EPA's Response to Selected Major Interagency Comments on the Interagency Science Discussion Draft IRIS Toxicological Review of Dichloromethane

November 18, 2011

Purpose: The Integrated Risk Information System (IRIS) assessment development process of May 2009 includes two steps (Step 3 and 6b) where White House offices and other federal agencies can comment on draft assessments. The following are EPA's responses to selected major interagency review comments received during the Interagency Science Discussion step (Step 6b) for the draft IRIS Toxicological Review of Dichloromethane (dated June 2011). All interagency comments provided were taken into consideration in revising the final draft assessment prior to posting on the IRIS database. The complete set of interagency comments is available on the IRIS website (www.epa.gov/iris) and includes comments from the Office of Management and Budget (OMB), the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP), and the Department of Defense (DoD).

For a complete description of the IRIS process, including the Interagency Science Discussion review step, visit the IRIS website at www.epa.gov/iris.

June 2011 Interagency Science Discussion Draft IRIS Assessment—Selected Major Comments and Responses:

Topic #1: Selection of the Cancer Descriptor – *OMB commented that EPA did not adequately respond to the peer reviewers who did not support the cancer weight of evidence descriptor of "likely to be carcinogenic to humans" by all routes of exposure for dichloromethane, and recommended that EPA provide additional scientific rationale in response to each of the reviewers' comments.*

> **EPA Response:** EPA considered all the recommendations from the peer reviewers including issues raised regarding the cancer descriptor and addressed these comments in Appendix A of the Toxicological Review of Dichloromethane. EPA's selection of the cancer descriptor of "likely to be carcinogenic to humans" is consistent with the recommendations of the majority of the peer reviewers. Some reviewers recommended that EPA select a cancer descriptor other than "likely to be carcinogenic to humans" based primarily on questions related to the use of evidence in mice for

characterizing human carcinogenic potential. Each issue raised by these reviewers was considered by EPA and responses to these issues were included in the Toxicological Review (see pages A-26 to A-29). The specific issues raised by these reviewers are briefly summarized and addressed below.

1) With regard to the individual concerns raised about the relevance of liver tumors in male B6C3F_1 mice to humans, EPA acknowledges that male B6C3F_1 mice are relatively susceptible to liver tumors, that mouse liver tumors can occur with a relatively high background rate, and that use of mouse liver tumor data in risk assessment has been a subject of scientific controversy. With respect to dichloromethane, however, increased incidence of liver tumors was seen in female as well as male B6C3F_1 mice in the 1986 National Toxicology Program (NTP) inhalation study of dichloromethane, and the background rate of these tumors in females is low, indicating that the tumors are likely to be related to exposure to dichloromethane rather than spontaneous formation. The cancer descriptor is also based on evidence of lung tumors in male and female B6C3F_1 mice following inhalation exposure, an increased incidence of benign mammary tumors in two strains of rats following inhalation exposure, and the presence of a relatively rare tumor in rats—astrocytoma or glioma (mixed glial cell) tumors. In the absence of mode-of-action data or other information that would indicate a lack of human relevance, EPA considers rodent tumors, including mouse liver tumors, to be relevant to humans.

2) Two reviewers noted that the greater glutathione S-transferase (GST) metabolic activity in the mouse compared to humans and other physiological differences between mice and humans could result in much greater susceptibility of the mouse to dichloromethane-induced liver tumors. The activity of the enzyme GST (i.e., the enzyme thought to be responsible for metabolizing dichloromethane to a carcinogenic metabolite) is relatively high in the mouse compared with humans. On the surface, this difference could be interpreted as indicating greater susceptibility of mice compared with humans and thus using a mouse model to estimate carcinogenic risk in humans could lead to an overestimation of risk. However, interspecies differences in the metabolic activity of GST do not indicate a lack of human relevance but rather a quantitative difference in the amount of metabolite(s) formed in the various species. The physiologically based pharmacokinetic (PBPK) modeling utilized in the assessment accounts for the interspecies differences in the amount of the relevant metabolite(s) formed. Similarly, the majority of other physiological differences between mice and humans were accounted for by the rodent and human PBPK models.

3) Two reviewers stated concerns with EPA's "reanalysis" of a 2-year mouse drinking water study published by Serota et al. (1986b) and its use in support of the cancer descriptor of "likely to be carcinogenic to humans." This study was conducted by Hazleton Laboratories (1983) and reported, in part, in Serota et al. (1986b). EPA did not perform a "reanalysis," but relied on the analysis presented in the complete Hazleton Laboratories report, rather than the summary presented by Serota et al, 1986b because the summary in the 1986 publication by Serota et al. omitted some important details. For a complete discussion of the study results see Section 4.7.2 (Synthesis of Human, Animal, and Other Supporting Evidence; pages 73, 143-144, 210-211, and 267) and Appendix A (pages A-28 to A-29) of the Toxicological Review.

