Methanol Institute

Comments on the U.S. EPA Draft Toxicological Review of Methanol (IRIS)

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Executive Summary

The Environmental Protection Agency has proposed that methanol be classified as "Likely to Be Carcinogenic to Humans"[1] under the Agency's 2005 Cancer Risk Guidelines. The Methanol Institute believes that the underlying science demonstrates instead that methanol merits a classification of "Inadequate Information to Assess Carcinogenic Potential" under these Guidelines.

These comments from the Methanol Institute[2] focus almost exclusively on EPA's proposed cancer classification because of the overwhelming importance of this issue for the future of methanol. Methanol promises to be a major component of this country's effort to reduce its dependence on oil as an alternative transportation fuel, and to help usher in the use of fuel cells to replace batteries in a wide variety of consumer and industrial uses. A (mis)characterization of methanol as being "Likely to Be Carcinogenic to Humans" would unjustifiably restrict the public's use of this vital chemical, which is an essential building block for thousands of products that touch our lives each day.

In classifying chemicals with regard to their carcinogenic hazard, EPA is required to carefully apply its own classification guidelines contained in the Agency's 2005 Cancer Risk Guidelines.[3] We believe that these criteria were misapplied to the available body of science regarding methanol. In proposing to classify methanol as "Likely to Be Carcinogenic to Humans," EPA apparently relied on the following two criteria:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;" and

- "a positive tumor study that is strengthened by other lines of evidence, for example … an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

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[1] Federal Register, January 12, 2010. 75 Fed. Reg. 1617.
[2] The Methanol Institute is the trade association for the global methanol industry, representing the world's leading methanol producers, distributors, and technology companies.
[3] The classification criteria under the 2005 Cancer Risk Assessment Guidelines are reprinted in Appendix B of these comments.

With regard to the application of the first criterion, there are no human studies of methanol and only four available animal studies. The Methanol Institute's comments address each of these four animal studies in detail and show that they simply do not support a classification of "Likely to Be Carcinogenic to Humans" and instead support a classification of "Inadequate Information to Assess Carcinogenic Potential," at best.

In short:

- The Ramazzini Foundation (ERF) study in rats: This study, upon which EPA relies most heavily for its proposed classification, has been seriously called into question by government agencies and scientific experts in the published literature. There is testimony under oath by laboratory officials that confirms the existence of respiratory infections within the laboratory rat colony. This type of infection is known to produce results that are not cancerous but can be misdiagnosed as lymphoma.

  The only conclusive way to confirm that this infection led to a misdiagnosis of lymphoma in the methanol study or, alternatively, that the original diagnosis was correct is to re-examine the preserved tissue slides for evidence of this infection and independently review the initial diagnosis of lymphoma. Government agencies and members of the scientific community have been calling for such a "Pathology Working Group" review of these studies since 2006. A Pathology Working Group would be a key contribution to the toxicological reviews that EPA is now conducting on ethanol, MTBE, and formaldehyde. These reviews involve similar findings of "lymphoma" made by the ERF Laboratory. If and until such a review of the slides is undertaken, the Ramazzini study's finding of lymphoma cannot legitimately be used for cancer risk assessment purposes.

- The New Energy Development Organization (NEDO) studies in rats and mice (2 studies): EPA agrees with the study authors that the NEDO mice study shows no cancer effects from exposure to methanol. In the case of the rat study as well, the Japanese authors concluded that there were no cancer effects. In examining the effects in the male rats, even the EPA considers the results to be only "suggestive." All but one of the lung tumors were benign adenomas; one high exposure rat had a carcinoma. While the incidence of combined adenoma and carcinoma is statistically significant when compared to the concurrent controls, the laboratory pointed out that its historical controls have sustained just as high an incidence. The laboratory concluded that methanol did not increase lung tumors in male rats, and four of five of EPA's independent peer review panel members did not raise increased lung tumors as an issue. It is the Methanol Institute's view that this weak response should not be used to support a classification of "Likely to Be Carcinogenic to Humans."

  EPA's use of the female rats' result also merits revision. EPA itself had this study independently reviewed. Three of the five reviewers agreed with the authors that there was no conclusive evidence of carcinogenicity in the study. Of the other two reviewers, one concluded that additional analysis—if performed—might have provided positive results, while the other reviewer stated that the tumors could not be conclusively dismissed. Inexplicably, the draft EPA assessment mentions this independent peer

review in connection with the NEDO studies, but never indicates whether the peer review agrees or disagrees with EPA conclusions. These female rat results, too, should not be used to support a classification of "Likely to Be Carcinogenic to Humans."

- The Apaja study in mice: This graduate student thesis is not of the quality that EPA should use for cancer classification purposes. There were no concurrent controls against which to compare the study results, and there is also some reason to suspect involvement of the same kind of respiratory infection seen in the Ramazzini rat colony that can lead to misdiagnoses. The author concluded that the effects seen were in the normal range. However, EPA selected only some groups of the animals to analyze and concluded that the effects shown were statistically significant. This picking and choosing of which animals to analyze is inappropriate and is contrary to EPA's benchmark dose analysis procedures. A proper analysis of all the animals exposed to methanol shows that there was no increase in lymphoma. This study should not be used for a "Likely to Be Carcinogenic to Humans" classification.

With regard to the second criterion dealing with metabolism, EPA suggests that since methanol metabolizes to formaldehyde, methanol may cause the same health effects as formaldehyde. While it is true that methanol does metabolize to formaldehyde, in order to meet this criterion in the Guidelines, EPA must show that this metabolism is "likely to be related to the tumor response in this case."

As indicated above, the ERF and Apaja studies provide no sound scientific basis for determining that there are tumors caused by methanol in the first place. Putting that aside, the metabolism of methanol is of such a nature that it is highly unlikely that the metabolism of methanol causes there to be any additional formaldehyde in animal cells, beyond what is naturally occurring in the absence of methanol. Further, the EPA in its draft assessment has given no evidence that exposure to methanol leads to a buildup of intracellular formaldehyde as a result of metabolism.

The Agency also appears to be making assumptions about the carcinogenicity of formaldehyde in this methanol review before any such conclusions have been peer reviewed by the National Academy of Sciences as prescribed for the forthcoming formaldehyde toxicological review. For all these reasons, this metabolic relationship between methanol and formaldehyde does not meet the "likely to be related" requirement in the Guidelines.

All of the above matters are discussed in greater detail in the Methanol Institute's comments that follow. Objective consideration of the issues raised should lead to the conclusion that the draft EPA assessment has misclassified methanol as "Likely to Be Carcinogenic to Humans" and that the proper classification is "Inadequate Information to Assess Carcinogenic Potential."

In summary, these comments by the Methanol Institute constitute a request that **EPA revise** its classification of methanol and that the **Science Advisory Board confirm**, particularly in the absence of verification of the accuracy of the diagnoses in the Ramazzini study, that these four animal studies do not satisfy the criteria of the Agency's 2005 Cancer Risk Assessment Guidelines for "Likely to Be Carcinogenic to Humans."

Preface

How these comments are organized

Focus on cancer: These comments from the Methanol Institute deal almost exclusively with the issue of cancer. While the EPA draft toxicological review addresses both cancer and non-cancer issues, we chose to focus on cancer because of the overwhelming importance of the cancer issue for the methanol industry. We understand other organizations may submit comments on the non-cancer aspects of the EPA assessment.

Use of cross-references: While these comments are quite lengthy, we have attempted to make them accessible. In addition to the normal Table of Contents, we have also included numerous cross-references throughout the document (in the form of "See discussion at page X") in order to make it as easy as possible for readers to find specific portions of the comments that are of interest to them.

Answers to the Charge Questions: Our comments then address the draft Charge Questions posed by EPA's National Center for Environmental Assessment. Rather than repeat our extensive discussion of issues in our answers to these questions, we instead summarize our answers in this section and provide references to the complete discussion located elsewhere in the paper.

Necessary Changes to the Draft Toxicological Review Document: Throughout these comments we indicate the necessary changes to make the EPA draft toxicological review document conform to the underlying scientific data as discussed in these comments. These necessary changes are summarized at the end of the comments beginning at page 71.

Resources Available to Assist in the Review of Key Studies

Independent Peer Reviews of the Four Key Animal Studies

Two independent peer reviews have been conducted of the four animal cancer studies relied upon by EPA in its Toxicological Review—the peer reviews by the Eastern Research Group (ERG) and by Toxicology Excellence for Risk Assessment (TERA).

In 2009, EPA decided to have the NEDO studies independently peer reviewed by a panel to be assembled by their contractor ERG. The Methanol Institute had previously obtained the original research data from NEDO officials in Japan, had it translated, obtained Japanese certification regarding the accuracy of the translation, and then made the data available to EPA. With these detailed data in hand, and under contract with EPA, ERG selected 5 reviewers, conducted the

review, and published a 48-page review in June 2009[4]. In the preface, the ERG report indicates that these studies had not previously been peer reviewed and that "The IRIS Program has a strong preference for use of peer-reviewed, published studies as principal or influential studies. Such a peer review process is important to establishing the appropriateness, validity, and robustness of the study design, conduct, and interpretation of findings of the reported investigation."

ERG chose the reviewers according to selection criteria provided by EPA, and EPA confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled EPA's selection criteria. The reviewers were Drs. Judith Buelke-Sam, David Dorman, David Gaylor, Kenneth McMartin, and David "Alan" Warren. Strangely, having paid for this review, EPA chose not to mention whether EPA's conclusions are consistent with the opinions of these reviewers. These reviewers' views are discussed elsewhere in these comments at page 43.

Upon learning of this peer review, the Methanol Institute formally requested EPA to ask ERG to convene the same or similar panel in order to conduct a comparable peer review of the Soffritti (ERF) study on methanol because it, like the NEDO studies, had never been peer reviewed. One would presume that if EPA were concerned that the NEDO study had not been independently peer-reviewed, the Agency should also have been equally concerned that the Soffritti study had not been peer reviewed and would have included that study in the peer review it funded with ERG. However, even in response to the Methanol Institute's request that the Agency rectify this failure, EPA refused to conduct this peer review of the ERF study, despite the considerable controversy that has surrounded the study's findings and the considerable weight that the Agency ultimately placed on the study in reaching its proposed classification of methanol.

Given EPA's refusal, the Methanol Institute turned to ERG and asked them, at the Institute's expense, to conduct such a peer review of the Soffritti study. ERG declined, citing its contract with EPA as a possible conflict. However, ERG recommended that the Methanol Institute instead ask TERA to conduct the peer review. Subsequently, the Methanol Institute contracted with TERA to conduct a peer review of the Soffritti study. (At a later date, the Apaja study cited in the EPA toxicological review was also added to this TERA peer review.)

TERA and the Methanol Institute went to extraordinary lengths to ensure the complete independence of the peer review. TERA chose the reviewers and conducted the review without consultation with the Institute. The Methanol Institute did not know the identity of the reviewers, the nature of the charge questions, or the content of the review until it was published on the TERA website. As it happens, TERA chose to ask some of the same reviewers who had conducted the ERG peer review of the NEDO study to also serve on its peer review of the Soffritti study. Drs. Judith Buelke-Sam, David Dorman, David Gaylor, and David "Alan" Warren served on the Soffritti panel as they did on ERG's NEDO panel and were joined by Dr.

[4] Eastern Research Group, "External Letter Peer Review of Reports Documenting Methanol Studies in Monkeys, Rats and Mice Performed by the New Energy Development Organization (NEDO): Peer Reviewer Comments", June 16, 2009.

Janis Eells for the Soffritti panel. TERA's Report[5], like the ERG report, re-prints the reviewers' comments to the charge questions without any editorial additions from TERA. Unlike the ERG report, TERA, as a matter of organizational policy, did not specify which comments come from which reviewers.

Both of these two independent peer reviews are excellent sources of insight into the strengths and weaknesses of these four cancer studies that are the subject of the EPA toxicological review.

Complete data files from the Soffritti methanol study

The Ramazzini Foundation made the complete data files (but not the tissue slides) for the Soffritti/ERF study on methanol available to the National Institute of Environmental Health Sciences who in turn made them available to EPA. After considerable effort, the Methanol Institute was able to obtain these files and to review them in detail. The results of this examination are discussed in a journal article entitled "Assessment of the cancer potential of methanol[6]." It is rare that complete file sets are made available by the Ramazzini Foundation, and the analysis of the data revealed considerable information that we have included in these comments.

About Methanol and the Methanol Institute

Methanol is one of the most widely used chemicals in commerce, with annual global consumption of more than 14 billion gallons. Methanol is a basic building block for hundreds of chemical compounds and products that touch our daily lives. Methanol is also an emerging energy fuel, with the potential to fuel our vehicles, electric power plants, homes and consumer electronics.

As the trade association for the global methanol industry, the Methanol Institute represents the world's leading methanol producers, distributors, and technology companies. Since the EPA began its assessment of methanol, the Institute has worked cooperatively with EPA staff to assist the Agency in obtaining the best available science for its assessment. The Methanol Institute:

- provided a large number of research documents to EPA via the IRIS Submission Desk;
- obtained detailed data files from the Ramazzini methanol study so that outside scientists could access them for review; initiated a four-year research project with the University of Toronto to address a basic research question related to the metabolism of methanol that was identified as needed by EPA staff;
- obtained the original research data from Japan for the NEDO studies, and translated and provided this information to EPA;

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[5] TERA, "Report on the Peer Review of the Methanol Bioassays: Soffritti et al. (2002) and Apaja (1980) 2010,http://www.tera.org/peer/meetingreports/MethanolFinalReportRevised.pdf

[6] Cruzan G. (2009). Crit Rev Toxicol, 39: 347-363.(196354)

- commissioned a Peer Review of the Ramazzini methanol study by independent experts after the Agency refused to conduct a peer review;
- engaged an expert modeler to review the PBPB model employed by the EPA in its methanol assessment;
- initiated a research project to determine the role of formaldehyde in the metabolism of methanol; and
- repeatedly called for a Pathology Working Group review of the Ramazzini methanol study.

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Methanol Institute's Comments on the U.S. EPA Draft Toxicological Review of Methanol (IRIS)

Introduction to Comments

The Required Criteria for Cancer Classification

In classifying chemicals with regard to their carcinogenic hazard, EPA is required to carefully apply its own classification guidelines contained in the Agency's 2005 Cancer Risk Guidelines.[7] We believe that these criteria were misapplied to the available body of science regarding methanol. In proposing to classify methanol as "Likely to Be Carcinogenic to Humans," EPA apparently relied on the following two criteria:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;" and

- "a positive tumor study that is strengthened by other lines of evidence, for example… an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

Our comments contained in this document address in detail the studies and mode of action hypotheses that underlie EPA's proposed classification of methanol. A thorough and objective consideration of these studies and hypotheses should lead to the conclusion that the proper classification for methanol is "Inadequate Information to Assess Carcinogenic Potential" under the EPA cancer guidelines.

The following are the Methanol Institute's comments on the EPA draft toxicological review document published on January 12, 2010 in the Federal Register for comment.

[7] The classification criteria under the 2005 Cancer Risk Assessment Guidelines are reprinted in Appendix B of these Comments.

I. Cancer

I.A. Lymphoma

I.A.1. Ramazzini Foundation Study of Methanol in Rats

Even though methanol—also known as wood alcohol—has been used for hundreds of years, it can be distinguished from many other major chemicals in commerce by the fact that there are very few scientific studies that shed any light directly on the possible carcinogenicity of methanol. Within the literature on methanol:

- There are no epidemiological studies suggesting cancer in humans; and
- There are only four animal studies:
    - The Ramazzini Foundation (ERF) study in rats[8];
    - The Apaja study in mice[9]
    - The New Energy Development Organization (NEDO) study in rats[10]; and
    - The NEDO study in mice[11].

This scarcity of evidence argues for a very careful review of each of these four studies since an incorrect interpretation of any one study could greatly skew any overall conclusion regarding the carcinogenicity of methanol. We note that no authoritative body across the international community has found any link between exposure to methanol and carcinogenicity.

The Ramazzini Foundation (ERF) study of methanol (Soffritti et al., 2002) reports increased total combined leukemias, histiosarcomas and lymphomas, referred to by the ERF as lymphohemoreticular cancers. In its report, ERF linked this study of methanol with similar ERF studies of aspartame[12], MTBE[13], and formaldehyde[14], stating that all four chemicals showed similar cancers and suggesting a possible common mode of action through the metabolism of the first three chemicals to formaldehyde[15]. In its assessment, EPA relies primarily on this

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[8] Soffritti et al., 2002 (091004)
[9] Apaja, 1980 (191208)
[10] NEDO, 2008 (196316)
[11] NEDO, 2008 (196315)
[12] Soffritti (2006) (196735)
[13] Belpoggi (1997) (047984)
[14] Soffritti (2002) (196211)
[15] This hypothesis linking the health effects of methanol and formaldehyde is addressed separately at page 56 in these comments.

Ramazzini methanol study to support its proposed classification of methanol as "likely to be carcinogenic to humans."

These four ERF chemical studies have received considerable criticism in both the peer-reviewed literature and in determinations by authoritative regulatory bodies both in the United States and in Europe. These criticisms have challenged the studies' accuracy and the appropriateness of their use in hazard and risk assessments. Because of the use of aspartame in food, the ERF study of aspartame has received more attention than the other three studies, but the key issues raised are common among the four studies.

For the reasons detailed below, we believe the ERF methanol study should be afforded very little scientific weight in any hazard assessment of methanol. Because EPA's proposed classification of methanol relies so heavily on this single study, we devote a large portion of our comments to an analysis of this study.

I.A.1.1. Types of Lymphoma Reported in ERF's Methanol Study

A major problem that pervades all of EPA's discussion of the Soffritti/ERF (Ramazzini) study on methanol is that all lymphomas in the study are combined. This can be very misleading in that it suggests that more than one type of lymphoma contributes to the reported increase in dose response. This is not the case. As noted in Cruzan (2009), there were only three types of lymphoma reported in the Soffritti/ERF methanol study:

- The incidences of "lymphoblastic lymphoma" were 1, 3, 1, 0 for males and 0, 1, 1, 1 for females at 0, 500, 5000, and 20000 ppm in drinking water.
- One female at 500 ppm was reported to have a lymphocytic lymphoma.
- The incidences of lympho-immunoblastic lymphoma (LIL) were 16, 25, 27, 37 (of 100) in males and 9, 17, 19, 21 in females at 0, 500, 5000, and 20000 ppm.

Although the EPA draft assessment correctly states that leukemias, histosarcomas and lymphomas are derived from different stem cells and should not be combined, EPA proceeds to combine all lymphomas, despite the fact that only one diagnosed lymphoma type is reported as increased, and despite the fact that no other laboratory in the world has ever reported a diagnosis of LIL in any rat. Tumor types should be considered separately because they have different cells of origin, organ specificity and mode of action. The National Toxicology Program (NTP)[16] has developed guidelines for combining tumor types that EPA should follow in its assessments.

