCUSSION

1. **Is the n of 60 for this study sufficiently powerful to find the expected no effect level?**
2. There was significant discussion about the sample size needed for this study and the best way to calculate the sample size and order of dose administration.

   To start the discussion, one reviewer asked for a definition of the term NOAEL or No Observed Adverse Effect Level. Dr. Dourson of *TERA* explained that the NOAEL is the dose or concentration at which there is no statistical or biological increase of incidence and/or severity of effect in comparison to its appropriate control. A pairwise comparison to the control can be made or sometimes to the other dose groups. It is not usually determined by trend tests.

   Dr. Araya clarified that in the proposed protocol each person will serve as his or her own control because he or she will receive each dose, including a control dose (no copper), during the six weeks of the study. This will avoid the criticism that many people are prone to nausea, by determining each individual's nausea threshold.

   Reviewers were concerned that the sample size of 60 would not be powerful enough and if the results show no difference, it might be attributable to insufficient sample size to detect differences. Several reviewers raised questions about how the sample size was calculated. They indicated that the question that needs to be answered is "what is the increased incidence of nausea above which is clinically meaningful?" What needs to be defined *a priori* is what response will be

considered no effect. Or, put another way "what is the minimum positive response that the investigators would consider significant?" To calculate the sample size the investigators assumed that a concentration of 4 mg/L would be the cut off point, or threshold, and that they could expect between 50 and 60% response at the highest concentrations, which was considered the worst case scenario. Using a chi-square design, the study would need a sample size of 52 people to detect a 20% response between two doses.

A number of reviewers saw problems with assuming a response rate of 20% as the determinant, indicating that a 20% response would be considered quite high by regulators.

One reviewer suggested unbalancing the design, using larger numbers of subjects at the lower doses and fewer at the higher doses where one would expect greater response and therefore need fewer people to detect the response. Another reviewer thought this approach could be valuable and the investigators might choose to increase the dose spacing. The latter reviewer liked the concept of this being the first study in a larger program, as this first study will not provide answers to satisfy everyone. Another reviewer pointed out that this study design will not provide a NOAEL, but the dose-response that will cluster around where the NOAEL is likely. He suggested putting more power into a second study.

One reviewer referred to the study by Pizzaro et al. (unpublished) for guidance and thought that about 100 people would be needed, assuming 2% response at the 0 ppm dose level and expecting response to go up 10% for each dose. This study, however, was not acute single doses, but was a constant concentration in drinking water for two weeks. One does not know whether adaptation played a role. Other case studies of acute situations indicate that around 4 mg/L is the response area.

Reviewers agreed that a latin square design would be the best design to estimate carryover effects. The panel suggested that 100 subjects should be used to increase the power of the study and that both an ascending and descending order of doses should be used to address the possibility of carryover effects. If this study is the first of many, then perhaps 60 subjects would be adequate if more power were used in later studies.

Reviewers asked whether the results from the three studies would be combined or blended for statistical analysis. Dr. Baker indicated that they plan to combine the results and analyze the threshold as one combined set of data. However, there may be some "cultural" aspect which confounds the results; in which case, the data may not be combined, but used perhaps as a way to analyze for trends in results**.**

3. **Does the study need six doses?**

4. The panel discussed whether all six concentrations were needed. They agreed that it was unclear which dose would be the no effect level and that they should all be included.

   One reviewer examined the latin square design provided as an example, for order of doses and noted that the doses were always in a descending order. Several reviewers suggested providing doses in both an ascending and descending order to protect against carryover effects.

5. **An alternate study design would be to randomly subdivide 60 subjects into at six different exposures groups (~5 subjects per sex per exposure). This study design would not be confounded with the multiple individual exposures of the current design, but would suffer because of a fewer number of subjects per group. Also the control groups would be different. In the current study design, everyone serves as his/her control. In the alternate design, one group serves as control. Is the alternative study design superior for the purposes of the stated objectives?**
6. The panel did not think that the alternate design was superior.
7. **This study specifically excludes age stratification, based in part on the results of recent work (26) which indicates that children may not be as sensitive to the effects of copper in drinking water as perhaps previous work indicates, and in part on the expected similarities among young and old adults on gastric physiology. Is this exclusion reasonable?**
8. The panel did not think that the recent work cited was relevant to acute exposure, but agreed that children should not be included because of the difficulty of obtaining meaningful responses from children and ethical reasons leading one to do the initial work in healthy adults. The panel agreed that a study with children would be desirable at a later point. Dr. Baker asked how the issue of children might be addressed.
9. **Does chronic exposure to copper at concentration just below the gastric response level lead to elevated positive copper balance in healthy individuals? In healthy infants?**
10. Reviewers indicated that the evidence is not convincing. At high concentrations, the fraction of absorption goes down, but the physiological mechanism is not established. There is a negative copper balance throughout the lifetime, starting with a high copper level in the liver during the first year and then it goes down and stays the same for the remainder of life.
11. **Is the one-hour observation period sufficient time for the development of potential gastrointestinal problems?**

A reviewer summarized the animal and human case study data, which indicate that a one-hour observation period would be sufficient. Adding a 24-hour recall would also be helpful. Other reviewers noted that fasting for too long might also cause problems with nausea. Additional points are discussed under question 7 below.

