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U.S. Environmental Protection Agency
EPA Docket Center (ORD Docket)
Mail Code: 28221T
1200 Pennsylvania Avenue, NE
Washington DC 20460

Re: Supplemental Comment of The Styrene Information and Research Center Protocol for the Ethylbenzene IRIS Assessment (Preliminary Assessment Materials) (CASRN 100-41-4); EPA Docket No. EPA-HQ-ORD-2014-0526

Ladies and Gentlemen:

The Styrene Information and Research Center (SIRC) appreciates the opportunity to submit these supplemental comments on the Protocol for the Integrated Risk Information System (IRIS) Assessment of Ethylbenzene. 88 Fed. Reg. 10,320 (Feb.17, 2023).

As noted in our comments submitted March 16, 2023, SIRC recently sponsored a research project to provide a further perspective on the exposure concentrations that were found in the National Toxicology Program (NTP) studies to produce tumors in rodents (National Toxicology Program (1999) NTP Technical Report on the Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS NO. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies) National Toxicology Program, Research Triangle Park, NC). This study explored the kinetically-derived maximal dose (KMD) concept for ethylbenzene inhalation exposure.

As detailed in the enclosed Extended Abstract, Burgoon *et al.*, tested the hypothesis that the neoplastic lesions seen in the NTP's ethylbenzene rodent cancer bioassay are a high dose phenomenon secondary to saturation of elimination kinetics, which can be identified by estimation of the KMD. The key findings of the study are that (1) using the method of Burgoon et al. (2022),[1] the authors defined KMD ranges for ethylbenzene inhalation in rodents (8-17mg/L venous ethylbenzene concentration) and in humans (10-18mg/L venous ethylbenzene concentration); and (2) these KMD ranges are near an inhalation concentration of 200ppm ethylbenzene. The authors conclude that typical human ethylbenzene exposures are well below the KMD and will not result in cancer; cancer endpoints in rodents exposed to ethylbenzene above the KMD are not relevant to toxicological testing and risk assessment focused on protecting human health at foreseeable

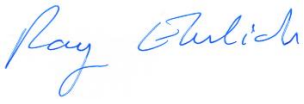
[1] Burgoon, L.D. et al. (2022) A novel approach to calculating the kinetically derived maximum dose. Arch Toxicol, 96, 809–816.

exposures; and ethylbenzene is not a human cancer hazard and does not pose a credible cancer risk to humans under foreseeable exposure conditions.

A manuscript for this study has been submitted for publication in a peer-reviewed journal and is summarized in more detail in the attached Extended Abstract, Bugoon LD, Borgert CJ, Fuentes C, Klaunig JE. (2023, submitted for peer review). Kinetically-derived maximal dose (KMD) indicates lack of human carcinogenicity of ethylbenzene. Extended Abstract provided ahead of publication.

Thank you for considering this additional work.

Sincerely,



Ray Ehrlich
Executive Director

Attachment: Bugoon LD, Borgert CJ, Fuentes C, Klaunig JE. (2023, submitted for peer review). Kinetically-derived maximal dose (KMD) indicates lack of human carcinogenicity of ethylbenzene. Extended Abstract provided ahead of publication.

EXTENDED ABSTRACT

Of

Embargoed Publication Under Peer-Review

Kinetically-Derived Maximal Dose (KMD) Indicates Lack of Human Carcinogenicity of Ethylbenzene

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Summary

- The kinetically-derived maximal dose (KMD) is defined as the maximal external dose where elimination kinetics are likely unchanged relative to lower doses.
- We and others have observed that toxicity above the KMD can be much different from toxicity below the KMD.
- We tested the hypothesis that the neoplastic lesions seen in the National Toxicology Program's rodent cancer bioassay are a high dose phenomenon secondary to saturation of elimination kinetics, which can be identified by estimation of the KMD.
- We used the method of Burgoon et al. (2022) to define KMD ranges for ethylbenzene inhalation in rodents and in humans.
- KMD estimates range from 8-17mg/L venous ethylbenzene concentration in the rat, and from 10-18mg/L venous ethylbenzene concentration in humans.
- These KMD ranges are near an inhalation concentration of 200ppm ethylbenzene.
- Our results indicate that:
    - typical human exposures are well below the KMD and will not result in cancer;
    - cancer endpoints in rodents exposed to ethylbenzene above the KMD are not relevant to toxicological testing and risk assessment focused on protecting human health at foreseeable exposures.
    - ethylbenzene is not a human cancer hazard and does not pose a credible cancer risk to humans under foreseeable exposure conditions.