Since we can dismiss the negligible lymphoblastic lymphoma and the lymphocytic lymphoma incidences in the Soffritti/ERF study, we are left with only the LIL reported as a lymphoma and having a dose response. As will be clearly documented in these comments (beginning on page 16), this finding of LIL has been the subject of serious controversy in the peer-reviewed literature. If in fact, as has been widely suggested, the reported LIL is not a cancerous tumor,

[16] McConnell et al., 1986 (073655)

then the conclusion of the Soffritti/ERF methanol study that exposure to methanol in rats leads to cancer is actually false. Because the EPA has based its classification of methanol as "Likely to Be Carcinogenic to Humans" primarily on this suspect LIL diagnosis, then that proposed classification cannot be supported.

Another example of where EPA combines tumor types is EPA's analysis of the ERF study on MTBE in the draft toxicological review of methanol. EPA presents combined lymphomas and leukemias, and does not analyze the incidences of lymphomas only. The cited publications do contain a breakdown by tumor type. If MTBE is to be included, the document should compare the chemicals "apples to apples," fairly and consistently; i.e., the document should present and analyze the MTBE lymphoma data.

As found in the MTBE individual pathology data, the incidences of each type of lymphohematoreticular* cancers were:

Table 1. Individual Pathology Data in the ERF MTBE Study

	Males				Females			
Dose	LIL	LL	LM	HS	LIL	LL	LM	HS
0	2	5	1	1	1	1	0	0
250	5	1	0	1	6**	0	0	0
1000	3	3	0	1	8	3	0	0

*LIL = lympho-immunoblastic lymphoma
LL = lymphoblastic lymphoma
LM = myeloid leukemia
HS = histiocytic sarcoma
** All LIL were found in the lung except one in this group, only in spleen.

From these data, it is clear that only LIL was increased in the MTBE study. We note that no other study of MTBE reported increased lymphoma.

Necessary Changes to the Draft Toxicological Review:

#1. Combining Disease Types: The document should be reworded to avoid combining leukemias and lymphomas as well as combining all lymphomas, treating separately those lymphomas that are reported as increased and those for which no increase was observed. There was only one type of reported lymphoma in the ERF methanol study that was increased and showed any dose response. That is the only "lymphoma" that should be analyzed; to include the other minor types confuses the issue and appears to indicate that all types of lymphoma were increased.

I.A.1.2. The *Mycoplasma pulmonis* Hypothesis for ERF studies

EPA places its principal reliance on the lymphoma results from the methanol study by Ramazzini (Soffritti, et al. 2002) to support its proposed classification of methanol as "Likely to Be Carcinogenic in Humans." Over a period of several years, several governmental bodies and independent scientists have developed what we term here the *Mycoplasma pulmonis* hypothesis related to four studies (aspartame, MTBE, methanol, and formaldehyde) conducted by the Ramazzini Laboratory (ERF) whose findings included the unusual lymphoma diagnosis of lympho-immunoblastic lymphoma(LIL). The hypothesis is that the lymphoma findings of these ERF studies likely represent a misdiagnosis of what is instead an immune response to a *Mycoplasma pulmonis* infection in the test animals.

In our comments below, we lay out the reasons why we believe this hypothesis is sound and should be investigated and resolved through a review of the actual tissue slides form the Soffritti/ERF study. These reasons can be summarized as follows:

- Authoritative government bodies (Food and Drug Administration, European Food Safety Authority) other U.S. Federal agencies (Department of Defense, Office of Management and Budget), and respected members of the scientific community have rejected the findings of the group of ERF studies showing reported LIL. Many of these have called as well for a Pathology Working Group to determine the accuracy of the ERF diagnoses *[See discussion beginning at page 17]*;

- The type of lymphoma (Lympho-immunoblastic lymphoma—LIL) reported has not been seen by any other laboratory and affects a completely different spectrum of organs than any known lymphoma *[See discussion at page 19]*;

- The LIL reported occurs at a much higher frequency than any other lymphoma in rats and has a unique cellular morphology *[See discussion at page 19]*

- An independent peer review panel of scientists convened by TERA raised serious questions about the accuracy of the Soffritti study including a statement by one reviewer that EPA should bear in mind that "'an absence of proof is not the proof of absence' when it comes to a *M. pulmonis* infection" *[See discussion at page 20]*;

- An ERF laboratory official has testified under oath confirming the existence of *Mycoplasma pulmonis* antibodies in the laboratory's rat colony, thereby showing that infections have occurred in the colony despite earlier denials by the Laboratory in personal communications to EPA. *[See discussion at page 22]*;

- With only two exceptions, the reported LIL in the Soffritti methanol study occur in the lung and associated organs (lymph nodes and spleen), consistent with a respiratory disease explanation. [See discussion at page 23]

- EPA's citation of the National Institute of Environmental Health Sciences (NIEHS) panel of pathologists as support for the LIL finding failed to point out that the panel only examined slides from 2 of the 149 animals with purported LIL and that this panel meeting took place before serious questions had been raised about a possible misdiagnosis arising from an infection with *Mycoplasma pulmonis [See discussion at page* 25];

- The reported LIL shown in photomicrographs has been identified by scientists, who are experts in respiratory disease in test animals, as *Mycoplasma pulmonis[See discussion at page 26]*; and

- The National Toxicology Program is planning a visit soon to the ERF Laboratories that may result in an independent review of the tissue slides from the Soffritti study. If this review is done correctly, then it could resolve the issue of possible misdiagnosis *[See discussion at page 28]*.

I.A.1.2.1. The Evolution of the Hypothesis

I.A.1.2.1.1. The European Food Safety Authority

Of the four studies that ERF (Ramazzini) suggested showed similar cancers (aspartame, MTBE, methanol, and formaldehyde), the ERF study of aspartame has received the most attention because of the use of aspartame in food. However, the key issues raised are common among all four studies.

In 2006, the European Food Safety Authority (EFSA) reviewed the ERF study of aspartame reported in publications in 2005 and 2006, as well as additional data it received from ERF. EFSA concluded as follows:

> *"After its evaluation the Panel considers that the study has flaws which bring into question the validity of the findings, as interpreted by the ERF…."*
>
> *"The increased incidence of lymphomas/leukaemias [sic] reported in treated rats was unrelated to aspartame, given the high background incidence of chronic inflammatory changes in the lungs and the lack of a positive dose-response relationship. It is well-known that such tumours [sic] can arise as a result of abundant lymphoid hyperplasia in the lungs of rats suffering from chronic respiratory disease. The most plausible explanation of the findings in this study with respect to*

lymphomas/leukaemias is that they have developed in a colony suffering from chronic respiratory disease."[17]

In April 2009, EFSA reviewed a second ERF study on aspartame and concluded that there was no reason to revise its previous findings.[18]

I.A.1.2.1.2. The U.S. Food and Drug Administration

In April 2007, the U.S. Food and Drug Administration (FDA) reviewed the ERF study on aspartame and issued the following statement[19]:

> *"FDA could not conduct a complete and definitive review of the study because ERF did not provide the full study data. Based on the available data, however, we have identified significant shortcomings in the design, conduct, reporting, and interpretation of this study. FDA finds that the reliability and interpretation of the study outcome is compromised by these shortcomings and uncontrolled variables, such as the presence of infection in the test animals.*
>
> *Additionally, the data that were provided to FDA do not appear to support the aspartame-related findings reported by ERF. Based on our review, pathological changes were incidental and appeared spontaneously in the study animals, and none of the histopathological changes reported appear to be related to treatment with aspartame."*

FDA concluded a detailed review of the tissue slides themselves would also be desirable:

> "*FDA believes that additional insight on the study findings could be provided by an internationally-sponsored pathology working group examination of appropriate tissue slides from the study."*

The rejection of this ERF study by both EFSA and FDA are in sharp contrast to EPA's use of the related ERF study on methanol as the principal support for its proposed classification of methanol as "Likely to Be Carcinogenic to Humans."

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[17] EFSA, Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request form the Commission related to a new long-term carcinogenicity study on aspartame, EFSA-Q-2005-122, May 3, 2006. (196098)

[18] EFSA, "Opinion on a request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame, EFSA-Q-2008-746, January 29, 2009 (196103)

[19] FDA, FDA Statement on European Aspartame Study, CFSAN/Office of Food Additive Safety, April 20, 2007.

Further, the EPA incorrectly points to the often-challenged aspartame study as additional evidence that the methanol study is sound.

I.A.1.2.1.3. Discussion of ERF and *Mycoplasma pulmonis* in the Scientific Literature

Responding to some of these expressed concerns, several EPA scientists, including the chemical managers on the IRIS toxicological reviews of MTBE and methanol, Drs. Caldwell and Gift, published a journal article[20] in 2008 attempting to rebut the hypothesis that the results of these ERF studies had been affected by a respiratory infection. In particular, they argued that there is no scientific support to the proposition advanced by the EFSA that the lesions interpreted as lymphomas were caused by a lung infection.

Subsequently, in 2009, a group of scientists, including acknowledged experts in the area of respiratory infections in test animals and the former Director of the Carcinogenesis Program of the National Toxicology Program in NIEHS (McConnell), published a peer-reviewed journal article addressing the likely role of a respiratory infection of *Mycoplasma pulmonis* in these ERF bioassays (Schoeb et al. 2009)[21]. The authors agreed with Caldwell and Gift that there is not much support for the proposition that *Mycoplasma pulmonis* causes lymphoma, but they concluded that ERF scientists had misdiagnosed as lymphoma what were likely lesions from the disease instead.

Specifically, these scientists reviewed the tumor data for ERF's bioassays on aspartame, MTBE, and methanol. For all three studies, the most frequent reported hematopoietic neoplasm was lympho-immunoblastic lymphoma (LIL), the most frequently affected organ was the lung, and, in almost half of the rats with this diagnosis, the lung was the only affected organ. Lesions diagnosed as lymphoma in published illustrations had pleomorphic cellular morphology and appeared to contain neutrophils. [In other words, the lesions do not appear to have the same structure as a lymphoma.] Information from these reports and other sources indicated that lesions typical of *Mycoplasma pulmonis* disease were prevalent among the study rats. Additionally, it is acknowledged that the ERF colony of rats is not Specific Pathogen Free, and there is little documentation regarding the environmental controls for animals housed at the ERF laboratory.

The authors of the Schoeb et al. article summarized their findings in their abstract:

> "Because the lymphoma type, cellular morphology, and organ distribution reported in these studies are atypical of lymphoma in rats, because lymphocyte and plasma cell accumulation in the lung is characteristic of *M. pulmonis* disease, and because *M. pulmonis* disease can be exacerbated by experimental manipulations, including chemical treatment, we suggest that a plausible alternative explanation for the reported results of these bioassays is

[20] Caldwell et al. (2008) (196182)
[21] Schoeb et al. "*Mycoplasma pulmonis* and Lymphoma in Bioassays in Rats, Vet Pathol 46: 952-959 (2009) (196192)

that the studies were confounded by *M. pulmonis* disease and that lesions of the disease were [mis]interpreted as lymphoma."

In the face of the Schoeb et al. positing that misinterpretation is the likely reason for the lymphoma diagnosis, the argument in the Caldwell and Gift paper that one must show that *Mycoplasma pulmonis* actually causes lymphoma is mooted.

The following non-neoplastic findings in the study further support a conclusion of *Mycoplasma pulmonis* infection in the rats in the ERF methanol study:

1. Respiratory tract or "ear" (assume otitis media) inflammatory lesions (at least one of the following: rhinitis, otitis, laryngitis, tracheitis, bronchitis, pneumonia) were reported in 688/800 rats—86% have one or more of these lesions. This inflammation is a strong indicator of *M. pulmonis* disease.

2. Either or both oophoritis (a disease of the ovaries) and endometritis were reported in 60/400 females (15%), which are characteristic of *M. pulmonis* disease in female rats.

3. Mediastinal lymph nodes (those draining the lungs) would be expected to have inflammation if there was a bronchial or lung infection. In the lymph nodes not draining the lung, inflammation was reported in 500 of the 800 rats in the study (62.5%).

I.A.1.2.1.4. Comments of Independent Reviewers (TERA) on the *Mycoplasma pulmonis* hypothesis

The independent peer reviewers assembled by TERA[22] had a number of informative comments about the *Mycoplasma pulmonis* hypothesis. For example, Reviewer 4 said:

> *"…In the EFT supplemental table detailing statistical analyses of non-neoplastic lesions, 57 to 80% of females and 60-71% of males reportedly had ear inflammation. The occurrence of ear inflammation exhibited a reverse dose trend in both sexes (statistically significant in females), implicating a causal factor other than methanol. This is of interest considering that the middle ear is a typical site of colonization with M. pulmonis and inflammation is commonplace under such conditions[23]."*
>
> * * * * * *
>
> *"I have reviewed the tumor patterns in the Ramazzini methanol study, commentaries by others on tumor patterns in the Ramazzini studies of aspartame and MTBE, several publications on M. pulmonis infection*

[22] See discussion regarding the conduct of the peer review at page 11 of these comments.
[23] TERA at 29.

and its relationship to cancer, and the advocacy pieces mentioned in the question above. Commendable arguments for and against involvement of M. pulmonis infection have been made, but I believe evidence for infection exists, albeit circumstantial. I am most impressed with the effort of Schoeb et al. (2009) that argues infection is a plausible explanation for the lymphoma excesses reported. These authors appear to have a level of expertise on the issue of M. pulmonis not shared by others participating in what has seemingly become a counterpoint argument. Nonetheless, not unlike others who have examined the issue, I have no direct evidence with which to resolve the M. pulmonis issue with any degree of scientific certainty. Under such circumstances, I believe the issue rightfully becomes a philosophical one. ***In a recent publication, USEPA employees (Caldwell et al., 2008) evaluated evidence for infection as a mode of action for rat lymphomas and conclude with a paragraph that suggest the absence of direct evidence supporting the assertion of M. pulmonis involvement justifies concluding otherwise. I disagree and argue essentially the opposite – studies which fail to take reasonable steps to ensure the validity of so-called causal relationships (by the application of serological testing and other widely accepted QA procedures) should be viewed as suspect and deemed unfit for regulatory purposes. After all, it is the USEPA that is considering Soffritti et al. (2000) for the purpose of cancer risk assessment, and thus the Agency should bear the burden of proving its validity prior to use. Such validation would come with evidence that animals used in the Ramazzini methanol study (or perhaps animals used in the study of aspartame and/or MTBE) were M. pulmonis free. I encourage the USEPA to bear in mind the old adage that "the absence of proof is not the proof of absence" when it comes to M. pulmonis infection***[24] [Emphasis added]

I.A.1.2.1.5. Comments of Scientists in Other Federal Agencies on the EPA Draft Toxicological Review

As part of the development process for draft Toxicological Review documents, EPA circulates them to other Federal agencies for peer review by knowledgeable scientists not involved in the initial reviews. In the case of the methanol toxicological review, scientists in both the Department of Defense (DOD) and the Office of Management and Budget (OMB) in the Executive Office of the President submitted detailed comments[25]. Both groups of scientists raised serious questions about EPA's justification for a classification of "likely to be

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[24] TERA, at 30-31

[25] Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=58015. (The Office of Management and Budget comments also incorporated comments from the Centers for Disease Control in the Department of Health and Human Services.)

carcinogenic to humans." OMB identified the contentiousness of ERF data as well and the fact that the EPA had not clearly identified MTBE and formaldehyde as carcinogens but yet the document implied that because they are carcinogens, methanol should also be so classified. With special emphasis on the Soffritti/ERF study, the Department of Defense concluded:

> "We recommend U.S. EPA re-evaluate the designation of methanol as "*likely to be a human carcinogen*," and consider revising to "*suggestive evidence of carcinogenic potential*." We do not believe that EPA presents compelling weight of evidence to support the descriptor.[26]

Unfortunately, EPA chose to ignore the comments of DOD and OMB and, rather than revising the document, the EPA choose to publish essentially the same draft document for public comment and ultimate transmittal for peer review to the Science Advisory Board.

I.A.1.2.2. EPA Staff's Response to the Mycoplasma pulmonis Hypothesis

It is distressing that EPA places so much reliance on this ERF Soffritti study of methanol, despite the widespread criticism of the laboratory procedures employed by ERF and the reliability of this set of studies in particular, as detailed above. Nevertheless, EPA does apparently recognize how important addressing this *Mycoplasma pulmonis* hypothesis is to the credibility and interpretation of the ERF methanol study, because the draft IRIS assessment addresses the issue at length:

EPA staff's key arguments against the hypothesis are as follows:

I.A.1.2.2.1. Lack of confirmation: EPA staff argue in the draft assessment that although a *Mycoplasma pulmonis* infection in the ERF colony is plausible since the colony is not Specific Pathogen-Free (SPF), "the existence of an *M. pulmonis* infection in the rat colony used for the ERF methanol study has not been confirmed (Caldwell et al., 2008, 196182)."[27]

The journal article cited by EPA in the draft assessment (Caldwell et al.) was written by EPA scientists, including the principal author of the IRIS Draft Toxicological Review of methanol. The article relies on **a personal communication from ERF staff** to assert that the existence of a *M. pulmonis* infection in the rat colony is not confirmed. **There are no data presented to support the statement.**

EPA's mistake in relying on this personal communication has now been revealed by a deposition, conducted pursuant to a law suit, of Fiorella Belpoggi, the pathologist for the ERF study of MTBE. In that deposition, she acknowledged, under oath, that **antibodies for *Mycoplasma pulmonis* have been detected in routine veterinary screening of the ERF colony**

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[26] Department of Defense Detailed Comments, page 5. (Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=58015)
[27] Page 5-48 of EPA Toxicological Review of Styrene

rats. Although Ms. Belpoggi attempted to draw a distinction between the presence of antibodies and the presence of the disease, the fact is that antibodies can only be present in the blood of an animal if that animal has been exposed to the organism. Therefore, the rats of the ERF colony have been exposed to *Mycoplasma pulmonis*.[28] Any manager of an SPF animal colony would assume that the presence of antibodies to *Mycoplasma pulmonis* indicated an infection and would take steps to get rid of the disease. This confirmation of the existence of a *Mycoplasma pulmonis* infection in the ERF rat colony removes one of the major arguments EPA staff have asserted in opposition to the *Mycoplasma pulmonis* hypothesis. Further evidence of *Mycoplasma pulmonis* infection is found in the high incidence of inflammatory responses in respiratory, ovarian and lymph tissues in the methanol study. [See discussion at page 24].

The Laboratory's response to EPA's inquiries about a possible *Mycoplasma pulmonis* infection has the appearance of a simple denial rather than a full examination of the issue. EPA also failed in its duty to conduct the proper due diligence in pursuing this issue with ERF. The recent example of the National Highway Traffic Safety Administration's dealing with the Toyota acceleration issue illustrates the need for government agencies charged with protection of public health to look behind the assurances of parties involved in a controversy to establish the truth through examination of verifiable data.

Necessary Changes to the Draft Toxicological Review:

#2. Confirmation of *Mycoplasma pulmonis*: The statement at page 5-48 that *Mycoplasma pulmonis* has not been confirmed in the ERF rat colony need to be removed from the document and in their place the document should acknowledge that ERF staff have confirmed—under oath—that *Mycoplasma pulmonis* infection has occurred within the rat colony.

