June 3, 2014
Lori White, Designated Federal Officer for the BSC
Office of Liaison, Policy and Review, Division of NTP
NIEHS
P.O. Box 12233, K2-03
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Re: Comments regarding NTP Research Concept for Xylenes for the NTP Board of Scientific Counselors Meeting June 17-18, 2014

Dear Dr. White:

The American Chemical Council's Toluene and Xylenes Panel (the "Panel") appreciates the opportunity to comment on NTP's Research Concept for Xylenes, which will be presented at the June 2014 meeting of the Board of Scientific Counselors (BSC). After evaluation of the specific aims and proposed approach, the Panel does not believe conducting additional studies will provide additional meaningful data given the studies that have already been conducted adequately address questions of carcinogenicity and toxicity of xylene.

- The proposal states "Given the carcinogenicity of ethylbenzene and that the human dataset for mixed xylenes is limited by co-exposures to other compounds, a test agent free of ethylbenzene is needed in order to allow assessment of the toxicity or carcinogenicity of xylenes." However, in 1986, NTP conducted a 2-year oral gavage carcinogenicity study in which groups of B6C3F1 mice were dosed at 0, 500, or 1000 mg/kg and F344/N rats at 0, 250, or 500 mg/kg of mixed xylenes containing approximately 17% ethyl benzene. The study did not demonstrate any treatment-related increase in the incidence of any tumors.[1] Therefore, further study to exclude ethyl benzene in a xylene mixture would be expected to produce a similar outcome and if conducted, would be not only unnecessary but also a wasteful use of animals and government resources.

- The proposal does not adequately consider dose and kinetics with regard to study relevance. It is likely that internal blood levels achieved by oral gavage exposures are higher on both a peak and cumulative concentration basis than could be achieved by an inhalation study. Thus, inhalation studies could be expected to be conducted at a lower internal dose that has already been demonstrated not to cause carcinogenic effects. Additionally, if one considers the availability of published physiology-based pharmacokinetic mode useful for xylenes, there is even less justification for conducting inhalation experiments, as the internal dosimetry can be readily estimated for both rats and humans using the available models. [2,3] Finally, given the

failure to observe portal-of-entry specific effects in homologous oral and inhalation studies, there is even less rationale to support use of additional animals to generate data.

- A test agent free of ethylbenzene is not commercially available. Thus, the development of subchronic and chronic toxicity data on a test material that is unlikely to reflect any real world product would be not be a valuable exercise.

- Additional studies evaluating the developmental toxicity, reproductive toxicity and neurotoxicity of xylenes following whole body inhalation exposures are not warranted, as sufficient data are already available to address these endpoints in detail. Overall, sufficient data exists for the evaluation of individual xylene isomers through the inhalation route of exposure showing no significant difference between the effects seen with the technical isomeric xylene mixture (containing ethylbenzene) and individual xylene isomers.
    - **Developmental toxicity –** In September 2012, the California Environmental Protection Agency (Cal EPA), Office of Environmental Health Hazard Assessment (OEHHA) published a detailed evaluation of the available data on the developmental and reproductive toxicity of xylene. This document not only evaluated data obtained in rodents and other animal species, but also evaluated existing epidemiological evidence in humans. **With regard to developmental toxicity, the OEHHA report identified fifteen (15) studies, thirteen (13) of which were performed through the inhalation route.**[4]
        - The most recent study by Saillenfait et al. is perhaps the most relevant as it made a direct comparison between effects with the technical xylene isomeric mixture and the individual xylene isomers. In this study, Sprague-Dawley rats were exposed to 100, 500, 1000 or 2000 ppm (433, 2165, 4330, 8660 mg/m^3) of *o-, m- p*-xylene, ethylbenzene and technical xylene (containing 15.3% ethylbenzene, 21.3% o-xylene, 43.9% m-xylene and 19.4% p-xylene), for 6h/day on gestation days 6-20. There was a significant decrease in fetal body weight and a decrease in mean percentage of fetuses/litter with skeletal variations at 1000 and 2000 ppm. However, these changes were minimal and occurred in association with significant maternal toxicity. Overall, the authors concluded that *"the only indication of a treatment-related effect was a slight decrease in fetal weight" and that "no evidence of teratogenic effects was found after exposure to any of these agents up to 2000 ppm".*[5]

        - In a study by authors Ungvary and Tatrai, New Zealand rabbits were exposed to 500 or 1000 mg/m^3 technical xylene or single isomers for 24h/day on gestation days 7-20. The study reported an increase in spontaneous abortions and resorptions at 1000 mg/m^3 with exposure to *p*-xylene and technical xylene. This was associated, however, with 13% and 30% mortality in dams at 1000 mg/m^3 *p*-xylene and technical xylene respectively.[6]