7. Are the questionnaires sufficient to address the potential effect? Are the approaches to questioning needed in this protocol?

The panel discussed how to determine the subject's symptoms. The proposed protocol called for a questionnaire prior to the first dose, with a follow up questionnaire administered one hour after dosing and in addition asking subjects to recall symptoms for next 24 hours. For the first fifteen minutes the subjects would be isolated and monitored individually for signs and symptoms of nausea and other GI effects.

Because nausea is a particularly subjective symptom, the investigators recognize the need to use the best survey techniques to reduce as much subjectivity as possible. Subjects in three different cultures are being tested with the objective of determining the nausea threshold independent of culture. There are known differences between cultures concerning individual's perception of physical and mental health conditions. The investigators indicated that the exact wording of the questionnaires has not been finalized but they will be utilizing experts to word the questions to insure that they will mean exactly the same thing in all three cultures.

The panel suggested that a baseline questionnaire should be administered prior to each dose. They suggested the subjects should first be asked about their general feeling at that time with questions such as "How do you feel?" and "Do you have anything to report?" perhaps asking for responses on a scale of 1-5. A written checklist of many types of symptoms would then follow this. These same general questions and checklist should be administered multiple times while at the testing center and later that day. The panel suggested having subjects fill out questionnaires at 15 minutes, 60 minutes, and later recall of the day's symptoms prior to retiring for night (or 24-hour recall).

One reviewer suggested no human intervention in the questioning, instead using a computer for subjects to fill out the questionnaire at specific time intervals.

One reviewer noted that it would be difficult to observe effects during the proposed 15-minute observation period since the dosing is in a range near the threshold and symptoms could be quite mild and hard to observe. A number of reviewers commented on the subjects being kept in a common room for the remainder of the first hour and expressed concern that they might discuss symptoms, resulting in a greater recall of symptoms. It was suggested that subjects be separated so as to reduce the potential for discussing symptoms.

Reviewers suggested that a pre-questionnaire is needed to elicit information on background levels of copper intake. Additional questions are also needed about existing health conditions such as ulcer, indigestion, and chronic gastrointestinal problems, which may produce similar symptoms. Because nausea is a frequent symptom in early pregnancy, subjects should be tested rather than rely on their knowledge. Occupational exposure to copper should also be determined. While the proposed protocol did not specify inclusion or exclusion criteria, the panel suggested that the conditions mentioned above be considered, along with others the researchers identify.

8. **Should subjects receive the same copper solutions described in protocols 1 and 2, plus the addition of aspartame to each solution in a sufficient quantity in order to mask the taste of copper?**
9. The panel agreed that some of the concentrations are above the taste threshold, and the investigators should therefore use something to mask the taste and prevent a learning response in the subjects. It was noted by several reviewers that if the taste is masked, what is being measured is the direct effect of copper and not the effect of copper in drinking water, since in drinking water consumers would be able to taste the copper. Dr. Baker indicated that they plan to do tap water studies later and would prefer to mask the taste now to determine the true nausea threshold. Several alternatives were discussed, including aspartame and sugar, and banana flavor was mentioned. A reviewer noted that aspartame will upset some people's stomach, and Dr. Araya indicated that sugar contains many contaminants. The panel suggested that the investigators contact the Monell Chemical Senses Center for suggestions on masking the copper taste.
10. **Does the presence of solid foods in the GI tract influence the response to copper in drinking water? How should this potential confounder be addressed?**
11. The panel agreed that the presence of food in the gastrointestinal tract would likely influence the response. Reviewers suggested that follow up studies could test this directly with several different types of meals, perhaps a "standard" meal, a high carbohydrate meal, and a high protein meal. All of these could be at the same dose level about the threshold. Each person could serve as his or her own control. The study could test both copper in water given right before the meal and copper in water given right after the meal. Two reviewers noted that the location of copper absorption in the gastrointestinal tract might change with the presence of food.
12. **Does a person become acclimatized to elevated copper concentrations in his/her drinking water? Will a person respond on first exposure to a lower concentration than would cause a problem with repeated exposures?**
13. Dr. Baker indicated that there is evidence obtained among well-studied populations in two Massachusetts towns, where residents had adjusted to the presence of copper in their drinking water, while visitors to the towns found the water distasteful, sometimes making them ill. Reviewers indicated that for other metals this is the case, but for copper it is unknown.
14. **The results of this study have potential use in two ways: as a way of judging the appropriate amount of copper in drinking water and as an initial study to support future development of guidance on copper supplementation. Is this dual purpose evident? Will the results be meaningful for both purposes?**
15. Dr. Baker indicated that the purpose of this first study is to examine effects from copper in drinking water. Nutritional supplements and how their use affects an individual's responses to copper present in drinking water will also be addressed in the research program.
16. **Will the current univariate study provide sufficient information to supplant weaker existing studies, and will it provide the necessary preliminary information to enable the subsequent multivariate study?**