I.A.1.2.2.2. Effect on Dose-response: The EPA staff argues that a lung infection caused by *Mycoplasma pulmonis* cannot explain lymphoma incidences reported in the ERF methanol study involving other organ systems. They suggest that removing the lung-only lymphomas from consideration and looking only at the non-lung lymphomas does not significantly alter the dose-response reported in the study:

> "Further, 60% of the lymphoma incidences reported in the ERF methanol study involved organ systems other than the lungs (Schoeb et al., 2009, 196192). The incidence of "lung-only" lymphomas is evenly distributed across the control and dose groups of the methanol study such that removing "lung-only" lympho-immunoblastic lymphomas from consideration (i.e., using only lymphomas from other organ systems) does not significantly alter the dose-response for this lesion (see Section 5.4.3.2)."
>
> **p. 4-118, EPA Draft Toxicological Review of Methanol**

[28] June 27, 2008 deposition of Fiorella Belpoggi in lawsuit dealing with MTBE (p.259, 260)

The EPA staff's argument appears to rest on the proposition that if a *Mycoplasma pulmonis* infection and immune response were mistakenly diagnosed as a lymphoma by Ramazzini Foundation pathologists, as is suggested by the *Mycoplasma pulmonis* hypothesis, this misdiagnosis would involve only the purported lympho-immunoblastic lymphoma (LIL) that is found in the lungs. The presence of non-lung LIL, they believe, therefore argues against a *Mycoplasma pulmonis* infection in these animals. This proposition ignores the fact that infections in the lung would also affect the draining lymph nodes and other immune response organs, such as the spleen. Immune responses to infections involve both the primary site of the infection (in this case, the lung) and related lymphoid tissue, such as lymph nodes and spleen, so that reported lymphomas found in related regions outside the lung could be the result of *Mycoplasma pulmonis*.

What EPA analysts failed to notice is that all of the non-lung LIL in this study were found only in the lymph node and/or spleen with the exception of 1 male LIL and 1 female LIL[29]. Thus, rather than showing that there are "60%" of the lymphomas that drive the dose-response without the possibility of *Mycoplasma pulmonis* interference, the data show that the *Mycoplasma pulmonis* hypothesis is consistent with all but 2 of the 171 incidences of LIL reported in the study.

Thus, excluding the "lung-only lymphomas" from the analysis as suggested by EPA does nothing to rebut the hypothesis that these lesions were the result of a respiratory infection. In fact, a close look at the sites reported for LIL, as well as other sites of inflammation, such as the middle ear, ovaries and other lymph nodes, actually reinforces the hypothesis that the ERF rats in the methanol study were suffering from *Mycoplasma pulmonis*, misdiagnosed as lymphoma by ERF.

While lymphocytic or lymphoblastic lymphomas in rodents are typically found associated with the intestinal tract, LIL is reported primarily in lungs and related organs. Furthermore, in historical databases of Sprague-Dawley rats, less than 1% of control animals develop lymphomas. Similar incidences were reported in the Ramazzini methanol study for lymphocytic and lymphoblastic lymphomas. The incidences of LIL in male and female control groups, respectively, in the methanol study were 16 and 9%. It would appear that the ERF researchers have found a type of lymphoid-specific lymphoma in rats that no other researcher has seen before, which explains why they gave it a designation of LIL which no other laboratory has used.

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[29] 105 males from all dose groups (out of 400 males) were reported to have LIL. LIL was reported in the lung of 100 of these males. In 45 of the 100, LIL was reported only in the lungs. In 96 of the 105 male rats, LIL was reported in lung and draining lymph nodes. Another organ where LIL was frequently reported was the spleen. There was only 1 male where LIL was reported that did not include lung, lymph node or spleen. In females, there were a total of 66 rats reported to have LIL. LIL was reported only in the lung in 23 female rats and in lung plus lymph nodes in an additional 33 females. LIL was not reported in the lungs of 12 females, but reported in other organs. In 11 of these 12, LIL was reported in lymph nodes and or spleen. *Cruzan G. (2009). Assessment of the cancer potential of methanol. Crit Rev Toxicol, 39: 347-363. (196354)*

Normally, such a significant finding by a laboratory would prompt the researchers to welcome other outside experts to view the slides and confirm this new diagnosis. Unfortunately, to date, the ERF laboratory has refused entreaties by outside experts and even spurned requests by authoritative bodies to view their slides and review this unique diagnosis.

If the incidence of LIL is removed from the ERF methanol study, this research shows that exposure to methanol exhibits no dose response related to lymphoma. Given the serious questions as to the validity of the study's findings of LIL, there is no basis to conclude, using the Soffritti/ERF study, that methanol is "Likely to Be Carcinogenic to Humans," and even a lesser classification of "Suggestive Evidence of Carcinogenic Potential" becomes difficult to justify.

Necessary Changes to the Draft Toxicological Review:

#3. Effect on Dose-Response: EPA's arguments at page 4-118 and related pages that the examination of non-lung lesions taken alone demonstrates that the *Mycoplasma pulmonis* hypothesis is invalid should be removed from the draft document.

I.A.1.2.2.3. NIEHS Review Panel: EPA staff argue that the ERF diagnosis of LIL should be accepted based on a review of some of the aspartame slides by an independent panel convened by the National Institute of Environmental Health Sciences (NIEHS):

> "However, several ERF lymphoma diagnoses in multiple rat organ systems, including the lung, have been confirmed by an independent panel of six NIEHS pathologists (Hailey, 2004, 089842).
>
> **p. 4-118, EPA Draft Toxicological Review of Methanol**

Here EPA staff fail to point out that they are referring to a very limited review of slides from an ERF study of aspartame by a NIEHS Panel. The introduction in the Panel's Report makes this point about its limitations quite explicitly. The Report indicates that the Panel's review was not a peer review of the aspartame study nor was it conducted in accordance with normal Pathology Working Group procedures:

> "…[T]his review was not considered a "peer review" of the pathology data from this study. A peer review would necessitate a review of the study data by a second party, and selection and examination of lesions based upon that data review. Also, as a result of a "pathology peer review," PWG consensus diagnoses different from original data would result in a change of the original data. There would also have to be verification that any changes made were correctly made."

In fact, this Panel reviewed only 75 slides out of what would be expected to be thousands of total slides from the study **and these 75 slides were hand-picked by Ms. Belpoggi, ERF's lead author of the study under review**. In addition, a review of the Report reveals that the Panel examined only **three slides** from two animals out of 149 animals for which there was a LIL diagnosis in the study, and **only one of these slides was from the lung**. The NIEHS panelists did not indicate a disagreement with the diagnosis for those three slides although they did disagree with the diagnosis for approximately 50% of the slides that ERF had stated indicated head (ear) carcinomas. (See discussion at page 39.)

It should also be noted that this NIEHS review took place before serious questions had been raised about the possible misdiagnosis of these LIL lesions based on *Mycoplasma pulmonis*. The panel members also did not indicate any attempt to question the unique LIL terminology used by ERF. One of the first steps of a Pathology Working Group is to ensure the use of consistent diagnosis terminology that comports with current scientific standards. In contrast, the authors of the Schoeb et al. (2009) article, who include experts in respiratory disease diagnosis as well as the former head of the National Toxicology Program's Chemical Carcinogenesis Program, note that photomicrographs presented in ERF publications appear to represent *Mycoplasma pulmonis* infection. There is no indication that the three hand-picked slides examined by the National Institute of Environmental Health Sciences (NIEHS) were the same as those pictured in the publications or that they were representative of the more than 149 diagnosis of LIL in the aspartame study.

Necessary Changes to the Draft Toxicological Review:

#4. NIEHS Panel: The statement on page 4-118 that several ERF lymphoma diagnoses have been confirmed should be removed from the document.

I.A.1.2.3. How Should the Mycoplasma Pulmonis Controversy Be Resolved?

The European Food Safety Authority, the U.S. Food and Drug Administration, the authors of the Schoeb journal article, the Department of Defense, and a TERA peer reviewer have all concluded that the way to resolve this controversy is to examine the slides from one or more of these related ERF studies.

The Schoeb authors, for example, suggest specific steps that they believe should be taken to determine the validity of the ERF findings:

> "*Questions regarding the role of M. pulmonis disease and the histopathologic diagnosis of the lung lesions in the studies in question could be settled by two straightforward measures. First, the histologic slides from the studies in question could be subjected to full and independent review by a qualified Pathology Working Group. Examination of respiratory tract sections in addition to those of lung*

would be helpful, because suppurative respiratory mucosal inflammation and epithelial hyperplasia and metaplasia would be present in M. pulmonis disease, but would not be characteristic of lymphoma. Second, tissues from the studies in question, and samples from animals in studies currently in progress, could be tested for M. pulmonis. Several methods for detection of M. pulmonis infection in rodents exist…."[30]

"Several issues that have been raised by detractors of the ERF can admittedly be resolved by the ERF-furnished supplementary data tables that provide details beyond what a typical journal article can accommodate. Unfortunately, the one issue that cast the most doubt as to the study's validity (M. pulmonis infection) is the most difficult (if not impossible) to resolve. Of interest would be whether USEPA or ERF scientists have attempted to apply state-of-the-science techniques to paraffin embedded or frozen tissues to once and for all lay the issue to rest. If not, why not?"[31]

We believe that EPA must squarely confront this issue of the interpretation of the lymphomas in the Soffritti study:

The Agency must conclude <u>either</u> that:

→ **The Ramazzini Foundation has observed a type of lymphoma (LIL) that is:**

- **not seen in any other laboratory,**
- **has a completely different spectrum of organs affected than any known lymphoma,**
- **occurs at a much higher frequency than any other lymphoma in rats, and has a unique cellular morphology, and**
- **occurs in the presence of inflammation in lymph nodes, middle ear, and female reproductive organs that have been identified by experts in respiratory disease as characteristic of *Mycoplasma pulmonis*,**

[30] Schoeb (2009)
[31] TERA, at 35 (Reviewer 4)

or that

→ **The observations reported as LIL by ERF in the methanol study likely represent accumulations of lymphocytes and plasma cells characteristic of a *Mycoplasma pulmonis* infection and have been so identified in photomicrographs by experts in respiratory disease, and**

→ **EPA cannot legitimately use the Soffritti ERF study to support a classification of "Likely to Be Carcinogenic to Humans."**

To acknowledge many of these weaknesses in the ERF study, as EPA does, AND STILL use the study as the principal basis for classifying methanol as "Likely to Be Carcinogenic to Humans" represents a clear failing of the Agency to meet its obligations to employ the best available science in its hazard assessments.

Given the strong supporting evidence detailed above that support the *Mycoplasma pulmonis* hypothesis, the Methanol Institute, on two occasions has asked EPA to conduct a Pathology Working Group (PWG) peer review of the ERF methanol slides. The Institute's 2006 request was denied on the basis that the Agency saw no need for such a PWG review of the slides. Based on the statements made in the subsequently published Caldwell et al. journal article, we can now speculate that the EPA staff had been falsely assured by ERF laboratory personnel that there was no basis for concern about a respiratory infection among the rat colony. It is unfortunate that the EPA staff trusted the representations of the ERF staff who would be naturally inclined to defend their published conclusions and did not investigate further at the time of the Institute's initial request.

Any EPA decision not to pursue an independent PWG of the methanol slides has implications far beyond methanol. The diagnosis of lymphoma (LIL) was made also in the ERF studies of **ethanol, MTBE, and formaldehyde**, chemicals for which EPA has ongoing re-assessments under the IRIS program (as well as the ETBE draft assessment that has already undergone external peer review.). Of particular importance, perhaps, in the public's mind would be the assessment of **ethanol**, a chemical which has figured prominently in public policy decisions in recent years.

On May 22, 2009, the Methanol Institute for the second time requested that EPA conduct a Pathology Working Group (PWG) of the ERF methanol slides, this time in the form of a written petition to the Administrator of EPA. Although the Institute has had several conversations and direct meetings with key EPA officials concerning this petition, no action has been taken by the EPA on this petition, to our knowledge.

We understand that the National Toxicology Program (NTP) may be sending a team to the ERF Laboratory to review their procedures and to examine some of the slides from the methanol study. According to Dr. John Bucher, NTP, this will not be a Pathology Working Group but rather a "data review" as part of a "broader effort to help the lab gain wider acceptance of its

data."[32] The Methanol Institute has asked NTP to include outside pathologists who have considerable expertise in *Mycoplasma pulmonis*, but so far they have not agreed to this. Given the fact that toxicology testing conducted by NTP for the last 30+ years has involved the use of Specific Pathogen Free (SPF) rats and mice, it is unlikely that NTP pathologists have much experience examining slides from animals with *Mycoplasma pulmonis*. Therefore, it is essential that experts in *Mycoplasma pulmonis* infection be included on the pathology review. Without such expertise and a clear protocol calling for a rigorous review of the LIL diagnosis, any NTP report on the ERF methanol study would lack credibility.

It is our view that the *Mycoplasma pulmonis* hypothesis is well supported even by the limited facts that are available about the ERF studies in question. Because the ERF study of methanol is so central to EPA's current cancer classification proposal for methanol, **we recommend that the Federal government conduct an independent PWG and that EPA then return to the Science Advisory Board panel for a review of the results and any resulting assessment of the toxicology of methanol**. If EPA chooses not to pursue an independent PWG, then EPA should not rely on the LIL results from the Soffritti study to support a cancer classification of methanol under the IRIS program.

Necessary Changes to the Draft Toxicological Review:

#5. *Mycoplasma pulmonis* Hypothesis*:* Given the current serious uncertainties that surround the accuracy of the Soffritti/ERF findings of lymphoma, the Weight of the Evidence section should be revised to place little or no scientific weight on that study's findings.

I.A.1.3. Analysis of Other Chemicals Tested at ERF

I.A.1.3.1. "Limited" Number of ERF Studies with Hemolymphoreticular Tumors

In Section 4.6.5.2. of the Draft Toxicological Review, EPA points out what it believes is a pattern of lymphoma responses reported in ERF bioassays of compounds related to methanol, including an analogue (ethanol), a precursor (aspartame), a metabolite (formaldehyde) and another chemical metabolized to formaldehyde (methyl tertiary butyl ether):

> "The ERF or the European Foundation of Oncology and Environmental Sciences have conducted nearly 400 experimental bioassays on over 200 compounds/agents, using some 148,000 animals over nearly 4 decades. Of the over 200 compounds tested by ERF[55], 8 have been associated with an increased incidence of hemolymphoreticular tumors in Sprague-Dawley rats, suggesting that it may be a rare and potentially species/strain-specific finding. These

[32] Risk Policy Report, March 2, 2010.

eight chemicals are: methanol, formaldehyde, aspartame, MTBE, DIPE, TAME, mancozeb, and toluene. Methanol, formaldehyde, aspartame, and MTBE share a common metabolite, formaldehyde, and DIPE, TAME, methanol and MTBE are all gasoline-oxygenate additives (Caldwell et al., 2008, 196182)."

Footnote 55: While ERF has tested over 200 chemicals in 398 long-term ERF bioassays, only 112 of their bioassays have been published to date (Caldwell et al., 2008, 196182). The extent to which the unpublished studies are documented varies.

p. 4-87, EPA Draft Toxicological Review of Methanol

First, it is not appropriate to combine leukemias, lymphomas, and histiosarcomas for statistical analysis or evaluation of carcinogenic potential as EPA's statement does in this instance and as EPA indicated should not be done for sound scientific reasons in other sections of the draft toxicological review. Tumor types should be considered separately because they have different cells of origin, organ specificity and mode of action. NTP[16] have developed guidelines for combining tumor types and EPA should follow them.

More importantly, EPA's assertion that there is limited number of ERF studies with an increased incidence of hemolymphoreticular (LHR) tumors [implying that it must therefore be a correct diagnosis] is based on representations by the laboratory and cannot be independently verified. Its validity is challenged, as noted below, by, an increase in LHR tumors in the ethanol study that the laboratory failed to mention. In addition EPA independently concluded that there were increases in LHR tumors in the ETBE study although they were not reported by ERF. We note that another commenter has indicated that increased LHR has been reported in an additional 5 ERF publications of 31 examined.

There is no database of studies and results (such as the NTP study database) for research conducted by ERF. Except for a few studies, reporting of ERF studies is limited to non-peer reviewed journals only and the occasional press conference. The lack of reporting by the ERF makes it virtually impossible to replicate, and thereby verify, their studies.

The ethanol study[33] is a good example of identifying how many ERF studies actually found LHR tumors. Although the manuscript does not separate lymphomas, the incidence of combined leukemia and lymphomas suggests that there were increased lymphomas in the ethanol study. For female breeders exposed to 10% ethanol in the drinking water, the incidence of LHR tumors was 41.8% compared to 15.5% in the breeder controls. The incidences in male breeders were 35.5% and 31.8% for exposed and controls, respectively. In the male offspring exposed to 10% ethanol in the drinking water the incidence of LHR tumors was 30% versus 20% in the control. In female offspring, the incidences were 20.5% and 20.0% for exposed and controls, respectively. Thus, in contrast to the statements in the EPA methanol document, the ERF ethanol study also had increased LHR cancers and must be counted as one of the ERF studies with LHR tumors.

[33] Soffritti (2002) (091004)

The identification of additional studies with increases in LHR tumors in ERF studies raises the possibility that there is a problem of respiratory disease in the laboratory's rat colony as hypothesized and discussed earlier in these comments at page 16. However, because of the publication policies of ERF and the frequent refusal by the laboratory to reveal further data about their studies, it is impossible to determine the total number of studies in which LHR tumors were diagnosed.

Therefore EPA's statement that the occurrence of hemolymphoreticular tumors in Sprague-Dawley rats in only 8 of the 200 compounds tested by ERF may represent a rare and potentially species/strain-specific finding [e.g. LIL] is based on a false assumption that there were only 8 such studies. The so-called LIL, or alternatively, the *Mycoplasma pulmonis* infection may not be so rare among the Laboratory's studies after all.

Necessary Changes to the Draft Toxicological Review:

#6. Number of Studies with LHR tumors: The statement at page 4-87 and related pages that there are only a few studies with increased incidence of hemolymphoreticular tumors is unsupportable, and the suggestion that these tumors only occur with chemicals linked in some way with methanol should be deleted from the draft toxicological review of methanol.

I.A.1.3.2. Do Formaldehyde, MTBE, Methanol, and Aspartame Have Similar Effects?

EPA addresses in more detail four of chemicals with an increased incidence of LHR tumors in ERF studies in an attempt to make a case for the existence of a common link among the chemicals in terms of their effects and their mode of action. All of the four chemicals discussed have a link through their metabolism. ERF suggested that this common metabolic link could represent a common mode of action in which the toxic entity would be formaldehyde. EPA has emphasized this possible link between methanol and formaldehyde in its draft assessment.

