Charge to External Reviewers for the
Toxicological Review of Carbon Tetrachloride
May 2008

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of carbon tetrachloride that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). An existing IRIS assessment of carbon tetrachloride was posted to the database in 1987.

The current draft health assessment includes a chronic Reference Dose (RfD) and Reference Concentration (RfC) and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of carbon tetrachloride. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

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

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of carbon tetrachloride.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of carbon tetrachloride.

4. Please comment on the identification and characterization of sources of uncertainty in Sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for carbon tetrachloride

1. A 12-week oral gavage study in the rat by Bruckner et al. (1986) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Has this study been transparently and objectively described in the document? Are the criteria and rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. An increase in serum sorbitol dehydrogenase (SDH) activity was selected as the most appropriate critical effect for the RfD because it is considered by EPA to be an indicator of hepatocellular injury and a biomarker of an adverse effect. Please comment on whether the rationale for the selection of this critical effect is scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. Benchmark dose (BMD) modeling methods were applied to SDH data to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., an increase in SDH activity two times the control mean) scientifically justified? Has it been transparently and objectively described? Please identify and provide rationales for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfD because the available quantitative information on the variability in human response to carbon tetrachloride is considered insufficient to move away from the default uncertainty factor of 10.
- A subchronic to chronic uncertainty factor of 3, rather than a default of 10, was used in light of limited chronic oral study data and more extensive inhalation study data that informed the progression of toxicity from subchronic to chronic exposure durations.
- A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for carbon tetrachloride, and in particular absence of a multigeneration reproductive toxicity study.

Are the criteria and rationale for the selection of these uncertainty factors transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified?

(B) Inhalation reference concentration (RfC) for carbon tetrachloride

1. The JBRC et al. (1998) 2-year inhalation bioassay in the rat was selected as the basis for the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Has the rationale for this selection been transparently and objectively described in the document? Are the criteria and rationale for this selection transparently and

objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Fatty changes in the liver were selected as the critical effect for the RfC because it is considered by EPA to be an adverse effect. Please comment on whether the selection of this critical effect is scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please comment on whether EPA's rationale about the adversity of the critical effect has been adequately and transparently described and is supported by the available data. Please provide a detailed explanation. Please identify and provide the rationales for any other endpoints that should be considered in the selection of the critical effect.

3. An increase in the severity (but not incidence) of proteinuria in low-dose male and female rats was reported in the 2-year JBRC (1998) bioassay. Because the biological significance of this finding in F344/DuCrj rats was considered unclear (see Section 4.6.2 of the Toxicological Review), proteinuria was not used as the critical effect for the RfC. Please comment on whether the decision not to use proteinuria as the critical effect is scientifically sound and has been transparently and objectively described in the document.

4. BMD methods were applied to incidence data for fatty changes in the liver to derive the POD for the RfC. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the BMR selected for use in deriving the POD (i.e., 10% extra risk of fatty liver) been scientifically justified? Has it been transparently and objectively described? Please identify and provide rationales for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA’s approach.

5. PBPK modeling was used to extrapolate the POD from rats to humans and from inhalation to oral dose estimates. Please comment on whether the PBPK modeling for interspecies and route-to-route extrapolation is scientifically justified. Has the modeling been transparently and objectively described in the document? Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported? Has the sensitivity analysis been clearly presented, and appropriately characterized and considered? Has the uncertainty been accurately captured and considered?

6. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfC. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfC because the available quantitative information on the variability in human response to carbon tetrachloride is considered insufficient to move away from the default uncertainty factor of

10.

- An interspecies uncertainty factor of 3 was used to address pharmacodynamic uncertainty only, with PBPK modeling used to address pharmacokinetic extrapolation from rodents to humans; this contrasts with the full default interspecies uncertainty factor of 10 used for the RfD where an oral PBPK model to support interspecies extrapolation was not available.
- A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for carbon tetrachloride, and in particular absence of a multigeneration reproductive toxicity study.

Are the criteria and rationale for the selection of these uncertainty factors transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified?

(C) Carcinogenicity of carbon tetrachloride

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that carbon tetrachloride is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described? Do the available data for both liver tumors in rats and mice and pheochromocytomas in mice support the conclusion that carbon tetrachloride is a likely human carcinogen? Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described?