In response to the first part, the reviewers noted a number of benefits to the univariate study. Previous studies were case reports and the proposed study will provide much better information. This design will eliminate confounders which may lessen the effect (e.g., with food or characteristics of drinking water). One reviewer asked whether by doing the worst case first, will it be harder to backtrack later and introduce other variables. Dr. Baker acknowledged this concern and recognized that they were starting with the worst case. Reviewers noted that with the results of this first study, a subsequent study could examine the influence of the volume of water containing the dose.

Other Comments

One reviewer raised the issue of considering seasonal differences and attempting to reduce any impact by conducting the study in the same season in each location. Dr. Baker indicated they plan to start the studies in several months, which will be spring in the Southern Hemisphere and fall in the northern.

One reviewer asked whether future studies would identify classes of adults who are more susceptible than other groups and how this study might be used for that end. Another reviewer indicated that one would need to know the mechanism of action to identify susceptible groups. Dr. Dourson suggested another approach. With a well defined no effect level one would still need an uncertainty factor for intraspecies variability in kinetics and dynamics. One could design a study for kinetic variability and replace part of the uncertainty factor. Dr. Baker indicated that ICA will start a physiologically based pharmacokinetic (PBPK) project next year and he believes the answer to why some people react to low levels lies in mechanistic work. Dr. Baker asked the panel that if the bottom of the dose/response curve is a "V" shape rather than a "U" shape, then is it worth doing susceptibility studies? There might be a possibility that the lower level for toxicity crosses over the upper level of essentiality.

REFERENCES

Pizarro, F., M. Olivares, R. Uauy, P. Contreras, A. Rebelo and G. Gidi. Acute gastrointestinal effects of graded levels of copper in drinking water. Unpublished.

Attachment A
Managing Potential Conflicts of Interest
ITER Peer Review Meeting
June 29 and 30, 1998

TERA peer reviewers donate their time and talents to this effort. They are 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 are representing their own expertise and views, not those of their employer.

TERA had requested that each peer reviewer identify potential conflicts of interest related to the review of the health risk assessment of 1,3-dichloropropene and the protocol for the acute copper study and/or the sponsors. Each reviewer has signed a statement indicating that he or she does not have a conflict of interest concerning these chemicals. *TERA* discussed any issues concerning conflicts or the potential or appearance of a conflict with the reviewers, and if necessary with the *TERA* Board of Trustees, and made a recommendation concerning each individual's participation. These recommendations were discussed at the beginning of the June 29 and 30, 1998 meeting and the panel agreed to the text below. Because of the diverse nature of the two reviews and need for specialized expertise, several reviewers participated in one part of the other of the meeting, but not the other, therefore, the names are listed as two different panels.

1,3-Dichloropropene Panel

Michael P. Carty– Dr. Carty is a Research Assistant Professor in the Department of Environmental Health of the University of Cincinnati. He has been asked to participate as an *ad hoc* reviewer for the 1,3-dichloropropene assessment because of his experience in genetic toxicology. He does not have any conflicts and will participate fully in the discussions and consensus for the 1,3-dichloropropene review.

Joyce M. Donohue – Dr. Donohue works for the U.S.EPA, Office of Water. She does not have any conflicts and will participate fully in both discussions and consensus.

Michael L. Dourson - Dr. Dourson works for Toxicology Excellence for Risk Assessment (*TERA*). He will chair the 1,3-dichloropropene review. He does not have any conflicts and will participate fully in the 1,3-dichloropropene discussion and consensus.

Marvin A. Friedman – Dr. Friedman is a private consulting toxicologist, retired from Cytec Industries, Inc. He does not have any conflicts and will participate fully in both discussions and consensus.

George Leikauf – Dr. Leikauf is on the faculty of the Department of Environmental Health of the University of Cincinnati. He does not have any conflicts and will participate fully in the 1,3-dichloropropene discussion and consensus.

Peter R. McClure – Dr. McClure works for Syracuse Research Corporation. He does not have any conflicts and will participate fully in both discussions and consensus.