Many foods contain methanol (e.g., orange juice, alcoholic beverages), and both methanol and formaldehyde are synthesized from a variety of foods. Therefore, all cells contain naturally occurring methanol and formaldehyde. Increased LHR tumors from formaldehyde would be expected only if formaldehyde increased LHR cancers and the dose of aspartame, MTBE or methanol increased the cellular level of formaldehyde in bone marrow or other lymphoid stem cell populations. No attempt has been made in the above comparison to account for the rate of metabolism to formaldehyde or its metabolism to formate.

It would be better to compare the incidence of lympho-immunoblastic lymphoma (LIL) in each study to the increase (if any) of the intracellular level of formaldehyde caused by the external doses of the respective chemicals. EPA's assessment itself begins with the statement:

> "*The primary purpose of this assessment is for the determination of noncancer and cancer risk associated with exposures that* ***increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde****) above prevailing, endogenous levels."* [emphasis added]

Unfortunately, for some of the ERF studies the incidence of LIL is not reported separately from total LHR tumors and the EPA PBPK model is not capable of making that comparison. (The shortcomings of the EPA PBPK model are discussed in these comments at page 53.)

To test this theory of common effects among the four chemicals, one can examine each of these four ERF studies to determine whether they individually show similar increases in tumors at equivalent dose levels of formaldehyde. The only comparison that can be made across all studies from ERF is total LHR tumors, although that combination is not appropriate for cancer assessment. Equivalent dose levels of formaldehyde can be determined for each chemical by identification of that portion of the molecular weight of the chemical which metabolizes to formaldehyde. Table 2 below shows formaldehyde equivalent doses and whether there was a statistically significantly increase in LHR tumors.

Table 2. Formaldehyde Equivalent Doses of Aspartame, Methanol, MTBE, and Formaldehyde in ERF Studies and Reported Significant Increases in LHR.

Chemical	Formaldehyde	Methanol	MTBE	Aspartame #1	Aspartame #2
Formaldehyde Equivalent[34]	100%	10%	36%	10%	10%
	Males (mg/kg)				
				2 ns	
	5 ns				
	10 *			10 ns	
					20 ns
	50 *	53 ns	52 ns	50 ns	
	100 *				100 *
	150 *				
			208 ns	250 ns	
		500 ns		500 ns	
		1780 ns			
	Females (mg/kg)				
				2 *	
	5 ns				
	10 ns			10 *	
					20 ns
	50 ns	66 ns	52 *	50 *	
	100 *				100 *
	150 *				
			208 *	250 *	
		624 ns		500 *	
		2177 *			

[34] The "Formaldehyde Equivalent External Dose" was calculated as follows: Aspartame is known to be metabolized to methanol. The methanol dose from this metabolism is 10% of the aspartame dose. So, if one divides the aspartame dose by 10, one gets the equivalent methanol dose. Methanol is somewhat slowly metabolized to formaldehyde; the molecular weights of methanol and formaldehyde are nearly identical so an external dose of methanol is equivalent to the same external dose of formaldehyde. MTBE is metabolized to formaldehyde (and t-butanol). The formaldehyde equivalent dose is 36% of an MTBE dose. Use of external equivalent doses ignores the dynamic of the normal formation and metabolism of formaldehyde, its presence in all animal cells, and whether external doses of any of these compounds increases the intracellular level of formaldehyde.

Given that these chemicals are metabolized relatively slowly to formaldehyde and that formaldehyde is rapidly metabolized to formate, the comparison in Table 2 above most certainly overstates any relationship to formaldehyde. In males, increased LHR tumors were reported at formaldehyde doses of 10, 50, 100 and 150 mg/kg/day. Only in the second aspartame study was there an increase in LHR tumors in males exposed to aspartame, MTBE or methanol. In females exposed to formaldehyde, LHR tumors were increased at only 100 and 150 mg/kg/day. LHR tumors were reported to be increased at lower formaldehyde-equivalent doses in females exposed to MTBE and aspartame. No increase in LHR tumors was reported in females exposed to methanol at doses 600-fold higher than the lowest increase reported for formaldehyde.

In the section of these comments dealing with mode of action, we present the percentage increase in total LHR vs. the formaldehyde-equivalent dose, using the EPA PBPK model, for the methanol and formaldehyde ERF studies. This comparison clearly refutes EPA's claim of linearity of response between methanol and formaldehyde [See discussion at page 53].

The draft document devotes a lot of space discussing and highlighting the lymphomas and leukemias in the ERF studies of formaldehyde but does not separate the lymphomas from the leukemias within the broad category of LHR tumors. Until recently, we believed that no one outside of the ERF laboratory had information about the breakdown of the various LHR tumors in the ERF formaldehyde study that would allow one to study the various types of tumors and draw comparisons between formaldehyde and methanol in the two ERF studies. But apparently, EPA has been in possession of the breakdown of LHR types in the formaldehyde study since last summer (2009) in the form of NTP data files. This was revealed when Dr. DeVoney of EPA presented a poster at the Society of Toxicology Meeting on March 8, 2010 (abstract #520). In this short time since seeing that poster, we have not been able to obtain those ERF formaldehyde files for analysis. However, the data presented by Dr. DeVoney indicates two things of interest about the ERF formaldehyde study:

1. The incidences of LHR presented in the preliminary (1989) and final (2002) ERF publications do not match those now apparently in the NTP files.

2. The NTP data files apparently indicate that exposure to formaldehyde resulted in increased lymphatic leukemia, lymphoblastic lymphoma as well as LIL. Since this pattern does not match that reported for methanol (only LIL), the formaldehyde data do not support the hypothesis that methanol has the same tumor effect as formaldehyde.

It is clear from all these data that there is NO consistent pattern that would suggest that LHR tumor responses of methanol and these other chemicals are related to metabolism to formaldehyde.

Necessary Changes to the Draft Toxicological Review:

#7. No Consistent Pattern Among ERF Studies: EPA should either remove the discussion of these other ERF studies on aspartame, MTBE, ethanol, and formaldehyde or show that there is no support within the data, but, rather, only speculation, for a consistent pattern of LHR among these chemicals.

I.A.2. Lymphoma in Mice Exposed to Methanol (Apaja)

A graduate thesis by Apaja (1980) describes studies performed to evaluate the toxicity and carcinogenicity of malonaldehyde, which is suspected of being carcinogenic because it produces reactive-oxygen species. (This thesis is not referenced on ToxLine or MedLine and is not in the peer-reviewed literature.) Malonaldehdye is not stable and is available commercially as malonaldehyde bis (dimethylacetal). For use in the drinking water study, Apaja hydrolyzed the acetal to malonaldehyde and methanol. He exposed three groups to the malonaldehdye/methanol mixture and three groups to methanol only. It is important to note that there were **no** unexposed controls in this study for comparison. **The author concluded that the effects seen were all within the normal range.**

In its analysis of the Apaja study, EPA inexplicably chose to ignore the animals exposed to methanol with malonaldehyde and focused selective on those groups exposed to methanol alone:

> "As is described in Section 4.2.1.3 (Table 4-3), Apaja (1980, 191208) found an increase in malignant lymphomas in mid-dose ($p = 0.06$) and high-dose ($p < 0.05$) female and mid-dose ($p < 0.05$) male Eppley Swiss Webster mice exposed for life via drinking water. The lack of a concurrent unexposed control data limit the confidence that can be placed on the relevance of the increased lymphoma responses noted in this study. However, while controls were not concurrent, they were from proximate (within 3 years) generations of the same mouse colony, lymphomas were evaluated via the same classification criteria and, in the case of the Hinderer (1979) controls, the histopathological analysis was performed by the same author (Apaja, 1980, 191208). In addition, this is a late developing tumor, as noted by the author, suggesting the possibility of a higher tumor response in the females of all exposure groups had their survival not been significantly lower than untreated historical controls."
>
> **p. 4-113-114, EPA Draft Toxicological Review of Methanol**

EPA's interpretation of this study is not sound. It is inappropriate to ignore the totality of the results from the study in reaching valid conclusions. EPA's Benchmark Dose Modeling

procedures indicate that all data in a study should be included in the analysis. There is no reason to eliminate certain groups from consideration unless there are data to indicate the groups are different. All the groups exposed to malonaldehyde in the Apaja study were also exposed to methanol. Therefore, these groups exposed to methanol should be included in the analysis of the study (unless there is some reason to suspect that malonaldehyde is tumor-protective). EPA emphasizes the use of all animals, for example, in its benchmark dose analysis and should follow the same procedure here.

Table 3 below summarizes the results from all the animals:

Table 3: Incidence of Lymphoma and Pneumonia in Eppley Swiss Mice Exposed to Methanol (Apaja)

Group	Malonaldehyde dose	Methanol dose	% pneumonia	% lymphoma
Males				
4	0	82.7	8	17
1	28.0	49.8	24	0
5	0	43.5	12	24
2	17.8	31.5	17	12
6	0	24.6	8	4
3	10.3	18.2	17	25
Females				
4	0	84.5	24	40
1	23.5	41.8	20	4
5	0	40.8	28	36
2	14.8	26.2	12	28
6	0	22.6	28	16
3	8.6	15.3	12	36

Based on an evaluation of all 6 groups exposed to methanol, there was no dose-response relationship for lymphomas and methanol exposure. Further, the thesis acknowledges that "In Groups 4 and 5 of the feeding study among females and males, respectively, there was an increased ($p < 0.05$ in both) occurrence of malignant lymphomas (40.0% and 24.0% of the effective animals, respectively) compared to the historical data of untreated controls (Table 9) according to the author himself. The percentages are within normal range in regard to occurrence of malignant lymphomas in Eppley Swiss mice."

Therefore, an appropriate conclusion for EPA to reach in its Toxicological Review is: **When all the groups exposed to methanol are considered, there is no increase in lymphoma from methanol in this study. Three of the four members of the TERA peer review panel who**

reviewed the Apaja study stated that the study should not be used for cancer classification or derivation of a cancer slope factor.[35]

We also note that the Apaja study was performed in the late 1970s with mice from a university breeding colony, prior to the development of Specific Pathogen Free animals. There is also a high level of pneumonia reported in this study (8-28%). Therefore, it is also possible that at least some of the lymphomas reported were in fact immune responses to *Mycoplasma pulmonis* that were incorrectly diagnosed by the graduate student author. We further note the comments of one of the TERA reviewers: "Tumor nomenclature is poorly defined. Histological descriptions of tumor types are lacking. Apaja uses a variety of terms to describe the neoplasia observed in the study."[36]

Necessary Changes to the Draft Toxicological Review:

#8. Apaja: Either the Apaja study should not be included in the Review or the interpretation of the Apaja study should show that it does not support a relationship between methanol exposure and lymphoma.

I.A.3. Conclusions regarding Lymphoma from Methanol Exposure

The totality of the evidence does not support a conclusion that methanol increases the incidence of lymphoma in animals. The Apaja study does not provide evidence of increased lymphoma in mice if all dose groups are included in the evaluation and, what is more, the author claimed the incidences were within the expected range, so these data should not be used to suggest an increase in cancer and should be stricken from the report.

The reported increase in LIL in the ERF methanol study is most likely an immune response to *Mycoplasma pulmonis* infection and not a lymphoma. To believe otherwise requires acceptance that this lymphoma is unique to the ERF, occurs at a much higher frequency than any other lymphoma, has a very different organ distribution than any other lymphoma, has cellular morphology different from all other lymphomas and is accompanied by non-neoplastic lesions characteristic of *Mycoplasma pulmonis* infection. Since 2006, the Methanol Institute has repeatedly suggested that EPA obtain definitive information about the occurrence of *Mycoplasma pulmonis* in the ERF test animals in the methanol study through a Pathology Working Group review. To date, EPA has chosen not to do so. **Until more definitive evidence is obtained and the very credible *Mycoplasma pulmonis* hypothesis is disproved, EPA cannot legitimately use the ERF methanol study—and the SAB should not approve its use—to support a classification of methanol as "Likely to Be Carcinogenic to Humans."**

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[35] TERA

[36] TERA at 46 (Reviewer 2)

I.B. Liver Carcinoma

EPA cites findings from the ERF methanol study suggesting a low incidence of hepatocellular carcinomas in the livers of male rats and compares the incidences to a "historical control" comprised of data from five studies from the ERF laboratory. EPA states:

> "As is described in Section 4.2.1.3 (Table 4-2), Soffritti et al. (2002, 091004) reported a number of tumors in methanol-exposed Sprague-Dawley rats. EPA reanalyzed the tumor findings from this study using individual animal pathology available from the ERF website (see Section 5.4.1.1). As indicated above, the increase in a relatively rare hepatocellular carcinoma in males compared to historical controls (Fisher's exact $p < 0.05$ for all doses and $p < 0.01$ for the high-dose group) is potentially related to methanol dosing."
>
> **p. 4-113, EPA Draft Toxicological Review of Methanol**

The incidences in the ERF methanol study were 0, 2, 2, and 3 for the males at 0, 500, 5000, and 20000 ppm, respectively. The comparison of these findings to such a small sample of five studies should not constitute the use of an "historical control." Historical controls should be based on a larger number of studies conducted at the same laboratory at approximately the same time. The four other studies chosen were conducted at different times and did not include all studies conducted at the time of the methanol study. Furthermore, hepatocellular carcinomas that are chemically induced are usually accompanied by increases in hepatocellular adenomas, and, in the ERF methanol study, there were no adenomas reported. None of the TERA reviewers of this study suggested increased liver carcinoma as a methanol-induced lesion.[37] A sound interpretation of the data, we believe, is that **there is no evidence of increased liver tumors in rats from methanol exposure**.

Necessary Changes to the Draft Toxicological Review:

#9. Liver Carcinomas: The paragraph on page 4-113 related to liver carcinomas should be removed from the toxicological review.

[37] TERA [See discussion at page 11 of these comments]

I.C. Ear Duct Carcinomas

EPA cites increased "ear duct carcinomas" in males and females in the ERF methanol study:

> "NTP pathologists interpreted a majority of such head pathologies, including in the ear duct, as being hyperplastic in nature, not carcinogenic (EFSA, 2006, 196098; Hailey, 2004, 089842)."
>
> "A significant increase in the incidence of ear duct carcinoma was also reported by Soffritti et al. (2002, 091004). However, the high incidence for this tumor in controls of the Soffritti et al. (2002, 091004) study relative to other studies of Sprague-Dawley rats (Cruzan, 2009, 196354) and the results of an NTP evaluation of pathology slides from another bioassay (EFSA, 2006, 196098; Hailey, 2004, 089842) raise questions about the ear duct pathological determinations of Soffritti et al. (2002, 091004)."
>
> **p. 4-19, 113 EPA Draft Toxicological Review of Methanol**

> "For increases in 2 other tumor types (ear duct and head/oral cavity tumors) reported in the ERF methanol study (Table 4-2), an independent review of the 75 pathology slides from the ERF aspartame study has suggested differences in interpretation. After reviewing these slides, the NTP PWG noted that "about half" of hyperplastic and neoplastic lesions in the ear duct or oral cavity were more severely classified by ERF study pathologists, compared with diagnosis from the PWG (EFSA, 2006, 196098). Though a similar review has not been conducted of the Soffritti et al. (2002, 091004) ERF methanol bioassay results, there is uncertainty regarding the ERF interpretation of these lesions. For this reason, these lesions were not considered in the derivation of the oral CSF."
>
> **p. 5-50,51, EPA Draft Toxicological Review of Methanol**

EPA's treatment of ear duct carcinomas in the Toxicological Review can be confusing at best. In the first quote, included above, taken from the overall weight of the evidence section of the Review, EPA indicates that questions have been raised about the validity of these findings from the ERF study, but does not explicitly discount them. However, the inclusion of this mention of ear duct carcinomas in the overall weight of evidence discussion would certainly indicate some importance of these findings. In contrast, in the section of the report dealing with Points of

Departure, EPA's statements, shown in the second quotation above, indicate that these findings are so unreliable that they were not considered in the derivation of the oral CSF.

We agree with the second characterization of the study. If the findings are so unreliable to be of no value to the derivation of the oral CSF, then they should not be included in the discussion of the overall weight of evidence for fear of misleading readers. Furthermore, these ear duct lesions may be further proof of the *Mycoplasma* infection hypothesis, as indicated by one of the TERA Peer reviewers:

> *"One clinical effect associated with Mycoplasma infection in rats is chronic otitis interna. Whether this could contribute to ear duct carcinomas is of possible concern. For example, chronic otitis media can lead to bony proliferation and other changes that may confound a diagnosis of ear duct carcinoma."*[38]
>
> *And "The tumor type seen in the head is primarily defined as an osteosarcoma – it's curious to note that osteosarcoma incidence at other sites did not appear to be affected by methanol treatment."*[39]

Necessary Changes to the Draft Toxicological Review:

#10. Ear Duct Carcinomas: The paragraph on ear duct carcinomas on page 4-19 needs to state clearly that the ear duct carcinomas are not considered reliable and are not included in the weight of evidence. The potential relationship to *Mycoplasma pulmonis* infection should also be pointed out.

I.D. Lung Tumors in Male Rats by Inhalation (NEDO)

The draft document reports on lung tumors in male rats from the NEDO study as follows:

> "The lung response of the male rats as shown in Table 4-5 **suggests** [emphasis added] a proliferative change in cells of the alveolar epithelium involving a progression towards adenoma and adenocarcinoma that appears to be more pronounced with increasing methanol exposure and considerably elevated over historical controls."
>
> [In the overall weight of evidence section of the review] "The available chronic inhalation studies of methanol (NEDO, 2008,

[38] TERA, at 24 (Reviewer 2)
[39] TERA, at 23 (Reviewer 2)

196315; NEDO, 2008, 196316) reported slight but statistically significant tumor responses in F344 rats at 24 months."

p. 4-34, 111 EPA Draft Toxicological Review of Methanol

The NEDO study provides, at best, only a very weak indication of lung tumors. All but one of the 7 lung tumors were benign adenomas; one high exposure rat had a carcinoma. While this incidence is statistically significant when compared to the overall NTP historical controls, it is also appropriate to compare this incidence to more current controls in F344 rats in NTP studies conducted at about the same time as the NEDO study. This comparison shows that in 12 of such NTP studies (out of 50) one male control rat had a carcinoma and in 9 of the studies, 2 male control rats had carcinomas. Furthermore, NEDO indicates that in another study conducted at the same laboratory at the same time, the male controls had a 14% incidence of lung adenomas[40]. This compares to the 13% found in the methanol study. Thus, neither the incidence of adenomas, carcinomas, nor adenoma plus carcinoma combined is outside the historical control range.

There was a slight but statistically significant increase in lung tumors in male rats; however, the response was weak, within the range of historical controls, and involved nearly all benign tumors, except for one. No such increase was found in female rats. These data should not be given weight in the overall assessment of methanol.

Necessary Changes to the Draft Toxicological Review:

#11. NEDO Lung Tumors: The sentences at page 4-111 and related pages on the NEDO lung tumor data should be dropped from the weight of the evidence section of the Toxicological Review.

