Peer Review Workshop
for EPA's Draft
Toxicological Review of Dichloromethane

Reviewer Post-Meeting Comments

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Contents

Reviewer Biographies

James V. Bruckner, Ph.D.

Professor of Toxicology and Pharmacology
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Dr. Bruckner is a professor of pharmacology and toxicology at the University of Georgia. His research includes validation of PBPK models to assist in predicting administered dose/exposure levels anticipated to produce effects in humans, including children. He has served on numerous state and national panels, including the National Academy of Sciences' Board on Environmental Studies and Toxicology and the Life Sciences Institute's Working Group on Physiological Parameters for Children. He has published in numerous professional journals, including the Journal of Toxicology and Environmental Health, Toxicology and Applied Pharmacology, and Toxicological Review.

David Gaylor, Ph.D.

President
Gaylor and Associates, LLC

Dr. Gaylor received a B.S. and M.S. degree in Statistics from Iowa State University and a Ph.D. in Statistics from North Carolina State University. Dr. Gaylor, whose expertise is in the fields of biometry, statistics, and health risk assessment, currently is president of Gaylor and Associates, LLC. He retired from the National Center for Toxicological Research (NCTR), FDA, where he served as the principal advisor to the NCTR Director/FDA Associate Commissioner for Science on matters related to the planning, development, implementation and administration of health risk assessment policies reaching across a wide range of FDA's activities. In a prior position with the NCTR, he was Director of the Biometry and Risk Assessment Division where he was responsible for the administration and scientific direction of the Biometry and Risk Assessment program. In that position, he developed experimental protocols and provided statistical analyses of experiments in carcinogenesis, teratogenesis, mutagenesis, and neurotoxicity, and developed techniques to advance the science of quantitative health risk assessment.

Dr. Gaylor also serves as an Adjunct Professor of Statistics at the University of Arkansas for Medical Sciences and the University of Arkansas, Little Rock. He is a Fellow of the American Statistical Association, the Society for Risk Analysis, and the Academy of Toxicological Sciences and is a member of the Biometric Society, the Society for Regulatory Toxicology and Pharmacology, and the Society of Toxicology. Dr. Gaylor has served on more than 70 national and international work groups and committees on many aspects of biometry, toxicology, and risk assessment. He is currently a member of the editorial board of four professional journals: Risk Analysis, Human and Ecological Risk Assessment, Toxicology and Industrial Health, and Regulatory Toxicology and Pharmacology. Dr. Gaylor has also authored or coauthored more than 160 journal articles, 25 book chapters, and made over 100 presentations at scientific meetings on bio-statistics and a wide range of health risk assessment issues.

Lisa Kamendulis, Ph.D.

Assistant Professor
Department of Pharmacology and Toxicology
Indiana University School of Medicine

Dr. Lisa M. Kamendulis is an Assistant Professor in the Department of Pharmacology and Toxicology at Indiana University School of Medicine. After completing her undergraduate degree in Biology from the University of Massachusetts at Amherst, Dr. Kamendulis received her Ph.D. Toxicology from the University of New Mexico in 1994, where she studied mechanisms of hepatotoxicity, specifically focusing on the induction of apoptosis. Following her Ph.D. work, Dr. Kamendulis completed post-doctoral studies within the Departments of Pathology and Biochemistry at Indiana University School of Medicine, then acquired additional training as an Assistant Scientist in the laboratory of Dr. James Klaunig at Indiana University. Her current research focuses on examining mechanisms by which environmental agents and therapeutic drugs elicit liver cancer, and uses both in vivo and in vitro rodent models to examine the role of oxidative stress in modulating growth regulatory signaling networks and gene expression elicited by nongenotoxic carcinogens. She has published over 40 peer-reviewed manuscripts, and book chapters in the areas of Toxicology and chemical carcinogenesis. Dr. Kamendulis has served the science of toxicology both locally and nationally by serving as Councilor and Secretary-Treasurer of the Ohio Valley Society of Toxicology, as a member and chair of the Placement Committee, and as Councilor in the Carcinogenesis Specialty Section of the Society of Toxicology.

Kannan Krishnan, Ph.D.

Professor, Occupational and Environmental Health
University of Montreal

Dr. Kannan Krishnan is Professor of Occupational and Environmental Health at the University of Montreal (Canada). He obtained his Ph.D. in Toxicology from the University of Montreal and completed his post-doctoral training at The Hamner Institutes (formerly CIIT Centers for Health Research), Research Triangle Park, NC. An expert in the areas of health risk assessment methods, PBPK modeling, chemical mixtures and computational toxicology, Dr. Krishnan has held visiting scientist/faculty appointments at the Karolinska Institutet, Sweden (2004), Toxicology Excellence for Risk Assessment (TERA, Cincinnati, OH) (2007) as well as Environmental & Occupational Health Sciences Institute of UMDNJ-Rutgers University, NJ (2007). Listed in Canadian WHO's WHO since 2006, Dr. Krishnan has served in various capacities at the national and international levels including: U.S. National Academy of Sciences' (NAS) Sub-committee on Acute Exposure Guideline Levels (2001-'04), Risk Assessment, Mixtures and Biological Modeling Specialty Sections of the Society of Toxicology (U.S.A.; 2004- todate), International Programme on Chemical Safety (IPCS) of the World Health Organization (2003-'09), Human Studies Review Board of U.S. EPA (2006-'08) and Workgroup of the International Agency for Research on Cancer (IARC/WHO; 2007). He has also served on the editorial boards of *Toxicological Sciences*, *International Journal of Toxicology*, *Journal of Applied Toxicology*, *Journal of Child Health* and the public access e-*Journal of Toxicology*. A *Fellow* of the Academy of Toxicological Sciences and *Diplomate* of the American Board of Toxicology, Kannan received the prestigious *Veylian Henderson Award* (2000) of the Society of Toxicology of Canada and the *Best paper award in Toxicological Sciences (2003)* of the Society of Toxicology (U.S.A.) for a land-mark publication on the PBPK modeling of metabolic interactions and health risk assessment of chemical mixtures.

Harihara M. Mehendale, Ph.D.

Professor and Kitty DeGree Chair in Toxicology
The University of Louisiana at Monroe

After obtaining his M.S. and Ph.D. degrees from the Toxicology Program at North Carolina State University, Dr. Mehendale received his postdoctoral training at the University of Kentucky and at the National Institute of Environmental Health Sciences (NIEHS), before joining NIEHS as a Staff Fellow. In 1975, he joined the University of Mississippi Medical Center as Assistant Professor, rose through the academic ranks to full Professor in 1980. He joined the University of Louisiana at Monroe (ULM) in 1992. His research interests span across pulmonary, hepatic, renal and general toxicology of medicinal, industrial and environmental chemicals.

Dr. Mehendale's current area of research emphasis involves understanding the role of tissue repair in the ultimate outcome of tissue injury, the mechanisms in control of cell division and tissue repair as well as the molecular events keying these mechanisms. His current research is focused on the impact of age, diabetes, and diet restriction on toxic effects, mechanisms of progression and regression of injuries, and potential adverse health effects of exposure to combinations of chemicals. Molecular, cellular, organ system and whole animal models are employed in genomic and proteomic investigations of the mechanisms of chemically induced toxicities. He has authored over 260 peer-reviewed original research and review articles, as well as book chapters. Dr. Mehendale serves on Editorial Boards of several journals, and reviews manuscripts for several journals. He is Editor-in-Chief of the International Journal of Toxicology, published by Taylor & Francis. He has served on the N.I.H. Toxicology Study Section and continues to participate in the peer-review process for NIH, EPA, OSHA and other funding agencies. He has served on the scientific Advisory Panel of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) administered by USEPA as well as on the Council of the American College of Toxicology. He completed (2002-2002) his service on the National Academy of Science's Committee on Toxicology (COT) and currently (2002-2005) serves on FDA's Dietary Supplements Sub-committee. He has served on the Board of Directors of the American Board of Toxicology. Dr. Mehendale has received several Honors and Awards for his research and scholarly contributions. In 1988, he received the "Burroughs Wellcome Scholar in Toxicology" award given by the Society of Toxicology, U.S.A. In 1993, he received the Zeneca International Travel Award. In 1995, he was named outstanding researcher at ULM and received the "Researcher of the Year" Award. In 1996, the American Association for the Advancement of Science (AAAS) elected him "Science Fellow". In 1999, he received the Best Paper Award for best paper published in Toxicology and Applied Pharmacology. In 2001, received the Society of Toxicology's Education Award for his eminent contributions to education in toxicology. His current research funding comes from ATSDR, NIEHS, NIA and NIDDK. Dr. Mehendale is a certified Diplomate of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences. He is a member of a dozen learned societies.

Martha Moore, Ph.D.

Director, Division of Genetic and Molecular Toxicology
National Center for Toxicological Research
Food and Drug Administration

Dr. Martha M. Moore is the Director of the Division of Genetic and Molecular Toxicology, National Center for Toxicological Research (NCTR), Food and Drug Administration (FDA), Jefferson, Arkansas. Prior to her appointment at NCTR, Dr. Moore was the Chief of the Genetic and Cellular Toxicology Branch, Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency (EPA), Research Triangle Park, North Carolina. Dr. Moore received a BA degree in Biology from Western Maryland College, Westminster, Maryland and a Ph.D. in Genetics from the University of North Carolina at Chapel Hill. She has served on numerous EPA, FDA and other Government Agency advisory groups and committees. She is the chair of an international effort for protocol harmonization for the in vitro gene mutation mouse lymphoma assay. She is a member of the Society of Toxicology, the Environmental Mutagen Society, the Society for Risk Analysis, the European Environmental Mutagen Society, the United Kingdom Environmental Mutagen Society, the Genotoxicity and Environmental Mutagen Society and the Genetic Toxicology Association. Within SOT, Dr. Moore is a member of the Risk Assessment, Carcinogenesis, Regulatory and Safety Evaluation, and the Occupational and Public Health Specialty Sections. Journals in which she has published include; Mutagenesis, Environmental and Molecular Mutagenesis, Mutation Research, Regulatory Toxicology and Pharmacology, and Toxicological Science. Her research interests include: (1) the development and utilization of mechanistically based in vitro and in vivo gene mutation assays (2) the interpretation and use of genetic toxicology data in cancer risk assessment and (3) the integration of rodent and human mutagenicity data in regulatory decision making.

Andrew Salmon, M.A, D.Phil.

Senior Toxicologist and Chief
Air Toxicology and Risk Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Dr. Salmon is a Senior Toxicologist and Chief of the Air Toxicology and Risk Assessment Section at the Office of Environmental Health Hazard Assessment, which is part of the State of California's Environmental Protection Agency (Cal/EPA). As a research toxicologist in industry and academia, Dr. Salmon has worked on cancer mechanisms, metabolism and pharmacokinetics, inhalation toxicology and safety evaluation for environmental and occupational exposures. His current activities include application of benchmark dose methodology and evaluation of special impacts on children's health in air toxics risk assessment. In addition to editing and contributing to numerous chemical-specific risk assessment documents and procedural guidance documents for the State of California, he is a contributing author on a number of papers published in journals such as Preventive Medicine, Environmental Health Perspectives, and the Journal of Toxicology and Environmental Health, and has made several presentations at meetings of the Society of Toxicology and Society for Risk Analysis. Dr. Salmon received his bachelor's and doctoral degrees in Biochemistry from Oxford University, U.K.

Responses to Charge Questions

General Charge Questions

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Reviewer	Comments
Bruckner	This Toxicological Review is very comprehensive, accurate and well written. It is obvious that considerable time and effort were expended in compiling and writing it. The accounts of pertinent studies and PBPK modeling exercises are clear and concise, yet there is a great deal of repetition throughout the document. Although formatting requirements likely dictated much of the redundancy, it is so excessive it detracts from the document. The accounts of relevant scientific investigations are presented objectively, yet the summary sections and rationales for decisions do not provide balanced overviews for the reader to consider in assessing the weight of scientific evidence on particular questions or subjects. Only findings/evidence in support of EPA's judgements and courses of action are presented.
Gaylor	The Toxicological Review is presented in a logical order, it is well written, and each issue is presented concisely. The Review considered a large number of cancer and non-cancer studies. The choices of the most relevant studies for the derivation of the RfD, RfC, OSF, and IUR and critical endpoints were clearly and objectively synthesized.
Kamendulis	Overall this toxicological review is well written, clear and concise. The science used to assess noncancer and cancer hazards for the most part, is objectively presented. Areas of concern are detailed in the appropriate sections below.
Krishnan	This document presents the background information and justification for the hazard and dose-response assessment of dichloromethane. It includes the derivation of an oral reference dose, inhalation reference concentration as well as a cancer assessment. This draft document summarizes a lot of relevant literature of pertinence to this assessment. In general, the synthesis of published information would appear to be done well and presented clearly – as it relates to the cancer and non-cancer hazards, mode(s) of action (MOA) as well as sources of uncertainty. State-of-the-art models and modeling techniques have been used for conducting the dose-response assessment of cancer and non-cancer endpoints. However, there are a number of areas related to the dose-response assessment that require clarification or improvement to ensure that their intended use does not contradict or overlap among themselves. A major concern for this reviewer is the use of a pharmacokinetic scaling factor for obtaining human-equivalent metabolite dose (concentration), to account for the fact that the DCM PBPK model only allows the prediction of metabolite formation and not metabolite concentrations. This then leads to a computation of a clearly more uncertain dose metric that is desired based on its closeness to the response; its preferred use over a more certain (but somewhat less desirable) dose metric obtained with the PBPK model is questionable. To illustrate this point, Figures 1 and 2 are attached to this report (see pages 69-70). Figure 1 indicates that the desired dose metric is the one that is closer to

	the response whereas the feasible one (that can be obtained with the model in hand) is somewhat removed from it. In the case of DCM for example, the current risk assessment desires to have information on metabolite concentration (i.e., formation minus clearance) whereas the PBPK model can only provide confident estimates of metabolite formation in the target organ. There is a dilemma here in terms of whether a more certain dose metric simulated by the model is more reliable, or a less certain dose metric obtained with the application of a correction factor applied to the model-simulated dose metric is more reliable for use in assessment. In essence then, the residual uncertainty associated with the default approach is reduced but not eliminated totally by the application of currently available DCM PBPK models (Figure 2) whereas the additional application of allometric correction as a way of eliminating residual uncertainty associated with the PBPK models, will reintroduce the source of uncertainty into the process. The intent of computing the metabolite concentration is superior to that of using metabolite formation as the dose metrics. However, given the limitations of the current models and lack of relevant data on metabolite kinetics, it is not realistic defensible to focus on a dose metric that cannot be predicted or measured with confidence at this time. In this context, it is pragmatic to balance the "ideal dose metric" needed for risk assessment with the "reliable dose metric" that can be obtained with the peer-reviewed PBPK model (Figure 1).
Mehendale	1. Page iv. Item 4.3.2.1 Gavage Studies and Culture Studies can be organ, tissue or cell culture. Specify which. Rat embryo culture? 2. Page iv. Item 4.6.1.2 Summary of Animal <u>Data</u> To be consistent with other side headings, should this not be Animal <u>Studies</u>? 3. Items 4.6 and 4.7.2. Synthesis 4.6 Synthesis of major noncancer effects Synthesis does not sound right here. You are not synthesizing effects are you? Effects are observed. 4.7.2. Synthesis of Human, Animal and other Supporting Evidence? 4. Table 4.16, page 118 and ix. Specify in the title if the data are from rats, mice, or humans. 5. Page 4, last sentence, biodegrade to toxic or nontoxic products? Specify. 6. Page 8, lines 6-7. Only three values are given for four tissues. Liver, kidney, adrenal gland, and brain. What gives? 7. Page vi, item 6. Conclusions in or on the characterization? 8. Page vii D.1. and D.2. 1986a not 1986A; likewise, 1988a not 1988A. Since you are referring to the respective publications listed in references. 9. Same in D2 on page vii. 1986b not 1986B. 10. Page 47, lines 1-3 bottom up. This statement may confuse the reader. What is not

	clear is a quantitative sense. "The systematic discrepancy …..is much less obvious….." The question is not whether the difference is less or more obvious in a graphical presentation. Is there a difference? How much is it? How can the difference be explained? Resolved? If it cannot be resolved, why not. Rewrite. *See also Mehendale comments 11–17 in response to question A1.a; comment 18 in response to question A2a; and comment 19 in response to question A3a, below, all of which also respond, in part, to question G1.*
Moore	Yes, I believe that the document is thorough and is well written. The authors have done a good job of using tables to present the summaries of available data. The tables are constructed to provide useful information such as endpoint, exposure and dose. The descriptions of the various studies generally include enough information to provide the reader with the information necessary to draw independent conclusions.
Salmon	In general, the document represents a logical, clear and objective presentation and analysis of the scientific evidence on the non-cancer and cancer hazards of exposure to dichloromethane. Although I have some points of disagreement in detail, as noted below, in general the Agency is to be commended for its thorough and thoughtful analysis of this large and complex data set. A document of more than 300 pages with substantial appendices can scarcely be characterized as "concise", but considering the volume of material needing to be covered the authors have done a good job of avoiding unnecessary prolixity.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Reviewer	Comments
Bruckner	Additional studies that provide relevant information are referenced at the end of my review.
Gaylor	Not aware of any additional studies.
Kamendulis	I did not identify additional studies that would impact the conclusions from this review.
Krishnan	None. See individual sections below for additional, relevant references.
Mehendale	*No comments.*
Moore	I am not aware of any additional studies that should be included.
Salmon	None identified.

