

CHARGE TO EXTERNAL REVIEWERS FOR THE IRIS ASSESSMENT OF NITROBENZENE

The U.S. EPA is conducting an external peer review of the human health risk assessment of nitrobenzene that will replace the assessment that currently appears on the Agency's online database, the Integrated Risk Information System (IRIS). The draft Toxicological Review contains derivations of the oral reference dose (RfD), inhalation reference concentration (RfC), cancer inhalation unit risk, and a cancer weight of evidence descriptor. Please provide detailed responses to the charge questions below.

General:

1. Is the Toxicological Review logical, clear and concise? Has EPA objectively and transparently represented and synthesized the scientific evidence for noncancer and cancer hazard?
2. Are you aware of additional studies that should be considered in the assessment of the noncancer and cancer health effects of nitrobenzene?

Oral reference dose (RfD) for Nitrobenzene

For the RfD, the draft reassessment uses a 90-day gavage study in rats by the National Toxicology Program (NTP, 1983) that was reviewed by the NTP Pathology Working Group. The critical effects used were splenic congestion, increased methemoglobin levels, and increased reticulocyte count. Alternate derivations for points of departure are presented in Appendix B-1 of the draft Toxicological Review.

1. Is the selection of the NTP (1983) study as the principal study scientifically justified? Is the rationale for selecting this study transparently and objectively described?
2. Splenic congestion (increased by 10%), methemoglobin levels (increased by 1 SD), and reticulocyte count (increased by 1 SD) relative to the control values serves as the basis for the RfD. Is the selection of splenic congestion, methemoglobin levels, and reticulocyte count as the co-critical effects for deriving the RfD scientifically justified? Has the rationale for selection of these critical effects been transparently and objectively described? Is it appropriate to derive the point of departure by averaging BMDLs across sexes and co-critical effects?

3. Are the uncertainty factors applied to the point of departure for the derivation of the RfD scientifically justified and transparently and objectively described?

4. An uncertainty factor of 3 was selected to account for less-than-lifetime exposure in the principal oral study. Is the choice of this UF scientifically justified and transparently and objectively described?

5. An oral database uncertainty factor of 3 was applied. The database of oral studies includes the principal study (NTP, 1983b), a 90-day gavage study in two species and both sexes; high quality reproductive/developmental studies (Mitsumori et al., 1994; Morrissey et. al., 1988; Bond et al., 1981); structure-activity relationship studies comparing nitrobenzene to dinitro- and trinitrobenzene; and a multidose immunological study in mice (Burns et al, 1994). However, due to a lack of an oral multigeneration reproductive toxicity study and in light of evidence of male reproductive toxicity, a factor of 3 was applied. Is the choice of an UF of 3 scientifically defensible given the available oral and inhalation databases? Does the available data suggest that oral exposures may result in new adverse effects at oral doses equivalent to or lower than the inhalation concentrations used in the multigeneration reproductive and developmental study by Dodd et al. (1987)?

Inhalation reference concentration (RfC) for Nitrobenzene

The draft reassessment of nitrobenzene uses a 2-year inhalation study for deriving the RfC. Several endpoints were identified as potential critical effects, including bronchiolization of the alveoli (mice), olfactory degeneration (mice), methemoglobin levels (rats), and splenic congestion (rats). Bronchiolization of the alveoli was chosen as the critical effect for the following reasons: 1) bronchiolization of the alveoli, a metaplastic lesion, occurred in $\geq 87\%$ of male and female mice at the lowest exposure concentration and none of the controls (olfactory degeneration occurred in 1.5% of males and 32% of females at the lowest concentration; methemoglobin levels were $\sim 3\%$ in both male and female rats at the lowest concentration tested); 2) the severity of bronchiolization of the alveoli was consistent in both male and female mice; 3) bronchiolization of the alveoli is a portal of entry effect that is relevant to oronasal breathers (e.g., humans); and 4) this endpoint was obtained from a chronic inhalation study in which 43% of male mice developed bronchio-alveolar adenomas or carcinomas at the highest concentration tested. Alternate derivations of the RfC are presented in Appendix B-2 of the draft Toxicological Review.

1. Is bronchiolization of the alveoli the most scientifically justifiable endpoint on which to base the RfC? Have the rationale and justification for this selection been transparently and objectively described? Are there any other studies that you believe would be justified scientifically as the basis for the RfC?
2. If bronchiolization of the alveoli is the most scientifically justifiable endpoint on which to base the RfC, is the LOAEL-to-NOAEL approach the best method for deriving the RfC?
3. A database UF of 1 was applied in deriving the RfC because the database includes a two-year (lifetime) chronic inhalation study with an interim (15-month sacrifice), two-generation reproductive and developmental inhalation studies, a subchronic (10-week) inhalation neurotoxicity study, and two 90-day inhalation studies. Is the application of a database UF of 1 scientifically defensible and transparently and objectively described given the available data for nitrobenzene?

Carcinogenicity of Nitrobenzene

1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), nitrobenzene is classified as *likely to be carcinogenic to humans*. Have the rationale and justification for this designation been transparently and objectively described? Do the available data support the conclusion that nitrobenzene is a likely human carcinogen? If the weight of the evidence supports the descriptor *likely to be carcinogenic to humans*, is it appropriate to describe nitrobenzene as a case that lies on the low end of the range of this descriptor?
2. The two-year inhalation cancer bioassay (CIIT, 1993; published as Cattley et al., 1994) was used for development of an inhalation unit risk (IUR). Is this study the most appropriate selection for the principal study? Has the rationale for this choice been transparently and objectively described?
3. Data on hepatocellular tumors in F344 rats were used to estimate the IUR. Are the reasons for basing the quantitative assessment on hepatocellular tumors in male F344 rats scientifically justified and transparently described? For calculating the IUR, adenomas and carcinomas were combined. Has EPA's justification for this approach been objectively and transparently presented? Is combining adenomas and carcinomas the most scientifically justifiable approach for these tumors? Please suggest any other scientifically justifiable approaches for calculating the IUR.

4. The IUR was calculated from hepatocellular tumors in male F344 rats. The recommended upper bound estimate on human extra cancer risk from continuous lifetime exposure to nitrobenzene was calculated to be $3 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$. Is it scientifically defensible to base the IUR on liver tumors alone? Have the rationale and justification for this analysis been transparently and objectively described? Is it more appropriate to calculate the IUR using combined tumor incidence of liver, thyroid, and kidney tumors in male F344 rats as done in the alternate derivation of the IUR in the Appendix? If summing of tumors is scientifically justified, is the method used to sum the tumors supported by the science and the data? If not, what alternative method should be used?