I.E. Adrenal Pheochromocytomas in Female Rats by Inhalation (NEDO)

"As is described in Section 4.2.2.3, individual tumor responses from the rat study were not significantly increased over concurrent controls, but the response in the high-dose (1,000 ppm) group for pulmonary adenomas/adenocarcinomas in male rats was increased over concurrent controls (Fisher's exact $p < 0.05$), and the dose-response for both pulmonary adenomas/adenocarcinomas in male rats and pheochromocytomas in female rats represent increasing trends (Cochran-Armitage trend test $p < 05$). Further, the high-dose responses for both of these tumor types were elevated ($p < 0.05$) over historical control incidences within their respective sex and strain. As can be seen from Table 4-5, the severity and combined incidence of effects reported in the alveolar

[40] NEDO, 2008, at 59.(196316)

epithelium of male rat lungs (epithethial swelling, adenomatosis, pulmonary adenoma and pulmonary adenocarcinoma) and the adrenal glands of female rats (hyperplasia and pheochromocytoma) were increased over controls and lower exposure groups. This pathology and the appearance of a rare adenocarcinoma in the high-dose group are suggestive of a progressive effect associated with methanol exposure. The increased pheochromocytoma response in female rats is considered to be potentially treatment related because this is a historically rare tumor type for female F344 rats (Haseman et al., 1998, 094054; NTP, 1999, 196291; NTP, 2007, 196299)[65] and because, when viewed in conjunction with the increased medullary hyperplasia observed in the mid-exposure (100 ppm) group females, it is indicative of a proliferative change with increasing methanol exposure."

"[S]everity of the pheochromocytoma response reported by NEDO (2008) is uncertain, potentially ranging from mischaracterized hyperplasia to highly proliferative and potentially metastatic malignancies. 196316 Finally, the NEDO study was a two-year study, and these lesions, which include diffuse hyperplasia, nodular hyperplasia, and pheochromocytoma, progress with age. Thus, it is possible that a life-span study would have detected a more severe carcinogenic response (e.g., progression of the mid-dose group hyperplastic responses as reported in Table 4-5)."

p. 4-114; 5-51, EPA Draft Toxicological Review of Methanol

In the analysis of the Soffritti et al. oral methanol study, the draft asserts that the concurrent control is the most important comparison, yet in its analysis of this NEDO study, the draft asserts that comparison with historical controls is more important. This approach gives the inappropriate appearance that EPA would choose to use whichever control provides any evidence of cancer by comparison. EPA should offer some sound scientific reason for ignoring the concurrent controls and using historical controls as the determining comparison with the test results instead.

Based on comparison to the concurrent control, there was no increase in pheochromocytomas. Furthermore, while the incidence may be different from the overall pre-1995 NTP database of historical controls, we checked the incidence in controls in NTP studies TR-255-327, which included 50 studies in F344 rats conducted about the same time as the NEDO study. In eight of the 50 studies, the female control incidence of pheochromocytomas was 12% or greater, while in 4 of those the incidence was 16% or greater as compared to the results in this NEDO study of 14% in high dose females. Furthermore, a significant increase in the trend with dose seems to have no real meaning given the responses which were 2/50, 3/52, 2/49 for 0, 500 and 5000 mg/l female groups, and 7/51 at 20,000. The only group that is possibly different from control is the high dose group; biologically, that does not make a trend.

Pheochromocytomas occur to a much greater extent in males than in females, probably related to differing hormonal influences in the adrenals of males and females; we note that the incidence in the high-dose males is below the historical control incidence for F344 rats. Based on the above

mentioned 50 studies, the average control incidence of pheochromocytomas in male F344 rats was 24% compared to 8% of the high-dose males in the NEDO study. Thus, if methanol caused increased pheochromocytomas in females, it was tumor **protective** in males.

Finally, EPA addresses the issue of severity of the pheochromocytoma response. Severity of pheochromocytomas was not discussed in NEDO study. It is purely speculation by EPA that the incidence could have been worse if the study was conducted for a "whole life"; it is equally possible that more controls would have developed pheochromocytomas. Furthermore, there would be no NTP historical database with which to compare the results, and there would be no basis for concluding there was a treatment effect.

EPA contracted for a peer review of the NEDO studies by the Eastern Research Group (ERG). (See discussion at page 8.) Three of the five reviewers agreed with the NEDO study conclusion that there was no conclusive evidence of carcinogenicity in the rat study. Of the other two reviewers, one concluded that histopathology should have been performed on all animals and different statistical analysis might have provided positive results, while the other reviewer concluded that the pheochromocytomas could not be **conclusively** dismissed.

The ERG Peer Review Panel members' conclusions included the following:

> "The methanol exposure paradigm used in this study (very high dose and round the clock), coupled with good survival and the minimal questionable high-dose finding suggests to me that such long-term chronic exposure is not a high cancer risk, and that acute toxicity and further ramifications of that in humans would be much more problematic."[41]
>
> "*From the test results in rats and mice, exposure to methanol at 1,000 ppm or lower does not cause cancer.*" I agree that the tumor data are not robust enough to support methanol as a carcinogen." [42]
>
> "The specific tumor data on lung adenomas and pheochromocytomas are likely to be inappropriate for cancer slope factor derivation as the relation of these tumors to methanol exposure is not clearly established and whether excesses even existed at all is debatable."[43]

Conclusion on Pheochromocytomas: The NEDO inhalation study of methanol does not provide evidence of increased pheochromocytomas.

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[41] Eastern Research Group, at 42 (Dr. Judy Buelke-Sam)
[42] Id. at 41 (Dr. David Warren)
[43] Id. at 42 (Dr. David Allen)

Necessary Changes to the Draft Toxicological Review:

#12. Pheochromocytomas: The discussion of pheochromocytomas should be deleted from the section of the paper dealing with the overall weight or synthesis of the evidence (Section 4.9.2).

I.F. Other Potential Tumor Cites from the NEDO Study

EPA suggests that the NEDO study contains evidence of additional tumors caused by methanol:

> "Other examples of tumor formation that were increased in high-concentration animals versus controls included an increased incidence of pituitary adenomas in high-concentration males (17/52 compared to 12/52 controls), hyperplastic change in the testis in high-concentration males (10/52 compared to 4/52 controls), and chromaffinoma (pheochromocytomas)[37] in the adrenals of high-concentration females (7/52 compared to 2/52 controls). Individually, these changes did not achieve statistical significance, and in general, the authors concluded that few if any of the observed changes were effects of methanol."
>
> **p. 4-32, EPA Draft Toxicological Review of Methanol**

> "However, the fact that the study duration was limited to 18 months rather than the traditional 2-year bioassay makes it difficult to draw a definitive conclusion, particularly regarding pulmonary adenomas…"
>
> **p. 4-30, EPA Draft Toxicological Review of Methanol**

This document ignores sampling theory and statistical principles. The only way to know for sure if a treatment causes an effect is to examine all cases both without the treatment and with the treatment while keeping all other parameters the same. It would be impossible to observe all F344 rats until 110 weeks of age to determine the true rate of all cancers, as well as treat those same F344 rats with methanol from age 6 weeks to 110 weeks. Thus, we can never know the true rate in unexposed rats that have been exposed to methanol. To compensate for this, procedures have been developed to estimate the likelihood of two samples being different. The basic principle is that groups are not different (no matter how large the numerical difference) unless they are more than 95% likely to be different. The draft toxicological review of methanol lists "examples of tumor formation that were increased" from the NEDO studies, and then acknowledges that they were not really increased; i.e., **based on statistical analysis, there is no difference between the control and high-dose groups for the hyperplasias and tumors cited by EPA on page 4-32. EPA appears to imply that results which are not statistically significant individually are somehow made significant by the fact that there is more than**

one such result collectively. This approach is not scientifically sound and, if followed by the scientific community, would result in numerous statistically non-significant effects being elevated to be matters of concern.

Secondly, "hyperplastic changes in the testis" are not tumors. They may be considered pre-neoplastic changes, but they are not tumors. What is really misleading about EPA statement on the testes is the unfounded implication that methanol increases testicular tumors. That is NOT the case: 44 of 52 male control rats had Leydig cell tumors, while 43 of 52 high exposure males had such tumors. **Thus, there is no increase in testicular tumors and EPA should not imply that there might be by discussing hyperplastic changes in the testis.**

In the second quotation above, EPA finds another inappropriate way to suggest there may be tumors caused by methanol. EPA suggests that the NEDO mouse study was inadequate to identify pulmonary adenomas because it was only 18 months in duration. However, EPA's own guidelines for carcinogenicity studies indicate that 18-month mouse studies are satisfactory. Since the EPA guidelines say 18 months is long enough, it is improper for EPA to suggest that further effects might have been found if the study had been for conducted for a longer time. The reason that these types of guidelines are created by EPA in the first place is to remove from hazard assessment practice exactly this kind of speculation about what might have been found had the study only been conducted in another fashion. Speculation is a basis for hypothesis generation, but is **not** the basis for sound science.

Necessary Changes to the Draft Toxicological Review:

#13. Non-statistically Significant Results: The document must not refer to differences from control that are not statistically significant as "increases" unless they provide the measuring stick for defining an increase. The section quoted above from page 4-32 must be deleted. Secondly, the document must delete the comment at page 4-30 that the mouse study was too short in duration.

I.G. Conclusion on Cancer Characterization in the Draft Assessment

There is no scientific basis to conclude that methanol causes increased lymphoma in rats (ERF study) or mice (Apaja study), or increased hepatocellular carcinoma or ear duct carcinomas in rats (ERF study). Although EPA proposes that methanol caused pheochromocytomas in female rats and lung tumors in male rats (NEDO study), the majority of an EPA-commissioned peer review panel found no evidence of cancer in the NEDO study. Curiously, EPA gives no explanation for rejecting the views of these reviewers.

The studies cited by EPA do not support a classification of methanol as "Likely to Be Carcinogenic to Humans." Combining several pieces of weak or questionable data, do not make the data adequate for assessment purposes. EPA apparently believes that the existence of several questionable differences from control tumor incidences, which by themselves do not even constitute "limited evidence" constitute "adequate evidence" under the cancer guidelines.

As we have demonstrated here, there is not even strong suggestive evidence of a dose response from exposure to methanol related to any tumors in rodents. Therefore, the proper cancer classification for methanol must be "Inadequate Information to Assess Carcinogenic Potential." This is the only classification that is justified by the current body of science on methanol.

The EPA 2005 Cancer Risk Guidelines' criterion that EPA argues that methanol meets is as follows:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;"[44]

EPA has examined the four animal studies on methanol and posited tumors by two routes of exposure, two sexes and several sites. However, in these comments we have shown that these studies do not support conclusions of tumors, with the one exception of the very weak finding of lung tumors in the NEDO study. This finding is too weak to be used to meet this criterion, and in any case, it is only one study when more than one is needed justify classification under this criterion. In short, EPA has failed to make its case for a classification of "Likely to Be Carcinogenic to Humans" under its guidelines. Even if the reader disagrees with some of our assessments of these four underlying studies contained in these comments, we would ask whether the quality of these studies, the strength of their findings, and the confidence one can place in these findings, taken together or singly, can justify a cancer classification of such gravity as "Likely to Be Carcinogenic to Humans."

[44] See Appendix B for the full text of the criteria for cancer classification.

II. Mode of Action

II.A. EPA's Approach to Mode of Action (MOA)

In its Toxicological Review of methanol, EPA devotes considerable space positing two theories about the means by which methanol might cause cancer. These two theories are: (1) because methanol metabolizes to formaldehyde and formaldehyde is argued to cause cancer, methanol may have cause cancer; and (2) oxidative stress caused by methanol results in oxidative DNA damage leading to tumors:

> In considering the dose-response relationship for methanol-induced carcinogenesis, a key factor is the saturation of metabolism since metabolic transformation to formaldehyde and generation of oxidative stress are considered likely candidates in the mode of action.
>
> **p. 5-55, EPA Draft Toxicological Review of Methanol**

Yet, when it came time for EPA to calculate a cancer slope factor (CSF), EPA declared that "there is no information to inform the MOA for carcinogenicity":

> "In the case of methanol, there is no information to inform the MOA for carcinogenicity. As recommended in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), "when the weight of evidence evaluation of all available data is insufficient to establish the MOA for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach." Accordingly, for the derivation of a quantitative estimate of cancer risk for ingested methanol, a linear extrapolation was performed to determine the CSF."
>
> **p. 5-39, EPA Draft Toxicological Review of Methanol**

As one can see from the above-quoted passages, EPA appears to be of two minds regarding mode of action. On the one hand, EPA sets a high bar to justify a deviation from its default linear extrapolation of a cancer slope factor. On the other hand, the Agency sets very low bar indeed when it allows itself to suggest two modes of action to shore up its arguments that methanol should be classified as "Likely to Be Carcinogenic to Humans."

In fact, the Agency devotes considerable space to expounding on the purported formaldehyde common mode of action. We would suggest that the application of a criterion for classifying a chemical as a carcinogen should be at least as strict as the criterion used to determine the cancer slope factor. The public health and potential market disruption implications of a classification decision far outweigh the impact of any particular numeric cancer slope factor. In short, we believe that EPA's speculation about possible modes of action does not rise to the level of scientific evidence that should be used to classify a chemical as a carcinogen and therefore should be deleted from the document.

EPA's guidelines criterion reads a follows:

- "a positive tumor study that is strengthened by other lines of evidence, for example … an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

We believe that there is considerable information demonstrating that the mode of action hypotheses advanced by EPA do not meet the requirement of being "likely to be related to the tumor response in this case." We discuss this information below.

II.B. EPA's Oxidative Stress Hypothesis

EPA summarizes its oxidative stress hypothesis as follows:

> "As discussed in Section 4.6.3, evidence of oxidative stress following methanol exposure has been reported in several organ systems. Studies of Wistar rats suggest that methanol exposure can cause the production of free radical formation, lipid peroxidation, and protein modifications in the liver (Skrzydlewska et al., 2005, 196205) and brain (Rajamani et al., 2006, 196157), and adversely impact the oxidant/antioxidant balance in the brain (Dudka, 2006, 090784) and lymphoid organs (Parthasarathy et al., 2006, 089721)."
>
> **p. 4-118,119, EPA Draft Toxicological Review of Methanol**

In early discussions between EPA and the Methanol Institute concerning the potential health effects of methanol, EPA scientists suggested that the Methanol Institute undertake research on this issue of mechanisms of methanol metabolism across species and the role of oxidative stress. The result was a grant to the University of Toronto to conduct a four-year research program.

These scientists have now found: While there is some evidence that near lethal doses of methanol (over 2000 mg/kg) may increase free radical production in circulating lymphocytes and liver[45], no methanol-dependent increases in 8-oxodG in bone marrow, spleen, kidney, lung or liver of any species were found[46]. Chronic treatment of CD-1 mice with methanol (2.0 g/kg ip) daily for 15 days also did not increase 8-oxodG levels in these organs. Further studies in DNA repair-deficient oxoguanine glycosylase 1 (***Ogg1***) knockout (**KO**) mice support these findings. Fibroblasts from *Ogg1* KO mice accumulated 8-oxodG at 6 hr following acute exposure to the ROS-initiating renal carcinogen potassium bromate (**$KBrO_3$**; 2.0 mM), but did not accumulate 8-oxodG following exposure to 125 mM methanol. In vivo exposure of *Ogg1* KO mice to $KBrO_3$ (100 mg/kg ip) doubled renal 8-oxodG levels 24 hr post-dose. In contrast, methanol (2.0 g/kg ip) did not alter renal levels of 8-oxodG at 6 or 24 hr post-dose in *Ogg1* KO mice.

Taken together, these observations suggest that methanol exposure does not promote the accumulation of oxidative DNA damage, and therefore it is unlikely that environmental exposure to methanol would lead to carcinogenesis via this mechanism.[47] This mode of action hypothesis does not meet the requirements of the criterion—"likely to be related to the tumor response."

II.C. EPA's Formaldehyde Hypothesis

Most of EPA's focus regarding mode of action is formaldehyde. In fact, the EPA draft document references formaldehyde a total of 255 times. In addition to arguing that there is a linear pattern of effects among a number of the chemicals studied by ERF (an argument we address in detail at page 31), EPA posits that a metabolic link between formaldehyde and methanol suggests a toxicological link between the two chemicals.

> "Additional support for the designation of methanol as a likely carcinogen is provided by the fact that methanol is metabolized to formaldehyde, which has been associated with increased incidences of **lymphoma** and leukemia in humans (IARC, 2004 196244)…. In addition, epidemiological studies have associated formaldehyde exposure with increases in the incidence of related **lymphohematopoietic** tumors. While lymphomas are a rare finding in chronic laboratory bioassays, NCI (Hauptmann et al., 2003, 093083) and NIOSH (Pinkerton et al., 2004, 093085) have reported increased **lymphohematopoietic** cancer risk, principally **leukemia**, in humans

[45] Parthasarathy et al. 2006 (196735)
[46] McCallum, G. P., Siu, M., Ondovcik, S. L., and Wells, P. G. Methanol exposure does not lead to accumulation of oxidative DNA damage in mice, rabbits or primates., submitted; also The Toxicologist, 2010, abstract #708
[47] McCallum et al., submitted for publication; SOT abstract: METHANOL EXPOSURE DOES NOT LEAD TO ACCUMULATION OF OXIDATIVE DNA DAMAGE IN MICE, RABBITS OR PRIMATES
Gordon P. McCallum, Michelle Sui, Stephanie L. Ondovcik and Peter G. Wells. Abstract #708, 2010

from occupational exposure to formaldehyde." [Emphasis added]

Footnote 66 states "IARC (2004, 196244) concluded that there was sufficient epidemiological evidence that formaldehyde causes nasopharyngeal cancer in humans but, also, that there was strong evidence for a causal association between formaldehyde and the development of leukemia in humans."

p. 4-114, 115, EPA Draft Toxicological Review of Methanol

Our response to this "formaldehyde hypothesis" is as follows:

We must first emphasize our view that EPA's Toxicological Review of methanol is not the appropriate place to debate whether or not formaldehyde is a human carcinogen under the EPA IRIS guidelines. EPA is preparing a full Toxicological Review of formaldehyde and plans to submit that review to the National Academy of Sciences for peer review. That is the appropriate forum in which the issue of the carcinogenicity of formaldehyde should be discussed and decided. For the EPA to suggest in the methanol draft assessment that it has been established that formaldehyde causes lymphohematopoietic tumors and that methanol through its metabolism is likely to exhibit the same health characteristics is a serious deviation from an orderly assessment process and undermines the scientific process for both chemicals. The methanol assessment should not be used to establish EPA's position on formaldehyde before it completes its assessment of that chemical and obtains the views of the National Academy of Sciences on its views.

While we do not believe it is appropriate to address whether or not formaldehyde causes lymphohematopoietic or related tumors in the context of this methanol assessment, we do want to address the portion of EPA's hypothesis that suggests that whatever the health effects of formaldehyde, methanol may exhibit the same effects because it metabolizes to formaldehyde.