- **Reproductive toxicity –** Although a standard 2-generation reproductive toxicity study could not be located for xylenes, several animal studies are available indicating that xylenes do not cause any adverse effect on the reproductive organs and fertility of male and female animals. [4]
    - Nylen et al. evaluated the effects of xylene inhalation exposure to the reproductive organs and fertility of male Sprague-Dawley rats exposed for 18 h/day, 7 days/week for 61 days. Rats were then evaluated 2 weeks, 10 months and 14 months after cessation of exposure. There was no evidence for adverse effects in the histopathology evaluations of the testis, hormone levels, sperm count, sperm morphology and fertility.[7]
    - A summary of reproductive performance following inhalation exposure to mixed xylene is available. The results indicate no adverse systemic or reproductive toxicity in male and female Crl-CD® (SC) BR rats exposed to 500 ppm mixed xylene (highest dose tested) for 6 h/day for 131 days prior to mating, during mating and continuing through gestation and lactation.[8]
    - The published hazard information on ethylbenzene is also informative, which indicates no adverse effects on F0 and F1 reproductive toxicity and offspring developmental endpoints exposures of 500 ppm ethylbenzene (highest dose tested).[9]
- **Neurotoxicity –** The neurological effects of xylene are well documented and studies show acute effects with both short term and long term exposures ranging from 100 – 2000 ppm. These acute central nervous system (CNS) effects observed in animals are consistent with those seen in humans and include narcosis, prostration, respiratory depression, hyperactivity and tremors.[10] Overall, the neurotoxicity of xylenes (either as individual isomers or as the isomeric mixture containing ethylbenzene) has been sufficiently documented in literature. The effects observed in humans are identical to those in animals and typically involve acute depression of the central nervous system. No persistent neurological effects have been observed following xylene exposure. Developmental neurotoxic effects were not found when pups were evaluated following prenatal exposure.
    - In one human study, authors Riihimaki and Savolainen exposed human volunteers to 100 or 200 ppm m-xylene, 6 h/day, for 5 days. The human volunteers reported acute CNS effects, including impaired body balance, but reported no persistent neurological effects.[11] Klaucke et al. reported as summarized by EPA, acute CNS symptoms such as headaches, vertigo and nausea in 15 workers occupationally exposed by inhalation to up to 700 ppm xylenes.[10] Similar complaints were also noted for paint workers using xylene-based products.[10]
    - The long-term occupational study by Uchida et al. is perhaps the most significant human study conducted to date on occupational exposure to xylene and has been used by California EPA to develop a chronic inhalation Reference Exposure Level (REL) and by ATSDR to develop a chronic Minimal Risk Level

(MRL). In this study, a total of 175 workers exposed to mixed xylenes at an estimated concentration of 14 ppm over a more than 7-year period were evaluated. This cohort included workers involved in the production of rubber and printing ink workers. The authors reported an increase in the prevalence of subjective symptoms related to acute CNS effects including dizziness, headache and a heavy feeling in the head. There was no evidence of prolonged neurotoxic effects.[12]

- Authors Gralewicz and Wiaderna exposed rats to 100 ppm m-xylene, 6 h/day, and 5 days/week for 4 weeks. There were no effects in radial maze tests conducted 14-18 days post-exposure, open field activity tests conducted 25 days post-exposure and active avoidance tests in rats evaluated 54-60 days post-exposure. A significant effect in one out of six (1/6) trials was obtained in passive avoidance tests conducted 39-48 days post-exposure. Paw lick latency tests were negative unless footshock was employed 50-51 days post-exposure.[13] Two neurotoxicity studies in rats exposed to m-xylene, 6 h/day and 5 days/week for 3 or 6 months (100 ppm for 6 months or 1000 ppm for 3 months in the 1st study and 100 ppm for 3 months in the 2nd study) showed effects on motor coordination and pain sensitivity when tested 24-hours after cessation of exposure.[14,15] One developmental neurotoxicity study was also available for review. In this study, pregnant rats were exposed to 3500 or 7000 mg/m3 p-xylene, 6 h/day on gestation days 7-16. Tests on locomotor activity and acoustic startle response were negative in the pups.[16]

In conclusion, the proposal aims to assess the toxicity of a mixture which does not appear to be in commerce, has already been assessed at higher internal exposures, and proved by NTP to lack carcinogenic effects even when it contained ethylbenzene. A substantial database already exists to characterize the potential risks from exposure to xylene isomers and mixed xylenes. It is unlikely that the proposed studies would result in any meaningful contribution to the overall risk characterization or risk assessment of xylenes. Given those considerations, the proposal would appear to be a low priority for research and thus does not warrant the expenditure of limited government resources and animal lives.

The American Chemistry Council Toluene and Xylene Panel appreciates the opportunity to submit these comments. If you have any questions, please contact Angela Lynch at 202-249-6708 or Angela_Lynch@americanchemistry.com.

Sincerely,

[Redacted]

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