Chemical-Specific Charge Questions

(A) PBPK Modeling

A1. A rat PBPK model was used for calculating the internal dosimetry for the RfD and RfC. EPA evaluated several versions of previously published rat PBPK models and modified the Andersen et al. (1991) model for use in the reference value calculations.

a. Does the chosen model with EPA's modifications adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

Reviewer	Comments
Bruckner	The rat PBPK model of Andersen et al. (1991), as modified by EPA, appears to adequately represent dichloromethane (DCM) toxicokinetics (TK) in the rat. The model assumptions, parameters and uncertainties have been clearly presented and discussed. The model expansion was done by competent individuals, though additional review/input by other modelers, including those who originally developed the models would provide more assurance of the validity of the model extensions/revisions.
Gaylor	Outside my area of expertise.
Kamendulis	To the extent of my knowledge, this model appears to have been updated using known human variability in metabolism through both the CYP2E1 and GST-T1 pathways. Many changes were apparently incorporated into the current PBPK model used in this document, it might be useful to include a table listing the changes from the model used in the previous assessment, as well as the justification for the change. The model appears to have been applied appropriately, however, the model assumptions for how variability in CYP2E1 was incorporated into the model. Throughout the document, EPA clearly states that CYP2E1 variability was incorporated into the model, however, specific information on this is included only in the appendix. Including information on how CYP2E1 variability was incorporated into the PBPK model in section 3 would be useful, as it would assure reviewers that this variable was accounted for in the model prior to reviewing the appendices.
Krishnan	From the material presented in the document, the rat model would appear to be deficient in some aspect related to the description of metabolism or another key aspect. Based on the arguments and simulations presented, it would appear that the model version D is the best. Such a conclusion should preferably be based on comparative simulations of dose metrics as well as some assessment of quantitative fitting analysis. In this regard, there does not appear to be a priori strategy of model averaging or a quantitative method for choosing the best model, it seems. Also it is totally unclear as to why version A in the rat was developed (reflecting the flux via GST pathway in lung tissue of *mouse*, a species that is thought to be more sensitive than rat) – even though all version of the

	model are described well and appear to be consistent with the model files provided in this document (in Appendix). Ideally, the strategy should be to evaluate a published model in its entirety for its potential usefulness in risk assessment – i.e., whether the capability, structure and parameters of a model correspond to the needs of the risk assessor. Whereas it is likely that some models in peer-reviewed literature just do not meet the requirements of an assessment, there has to be a strong case to significantly rework the model (or re-parameterize) during the evaluation and use in risk assessment, as is the case here. Even though visual inspection is a commonly used approach to assess how model simulations compare with experimental data, it is advisable to take into account the variability in experimental data. This is especially important if a version of a model is to be compared with another – particularly while assessing their fit to experimental data (without the use of any statistical method). In this regard, Figure C4 could make use of the variability/bounds given in Figure 1 of Angelo et al. (1986). Also, the Figure C-10 presents % dose expired in the rat; but is unclear as to whether both the experimental data and simulations reflect the same measure (with/out variability). It might be that the rat PBPK model, in the present form, is inadequate to simulate CO kinetics as described in the document. However, it is difficult to follow the argument in the absence of reproduction of the simulations of the original publication (Andersen et al. 1991) regarding COHB in the rat (Figures 3A, 3B, 4). Specifically Figure 4b from the original paper is not included in the EPA document. In the opinion of this reviewer, it is important to, first, reproduce the simulations reported in the original study before attempting to refine the parameter estimates of the rat model. In doing so, it is important to observe the following: 1. VPR which is initially set to 0.42 in the rat model should be reset to an appropriate value during model runs (e.g., 0.94; page H23). 2. The Kf value of 2.2 in Andersen et al. (1987) is given for a rat of specific body weight (233 g) and not for 1 kg rat. However in the 1991 publication it would appear to relate to an animal of 1 kg. These aspects should be carefully verified to ensure that the value of this parameter used in the model essentially allows the reproduction of the kinetic profiles reported in the original publications.
Mehendale	1. Page 9, line 11, bottom up. Why do you say "Dichloromethane is thought to …..," when in the very next sentence, you provide evidence for it? Do you need any more evidence? 2. Page 11. Does CYP2E1 pathway saturate at higher CYP2E1 levels such as may be encountered in alcohol-dependent and in individuals taking chronic antiepilepsy drugs? Barbiturates? Clarify. 3. Page 12. McKenna and Zempel, 1981 reported inhibition of CYP2E1 pathway when dichloromethane exposure was increased from 1 mg to 50 mg/kg in rats. Please comment for not considering this observation (lines 18-19 bottom up). How does this finding relate to saturation of CYP2E1 pathway in humans at 400

	ppm dichloromethane (Ott et al., 1983e)? 4. Pages 11-13. One would expect CO to bind to CYP2E1 and prevent further metabolism of dichloromethane (DCM). This has to affect the dynamics and kinetics of dichloromethane metabolism by CYP2E1. This would also affect the availability of DCM for metabolism by the GSH pathway. None of this has been considered or discussed in the document. As soon as CO is formed via CYP2E1 catalysis, it is highly likely to bind to CYP2E1, thereby disengaging any further DCM metabolism via CYP2E1. What is the impact of CO.CYP2E1 on overall metabolism of DCM? Likewise, the impact on the quantitative aspects of DCM metabolism via GSH pathway is not discussed. The method for quantitation of CYP2E1 or any cytochrome P450 involves reduction of CYP450 by adding a pinch of dithionite (to reduce P450 from the oxidized state) and bubbling CO with a small stream of the gas so that it binds to the cytochrome P450 to form carboxy cytochrome P450 (CO.CYP2E1 in the present context) to quantitate the P450 content from the differential spectrum obtained by scanning at 450 nm. Carbon monoxide avidly binds to cytochrome P450 and forms CO.CYP 450, indicating the tenacity of CO binding to CYP 2E1. 5. The generation of CO may be expected to increase with higher P-450 CYP2E1 content in the liver. The impact on CYP2E1 catalysis is not considered. Because the affinity of CO to bind to hemoproteins is very high, and any interference with ongoing DCM metabolism via CYP2E1 may be mistakenly interpreted as saturation of CYP2E1 pathway of metabolism of DCM. How much of DCM metabolism by CYP2E1 is likely to be affected by inhibition of CYP2E1 catalysis of DCM oxidation by CO.CYP2E1? Such spillover of DCM to the GST pathway as a consequence of inhibition by CO is highly likely to be counted as “spillover due to saturation”, thereby adding to errors in any estimates of the products formed via the two pathways. No matter how sophisticated the PBTK model is for DCM, it is fraught with daunting errors, unless the inhibition of CYP2E1 by CO is fully taken into account. 6. Page 14, lines 7-8 bottom up. Metabolism by GST pathway may increase for another reason when DCM concentration increases. As CO.CYP2E1 increases, proportionately greater amount of DCM is likely to enter the GST pathway. The document needs to include a section to consider this and other impacts (is “saturation of DCM oxidation by CYP2E1 pathway” not really inhibition of CYP2E1 pathway by CO.CYP2E1?). 7. Page 46 and throughout. The two-fold difference in km value for DCM oxidative metabolism to CO is bothersome. This introduces considerable error-prone stress and uncertainty on the PBTK model for DCM. The potential reasons for the discrepancy (2-fold difference) and the overall impact on the PBTK model derived for DCM should be discussed as close to resolution as possible. (a) What, if any, influence would CO.CYP2E1 formation have on the high affinity and low affinity kms, on the overall km? (b) Both in vitro and in vivo estimates involve some unrealistic DCM exposure concentrations. (c) At very high DCM concentrations, these parameters tend to be skewed. (d) How realistic are the assumed DCM

	exposure concentrations? (e) What, if any, is the impact of formaldehyde produced during the metabolism of DCM, on the oxidative metabolism of DCM? (f) Does formaldehyde affect the GST pathway of DCM metabolism? The answers to these questions and discussion of these factors are missing from the document. If these questions cannot be answered, how will the IRIS document be affected? *See also Mehendale comment 18 in response to question A2.a below.*
Moore	PBPK modeling is outside of my specific expertise.
Salmon	The model chosen is the latest development of a basic model structure which has been around for quite a long time now, and has benefited from various revisions and updates both in terms of model structure and parameter values. As a result of these progressive refinements the model appears to describe the toxicokinetics in animals well, and is able to fit independently observed kinetic data adequately. Description of the model is thorough and the basis for the various parameter values chosen is clearly presented. In particular, EPA is to be commended for providing full documentation of the model and the parameter values used, in the descriptive section and Appendices. The rat model is less advanced than those applied for the mouse and human, in that it uses single deterministic values for model parameters rather than the probabilistic approach where distributions of values are estimated to reflect uncertainty and/or variability. This is inevitable given the lack of sufficient data to support a probabilistic analysis for the rat. However, the deterministic version of the model has been reasonably validated, and the variability inherent in the laboratory strain of rat is at lot less than what is expected in the "wild type" human so the probabilistic aspect of the model is less essential. Confidence in the model is enhanced by the inclusion of a sensitivity analysis for key parameters. Remaining uncertainties are appropriately identified. The major uncertainty affecting the rat model is the finding that the CYP2E1 pathway appears to not show simple Michaelis-Menten kinetics, but rather some other behavior, perhaps because of multiple CYP isoenzymes with different affinities but more probably based on cooperative binding of substrate. This has not been incorporated into the current version of the model: as noted by EPA, it would be desirable to deal with this once sufficient additional data (e.g. enzyme kinetic data *in* vitro including a Hill coefficient if the cooperative binding result is confirmed) are available to support the change objectively. EPA's analysis shows that although there are currently inconsistencies between experimental observations of CO formation and model predictions for the CYP2E1 pathway, the impact of this uncertainty on the dosimetry used in the derivation of reference levels and cancer potencies is not large compared to some of the other inevitable uncertainties.

b. The internal dose metric used in the RfD and RfC derivations was based on total hepatic metabolism via the CYP2E1 pathway. Because the metric is a rate of metabolism, and the clearance of metabolites is generally expected to be slower in the human compared with the rat (assuming clearance scales as $BW^{3/4}$), the rat internal dose metric is adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric selection and calculations appropriately considered and discussed?

Reviewer	Comments
Bruckner	The choice of total hepatic metabolism by the CYP2E1 pathway is reasonable, given the modest hepatocellular changes DCM produces and the lack of knowledge of the putative toxic moiety. I do not necessarily concur that scaling of metabolism is preferable, given the availability of experimentally-derived or model predicted, species-specific metabolic rate constants. Adjustment of the rat internal dose metric by an arbitrary scaling factor yields a human-equivalent dose that is excessive. Clearance of unidentified CYP metabolites in rodents versus humans remains an unknown.
Gaylor	Outside my area of expertise.
Kamendulis	I agree with the dose metric selected (total hepatic metabolism via the CYP2E1 pathway) to derive RfD's and RfC's. While human data does not support liver toxicity as a primary effect, there is not always concordance in target sites between rodents and humans. Thus, liver effects appear as the principal effect is the available literature and therefore, CYP2E1 metabolism is expected as the pathway that would contribute towards liver effects. EPA states that the clearance of metabolites is generally expected to be slower in the human compared with the rat, so it is proposed to adjust the rat internal dose metric by dividing by a toxicokinetic scaling factor ($BW^{3/4}$) to obtain a human-equivalent internal dose. The application of this scaling factor was used to characterize the uncertainty of the metabolite concentration (effective concentration) at the target site. EPA did not discuss why a scaling factor was used rather than applying an uncertainty factor. The document should show outcomes if a UF of 3 was applied to characterize the uncertainty in not knowing the metabolite concentration at the target tissue vs. using the allometric scaling factor selected. In addition, the scientific rationale for selecting the scaling factor ($BW^{3/4}$) rather than another scaling factor (BW0.9 or other) was not presented.
Krishnan	The dose metric based on CYP pathway has been justified in a limited manner. This dose metric adjustment factor (based on ratios of $BW^{0.25}$ based on the assumption that the metabolite clearance might scale to $BW^{0.75}$ and the volume of its distribution might scale to BW^{1}) is questionable. In fact, there is no confidence in applying any of these allometric rules for predicting DCM metabolite concentration in target tissues of rats and humans, given that the qualitative and quantitative difference in mechanisms

	across species is not known or presented. Normally, the application of the body surface scaling as done would be appropriate when the chemical entity itself is the active moiety, the metabolism/reaction renders it inactive, and the rate of the metabolism/reaction process is proportional to the liver perfusion rate, cardiac output or to the body surface. The draft document does not clearly provide scientific support along these lines to justify the use of a pharmacokinetic scaling factor – which actually is used as a "dose metric extrapolation factor" for predicting a dose metric (i.e., metabolite concentration) based on a PBPK model not tested or intended to do that. It would appear then that the application of a dose metric extrapolation factor (in addition to PBPK modeling) will only add to and not reduce the residual uncertainty related to the use of PBPK-derived dose metric. In this regard, the use of a pharmacokinetic scaling factor (i.e., dose metric extrapolation factor) for the metabolite dose, without regard to its mechanism of clearance and reactivity, only with the PBPK modeling approach and not the default approach will draw further criticism and/or require unproductive re-evaluation of the magnitude of the default uncertainty factor both for interspecies and intraspecies extrapolations. An alternative would be to use both the dose metrics (i.e., PBPK-derived and pharmacokinetically-scaled) in relation to the default approaches and then at the end, choose the most appropriate one on the basis of residual uncertainty (or level of confidence) as well as relevance to mode of action (i.e., most desired measure of internal dose), i.e., balance the prediction uncertainty and relevance to MOA.
Mehendale	*See Mehendale comments 11–17 in response to question A1.a above, and comment 18 in A2.a below, all of which also respond in part to question A1.b.*
Moore	PBPK modeling is outside of my specific expertise.
Salmon	The total hepatic metabolism via the CYP2E1 pathway is an appropriate choice for the internal dose metric in derivation of the RfC and RfD. It appears that this pathway is predominant in the mechanism of induction of liver lesions in the rat, which are the critical effect used in these derivations. However there appears not to be clear evidence to identify a specific metabolite or intermediate as the causative agent, at least with sufficient confidence to justify selection of a metabolite AUC or peak concentration as the dose metric. Given this choice, the assumption that is sometimes made that equivalent tissue levels or process throughputs would have equivalent toxicological effects has to be modified to allow for possible allometric scaling of processes such as Phase II metabolism. This would change the relationship between the rates or concentrations actually modeled and the rates or concentrations for the actual proximate toxicant (whose identity is unknown), with differences in species. If such protective mechanisms are active and scale with $BW^{3/4}$, it is appropriate to apply this scaling factor. The rationale for the decision to apply this correction is described in the document, but further clarification as to why this was considered appropriate and whether any alternative approaches were considered would be helpful. Additionally, some discussion of whether this adjustment overlaps to any extent with any of the uncertainty factors used in the RfD/RfC derivation, or the body weight scaling ratio used in carcinogenic potency calculations, is necessary. Arguments can be made that

	some such overlap may exist, but it is important to note that this adjustment is strictly confined to toxicokinetic modeling considerations and does not address the toxicodynamic differences which are also addressed by uncertainty factors or scaling ratios.

