April 28, 2000

EPA-SAB-EC-00-009

Honorable Carol Browner
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: Review of the Draft Chloroform Risk Assessment

Dear Ms. Browner:

The Chloroform Risk Assessment Review Subcommittee (CRARS) of the US EPA Science Advisory Board (SAB) met on October 27-28, 1999, in Washington, DC. The purpose of the review was to determine if either the Office of Water's draft chloroform risk assessment or the Office of Research and Development's proposed Cancer Risk Assessment Guidelines' section on Mode of Action required revision before they were finalized. The Agency requested that the Subcommittee provide a response to the questions pertaining to the Guidelines within three weeks of the public meeting. The Subcommittee consequently developed a letter report (EPA-SAB-EC-LTR-00-001, final issued on December 15, 1999), incorporating its findings on this issue. These findings are also summarized in the enclosed report (Section 3.1)). The Subcommittee also addressed the draft chloroform risk assessment's conclusions as to chloroform's mode of action; the strength of the analyses supporting the choice of a non-linear approach to dose-response; epidemiological issues, and the adequacy (given the data available) of the assessment of children's risk from exposure to chloroform in drinking water (the complete Charge is provided in section 2.2 of the enclosed report).

The Subcommittee agrees with EPA that sustained or repeated cytotoxicity with secondary regenerative hyperplasia in the liver and/or kidney of rats and mice precedes, and is probably a causal factor for, hepatic and renal neoplasia. In considering this potential mode of action for chloroform-induced carcinogenicity, the Subcommittee expressed concern that a cytotoxicity/regenerative cell proliferation mode of action may not be the exclusive mode, and that alternative modes of action have not been rigorously studied.

Although cytotoxicity/cell proliferation appears to be a major factor driving the observed chloroform-induced carcinogenesis in some studies, these findings do not address the underlying mechanism(s) of the responses. The data available on chloroform metabolism generally are consistent with the mode of action proposed by EPA. This mode of action, as well as all other potential modes of action identified, required that chloroform be metabolized by cytochrome P450. The Subcommittee was unanimous on these points.

An unresolved critical question is the extent to which genotoxicity plays a role in chloroform tumor induction. If it does, this has s implications for risk assessment, particularly if these effects occur at low doses. Most Members felt that there was little evidence that genotoxicity plays a role in the tumorigenic responses. Other Members felt that, although the weight of evidence indicates that chloroform is not strongly mutagenic, some evidence suggests a potential genotoxic contribution. The data supporting this view are identified in the report. The bulk of the database on chloroform was analyzed and summarized by an International Life Sciences Institutes (ILSI) Expert Panel and can be found in their report, so these data are not recapitulated in our report. It would have been preferable for EPA to systematically discuss the genotoxicity findings in their own document rather than relying completely on the ILSI Panel report cited in their draft document.

After examining the data, most Members agreed that the dose-response for both liver and kidney neoplasia appears to be determined by cytotoxicity, and that a margin of exposure approach (MOE) or non-linear approach is most appropriate. In coming to this conclusion, it was recognized that although cytotoxicity and reparative cell division can be a cause of cancer in a particular organ within a given species or strain of animal, such effects do not inevitably lead to cancer. Therefore, exploration of cases where cancer occurred in the absence of cytotoxicity could provide evidence of multiple modes of action. Some Members noted the possibility that genotoxicity could contribute to the kidney response at low doses.

The Subcommittee was supportive of the Agency's attempt to incorporate the scientific literature on chloroform and to address the complex scientific issues involved in assessing the dose-response relationship for chloroform. However, we found it somewhat difficult to track the scientific bases for decisions made in the risk characterization document. The Subcommittee recommends revision of the risk characterization to incorporate critical data on the dose-response assessment and allow the consistency of the data to be more readily evaluated.

The extensive epidemiologic evidence relating drinking water disinfection (specifically chlorination) with cancer has little bearing on the determination of whether chloroform is a carcinogen or not. The goal of the draft risk assessment was to isolate the health effects of chloroform in drinking water. Although the literature is not definitive, the epidemiologic evidence is pertinent to a broader question, i.e., the effect of disinfection by-products in the aggregate. There are several disinfection by-products that are more plausible as causes for some of these effects. However, the Agency should have provided some context that explains the potential meaning of these data. A brief discussion that

acknowledges the importance of the epidemiologic research to the broader and more important question of disinfection by-products and an indication of how EPA is addressing those concerns should be provided. EPA should provide a brief overview of the key endpoints that have been identified as a result of epidemiologic research on disinfection by-products, and cite the pertinent reviews to be certain that this point is not lost. This makes the dismissal of these data as they apply to chloroform more explicit.

The Subcommittee found that the draft document addressed children's risks quite adequately, based on the scientific information that is currently available. The document's major conclusions are correct, but that they could be stated with slightly more caution. Although we agree that the enzyme metabolizing chloroform at low doses (CYP2E1) plays an important role in the production of tissue injury, cell death, and tumor development in the studies reviewed, its definitive role in the developing human or mammal has yet to be confirmed. The idea that children on occasion may be less sensitive needs to be expanded upon. In fact, children may be more -- or less -- sensitive for a variety of reasons, including exposure latency, differential chemical exposure, absorption, metabolism, factors that could contribute to the sensitivity of specific subpopulations, chronic low level exposures, perinatal imprinting, and target-organ susceptibility.

In future mode of action determinations, the Subcommittee believes that issues of susceptibility need to be discussed more systematically. Essentially a mode of action determination provides the type of information that is necessary to identify factors that lead to increased susceptibility as a matter of course. EPA's regulatory program offices need guidance in making such determinations. Therefore, the Guidelines should include a "check off" format for each agent to determine whether the mode of action identified is of a type that would place particular populations at heightened risks. Such a check-off should include, but not necessarily be limited to the following considerations:

a) age-related susceptibilities – fetuses, infants and children, pubescent adolescent, adults, elderly

b) gender-related -- including pregnant females and lactating females

c) genetic polymorphisms/deficiencies

d) drug-drug interactions (xenobiotics, environmental chemicals)

e) disease states

f) foods and diets

The Subcommittee feels strongly that the documentation for future applications of the Cancer Risk Assessment Guidelines to a mode of action determination should be more concisely and

systematically developed. It was necessary for the Subcommittee to assemble the key data into an understandable form to determine how consistently the data supported the argument for the determination of the cytotoxic mode of action. Although the ILSI document relied upon by the Agency contained much of these data, extracting the key information was quite unwieldy. As noted in several places in our report, this key information could have been displayed much more systematically and described in more objective language.