2. EPA has discussed a mode of action (MOA) for liver cancer involving metabolism, cytotoxicity, and regenerative proliferation leading to tumor induction as key events occurring at relatively high exposure levels. EPA has also discussed that carbon tetrachloride carcinogenicity may not be explained by a cytotoxic-proliferative mode of action only and that a MOA involving genetic damage may also be operative at high exposure levels and may predominate at noncytotoxic (low) exposures. Please provide detailed comments on whether this analysis regarding carbon tetrachloride's MOA(s) is scientifically justified. In particular, please provide comments on EPA's evaluation of the carbon tetrachloride genotoxicity database and EPA's judgments about potential low-dose genotoxicity given the limited information at low doses. Has the MOA for liver cancer been transparently and objectively described in the document? Considerations should include the scientific support regarding the plausibility for each of the hypothesized MOAs, and the characterization of uncertainty regarding these MOAs.

3. Regarding liver cancer, two approaches to dose-response assessment for the inhalation exposure route are presented in the Toxicological Review—a nonlinear low-dose approach and linear low-dose extrapolation approach. Do you agree with EPA regarding the support for a nonlinear extrapolation approach consistent with a mode of action involving hepatocellular cytotoxicity and regenerative hyperplasia? Do you agree with EPA regarding the support for applying the default linear extrapolation approach due to uncertainty in understanding the cancer mode of action at low doses? Please provide detailed comments on whether the inclusion of both approaches to dose-response assessment is scientifically sound and transparently and objectively described in the document.

4. Is EPA's characterization of mouse pheochromocytomas, including their relevance to human cancer risk, transparently and objectively described in this document? EPA has applied a linear extrapolation approach to pheochromocytoma data from the JBRC inhalation bioassay in mice in the absence of mode of action information. Please comment on the scientific justification for quantification of cancer risk for this tumor type, considering relevance to humans. Has the dose-response modeling been appropriately and objectively conducted? Are the results objectively and transparently described?

5. Nonlinear approach: The Toxicological Review finds that the RfD of 0.004 mg/kg-day and an RfC of 0.1 mg/m^3 be used to assess liver cancer risk for carbon tetrachloride under the assumption of a MOA consistent with low-dose nonlinearity. Please provide detailed comments on whether this nonlinear approach is scientifically justified. Has this approach been transparently and objectively described in the document? Are there other nonlinear approaches to evaluating liver cancer risk for carbon tetrachloride that should be presented in the Toxicological Review? Please comment on the utility of including these alternative nonlinear approaches. Please comment on the confidence that EPA should have that there is not a cancer risk for exposures below the level of the RfD/RfC.

6. Linear extrapolation: The Toxicological Review describes alternative approaches incorporating low-dose linearity that were applied to four tumor datasets from JBRC (1998) (female rat and mouse liver tumors and male and female mouse pheochromocytomas). These included (1) point of departure (POD)-based straight line risk calculations and (2) similar risk calculations (for liver tumor data sets only) that examined the effect on risk estimates of using only data on carbon tetrachloride cancer response at exposure levels below those for which increased cell replication was reported. In addition, a Bayesian approach was applied to male mouse pheochromocytoma data to investigate the distribution of the slope parameter in the log-probit model. Please comment on whether the linear extrapolation approaches are scientifically plausible given potential for a cytotoxic MOA at higher doses and other MOAs at lower doses. Please comment on EPA's choice of using data for pheochromocytomas in the male mouse as the basis for the inhalation unit risk and data for female mouse liver tumors as the basis for the oral slope factor. Has the rationale for including a low-dose linear extrapolation been transparently and objectively described in the document? In the above analyses, a benchmark response (BMR) of 5% was used for the female rat liver tumor data set, and a BMR of 10% was used for other tumor data sets. Please comment on the scientific justification for the selection of these BMRs. Is the rationale transparently and objectively described in the document?

7. The conclusion was reached that studies of carbon tetrachloride carcinogenicity by the oral exposure route are not sufficient for the derivation of a quantitative estimate of cancer risk using oral cancer response data and low-dose linear approaches. Please provide detailed comments on whether this judgment is scientifically justified. Has EPA's judgment been transparently and objectively described in the document? EPA used a PBPK model to extrapolate inhalation data to derive an oral cancer risk estimate. Please comment on EPA's application of a PBPK model for route-to-route extrapolation to derive an oral cancer risk estimate from the inhalation data. Please provide detailed comments on whether this approach is scientifically justified. Has EPA's judgment been transparently and objectively described in the document?

8. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* provides guidance on choosing an approach for dose-response extrapolation below the observed data. Relevant language related to choosing an extrapolation approach is provided in Section 5.4.3 of the Toxicological Review. In this section of the Toxicological Review, a linear low-dose extrapolation approach is recommended for assessing carbon tetrachloride cancer risk over a nonlinear approach due to uncertainty in understanding the cancer MOA as well as some bioassay evidence inconsistent with a nonlinear mode of action at low exposure levels. Please comment on the scientific justification for this recommendation. Has this recommendation been transparently and objectively described in the document?