

**NCEA's Proposed Charge to External Reviewers for the
IRIS Toxicological Review of Methanol
December, 2009**

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of methanol 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). There is a current assessment on the IRIS database for the health effects associated with methanol exposure which was first available in 1988.

Publication of significant new toxicological studies since 1988 prompted the Agency to update the methanol IRIS assessment. Newly published research regarding methanol includes toxicokinetic and metabolism studies, development of pharmacokinetic models, inhalation and oral developmental toxicity studies in mice, inhalation developmental and toxicokinetic studies in monkeys, and cancer studies. To develop the 2009 IRIS assessment, EPA collaborated with the National Institute of Environmental Health Sciences (NIEHS) to obtain individual animal pathology and consumption data for a European Ramazzini Foundation (ERF) study for use in the cancer assessment. In March, 2008, the Methanol Institute (MI) submitted to EPA a detailed English translation from Japanese of the original 1985 New Energy Development Organization (NEDO) chronic rat and mouse inhalation studies.^{1,2}

The 2009 draft IRIS reassessment for methanol is based on a comprehensive review of the available scientific literature on the human health effects of methanol and was developed in adherence with general guidelines for risk assessment set forth by the National Research Council in 1983 (NRC, 1983) and numerous guidelines and technical reports published by EPA (see Section 1 of the assessment). Specifically, this IRIS reassessment provides an overview of sources of exposure to methanol, reviews the data on the toxicokinetics of methanol and its metabolites, describes the development of an updated physiologically based pharmacokinetic (PBPK) model of methanol and metabolites, characterizes the hazard posed by methanol exposure for carcinogenicity and non-cancer health effects based on the available scientific evidence, and presents a quantitative risk assessment for methanol health effects, including the derivations of a chronic inhalation reference concentration (RfC) and chronic oral reference dose (RfD) for non-cancer effects and an inhalation unit risk and oral slope factor for carcinogenic effects.

¹ Obtaining individual animal data from both the ERF and NEDO chronic bioassays enabled EPA to independently analyze the tumor data from these studies.

² This translation was certified by NEDO (2008 letter) as being accurate.

Charge Questions

Below is a set of charge questions that address scientific issues in the assessment of methanol. Please provide detailed explanations for responses to the charge questions, and focus any recommendations on improving the accuracy, objectivity, transparency, and utility of EPA's current analyses and conclusions.

(A) Toxicokinetics and PBPK Modeling

A PBPK model developed by EPA based on models by Ward et al. (1997) and Fisher et al. (2000) was utilized in the Toxicological Review of methanol. This model is described in Section 3 and a detailed description of the EPA model modifications, evaluation, and application are found in Appendix B. The PBPK model modified by EPA can estimate internal dose levels due to exogenous methanol exposure (i.e., doses above background). This modified methanol PBPK model was first applied to predict internal doses in experimental animals under bioassay conditions. Benchmark dose (BMD) modeling, using internal doses as exposure metrics, was then used to identify internal-dose points of departure (PODs) from the animal data. Finally the human PBPK model was used to identify human equivalent concentrations (HECs) or doses (HEDs) for each internal-dose POD.

Note: Background methanol levels have been subtracted by study authors from most of the mouse and rat pharmacokinetic data and those background levels are not reported. Since the goal is to predict risk above background, the EPA subtracted background levels from the pharmacokinetic data where it was otherwise included, to obtain a consistent total data-set for use in developing the PBPK models. The underlying assumption is that noncancer and cancer risks from methanol exposure are due to increases in the levels of methanol or its metabolites above background.

1. Please comment on the scientific soundness of the PBPK model used in this assessment.
2. Please comment on the scientific justification for the subtraction of background levels of methanol from the data in relation to the quantification of both the noncancer and cancer risks.
3. The PBPK modeling effort assumed similar methanol pharmacokinetics between pregnant and non-pregnant animals. Please comment on the adequacy of the dose-metric extrapolation based on a PBPK model for non-pregnant adults (i.e., no fetal compartment) for predicting risks associated with fetal/neonatal brain concentrations of methanol.
4. EPA assumes limited methanol metabolism in the fetus because of limited alcohol dehydrogenase (ADH) activity in the human fetus, limited catalase and ADH activity in fetal rodents, and existing pharmacokinetic data that show nearly equal concentrations in maternal blood vs. the fetal compartment. Please comment on the validity of this assumption given the lack of data regarding potential alternate

metabolic pathways in the fetus.

5. Please comment on the scientific justification of the extrapolation approach from rats to humans for in-utero and neonatal lactational and inhalation exposures.