We appreciate the opportunity to review these documents, and look forward to receiving your response to the issues raised.

Sincerely,

/signed/
Dr. Morton Lippmann, Interim Chair
Science Advisory Board

/signed/
Dr. Mark Utell, Co-Chair
Chloroform Risk Assessment
Review Subcommittee
Science Advisory Board

/signed/
Dr. Richard Bull, Co-Chair
Chloroform Risk Assessment
Review Subcommittee
Science Advisory Board

United States Environmental Protection Agency
Science Advisory Board (1400A) Washington DC
EPA-SAB-EC-00-009 April 2000 *ww.epa.gov/sab*

REVIEW OF THE EPA'S DRAFT CHLOROFORM RISK ASSESSMENT

REVIEW OF THE DRAFT CHLOROFORM RISK ASSESSMENT BY A SUBCOMMITTEE OF THE SCIENCE ADVISORY BOARD

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1. EXECUTIVE SUMMARY

The Chloroform Risk Assessment Review Subcommittee (CRARS) of the US EPA Science Advisory Board (SAB) Executive Committee met on October 27-28, 1999, in Washington, DC. The purpose of the review was to determine if significant changes need to be made to the chloroform risk assessment before it is finalized, or to the proposed Cancer Risk Assessment Guidelines' section on Mode of Action.[1] The Subcommittee also addressed the draft chloroform risk assessment's conclusions as to chloroform's mode of action; the strength of the analyses supporting the choice of a non-linear approach to dose-response; epidemiological issues, and the adequacy (given the data available) of the assessment of children's risk from exposure to chloroform in drinking water (the complete Charge is provided in section 2.2 of this report).

The Subcommittee expressed overall support for the GLs (July, 1999 draft) framework for determining the importance of different modes of action and encouraged the Agency to publish the Guidelines expeditiously. Several suggestions were offered for the implementation of the guidelines, including advising the Agency to: include a step that identifies gaps in knowledge when presenting conclusions in the human relevance section; amplify the description of what the term 'sufficient' information means when making a mode of action determination; point out that the carcinogenic activity of some chemicals appears to involve both modifications of cell division and cell death processes; consider establishing a checklist addressing populations of concern to be considered in each mode of action analysis; and define more clearly the terms "linear" and "non-linear" as applied to dose-response curves in the Guidelines.

Because of the close relationship between the question of chloroform's mode of action and the relationship of low-dose pathology to the doses that induce tumors, these issues were addressed together in this report. The Subcommittee agrees with EPA that sustained or repeated cytotoxicity with secondary regenerative hyperplasia in the liver and/or kidney of rats and mice precedes, and is probably a causal factor for, hepatic and renal neoplasia. In considering this potential mode of action for chloroform-induced carcinogenicity, the Subcommittee expressed concern that a cytotoxicity/regenerative cell proliferation mode of action may not be the exclusive mode, and that alternative modes of action have not been rigorously studied. Although cytotoxicity/cell proliferation appears to be a major factor driving the observed chloroform-induced carcinogenesis in some studies, this does not address the underlying mechanism(s). Multiple mechanisms may be operating concurrently to produce the tumor responses. The data available on chloroform metabolism generally are consistent with the mode of action proposed by EPA. Both oxidative and reductive cytochrome P450 mediated metabolism occurs, resulting in the production of tissue reactive metabolites that in turn

[1] The Agency requested that the Subcommittee provide a response to this Charge element within three weeks of the public meeting. The Subcommittee consequently developed a letter report (EPA-SAB-EC-LTR-00-001), 12/15/99, incorporating its findings on this issue. These findings are also summarized in this report (Section 3.1).

leads to tissue injury and cell death. The argument that phosgene is the metabolite essential to the proposed mode of action is not

compelling. However, the Subcommittee did not see this element as critical to the mode of action argument made by the Agency.

A more critical question is the extent to which genotoxicity plays a role in the induction of tumors by chloroform. If it does, the question for risk assessment is to determine the extent to which genotoxic mechanisms may be operating at low doses. Most Members felt that there was little evidence that genotoxicity plays a role in the tumorigenic responses. Other Members felt that, while the weight of evidence indicates that chloroform is not strongly mutagenic (ILSI, 1997), some evidence suggests a genotoxic contribution to the response. The data supporting this view are identified in the report. The bulk of the database on chloroform was analyzed and summarized by an International Life Sciences Institutes (ILSI) Expert Panel and can be found in their report, so these data are not recapitulated in our report. Ultimately, it would have been useful for EPA to more systematically discuss the genotoxicity findings in their own document rather than relying completely on the ILSI Panel report cited in their draft document.

The Subcommittee was supportive of the Agency's attempt to incorporate the scientific literature on chloroform and to address the complex scientific issues involved in assessing the dose-response relationship for chloroform. However, the Subcommittee found it somewhat difficult to track the scientific bases for decisions in the risk characterization document. The Subcommittee recommends revision of the risk characterization to incorporate critical data on the dose response assessment and allow the consistency of the data to be more readily evaluated. After examining the data, most Members agree that the dose response for both liver and kidney neoplasia appears to be determined by cytotoxicity, and that a margin of exposure approach (MOE) or non-linear approach is most appropriate. In coming to this conclusion, it was recognized that while cytotoxicity and reparative cell division can be a cause of cancer in a particular organ within a given species or strain of animal, such effects do not inevitably lead to cancer. Therefore, exploration of cases where cancer does not occur in the presence of cytotoxicity would provide evidence of multiple modes of action. Some members noted the possibility that genotoxicity could contribute to the kidney response at low doses.