Introduction

Ethylbenzene is a common feedstock for the synthesis of styrene. Ethylbenzene may also be found in paints, varnishes, and in many other products, and it is a component of mixed xylenes (Saghir *et al.*, 2010). Our interest in ethylbenzene is focused on whether rodent tumors observed at doses up to the Maximum Tolerated Dose (MTD) of ethylbenzene – 750 ppm by inhalation exposure (National Toxicology Program, 1999) – are relevant for human risk assessment. Typically, the MTD not only exceeds anticipated human exposure levels but frequently exceeds dose levels that saturate drug and chemical metabolism and elimination pathways in the test species, leading to a host of toxic effects that are irrelevant to the effects expected from lower concentrations where elimination kinetics are not saturated (Borgert *et al.*, 2021; Burgoon *et al.*, 2022; Bus, 2017; Andersen, 1981).

We have argued that MTD testing is biologically irrelevant to human exposures and therefore an unethical waste of animals and resources (Borgert *et al.*, 2021). We contend that instead, toxicity testing should be constrained to the Kinetically-Derived Maximum Dose (KMD). We define the KMD as the maximal external dose where elimination kinetics are likely unchanged relative to lower doses (Borgert *et al.*, 2021). In practice, we estimate the KMD as a range to represent our uncertainty about the precise location of the KMD (Burgoon *et al.*, 2022). The KMD has practical importance for the identification of safe exposure levels to protect against tumorigenesis and cancers.

Hypothesis

The National Toxicology Program (NTP) bioassay in rats showed a lack of excess tumors at concentrations of 75ppm and 250ppm. Based on this finding and previous physiologically-based toxicokinetic modeling (PBTK), we hypothesized that the increase in tumor incidence observed at 750 ppm ethylbenzene is consistent with toxicity that occurs secondary to saturation of elimination kinetics rather than to frank carcinogenicity of ethylbenzene, and that a KMD likely exists between 200-500ppm (Saghir *et al.*, 2010; Charest-Tardif *et al.*, 2006). A KMD estimate below 750ppm would suggest that the tumors and cancers observed in the NTP bioassay are likely not relevant to lower exposure levels, whereas a KMD estimate greater than 750ppm would refute our hypotheses and would suggest that saturation of elimination kinetics does not contribute to tumorigenicity and carcinogenicity.

Methods

Literature Search

We conducted a comprehensive literature search to identify studies that may contain pharmacokinetic data for ethylbenzene in mice, rats, and humans and from this comprehensive search, identified 52 potentially useful peer-reviewed articles and reports. Upon review of those publications, we narrowed this list to five peer-reviewed articles that contain sufficient pharmacokinetic data to support a valid model. Those data are from rats and humans. We found the available mouse data (Charest-Tardiff et al., 2006; Fuciarelli et al., 2000) to be insufficient for a reliable estimation of the Michaelis constants *Vmax* and *Km* for mice, but that rat kinetic data are applicable to mice, which is consistent with determinations made previously by Nong et al. (2007).

KMD Range Estimation

We used a Bayesian approach to estimate the Km and Vmax for the system-wide Michaelis-Menten function that governs the toxicokinetic curve (Burgoon et al., 2022). We then calculated the Michaelis-Menten curve from the estimated Km and Vmax. We define the KMD as the maximal curvature in the Michaelis-Menten curve, identified using the “kneedle” algorithm (Burgoon et al., 2022; Satopaa et al., 2011).