This is not a sound hypothesis and is not supported by what is known about the metabolism of methanol. Since it is true that formaldehyde is a metabolic product of methanol, we address below, *without debating the toxicological effects of formaldehyde,* the soundness of the link between methanol and formaldehyde effects:

II.C.1. Human Data

As the above quotation illustrates, the draft assessment attempts to make a connection between methanol exposure and lymphohematopoietic cancer from formaldehyde in humans. **The first statement regarding lymphoma in the quotation is false because the International Agency for Research in Cancer (IARC) did not find increased lymphoma in humans exposed to formaldehyde, only leukemia. Therefore, the cited human formaldehyde data do not support a lymphoma risk from methanol.** In addition, the EPA document previously stated that leukemias and lymphomas need to be considered separately, but this analysis improperly groups them together.

Necessary Changes to the Draft Toxicological Review:

#14. IARC and Lymphoma: The inaccurate statement at page 4-114 that IARC found that formaldehyde exposure is associated with lymphoma needs to be deleted and, throughout the document, leukemias and lymphomas need to be treated separately.

II.C.2. Exogenous vs. Endogenous Formaldehyde and Their Effects:

Formaldehyde is, of course, a naturally occurring chemical that is present in all cells in all animals, including humans. This endogenous background level of formaldehyde produces adducts to adenine and guanine bases of DNA[48] (Lu et al., in press) in rats.

The central question of EPA's formaldehyde hypothesis, on which EPA and we agree, is whether exogenous formaldehyde from direct exposure to formaldehyde or from the metabolism of methanol results in additional levels of formaldehyde in the body—as EPA puts it, a "saturation" of formaldehyde in the body. A recent study sheds substantial light on this question: In this study (see Table 4 below), inhalation of 10 ppm formaldehyde 6 hours/day for 1 or 5 days produced increased guanine, but not adenine, adducts in the nasal epithelium of rats (Lu et al., in press); furthermore, there was no increase in adenine or guanine adducts in liver, spleen, lymphoid tissue, or bone marrow. These data indicate that the level of formaldehyde is not increased in distant tissues following inhalation exposure of a very high concentration of formaldehyde.

[48] Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA. (2010). Distribution of DNA Adducts Caused by Inhaled Formaldehyde is Consistent with Induction of Nasal Carcinoma but not Leukemia. Tox. Sci., in press.

Table 4. Data from Lu et al. (in press) Table 2.
Formaldehyde-induced monoadducts and dG-dG cross-links in rats exposed to 10 ppm formaldehyde for 1 day or 5 days*

Exposure Period	Tissues	N_2-$HOCH_2$-dG (adducts/10^7 dG)		N_6-$HOCH_2$-dA (adducts/10^7 dA)		dG-CH_2-dG (adducts/10^7 dG)	
		exogenous	endogenous	exogenous	endogenous	exogenous	endogenous
	Nose	1.28±0.49#	2.63±0.73	n.d.	3.95±0.26	0.14±0.06§	0.17±0.05
	Lung	n.d.+	2.39±0.16‡	n.d.	2.62±0.24	n.d.	0.20±0.04¶
	Liver	n.d.	2.66±0.53	n.d.	2.62±0.46	n.d.	0.18±0.05
1 day	Spleen	n.d.	2.35±0.31	n.d.	1.85±0.19	n.d.	0.15±0.06
	Bone Marrow	n.d.	1.05±0.14	n.d.	2.95±1.32	n.d.	0.09±0.01
	Thymus	n.d.	2.19±0.36	n.d.	2.98±1.11	n.d.	0.10±0.03
	Blood**	n.d.	1.28±0.38	n.d.	3.80±0.29	n.d.	0.12±0.09
	Nose	2.43±0.78	2.84±1.13	n.d.	3.61±0.95	0.26±0.07	0.18±0.06
	Lung	n.d.	2.61±0.35	n.d.	2.47±0.55	n.d.	0.20±0.03
	Liver	n.d.	3.24±0.42	n.d.	2.87±0.65	n.d.	0.21±0.08
5 days	Spleen	n.d.	2.35±0.59	n.d.	2.23±0.89	n.d.	0.16±0.08
	Bone Marrow	n.d.	1.17±0.35	n.d.	2.99±0.08	n.d.	0.11±0.03
	Thymus	n.d.	1.99±0.30	n.d.	2.48±0.11	n.d.	0.19±0.03
	Blood**	n.d.	1.10±0.28	n.d.	3.66±0.78	n.d.	0.10±0.07

**The limit of detection for dG monoadducts, dA monoadducts and dG-dG cross-links was ~240, ~75 and ~60 amol, respectively.*
#n=5-8 nose samples for the analysis of monoadducts in 30-50 μg of DNA; data represent mean ± SD; + not detectable in 200 μg of DNA; ‡n=4-5 for distant tissues;
§ n=4-5 nose samples for the analysis of cross-links, artifacts have been subtracted from the data;
¶ n=3 for distant tissues;

Therefore, it is not reasonable to expect that formaldehyde has a role in the carcinogenicity of methanol, **unless there is evidence that the metabolism of methanol increases the intracellular level, or body burden, of formaldehyde**. No such evidence is presented by EPA in its draft methanol assessment. In addition, given that methanol is slowly metabolized to formaldehyde and formaldehyde is rapidly metabolized to formate, it is very unlikely that methanol metabolism can increase the intracellular level of formaldehyde. The Draft EPA document reports several studies showing increased blood levels of methanol following exogenous methanol exposure without an accompanying increase in blood formate. These data suggest that exogenous methanol exposure does not lead to an increase in the intermediate formaldehyde. With this kind of metabolic pattern, it is not clear how methanol would cause a "saturation" of formaldehyde in the body.

II.C.3. Modeling of Formaldehyde Doses Resulting from Methanol Metabolism

Some of EPA's justification for linking methanol effects with those of formaldehyde is based on modeling conducted by the Agency. As an example:

> "The primary purpose of this assessment is for the determination of noncancer and cancer risk associated with exposures that increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde) above prevailing, endogenous levels. Thus, the focus of model development was on obtaining predictions of increased body burdens over background following external exposures. To accomplish this, the PBPK models used in this assessment do not account for background levels of methanol, formaldehyde or formate."
>
> **p. 3-30, EPA Draft Toxicological Review of Methanol**

However, the current PBPK model used in the assessment did not predict the increased body burdens of the metabolites of methanol; only blood, tissue, and urine concentrations of methanol. While the model does include the metabolic clearance of methanol, this is not analogous to predicting the body burden of formaldehyde or formate since the body burden will be a balance of the naturally-occurring and exogenous-source process of formation and removal.

In addition, the wording leads readers to believe that the model predicts both background and any additional increase in both methanol and its metabolites. However the model mainly focuses on predicting the pharmacokinetics of the exogenous methanol. Background levels were only included as either a constant concentration over time or a linear increase over time. One important test of the model not performed was the ability to predict endogenous methanol. A model that can predict the pharmacokinetics of exogenous exposure to methanol should also be able to predict the pharmacokinetics of uptake from natural sources, and endogenous production of methanol and its metabolites. Simply put, one cannot understand at what point exposure to a chemical "saturates" the body's ability to process that chemical and may begin to cause harm, unless one first has a solid understanding of the body's on-going metabolic disposition of that chemical and whether exogenous methanol changes that disposition. EPA failed to take that critical step.

A more robust model would have attempted to account for endogenous and all exogenous input of methanol in the animals. Since the body is naturally exposed to methanol, it has an ability to detoxify methanol within a range of concentrations that would not lead to toxicity. This approach would be consistent with that of Andersen and Krewski's "Toxicity Testing in the 21st Century" [49] advocating that biological systems operate normally within a range of environmental

[49] Andersen ME; Krewski D (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life Toxicol. Sci. 2009 107: 324-330.

exposures until the perturbation becomes large enough that the adaptive capacity of the system is exceeded and thus progresses to a disease state.

For example, in a paper by Dhareshwar and Stella (2007) assessing potential formaldehyde release from prodrugs, they used estimates for the steady state concentrations of total formaldehyde products, total body water, and half-life of formaldehyde removal to determine that the background production of formaldehyde would be approximately 41 mg/min to maintain the steady state concentrations. This can be compared to the amount of methanol metabolized that was used to determine the oral $BMDL_{10}$ (104.4 mg/day*kg^0.75) and inhalation $BMDL_{10}$ (39.4 mg/day*kg^0.75). These BMDL levels would be equivalent to the production rate of 1.75 mg/min and 0.66 mg/min in a 70 kg reference man. These are only 1.6% to 4.2% of the estimated background production. EPA acknowledges in the review (Pg 4-119) that the metabolized methanol estimate is not an accurate representation of formaldehyde distribution; however, the risk assessment dose metrics (total metabolized methanol and methanol blood) presume that the methanol pharmacokinetics is an indicator of formaldehyde pharmacokinetics in the body.

As demonstrated below (Tables 5 and 6), the EPA assertion that there is a linear relationship between methanol metabolized and formaldehyde administered with LHR tumor response is not valid. First of all, the EPA document previously concluded that it was inappropriate to perform analyses of combined leukemias, lymphomas and histiosarcomas, but for this analysis, the EPA ignored its own conclusion and combined them anyway. Furthermore, the model does not accurately account for methanol metabolism and the increase in LHR tumors over control value in each study does not follow a linear response.

The Methanol Institute asked Dr. Teresa Leavens of the College of Veterinary Medicine of North Carolina State University to verify the calculations and conclusions of the EPA PBPK model. For methanol the EPA model predicts the amount of methanol exhaled, excreted, metabolized and retained in the body unchanged. Those values are shown below:

Table 5. Calculated Methanol Consumed in the ERF Methanol Study

Water Concentration (ppm)	**TWA weight (kg)**			**Water consumption (L/d)**			**Calculated Amt consumed (mg/kg/d)**	
	Male	Female		Male	Female		Male	Female
0	0.5	0.33		0.05257	0.04255		0.0	0.00
500	0.49	0.33		0.05206	0.04305		53.12	65.23
5000	0.5	0.33		0.05258	0.04111		525.80	622.88
20000	0.54	0.34		0.04832	0.03726		1789.63	2191.76

Table 6. Male Model Predictions of Methanol Disposition based on Draft IRIS Document's Table E-1 Values for the Soffritti et al, 2002 Methanol Study

Water Concentration (ppm)	total dose (mg)	Met (mg)	Exh (mg)	Urine (mg)	Feces (mg)	Tbody (mg)
0	0.00	0.00	0.00	0.00	0.00	0.00
500	26.07	19.66	0.35	0.00	2.67	3.40
5000	262.00	146.92	24.83	0.00	26.97	63.28
20000	961.20	190.06	168.17	0.00	100.85	502.12

If the model is correct, the males of the 20,000 ppm dose group retained 502 mg unchanged methanol the first day. As this methanol builds up on a daily basis, the amount of methanol exhaled unchanged increases. From approximately one week on, the daily accumulation is 160 mg/day. Since the rats in this group received methanol every day for 730 consecutive days, at the end of the 730 days the rats would contain (.160 g/day * 730 days) = 116 g of methanol. In other words, the model predicts that for the high dose male rat, 21% of its body weight would be unchanged methanol at the end of the 730 day dosing period. Similar results are estimated, but not shown here, for the females. This body burden is approximately 10 times the acute LD_{50} of methanol in rats.

Furthermore, the model predicts that no methanol, which is extremely water soluble, is eliminated via urine, but ~12% is eliminated in the feces. No mechanism is proposed for fecal elimination of methanol.

Thus, the first step in the EPA assumption of linearity of methanol metabolized and external formaldehyde is questionable, because the PBPK model is not valid; i.e., the amount of methanol metabolized as predicted by the model is too low to account for the methanol dosed. If the model accounted for all methanol dosed and did not predict a dramatic storage of unchanged methanol in the rats, the formaldehyde equivalent dose used for methanol in EPA Figure 4-1 would be considerably higher and there would not be linearity.

Secondly, the comparison is based on the absolute value of LHR tumors reported for each dose group in the methanol and formaldehyde studies. However, this comparison is not valid as the control responses were very different in the two studies. In the formaldehyde study, 8% of the control males were reported to have LHR tumors, while 28% of the controls in the methanol study were reported to have LHR tumors. Similarly, the incidences of LHR tumors in females were reported as 7% in the formaldehyde study and 13% in the methanol study. EPA apparently combined the incidences in the control groups for comparison to the treated groups. This is not appropriate for studies that were conducted several years apart and had very different incidences in the control groups. A more appropriate comparison would be the percent increase over control in each study, as shown in Table 7 below.

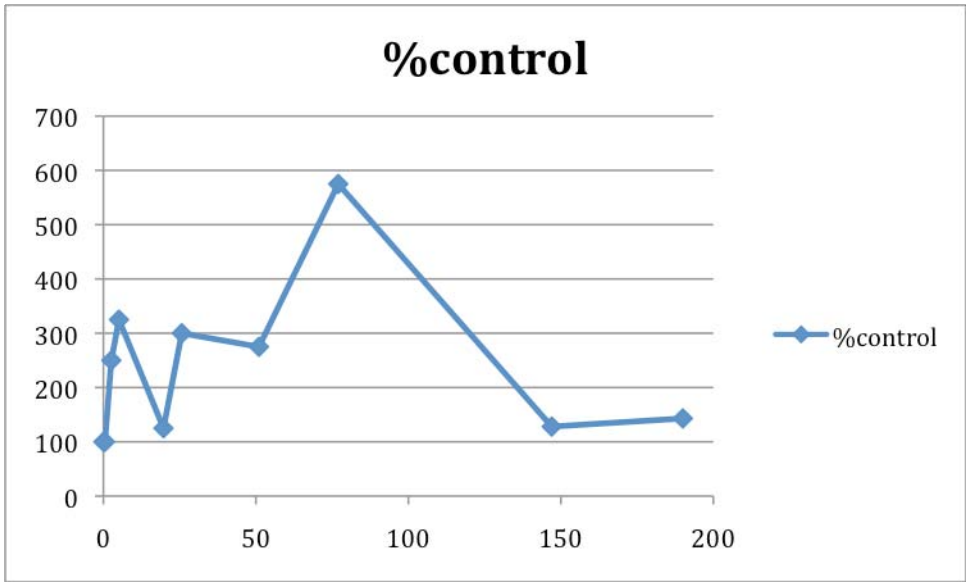
Table 7. Reported Incidences of LHR from Methanol and Formaldehyde in ERF Studies and Percent of Respective Control Values

Chemical dosed	**FA eq**	**LHR % reported**	**% control**		**FA eq**	**LHR % reported**	**% control**
	Males				**Females**		
FA	0	8	100		0	7	100
MeOH	0	28	100		0	13	100
FA	0.51	8	100		0.41	10	143
FA	2.6	20	250		2	14	200
FA	5.1	26	325		4.1	16	229
MeOH	19.7	35	125		16.5	24	186
FA	25.7	24	300		20.5	14	200
FA	51	22	275		41	22	314
FA	77	46	575		61	20	286
MeOH	147	36	128		112	24	186
MeOH	190	40	143		135	28	215

The graph of these data does not look like a linear relationship.

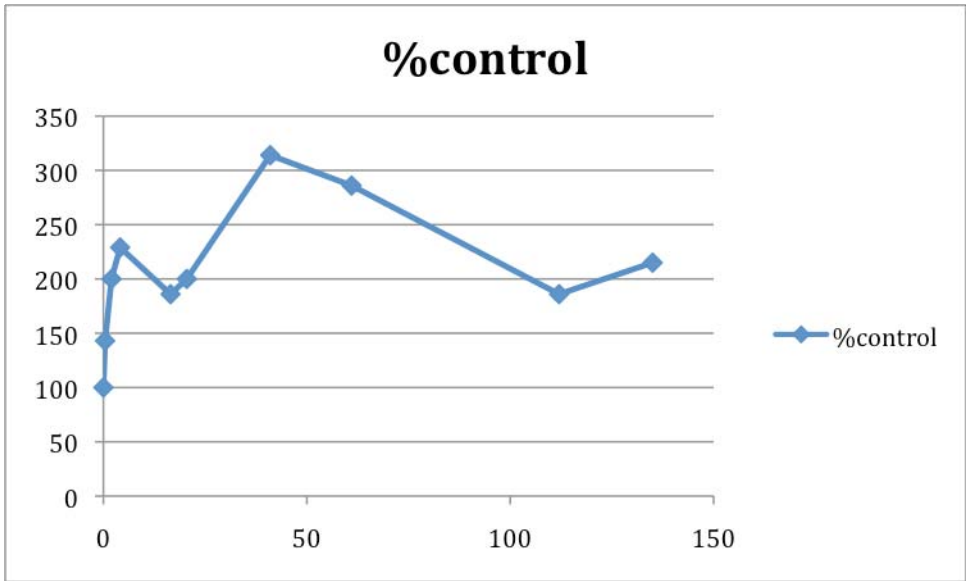
Figure 1. Percent Increase in LHR Cancers Reported vs. Calculated Formaldehyde Equivalent Dose, Using EPA PBPK Model

A. Males



Formaldehyde dosed or methanol metabolized by EPA model

B. Females



Formaldehyde dosed or methanol metabolized by EPA model

The above comparison was developed using total LHR, which is not appropriate since only LIL was reported to be increased in the methanol study, while lymphatic leukemia and lymphoblastic lymphoma were also reported to be increased in the formaldehyde study. EPA has been in possession of these data since mid-2009, and could have presented that analysis, but chose not to. Since we found out about this breakdown of LHR only recently on March 8, 2010, we have not been able to obtain the data and conduct the proper comparison. In the previous section of these comments on cancer we similarly demonstrated a lack of consistent statistically significant increase in LHR in studies of methanol, formaldehyde, MTBE, and aspartame. [See discussion at page 31.]

Other issues with the PBPK modeling and its use in human risk estimates are discussed in Appendix A.

Conclusion: The data and PBPK model do not support a linear relationship between methanol metabolized or formaldehyde administered and incidence of LHR tumors. Thus, the basic premise of EPA's hypothesis for a common mode of action between methanol and formaldehyde because of metabolism must be dismissed.

Necessary Changes to the Draft Toxicological Review:

#15. Correction of the PBPK model: The mistakes in the PBPK model must be corrected to account more accurately for methanol metabolism to formaldehyde and the further metabolism of formaldehyde to formate. The section of the IRIS document asserting a linear relationship between methanol metabolism, formaldehyde and LHR tumors must be removed or corrected to indicate there is no such relationship.

Overall Conclusion Regarding the Mode of Action of Methanol:

There is insufficient evidence of increased cancer from methanol exposure in rats and mice; however, a complete assessment queries whether there are modes of action (MOAs) that would increase the weight of evidence for cancer from methanol exposure. Two MOAs have been proposed by EPA:

1. Increased oxidative DNA damage from hydrogen peroxide, a byproduct of methanol metabolism to formaldehyde by catalase in rodents:

A recent study has demonstrated that dosing 2000 mg/kg/day for 15 days did not increase oxidative DNA damage in mice. Thus, this proposed MOA does not add to the weight of evidence that methanol causes cancer.

2. Increased body burden of formaldehyde and a linear relationship of LHR response for dosed formaldehyde or total methanol metabolized to formaldehyde.