The extensive epidemiologic evidence on drinking water disinfection by-products largely irrelevant, given the goal of the draft risk assessment to isolate the health effects of chloroform in drinking water. Although that literature is not definitive, the epidemiologic evidence is pertinent to a broader question, i.e., the effect of disinfection by-products in the aggregate. A brief discussion that acknowledges the importance of the epidemiologic research to the broader question of disinfection by-products and an indication of how EPA is addressing those concerns should be provided. It might also be noted that the reason for a lack of epidemiologic research on chloroform in drinking water and cancer is that humans are not exposed to chloroform alone in chlorinated drinking water so it cannot possibly be studied. EPA should provide a brief overview of the key endpoints that have arisen as a

result of the epidemiologic research on disinfection by-products more generally, citing some reviews, to reinforce this point.

The Subcommittee found that the draft document addressed children's risks quite adequately, based on the scientific information that is currently available. The document's major conclusions are correct, but they could be stated with slightly more caution. Although we agree that the enzyme metabolizing chloroform at low doses (CYP2E1) plays an important role in the production of tissue injury, cell death, and tumor development in the studies reviewed, its definitive role in the developing human or mammal has yet to be confirmed. The idea that children on occasion may be less sensitive needs to be expanded upon. In fact, children may be more -- or less -- sensitive for a variety of reasons, including exposure latency, differential chemical exposure, absorption, metabolism, are factors that could contribute to the sensitivity of specific subpopulations, chronic low level exposures, perinatal imprinting, and target-organ susceptibility.

2. INTRODUCTION

2.1 Background

A number of national drinking water surveys performed in the United States between 1975 and 1981 revealed that chloroform (an unwanted by-product of the disinfection process) was detectable in a majority of water supply systems using a surface water source. These findings raised concerns about the possibility of chloroform producing adverse health effects, including cancer. EPA undertook a number of studies of both the disinfection process and the toxicology and health effects of chloroform ingestion. Among the activities addressing the latter area, EPA co-sponsored an International Life Sciences Institute (ILSI, 1997) project in which an expert panel was convened and charged to (among other objectives) to:

a) review the available database relevant to the carcinogenicity of chloroform

b) consider how end points related to the mode of carcinogenic action can be applied in the hazard and dose-response assessment

c) use guidance provided by the 1996 EPA Proposed Guidelines for Carcinogen Assessment to develop recommendations for appropriate approaches for risk assessment

d) provide a critique of the risk assessment process and comment on issues encountered in applying the proposed EPA Guidelines

EPA subsequently used information from the ILSI panel and other sources to produce a draft risk assessment document for chloroform. The review of this document (and the pertinent section of the revised Draft Cancer Risk Assessment Guidelines, as described below) was the subject of the October 27-28, 1999 meeting of the SAB's Chloroform Risk Assessment Review Subcommittee.

2.2 Charge

The Charge's general purpose was to review the Mode of Action determination and the selection of a nonlinear dose-response approach for the risk assessment of chloroform under EPA's Proposed Cancer Risk Assessment Guidelines Revisions.

The specific questions are:

a) Based on its application to the chloroform risk assessment, please identify any specific text in the draft Cancer Risk Assessment Guideline's

framework for mode of action analysis (section 2.5) which you would advise be changed prior to their publication[2]

b) In the draft chloroform risk assessment document, are the conclusions as to the following issues adequately supported by the analyses presented in the health risk assessment/characterization (as supported by the ILSI report) and the framework analysis?

i) chloroform's mode of action

ii) consideration of a nonlinear approach to dose-response, and the possibility that mutagenesis might play a role in the carcinogenic response.

iii) the relationship of low-dose pathology to the doses that induce tumors.

iv) epidemiologic evidence on chlorinated drinking water as to the carcinogenicity of chloroform, including comment on any conclusion to be drawn from the epidemiologic data about mode of action.

c) Does the assessment of children's risk for chloroform appropriately address the risk concerns, including ontogeny of drug metabolizing enzymes, given the data available?

[2]The Agency requested that the Subcommittee provide a response to this Charge element within three weeks of the public meeting. The Subcommittee consequently developed a letter report (EPA-SAB-EC-LTR-00-001), 12/15/99, incorporating its findings on this issue. These findings are also summarized in this report (Section 3.1).

3. DETAILED FINDINGS

3.1 Framework for Mode of Action Analysis

The first element of the Charge (a) asked the Subcommittee to determine if, based on its application to the chloroform risk assessment, it recommended that any specific text in the draft Cancer Risk Assessment Guideline's (GLs) framework for mode of action analysis (section 2.5) be changed prior to their publication.[3]

The Subcommittee expressed overall support for the GLs (July, 1999 draft) framework for determining the importance of different modes of action and encouraged the Agency to publish the guidelines expeditiously. A few suggestions were offered for the implementation of the guidelines:

a) include a step that identifies gaps in knowledge when presenting conclusions in the human relevance section (gaps that relate to the potential for effects in sensitive populations and or subpopulations are particularly important in this regard)

b) amplify on what the term 'sufficient' information means when making a mode of action determination

c) pay attention to the specific terms that are used in describing a mode of action

d) point out that the carcinogenic activity of some chemicals appears to involve both modifications of cell division and cell death processes

e) consider establishing a checklist addressing populations of concern (such as pregnant women, children, and individuals with particular disease states or genetic susceptibilities, etc), similar to that developed by FDA, to be considered in each mode of action analysis

f) incorporate a statement to the effect that "Consistency between endpoints related to mode of action and carcinogenic responses should be sought in experiments that give both positive and negative results. Findings that show that other chemicals having parallel toxicological properties also result in a carcinogenic response strengthen the conclusion that a particular mode of action is causal."