4) Two reviewers stated the epidemiological data were largely negative. The findings as well as the strengths and limitations of the available epidemiologic studies of dichloromethane are discussed in Sections 4.1.3 (Cancer Studies) and 4.7.2 (Synthesis of Human, Animal, and Other Supporting Evidence) and Appendix D (Summary of Epidemiology Studies) of the Toxicological Review. Based on the available evidence, EPA concluded that these studies suggest an association between occupational exposure to dichloromethane and increased risk of liver (and biliary) cancer, brain cancer, and specific types of hematopoietic cancers (specifically non-Hodgkin lymphoma and multiple myeloma). The primary limitation of all of the available dichloromethane cohort studies is the limited statistical power for the estimation of effects relating to relatively rare cancers (such as brain cancer, liver cancer, and subtypes of hematopoietic cancers). Although this collection of studies does not establish dichloromethane as a human carcinogen, the data are not consistent with a conclusion of being predominately negative. For a more detailed discussion of this issue see Appendix A of the Toxicological Review (pages A-27 to A-29).

As noted above, the reviewers' comments were summarized and addressed in Appendix A of the Toxicological Review; however, to improve clarity and transparency, a discussion of these issues was also added to Section 4.7.2 (Synthesis of Human, Animal, and Other Supporting Evidence) as part of the cancer weight-of-evidence evaluation.

Topic #2: Cancer Mode of Action – *OMB and DoD commented on EPA's determination that dichloromethane operates by a mutagenic mode of carcinogenic action. Specifically, OMB directed EPA to comments of one of the peer reviewers who is an expert in genotoxicity who offered the view that while there is evidence to indicate that the mode of action for*

dichloromethane might be a mutagenic, there is insufficient data to prove a mutagenic mode of action. OMB recommended that EPA consider revising its conclusions regarding a mutagenic mode of action in light of this reviewer's comments. DoD commented that the available genotoxicity data do not demonstrate that dichloromethane is mutagenic at lower levels of exposure and recommended that EPA differentiate the mode of action for higher and lower levels of exposure, and include a statement that a mutagenic mode of action might occur at occupational levels but is not likely to occur at ambient environmental levels absent a nearby source, and calculate toxicity values for high and low exposure levels separately.

EPA Response: EPA concluded that dichloromethane acts through a mutagenic mode of carcinogenic action in accordance with a majority of the external peer reviewers. The reviewer referenced by OMB commended the presentation in the Toxicological Review of Dichloromethane of the series of tables of genotoxicity data but also suggested additional table(s) that could be useful in summarizing the available mode of action data for particular rodent species, target tissues, and doses. This reviewer described a framework for assessing the evidence pertaining to a mutagenic mode of action, with a hierarchy of types of evidence from (1) assays detecting primary DNA damage (e.g., DNA breakage, unscheduled DNA synthesis, sister chromatid exchanges); (2) assays detecting chromosomal breakage (e.g., chromosomal aberrations, micronuclei tests); and (3) gene mutation assays. This reviewer observed that the in vitro data are probably sufficient to conclude that dichloromethane is an in vitro mutagen, but there was a lack of data from the third tier of the framework, i.e., in vivo data demonstrating induction of mutations in either target or nontarget tissues. This reviewer's conclusion was that the available data were insufficient to conclusively define a mode of action for dichloromethane-induced tumors.

Consistent with peer reviewer suggestions, EPA incorporated four new evidence tables into the Toxicological Review (Tables 4-35 to 4-38) that summarize the relevant genotoxicity data by type of assay, species, and target organ, and discuss the strength of the evidence, target-tissue specificity, dose-response concordance, and temporality associated with the available data for dichloromethane. EPA also re-evaluated the mode-of-action information and revised Section 4.7.3 (Mode-of-Action Information) of the Toxicological Review. Using the suggested framework for assessing the available evidence, the database for dichloromethane provides support for the mutagenicity of dichloromethane and the key role of GST metabolism and the formation of DNA-reactive GST-pathway metabolites along each of these lines: 1) in vivo evidence of chromosomal mutations (chromosomal aberrations and micronuclei)

in the mouse lung and peripheral red blood cells (but not in the more bone marrow, a site that would be expected to be much more limited in terms of degree of dichloromethane metabolism; liver tissue, another site of tumor response, has not been examined in these assays) 2) in vitro chromosomal instability evidence in human cells, other mammalian cells (i.e., CHO), and in bacterial systems; and 3) positive DNA damage indicator assays in numerous vivo and in vitro studies. EPA concluded that the overall weight of the evidence supports the conclusion that dichloromethane induces cancer by a mutagenic mode of action. This weight-of-evidence analysis includes explicit acknowledgement of the lack of in vivo demonstration of mutations in critical target genes for carcinogenesis, and notes specific limitations in the two available studies that have examined this question (see Section 4.7.3.1.1). The relative weighting of different types of evidence (i.e., the greater weight given to data pertaining to chromosomal instability compared with genotoxicity indicator assays of DNA damage) is also described in these revisions.