We validated our Km and Vmax using “out of sample data”. This involves comparing the toxicokinetic measurements for a concentration of ethylbenzene not used to estimate the Km and Vmax for the system-wide Michaelis-Menten function (we call those data and resulting curve “ground truth”) with the curve generated from the estimated Km and Vmax for the system-wide Michaelis-Menten function. Specifically, we used toxicokinetic data for 50ppm ethylbenzene to estimate a system-wide Km and Vmax and then compared the toxicokinetic curve generated from those parameters with the toxicokinetic curve for 100ppm ethylbenzene at 4, 4.5, 5, 5.5, and 6hrs post exposure (ground truth). If the toxicokinetic curve generated from the estimated Km and Vmax fit the ground truth measurements sufficiently well, we would conclude that the model produced a valid result. We would conclude the model to be invalid if the toxicokinetic curve generated from the estimated Km and Vmax did not fit the ground truth measurements.

Results

The five peer-reviewed publications used to determine the KMD for rat and humans are listed in Table 1.

Table 1. Peer-reviewed publications used to determine the KMD for ethylbenzene in rat and humans.

Publication	Species
Haddad et al. (1999) Toxicology and Applied Pharmacology 161: 249-257.	Rat
Haddad et al. (2000) Toxicology and Applied Pharmacology 167: 199-209.	Rat
Freundt et al. (1989) Bull. Environ. Contam. Toxicol. 42: 495-8.	Rat
Tardif et al. (1997) Toxicology and Applied Pharmacology 144: 120-134.	Rats and Human
Marchand et al. (2015) Toxicological Sciences 144(2): 414-24.	Human

We used the male rat venous ethylbenzene concentration data at an external exposure of 50ppm (Haddad *et al.*, 1999, 2000) to estimate the Km and Vmax of the global pharmacokinetic system (Burgoon *et al.*, 2022). Blood measurements were taken at 4, 4.5, 5, 5.5, and 6hrs post exposure.

The estimated Km ranged from 3.49-5.01 mg/L, while the estimated Vmax ranged from 3.39-7.64 mg/(L*30 min) or 0.11-.25mg/L*min (note: our model runs in 30 minute increments, so 3.39-7.64 mg/(L*30min) is the output from our model; however, we are reporting mg/L*min for reader convenience). The estimated Km and Vmax for rat at the 50ppm exposure in rats resulted in a toxicokinetic curve that matched the toxicokinetic measured data (i.e., the measured data as blue dots overlay the yellow line, which is the mean of the 100 model runs; Figure 1A), indicating a valid model result.

We validated the Km and Vmax estimates from the 50ppm ethylbenzene exposure group by comparing those to the out of sample ground truth data for rats exposed to 100ppm ethylbenzene (Figure 1B). Again, we see that the measured data (the blue dots in Figure 1B) overlay the mean of the 100 model runs (the yellow line in Figure 1B). This provides increased confidence that the rat Km and Vmax estimates are valid estimates independent of the exposure concentrations.

We used the method of Burgoon et al. (2022) to estimate the rat KMD range. Using the validated and estimated Km and Vmax values, we estimated the rat KMD range to be from 8-17mg/L venous ethylbenzene concentration, which translates to a 200ppm exposure over a 4hr period based on data in Haddad et al. (1999). We used the Km and Vmax estimates to generate the Michaelis-Menten curve that allowed us to calculate the KMD range using the kneedle algorithm (Burgoon et al., 2022; Satopaa et al., 2011).

We estimated the human Km and Vmax using the same approach used for estimating the rat Km and Vmax (Burgoon *et al.*, 2022). We used the venous ethylbenzene concentration data from 6.25, 6.75, 7.50, and 8.0hrs post 12.5ppm inhalation exposure provided by Marchand *et al.* (2015). The human data also closely fit the estimated curve, indicating a valid model result (Figure 1C). The y-axis is particularly important in considering the human toxicokinetic curves in Figure 1C/D. Typically, the model overpredicts by 0.01mg/L or less, which is well within our stated tolerances for a valid model result. Fewer data were used to estimate the human Km and Vmax compared to the rat, so it is possible we would see less overprediction by the model if more human data were available.