[3] The following section summarizes the Subcommittee's findings. Full details may be found in the Subcommittee's separate letter report (EPA-SAB-EC-LTR-00-001) of 12/15/99. See also footnote 1 of this report.

f) define more clearly the terms “linear” and “non-linear” as applied to dose-response curves in the Guidelines; the current usage creates some confusion

3.2 Mode of Action and Low-dose pathology

3.2.1 The Role of Cytotoxicity in Chloroform-Induced Neoplasia

Because of the close relationship between the second and fourth Charge elements, they are addressed together below. The Subcommittee has assumed that what is meant by low-dose pathology is evidence of cytotoxicity in the target tissues that ultimately developed a neoplastic response.

The second Charge element (b)(i) asked if the conclusions as to chloroform’s mode of action are adequately supported by the analyses presented in the health risk assessment/characterization (as supported by the ILSI report) and the framework analysis. The fourth Charge element (b)(iii) asked if the conclusions as to the relationship of low-dose pathology to the doses that induce tumors are adequately supported by the analyses presented in
the health risk assessment/characterization (as supported by the ILSI report) and the framework analysis.

The Subcommittee agrees that sustained or repeated cytotoxicity with secondary regenerative hyperplasia in the liver and/or kidney of rats and mice precedes, and is probably a causal factor for, hepatic and renal neoplasia as observed in some of the reported rodent cancer bioassays. In considering this potential mode of action for chloroform-induced carcinogenicity, the Subcommittee expressed concern that a cytotoxicity/regenerative cell proliferation mode of action may not be the exclusive mode, and that alternative modes of action have not been rigorously studied. The statement "Other modes of action have been well studied and are not supported by the evidence" (page 7of the draft document) implies that several alternative modes of action have been studied. The draft document should state what other modes of action have actually been well studied. Although cytotoxicity/cell proliferation appears to be a major factor driving the observed chloroform-induced carcinogenesis in some studies, this does not address the underlying mechanism(s). Multiple mechanisms may be operating concurrently to produce the tumor responses.

The data available on chloroform metabolism generally are consistent with the mode of action proposed by EPA. Both oxidative and reductive cytochrome P450 mediated metabolism occurs, resulting in the production of tissue reactive metabolites that in turn leads to tissue injury and cell death. The argument for phosgene being the metabolite essential to the proposed mode of action is not compelling. The Agency's conclusion that CYP2E1 (the enzyme responsible for metabolizing chloroform at low dose levels) metabolism is a critical step in toxicity is supported by the recent study in CYP2E1-null mice (Constan *et al*., 1999). A carcinogenesis assay with CYP2E1-null mice would provide even more definitive evidence that this is a primary pathway leading to cancer.

The data provided in Tables 1 and 2 of this report (see section 3.3) identify rodent studies that form the basis for the proposed linkage between enhanced cell proliferation and target tissue neoplasia. When measured, cytotoxicity/regenerative tissue hyperplasia and ultimate neoplasia is present in a limited set of experimental conditions in short-term studies and follows a similar species and gender pattern as induction of neoplasia by chloroform in chronic bioassays.

A clearer relationship is seen with the Osborne-Mendel male rat kidney tumor response as reported by Jorgenson *et al*. (1985). This result is linked to a truly sustained cytotoxicity/cell proliferation as measured by histopathology and reported by Hard *et al*. (2000).

The induction of renal tumors was also observed in male BDF1 mice treated with chloroform by inhalation (Matsushima *et al.*, 1994) (see Table 1). It was necessary to acclimatize male mice of this strain to chloroform by gradually increasing the dose since they would otherwise not survive inhaled concentrations of 30 and 90 ppm. Renal tumors, but no liver tumors, were induced in mice treated at these two high doses, and not at lower doses. Templin *et al.*, (1998) duplicated this treatment (including the acclimatization period) and observed cytotoxicity and reparative cell division in the kidneys of mice treated with 30 and 90 ppm throughout a 90 day exposure period. Therefore, the renal tumor responses in two experiments (in which responses were measured over an extended period) support the finding that cytotoxicity and reparative hyperplasia is consistently associated with those doses that produce renal tumors. In the single case where a strain of mouse (ICI) was shown to be responsive to renal carcinogenesis, the relationship was less clear. Moore *et al*. (1982) found no tumors, but indications of toxicity and increased cell replication at a dose of 199 mg/kg (but not at 60 mg/kg, a dose in which renal tumors were produced with the same vehicle (i.e. a toothpaste base) in a previous cancer bioassay (Roe *et al*., 1979)). This bioassay had some intrinsic deficiencies that limit its usefulness (e.g., short duration, concurrent respiratory and renal disease). It must also be noted that the method of measuring cell replication was indirect in the Moore study (simply 3H-thymidine incorporation, not a labeling index) and has a limited specificity and sensitivity for measuring cell division.

Limited but potentially relevant linkage is seen between the liver tumor response in B6C3F1 mice (NCI, 1976) (see table 2) and subsequently conducted short-term cell proliferation studies (Larson *et al.*, 1994b) (The other sets of studies are either not relevant (missing cell proliferation data) or non-informative (cell proliferation carried out in different strains or for very short intervals only)). It is also apparent that important data gaps make it difficult to formulate firm conclusions regarding any mandatory linkages between cytotoxicity/regenerative cell proliferation and neoplasia. Nevertheless, within the data sets available, cytotoxicity/regenerative cell proliferation track together with liver tumorigenicity in a variety of experimental situations. Differential tumorigenic effects of chloroform are seen with variations in the vehicle used, the route of chloroform administration, and the strain and species differences. The short-term data on cytotoxicity/regenerative hyperplasia (2 to 21 days of treatment) consistently predict circumstances under which chloroform will or will not induce liver cancer in mice.

With respect to liver cancer, there is a body of data that consistently shows that carcinogenic responses are not seen when chloroform is administered in drinking water (e.g., the Jorgensen *et al*. study). Chloroform in corn oil induces liver cancer even in rodents (Deml and Osterle, 1985). In parallel to the Jorgenson study, when administered in drinking water to mice at similar daily doses, chloroform does not induce liver cancer. These latter studies of chloroform in drinking water show that it actually inhibited cancer induced by initiating doses of two well-established initiators, ethylnitrosourea or diethylnitrosamine (Pereira, 1985; Klaunig *et al*., 1986). These effects can be associated with the ability of chloroform provided in drinking water to suppress cell replication within the liver (Pereira, 1994). Most interesting is the finding that relatively modest concentrations of chloroform in drinking water suppressed both the hepatotoxicity and reparative cell division produced by chloroform administered in corn oil by stomach tube (Pereira and Grothaus, 1997). These data support the conclusion that low levels of chloroform in drinking water are not likely to be carcinogenic in the liver. At the same time, these data reinforce the fact that the underlying mechanisms responsible for chloroform-induced liver cancer are not well understood.