A2. The mouse PBPK model used in deriving the cancer risk estimates was based on the published work of Marino et al. (2006).

a. Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

Reviewer	Comments
Bruckner	The PBPK model of Marino et al. (2006) is the appropriate model to use to predict internal dosimetry in the mouse. The model appears to have been properly applied. The model assumptions, parameters and uncertainties were clearly discussed and considered.
Gaylor	Outside my area of expertise.
Kamendulis	I have no concerns regarding the PBPK model used. To the extent of my knowledge, this model appears to have been updated using known human variability in metabolism through both the CYP2E1 and GST-T1 pathways. The model appears to have been applied appropriately, however, the model assumptions for how variability in CYP2E1 was incorporated into the model. As indicated in response to A1 above, throughout the document, EPA clearly states that CYP2E1 variability was incorporated into the model, however, specific information on this is included only in the appendix. Including information on how CYP2E1 variability was incorporated into the PBPK model in section 3 would be useful, as it would assure reviewers that this variable was accounted for in the model prior to reviewing the material in the appendices. Similar to the rat modeling section, it would be useful to include a table listing the changes incorporated in the current PBPK model from the model used in the previous assessment, as well as the justification for the changes.
Krishnan	Yes. The difference in Pb value compared to the previous version is striking (23 vs 8.29); however, it seems that it is supported by experimental measures (pages 27-28).
Mehendale	8. Pages 9, 12, 17, and 24. The document states that, of the two pathways for dichloromethane (DCM) metabolism, the CYP pathway (page 9) "*is predominating at low exposure levels*" and "*At higher exposure levels, the CYP pathway becomes saturated and a second pathway begins to predominate*." The second pathway is the glutathione-S-transferase (GST) pathway. On page 12, it states that in humans, the saturation of the CYP pathway "*appears to be approached in the 400-500 ppm range*." Is it saturation or inhibition by CO.CYP2E1? Since EPA asserts the mechanism for tumors requires the GST pathway, DCM levels below the saturation

	(if it really exists) of the CYP pathway should have either (a) no risk or (b) a highly attenuated risk relative to that calculated from the point of departure (POD) of the mouse data where tumors were observed, i.e., above the saturation of the CYP pathway where the additional exposure to dichloromethane will end up being all metabolized by GST, the riskiest pathway.
Moore	PBPK modeling is outside of my specific expertise.
Salmon	Description of the model is thorough and the basis for the various parameter values and distributions chosen is clearly presented. In particular, EPA is to be commended for providing full documentation of the model and the parameter values used, in the descriptive section and Appendices. This probabilistic model is a useful way of quantitatively incorporating the variability and uncertainty observed in the various sources of published data, providing not only a best overall estimate for the dosimetry of interest but also an objective measure of the uncertainty. Presentation and analysis of the model results and uncertainties is thorough. As noted in the response to charge question A1a, the main uncertainty in the model structure is the divergence from simple Michaelis-Menten kinetics for the CYP2E1 pathway also noted in the discussion of the rat model. This is a source of concern, but will probably have less impact on the cancer risk assessment based on the mouse model, where the focus is on the glutathione pathway and the CYP2E1 pathway is probably approaching saturation in the dose ranges of interest for interpretation of the bioassay results.

b. The internal dose metric used in the cancer quantitation was based on tissue-specific GST metabolism. To account for potential clearance rate differences, the mouse internal dose metric was adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric selection and calculations appropriately considered and discussed?

Reviewer	Comments
Bruckner	No comment.
Gaylor	Outside my area of expertise.
Kamendulis	I agree with the selection of tissue-specific GST metabolism as the dose metric used for deriving cancer values. As indicated in response to A1 above, the use of a scaling factor to obtain a human-equivalent internal dose, based on the presumption that the clearance of metabolites is generally expected to be slower in the human compared with the rat is not scientifically justified. See comments under A1 on use of scaling factor.
Krishnan	The use of a toxicokinetic scaling factor is somewhat similar to the use of a allometric scaling factor previously used in DCM assessment to account for interspecies differences in sensitivity. In the present case, however, the use of the scaling factor is

	not justified on the basis of existing scientific database on DCM or for another relevant surrogate chemical (or its metabolite). Assuming all of the formed metabolite becomes available and all of it is cleared by a process that scales to $BW^{0.75}$ is not supported by the literature (e.g., Mahmoud I and Sahajwalla C. 2002. Interspecies scaling of biliary excreted drugs. J Pharm Sci 91: 1908-1914; Mahmood I. 2005. Interspecies Pharmacokinetic Scaling: Principles And Application of Allometric Scaling, Pine House Publishers, Maryland) or the present draft document. The application of the toxicokinetic scaling as done would be appropriate when the chemical entity (metabolite) itself is the active moiety (which is the case), further metabolism/reaction renders it inactive (which is likely the case), and the rate of the metabolism/reaction process is proportional to the liver perfusion rate, cardiac output or to the body surface (which is not known to be the case). The draft document does not clearly provide scientific support along these lines to justify the use of a pharmacokinetic scaling factor – which actually is used as "dose metric extrapolation factor" for computing a dose metric from the results of a PBPK model that is not tested or parameterized to simulate that very dose metric. *See also response to Question A1b, above.*
Mehendale	*See response 18 to Question A1b, above.*
Moore	PBPK modeling is outside of my specific expertise.
Salmon	As far as the analysis of the carcinogenic response in the mouse is concerned, the choice of dose metric as the tissue-specific GST metabolism is justified, since the target tissues are clearly established in this species and there is supporting evidence for the assumption that the GST pathway is the greatly predominant (although not necessarily exclusive) source of the genotoxicity. Extrapolation of this conclusion to the human is however somewhat more problematic. Since the percentage of metabolism in the human by the CYP2E1 pathway is much greater, even a small contribution to genotoxicity from this pathway, which would not be noticeable in the animal experiments or studies *in vitro*, might make a significant contribution. Moreover, the whole interspecies extrapolation approach is contingent on the assumption that the target tissues in the mouse are the same, exclusively, in humans. There are some grounds for supposing that this latter assumption is not necessarily valid, specifically in relation to possible observations of leukemia, which will be addressed in later comments. There are therefore some significant uncertainties in the animal to human extrapolation of the internal dose metric from the mouse model. It is certainly appropriate to include a scaling factor to allow for possible interspecies differences associated with choice of GST metabolism as the dose metric, and the EPA has appropriately explained their rationale based on possible clearance rate differences. However, the questions of site concordance and possible minor contributions from the CYP2E1 pathway have not been explicitly factored into the determination of a human equivalent dose metric, although they are referenced elsewhere in the discussion. Thus this calculation may underestimate the human exposure to reactive metabolites at relevant tissue sites, even after making the adjustments described.

A3. A probabilistic human PBPK model (David et al., 2006) was used to estimate a distribution of human equivalent doses and concentrations for the points of departure (PODs) for the RfD and RfC, respectively. The 1st percentile of these distributions was selected to represent the most sensitive portion of the population. For the derivation of the oral and inhalation cancer risk estimates, the probabilistic human PBPK model was used to calculate the distribution of human internal doses (mg dichloromethane metabolized via the tissue-specific GST pathway per unit volume of tissue) that would be expected from a 1 mg/kg-day oral dose or a 1 µg/m3 inhalation concentration. This distribution of human internal doses was used with the tumor risk factor to generate a distribution of oral slope factors or inhalation unit risks.

a. Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

Reviewer	Comments
Bruckner	One key assumption in this TK prediction is subject to question (i.e., some DCM is metabolized by the GST pathway, no matter how low the DCM dose). What scientific evidence is there to support this assumption?
Gaylor	Outside my area of expertise.
Kamendulis	To the extent of my knowledge, I have no concerns regarding the PBPK model used. It has used modern science to better address rodent and human values and variations in metabolism. Some questions are raised concerning the rationale and whether scaling factors and other factors applied for the derivation of RfC's and RfD's were scientifically justified. While it is clear that that intent is to derive toxicity values that are protective of the most sensitive populations, it appears that the estimates may be overly conservative. 1) See comment in A1 concerning the use of scaling factors, 2) More information needs to be included in the BMD section on the EPAs rationale/reasoning on the selection of the model used in calculations – (Tables 5-2 and 5-6), such as, how (or) do these models relate to the available scientific data? would one model be more scientifically credible? 3) For the derivation of the oral and inhalation cancer risk estimates, the probabilistic human PBPK model was used to calculate the distribution of human internal doses and the 1st percentile was selected to represent the most sensitive portion of the population. Using this value will indeed represent the most sensitive populations, and as indicated in the footnotes of tables 5-3 and 5-7 , replaces the use of a UF for human toxicokinetic variability. At least in the case of the RfD derivations, using the 1st percentile provides a HED value that is well below (~7-fold) that which would be derived if an uncertainty factor of 10 was applied (1.51 versus 0.216). In addition, the PBPK models used incorporate population variability into the modeling, thus using the 1st percentile is overly conservative since the models already incorporated measures of variability. I do not agree that the 1st percentile should be used in this calculation, using the 1st percentile on top of the use of a scaling factor, and modeling using the most sensitive GST genotype over adequately accounts for human variability.

Krishnan	Yes – with respect to the questions on the human PBPK model; see also responses to Qs in the section on cancer risk assessment.
Mehendale	9. Pages 9, 12, 17, and 24. The review document repeatedly (e.g. page 17) states that, with regard to the GST pathway, the activity is greater in mice than rats, and greater in rats than the most sensitive human, i.e., those who are GST (+/+). It states that GST +/- people have less activity, and GST (-/-) the least. The data demonstrate that rats exposed to DCM do not have statistically significant increases in carcinogenic tumors. In 1987 EPA (page 24) applied an interspecies scaling factor "*to account for the* presumed *higher human responsiveness, relative to mice, to dichloromethane-induced cancer*." However, the differences between the scaling factor used and presumed higher human responsiveness in 1987, versus the assumptions in this draft toxicological review are not discussed in the IRIS document. *See also Mehendale comment 18 in response to question A2.a above.*
Moore	PBPK modeling is outside of my specific expertise.
Salmon	The extent to which the model is representative of the situation in humans is always harder to establish precisely, but it appears that this has been validated to the extent possible. EPA's analysis provides an important improvement to the human model by evaluating and extending the Bayesian MCMC analysis to address likely human variability in the population as a whole, rather than the more restricted range of subjects examined in the previously published version of the model. With this new extension of the model, it is correctly applied for development of the cancer potencies, RfD and RfC. In general, the human model appears to be well constructed and validated, and the probabilistic approach is an effective way of addressing the variability and uncertainty inevitable in the effort to represent a diverse human population. There is one specific uncertainty which has a somewhat significant impact on the predicted dosimetry used in the risk assessment, and this is the value chosen for the blood:air partition coefficient (PB). The value used in the mouse model is updated to reflect experimentally determined value reported by Clewell (1993): EPA describes this new value and concludes it is preferable to the previous value reported by Andersen et al. (1987), in particular being more concordant with values reported for rats, and hamsters. The new value (23) is substantially higher than the older one (8.3). However, the human model continues to use the value reported by Andersen et al. (1987), which is much lower (9.7) and more comparable to the old mouse value reported by the same authors. One is prompted to wonder whether the increased values reported in Clewell (1993) reflect some underlying difference in the experimental methodology used by these authors compared to Andersen et al. (1987), and therefore if the higher values for PB are in fact correct then the human value chosen is a substantial underestimate. EPA does provide analysis of how the changed PB value for mice affects the relative dosimetry in humans *vs.* mice, but does not appear to have considered that the PB value chosen for humans is in fact in error. It is not entirely clear why the human PB value should be so substantially different from the other species, since this partition coefficient is

	presumably dependent more on physical chemistry rather on any biochemical parameters which would be expected to differ substantially between species. Indeed, this argument is used to justify the choice of the Clewell (1993) value for the mouse: at the very least it is necessary to explain why it does not have the same force for humans. This issue needs further examination, given that it potentially impacts determination of the health protective values. The issue of non-Michaelis-Menten kinetics of the CYP2E1 metabolism of DCM is also a potential confounder of the human model, as was described in relation to the rat and mouse models. There do not appear to be any specifically human data to address this question, but it needs to be noted in the description of the model. As noted in the comments responding to question A2b, there are issues connected with site concordance and possible minor contributions from the alternate metabolic pathway which complicate the definition of an appropriate dose metric for use in the human PBPK model. These add uncertainty in the use of this modeling approach to extrapolate risk predictions from the mouse to the human.

b. EPA modified the parameter distributions in the published David et al. model. Does the set of model parameter distributions adequately account for population variability and parameter uncertainty in estimating human equivalent doses? Are the human parameter values and distributions clearly presented and scientifically supported?

Reviewer	Comments
Bruckner	No comment.
Gaylor	Outside my area of expertise. Choice of the 1st percentile to account for population variability is supported.
Kamendulis	See comments in a. above.
Krishnan	• Why use GSD based on trichloroethylene model? Why not base it on the data on the distribution CYP protein? • This reviewer is not convinced of the adjustment based on BW (re: figures B2, B-3). The decision to not to make the adjustment based on liver volume is still unclear (pages B-6, B-7). • In determining the distribution of K_fC for U.S. population from the data of Swedish subjects, was the proportion of caucasians in Swedish population vs U.S. population accounted for? (p. B-2, line 56) • How was the mass balance of the flows and volumes ensured during the MC iterations? (re: Table B3). • On what bases the normal distribution for GST is set? (p. B-8, line 219)
Mehendale	*See Mehendale comment 18 in A2.a. and 19 in response to A3.a, above.*

Moore	PBPK modeling is outside of my specific expertise.
Salmon	The development and inclusion of these parameter distributions reflective of both parameter uncertainty and interindividual variation is an important addition to the earlier published version of this model. The distributions and their scientific basis are clearly explained. Although the distributions for some parameters rest on a relatively small number of individuals they represent a reasonable attempt to characterize the overall population variability. In particular, the use of the data from Lipscomb et al. (1997) to characterize the distribution of CYP enzymes activities in humans is an important improvement for this key parameter. Although there are inevitable uncertainties in measurements of enzyme activities or protein levels on samples of human tissue (especially based on disease status of donors and condition of samples), this dataset appears to offer a reasonable basis for predicting the overall distribution.

(B) Noncancer Toxicity of Dichloromethane

Oral reference dose (RfD) for dichloromethane

B1. A chronic RfD for dichloromethane has been derived from a 2-year oral (drinking water) study in the rat (Serota et al., 1986a). Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Reviewer	Comments
Bruckner	The study by Serota et al. (1986a) as the principal study is appropriate. It involved ingestion in drinking water, which is representative of actual human oral exposures, as opposed to gavage. Selection of this investigation is clearly described and supported scientifically.
Gaylor	No studies of human chronic oral exposures were available. The database of laboratory animal oral exposure included six studies that were considered. The most sensitive results were obtained in a 2-year drinking water study in rats (Serota et al., 1986a), as displayed in Table 4-35 and Figure 5-1. The selection of this study as the principal study is scientifically supported and clearly described.
Kamendulis	The Serota et al., 1986a study appears to be a well conducted scientific study and is appropriate to use as the principal study to derive oral RfDs for dichloromethane. While the rat toxicity profile is likely qualitatively and quantitatively different from human, the use of the current PBPK models take the known species differences into account. Other endpoints such as COHb that are observed in both rodents and humans following dichloromethane exposure might be considered, however, the studies are not as comprehensive as the Serota study.

Krishnan	As presented, the choice of the above study and endpoint are well justified in the draft document. However, the choice of the principal study would be strengthened by including a graph (similar to Fig 5.1), that is actually based on internal dose metrics. Such an analysis, in the opinion of this reviewer, would enhance the scientific basis of the choice of the critical study for the non-cancer assessment.
Mehendale	20. The selection of the Serota (1986a) 2-year drinking water study is appropriate for the oral RfD for DCM.
Moore	The calculation of RfDs is outside of my specific expertise.
Salmon	Serota et al., 1986a is a reasonable choice for the key study, as explained and justified in the narrative. There are no alternatives that would be a better choice. Some issues with the published reports by Serota et al. (1986a,b) of the Hazleton studies appear, including incomplete statistical analysis and dismissive analysis of some findings by the authors. In the case of the mouse cancer study (Serota et al., 1986b) the EPA analysts resorted to the original GLP report of the study from Hazleton Laboratories (1983) to resolve omissions and ambiguities, but apparently in the case of the rat study they were able to resolve any such issues without referring to the original report. It might be reassuring for EPA to confirm that they do in fact have access to the original Hazleton report on the rat study and that they did not identify any discrepancies with the published account. This is of some importance given the apparently uneven quality of reporting by Serota et al. and the fact that the GLP reports are not always readily available for review by independent analysts.

