Supplemental Analysis of Parameter and Model Uncertainty*: Overview & Context

- Focus of this peer review and possible next steps
- Rationale & context for EPA UA
- Chemical-specific vs. non-specific data
- Newly published papers of interest

*Supplement: Uncertainty Analysis of In Vitro Metabolic Parameters and of In Vitro to In Vivo Extrapolation (IVIVE) Used in a Physiologically Based Pharmacokinetic (PBPK) Model for Chloroprene

Focus of Peer Review

- Focus of current peer review: Is the chloroprene PBPK model sufficiently reliable for use in an IRIS Toxicological Review?
  - Prediction of chloroprene tissue concentrations in mice, rats, and humans
  - Prediction of chloroprene oxidative metabolism in lung, liver, and kidney of mice, rats, and humans
- If the model is found sufficiently reliable, then details of "how" it is applied would be addressed in a subsequent EPA analysis.
- That subsequent EPA analysis would include consideration of:
  - Multiple tumor sites identified and considered in the 2010 IRIS assessment
  - Appropriateness of specific internal dose metrics vs. use of external exposure to extrapolate risk for each site
  - Factors to address other possible animal-human differences
- For now, EPA seeks to evaluate the broadest possible use of the model
  - Prediction of metabolism depends on accurate tissue concentration

Rationale & Context for EPA UA

- **Expectation of quantitative UA for PBPK models**
  - When PBPK models are used in IRIS Toxicological Reviews and other EPA assessments there is an increasing expectation for a quantitative UA
  - Rigorous UA requires robust measures of uncertainty for all parameters
- **Observations re. confidence intervals (CIs) presented in Ramboll report**
  - CIs appear to be very narrow
    - Example: Vmax for female mouse liver has 95% CI ~ ± 20% of mean
  - Experiments involve repeated measures (samples) from incubation vials
    - If 5 vials are each sampled 6 times, those are not 30 independent measurements
    - Variability between vials may occur due to exact volume of microsomes added, temperature (location in incubator), septum seal, etc.
  - Ramboll analysis appears to treat each data point as independent, which *could* result in overly narrow CIs

Rationale & Context for EPA UA (2)

- **Difference between Himmelstein et al. (2004) and Yang et al. (2012)**
  - Vmax for male vs. female mouse lung appears quite different
    - Male data: Himmelstein et al. (2004); female: Yang et al. (2012)
    - Same lab, same experimental PI (M. Himmelstein), but 8 years apart
  - Similar tumor response rate for male vs. female mice in the NTP bioassay
    - If risk is proportional to metabolic rate, then tumor response rates should be different
  - Could be due to pharmacodynamic differences between males & females
  - But could this reflect an underlying uncertainty in the in vitro metabolism results, due to unrecognized differences in the two sets of experiments?
- **Ramboll analysis assumes control incubation data from Yang et al. (2012) are applicable to Himmelstein et al. (2004) studies**
  - Apparent significance of difference between male & female mice depends on assumptions in the statistical modeling

Rationale & Context for EPA UA (3)

- **U.S. EPA Uncertainty Analysis (UA) (initial results shown)**
  1. Parameters fit separately to data for each vial (experimental unit)
  2. Vial-to-vial variation assumed due to irreducible sources of experimental variation ➔ uncertainty in parameters
  3. Parameters for each vial then combined in a way that maintains this measure of uncertainty
  4. *Considering* option of analyzing Himmelstein et al. (2004) data using only concurrent controls (limited #) vs. all controls (much more data)

- **Technical public comment on likelihood derivation (Ramboll)**
  - EPA considered & responded to comment on a prior version
  - Additional slides available, if requested, with details of EPA's derivation

Chemical-Specific vs. Non-Specific Data

- **Ramboll report suggests use of relative CYP activity in human lung vs. liver to estimate human lung metabolism for chloroprene (parameter "A1")**
- **This approach was used previously by the U.S. EPA for dichloromethane (DCM)**
- **However, A1 is determined using data for a marker substrate, 7-ethoxycoumarin**
  - Relative affinity/activity of various CYPs for this substrate is likely to be different from chloroprene and DCM
- **For DCM, chemical-specific data was not available for human lung metabolism**
  - Use of A1 was best alternative
- **For chloroprene, we have chemical-specific Himmelstein et al. (2004) data**
  - All else being equal, chemical-specific data are preferred
  - But these data are challenging to analyze because activity is close to background
  - EPA believes this challenge can be overcome through appropriate, careful statistical analysis
  - Estimation of background losses (and the uncertainty in those), depends on which control incubation data are used
- **Ultimately, EPA's approach should provide an alternate estimate, *and upper confidence bound*, of the human lung metabolic rate, and all other metabolic parameters**

New IVIVE Studies

- **Additional studies have recently been published on IVIVE**
  - Benet, L.Z., Sodhi, J.K. Investigating the Theoretical Basis for *In Vitro–In Vivo* Extrapolation (IVIVE) in Predicting Drug Metabolic Clearance and Proposing Future Experimental Pathways. *AAPS J* **22,** 120 (2020). https://doi.org/10.1208/s12248-020-00501-9
  - Kenyon EM, Eklund C, Pegram RA, Lipscomb JC. Comparison of in vivo derived and scaled in vitro metabolic rate constants for several volatile organic compounds (VOCs). Toxicol In Vitro. 2020 Sep 15:105002. https://doi.org/10.1016/j.tiv.2020.105002
- **These papers were brought to attention of the peer review panel for their consideration**
- **EPA scientists are evaluating these studies and what they might suggest for the chloroprene IVIVE PBPK model**