This MOA is based on pure speculation. We have noted that this linear relationship does not exist, that the EPA PBPK model does not predict increased body burden of formaldehyde from methanol exposure, and that the comparison of total LHRs across several ERF-studied chemicals is not valid. This proposed MOA does not add to the weight of evidence that methanol causes cancer.

In conclusion, EPA's suggested modes of action are not supported by the body of knowledge regarding the metabolism of methanol and do not meet the requirement of the EPA cancer guidelines that the metabolism is "likely to be related to the tumor response in this case."

III. Comments on EPA's Charge Questions to the Panel

In the following section the Methanol Institute (MI) addresses some of the charge questions that were submitted to the SAB by the EPA.

III. A. Charge Questions on Toxicokinetics and PBPK Modeling

> A.1. Please comment on the scientific soundness of the Physiologically-based pharmacokinetic (PBPK) model used in this assessment."
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

A.1. MI Comment: The model is based on an assumption that could be tested using the model, but was not: that methanol metabolism increases the intracellular level of formaldehyde. This is extremely unlikely given the relatively slow metabolism of methanol (half-life about 3 hours) and the rapid conversion of formaldehyde to formate (half-life about 1 minute). In addition, the model does not appear to account for all of the methanol dosed. If the model is incapable of predicting the intracellular level of formaldehyde or all of the methanol dosed, it is useless for assessing the "body burden" of methanol and its metabolites, e.g. formaldehyde and formate, and the EPA's assessment is not valid.

The model has additional flaws that have been identified in a review conducted for the Methanol Institute by Dr. Teresa Leavens of North Carolina State University and are detailed in Appendix A of these comments.

> "A. 2. Please comment on the scientific justification for the subtraction of background levels of methanol from the data in relation to the quantification of both the noncancer and cancer risks."

A.2. MI Comment: All mammalian cells contain background levels of methanol and formaldehyde. Dr. Swenberg has demonstrated both guanine- and adenine-DNA adducts from endogenous formaldehyde. Thus, there is a background level of formaldehyde that has some reaction with DNA. The background level is not without impact and to ignore it is wrong. Exogenous formaldehyde or methanol can only increase the potential cancer risk through formaldehyde if it increases that level of formaldehyde in the cells. Methanol is metabolized to

formaldehyde rather slowly (half-life = 3 hours, but formaldehyde is metabolized to formate very rapidly (half-life = ~1 minute).

The current model assumes that any methanol metabolized to formaldehyde remains as formaldehyde—ignoring the secondary step to formate—and this excess burden of formaldehyde is said potentially to cause DNA adducts and cancer. Methanol metabolism to formaldehyde would only cause an increase in formaldehyde-related toxicity if the intracellular level of formaldehyde is increased. Assuming that there is no background level and failing to incorporate the metabolic removal of formaldehyde changes the whole quantification of dose-response. Subtraction of background levels is not appropriate.

III. B. Charge Questions on Inhalation Reference Concentration (RfC) for Methanol

No specific comments on these questions are included in this comment document.

III.C. Charge Questions on Oral Reference Dose (RfD) for Methanol

No specific comments on these questions are included in this comment document.

III. D. Charge Questions on Carcinogenicity of Methanol

> "D.1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that methanol is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the weight of evidence characterization scientifically justified and adequately described?"
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

D.1. MI Comment:

The weight of evidence characterization is **NOT** scientifically justified. In classifying chemicals with regard to their carcinogenic hazard, EPA is required to carefully apply its own classification guidelines contained in the Agency's 2005 Cancer Risk Guidelines.[50] We believe EPA misapplied these criteria to the body of science on methanol.

[50] The classification criteria under the 2005 Cancer Risk Assessment Guidelines are reprinted in Appendix B

In proposing to classify methanol as "Likely to Be Carcinogenic to Humans" EPA apparently relies on two criteria for this classification:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" and

- "a positive tumor study that is strengthened by other lines of evidence, for example … an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

With regard to the first criterion, there are no human studies of methanol and only four animal studies which EPA can use in applying this criterion. The detailed comments provided in the Methanol Institute's submission address each of these four animal studies in detail and show that they do not support a classification of "Likely to Be Carcinogenic to Humans" and instead support a classification of "Inadequate Information to Assess Carcinogenic Potential," at best.

Neither the Soffritti et al., 2002, nor the Apaja, 1980 studies provide evidence of increased lymphoma, or any other cancer from oral exposure. The NEDO studies provide no evidence of increased lung tumors in males or pheochromocytomas in females that are clearly different from concurrent controls or are outside the historical control range.

Specifically:

- The Soffritti/ERF study has little value and should not be considered for classification purposes:

    - The lymphomas most likely represent an immune response from *Mycoplasma pulmonis* infection because:

        - Authoritative government bodies (Food and Drug Administration, European Food Safety Authority) other U.S. Federal agencies (Department of Defense, Office of Management and Budget), and respected members of the scientific community have rejected the findings of the group of ERF studies showing reported LIL. Many of these have called as well for a Pathology Working Group to determine the accuracy of the ERF diagnoses *[See discussion beginning at page 17]*;

        - The type of lymphoma (Lympho-immunoblastic lymphoma—LIL) reported has not been seen by any other laboratory and affects a completely different spectrum of organs than any known lymphoma *[See discussion at page 19]*;

        - The LIL reported occurs at a much higher frequency than any other lymphoma in rats and has a unique cellular morphology *[See discussion at page 19]*

- An independent peer review panel of scientists convened by TERA raised serious questions about the accuracy of the Soffritti study including a statement by one reviewer that EPA should bear in mind that "'an absence of proof is not the proof of absence' when it comes to a *M. pulmonis* infection" *[See discussion at page 20]*;

- An ERF laboratory official has testified under oath confirming the existence of *Mycoplasma pulmonis i*nfection antibodies in the laboratory's rat colony, thereby showing that infections have occurred in the colony despite earlier denials by the Laboratory in personal communications to EPA. *[See discussion at page 22]*;

- With only two exceptions, the reported LIL in the Soffritti methanol study occur in the lung and associated organs (lymph nodes and spleen), consistent with a respiratory disease explanation *[See discussion at 23]* ;

- EPA's citation of the NIEHS panel of pathologists as support for the LIL finding failed to point out that the panel only examined slides from 2 of the 149 animals with purported LIL and that this panel meeting took place before serious questions had been raised about a possible misdiagnosis arising from an infection with *Mycoplasma pulmonis [See discussion at page* 25];

- The reported LIL shown in photomicrographs has been identified by scientists, who are experts in respiratory disease in test animals, as *Mycoplasma pulmonis[See discussion at page 26]*; and

- The National Toxicology Program is planning a visit soon to the ERF Laboratories that may result in an independent review of the tissue slides from the Soffritti study. If this review is done correctly, then it could resolve the issue of possible misdiagnosis *[See discussion at page 28]*.

- For all these reasons, the lymphoma findings of the Soffritti study should not be used for hazard assessment until and if an independent Pathology Working Group review of the tissue slides confirms the reported finding of lymphoma and disproves the Mycoplasma pulmonis hypothesis of a misdiagnosis.

- an NIEHS panel review of selected slides from an aspartame study at the laboratory concluded that most of the ear duct lesions diagnosed in that study were not tumors, so the reported ear duct carcinomas in the methanol study are not likely to be tumors either *[See discussion at page 39]*;and

- the reported liver carcinomas are not likely related to methanol, as there are no accompanying adenomas or foci, and the historical control group is very small and artificial. *[See discussion at page 38].*

- In the Apaja study, when all the groups exposed to methanol are considered, there is no increase in lymphoma from methanol in this study, as the author himself concluded. *[See discussion at page 35]*

- The pheochromocytomas in the NEDO study are not increased relative to the concurrent control and are not outside the range found in NTP studies conducted at approximately the same time. Furthermore, if methanol causes increased pheochromocytomas in females it causes decreased pheochromocytomas in males. *[See discussion at page 41]*

- The only possible increase in tumors is a small, but statistically significant increase in lung adenomas and carcinomas combined at the highest exposure concentration in males only in the NEDO study. However, the response was weak, with the range of historical controls, and involved nearly all benign tumors, except for one. *[See discussion at page 40]*

In short, the combining of several weakly suggestive and non-significant differences from control incidences does not make the data become "adequate" evidence under the Agency's cancer guidelines.

In summary, the studies cited by EPA as justification for the classification of methanol under its guidelines as "Likely to Be Carcinogenic to Humans" do not, upon careful examination, support this classification recommendation and instead support, at best, a classification of "Inadequate Information to Assess Carcinogenic Potential."

> "D.2. EPA has determined that the mode of action of the carcinogenicity of methanol is not known. Has the discussion of the mode(s) of carcinogenic action been accurately and clearly described?"
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

D.2. MI Comment: No. The modes of carcinogenic action have NOT been accurately and clearly described.

The Agency speculates at length about two possible modes of action for methanol as a way of shoring up its support for its proposed classification of methanol as "Likely to Be Carcinogenic to Humans." However, when it comes to a judgment of whether the Agency knows enough about mode of action to deviate from its linear extrapolation of a cancer slope factor, the Agency declares it has "no information to inform the MOA for carcinogenicity."

The Agency's approach to mode of action in support of its classification decision lacks rigor. With regard to its speculation concerning oxidative stress as a mode of action, recent data from

the University of Toronto study indicate that methanol exposure does not promote the accumulation of oxidative DNA damage, and therefore it is unlikely that environmental exposure to methanol would lead to carcinogenesis via this mechanism. *[See the discussion at page 48]*

With regard to the lengthy discussion by EPA regarding its formaldehyde MOA hypothesis, EPA poses and then answers the wrong question. The issue is not whether methanol is metabolized to formaldehyde, but whether that metabolism results in an increase in the intracellular level of formaldehyde:

- Based on metabolic data and preliminary DNA adduct work, exposure to high levels of methanol does not increase intracellular levels of formaldehyde. *[See discussion at page 51]*

- There is no consistent pattern among the ERF studies of chemicals with a metabolic link to formaldehyde that would suggest that the LHR tumor responses of these chemicals are related to metabolism to formaldehyde. *[See discussion at page 31]*

This hypothesized mode of action assumes that any excess body burden of formaldehyde leads to cancer. In effect, the EPA is prejudging it own upcoming IRIS review of formaldehyde, which is to be submitted for review to the National Academy of Sciences before it becomes final. The methanol IRIS assessment is NOT the appropriate venue to, in effect, classify formaldehyde as to its carcinogenicity.

> "D.3. Specific to the cancer assessment, EPA has chosen to use the total rate of methanol metabolism as a measure of formaldehyde production from exposure to methanol. In part, the rate of metabolism is used due to the difficulty in determining levels of formaldehyde in the blood, since without data on formaldehyde blood concentrations it is not possible to model those concentrations. This metric of formaldehyde production is uncertain because metabolic processes may differ between species (EPA has attempted to account for expected interspecies differences in the clearance of formaldehyde by normalizing the total rate of metabolism by BW•). Are there alternative approaches which could be readily applied in conjunction with the existing PBPK model to estimate formaldehyde production from methanol metabolism that would be preferred? If so, please provide the rationale and a detailed explanation of how the alternative formaldehyde dose could be implemented in the PBPK model."
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

D.3. MI Comment:

- **The EPA draft's approach to methanol metabolism is totally wrong and the error is carried over to this charge question that seeks possible alternatives to "estimate formaldehyde production from methanol metabolism." The correct issue is whether methanol metabolism increases formate or formaldehyde levels above the levels that occur in the body naturally.**

- At the beginning of EPA's draft document the Agency seems to agree:

> *"The primary purpose of this assessment is for the determination of noncancer and cancer risk associated with exposures that* ***increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde****)* [emphasis added by MI] *above prevailing, endogenous levels. Thus, the focus of model development was on obtaining predictions of increased body burdens over background following external exposures. To accomplish this, the PBPK models used in this assessment do not account for background levels of methanol, formaldehyde or formate."*

However, when the document discusses the results of the PBPK model and the Agency's formaldehyde hypothesis for a common mode of action, the Agency reverts to discussion of how much formaldehyde is produced through metabolism of methanol, not what the increased body burden would be. The PBPK model is not designed to answer the body burden question:

- The EPA PBPK model offers no evidence of increased levels of formaldehyde or formate from methanol exposure, nor does it demonstrate increased body burden above prevailing, endogenous levels.

- For example in a paper by Dhareshwar and Stella (2007) assessing potential formaldehyde release from prodrugs, they used estimates for the steady state concentrations of total formaldehyde products, total body water, and half life of formaldehyde removal to estimate that the background production of formaldehyde would be approximately 41 mg/min to maintain the steady state concentrations. This can be compared to the amount of methanol metabolized that was used to determine the oral $BMDL_{10}$ (104.4 mg/day*kg^0.75) and inhalation $BMDL_{10}$ (39.4 mg/day*kg^0.75). These would be equivalent to the production rate of 1.75 mg/min and 0.66 mg/min in a 70 kg reference man. These are only 1.6 to 4.2 % of the estimated background production. One would not expect this de minimis level of added formaldehyde to be responsible for added cancers;

- There appear to be a number of issues with the input parameters, which affect the outcome predictions of the model *[See discussion in Appendix A]*. In addition, the model assumes that there is no removal of formaldehyde by metabolism. Formation of formaldehyde does not mean increased level of formaldehyde. There is no indication that this minor increase in formaldehyde production would increase the intracellular level of

formaldehyde; and

- **If methanol produces cancer through its metabolism to formaldehyde, the PBPK model must demonstrate increased body burden (increased intracellular level of formaldehyde) for formaldehyde. EPA provides no data indicating that the body burden is increased. The nature of methanol metabolism and recent data on the formation of adducts from formaldehyde exposure suggest that that the metabolism of methanol will not increase the body burden of formaldehyde. As we have stated above, it is inappropriate for EPA to conclude that any additional formaldehyde in the body leads directly to the formation of cancer. This is especially true because EPA has not completed its own assessment of formaldehyde and submitted it to the NAS for review.**

For further discussion of all of these PBPK issues, see pages 51 through 58.

> "D. 4. A lifetime drinking water cancer bioassay in SD rats (Soffritti et al., 2002) was selected for the derivation of an oral slope factor. Please comment on the scientific justification for the selection of this study. Have the strengths and limitations of the study been adequately characterized? There are two main issues associated with the use of the European Ramazzini Foundation (ERF) bioassay results. One issue is the differences in protocol used by the ERF compared to more generally used study protocols such as those used by the National Toxicology Program. Another issue concerns the possibility of *Mycoplasma pulmonis* infection in the test animals. Please comment on whether these and any other issues associated with this study have been adequately and clearly described and addressed."
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

D.4. MI Comment: EPA should not develop an oral cancer potency slope based on the Ramazzini study.

The document attempts to establish the protocol used at the ERF as superior to that of the NTP because animals are allowed to live out their lifetime, not terminated after 104 weeks of exposure. While there are significant disputes as to the purported benefits of this practice, this is irrelevant for this study. The only diagnosis that had increased incidence in the methanol treated rats was lympho-immunoblastic lymphoma (LIL) and the percent of animals dying with LIL was no different before or after 104 weeks; i.e., the percentage of LIL did not increase in control or treated animals dying after 104 weeks. **The extended observation period did not increase the sensitivity of the study. The weaknesses of the study have not been made clear.**

Either Ramazzini Foundation personnel have found a new lymphoma that is not found in any other laboratory, has a different cellular morphology, different organ spectrum, and occurs at least 10 times as frequently in ERF control animals as other lymphomas, or the lesions are an immune response to *Mycoplasma pulmonis*. The laboratory has admitted that its health screening sometimes finds antibodies for *Mycoplasma pulmonis*; thus we know *Mycoplasma pulmonis* is present in the animal colony. **It is not likely that this lesion represents a lymphoma.**

We address all of these issues in our answer to Charge Question D.1 (above) and in the detailed discussions throughout this document as referenced. Please refer to these discussions for a more detailed response to this Charge Question D.4.

> "D. 5. The oral cancer slope factor was calculated by linear extrapolation from the POD (lower 95% confidence limit on the internal dose associated with 10% extra risk for lymphomas). Specifically, PBPK model estimates of total metabolized methanol/day (normalized to $BW^{3/4}$; i.e., the internal doses) for each bioassay exposure were used to establish the POD and extrapolate to a human equivalent oral dose. Please comment on the adequacy of this approach, including the choice of tumors and the manner in which the modeling was conducted."
>
> **NCEA's Proposed Charge to External Reviewers, December, 2009**

D.5. MI Comment: EPA's approach to the calculation of an oral cancer slope factor is not valid. First, as discussed at length in these comments, the Soffritti/ERF study is not appropriate for developing a cancer slope *[See the summary of our comments on the Soffritti study under Charge Question # D.1 above*.]

In addition, the dose metric should be based on increased intracellular level of formaldehyde, not total methanol metabolized. EPA has failed to show that exposure to methanol would result in an increased level of intracellular formaldehyde. *[See the summary of our comments and the related references in our response to Charge Questions #2 and 3 above.]*

"D. 6. A two-year inhalation cancer bioassay in F344 rats (NEDO et al., 2002) was selected for the development of an inhalation unit risk. Please comment on whether the selection of this study is scientifically justified. Have the strengths and limitations of the study been adequately characterized?"

NCEA's Proposed Charge to External Reviewers, December, 2009

D.6. MI Comment: No, the strengths and limitations of the NEDO study have not been adequately characterized and the NEDO study should not be used for the development of the inhalation unit risk. The pheochromocytomas in females should not be the basis of a slope factor. They are not increased relative to the concurrent controls; the only group that had a larger number of pheochromocytomas than the controls was the high dose females, so a "trend test" has no real meaning. The majority of the members of the EPA chartered Peer Review of the NEDO studies found no basis for methanol-induced cancer in the NEDO studies—a fact not mentioned in the EPA draft assessment. This is consistent with the findings of the authors of the study. **No inhalation unit risk is justified by the data in the NEDO studies.**

"D.7. The inhalation unit risk was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for pheochromocytomas). PBPK model estimates of total metabolized methanol/day (normalized to BW3/4) were used to establish the POD and extrapolate to a human equivalent inhalation concentration. Please comment on the adequacy of this approach, including the choice of tumors and the manner in which the modeling was conducted."

NCEA's Proposed Charge to External Reviewers, December, 2009

D.7 MI Comment: The approach taken by EPA, including the choice of tumors and the manner in which the modeling was conducted is not adequate. The data do not support developing an inhalation unit risk from the NEDO data or any other data; furthermore, the PBPK model contains many deficiencies that need correcting.

The EPA states in the draft review that

> *"The primary purpose of this assessment is for the determination of noncancer and cancer risk associated with exposures that increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde) above*

prevailing, endogenous levels. Thus, the focus of model development was on obtaining predictions of increased body burdens over background following external exposures. To accomplish this, the PBPK models used in this assessment do not account for background levels of methanol, formaldehyde or formate."