A single experimental study published in the peer reviewed literature wherein chloroform-induced cell necrosis, cell proliferation and resulting neoplasia were examined in parallel, with the use of tumorigenic and non-tumorigenic exposure levels, would provide a more convincing case. The proposed mode of action for chloroform suggests *sustained* cell proliferation from chronic persistent cell injury is needed. This is not firmly supported by the experimental evidence since most of the cell proliferation studies employed short term treatment protocols (some as short as 2 days) to support the linkage between the cell injury and the neoplasia development. Only in the case of the renal tumors induced in rats by Jorgenson *et al* (1985) has there been a clear association with chronic cell injury with tumorigenicity over a large range of doses (Hard *et al*., 2000).

Finally, this portion of the draft document would benefit from editorial revisions aimed at eliminating or de-emphasizing some global and dogmatic statements that are hard to defend. For example, the elimination of undefined judgmental modifiers such as “very strong,” “obligatory,” “clearly defined,” “persistent,” and “sustained” would improve the draft.

3.2.2 Chloroform's Mode of Action and the Role of Genotoxicity

As previously stated, the Subcommittee notes that cytotoxicity precedes and is likely to play a major role in chloroform carcinogenesis. This mode of action might not be exclusive, and alternatives may be at work. A critical question is the extent to which genotoxicity plays a role in chloroform tumor induction in the bioassay; if it does, the question for risk assessment is to determine the extent to which genotoxic mechanisms may be operating at low doses. Some Members expressed concerns about the adequacy of the genotoxicity database to answer these questions. Some (especially older) studies of genotoxic changes after chloroform exposure have shortcomings (such as inadequate control of volatility, the use of ethanol in U.S.P. chloroform as a preservative (resulting in formation of ethyl and diethyl carbonate, potent alkylating agents) (IPCS, 1994), or adequate selection of appropriate

cofactors). Also positive *in vitro* clastogenicity findings can result from severe cytotoxicity (Busick, 1986).

The EPA assessment relied heavily on the analysis of the ILSI (1997) Expert Panel. The assessment of this Panel included a quantitative weight of evidence evaluation of the chloroform genotoxicity studies. This Panel used an approach published by the International Commission for Protection against Environmental Mutagens and Carcinogens and found that it supports a non-genotoxic classification. The Panel noted that the database for chloroform is large, heterogeneous and contains conflicting test responses, and concluded that no subset of data points unequivocally pointed to a specific genotoxic mechanism associated with chloroform carcinogenicity. They concluded that the preponderance of evidence indicates that chloroform is not strongly mutagenic and that the chemical would not be expected to produce rodent tumors via a genotoxic mechanism.

Genotoxicity endpoints have to be interpreted cautiously when used as evidence for potential carcinogenicity. *In vitro* clastogenicity can be a product of severe cytotoxicity resulting from lysosomal or other releases (Brusick, 1986). This may be important with substances such as chloroform, where there is evidence of cytotoxicity and cell proliferation in target tissues. Also, cycles of cytotoxicity and cell proliferation could cause the expression of preexisting genetic damage in target tissues which, under normal conditions, have low mitotic indices (This is the basis for tumor promotion and for the proposed EPA mode of action for chloroform).

Some Members felt that, while the weight of evidence indicates that chloroform is not strongly mutagenic (ILSI, 1997), some evidence suggests a genotoxic contribution to the response. Findings from some more recent studies are noteworthy in this respect:

a) A 3-fold increase in micronucleated kidney cells in rats exposed orally to a high dose of chloroform (Robbiano *et al*., 1998). These findings are of particular interest because they report chromosome level damage in the species and tissue most relevant to the cancer risk assessment.

b) Dose dependent findings of sister chromatid exchange (*in vivo*) in mouse bone marrow (Morimoto and Koizumi, 1983) and chromosomal aberrations *in vivo* in rats treated orally or ip with chloroform (Fujie *et al*, 1990)

c) Positive mouse micronuclei assay with chloroform (Agustin and Lim-Sylianco, 1978).

d) Chloroform dose related induction of intrachromosomal recombination in yeast (Brennan and Schiestl, 1998), and reduction in recombination when the assay was performed in the presence of a free radical scavenger

e) Chloroform DNA binding *in vivo* (Colacci *et al.*, 1991)

f) Reductive metabolism of chloroform *in vivo* and *in vitro* (Gemma *et al*., 1996; Testai *et al*., 1990, 1995) leading to dichloromethyl radicals does occur at some level (although discounted by EPA)

Ultimately, it would have been useful for EPA to more systematically discuss the genotoxicity findings in their own document rather than relying completely on the ILSI report.

3.3 Approach to Low Dose Extrapolation

Charge element (b)(ii) asked if the conclusions as to consideration of a non-linear approach to dose-response is appropriate.

The Subcommittee was supportive of the Agency's attempt to incorporate the extensive scientific literature on chloroform and to address the complex scientific issues involved in assessing the dose-response relationship for chloroform. However, the Subcommittee found it somewhat difficult to track the scientific bases for decisions in the risk characterization document (EPA 815-B098-C). With the aid of the Agency, the Subcommittee constructed tables (below) displaying some of the key scientific data on regenerative hyperplasia and neoplasia that bear on the low dose extrapolation. The Subcommittee recommends revision of the risk characterization to incorporate critical data on the dose response assessment and allow the consistency of the data to be more readily evaluated. The ability for any scientific group to come to any judgments about potential shapes of the dose-response curve at lower doses critically depends upon such an evaluation.