Relevance of New Studies

- **Benet and Sodhi (2020)**
  - Focus on clearance of drugs
  - Binding to serum proteins is a significant factor
  - Distinguishes between net hepatic clearance (CL_H) and intrinsic clearance (CL_{nt}):

$$CL_H = Q_H \cdot f_{u,B} \cdot CL_{nt} / (Q_H + f_{u,B} \cdot CL_{int})$$

    where Q_H = hepatic blood flow and $f_{u,B}$ = fraction unbound in blood.
  - Suggests that commonly used values for Q_H may be too low
  - While serum binding is significant for some environmental chemicals (e.g., PFAS), it is not a factor for chloroprene and other VOCs ($f_{u,B}$ = 1)
  - Use of reported blood perfusion rates in PBPK models has generally been found to provide adequate predictions of VOC distribution and hepatic clearance
  - Significantly increasing Q_H is likely to lead to an over-prediction of hepatic clearance for VOCs

Relevance of New Studies (2)

- **Kenyon et al. (2020)**
  - Term "clearance" used in paper corresponds to intrinsic clearance
  - PBPK model only has metabolic clearance in the liver, but...
  - Compares hepatic Vmax or CL_{int} = Vmax/Km estimated by IVIVE to value obtained by fitting gas-uptake data from in vivo exposures
  - While in vivo CL_H also depends on hepatic clearance, a key focus of this review is the IVIVE estimation of Vmax
  - For the *lung*, metabolic clearance is generally less dependent on blood-flow:

$$CL_{lung} \approx Vmax(lung)/Km$$

  - For chloroprene metabolism in the *lung* two options are being considered:
    - $Vmax(lung) = A1*Vmax_{liver,in\text{-}vitro}$(IVIVE) [Ramboll's primary proposal]
    - $Vmax(lung) = Vmax_{lung,in\text{-}vitro}$(IVIVE) [alternate being considered by EPA]
  - Both options depend on the accuracy of IVIVE predictions for Vmax
  - Kenyon et al. (2020) evaluates Vmax(IVIVE) vs. Vmax(in vivo) in rats, for which extra-hepatic oxidative metabolism (including in the lung) is low.

Clarifications on Statistical Likelihood Derivation Comments Submitted by Ramboll

Comment on Likelihood

During the public comment period, Ramboll submitted the following comment:

p. 11, equation 4. The equation should have a factor of Cdat(tj) in the denominator on the right-hand side of that equation. The basis for that statement is in the line previous to that equation where it is stated that the data are {(tj, Cdat(tj)): j ϵ {1, …, N}}. As noted in Equation 3, USEPA specifies that log[Cdat(t)] is assumed to be normally distributed. Note that the integral over the support for any given data point should equal 1. The support for Cdat(t) is from 0 to infinity. The integral based on the expression given in Eq. 4 does not equal 1. An easy fix to this discrepancy might be to state that the data are {(tj, log[Cdat(tj)]): j ϵ {1, …, N}}. The support for log[Cdat(t)] is negative infinity to infinity, and integration over that support does indeed equal 1 as desired.

Comment on Likelihood (2)

- U.S. EPA (2020) states that the *residuals*, or differences between observations and model estimates, which are represented by ε in Eq. 3, are normally distributed. (Justification for this assumption is provided earlier in the U.S. EPA document, notably in Figures 3 and 4.)

$$\log[C_{\mathrm{dat}}(t)] = \log[C_{\mathrm{mod}}(t;q)] + \varepsilon \quad \text{(Eq. 3)}$$

$$\Updownarrow$$

$$\varepsilon = \log[C_{\mathrm{dat}}(t)] - \log[C_{\mathrm{mod}}(t;q)]$$

- U.S. EPA (2020) does <u>not</u> state, as Ramboll asserts, that the *observations* ($\log[C_{\mathrm{dat}}(t)]$) are normally distributed.

Comment on Likelihood (3)

- The likelihood function defined in Eq. 4 of U.S. EPA (2020) has been correctly stated based on the error model stated in Eq. 3.

$$\mathcal{L}(\theta|\mathcal{D}) = \prod_{j=1}^{N} \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{\left(\log[c_{\mathrm{dat}}(t_j)]-\log[c_{\mathrm{mod}}(t_j;q)]\right)^2}{2\sigma^2}\right] \quad \text{(Eq. 4)}$$

- If one makes the substitution $\varepsilon_j = \log[C_{\mathrm{dat}}(t_j)] - \log[C_{\mathrm{mod}}(t_j; q)]$ in Eq. 4, then the term in the product is, by definition, the probability density function for $N(0, \sigma)$ evaluated at ε_j. If one integrates the expression in the product over the domain of possible values of ε_j ($-\infty$ to ∞) the value of the definite integral is one, as shown here*.

*The hyperlink is to https://www.wolframalpha.com with the site search string: "integral(1/sqrt(2*pi*s^2) * exp(-x^2/(2*s^2))) from negative infinity to infinity"