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WORKPLAN TO PROVIDE A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL TO SUPPORT THE INHALATION UNIT RISK (IUR) FOR CHLOROPRENE

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1 INTRODUCTION

Multiple physiologically-based pharmacokinetic (PBPK) models available in the published, peer-reviewed scientific literature (Allen et al. 2014; Himmelstein et al. 2004; Thomas et al. 2013; Yang et al. 2012) have been evaluated and applied in the estimation of potential cancer risks following inhalation exposure to chloroprene (CAS No. 126-99-8). Several of these were identified by the U.S. Environmental Protection Agency's (USEPA) Integrated Risk Information System (IRIS) Toxicological Review of Chloroprene (USEPA 2010) and in a recent Request for Correction (RFC) of the Inhalation Unit Risk (IUR) submitted by Denka Performance Elastomer, LLC (DPE 2017). As noted in USEPA's Denial of the RFC (USEPA 2018), one of the key reasons for the denial was the lack of model validation, noting limitations and uncertainties that need to be addressed. Also lacking was the underlying code for these models to fully evaluate and consider them in the estimation of the IUR for chloroprene. All the published models rely upon the same underlying in vivo and in vitro data and PBPK models.

We outline below an approach for addressing the limitations and uncertainties raised by the USEPA that have prevented the use of these models in the development of the IUR for chloroprene, and provide the model code(s) needed to allow for full review of the available peer-reviewed models by USEPA and their application in the estimation of an IUR for chloroprene. This workplan primarily is intended to guide the process of scientifically evaluating and improving the PBPK model for chloroprene in support of an updated and more scientifically justifiable IUR. An ancillary objective is to provide USEPA a clear representation of the model refinement process and facilitate USEPA's possible review and input at each stage.

2 PROPOSED APPROACH

As noted in the response to the RFC dated January 25, 2018, USEPA was unable to locate and obtain the final code associated with the published PBPK models. USEPA (2018) noted that PBPK code is necessary for a quality assurance and quality control review by USEPA. Because the final code is not available, USEPA cannot evaluate the internal validity of the Yang et al. (2012) PBPK modeling methods or results, or results that are dependent on this model [i.e. Allen et al. (2014)]. Further complicating this, the software platform for these models (ACSL) is no longer available; therefore, migration to a new platform, such as R, will be necessary. The proposed approach to validating the PBPK model will be focused on addressing the comments that have been provided by USEPA in the IRIS (2010) assessment, as well as the Denial of the RFC (USEPA 2018), that were discussed as limitations and uncertainties with the PBPK model for chloroprene. The workplan further describes additional analyses to be conducted using the existing model to address these limitations and uncertainties, which will provide the USEPA with the necessary PBPK model code that would allow for a quality review and application of the model in the estimation of the IUR.

The uncertainties remaining in the application of the PBPK models that have been noted by USEPA in the IRIS Assessment (USEPA 2010) and the response to the RFC (USEPA 2018) are related to four specific areas:

- Justification for selected parameters in the in vivo/in vitro models
- Ability to reproduce in vivo pharmacokinetic data
- Estimation of uncertainty in the model using Markov Chain Monte Carlo (MCMC) analyses
- Reproduction of PBPK model code in an available operating platform

How we plan to address each of these areas of uncertainty is discussed in the following sections.

2.1 Justification for selected parameters in the *in vivo/in vitro* models

USEPA (2010) noted that the PBPK model reported in Himmelstein et al. (2004) currently predicts blood chloroprene and delivery of chloroprene to metabolizing tissues based on metabolic constants and partition coefficients based on in vitro data. Loss of chamber chloroprene is attributed to uptake and metabolism by test animals and was used to test the metabolic parameters and validate the model. However, Himmelstein et al. (2004) did not provide results of sensitivity analyses indicating whether chamber loss was sensitive to metabolism, and therefore it is uncertain whether chamber loss is useful for testing the metabolic parameters used in the model. We will conduct a sensitivity analysis using the current ASCL model in vitro and in vivo code and the results provided to USEPA for consideration.

The USEPA has further noted that the female mouse lung metabolism and internal doses in Yang et al. (2012) are not consistent with results for male mice. Vmax is approximately five times higher for male mice than for female mice, yet the tumor response is similar. This has implications for biological basis for the site-specific dose-response, and parameterization of extra-hepatic metabolism. Additional analyses will be conducted to evaluate the uncertainty in the Vmax estimates. The results of these analyses will determine if pharmacokinetic differences can explain the sex-specific differences in response in the mouse, or if there is evidence of pharmacodynamic differences or sex-specific sensitivity.