Figure 1

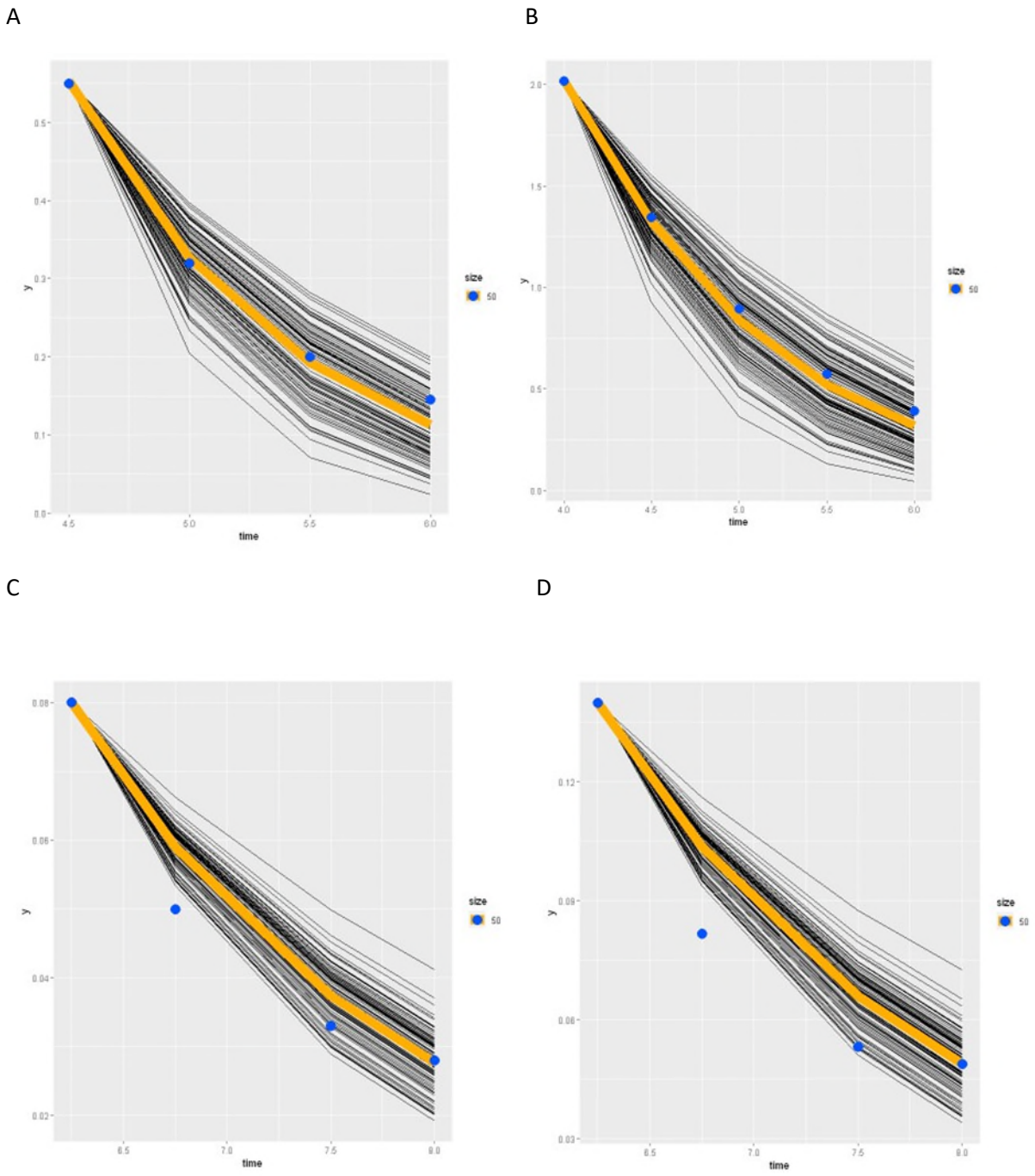

Figure 1: (A) Rat 50ppm exposure, (B) rat 100ppm exposure (validation), (C) human 12.5ppm exposure, (D) human 25ppm exposure (validation). Spaghetti plots show 100 different runs; the yellow line is the mean of the 100 runs, and the blue dots are the measured venous ethylbenzene concentrations.

We estimated the KMD range to be from 10-18mg/L venous ethylbenzene concentration for humans. We used the Km and Vmax estimates to generate the Michaelis-Menten curve, and the Kneedle algorithm was applied to estimate the KMD range (Burgoon *et al.*, 2022; Satopaa et al., 2011).