B2. An increase in the incidence of liver lesions (foci/areas of alteration) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Reviewer	Comments
Bruckner	It was reasonable to select liver fatty vacuolation in the rat as the critical effect, as DCM doesn't do much else. The document's authors go to some length to correctly conclude the altered hepatic foci likely represent a focal fatty change. Serota et al. (1986a) state this in their paper.
Gaylor	Hepatic vacuolation and liver foci are the primary dose dependent non-cancer effects associated with oral exposure to dichloromethane (Table 4-35), providing adequate data to describe dose response relationships for the incidence of liver lesions from four exposure groups and controls in the study by Serota et al. (1986a). This critical effect is scientifically supported and clearly described.
Kamendulis	The selection of an increase in the incidence of liver lesions (foci/areas of alteration) as the critical effect is appropriate. This finding was seen in both genders of rats, generally in a dose-responsive manner. Other studies observed similar finding supporting this as a

	toxic effect of dichloromethane exposure. While liver effects are not necessarily a concern in human exposure, this finding is consistently observed in rodents and can be used to assess an RfD. This effect was clearly described in the document.
Krishnan	The choice of endpoint, based on the key study, is supported by the information presented in the draft document. However, the choice of the critical endpoint (most sensitive) would be strengthened by including a graph (similar to Fig 5.1), that is actually based on internal dose metrics, as well as consideration of plausible mode of action.
Mehendale	21. The increase in the incidence of liver lesions as the critical effect for the RfD is scientifically supported. There are no other studies.
Moore	The calculation of RfDs is outside of my specific expertise.
Salmon	This is a reasonable choice for the critical effect. EPA thoroughly reviews both this and the various other available non-cancer endpoints from various studies and justifies the selection of the liver effect.

B3. Benchmark dose (BMD) modeling was applied to the incidence data for liver lesions to derive the POD for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in incidence of liver lesions) scientifically supported and clearly described?

Reviewer	Comments
Bruckner	The use of BMD modeling in combination with a rat PBPK model is appropriate to derive the POD for the RfD. This approach and several assumptions result in a quite conservative RfD.
Gaylor	BMD modeling is supported for deriving the POD based on the incidence of liver lesions as a function of internal liver dose. The results from the various BMD model options have been summarized in adequate detail in Table 5-2. The BMD modeling has been appropriately conducted and clearly described in Appendix D. Selection of a BMR of a 10% increase in the incidence of liver lesions is scientifically supported, clearly described, and follows EPA guidelines.
Kamendulis	The benchmark dose modeling that EPA applied to the incidence data for liver lesions to derive the POD for the RfD was clearly described. However, as indicated in response to A3 above, the use of the 1st percentile to calculate an HED and thus an RfD is not justified.
Krishnan	Yes. However, the use of the dose metrics for this modeling and as well as the application of the pharmacokinetic scaling to the internal dose have not been adequately justified. It is also unclear as to whether the rate of production was used as the dose metric as discussed in page 241 and page 244 (for justifying the use of a PK scaling

	factor on tissue dose) or only metabolite concentration was used as presented in the figure in Appendix D. It may be useful to show the results obtained with other dose metrics as well.
Mehendale	22. The BMD modeling applied to the incidence data is scientifically sound.
Moore	The calculation of RfDs is outside of my specific expertise
Salmon	The BMD analysis for the RfD was conducted using standard methodology, and is accurate and sufficiently described. However, the selection of a 10% BMR as the POD is inappropriate if the standard uncertainty factors (similar to those that would be used with a NOAEL) are to be applied. Extensive work using this technique by the State of California has shown that a 5% BMR (and the lower 95% confidence limit on the resulting BMC or BMD) produces a POD which more closely corresponds to a NOAEL in animal toxicity studies with quantal endpoints[1,2]. Although the EPA guidelines for use of benchmark dose methodology initially endorsed a default BMR of 10% the current version of the guidance is not dogmatic on this point and a number of EPA analysts have similarly concluded that a 5% BMR is more appropriate. As an alternative, if the observed data are spaced so as to require considerable extrapolation to a 5% BMR, a response closer to the observed data may be used with an uncertainty factor having similar scale and justification to that employed with a LOAEL. However, the analyst needs to determine whether this approach unnecessarily incorporates greater uncertainty in the result than a limited extrapolation of the data model.

B4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

Reviewer	Comments
Bruckner	The adoption of an interspecies uncertainty factor (UF) of 3 for lack of understanding of potential toxicodynamic (TD) differences is reasonable. An intraspecies UF of 3 for possible TD differences within human populations would account for possible neurodevelopmental changes produced by CO, though this appears unlikely at low exposure levels. It is clear from chronic studies that there is no progression to more serious, irreversible changes including carcinogenesis. A UF of 3 for database deficiencies is unnecessary. A great deal of research on a wide variety of endpoints has been conducted on DCM in several species. There was a two-generation reproductive inhalation study by Nitschke et al. (1988a), although there has

1. JF Collins, AG Salmon, JP Brown, DC Lewis, DE Dodge, MA Marty and GV Alexeeff (2003). Use of benchmark concentration methodology in risk assessment for air toxics. Presented at the Society of Toxicology Annual Meeting, Salt Lake City, UT, March 2003. Abstract #688: *The Toxicologist* **72**(S-1): 142.
2. OEHHA (2008). Air Toxics Hot Spots Risk Assessment Guidelines: Technical Support Document For the Derivation of Noncancer Reference Exposure Levels. http://www.oehha.ca.gov/air/hot_spots/2008/NoncancerTSD_final.pdf

	not been a similar oral study. This should not matter. DCM and other VOCs are extensively absorbed from the lungs and attain higher arterial blood levels than following oral dosing, since ingested VOCs are subjected to first-pass hepatic and pulmonary elimination. Bruckner et al. (2010), for example, recently administered equivalent inhaled and ingested doses of 1,1-dichloroethylene (DCE) to rats over the same time-frame. Inhalation resulted in higher arterial DCE levels and more pronounced injury of extrahepatic organs (e.g., kidney). It is very doubtful that exposure of infants or young children at the proposed RfD of 7 µg/kg-day (or even 3 – 10X higher) would generate brain/blood CO levels sufficient to perturb neuronal development. Run the PBPK model to learn what CO levels to expect at these very low DCM doses and compare these CO levels with CO levels known to inhibit CNS function or maturation in animals and/or humans.
Gaylor	The use of internal doses in rats and humans was impressive. Use of the 1st percentile of the human equivalent dose to account for human toxicokinetic variability is supported. The UFs are scientifically supported and clearly described in the document. A total UF of 30 is justified, resulting in an RfD of $7x10^{-3}$ mg/kg-d.
Kamendulis	An extensive body of scientific research is available for dichloromethane. The UF of 3 for database weaknesses is not needed.
Krishnan	• The animal – human UF only accounted for the toxicodynamic portion – which appears to be appropriate. • The first percentile of the value was chosen to be protective of essentially the population, including sensitive individuals. This has resulted in the use of only the toxicodynamic variability factor, which is adequate (based on comparisons with the median adult values divided by UF=3, as discussed elsewhere in the document). • The database deficiency factor is not well justified – particularly the case is not made to the effect that there is considerable magnitude of the uncertainty associated with the lack of a neuro-developmental assessment and that it could be a more sensitive endpoint than the one used in the present assessment. Furthermore, the statement that there is high confidence in the RfD (page 335) – given the stated uncertainty in the toxicological database (UF = 3) is somewhat inconsistent. • The use of the pharmacokinetic scaling factor is questionable. The PK scaling factor essentially introduces the same level of uncertainty in an assessment, as would the "default" approach lacking a PBPK model or mode of action understanding. • Given that the total uncertainty factor of 10, divided into PK and PD components of 3 each, only accounts for the clearance differences between animals and humans. In other words, the factor of 3 or body surface scaling could be argued to account for the unknown differences in interspecies clearance of the parent chemical such that equal blood concentration of the parent chemical is thought to result in both species. However, the proposed use of an additional BSA correction (or an equivalent allometric PK scaling factor) to account for metabolite clearance, would lead to re-

	definition of default uncertainty factors in which the PK portion would equal 3 x PK scaling factor (which is essentially equal to two times the BSA correction) in case of chemicals for which metabolite concentration is considered to be the appropriate dose metric. In other words, in the past, the use of a UF-PK factor of 3 or BSA correction has been used to account for PK uncertainty without explicitly addressing parent chemical or metabolite kinetics (and clearance). Now, the separate definitions and repetitive applications of BSA correction to these components will require serious reconsideration of the default approach, and development of scientific basis (along with definition of the valid application domain) before implementation. Whether the proposed manner of correcting for interspecies differences in the clearance of DCM metabolites is valid is not known – largely because of lack of relevant empirical data to support it as well as because of the lack of scientific knowledge to support the notion that the pathway-specific mechanisms of relevance to the present case also scale to body surface area across species.
Mehendale	23. Rationale for the selection of uncertainty factors. There is considerable uncertainty regarding the pharmacokinetics and those uncertainties impinge on the amount of DCM metabolized through the two pathways of DCM metabolism. I would suggest adding another uncertainty factor because of this.
Moore	The calculation of RfDs is outside of my specific expertise.
Salmon	The values selected for the uncertainty factors are appropriate (except in so far as this choice interacts with the selection of a BMR of 10%, as noted in the response to question B3 above). The decision to include factors of 3 as part of the UF_A and UF_H to reflect toxicodynamic uncertainty, although the toxicokinetic uncertainty is addressed by PBPK modeling, is particularly to be applauded since this type of uncertainty has often been ignored in earlier risk assessments using PBPK modeling as an approach to interspecies extrapolation. The inclusion of a UF_D of 3 is important to reflect data deficiencies, particularly in the area of neurodevelopmental effects, which are a serious concern for a chemical with demonstrable neurotoxic effects in adults.

Inhalation reference concentration (RfC) for dichloromethane

B5. A chronic RfC for dichloromethane has been derived from a 2-year inhalation bioassay in rats (Nitschke et al., 1988a). Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Reviewer	Comments
Bruckner	The 2-year inhalation bioassay of Nitschke et al. (1988a) is a good choice for the principal study on which to base the RfC calculation. Alternative investigations and the logic for selecting this study are clearly and logically explained.

Gaylor	Apparently, the occupational study in humans reported by Lash et al. (1991) was not selected as the primary study partly because of the uncertainty of early exposure levels. From Figure 5-5, the lowest LOAEL of approximately 200 ppm was obtained from this study. This study has the advantage of being conducted in humans. An RfC of 0.55 mg/m^3, based on neurological effects in humans reported in the study by Lash et al. (1991), is derived in Section 5.2.6 . Since actual early exposures to dichloromethane presumably may have been higher, the human RfC of 0.55 mg/m^3 is conservative. The recommended RfC of 0.2 mg/m^3 is based on liver lesions in rats reported by Nitschke et al. (1988a). Since liver lesions are not amenable to study in humans, it is more health protective to base the RfC on liver lesions in rats (Nitschke et al., 1988a). Hence, the selection of this study as the principal study is scientifically supported and clearly described.
Kamendulis	The selection of the chronic inhalation study by Nitschke et al. (1988) is not an appropriate study to derive an RfC for dichloromethane. The EPA reports that the study examined a range of concentrations, and identified dose-responsive effects in both mice and rats. The female rat data was then used to derive an RfC. However, as shown in Table 5-5, data for hepatocyte vacuolation (which also can be viewed as a minor toxicity that may not have any biological significance) was incomplete for the male rat and was not dose responsive in the female rat. Therefore, using this study does not appear appropriate. However, this reviewer would be satisfied if the limitations and deficiencies of this study and endpoint were sufficiently documented in the draft document. Other studies such as those identifying elevated levels of COHb, an effect also shown in humans, could be considered. The 2-year bioassays (Burek et al., 1984; Nitschke et al., 1988) showed increases (dose-related in the Nitschke study) in COHb, however, a NOAEL was not identified. The subchronic study by Savolainen et al showed neurological changes and identified a LOAEL of 1000 ppm and a NOAEL of 500 ppm. Since neurological (brain) is a target organ following exposure, selection of this study could also be considered.
Krishnan	The selection of the above study is supported and described in the draft document. However, the choice of the principal study would be strengthened by including a graph, that is actually based on internal dose metrics. How does the internal dose-based dose response curve compare between the critical studies underlying RfD and RfC derivations?
Mehendale	24. The selection of 2-year inhalation bioassay in rats (Nitschke et al 1988b) is scientifically well supported. An increase in the incidence of hepatic vacuolation as the critical effect is scientifically supported, and the BMD modeling applied to the incidence data for hepatic vacuolation to derive POD is scientifically sound. The rationale for the selection of UFs is generally sound. However, because of the uncertainties associated with suicidal inhibition of Cyp2E1 by avid binding of CO and the effects of formaldehyde on the oxidative and carcinogenic/mutagenic pathways of DCM metabolism, I would suggest adding another UF.

Moore	The calculation of RfCs is outside of my specific expertise.
Salmon	This is an appropriate choice for the critical study, as described in the document, which addresses the low-dose end of the dose range of interest in developing the RfC. It is unfortunate but perhaps unsurprising that EPA found the human studies unsuitable for deriving an RfC. Because of this, their effort in section 5.2.6 to derive a comparison RfC from human neurotoxicity data, demonstrating that the value based on rat hepatotoxicity is protective but not vastly disproportionate, is a useful contribution to the overall confidence in the chosen value for the RfC.

B6. An increase in the incidence of hepatic vacuolation was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Reviewer	Comments
Bruckner	Liver foci of fatty alteration is an appropriate critical effect, as it is the only non-cancer effect (other than CNS depression) which is reproducible and dose-dependent. Its selection is clearly described and justified.
Gaylor	As discussed in the response to Charge Question B5, the incidence of hepatic vacuolation in rats was the most sensitive endpoint (produced the lowest RfC). Selection of this critical effect is scientifically supported and clearly described.
Kamendulis	As indicated in B5 above, EPA reports that the Nitsche et al study examined a range of concentrations, and identified dose-responsive effects for liver lesions in both mice and rats. The female rat data was then used to derive an RfC. However, as shown in Table 5-5, the data represented is for hepatocyte vacuolation. The document does not describe whether there is any biological significance for this endpoint. In addition, the data was incomplete for the male rat and was not dose responsive in the female rat. It appears that hepatocyte vacuolation is a high dose effect observed in female (not male) rats. Correlates to human exposure do not exist. Therefore, using this endpoint does not appear appropriate. However, this reviewer would be satisfied if the limitations and deficiencies of this study and endpoint were sufficiently documented in the draft document.
Krishnan	The choice of endpoint, based on the crucial study, is supported by the information presented in the draft document. However, the choice of the critical endpoint (most sensitive) would be strengthened by including information (similar to Fig 5.8), that is actually based on internal dose metrics chosen for the analysis.
Mehendale	*See Mehendale comment 24 in response to question B5 above.*
Moore	The calculation of RfCs is outside of my specific expertise.

Salmon	This is an appropriate choice of critical effect, which was adequately described and supported in the document. It appears to be the most sensitive and best documented effect in the rodent model of DCM toxicity.

B7. BMD modeling was applied to the incidence data for hepatic vacuolation to derive the POD for the RfC. Has the BMD modeling been appropriately conducted and clearly described? Is the BMR selected for use in deriving the POD (i.e., a 10% increase in incidence of hepatic vacuolation) scientifically supported and clearly described?

Reviewer	Comments
Bruckner	BMD modeling is reasonable, but conservative approach to derive a RfC for DCM, a chemical that is relatively innocuous in terms of non-cancer effects.
Gaylor	As shown in Appendix D, the BMD modeling to derive the POD has been appropriately conducted and clearly described. The BMR (10% increase in incidence) follows EPA guidelines and is scientifically supported and clearly described.
Kamendulis	If this endpoint is selected, the benchmark dose modeling that EPA applied to the incidence data for liver lesions to derive the POD for the RfC was clearly described. However, as indicated in response to A3 above, the use of the 1st percentile to calculate an HED and thus an RfC is not justified.
Krishnan	Yes. However, as with the RfD, the use of the dose metrics for this modeling and as well as the application of the pharmacokinetic scaling to the internal dose have not been adequately justified. It is also unclear as to whether the rate of production as discussed in page 261 or metabolite concentration as presented in the Appendix D, was used as the dose metric. Because the arguments and justification of the use of a PK scaling factor is not likely to be the same in both cases.
Mehendale	*See Mehendale comment 24 in response to question B5 above.*
Moore	The calculation of RfCs is outside of my specific expertise.
Salmon	BMD modeling has been correctly applied and thoroughly described for the data on hepatic vacuolation. However, as also noted in the response to question B3, the choice of 10% increase as the BMR is inappropriate if the aim is to derive a POD to which the standard UFs appropriate to a NOAEL can be applied. A BMR of 5% is more appropriate for this type of data (animals study, quantal response).