The dose metrics relied upon in all the modeling publications have focused on metabolism in the liver, lung or kidney. The USEPA has noted that lung metabolism does not account for tumor responses at other sites outside the lung, which also need to be incorporated into a risk assessment. Additional analyses will be conducted to determine if data are available to suggest significant metabolic capability

in organ systems other than the liver, lung or kidney and how critical the potential contribution of this metabolism might be to the overall composite risk.

2.2 Ability to reproduce *in vivo* pharmacokinetic data

In the IRIS Assessment (USEPA 2010), the USEPA noted that the model's ability to reproduce in-vivo PK data [i.e. from Himmelstein et al. (2004)] has not been evaluated. In the chloroprene docket is a report in which blood chloroprene was measured in mice following single (6-hour) and repeated (5- or 15-day) inhalation exposures (unpublished). Chloroprene blood levels were higher following single exposures, which was postulated to be because of higher minute volume due to stress. The authors conclude that these blood data are suitable for validation of a PBPK model, but it is unclear whether the data were used for the validation of the PBPK model in Yang et al. (2012). The report did not investigate chloroprene levels in the organs of interest (namely the lungs, liver, or kidneys). Additional simulations will be conducted to determine if the in vivo model can be validated using the datasets in the mouse provided in the chloroprene docket (DuPont 2009).

Of additional concern in the IRIS Assessment (USEPA 2010) was that Himmelstein et al. (2004) had to reduce alveolar ventilation and total blood flow values predicted from the in vitro data by 50% to match the in vivo PK data presented. Mice are well known to suppress respiration (RD) and cardiac output in response to irritant gases. However, the response would be dose dependent. Change in respiration and cardiac output is necessary to fit the available data and has been observed with and incorporated into models for other compounds. Although there are no data specific to chloroprene to characterize respiratory and cardiac output suppression, additional analyses will be conducted to increase the confidence in this adjustment and to find additional scientific data to support this adjustment.

2.3 Estimation of uncertainty in the model using Markov Chain Monte Carlo (MCMC) analyses

In the 2010 IRIS assessment, USEPA noted the need to use distributions of the PBPK model parameters to represent variability in intra-population rates of chemical absorption, distribution, metabolism, and elimination to estimate human variability. The MCMC analyses conducted as part of the Yang et al. (2012) publication was to investigate potential variability in parameters, but also understand the potential uncertainty and its impact on estimating potential cancer risks from exposure to chloroprene. So, while Yang et al. (2012) addresses part of USEPA's (2010) comments, additional relevant comments were noted in the USEPA (2018) response to the RFC. USEPA (2018) questions the form of the log-likelihood function used in the MCMC analysis and suggests that the autocorrelation among repeated measures from a single experimental unit has not been considered. USEPA (2018) also noted that the female mouse kidney metabolism approaches zero in the MCMC optimization and that parameterization of extra-hepatic metabolism may be incorrect.

For liver metabolism, this is apparent on the log-scale for predictions of chloroprene headspace concentration data provided in Figure 2b of Yang et al. (2012), and Figures 5 and 25 of Study IISRP-17520-1388 (submitted to EPA-HQ-ORD-2009-0217). The underestimation occurs for both the point estimate results and the Monte Carlo results. Also, because the molecular form of enzymes does not vary between tissues within an individual, or males and females of a species, the Km for metabolism should be likewise constant across tissues and between sexes.

The MCMC analyses conducted by Yang et al. (2012) will be revisited to address these comments.

2.4 Reproduction of PBPK model code in an available operating platform

As noted in the USEPA (2018) response to the RFC, while several model code packages were shared with the USEPA by Dr. Harvey Clewell, these are poorly documented and do not provide sufficient instructions that allow the EPA to review or apply the available models now. Once the comments

previously outlined have been addressed, the final step in the workplan will be to provide a complete model code with adequate documentation and files to reproduce critical results needed for the quality review of the model and the application in the estimation of the IUR. Both the code for the in vivo and in vitro components of the model will be provided allowing the USEPA to reproduce the PBPK results from Himmelstein et al. (2004), Yang et al. (2012), Thomas et al. (2013) and Allen et al. (2014). The code will be provided in the R platform, with the necessary scripts to reproduce the analyses conducted as part of the workplan as well as the results provided in the publications.

3 SCHEDULE

We plan to communicate closely with the USEPA to ensure that the remaining questions and uncertainties associated with the review and application of the PBPK model for chloroprene have been addressed. We anticipate that we will be able to provide the needed model code, addressing the remaining uncertainties, to the USEPA within 4 to 6 months following acceptance of the workplan.

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