Comments on: The Draft IRIS Toxicological Review for Trichloroethylene (TCE) for Final Agency/Interagency Science Discussion

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Comments:

Although the document is lengthy, EPA has done a good job in presenting the body of evidence to support an RfD of 5 x 10^{-4} (and the RfC) based on the Keil et al. (*1*) data in female mice, the Peden-Adams et al. (*2*) decreased IgM antibody plaque-forming cell (PFC) response and increased delayed type hypersensitivity (DTH) response; Johnson et al. (2003) fetal heart malformations. Two important adjustments in the interpretation and use of immune data are strongly suggested.

1. Autoimmune effects from the Keil (*1*) study in B6C3F1 mice should have been used in support of the combined RfD and to derive the RfC in place of the data on decreased thymus weight.
   Both autoimmune effects and decreased thymus weight were observed at the lowest dose in the Keil (2009) drinking water study of adult B6C3F1 mice (0, 1400, 14000 ppb TCE). The thymus data have the uncertainty of being an unreliable indicator of immunotoxicity while the autoimmune data have greater biological significance and are linked to human TCE-autoimmune effects.
   a. Autoimmune effects:
      i. Autoantibodies to (double stranded) ds DNA at 1400 and 14000ppb
      ii. Autoantibodies to (single stranded) ss DNA at 1400 and 14000ppb
      iii. Dose-response- high dose (14000ppb)effects on ds DNA 6 weeks before low dose
      iv. Supported by renal pathology (may be associated with anti-dsDNA) observed at 1400ppb
      v. 1400ppb (EPA converts to 0.35 mg/kg/d POD) is LOAEL
      vi. EPA UF of 100 is too low (10-interspecies, 10-intrahuman, 1-LOAEL to NOAEL, 1-database)
      vii. The LOAEL to NOAEL UF cannot be reduced from 10 to 1 for autoimmune or other immune effects at 1400ppb TCE in B6C3F1 mice because of the three clear autoimmune effects *[1)autoantibodies to dsDNA, 2)autoantibodies to ssDNA, 3) renal pathology],* the stimulation or hypersensitivity observed at this dose *[4) increased DTH]* which supports human data and may be related to the autoimmunity as well, and then finally the 5) suppression of PFC (*1, 2*). If the LOAEL to NOAEL were reduced from 10 to 3 (which we advise against) then an increase in the database UF from 1 to 3 or 1 to 10 is warranted because it is clear the database demonstrates multiple immune effects a the 1400ppb dose and there is a lack studies with data at lower doses.
      viii. The total UF should be 1000 (10-interspecies, 10-human variation, 10-LOAEL to NOAEL)
      ix. Therefore, the RfD should be 0.00035, which supports the Peden-Adams and Johnson RfDs.
      x. Increased ds DNA is accepted biomarker for autoimmunity (*3*)
      xi. Human data support association between TCE and autoimmunity, with the strongest data for systemic sclerosis (as concluded in the EPA draft assessment and (*4, 5*)); the autoimmunity is also related to hypersensitivity in humans and supported by DTH data cited in point vii above
   b. Thymus weight was decreased at both doses (at 1400 and 14000ppb)
      i. 1400ppb (EPA converts to 0.35 mg/kg/d POD) is LOAEL
      ii. UF=1000 (10-interspecies, 10-human variation, 10-LOAEL to NOAEL)
      iii. Thymus weight is a relatively insensitive or “unreliable indicators of immunotoxicity” (*6*); therefore, it may be an indicator of immunotoxicity but its use to derive an effect level should be done with caution or avoided if there are other options.
      iv. Thymus weight change was not observed in the Peden-Adams study of B6C3F1 mice at the same dose (1400, and 14000ppb) in a developmental through 8 weeks of age

v. **Thymus weight is a non-specific marker of potential immunotoxicity and should not be used when there are better data, with functional significance and a clear link to the human data for effects of TCE.**

2. Several statements were made in the draft TCE document that downplays the significance or predictability of IgM antibody plaque-forming cell (PFC) response data for the determination of immunotoxicity. These statements are incorrect, because PFC is highly predictive for immunotoxicity and a preferred functional immune assay for use in risk assessment (*6, 8*). The statement that "decreased PFC response may not be considered adverse in and of itself" contradicts the EPA's immunotoxicity testing guidelines(*7*) and is counter to the strong support of PFC data by immunotoxicologists (*6, 8*), and they should be corrected or removed. The statement that on lines 29-31 page 5-53 is incorrect and should be deleted, and the document should be searched and similar statements removed throughout:

> 29 Although decreased PFC response may not be considered adverse in and of itself, a
> 30 LOAEL-to-NOAEL UF of 10 was used because of the increased delayed-type hypersensitivity at
> 31 the same dose.

In summary, the autoimmune effects (specifically increased autoantibodies to dsDNA and ssDNA with associated increase in renal pathology) in Keil et al. (*1*) study of B6C3F1 mice should have been used for the RfD and RfC in place of the thymus data because the thymus weight data is a less reliable indicator of immunotoxicity. Furthermore, the autoimmune data has clear support for functional significance and as strong link between the animal data on autoimmune endpoints and the evidence for TCE-related autoimmunity and hypersensitivity in humans. The statements discounting the reliability of the PFC should be deleted.

Best Regards,

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References:

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