After examining the data, most Members agree that the dose response for both liver and kidney neoplasia appears to be determined by cytotoxicity, and that a margin of exposure approach (MOE) or non-linear approach is most appropriate. In coming to this conclusion, the Subcommittee recognizes the principle that while cytotoxicity and reparative cell division can be a cause of cancer in a particular organ within a given species or strain of animal, such effects do not inevitably lead to cancer. Therefore, more weight is given to alternate modes of action to cytotoxicity and reparative cell division when tumors appear when there is no sign of cytotoxicity.

Nonetheless, taking all of the information into account, the Subcommittee concluded that

a) For the liver tumor response - because of the strong role cytotoxicity appears to play - a margin of exposure (MOE) assessment is a scientifically reasonable approach. In contrast, the application of the standard linear approach to the liver tumor data is likely to substantially overstate the low dose risk. In addition, there is considerable question about this response because it is not produced when chloroform in administered to mice in drinking water.

b) For the kidney response - because sustained cytotoxicity plays a clear role in the in the rat - a margin of exposure (MOE) is a scientifically reasonable approach. Most Members felt that there was some possibility that genotoxicity could contribute to the dose-response at low doses (i.e. below the range of observation in the animal studies). Several studies do suggest a role of genotoxicity for carcinogenesis (see Gemma, *et al*., 1996; Rossi, *et al*., 1999; Robianno *et al.*, 1998; and Brennan and Schiestl, 1998). Some Members of the Subcommittee questioned whether these effects would contribute to a tumor response at the doses that would be encountered in drinking water.

The Subcommittee would like to take this opportunity to point out that the chloroform case is a *relatively* simple example of how the cancer guidelines can be applied. Given more complex problems in the future, the Subcommittee would strongly suggest that the Agency take a more quantitative approach to evaluating the components of a compound's mode of action through applications of biologically based models. The classic case will be a chemical with consistent evidence of weak genotoxic activity, with strong evidence that virtually all of the activity in the observable range is due to a non-genotoxic mode of action. Since the Agency is invariably attempting to predict cancer risks outside the observable range, it is critical that they begin to develop a reasonable means of estimating the most likely and upper bound estimate of potential contribution of a "genotoxic" component to the carcinogenic activity. These estimates should be projected down to include conditions of environmental exposure. A beginning might be made by estimating the amount of a genotoxic form of a chemical that is likely to reach the target organ, coupled with some estimate of mutagenic potency. Full consideration should be made of the potential contribution of other processes of normal physiology that might produce a spontaneous contribution to such processes. The Subcommittee recognizes that most frequently data do not exist for these purposes for specific compounds, but a willingness to entertain such approaches will encourage the development of the data.

Having made this recommendation, the Subcommittee would like to tread lightly into the policy arena to point out that it is difficult to take the modeling approach suggested above in situations where general policy requires the simple assignment of carcinogens to two categories, one to be treated by a linear approach and the other by a MOE approach. In the drinking water program an modeling approach is essentially frustrated by the general policy that the MCLG must be set at zero for carcinogens. Consequently, recommendations now must simply rely on weight of the evidence arguments which are difficult because absolute knowledge is not possible.

3.4 Epidemiologic Evidence on the Carcinogenicity of Chloroform in Drinking Water

Charge element (b)(iv) asked if the conclusions relating to the epidemiologic evidence on chlorinated drinking water and carcinogenicity are adequately supported by the analyses presented in the health risk assessment/characterization (as supported by the ILSI report) and the framework analysis.

The goal of the draft risk assessment (the isolation of the effect of chloroform in drinking water) makes the extensive epidemiologic evidence on drinking water disinfection by-products largely irrelevant. While that literature is not definitive, the epidemiologic evidence is quite pertinent to the broader question of most direct regulatory concern, namely disinfection by-products in the aggregate.

The brief discussion of the epidemiologic literature on chloroform in drinking water and cancer is largely dismissive because chloroform cannot be isolated from other disinfection by-

Table 1. Observations of Kidney Neoplasia and Cytotoxicity

Species/strain/ Sex/vehicle	Dose or Concentration	Tumor incidence [a] (%)	Labeling index or other cytotoxicity indicator[f]	Bioassay ref.; cytotoxicity ref.
Corn Oil	**mg/kg:**			
B6C3F1 Mouse				
Male	0, 138, 277	1 (6), 2, 4	3, 29.5, 26.7 (4 day) 2, 6.9, 17.1 (3 wk).	NCI, 1976; Larson *et al*., 1994a
Female	0, 238, 477	0 (0), 0, 0	1.4, 0.4, 4.2 (4 day)[d] 2, 1.5, 1.5 (3 wk)	NCI, 1976; Larson *et al.*, 1994b
OM Rat				
Male	0, 90, 180	0 (0), 8, 24	0.42, 1.7, 1.86 (1 day)	NCI, 1976; Templin *et al*., 1996
Female	0, 100, 200	0 (0), 0, 4	NA. F344: 2.1, 3.2, 17.7 (3 day); 1.3, 22.4, 33.8 (4 wk)	NCI, 1976; Larson *et al*., 1995a
Drinking water	**ppm ad libitum:**			
B6C3F1 Mouse				
Female	0, 200, 400, 900, 1800	0 (0), 0, 0, 0, 0	No dose dependent increase in cortex, but increase in outer stripe, outer medulla at 4 days and 3 weeks	Jorgenson *et al*., 1985; Larson *et al*., 1994b
OM Rat				
Male	0, 200, 400, 900, 1800	1 (2), 1, 3, 6, 14	LI NA. F344 rats: increase. Sustained cytotoxicity observed in OM histopathology re-evaluation	Jorgenson *et al*., 1985; Larson *et al.*, 1995b (LI); Hard *et al*., 2000
Wistar Rats				
Male	0, 2900	0, 7[b]	NA	Tumansonis *et al.*, 1985
Female	0, 2900	0, 0[b]	NA	
Inhalation	**ppm:**		LI: 2, 1, 20, 38 (90 d)	
BDF1 Mouse			Histopath. score: 0,	
Male	0, 5, 30, 90	0, 2, 14, 25	0.25, 2.75, 2.75	Matsushima, 1994; Templin *et al*., 1998
Female	0, 5, 30, 90	0, 0, 0, 0	2, 1, 1.5, 1 (90 day)	
F344 Rat				
Male	0, 10, 30, 90	No increase	2, NM[c], 2, 3 (13 wk)	Matsushima, 1994;
Female	0, 10, 30, 90	No increase	1, NM, 1.5, 8 (13 wk)	Templin *et al*., 1996a
Toothpaste[e]	**mg/kg:**			
Male Mice: C57BL, CBA, or CF/I	0, 60	0, 0	NA	Roe *et al*., 1979
ICI	0, no vehicle, 60	2, 0, 11	No increase in thymidine incorporation or other indicators of cytotoxicity after single ip dose	Roe *et al*., 1979; Moore *et al*., 1982
ICI: arachis oil	0, no vehicle, 60	2, 0, 25		
ICI: without flavoring	0, 17, 60	0, 0, 21		