(B) Inhalation reference concentration (RfC) for methanol

1. A chronic RfC for methanol has been derived from a perinatal inhalation study of the effects from exposing rat dams and pups to methanol during gestation and lactation (NEDO, 1987). Reference values from mouse (Rogers et al., 1993) and monkey (Burbacher et al., 1999; 2004) developmental studies, were also derived and discussed, but were not chosen for the RfC. Please comment on whether the selection of the principal study has been scientifically justified.
2. Reduction of brain weight at 6 weeks postnatally as reported in the NEDO (1987) developmental rat study was selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Please identify and provide the rationale for any other endpoints (e.g., other reproductive and developmental effects reported in mouse and monkey studies) that should be considered in the selection of the critical effect.
3. Benchmark dose modeling of decreased pup brain weight relative to maternal internal methanol doses predicted by the PBPK model was used to derive the point of departure (POD) for the RfC. Has the BMD/PBPK approach been appropriately conducted? Has adequate justification been provided for the selected internal dose metric, i.e., area under the curve (AUC) for methanol, in the blood of dams? Please identify and provide the rationale for any alternative approaches for the determination of the POD, including choice of another dose metric (e.g., methanol metabolized), and discuss whether such approaches are preferred to EPA's approach.
4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. It is assumed that these UFs account for variability in methanol dosimetry among human newborns following gestational and lactational exposure, and for uncertainty regarding the ratio of newborn-dose to maternal-dose in humans. Please comment on these assumptions and on the scientific justification for the selected UFs.

(C) Oral reference dose (RfD) for methanol

1. EPA concluded that the oral RfD should be derived using a route-to-route extrapolation from the more extensive inhalation database given the paucity of oral toxicity data. Please comment on whether the rationale for this approach has been scientifically justified and clearly explained. Please identify and provide the rationale for any alternative approaches for the determining the RfD and discuss whether such approaches are preferred to EPA's approach.

2. A PBPK model was used to derive the RfD via a route-to-route extrapolation, in which the internal-dose POD used for the derivation of the RfC based on data from the NEDO (1987) study was extrapolated to human oral exposure levels using the human PBPK model. Please comment on whether the rationale for this approach has been scientifically justified. Has adequate justification been provided for the selected internal dose metric, i.e., AUC for methanol, in the blood of dams? Is the PBPK model suitable for extrapolation of fetal and neonatal endpoints to human oral exposures? Please provide a detailed explanation.
3. EPA applied the same UFs to the POD for the derivation of the RfD as for the RfC. Please comment on the rationale for the selection of the UFs.

(D) Carcinogenicity of methanol

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that methanol is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the weight of evidence characterization scientifically justified and adequately described?
2. EPA has determined that the mode of action of the carcinogenicity of methanol is not known. Has the discussion of the mode(s) of carcinogenic action been accurately and clearly described?
3. Specific to the cancer assessment, EPA has chosen to use the total rate of methanol metabolism as a measure of formaldehyde production from exposure to methanol. In part, the rate of metabolism is used due to the difficulty in determining levels of formaldehyde in the blood, since without data on formaldehyde blood concentrations it is not possible to model those concentrations. This metric of formaldehyde production is uncertain because metabolic processes may differ between species (EPA has attempted to account for expected interspecies differences in the clearance of formaldehyde by normalizing the total rate of metabolism by $BW^{3/4}$). Are there alternative approaches which could be readily applied in conjunction with the existing PBPK model to estimate formaldehyde production from methanol metabolism that would be preferred? If so, please provide the rationale and a detailed explanation of how the alternative formaldehyde dose could be implemented in the PBPK model.
4. A lifetime drinking water cancer bioassay in SD rats (Soffritti et al., 2002) was selected for the derivation of an oral slope factor. Please comment on the scientific justification for the selection of this study. Have the strengths and limitations of the study been adequately characterized? There are two main issues associated with the use of the European Ramazzini Foundation (ERF) bioassay results. One issue is the differences in protocol used by the ERF compared to more generally used study protocols such as those used by the National Toxicology Program. Another issue

concerns the possibility of *Mycoplasma pulmonis* infection in the test animals. Please comment on whether these and any other issues associated with this study have been adequately and clearly described and addressed.

5. The oral cancer slope factor was calculated by linear extrapolation from the POD (lower 95% confidence limit on the internal dose associated with 10% extra risk for lymphomas). Specifically, PBPK model estimates of total metabolized methanol/day (normalized to $BW^{3/4}$; i.e., the internal doses) for each bioassay exposure were used to establish the POD and extrapolate to a human equivalent oral dose. Please comment on the adequacy of this approach, including the choice of tumors and the manner in which the modeling was conducted.
6. A two-year inhalation cancer bioassay in F344 rats (NEDO et al., 2002) was selected for the development of an inhalation unit risk. Please comment on whether the selection of this study is scientifically justified. Have the strengths and limitations of the study been adequately characterized?
7. The inhalation unit risk was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for pheochromocytomas). PBPK model estimates of total metabolized methanol/day (normalized to $BW^{3/4}$) were used to establish the POD and extrapolate to a human equivalent inhalation concentration. Please comment on the adequacy of this approach, including the choice of tumors and the manner in which the modeling was conducted.

(E) General Charge Questions

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?
2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review and should be considered in the assessment of the noncancer and cancer health effects of methanol.
3. Please discuss research likely to substantially increase confidence in the database for *future* assessments of methanol.