B8. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

Reviewer	Comments
Bruckner	A 3-fold UF for extrapolation/uncertainty about potential interspecies TD differences is justified. A 3-fold interspecies UF is also justified, as the developing brain in neonates and young children may be more sensitive than the adult brain to CO. The recommended database deficiency UF of 10 is not warranted nor scientifically justifiable. It is true there has been a paucity of investigations of potential neurodevelopmental effects of DCM. I am unaware, however, of reports of any structurally-related VOCs being a problem in this regard. Exposures of sufficient magnitude do alter brain neurochemistry and functions reversibly. The existence of chronic solvent encephalopathy (i.e., residual effects) in highly-exposed workers is a subject of considerable debate (Bruckner et al. 2008). Pregnant rats were exposed to 4,500 ppm DCM to produce reported behavior changes in offspring (Bornschein et al. 1980). The presence of sufficient levels of CO in the developing brain can predispose to neurological dysfunction, though it appears such levels would require quite high maternal DCM exposures. Nitschke et al. (1988a) demonstrated that 50 ppm DCM increased maternal CO levels somewhat. This is obviously not the case at the proposed RfC of 0.056 ppm, or at a value 10-fold higher. Intraspecies TD uncertainty in already accounted for with the first UF of 3.
Gaylor	Use of internal liver tissue dose for rats and humans covers the toxicokinetics between species. Use of the 1st percentile of the HEC covers the human toxicokinetic variability. The UFs are scientifically supported and clearly described in the document.
Kamendulis	Further, a UF of 10 was included for database weaknesses due to lack of adequate developmental (neurodevelopmental) studies, and concern for immunological effects. The database for dichloromethane by inhalation exposures is relatively large and contains a two generational study, therefore, a UF of 3 (if not 1) is more appropriate.
Krishnan	• The animal – human UF only accounted for the toxicodynamic portion – which appears to be appropriate. • The first percentile of the value was chosen to be protective of essentially the population, including sensitive individuals. This has resulted in the use of only the toxicodynamic variability factor, which is adequate. • The database uncertainty factor (DBUF) is not well justified – particularly the case is not made to the effect that the lack of a neuro/immune assessment requires the application of a full factor. Based on the consideration that DCM is a systemic toxicant, it would be inconsistent to apply a factor of 10 for DBUF for one route and 3 for another route. The application of a full factor of 10 would is thought to be appropriate for chemicals with an incomplete database, particularly the lack of 2 chronic studies in mammals, 2 developmental toxicity studies and 1 multigeneration study. In light of the toxicological database for DCM, and the discrepancy between

	the RfC and RfD sections of this document, the magnitude of DBUF needs to be re-evaluated. • The use of allometric/pharmacokinetic correction factor used in this assessment needs to be reevaluated. The proposed use of an allometric correction to account for metabolite clearance would lead to re-definition of default uncertainty factors in which the PK portion would equal 3.16 times an allometric factor (instead of 3.16) - particularly for inhaled toxicants for which metabolite concentration is considered to be the adequate dose metric. As discussed elsewhere in this report, the redefinition of the default uncertainty factor for the PK component to account separately for parent chemical clearance and metabolite clearance requires serious reconsideration, and more extensive development of scientific basis before implementation. Whether proposed manner of correcting for interspecies differences in the clearance of DCM metabolites is valid, is not known – largely because of lack of relevant empirical data to support it as well as because of lack of arguments to support that pathway-specific mechanisms of relevance scale with body surface. • Figure 1 indicates that the desired dose metric is the one that is closer to the response whereas the feasible one (that can be obtained with the model in hand) is somewhat removed from it. In the case of DCM for example, the current risk assessment desires to have information on metabolite concentration (i.e., formation – clearance) whereas the model can only provide confident estimates of metabolite formation in the target organ. As discussed under G-1, there is a dilemma here in terms of whether a certain dose metric provided by the model is more relevant, or a less certain dose metric obtained with the application of a correction factor applied to the model-simulated dose metric is more relevant for use in assessment. In essence then, the residual uncertainty associated with the default approach is reduced but not eliminated totally by the application of currently available DCM PBPK models - and the additional application of BSA correction, as a way of eliminating residual uncertainty associated with the PBPK models, will reintroduce the source of uncertainty into the process. Whereas the intent of computing the metabolite concentration is superior to that of metabolite formation, given the limitations of the current models and lack of relevant data on metabolite data, it is not realistic to focus on a dose metric that cannot be estimated with confidence.
Mehendale	*See Mehendale comment 24 in response to question B5 above.*
Moore	The calculation of RfCs is outside of my specific expertise.
Salmon	The values selected for the uncertainty factors are appropriate (except in so far as this choice interacts with the selection of a BMR of 10%, as noted in the response to question B3 above). The description and justification for the choices made is thorough and accurate, except that there appear to be an error in the fourth bulleted paragraph on page 263 (section 5.2.4), which refers to extrapolation from a chronic drinking water study. In fact, the RfC was derived from data obtained in a chronic inhalation study (Nitschke et al., 1998a). The selection of the value of the corresponding uncertainty factor as 1 is nevertheless correct. As before, the areas of toxicokinetic uncertainty

	addressed by PBPK modeling are correctly distinguished from toxicodynamic uncertainty, and the concerns over the database on neurotoxicity (especially possible developmental effects) are justified.

(C) Carcinogenicity of Dichloromethane

C1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), dichloromethane is *likely to be carcinogenic to humans* by all routes of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

Reviewer	Comments
Bruckner	The document's authors have done a credible and thorough job of summarizing information on DCM carcinogenicity testing in rodents that supports their judgment that DCM should be classified as "likely to be carcinogenic in humans". Their account is clear and succinct. I do not believe, however, that they have given a full account of pertinent information for and against their rationale for deriving an OSF, so readers are not given a balanced perspective. The decision that DCM is likely to be carcinogenic in humans is based predominantly on findings that male and/or female B6C3F1 mice develop liver and lung tumors after chronically inhaling very high DCM concentrations and liver tumors after chronic ingestion of high doses of the chemical. The tentative findings of liver tumors in female F344 rats and malignant mammary tumors in female Sprague-Dawley rats are minimally supportive, but not confirmative for cancer in a second species. Hamsters are refractory. The relevance of the B6C3F1 mouse to humans is highly questionable and should be discussed openly in this section. It is widely recognized that each sex of this strain of mouse exhibits a very high incidence of spontaneous liver adenoma and carcinoma. Haseman et al. (1998) reported that male B6C3F1 NTP control mice had a 42.2% incidence of hepatocellular adenomas and carcinomas. NTP (1986) reported a 44% incidence in male controls. Secondly, it is pointed out in the current document that metabolites of the glutathione (GSH) pathway [e.g., S-(chloromethyl) GSH] are responsible for DCM's mutagenicity, and that mice have a substantially greater capacity to metabolize DCM via the GSH pathway than do rats or humans. It follows that mice should be much more susceptible to DCM-induced hepatocarcinogenicity than humans with equivalent doses. Hamsters have the lowest capacity to conjugate DCM with GSH, and do not develop cancers with DCM. It is assumed that rodents and humans absorb 90 – 100% of ingested VOCs. Internal dosimetry is not equivalent, however, for inhalation exposures of different species. Mice have a considerably high alveolar ventilation rate, cardiac output, DCM blood:air partition coefficient and DCM metabolic rate than do humans. These are the four primary determinants of systemic absorption of inhaled DCM and other VOCs (Bruckner et al., 2008). Rats are intermediate (Brown et al., 1997) in this regard. With equivalent inhalation exposures, internal doses of VOCs are markedly higher in mice than in rats, and in rats than in humans. Thus mice will

	absorb considerably more inhaled DCM than humans and convert considerably more of it to mutagenic GSH metabolites. Volkel et al. (1998), for example, found significantly higher urinary levels of reactive metabolites in rats than in humans subjected to equivalent perchloroethylene inhalation regimens. Another relevant species difference is the prevalence of Clara cells in the bronchioles of mice versus their sparcity in rats and rarity in humans. Clara cells, of course, contained relatively high levels of CYP2E1 and GSH transferases (GST-T) and are preferentially injured by inhaled VOCs that undergo metabolic activation. Metabolism of trichloroethylene to chloral is high in the mouse and undetectable in human lung preparations (Green et al., 1997). For the aforementioned scientific reasons, B6C3F1 mice would be expected to be much more susceptible than humans to DCM-induced liver and lung tumors. This information should be presented and discussed in the document and factored into the decision making process. Sound scientific judgment should be utilized in classifying potential human carcinogens and conducting cancer risk assessment, rather than consistently making worst case assumptions and reaching decisions based on entrenched policy. In light of knowledge available from the extensive human and animal database on DCM, I think it is a big "stretch" to classify DCM as a likely human carcinogen. Possible human carcinogen is much more appropriate for a chemical with limited evidence of animal carcinogenicity and largely negative epidemiology data. The stated intent of EPA's Guidelines for Carcinogen Risk Assessment (2005) is to use a weight of scientific evidence approach, rather than a "one size/classification scheme fits all" for a chemicals that may be carcinogenic in $\geq$ 2 sites in 2 species. It should be pointed out on page 212 that the workers studied by Heineman et al. (1994) were exposed to mixed solvents. There was no direct exposure information, and the probability of DCM exposure (i.e., work history) of subjects was apparently based upon recall by next-of-kin. Some additional information should be provided on page 220, in order to provide an accurate and balanced account. Nitschke et al. (1998a) and Burek et al. (1984) did not see an increased % of DCM-treated female rats with benign mammary tumors. There was a modest increase in the number of such tumors only in animals with the tumor. The incidences of this tumor in control and treated groups should be given, so the extremely high incidences in controls are apparent to the reader.
Gaylor	The strongest evidence that DCM is carcinogenic in humans comes from studies of workers exposed by inhalation. This is supported by evidence of carcinogenicity in two animal species, rats and mice. Since DCM is mutagenic, it is likely to be carcinogenic across routes of exposure. This is supported by evidence of carcinogenicity in rats and mice exposed to DCM both orally and by inhalation. Categorizing DCM as likely to be carcinogenic to humans by all routes of exposure is scientifically supported and clearly described in the document.

Kamendulis	I do not agree with EPA's classification. The 2-year drinking water study showed no induction of cancer in mice (Serota et al., 1986a). EPA performed a reanalysis of the study of Serota et al. 1986b, and concluded that dichloromethane induced a small, but statistically significant, increase in hepatocellular adenomas and carcinomas in male B6C3F1 mice. The original findings of the study authors had concluded that there were no statistical increased incidences and no dose-response trend by the oral route of exposure. The EPA's reanalysis used a different statistical approach and control groups than used by the authors, which lead to a very marginal statistical significant increase in the highest dose group. I do not agree with this approach and agree with the original interpretation by the authors who concluded that dichloromethane was negative for carcinogenicity by the oral route of exposure. For dichloromethane, several species have been tested for long-term carcinogenicity. No evidence of carcinogenicity by dichloromethane was seen in F344 rats (male and female) by both oral and inhalation routes of exposure, or in male and female Sprague-Dawley rats by inhalation, or in Syrian hamsters by inhalation. Dichloromethane therefore exerts a carcinogenic effect in mice by inhalation exposure only. Using the cancer guidance documents, since at least 2 species were negative for tumors is suggestive that the agent may not be of a carcinogenic concern for humans. The existing data better supports that dichloromethane is *suggested to be carcinogenic to humans*. Concerning all routes of exposure, the existing scientific data supports that dichloromethane is carcinogenic by inhalation, but not by other routes of exposure. Therefore, the descriptor that dichloromethane will be carcinogenic by all routes of exposure is not scientifically justified.
Krishnan	The weight of evidence characterization is presented clearly, even though additional information regarding the dose-dependency of the GST pathway and the relative differences across species (rat vs mouse vs human) of the flux through GST pathway might be mentioned. Given the document presents the argument that DCM is a systemic carcinogen, and not a route-specific local carcinogen, the concern due to the lack of oral route-specific data is somewhat less obvious to this reviewer. Despite the shortcomings, the information presented in this document situates DCM between the EPA descriptor categories of "carcinogenic to humans" and "suggestive evidence of carcinogenic potential"; accordingly the current determination of "*likely to be carcinogenic to humans*" would appear to be consistent with the Agency guidelines.
Mehendale	25. The current scientific literature does not support the cancer descriptor that EPA is proposing, that is, DCM is "likely to be carcinogenic to humans by all routes of exposure" and therefore, it is not justified. In fact, the literature indicates the opposite, that is, DCM is likely not carcinogenic to humans, especially at relevant environmental exposure levels as low exposures would be metabolized by a non-carcinogenic CYP2E1 pathway according to the EPA Interagency Review (IAR) draft document. DCM is unlikely to be carcinogenic to humans via the oral exposure pathway because: (a) In a well-performed 2-year drinking water ingestion carcinogenesis bioassay in rats (Serota et al., 1986a), the data did not exhibit any conclusive indication of a

	carcinogenic response. (b) In mice, it has only been reported to induce liver tumors in a strain with a very high incidence of spontaneous liver tumors, that is, Serota et al., 1986b. These tumors did not show a dose-response effect that is expected of a positive response, and therefore were considered negative by the scientists who analyzed the data. That some, but not all of the doses produced statistically significant findings as compared with the control, is a much weaker finding. This should be carefully considered and discussed in the document. (c) A very large number of consumers may be exposed to DCM in decaffeinated coffee (ATSDR 2000), yet the draft document states that the percentage of humans with liver cancer is small. Long-term occupational worker studies do not provide clear evidence of liver and/or lung tumors in DCM-exposed workers. For example, twelve epidemiological studies of cancer risk were identified in this review, of which seven were case control studies of specific cancers with data on DCM exposure and four that were cohorts for which the primary solvent exposure was to DCM. These studies did not provide clear, statistically significant evidence of hepatic and/or lung tumors in DCM-exposed workers. Should this finding not be weighed more? (d) DCM induces liver tumors by a mechanism related to GST pathway, and only after the primary pathway is saturated (or inhibited because of CO.CYP2E1 formation). Humans are much less sensitive than mice to this mode of action, as the IAR draft document states that mice are the most sensitive species, and humans are not as sensitive as rats and do not get tumors. (e) The significant uncertainty related to the scaling factor (7.0 for allometric scaling versus 1.0) results in a 7-fold decrease in the estimated cancer toxicity values. The draft text states that "Using a whole body GST metabolism dose metric, the resulting IUR (inhalation unit risk) for liver and lung cancer were approximately five-fold higher than when tissue-specific dose metrics were used." (f) The EPA's conclusions that the liver cancer data derived from human studies are debatable. The relevance to humans of the liver cancer data derived from animal studies has been and continues to be the subject of much scientific debate. This principle has not been accepted unequivocally. The presence of an increased yield of liver tumors in a strain of mouse with a high background incidence of liver tumors is not sufficient to suggest that a chemical is a human carcinogen. Suggestive evidence that a chemical is a human carcinogen would require at least an increased yield of tumors in another organ or in another species with a low yield of background liver tumors. In the absence of such data, the presumption is very weak. The results of the Serota et al., 1986b mouse study discussed in the draft toxicological review did not reveal any increase in the incidence of proliferative hepatocellular lesions in the DCM-treated female mice, despite the fact that the strain of female mice are reportedly more sensitive than males in exhibiting carcinogenicity. Thus, an increase in liver tumors in females would have been

	expected if DCM were carcinogenic via the oral route of exposure, especially by a mutagenic mode of action that EPA claims is the case. Although treatment-related toxic effects were observed in the male and female B6C3F mouse liver following ingestion of DCM in drinking water at levels up to 250 mg/kg/day for 104 weeks, only a slight increase in proliferative hepatocellular lesions were noted and then only in the male group. The lesions in the male group did not appear to be dose-related, and were within historical control ranges according to the original authors' data interpretation/conclusions. These observations lead this reviewer to think that the evidence for DCM being a carcinogenic ever weaker. (g) I have reservations on the EPA's classification of DCM as 'is likely to be carcinogenic to humans'. I do not feel that the scientific evidence for this is strong enough.
Moore	My primary expertise is genotoxicity rather than carcinogenicity. Given that caveat, I did find the document to provide a clear outline of the available information.
Salmon	The evidence for carcinogenicity of DCM, based primarily on the results of animal studies but with limited support from human studies, is clearly and adequately described and the weight of evidence is characterized according to the usual guidelines. The extensive studies of genotoxicity and DNA damage also reported provide additional support for the conclusion of carcinogenicity by all routes. There is one aspect of the overall interpretation of the cancer data which deserves comment, this being the question of the tumor sites observed in animal studies and the extent to which there is an expectation of site concordance for tumors in humans exposed to DCM. The sites affected in the animal experiments are the liver, lung and central nervous system. Of these the liver has attracted by far the greatest level of attention and mechanistic research, and the tissue dose calculations which were undertaken in the PBPK modeling exercise are largely directed towards enzyme levels and biotransformation events in the liver, and to some extent the lung. This certainly has provided a useful analysis of the dose-response relationships for both cancer and non-cancer responses in animals, but the extent to which this extrapolation can be made for predicting cancer responses in humans has not been sufficiently explored. In spite of the very limited nature of the evidence of carcinogenicity by DCM in epidemiological studies, it does seem that although the liver is an important site in the animal studies it is not a predominant site in humans, although some evidence of hepatocellular and biliary duct cancer was found by Lanes et al., (1990, 1993). Central nervous system tumors are possibly indicated in the human epidemiology data, and also, as noted in EPA's report, have some support in the animal bioassay data. Less convincing, but still possibly associated with DCM exposure in both human and animal data, are possible increases in mammary gland tumors. Finally, there are some indications of leukemia and other hematopoietic tumors in humans, although as for the other sites the epidemiological findings are occasional and equivocal due to the low power of most of the studies available. In this context is interesting that NTP (1986) reported reduced survival in female rats, possibly associated with leukemia. Increases in the cidence of mononuclear cell leukemia in mid- and high-dose female rats were statistically significant after mortality correction. Mennear et al. (1988) discounted the significance of this result,

	reportedly because of the high background rate of mononuclear cell leukemia in male rats. The exact logic behind this dismissive conclusion is not entirely clear. At the very least, it deserves further analysis and comment by EPA in considering the range of sites (other than liver and lung) at which DCM-associated tumors have been observed. This in turn has some implications for the rather strict focus on the two well established sites in the animal studies, when discussing appropriate potency comparisons between animals and humans.