The cancer mode of action discussion (Section 4.7.3) also specifically addresses the DoD comment that a mutagenic mode of carcinogenic action is not operative at low exposure concentration. As discussed in Section 4.7.3.1.1 (Experimental support for the hypothesized mode of action) of the Toxicological Review, although the majority of genotoxicity assays were conducted at relatively high exposure concentrations (i.e., the exposure concentrations that induced liver and lung tumors in mice), the available information suggests that mutagenicity would occur at low exposure concentrations. As noted above, dichloromethane is metabolized by GST to reactive metabolites that induce mutations in DNA leading to carcinogenicity. Even at very low exposure concentrations, the amount of dichloromethane metabolized in humans through the GST pathway is not zero. At low exposures, the rate of enzyme-catalyzed reactions becomes proportional to the concentration of the substrate(s) and enzyme, and the reaction will proceed at a non-zero rate as long as reduced glutathione, GST, and dichloromethane are present at non-zero concentrations. Thus, although the probability of events induced through the GST pathway is reduced at lower exposures, there is no evidence indicating that the proposed mode of action would not operate at lower exposure concentrations (see Section 4.7.3.2 of the Toxicological Review for discussion). EPA considered this potential issue; the PBPK model utilized in the assessment incorporates the GST dose attenuation with low exposure concentrations.

Topic #3: Physiologically based pharmacokinetic (PBPK) modeling and other analytic details – *OMB observed that EPA rejected certain comments of the peer reviewers specifically with respect to aspects of PBPK modeling, citing examples of comments related to the inhibition of CYP2E1 by carbon monoxide (CO) and two publications noted by a reviewer that questioned the justification of the interspecies scaling factor used to extrapolate data from experimental animals to humans.*

EPA Response: EPA followed all of the major recommendations from the peer reviewers related to PBPK modeling. For example, EPA implemented the approach for evaluation and selection of the rat PBPK model as recommended by a peer reviewer (i.e., that the evaluation should begin with a published model, followed by evaluation of modifications and alternatives to the model based on statistical comparison of model fit to available data). The details of this evaluation as revised per peer reviewer comments are provided in Appendix C. In response to another reviewer comment, EPA added a discussion of scientific evidence supporting the assumption that some dichloromethane is metabolized by the GST pathway at all levels of exposure. The support for this assumption was provided in Sections 3.3 (Metabolism) and 4.7.3.2 (General Conclusions About the Mode of Action for Tumors in Rodents and Relevance to Humans) of the Toxicological Review.

In the few instances where suggestions of the reviewers were explored but no revisions were made, an explanation is provided in detail in the response to comments in Appendix A of the Toxicological Review. For example, EPA investigated the issue of the hypothesized potential inhibitory effect of CO on CYP2E1 metabolism and the recommendation offered by one peer reviewer that this be incorporated in the PBPK model. EPA conducted a thorough review of the literature relating to this issue. No data were identified that could be used to support the hypothesis, and some data were found that contradicted the hypothesis. In addition, a semi-quantitative analysis of this issue by EPA suggests that CO generated as a result of dichloromethane metabolism would not be expected to have a significant effect on CYP2E1 activity. A detailed response is provided in Appendix A under charge question A1a (pages A-4 to A-5).

Another peer reviewer questioned the justification of the interspecies scaling factor (based on body weight raised to the 0.75 power [$BW^{0.75}$]) used to extrapolate data from experimental animals to humans and cited two publications that indicated the $BW^{0.75}$ scaling value was not supported by the literature. EPA reviewed the publications identified by this reviewer which evaluated clearance data for a variety of pharmaceutical compounds and provided ranges of estimated scaling coefficient

values. These results indicate the potential for a range of coefficient values and hence uncertainty in the scaling for clearance of dichloromethane metabolism; EPA acknowledges this uncertainty. However, these data were consistent with $BW^{0.75}$ scaling (a detailed scientific justification for the application of a scaling factor is provided in Section 5.1.2, Derivation Process for Noncancer Reference Values) as a most-likely estimate of clearance in humans compared to rodents. EPA's evaluation of these publications is discussed in detail in Appendix A (pages A-9 to A-10).