[a] Tumor incidence in colony control given, with incidence in matched control given in parentheses

[b] Other than the liver, histopathology was only performed on sections from tissues with gross lesions

[c] NM - Labeling index not measured for this dose group; LI – labeling index; NA – not available;

[d] Results for cortex estimated from graph; figures not otherwise provided in publication. Similar pattern seen for medulla.
[e]Toothpaste with peppermint oil and eucalptol except when dissolved in Arachis oil or no vehicle was used
[f]Larson *et al*., 1994a: by gavage 4 to5 ×/wk., with LI observations at 4 days and 3 weeks; Larson *et al*., 1994b: by gavage for 4 consecutive days or 5 days/wk for 3 weeks; BrdU label received for 3.5 days; Templin *et al*., 1996: single gavage dose .BrdU received ip 2 hour before killing, 48 hours after gavage dose; Templin *et al*., 1998: by inhalation, 6 hr/day, 5 d/wk, for 3, 7 or 13 weeks. Acclimatization of high dose males; Larson *et al*., 1995a: by gavage, for 4 consecutive days or 5 days/wk for 3 weeks; BrdU label received for 3.5 days; Larson *et al*., 1995b: by drinking water *ad libitum* for 4 consecutive days or 3 weeks; Matsushima 1994 6 hours/day, 5 days per week, for the mouse, after initial acclimatization period of 4 weeks to lower levels

Table 2. Observations of Liver Neoplasia and Cytotoxicity[a]

Species/strain/ Sex/vehicle	Dose or Concentration	Tumor incidence [a] (%)	Labeling index or other cytotoxicity indicator	Bioassay ref.; cytotoxicity ref.
Corn Oil	**mg/kg:**			
B6C3F1 Mouse				
Male	0, 138, 277	6 (11), 38, 98	0.4, 6.5, 29.3	NCI, 1976; Larson *et al*., 1994a
Female	0, 238, 477	1 (0), 80, 95	2.78, 20.3, 85.5 (4 day) 1.78, 11, 16.8 (3 week)	NCI, 1976; Larson *et al.*, 1994b
OM Rat				
Male	0, 90, 180	1 (0), 2, 6	No increase (1 day)	NCI, 1976; Templin *et al*., 1996b
Female	0, 100, 200	2 (10), 10, 6	NA. F344 rats: 1.6, 6, 11.7 (4 day); 0.6, 14, 11.8 (3 wk)	NCI, 1976; Larson *et al*., 1995a
Drinking water	**ppm ad libitum:**			
B6C3F1 Mouse				
Female	0, 200, 400, 900, 1800	5 (0), 4, 6, 0, 2	3.52, 1.99, 0.98, 0.97, 0.90 (4 day) 2.65,2.34,2.57,2.01,3.34 (3 week)	Jorgenson *et al*., 1985; Larson *et al*., 1994b
OM Rat				
Male	0, 200, 400, 900, 1800	No increase	NA. F344 rats: no increase at 4 days or 3 weeks	Jorgenson *et al.*, 1985; Larson *et al*., 1995b
Wistar Rats				
Male	0, 2900	23, 18	NA	Tumansonis *et al*., 1985
Female	0, 2900	0, 25	NA	
Inhalation	**ppm:**			
BDF1 Mouse				
Male	0, 5, 30, 90	30, 14, 24, 35	1.2, 0.5, 1.2, 5 (7 wk) 1, 0.8, 1, 1.2 (13 wk)	Matsushima, 1994; Templin *et al*., 1998
Female	0, 5, 30, 90	4, 4, 8, 12.5	4.2, 2.3, 4, 10.3 (3 wk) 0.8, 1, 1, 4 (13 wk)	
F344 Rat				
Male	0, 10, 30, 90	No increase	0.5, NM, 0.5, 0.5 (13 wk)	Matsushima, 1994; Templin *et al*., 1996a
Female	0, 10, 30, 90	No increase	1, NM, 0.5, 1.5 (13 wk)	

[a]See explanatory footnotes to Table 1

products. Instead, a brief discussion that acknowledges the importance of the epidemiologic research to the broader question of disinfection by-products and an indication of how EPA is addressing those concerns should be provided. It might be noted that the reason for a lack of epidemiologic research on chloroform in drinking water and cancer is that humans are not exposed to isolated chloroform in drinking water so it cannot possibly be studied. The review of the few studies that happened to

evaluate cancer risk in relation to chloroform as an index (as opposed to total trihalomethanes) should be omitted in that those studies are no more directly relevant to chloroform than any others in the series of reports. The choice of Doyle *et al*. (1997), Lawrence *et al*. (1984), and Hoff *et al*. (1992) is not explained, given that they are not necessarily the best or most recent studies. Similarly, the highlighting of Kramer *et al*. (1992), among the dozen or so studies of reproductive and developmental effects, seems arbitrary. The brief methodologic criticisms of the literature on disinfection by-products and cancer should also be omitted, in that a thorough analysis of that literature would require much more work and extensive evaluation. Also, mention could be made of the forum in which EPA would undertake such work.