C2. A mutagenic mode of carcinogenic action is proposed for dichloromethane. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for dichloromethane that may support an alternative mode of action.

Reviewer	Comments
Bruckner	As described in pgr. 3 on page 222, very high inhaled and ingested doses of DCM were required to cause DNA damage *in vivo*. This is consistent with the premise that the GST pathway, which is responsible for production of mutagenic metabolites, becomes predominant at high exposure levels. This implies that formation of such metabolites and associated mutagenic risks will be minimal or negligible at very low exposure levels, as well as in species (i.e., humans, hamsters) with very low GST-T activity. Graves et al. (1995) found DCM-induced DNA single strand breaks in mouse, but not in hamster or human hepatocytes. It is cited at the end of the second full paragraph that rats and hamsters have considerably lower GST activity than mice, and may be less sensitive to DCM genotoxicity. The same is true for humans. Why is this not mentioned in the mode of action discussion, when the document's purpose is to extrapolate from rodents to humans to predict human risks? This finding should be included at the bottom of page 225, in order to balance the summation of human genotoxicity data. The two paragraphs in the Section of Relevance of Rodent Tumors to Humans is largely silent on many of the points I have made above. It must be expanded to present a balanced overview of pertinent findings and species differences and similarities, from which the weight of scientific evidence can be evaluated. The current composition gives the impression of bias by selective inclusion and exclusion of relevant information.
Gaylor	Genotoxicity was demonstrated in a number of studies, as discussed in Section 4.5.1. A mutagenic mode of action is scientifically supported and clearly described in the document.
Kamendulis	Many in vivo and in vitro studies have been performed to assess the genotoxic and mutagenic potential of dichloromethane. Results from these investigations have shown that mutagenicity occurs following exposure to dichloromethane and operates through a mechanism involving metabolic activation by GST-T1. The scientific evidence for this is clear. It is also clear that this effect is observed at relatively high doses, when metabolism through CYP4502E1 is saturated. This is clearly described in the

	document. While it can be argued that carcinogenicity would be less likely to occur in humans based on metabolic activity of GST-T1 in humans compared with rodents (mouse > rat > human), the data clearly demonstrate that the mechanism for mutagenicity is through a GST-T1 pathway, and metabolism through this pathways does occur in humans.
Krishnan	The draft document clearly presents the available data in support of this mode of action vs other modes. The agency's determination is also supported by the absence of demonstrable non-mutagenic mode of action for this chemical. The available evidence on cell proliferation is well summarized and is not inconsistent with the current determination.
Mehendale	26. The mutagenic mode of action for potential carcinogenic action of DCM is scientifically justified and supported.
Moore	The authors have summarized the available genotoxicity data in a series of very useful tables. The tables include information on the test system, the endpoint, concentrations/doses used and a summary of the findings. The tables are organized to include (1) the in vitro assays in lower organisms: bacteria, yeast and fungi, (2) in vitro assays in mammalian cells, (3) in vivo assays in insects, (4) in vivo assays using mice, (5) in vivo assays using rats and hamsters. In addition there is a table to compare the in vivo results in the target tissues (lung and liver, by rodent species). This presentation is far superior to the summaries often done for genotoxicity in which the results are presented as a general summary of how many tests are positive or negative. It is particularly helpful to have the specific endpoint included in the table. Different assays use different endpoints and an overall weight of the evidence evaluation of the genetox data, needs to take this fact into consideration. The discussion concerning the type of metabolic activity used in the various studies, is also very useful, particularly in the discussion of interspecies differences. I do have a few specific comments on the details of the presentations. Table 4-29: It would be helpful to include the specific yeast strains that were used for the gene conversion, gene recombination studies. Table 4-30. For the mouse lymphoma assay (MLA) the locus used for mutant selection should be identified. Presumably the thymidine kinase (tk) gene was used and that is not an assay for point mutations as indicated in the table. In fact the majority of mutants generated in this assay will be chromosomal mutations including deletions, rearrangements, mitotic recombination, and some nondisjunction (aneuploidy). Also the negative result in the MLA is surprising in light of the fact that there is a positive result for hprt mutations in Chinese Hamster ovary cells (CHO). The concentration for this MLA study by Thilager et al. is not reported, which should cast serious doubt on the results. Listed under the Hamster without GST activity studies is a Chinese hamster epithelial cell assay. The assay is listed as a forward mutation assay. It would be important to identify the gene used. Presumably it is hprt. Table 4-32: The Allen et al. sister chromatid exchange (SCE) study using mouse lung and pheripheral blood lymphocytes is indicated to be positive at 8000 ppm, but it is not

	clear if both tissues are positive or only one is positive. Likewise, the Allen et al. chromosome aberration study using mouse lung and bone marrow is noted as positive at 8000 ppm, but it isn't clear which tissue is positive. The last statement on page 174: "Crebelli et al…….should not offset the consistently positive in vitro results (Dearfield and Moore)". The Dearfield and Moore reference is taken out of context for the discussion in this paragraph. The subject of this discussion is the variability of in vivo results. The statement from the Dearfield and Moore paper refers to determining whether a chemical is a mutagen—that is hazard identification for mutagens. When one moves to discussing mode of action (MOA) for tumors, the in vivo data has to be utilized in the context of other in vivo data—including genotoxicity, preneoplastic lesions and other biological effects such as cell proliferation. Positive in vitro data, while it informs the overall analysis, does not take precedent over well conducted in vivo studies for MOA analysis. In considering the MOA for tumorigenesis, the results in the target tissues are particularly important, so the inclusion of a table summarizing the genotoxicity results in vivo in the target tissues is particularly useful. One thing missing from the analysis is a table looking at the body of data available for a particular rodent species and target tissue (for instance the mouse lung) and putting what data are available into a framework looking at specific doses by exposure route and target tissues. This should be put into an overall timeframe that outlines the "key events" that are induced by the chemical. This MOA analysis framework should look at both "genotoxic" and nongenotoxic endpoints such as cell proliferation. Once this is done, issues of temporality and dose response concordance can be evaluated to assess the proposed and other possible MOAs. I would strongly encourage the authors to do this sort of MOA framework analysis in their revision. Once this is done, it will be much easier for the reader to assess the strengths of the various possible MOAs. Even in the absence of this MOA framework, it is possible to assess the general strengths of the evidence that DCM is causing tumors by a mutagenic MOA. The endpoints available for making a determination as to whether a chemical is an in vitro mutagen, an in vivo mutagen and conducting a subsequent analysis as to whether the MOA for tumors is a mutagenic MOA include events such as (1) primary DNA damage (various DNA breakages assays, unscheduled DNA synthesis, DNA adducts, cross links, SCE and Comet), (2) Chromosomal breakage assays and (3) gene mutation assays. For assessing whether a chemical is a "mutagen" the gene mutation assays are the most definitive. All of the other assays, while useful, do not really provide a definitive answer. The vast majority of the data for DCM is with endpoints other than actual induction of mutation. The in vitro data is probably sufficient to conclude that DCM is an in vitro mutagen. There is NO in vivo data in rodents addressing whether DCM can induce mutations either in target or non target tissues. Therefore, I do not believe that there is sufficient data to prove a mutagenic MOA for DCM. In looking at the alternative MOAs, there appears to be no evidence to strongly conclude that the MOA has a nonmutagenic MOA. So, unfortunately, one must conclude that while there is evidence to indicate that the MOA for DCM <u>might</u> be a

	mutagenic MOA, it is not possible to conclusively define a MOA for tumor induction. One then has to conclude that the MOA for DCM induced tumors is unknown.
Salmon	Identification of a "mutagenic mode of action" is complicated by EPA's failure so far to present a consistent and scientifically justifiable analysis of exactly what they mean by this phrase, and what its implications for cancer risk assessment should be. However, the nature and extent of the evidence of carcinogenicity and genotoxicity for DCM are such that the conclusion of a mutagenic mode of action, by any reasonable definition, is inevitable. One of the problems with this analysis is that virtually all carcinogens produce non-cancer toxicity as well as carcinogenicity, and this interacts with the process of carcinogenesis in various ways, depending on the type of damage involved. Thus most carcinogens have, in effect, multiple modes of action, but this does not detract from the central role of somatic mutational events in the whole process. Nor does it argue against the assumption of low-dose linearity, or the need to apply age-related sensitivity factors, which are sometimes claimed to be dependent on the finding of a mutagenic mode of action.

Quantitative cancer assessment - oral exposure

C3. A 2-year drinking water study in mice (Serota et al., 1986b) was selected for the derivation of an oral slope factor (OSF) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

Reviewer	Comments
Bruckner	The 2-year drinking water study by Serota et al. (1986b) is the best choice, as its mode of DCM administration is relevant to human oral exposure scenarios. The doses, while substantially higher than doses that would normally be received by humans, did not approach "megadose levels" frequently employed in many cancer bioassays. My concerns about relying on B6C3F1 mouse liver and lung tumors are described above. It is also noteworthy that the tumor incidences in these DCM-treated mice and the F-344 rats were of marginal statistical significance.
Gaylor	No human data are available for the quantification of neoplastic effects from oral exposures to DCM. The 2-year drinking water study in mice (Serota et al., 1986b) provided the strongest statistical data for the carcinogenicity of DCM in animals via oral exposure. The selection of this study for derivation of the OSF is scientifically supported and clearly described in the document.
Kamendulis	This study is not appropriate for the derivation of an oral slope factor for dichloromethane. The findings reported in the 2-year drinking water study concluded that there was no induction of cancer in mice (Serota et al., 1986a). EPA reanalyzed the study of Serota et al 1986b, and concluded that dichloromethane induced a small, but statistically significant, increases in hepatocellular adenomas and carcinomas in male B6C3F1 mice (p=0.058), in contrast to the original findings of the study authors (no

	statistical increased incidences and no dose-response trend by the oral route of exposure). The EPA's reanalysis used a different statistical approach and control groups than used by the authors, which lead to a very marginal statistical significant increase in the highest dose group. I do not agree with this approach and agree with the original interpretation by the authors who concluded that dichloromethane was negative for carcinogenicity by the oral route of exposure. Therefore, this study is inappropriate to use for the derivation of an OSF for dichloromethane. The chronic inhalation study by NTP could be considered as an alternative.
Krishnan	The study would not appear to be appropriate for the derivation of OSF. The route to route extrapolation based on PBPK model should be given priority. Furthermore it is not clear as to why the Agency did not conduct a BMD analysis on combined datasets from the oral and inhalation routes of exposure (based on internal dose), or did not determine the arithmetic mean of slope factors for the two routes (as done in the previous DCM assessment).
Mehendale	27. The 2-year drinking water study (Serota 1986b) for the derivation of Oral Slope Factor (OSF) is scientifically well supported and well described.
Moore	Because my primary expertise is genotoxicity as it relates to carcinogenicity, I am not aware of other studies. I did find the discussion to be clearly described.
Salmon	The drinking water studies described by Serota et al. are the most suitable source for data to use in determination of an oral slope factor, particularly since the only obvious alternative series using this route (by Maltoni et al., 1988) appears to have failed due to high mortality in both mice and rats. Of the studies by Serota, the male mice showed the clearest and most sensitive response (hepatocellular adenoma/carcinoma).

C4. The OSF was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for liver tumors in male mice). The OSF is based on an analysis of the most sensitive of the human subgroups, the GST-T1 +/+ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

Reviewer	Comments
Bruckner	The linear multistage extrapolation approach utilized here is based on a series of conservative assumptions. The net result (the cancer risk estimate) is <u>much</u> more health protective than necessary for DCM. This approach ignores protection and repair systems known to be operative in cells and organ systems, as well as the likelihood of minimal or negligible GST-mediated metabolism in humans at low/trace exposure levels.

Gaylor	Liver tumors in male mice provided the most sensitive tumorigenic effect. Among the various internal dose metrics, scaling factors, and human population genotypes, allometric scaling for the internal liver-specific dose applied to the GST-T1$^{+/+}$ human population genotype produced the largest, most potent, OSF (Table 5-13). This value is scientifically supported and clearly described in the document.
Kamendulis	I do not agree with the selection of this study or endpoint for calculating an OSF for dichloromethane – the justification for this is included in responses C1-C3 above. I do agree however that to derive an OSF, modeling based on the GST-T1+/+ genotype should be used as this represents the most susceptible population. The linear extrapolation from the POD ignores biology, in that, due to repair, detoxification, and other biological processes, dose levels for any chemical exposure exist that are below a level that will exhibit a response. Using the most sensitive population, and the 1st percentile is already sufficiently conservative.
Krishnan	The derivation of OSF for most sensitive population is not clearly justified. Clarification is needed as to the realism and scientific validity of this approach in light of the use of a probabilistic PBPK model that already accounts for the population distribution of parameters of relevance.
Mehendale	28. The OSF calculation and the selection of the human subgroup of GST-T1+/+ genotype using internal dose predictions is scientifically supported. However, accuracy of those predictions is somewhat questionable because of uncertainties related to CO.CYP2E1, etc.
Moore	This is outside my primary expertise, so I cannot provide comments.
Salmon	This calculation follows standard guidance as far as the modeling and extrapolation methods are concerned. The application of PBPK modeling to the mouse liver carcinogenicity data is well described and provides a logical and scientifically justified interpretation of the data. Extrapolation of the animal potency to provide a human estimate based on the tissue-specific dose level in the liver (for the GST-T1 +/+ subpopulation) is not an unreasonable way of handling the interspecies extrapolation necessary, but the approach taken in the analysis largely ignores the possibility of tumor sites other than the liver being important. It is likely that there are such sites in both mouse and human, and moreover that they are proportionately more significant in the human than the mouse due to intrinsic differences in sensitivity between the tissues of mice and humans. It may be that there are no good data or methods to address this question from a quantitative point of view, but the problem at least deserves discussion even if it is eventually decided that the approach used by EPA is the best available.