Bruce D. Naumann - Dr. Naumann works for Merck & Co., Inc. He does not have any conflicts and will participate fully in both discussions and consensus.

Kenneth A. Poirier – Dr. Poirier works for The Procter & Gamble Company. He does not have any conflicts and will participate fully in both discussions and consensus.

Karl H. Summer – Dr. Summer is Head of the Metal Toxicology Unit of the GSF-National Research Center for Environment and Health, Neuherberg, Germany. He has

been asked to participate as an *ad hoc* reviewer for both reviews because of his familiarity with copper toxicity and expertise in toxicology. He does not have any conflicts and will participate fully in both discussions and consensus.

Marcia van Gemert – Dr. van Gemert works for van Gemert & Hauswirth, L.L.C.. She does not have any conflicts and will participate fully in the 1,3-dichloropropene discussions and consensus.

Vanessa T. Vu – Dr. Vu is Associate Director for Health at U.S. EPA's National Center for Environment Assessment (NCEA). She has management oversight of the Agency's IRIS (Integrated Risk Information System) program which develops health assessments of environmental agents. NCEA is presently developing a hazard and dose-response assessment for 1,3-dichloropropene, but at this time Dr. Vu has no direct input into the development of this particular assessment, nor has she seen a draft. Dr. Vu believes she can provide impartial comments and opinions on the assessment. The panel agreed and recommended that Dr. Vu participate fully in the 1,3-dichloropropene discussion and consensus.

Copper Protocol Panel

Pamela Dalton – Dr. Dalton works for the Monell Chemical Senses Center at the University of Pennsylvania School of Medicine. She has been asked to participate as an *ad hoc* reviewer for the copper protocol because of her expertise in designing studies. She does not have any conflicts and will participate fully. Dr. Dalton was not able to attend the meeting, but her written comments have been provided to the panel.

Robert Devlin – Dr. Devlin is the Chief, Clinical Research Branch of the U.S. EPA National Health and Environmental Effects Research Laboratory. He has been asked to participate as an *ad hoc* reviewer for the copper protocol because of his experience in designing and conducting studies of the effects of metals and chemicals on humans. He does not have any conflicts and will participate fully in the discussions and consensus for the copper protocol.

Joyce M. Donohue – Dr. Donohue works for the U.S. EPA, Office of Water. She does not have any conflicts and will participate fully in both discussions and consensus.

Ernest C. Foulkes – Dr. Foulkes is on the faculty of the Department of Environmental Health of the University of Cincinnati. He has been asked to participate as an *ad hoc* reviewer for the copper protocol because of his expertise in metals toxicology. He does not have any conflicts and will participate fully in the discussion and consensus for the copper protocol.

Marvin A. Friedman – Dr. Friedman is a private consulting toxicologist, retired from Cytech Industries, Inc. He does not have any conflicts and will participate fully in both discussions and consensus.

Heidi J. Kalkwarf – Dr. Kalkwarf is a Research Assistant Professor at the Children's Hospital Medical Center in Cincinnati, Ohio. She has been asked to participate as an *ad hoc* reviewer for the copper protocol because of her expertise in designing and conducting human studies and in nutrition. She does not have any conflicts and will participate fully in the discussion and consensus for the copper protocol.

Peter R. McClure – Dr. McClure works for Syracuse Research Corporation. He does not have any conflicts and will participate fully in both discussions and consensus.

David W. Morry – Dr. Morry works for the California Environmental Protection Agency. He has been asked to participate as an *ad hoc* reviewer for the copper protocol because of his familiarity with copper toxicity. He does not have any conflicts and will participate fully. Dr. Morry was not able to attend the meeting, but his written comments have been provided to the panel.

Bruce D. Naumann - Dr. Naumann works for Merck & Co., Inc. He does not have any conflicts and will participate fully in both discussions and consensus.

Kenneth A. Poirier – Dr. Poirier works for The Procter & Gamble Company. He does not have any conflicts and will participate fully in both discussions and consensus. He chaired the copper review panel.

Bonny L. Specker – Dr. Specker is Director and Chair of the Ethel Austin Martin Endowed Program in Human Nutrition at the South Dakota State University. She has been asked to participate as an *ad hoc* reviewer for the copper protocol because of her experience in designing and conducting human studies and in nutrition. She does not have any conflicts and will participate fully in the copper discussion and consensus.

Karl H. Summer – Dr. Summer is Head of the Metal Toxicology Unit of the GSF-National Research Center for Environment and Health, Neuherberg, Germany. He has been asked to participate as an *ad hoc* reviewer for both reviews because of his familiarity with copper toxicity and expertise in toxicology. He does not have any conflicts and will participate fully in both discussions and consensus.