What should be provided is a brief overview of the key endpoints that have arisen as a result of the epidemiologic research on disinfection by-products more generally, citing some reviews. Those endpoints would include bladder cancer, colon cancer, rectal cancer, and more recently, spontaneous abortion and fetal growth retardation. It is important to indicate that the substantive findings and methodologic issues are being addressed elsewhere, because reviewers have interpreted the omission of a serious discussion of the epidemiologic literature as an
indication of lack of appreciation for epidemiology in general or of the relevance of this body of research to regulatory decisions pertaining to disinfection by-products.

3.5 Children's Risk Concerns, Including Ontogeny of Drug Metabolizing Enzymes

Charge element (c) asked if the conclusions relating to the assessment of children's risk for chloroform appropriately address the risk concerns (including ontogeny of drug metabolizing enzymes) and are adequately supported by the analyses presented in the health risk assessment/characterization (as supported by the ILSI report) and the framework analysis.

Before addressing the specific Charge question, some general comments are in order. The idea that children on occasion may be less sensitive needs to be expanded upon. In fact, children may be more -- or less -- sensitive for a variety of reasons, including differential chemical exposure, absorption, metabolism, and target-organ susceptibility. Therefore, in some cases with some chemicals and drugs, children can be less susceptible to the toxicant, but tragedies have occurred when children are more susceptible to the toxicant, which is probably more often the case than not. (e.g., methyl mercury poisoning in Japan (Harada and Moriyama, 1976; Amin-Zaki *et al*, 1974)).

EPA provided a comprehensive review of the available data, with the drinking water risk assessment document being the most thorough. However, even when all the data are taken into consideration, the Subcommittee has identified areas that could be improved. These are:

a) The data support the supposition that children, when compared to adults, are not at increased risk when exposed to similar dose levels. The incidence of renal cancer in the human population from causes other than genetic predisposition is very low, and the low incidence of liver cancer in children would support EPA's conclusion. However, in

assessment it is necessary to discuss the issue of exposure latency. Exposure to an agent during development may not result in cancer during childhood, but only manifest itself when the subject becomes an adult. Consequently, using the data from children noted above as the indicator of "true risk" and positing that exposed children remain at risk levels similar to non-exposed children over their life span may not be as conservative as the Agency believes, and may even be slightly misleading.

b) The Agency's supporting documents discuss issues of differential exposure, noting that the child drinks more water on a per kg of body weight basis than does an adult, and inhales more air on a body weight basis than does the adult. In addition, however, powder-formula fed infants should be addressed as a special population. From birth to age 6 months these infants are sustained on a diet consisting mainly of tap water and powdered formula. The median drinking water intake for this group is roughly 150 mL/kg-body weight, nearly an order of magnitude greater than the median intake for adults. Formula fed infants consuming a greater number of calories on a body weight basis can drink roughly 50% more than the median infant. Their tap water and hence chloroform intake on a bodyweight basis can therefore be considerably greater than the baseline case on which the MOE comparison is made. Such exposure should also be considered when deriving the MCLG. The derivation of the MCLG should also consider inhalation and dermal exposures that result from the use of tap water for other purposes. As noted on p. 35 of the risk characterization document, inhalation exposure can be significant relative to oral exposure.

In addition, two important areas -- transplacental and transmamillary exposure to agents -- are not discussed

c) The documents address the issues of CYP2E1 activation of chloroform and the fact that CYP2E1 levels are lower in the child than the adult. The October 29, 1998 chloroform risk assessment document discusses the fact that organ susceptibility also has to be addressed and that the developing rodent does not seem to have a higher degree of susceptibility than the adult rodent on a acute or semi-acute basis. The discussion gives the impression that since the CYP2E1 level is lower during development, the developing mammal must be at less risk in developing cancer. Other factors influence the susceptibility of a tissue. It is possible that liver tissue of the young is, in fact, responsive to smaller amounts of the responsible metabolites. While there is no evidence of increased susceptibility of children to chloroform, it is important to systematically recognize all known factors that could contribute to the sensitivity of specific populations, especially children, pregnant or lactating females, etc.

d) EPA needs to address the issue of chronic low level exposures. It is possible that such exposure may alter cellular factors (e.g., inducing CYP2E1) and increase activation or decrease detoxification/protective capacity in the developing mammal as compared to

the adult. It is recognized, however, that there is no precedence for such effects at doses of chloroform that would normally be derived from drinking chlorinated water (generally less than 2 μg/kg per day even in a child). This may be possibly true even at doses that might be obtained at the proposed MCLG (about 30 μg/kg per day in a 10 kg child consuming 1 L per day).

e) The documentation does not discuss the issue of perinatal imprinting. Imprinting can occur as a result of exposure to a specific chemical or from a variety of other environmental factors. Multi-generational studies could be helpful in addressing this issue; i.e., since the one extant study did not compare adult vs developing animals, the issue remains unresolved.

In several other areas, the Subcommittee was unsure as to EPA's position or intentions. For example, when daily exposure levels are set, how is the Agency going to address issues with children who drink a larger volume portion of water per unit body weight than the adult? An example might be the formula-fed infant when formula is prepared from tap water. Is the Agency planning to invoke the 10x safety factor to deal with this issue?

We suggest that, to discuss and explore more fully possible subpopulations at risk subpopulations at risk, it would be informative for the Guidelines to include (provide) a "check off" format for each agent to identify and describe populations at heightened risks as in terms of:

a) age related susceptibilities – fetuses, infants and children, pubescent adolescent, adults, elderly

b) gender-related -- including pregnant females and lactating females

c) genetic polymorphisms/deficiencies

d) drug-drug interactions (xenobiotics , environmental chemicals)

e) disease states

f) foods and diets

The Subcommittee found that the draft document addressed children's risks quite adequately, based on the scientific information that is currently available. We believe that major conclusions are correct, but that they could be stated with slightly more caution. Although we agree that CYP2E1 may play an important role in the metabolism of chloroform to reactive metabolites that are involved in tissue injury, cell death, and tumor development, its definitive role in the developing human or mammal has yet to be confirmed.

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