Quantitative cancer assessment - inhalation exposure

C5. A 2-year cancer bioassay in mice (NTP, 1986) was selected for the derivation of an inhalation unit risk (IUR) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

Reviewer	Comments
Bruckner	The NTP (1986) inhalation cancer bioassay is the only logical choice to use as the critical study for cancer risk assessment of inhaled DCM. Other inhalation bioassays of rats and hamsters showed nothing or very little. Nevertheless, the use of such high vapor concentrations by NTP is troubling, considering the shift from the CYP to the GST pathway under such exposure conditions. This artificial experimental design certainly calls into question the validity of extrapolations to very low human vapor exposures in environmental settings. I am also troubled about the highly questionable human relevance of frequent liver tumors in control B6C3F1 mice, by the unique presence of numerous Clara cells in mouse bronchioles, and by the elevated GST-T1 activity in mouse hepatocytes and Clara cells.
Gaylor	Several studies of workers exposed to DCM via inhalation provide some evidence of carcinogenicity. However, these studies in humans do not provide adequate data to derive dose response relationships. Justification for selecting the 2-year bioassay study in mice (NTP, 1986) for quantification is provided in Section 5.4.2.1. The selection of this study for quantification is scientifically supported and clearly described in the document.
Kamendulis	I agree with the selection of the NTP (1986) 2-year inhalation study for the derivation of an inhalation unit risk for dichloromethane. The scientific justification for using this study is clearly presented in the document.
Krishnan	The NTP study appears to be adequate for the purpose of this assessment.
Mehendale	29. The selection of the NTP 1986) 2-year study for derivation of risk factor for inhalation unit risk (IUR) is scientifically justified.
Moore	This is outside my primary expertise, so I cannot provide comments.
Salmon	The selection of the NTP bioassay for mice is appropriate and clearly described. Human data are clearly inadequate for cancer risk estimation, although they do contribute to the weight of evidence for identification of DCM as a likely human carcinogen. Among animal studies, the NTP bioassays are generally regarded as among the best available in terms of design, execution and reporting, and this specific study in the mouse shows a clear dose response for both lung and liver tumors. It also benefits from the extensive PBPK analysis of DCM tissue doses in mice. Some consideration was also given to the other NTP results at different sites and in rats as well as mice, but it seems clear that the data in mice (and in particular the male mice) are the most suitable single data set to be

	used as the basis of an IUR. Selection of this particular site for estimation of human risk is appropriate following the guidelines' recommendation to use the most sensitive site, species and sex. However, it does not address the question of differential intrinsic sensitivity of tissue sites between species, for which there is some evidence, but not sufficient to present a reliable quantitative basis for analysis, in the human epidemiological data. Thus the exclusive consideration of lung and liver tumors both in the tumor incidence data and in the tissue-specific PBPK modeling is a source of uncertainty in the interspecies extrapolation. While this probably cannot be avoided, it needs to be better addressed in the discussion. For instance, simply dismissing the mammary tumor data (in rats) because there isn't a good mechanistic or PBPK analysis available for that site isn't sufficient.

C6. The IUR was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for lung or liver tumors in male mice) taking into consideration total cancer risk by determining the upper bound on the combined risk for male lung and liver tumors. The IUR is also based on the analysis of the most sensitive of the human subgroups, the GST-T1 +/+ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

Reviewer	Comments
Bruckner	My concerns about the almost universal use of linear extrapolation for cancer risk assessments, no matter the circumstances or weight of evidence for a given chemical, have been expressed above.
Gaylor	The last paragraph on page 304 describes the statistical procedure for estimating the upper 95% confidence limit for the combined risks of liver and lung tumors. This procedure assumes that the BMD is normally distributed, which is questionable. Also, this procedure assumes that the presence of lung and liver tumors are not correlated. Hence, the 95% confidence limit calculated for lung and liver tumors combined is approximate. An exact statistical procedure is to first note for each male mouse, from the experimental data, if the animal had a lung and/or liver tumor. Then, the incidence of lung and/or liver tumors simply can be calculated for each dose group and the Benchmark dose procedure can be applied correctly with these incidences. Basing the IUR on the most sensitive human genotype (GST-T1 $^{+/+}$) and internal dose estimates for the lung and liver is supported. This approach is scientifically supported and clearly described in the document.
Kamendulis	In general, the calculation of the IUR was scientifically based. However, the scientific justification for combining lung and liver tumors to derive the IUR is not clear, the scientific rationale for using combined data should be presented. It appears more scientifically justified to use the higher tumor risk to calculate an IUR (an analogy is the use of the GST-T1+/+ genotype to account for the most susceptible population). As discussed in the RfC and RfD derivation sections above, to derive a $BMDL_{10}$, a scaling

	factor was applied for rodent to human differences, see comments in A1 concerning the use of a scaling factor.
Krishnan	• Clarification is needed as to the validity and adequacy of this approach in light of the use of a probabilistic PBPK model that already accounts for the population distribution of parameters of relevance. Why is the slope factor determined for the most sensitive subpopulation and not for the entire population that also consists of this subpopulation (which would be more realistic)? • Similarly, since the distributions of parameters representative of children of various ages are used in the PBPK model, the need to use additional adjustment factor for early life exposures should be more clearly presented. • Finally, the cancer risk assessment is based on the mean value for the GST-T1 +/+ population whereas the non-cancer assessments are based on 1 pctle value of the dose metric in the entire population. This discrepancy may be clarified or resolved by doing similar analysis for both endpoints.
Mehendale	30. The IUR calculated by linear extrapolation from POD associated with 10% extra risk for lung or liver tumors in male mice taking into consideration total cancer risk in most sensitive human subgroups is scientifically supported and clearly described. The modeling has been appropriately conducted and well described.
Moore	This is outside my primary expertise, so I cannot provide comments.
Salmon	Calculation of the individual IUR vales for lung and liver, using the BMR methodology and the various possible dose metrics is presented clearly and thoroughly, and follows the standard procedures. Analysis of the various PBPK approaches, and selection of the human model representing the sensitive GST T1 +/+ subgroup is appropriate and well presented. Uncertainty associated with the focus of both tumor incidence data and PBPK modeling on these two sites, which may not be the only important sites in humans, is inevitable: this should be more clearly explained. There is an issue with the way that the distributional summation of the lung and liver tumors is undertaken. The addition of MLE values and recalculation of the 95% UCL by applying a combined SD only works if the probability density functions for the potency estimates for both sites are normal, or at least symmetrical. However, there are plenty of examples where this is not even approximately true. The narrative is also incorrect in stating (or assuming) that the MLE is a measure of the central tendency for such a distribution. For an asymmetrical distribution it is somewhat debatable what the appropriate measure of central tendency is, but usually this would be taken to refer to the mean of the distribution, not the mode (i.e. the MLE). Notably, the MLE is not the proper measure to which to apply a standard deviation (which is applied relative to a mean, and in any case only calculates percentile limits if the distribution is normal). The use of this incorrect procedure may result in significant underestimation of the

	combined 95% UCL potency value. This issue has been explored in detail recently (Salmon and Roth, 2010)[3].

[3] AG Salmon, LA Roth (2010). Cancer Risk Based on an Individual Tumor Type or Summing of Tumors. In: "Cancer Risk Assessment: Chemical Carcinogenesis from Biology to Standards Quantification": Ching-Hung Hsu and Todd Stedeford, Eds. John Wiley & Sons, Inc., Hoboken, NJ, 2010

Additional Reviewer Comments

Additional Comments Submitted by Dr. James Bruckner

p. 5, 3.1.1, lines 14 & 15: It is not necessary to mention the lack of data on a carrier (membrane transporter). As DCM and other volatile organic chemicals (VOCs) are small, uncharged, lipophilic molecules, it is well established that they freely pass though membranes by passive diffusion.

p. 9, 3.2, pgr. 2, lines 1 & 2: VOCs readily pass through the blood:brain barrier by passive diffusion.

p. 10, 3.3, pgr. 1, line 5: The GSH pathway does not necessarily predominate at higher doses, but does begin to participate in/contribute to the overall/net metabolism of DCM.

p. 12, 3.3.1, pgr. 2, lines 1 & 2: I doubt that metabolic saturation would be approached at a higher DCM concentration in humans than in rats. CYP2E1 in the fiber production workers was probably induced significantly by acetone.

p. 13, 3.3.1, pgr. 1: Parent VOCs are generally responsible for acute CNS depressant effects. Persons with low CYP2E1 activity should be subjected to higher DCM levels for longer durations, and therefore exhibit more pronounced CNS dysfunction, so long as DCM is present in high enough amounts in the blood and brain. Conversely, more CO would be present in individuals with high CYP2E1 activity. Sufficient circulating CO levels can inhibit CNS functions. Therefore, it is difficult to predict the net effect of CYP2E1 activity levels on neurological effects of DCM. Are there published data on neurotoxic GSH adducts of DCM?

p. 26, 3.4, pgr. 3, lines 7 – 13: Was the blood:air partition coefficient (PB) (i.e., 23) used by Clewell et al. (1993) for humans determined experimentally? This 1993 submission was apparently not published. It is not scientifically justifiable to adopt a PB from rodents for humans merely because the rodent value is higher. Gargas et al. (1989) determined the human PB for DCM to be 8.94 ± 0.13. These researchers found human PBs for over 30 VOCs were consistently lower than PBs for F344 rats. Therefore, it is not appropriate to utilize a rodent value for humans, when human data are available.

p. 28, 3.5, pgr. 1, lines 1 – 6: Marino et al. (2006) used a PB of 23, because they were modeling DCM toxicokinetics (TK) in mice. The same research group (David et al., 2006) subsequently utilized a posterior PB value of 9.7 for modeling DCM TK in humans.

p. 37, 3.5.2, lines 8 – 12: It is not clear why individuals under the age of 18 were "lumped" into one group for scaling V_{max}. Johnsrud et al. (2003) found that adult CYP2E1 expression (protein) values were reached by 90 days postnatal age, and that gender was not a factor. Blanco et al. (2000) reported similar findings upon measuring hepatic microsomal CYP2E1 activity in a smaller number of immature human subjects. These researchers pointed out that clearance of certain drugs is higher in young children (< 10 years), though this is not due to differences in P450 activity. Liver size (when normalized to body weight, but not to body surface area) is larger in young children (Murry et al., 1995). Higher liver blood flow would also contribute to more rapid metabolic clearance of well-metabolized chemicals (e.g., DCM), but there are virtually no data on liver blood flow as a function of age in children (Bjorkman, 2006) or laboratory animals.

p. 46, 3.5.5, pgr. 2: The document's authors should point out there that Korzekwa et al. (1998) merely proposed that CYPs may have 2 or more binding sites with different affinities. Have any data been published since 1998 to support or expand upon this hypothesis? The relevance of the high concentration *in vitro* data (fitted in Figure 3-6) to *in vivo* exposures, as pointed out, is limited.

p. 48, 3.5.5, pgr. 1, lines 5 – 7: Inclusion of the word "substantial" makes this sentence an overstatement of the degree of uncertainty in the PBPK model of David et al. (2006). Their model predicts the kinetics of DCM in human subjects quite well. The major uncertainty is what carcinogenic moiety to measure/model.

p. 51, 4.1.2.2, pgr. 2, lines 7 – 9: Did inhalation of 213 ppm for 1 – 2 hours result in COHb levels that exceeded those seen at the TLV of 500 ppm? This is implied the way this sentence is written.

p. 52, 4.1.2.2, pgr. 2: What is meant by "increased hemoglobin affinity for oxygen"? Is this an adverse effect?

p. 68, 4.1.2.9, pgr. 1, line 4: Substitute the word "potential" for "the" when referring to long-term effects of DCM.

p. 68, 4.1.2.9, pgr. 2, lines 8 – 10: Delete the word "other" in reference to hepatic enzymes, as bilirubin is not an enzyme. Delete the word "clear" in reference to evidence of hepatic damage. These serum enzymes are much more sensitive indices of hepatotoxicity than bilirubin levels. Extensive acute, subacute and chronic studies in rodents show that DCM has limited hepatotoxic potential.

p. 88, 4.1.3.6, pgr. 2, line 4: Another limitation is that subjects were exposed to multiple solvents which were potential human carcinogens.

p. 98, 4.1.3.7.2, pgr. 1, line 16: The word "some" should be inserted before "evidence". An increased SMR or OR for liver or biliary duct cancer has been reported in just one study.

p. 100, 4.2.1: This overview section on oral noncancer and cancer effects seems out of place at the beginning of this major section.

p. 101, 4.2.1.1, pgr. 2, lines 3 and 4: Did Kirschman et al. (1986) describe what they meant by elevated? A statistically-significant, reversible 2- or 3-fold elevation in ALT or AST, is of limited toxicological significance.

p. 101, 4.2.1.1, pgr. 3: A sentence, stating that the extent and incidence of hepatocellular vacuolation were not dose-related in male or female rats, should be added to this paragraph.

p. 103, 4.2.1.1, pgr. 1: It is not accurate to conclude/state there is an increased incidence in severity of generalized vacuolation with increasing exposure level, as there was no difference between the two higher doses in male or female mice.

p. 105, 4.2.1.2.1, pgr. 1: The document's authors should state here in the text that Serota et al. (1986a) administered DCM to rats in drinking water. Did the researchers measure water consumption in order to estimated oral dosages?

p. 106, 4.2.1.2.1: The authors of the document should clarify that the hepatic foci/areas of cellular alteration were areas of fatty change. See pgr. 1 on p. 955 of Serota et al. (1986a).

p. 108, 4.2.1.2.1, pgr. 1, line 6: The words "of neoplastic nodules" should be inserted after the first word in the sentence.

p. 111, 4.2.1.2.2, lines 1 and 2: The word "modest" should be inserted to clarify the EPA's conclusion that DCM ingestion in water produced a treatment-related increase in liver adenomas and carcinomas in male B6C3F1 mice.

p. 112, 4.2.2: Again, I believe the overview section should be omitted in favor of a succinct conclusion section at the completion of the description of inhalation studies.

p. 113, 4.2.2.1, pgr. 1: I do not find this summary paragraph to be useful.

p. 116, 4.2.2.1, line 1: What is cytochrome P-420?

p. 144, 4.3.2.2, pgr. 1, lines 8 – 10: One 1980 report (Borneschein et al., 1980), of altered behavior of immature rats in a novel environment, is insufficient to conclude that DCM induces development neurotoxic effects. The content of the remainder of the paragraph is sufficient to emphasize the potential for elevated COHb formed during high DCM exposures to cause adverse neurological effects.

p. 161, 4.4.3.2.3, pgr. 2, line 12: Was the 1% decrease in catecholamine levels in the 300-ppm exposure group sufficient to be statistically significant?

p. 162, 4.4.3.2.3, pgr. 2: What DCM exposure regimen did Karlsson et al. (1987) employ?

p. 163, 4.4.3.2.3, pgr. 2, lines 17 – 19: How did Karlsson et al. (1987) and Rosengren et al. (1986) determine that neurochemical changes they observed were not due to CO?

p. 163, 4.4.3.2.1, pgr. 1: It should be stated whether the reversibility of the neurochemical changes described here was determined.

p. 173, 4.5.1.1: An account of the observed interspecies differences (i.e., enhanced susceptibility of mice) should be included in this summary paragraph.

p. 178, 4.5.1.2: The relevance of these interspecies differences to humans should be mentioned here, and readers referred to information in the last paragraph in section 4.5.2.

p. 192, 4.5.3: A paragraph should be added to summarize information on interspecies differences in susceptibility to DCM lung tumors. The lack of Clara cells in bronchioles of other rodents and humans should be noted.

p. 192, 4.5.2, pgr. 1: I would insert the word "can" before "impair" in line 4 and remove the word "significant" from line 6. Studies of memory and learning deficits are meager. Almost all halocarbons, in sufficiently high inhaled concentrations, produce reversible CNS depression. The key question is reversibility. This has received relatively little attention in DCM-exposed subjects.

p. 194, 4.5.4: Again, it should be stated that the reversibility of the CNS neurochemical changes has apparently not been studied. A myriad of acute neurochemical changes would be anticipated following exposure to a sufficient dose of any CNS depressant. Animals recover from DCM-induced CNS depression within a relatively short period of time, so it would be anticipated neurochemical changes would also be transient.

p. 195, 4.6.1.2, lines 15 – 23: It should be noted that a trend towards liver tumors was seen in female F344 rats, not in Sprague-Dawley rats of either gender.

p. 207, 4.6.4.1, pgr. 1, line 4: Again, it should be emphasized that the foci/areas of alteration involved fat accumulation, not preneoplastic changes.

p. 208, 4.6.3.2: It should be stated that the mechanism of pulmonary carcinogenic action of DCM is unknown.

p. 209, 4.6.3.3, pgr. 1: The document's authors have relied too heavily on one study (Putz et al., 1979) in concluding that CO is primarily responsible for CNS depression at early times with low exposures. Although DCM is not as potent as more highly halogenated congeners (e.g., chloroform and carbon tetrachloride), the parent compound itself has some CNS depressant effect. Therefore, it seems likely that CO and DCM act in concert.

pp. 209 and 210, 4.6.3.4: It appears that DCM exerts little, if any adverse effects on reproduction, other than delayed ossification at high doses. Thus, speculation about "the mode of action of developmental effects" seems overdone. Although, rat and human fetuses are undoubtedly exposed to DCM, CYP2E1 is undetectable in fetal rat liver (Elbarbry et al., 2007), undetectable or very low in early human fetal liver (Johnsrud et al., 2003) and very low in human neonatal brain (Brzezinski et al., 1999).

p. 232, 4.8.1, pgr. 1, lines 8 & 9: CYP2E1 levels and activity do appear to be higher in human fetal brain microsomes than liver microsomes during the first two trimesters. The actual number of fetal brain donnors, however, was not stated by Brzezinski et al. (1999), so it is not possible to judge how representative their values are. These researchers expressed CYP2E1 activity in terms of pmol product/hour/mg of microsomal protein. As microsomal protein content/g tissue is much lower in brain than liver, it is not accurate to state in line that CYP2E1 activity in brain is high relative to the liver during the second and third trimesters.