The PBPK modeling needs to account for the normal intracellular level of formaldehyde and whether exposure to methanol increased that level. There is nothing in the draft IRIS document or PBPK modeling that suggests an increased body burden of formaldehyde or formate. The model accounts for metabolism of methanol to formaldehyde, but does not account for the metabolism of formaldehyde. Furthermore, as Dr. Leavens has demonstrated, there are a number of issues with both the parameters used and the modeling outcome (See specific comments in Appendix A). There is no evidence that methanol exposure increases pheochromocytomas, and therefore, unit risk should not be based on pheochromocytomas.

The data do not support developing an inhalation unit risk from the NEDO data or any other data; furthermore, the PBPK model contains many deficiencies that need correcting.

III. E. General Charge Questions

"E.2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review and should be considered in the assessment of the noncancer and cancer health effects of methanol."

NCEA's Proposed Charge to External Reviewers, December, 2009

E.2. MI Comment: There is nothing in the draft methanol assessment to indicate that the Agency considered the opinions of the expert reviewers it engaged through the Eastern Research Group to consider the two NEDO methanol studies (2 of only 4 animal studies on the carcinogenicity of methanol). Additionally, the Methanol Institute has supported an independent peer review of the Soffritti methanol study managed by TERA that provides important expert commentary on this study which forms the principal basis of EPA's proposed cancer classification for methanol. The latter peer review was received by EPA after the beginning of the comment period of its draft toxicological review. Both of these expert reviews have significant bearing on the methanol assessment, and should be carefully considered.

Additionally, there is one study that is so important to the determination of the proper classification of methanol that it should be undertaken BEFORE EPA completes its toxicological review of methanol and submits it to the Science Advisory Board for peer review. This is an independent Pathology Working Group (PWG) review of the slides in the Soffritti/ERF study on methanol. The PWG panel should have a carefully developed protocol and include

knowledgeable pathologists who have familiarity with non-Specific Pathogen Free rodents and with distinguishing lymphoma from lesions caused by *Mycoplasma Pulmonis* infections. The panel should also have as a member a scientist experienced in testing tissue slides for *Mycoplasma Pulmonis* infections. The panel must have the freedom to examine all slides they deem necessary to understand the results of the study.

If and until such a PWG is undertaken, reported on, and then peer reviewed, the Soffritti/ERF study on methanol cannot legitimately be relied upon for classification purposes by EPA. Because EPA, to date, has taken the position that such a PWG review is unnecessary and that the doubts about the Soffritti study should be resolved in favor using the study's reported results for classification purposes, we fear that the responsibility will fall to the Science Advisory Board to decide that such a PWG is necessary and to insist that the Soffritti study NOT be used for classification purposes until and if such a PWG is conducted and the truth about the tissue diagnoses is definitely determined.

IV. Summary

IV.A. Compilation of Necessary Changes to the Draft Toxicological Review

Throughout this comment document we have recommended specific changes in the EPA draft toxicological review that are necessary in order to conform the EPA document to the underlying body of science and to ensure compliance with the Agency's own Cancer Guidelines. These recommended changes are compiled below for the reader's convenience:

#1. Combining Disease Types: The document should be reworded to avoid combining leukemias and lymphomas as well as combining all lymphomas, treating separately those lymphomas that are reported as increased and those for which no increase was observed. There was only one type of reported lymphoma in the ERF methanol study that was increased and showed any dose response. That is the only "lymphoma" that should be analyzed; to include the other minor types confuses the issue and appears to indicate that all types of lymphoma were increased. 15

#2. Confirmation of *Mycoplasma pulmonis:* The statement at page 5-48 that *Mycoplasma pulmonis* has not been confirmed in the ERF rat colony need to be removed from the document and in their place the document should acknowledge that ERF staff have confirmed—under oath—that *Mycoplasma pulmonis* infection has occurred within the rat colony. 23

#3. Effect on Dose-Response: EPA's arguments at page 4-118 and related pages that the examination of non-lung lesions taken alone demonstrates that the *Mycoplasma pulmonis* hypothesis is invalid should be removed from the draft document. 25

#4. NIEHS Panel: The statement on page 4-118 that several ERF lymphoma diagnoses have been confirmed should be removed from the document**.** 26

#5. *Mycoplasma pulmonis* Hypothesis*:* Given the current serious uncertainties that surround the accuracy of the Soffritti/ERF findings of lymphoma, the Weight of the Evidence section should be revised to place little or no scientific weight on that study's findings. 29

#6. Number of Studies with LHR tumors: The statement at page 4-87 and related pages that there are only a few studies with increased incidence of hemolymphoreticular tumors is unsupportable, and the suggestion that these tumors only occur with chemicals linked in some way with methanol should be deleted from the draft toxicological review of methanol. 31

#7. No Consistent Pattern Among ERF Studies: EPA should either remove the discussion of these other ERF studies on aspartame, MTBE, ethanol, and formaldehyde or show that there is no support within the data, but, rather, only speculation, for a consistent pattern of LHR among these chemicals. 35

IV.B. Our Overall Conclusions

In proposing to classify methanol as "Likely to Be Carcinogenic to Humans," EPA apparently relied on the following two criteria:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;" and

- "a positive tumor study that is strengthened by other lines of evidence, for example … an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

The comments above have laid out in detail why the four studies on which EPA relies cannot be used together or separately to meet the first criterion to label methanol a chemical "Likely to Be Carcinogenic to Humans." Our comments also argue why the metabolite arguments that EPA advances to meet the second criterion are not consistent with what is known about the metabolism of methanol and formaldehyde. These comments begin at pages 13 and 47 respectively.

We reiterate our plea that an independent Pathology Working Group review of the tissue slides of the Soffritti/ERF study be conducted before the Soffritti study is used in any classification decision on methanol. A decision to classify methanol as a "Likely" carcinogen without this key information risks branding the chemical with study results that are very likely ultimately to be proven false. Because of the way the marketplace for chemicals operates, a reversal of this classification some years from now could not repair any damage that such an incorrect classification would do to the industry.

We are very concerned about the outcome of this review because:

- EPA, to date, has taken the position that such a PWG review is unnecessary;
- EPA scientists who are responsible for carrying out the assessment of methanol and related chemicals have published a scientific article defending Soffritti's findings (Caldwell, et al. 196182); and
- The EPA draft toxicological review, while acknowledging some of the shortcomings of the Soffritti study, still resolves all doubts in favor of using the study's reported results as the principal basis for classification.

We therefore fear that EPA may not want to change its mind about the necessity of a PWG, but we hope that these comments will persuade EPA's scientists to reconsider. However, if they do not, then the burden will shift to the Science Advisory Board to decide that such a PWG is necessary and to insist that the Soffritti study NOT be used for classification purposes until and if such a PWG is conducted and the truth about the tissue diagnosis is finally determined.

We cannot emphasize enough the need for a careful review of EPA's proposed classification of methanol. The gravity of its proposed classification of "Likely to Be Carcinogenic to Humans" and the potential impact of this classification on the beneficial use of methanol in the U.S. and the world requires that this decision not be taken lightly. This classification should not be considered to be "only" a matter of public information, with minimal or no impact on the choices that governments, corporations, and individuals make about the use of this chemical. On the contrary, the potential impact could be very significant and for that reason the criteria must be applied very carefully.

The “Likely to Be Carcinogenic to Humans” should NOT be the chosen classification. Combining several pieces of weak or questionable data, do not make the data adequate for assessment purposes. EPA apparently believes that the existence of several questionable differences from control tumor incidences, which by themselves do not even constitute “limited evidence” constitute “adequate evidence” in the aggregate under the cancer guidelines. Given the extremely weak possibility of carcinogenicity in the body of scientific evidence, we believe the proper classification is instead “Inadequate Information to Assess Carcinogenic Potential.”

Appendix A: Other PBPK Modeling Issues

Because the PBPK model is used for interspecies extrapolation of the point of departure to provide estimates of RfC and RfD for humans, below are some issues with the PBPK model that should be addressed because of impact on the model predictions:

1. While the modeling code and data files have been provided for evaluation, confidence in the model and results would have been stronger had the model been peer-reviewed for publication prior to use in the Toxicology Review.

2. As listed in Table 3-1 and 3-2, there are numerous studies measuring background methanol and formate as well as exposure to natural sources of methanol. Since the PBPK model developed for modeling exogenous, environmental sources of methanol should adequately describe the pharmacokinetics of methanol and its resulting metabolites from any source, the model should have been tested for its ability to predict methanol and formate concentrations from these studies (Stegink 1981; 1983; Davioli, 1986) to help further validate the model. This would provide a means of validating the metabolic constants in humans, which is needed.

3. The value in Table 3-10 for human fractional uptake value does not appear to be from either Ernstgard (2005) or Sevidec (1981). Ernstgard (2005) lists a relative uptake (% of net uptake) as 51.0 (49.1–52.9) and 49.3 (47.3–51.4) for males and females, while Sedivec et al. (1981) reported a slightly higher relative uptake of 53–61%. The value from Batterman et al. (1998) of 79% is higher but isn't comparable to the others because the analysis excluded the first 0.51 L of breath.

4. In addition, the fractional uptake reported in the cited studies was not a measure of the upper airway loss, which is what Perkins et al. (1996) was reporting and measuring for rats. Highly water soluble compounds with blood:air partition coefficients greater than 100 can deposit in the upper airways, lowering the effective alveolar concentration for absorption to blood. As pointed out below the relative uptake measured in Ernstgard (2005), Batterman (1998), and Sevidec (1981) represents the amount of methanol taken up both by the upper airways and diffusion to blood in the alveoli.

5. According to Table 3-10 footnote k: "For human exposures, the fractional availability was from Šedivec et al. (1981, 031154), was corrected for the fact that alveolar ventilation is 2/3 of total respiration rate" However don't need to correct as ventilation drops out of equation for relative uptake since it is calculated as $\%Rel\ Uptake = 100 * \frac{Q(C_{in} - C_{ex})}{Q C_{in}}$. The alveolar ventilation versus minute ventilation would only be a factor in calculating the net uptake.

6. The values for the fractional uptake, alveolar ventilation, and cardiac output are important because they affect the values of other parameters in the model, which were estimated from the literature data. The most important of these are the metabolic parameters, since

the dose metrics for the assessment are total metabolism of methanol and methanol blood concentrations. As shown in figure 1 below, lower metabolic parameters could adequately estimate the Ernstgard data, if lower fractional uptake and more realistic ventilation parameters (Table 1) from Astrand and Rodahl (1977) were used. The fit was visual but could be optimized; fits to other human data can be expected to be similar.

As shown in Table 2, the total predicted metabolism for the same exposure will be less when a higher fraction of methanol is available in the alveolar region for uptake to blood. Lower total metabolism would also be predicted for the ingestion exposures as well. Unfortunately, there is a lack of data following human ingestion, which could be used to provide limits on the metabolic parameters. Because EPA is using total metabolism of methanol as an indicator of formaldehyde, the parameter space for values should be explored carefully.

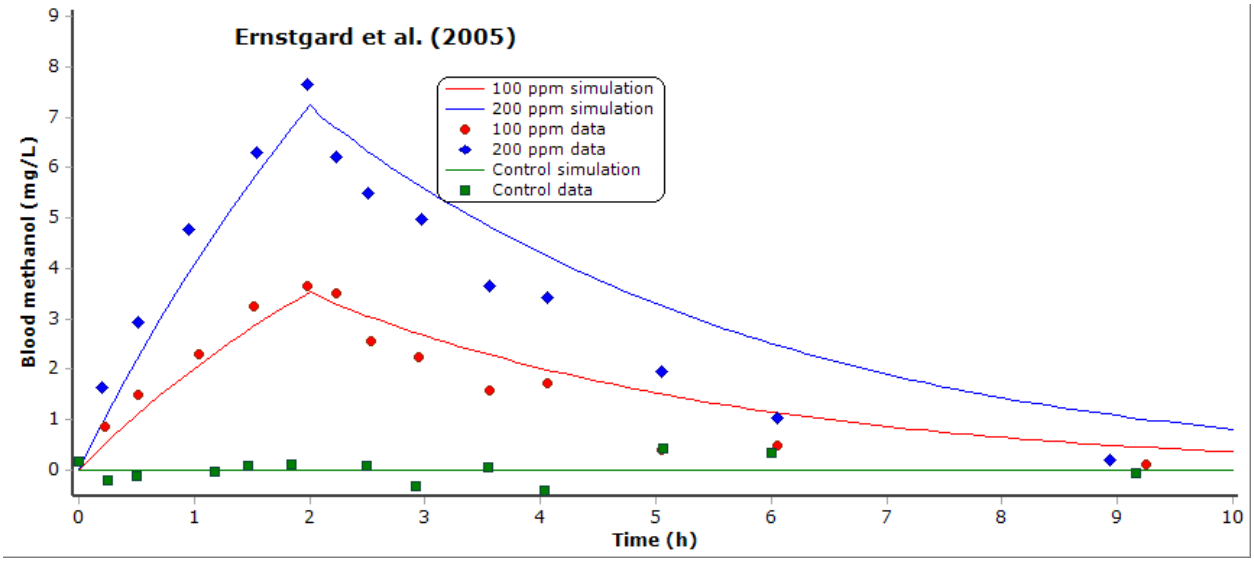


Figure A1: Preliminary fits of Ernstgard data (not corrected for background) with different fraction of exposure concentration available for diffusion in alveoli, alveolar ventilation, cardiac output, and metabolic parameters.

Table A1: Comparison of EPA values with adjusted values for simulations in Figure A1

	Qpc (L/h/kg^0.75)		Qcc (L/h/kg^0.75)		Fracin		Vmc (mg/h/kg^0.75)		Km (mg/L)	
	Rest	Work	Rest	Work	Rest	Work	Path 1	Path 2	Path 1	Path 2
EPA value	24	52.6	16.5	26	0.86	1.0	33.1	15	23.7	45
Modified value	9.9	55	12.4	17	0.6	0.6	15.1	NA	23.7	NA

*Note that the m files set metabolic constants for the fit of Ernstgard which did not match Table 3-10. Files seem to indicate when background was not included, saturable parameters are used and included a Vmaxc2, which is not shown in Table 3-10 in the Review. Figure 3-13 appears to have not included background so saturable parameters were used.

Table A2: Comparison of Predictions of Amount of Inhaled Methanol Eliminated

	Amount Methanol Eliminated (mg)*				
	Metabolism pathway 1	Metabolism pathway 2	Total Metabolized	Exhaled	Excreted
EPA value	449	114	576	11.2	0.93
Modified value	371	0	371	10.8	1.5
Difference=			205	0.4	-0.57

*Total amount metabolized, exhaled, or excreted from time 0 to 10 hours (the remainder is left in tissues) in the Ernstgard study for 200 ppm inhalation exposure. The majority of the actual absorbed dose is exhaled during the 10 hr period including and following exposure (97 and 88%, for the original and modified values, respectively)

PBPK References

Andersen ME; Krewski D (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life Toxicol. Sci. 2009 107: 324-330.

Batterman SA; Franzblau A; D'Arcy JB; Sargent NE; Gross KB; Schreck RM. (1998). Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int Arch Occup Environ Health, 71: 325-335.

Davoli E; Cappellini L; Airoldi L; Fanelli R. (1986). Serum methanol concentrations in rats and in men after a single dose of aspartame. Food Chem Toxicol, 24: 187-189.

Ernstgard L; Shibata E; Johanson G. (2005). Uptake and disposition of inhaled methanol vapor in humans. Toxicol Sci, 88: 30-38.

Sedivec V; Mraz M; Flek J. (1981). Biological monitoring of persons exposed to methanol vapours. Int Arch Occup Environ Health, 48: 257-271.

Stegink LD; Brummel MC; McMartin K; Martin-Amat G; Filer LJ Jr; Baker GL; Tephly TR. (1981). Blood methanol concentrations in normal adult subjects administered abuse doses of aspartame. J Toxicol Environ Health, 7: 281-290.

Stegink LD; Filer LJ; Bell EF; Ziegler EE; Tephly TR. (1989). Effect of repeated ingestion of aspartame-sweetened beverage on plasma amino acid, blood methanol, and blood formate concentrations in normal adults. Metabolism, 38: 357-363.

Dhareshwar S; Stella V (2009). Your Prodrug Releases Formaldehyde:Should You Be Concerned? No! American Pharmacists Association J Pharm Sci 97:4184–4193.

Appendix B: Excerpts from 2005 EPA Cancer Risk Assessment Guidelines: Classification Criteria

"Carcinogenic to Humans"

This descriptor indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.
- Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when all of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on mode of action and also an indication of the relative weight that each source of information carries, e.g., based on human information, based on limited human and extensive animal experiments.

"Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "Carcinogenic to Humans." Adequate evidence consistent with this descriptor covers a broad spectrum. As stated previously, the use of the term "likely" as a weight of evidence descriptor does not correspond to a quantifiable probability. The examples below are meant to represent the broad range of data combinations that are covered by this descriptor; they are illustrative and provide neither a checklist nor a limitation for the data that might support use of this descriptor. Moreover,

additional information, e.g., on mode of action, might change the choice of descriptor for the illustrated examples. Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments;
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

"Suggestive Evidence of Carcinogenic Potential"

This descriptor of the database is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples include:

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system (see discussions of conflicting evidence and differing results, below);

- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed. (When there is a high background rate of a specific tumor in animals of a particular sex and strain, then there may be biological factors operating independently of the agent being assessed that could be responsible for the development of the observed tumors.) In this case, the reasons for determining that the tumors are not due to the agent are explained; evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships); or
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.

"Inadequate Information to Assess Carcinogenic Potential"

This descriptor of the database is appropriate when available data are judged inadequate for applying one of the other descriptors. Additional studies generally would be expected to provide further insights. Some examples include:

- little or no pertinent information;
- conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative. Differing results, that is, positive results in some studies and negative results in one or more different experimental systems, do not constitute conflicting evidence, as the term is used here. Depending on the overall weight of evidence, differing results can be considered either suggestive evidence or likely evidence; or
- negative results that are not sufficiently robust for the descriptor, "Not Likely to Be Carcinogenic to Humans."

"Not Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each mode of action in experimental animals

does not operate in humans. In other cases, there can be convincing evidence in both humans and animals that the agent is not carcinogenic. The judgment may be based on data such as:

- animal evidence that demonstrates lack of carcinogenic effect in both sexes in well-designed and well-conducted studies in at least two appropriate animal species (in the absence of other animal or human data suggesting a potential for cancer effects),
- convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans,
- convincing evidence that carcinogenic effects are not likely by a particular exposure route (see Section 2.3), or
- convincing evidence that carcinogenic effects are not likely below a defined dose range.

A descriptor of "not likely" applies only to the circumstances supported by the data. For example, an agent may be "Not Likely to Be Carcinogenic" by one route but not necessarily by another. In those cases that have positive animal experiment(s) but the results are judged to be not relevant to humans, the narrative discusses why the results are not relevant.

Multiple Descriptors

More than one descriptor can be used when an agent's effects differ by dose or exposure route. For example, an agent may be "Carcinogenic to Humans" by one exposure route but "Not Likely to Be Carcinogenic" by a route by which it is not absorbed. Also, an agent could be "Likely to Be Carcinogenic" above a specified dose but "Not Likely to Be Carcinogenic" below that dose because a key event in tumor formation does not occur below that dose.