Due to limitations in the existing literature, we were unable to translate this venous ethylbenzene concentration to an external exposure concentration. However, assuming the kinetics of ethylbenzene absorption similar in humans and rats, as is suggested by Marchand et al (2015), then we would expect an external exposure of 200ppm over 4hrs to exceed the human KMD.

Conclusions

We used pharmacokinetic data for ethylbenzene from rats and humans to estimate KMD ranges. For the rat, we estimated a KMD range from 8-17mg/L venous ethylbenzene. For humans, we estimated a KMD range from 10-18mg/L venous ethylbenzene. These ranges correspond to an inhalation concentration of approximately 200ppm ethylbenzene. These KMDs, taken in a risk context, support the hypothesis that the neoplastic lesions seen in the NTP's rodent cancer bioassay are a high-dose phenomenon secondary to saturation of elimination kinetics. The evidence indicates that typical human exposures to ethylbenzene, which are well below the KMD, are non-carcinogenic.

Our results also indicate that cancer endpoints measured in rodents exposed to ethylbenzene concentrations above our estimated KMD range are not relevant to toxicological and risk assessments focused on protecting humans at typical human exposure levels. Thus, the evidence indicates that ethylbenzene is not a carcinogenic hazard for humans and does not pose a cancer risk to humans under foreseeable exposure conditions. Future work on this topic should either attempt to refute this hypothesis through better and higher quality studies or should focus on ethylbenzene effects below the KMD.

Mouse data were also considered in our evaluation because tumors have been reported in chronic toxicity/carcinogenicity studies in mice as well as in rats. The kinetic profile for ethylbenzene in mice clearly differs between 75 ppm (linear) and 750 ppm (saturated), prompting the authors who reported this phenomenon to conclude that the kinetics become saturated at an intermediate dose not greater than 500 ppm (Charest-Tardiff et al., 2006). Based on Nong et al.'s validation of a PBPK model for ethylbenzene in mice that used Michaelis constants derived from rat, our KMD estimate should be equally applicable for interpreting the results of toxicology and carcinogenicity studies conducted in mice and rats.

It has been argued that evidence of alveolar carcinoma was observed in mice at doses lower than those required to produce liver and kidney tumors. However, the incidence of frank alveolar carcinoma was not observed at any level of exposure in mice, and combined adenoma/carcinoma incidence was statistically elevated only at 750 ppm, but not at 250 ppm or 50 ppm exposure in studies conducted by the U.S. National Toxicology Program (NTP, 1999). Furthermore, the alveolar precursor lesions alleged by NTP to be observable at lower doses are dependent on mouse-lung-specific metabolism of ethylbenzene, as discussed in the literature (e.g., Nong et al., 2007). As with styrene (Cruzan et al., 2002), the higher conversion of ethylbenzene to CYP2E1 metabolites in mouse lung are likely responsible for changes observed in mouse lung at exposures below 200 ppm, but these do not appear to correspond with neoplasia or tumors at higher doses. Furthermore, such pulmonary effects in mice are highly unlikely to be relevant to humans since the pulmonary activity of CYP2E1 in mice is approximately 20-fold higher than in mouse liver, and 23 and 600 times higher in mouse versus rats and human lung microsomes.

Risk assessors should consider the KMD when evaluating the mode of action that underlies dose-response relationships (Borgert *et al.*, 2021; Burgoon *et al.*, 2022) because, as we and others have discussed (Andersen, 1981; Bus, 2017), toxicity is likely to change once exposure nears the point of metabolic saturation and/or saturation of clearance mechanisms. This is a critical point in the case of ethylbenzene risk assessment. At this time, the data clearly support the hypothesis that neoplastic lesions, and thus cancers, occur only when elimination kinetics are saturated, and this should now be the default assumption for risk assessment. Until and unless future work on ethylbenzene carcinogenesis convincingly falsifies that conclusion, research on the toxicity of ethylbenzene that is applicable for human risk assessment should be focused on ethylbenzene exposures that approximate foreseeable human exposure levels, which are likely to be below the KMD identified here.

Acknowledgements

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