It is questionable whether the amounts of CO, formed in the fetus of a woman exposed to trace levels of DCM environmentally or to low levels occupationally, would be sufficient to produce developmental neurological defects. Such exposure situations are radically different from those in which behavioral aberrations were reported in offspring of rats subjected repeatedly to 4,500 or 47,000 ppm.

p. 233, 4.8.1, pgr. 2, line 1: The word "young" should be inserted before "infants". Johnsrud et al. (2003) found that near-adult or adult hepatic CYP2E1 protein levels were attained by 90 – 100 days of age.

It is stated here that relatively low CYP2E1 activity in infants would tend to shift metabolism of DCM to the GST pathway. Were any papers found on the ontogeny of hepatic GST activity, notably GST-T1 activity in humans? McCarver and Hines (2002) reviewed data published on hepatic GSTA1/2, GSTM and GSTP1. These GSTs exhibited different developmental patterns, so it is unknown whether there would be a shift in DCM metabolism from CYP2E1 to GST during the first few months of an infant's life. GST activity surges between birth and postnatal day 10 in Sprague-Dawley rats (Lundquist and Morgenstern, 1995).

p. 234, 4.8.2: Hepatic CYP2E1 activity and protein levels have not been found to be gender-dependent in humans (Johnsrud et al., 2003).

p. 234, 4.8.3, pgr. 3, lines 5 – 7: To provide a balanced perspective, the document's authors should also note that individuals with high CYP2E1 activity should metabolize a smaller proportion of their DCM dose via the GST pathway and therefore be at lower risk of cancer.

p. 270, 5.3, pgr. 3, lines 10 – 13: Undue emphasis has been placed on a theory published by Koezewka et al. (1998), for which there has apparently been no follow-up work. David et al. (2006) conducted MCMC analysis on 5 human data sets (priors) to obtain a human V_{max} (posterior) value higher than values previously used in PBPK models.

p. 278, 5.3, pgr. 1: It may be worth noting that CYP-mediated xenobiotics metabolism in the liver decreases little with advancing age (Bebia et al., 2004; Schmucker et al., 2001).

p. 278, 5.3, pgr. 2: The possibility/likelihood of sensitivity of developing neurons in infants and children to CO could be considered a TD difference.

p. 320, 5.4.4, last sentence: It is assumed that there is some GST metabolism at all exposure levels. Is there experimental evidence to support this important supposition?

p. 331, 6.1, pgr. 2, line 2: The phrase "near steady-state level" is preferable to "steady-state saturation".

p. 331, 6.1, pgr. 2, line 21: Should "GST" be "GSH"?

p. 332, 6.1, pgr. 2, line 6: Substitute the word "limited" for "some".

p. 333, 6.1, pgr. 1, line 3: Changes in behavioral habituation were seen in the offspring, or progeny of pregnant Long-Evans rats.

pp. 331 – 334, 6.1: The document's authors have not provided a balanced perspective of findings in the many toxicological investigations of oral and inhaled DCM. In reality, relatively few adverse effects have been seen in any species despite chronic daily administration of high doses. There have been many negative studies and a lack of adverse effects on many organ systems examined. Hepatic fatty vacuolation and reversible CNS depression are about the only consistent non-cancer effects. Incidences of liver adenomas and carcinomas and mammary fibroadenomas have been of marginal significance, even in highly sensitive strains and species of rodents. There are several well-established scientific reasons that these modest findings are of limited relevance to humans, particularly under realistic (i.e., low) environmental exposure conditions. The reader should be presented a succinct, but comprehensive discussion of evidence for and against the likelihood of human risks for likely human exposure scenarios, rather than a blanket indictment of the chemical.

p. 333, 6.1, pgr. 3, line 3: In most instances the tumor dose-response relationships are problematic.

p. 336, 6.1, pgr. 3, line 8: Selection of the lowest RfC, because it is most health protective, does not seem justifiable, when orders of conservatism are inherent in the standard BMD modeling used to derive the value.

Additional References Submitted by Dr. James Bruckner

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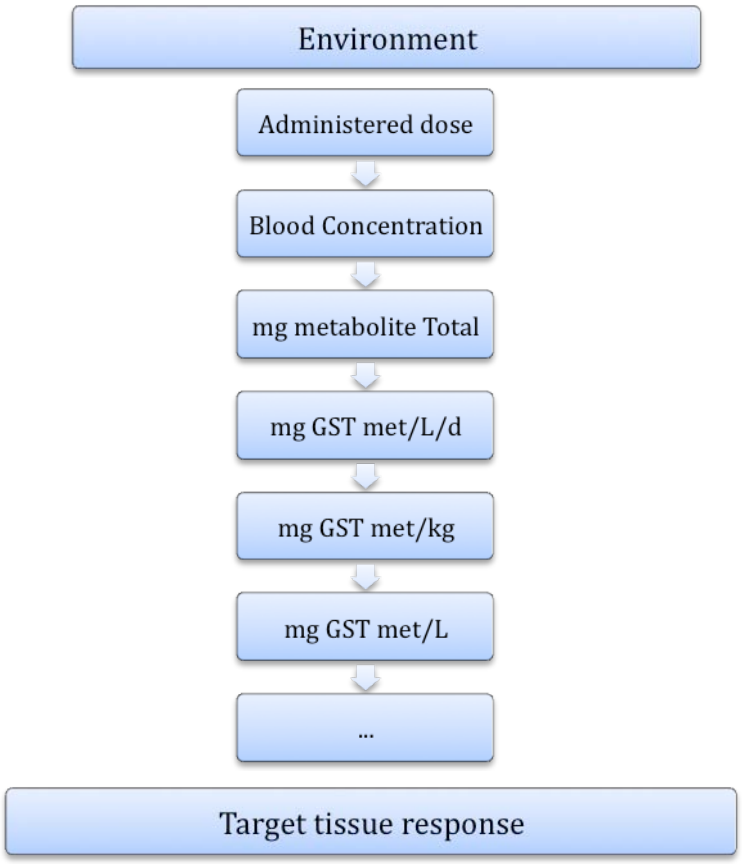

Desire	Feasible
L	H
L	H
L	H
M	M
M	L
H	N

Figure 1. Desirability (based on relevance and closeness to mode of action) as well as feasibility (based on available, peer-reviewed PBPK models) associated with the various dose metrics of relevance to DCM risk assessment (using GST metabolite as an example)

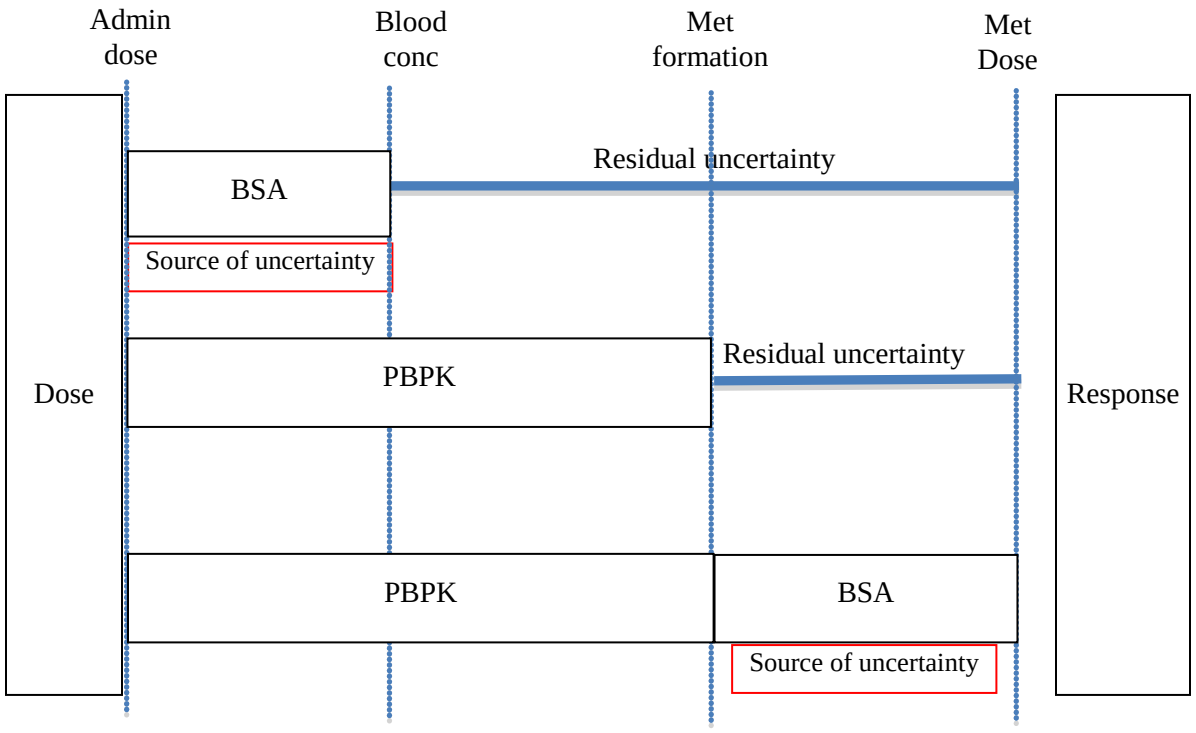

Figure 2. Residual uncertainty in relation to the various approaches. The three layers (horizontal lines) correspond to the default approach, PBPK approach, and the proposed approach (i.e., PBPK+PK scaling).

Appendix A

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National Center for Environmental Assessment

Workshop to Peer Review EPA's Draft Toxicological Review of Dichloromethane

September 23, 2010
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Bethesda, MD

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Appendix C

Agenda

United States
Environmental Protection Agency
National Center for Environmental Assessment

Workshop to Peer Review EPA's Draft Toxicological Review of Dichloromethane

September 23, 2010
Doubletree Bethesda
Bethesda, MD

Agenda

8:00 a.m. Registration/check in

8:30 a.m. **Welcome, Introductions, Meeting Purpose & Agenda**..... *Jan Connery, ERG (contractor)*

8:40 a.m. **EPA Welcome Remarks**..... *Abdel Kadry, IRIS Program Director, EPA NCEA*

8:45 a.m. **Public Comment** ..... *Jan Connery*

9:00 a.m. **General Questions**..... *Hari Mehendale* (*Chair) & Panel*

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

PBPK MODELING

9:20 a.m. **PBPK Modeling** ..... *Hari Mehendale & Panel*

A1. A rat PBPK model was used for calculating the internal dosimetry for the RfD and RfC. EPA evaluated several versions of previously published rat PBPK models and modified the Andersen et al. (1991) model for use in the reference value calculations.

a. Does the chosen model with EPA's modifications adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

b. The internal dose metric used in the RfD and RfC derivations was based on total hepatic metabolism via the CYP2E1 pathway. Because the metric is a rate of metabolism, and the clearance of metabolites is generally expected to be slower in the human compared with the rat (assuming clearance scales as $BW^{3/4}$), the rat internal dose metric is adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric

selection and calculations appropriately considered and discussed?

9:50 a.m. **PBPK Modeling** *(cont.)* ..... *Hari Mehendale & Panel*

A2. The mouse PBPK model used in deriving the cancer risk estimates was based on the published work of Marino et al. (2006).

a. Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

b. The internal dose metric used in the cancer quantitation was based on tissue-specific GST metabolism. To account for potential clearance rate differences, the mouse internal dose metric was adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric selection and calculations appropriately considered and discussed?

10:15 a.m. BREAK

10:30 a.m. **PBPK Modeling** *(cont.)* ..... *Hari Mehendale & Panel*

A3. A probabilistic human PBPK model (David et al., 2006) was used to estimate a distribution of human equivalent doses and concentrations for the points of departure (PODs) for the RfD and RfC, respectively. The 1st percentile of these distributions was selected to represent the most sensitive portion of the population. For the derivation of the oral and inhalation cancer risk estimates, the probabilistic human PBPK model was used to calculate the distribution of human internal doses (mg dichloromethane metabolized via the tissue-specific GST pathway per unit volume of tissue) that would be expected from a 1 mg/kg-day oral dose or a 1 µg/m^3 inhalation concentration. This distribution of human internal doses was used with the tumor risk factor to generate a distribution of oral slope factors or inhalation unit risks.

a. Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

b. EPA modified the parameter distributions in the published David et al. model. Does the set of model parameter distributions adequately account for population variability and parameter uncertainty in estimating human equivalent doses? Are the human parameter values and distributions clearly presented and scientifically supported?

CARCINOGENICITY OF DICHLOROMETHANE

11:00 a.m. **Carcinogenicity of Dichloromethane** ..... *Hari Mehendale* & *Panel*

C1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), dichloromethane is *likely to be carcinogenic to humans* by all routes of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

C2. A mutagenic mode of carcinogenic action is proposed for dichloromethane. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for dichloromethane that may support an alternative mode of action.

11:30 p.m. **Quantitative Cancer Assessment - Oral Exposure** ..... *Hari Mehendale* & *Panel*

C3. A 2-year drinking water study in mice (Serota et al., 1986b) was selected for the derivation of an oral slope factor (OSF) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

C4. The OSF was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for liver tumors in male mice). The OSF is based on an analysis of the most sensitive of the human subgroups, the GST-T1 +/+ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

12:00 p.m. **Quantitative Cancer Assessment - Inhalation Exposure** ..... *Hari Mehendale* & *Panel*

C5. A 2-year cancer bioassay in mice (NTP, 1986) was selected for the derivation of an inhalation unit risk (IUR) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

C6. The IUR was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for lung or liver tumors in male mice) taking into consideration total cancer risk by determining the upper bound on the combined risk for male lung and liver tumors. The IUR is also based on the analysis of the most sensitive of the human subgroups, the GST-T1 +/+ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

12:30 p.m. LUNCH

NONCANCER TOXICITY OF DICHLOROMETHANE

1:45 p.m. **Oral reference dose (RfD) for dichloromethane** ..... *Hari Mehendale & Panel*

B1. A chronic RfD for dichloromethane has been derived from a 2-year oral (drinking water) study in the rat (Serota et al., 1986a). Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

B2. An increase in the incidence of liver lesions (foci/areas of alteration) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

B3. Benchmark dose (BMD) modeling was applied to the incidence data for liver lesions to derive the POD for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in incidence of liver lesions) scientifically supported and clearly described?

B4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

2:40 p.m. BREAK

2:50 p.m. **Inhalation reference concentration (RfC) for dichloromethane** ..... *Hari Mehendale & Panel*

B5. A chronic RfC for dichloromethane has been derived from a 2-year inhalation bioassay in rats (Nitschke et al., 1988a). Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

B6. An increase in the incidence of hepatic vacuolation was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

B7. BMD modeling was applied to the incidence data for hepatic vacuolation to derive the POD for the RfC. Has the BMD modeling been appropriately conducted and clearly described? Is the BMR selected for use in deriving the POD (i.e., a 10% increase in incidence of hepatic vacuolation) scientifically supported and clearly described?

B8. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

3:45 p.m.	**Additional Discussion Issues**	*Hari Mehendale & Panel*
4:00 p.m.	**Reviewer Final Comments**	*Hari Mehendale & Panel*
4:15 p.m.	**Closing Remarks**	*Jan Connery & EPA/NCEA*
4:30 p.m.	ADJOURN	