

Comments of the ACC Hydrocarbon Solvents Panel
Attachment III

Justification for employing the Adenuga et al (2014) study (cited as Koch Industries, 1995b) as the basis for the derivation of a reference dose (RfD)

Chemical-Specific Charge Questions

B. Literature Search Strategy/Study Selection

1. Please comment on the whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported.

Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB.

ACC Comments:

The 90-day oral subchronic toxicity study of 1,3,5-trimethylbenzene (cited as *Koch Industries, 1995b* in the Draft Assessment) (Adenuga et al., 2014) was conducted in response to a TSCA Section 4(a) test rule (58 Fed. Reg. 59667 (1993) in support of the “EPA’s efforts to develop Health Advisories (HAs) for unregulated drinking water contaminants that are monitored under section 1445 of the Safe Drinking Water Act (SDWA)”. As an oral study, it is directly relevant for to an RfD determination compared to the inhalation studies the EPA has used since it does not require route-to-route extrapolation. **The principal reason this study was rejected was that it did not identify any adverse neurological or respiratory effects. As will be explained in more detail below, the respiratory effects observed with trimethylbenzenes (either as individual isomers are as complex substances) are local “portal of entry” effects that would not be associated with exposure in drinking water and are assessed in inhalation studies that are directly relevant to the RfC. In addition, evaluation of oral exposures causing acute central nervous system (CNS) effects with 1,3,5-trimethylbenzene and other structurally similar alkylbenzenes (such as xylenes) show that neurological effects are not expected at the highest dose employed in the study.** In other words, the study provides a more conservative NOAEL estimate that is also protective of systemic effects more appropriate to oral exposures. Specifically, our comments on the validity of this study for the determination of an RfD are as follows:

[1] Lack of objectivity in EPA independent peer-review

As the study report was not published at the time the Draft Assessment was developed, the EPA sought external peer review to assess study reliability. The EPA indicated that the results of the external peer review led them to “conclude that this study was not suitable to serve as a principal study with which to derive human health reference doses”¹ and that it provided only “limited toxicological information”². Although this was the conclusion of two of three peer reviewers, this conclusion is **not** based on the quality of the study itself but in context of the neurotoxicity endpoints evaluated in the inhalation studies. In essence, the EPA concluded that the TSCA test rule-mandated study, conducted in accordance with

¹ Section 2.6.1, lines 6-19, page 2-48 in Draft Assessment

² EPA response to public comments. Appendix F, lines 3-13, page F-4 of the Supplement to the Draft Assessment.

existing EPA guidelines, was not “suitable” only because it did not evaluate the EPA’s pre-determined critical endpoints that are more appropriate for inhalation exposure. This bias is reflected in the misleading charge question presented to the peer reviewers for their review of the 1995 study report. Rather than request that the external peer reviewers independently assess the quality of the study, the EPA framed charge question 1b as follows:

In consideration of the toxicological properties of trimethylbenzenes reported in the provided contextual references (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Korsak et al., 200a, b; Wiaderna et al., 1998; Gralewicz et al., 1997a; Gralewicz et al., 1997b; Korsak et al., 1997; Korsak and Rydzynski, 1996; Korsak et al., 1995), please comment on whether there are key physiological/toxicological endpoints that should have been assessed that were not part of the investigation³.

Considering that the existing EPA guideline at the time this study was conducted did not specifically call for neurotoxicity evaluation other than the standard clinical observations, this charge question could only have led to one conclusion. **As indicated by all three peer reviewers, the study quality was high and “all the elements required by the EPA 798.2650 guidelines were included”**. According to one of the peer reviewers (citing the current EPA OCSSP Harmonized Test Guideline 870.3100), such a study should include a functional observation battery (FOB), if the two-week repeat study had clinical signs of depression of the CNS. Indeed, a 2-week oral toxicity study of 1,3,5-trimethylbenzene was available (cited as *Koch Industries, 1995a* in the Draft Assessment). Clinical observations of treated rats in this study (administered 60, 150 or 600 mg/kg, 7 days/week for 2 weeks) revealed no signs of CNS depression, hence including a full functional observation battery in the 90-day subchronic toxicity study was not justified. The 90-day study has since undergone rigorous peer review and is now published as Adenuga et al., 2014.

[2] The NOAEL is a valid conservative estimate of safe exposure levels through the oral route

In Appendix F (response to comments)⁴, the EPA cited a concern raised by one of the external peer reviewers that the NOAEL identified in the study report was “*most likely an artifact of the study investigating insensitive endpoints (i.e., body weights, gross pathology)*”. We strongly disagree with this comment. Not only was the study conducted strictly according to existing EPA guidelines at the time, this statement implies that an endpoint is only “sensitive” when an adverse effect is observed. This statement also ignores that the goal of subchronic toxicity tests is not merely to identify adverse effects, but to determine levels at which exposure to a substance can be considered safe.

In the 1,3,5-trimethylbenzene oral toxicity study, several statistically significant effects were noted, particularly in the high dose group (600 mg/kg-day). These included clinical chemistry changes such as an increase in phosphorus levels, alkaline phosphatase, in high dose male rats and increased liver weights in males and females. In humans and rodents, sustained elevations of serum phosphorus are a sensitive

³ Peer Review Report – External Peer Review of the 1995 Koch Industries Study Report. 90-Day Oral Gavage Toxicity Study of 1,3,5-Trimethylbenzene in Rats with a Recovery Group. Page 2.

⁴ EPA response to public comments. Appendix F, lines 9-11, page F-14 of the Supplement to the Draft Assessment.

indicator of decreased renal elimination (such as would be expected in patients with renal insufficiency), increased phosphate load (such as could occur through hemolysis or muscle breakdown) and increased reabsorption, an indicator of hypoparathyroidism. In addition, other general clinical chemistry and gross pathological changes are highly sensitive indicators of adverse effects on tissues such as the liver or kidney while significant decrease in body weight is a sensitive indicator of adverse maternal systemic effects in developmental toxicity studies for example. The relevance of effects observed in rats in the oral 90-day study of 1,3,5-trimethylbenzene was rigorously adjudicated during the publication peer review process, especially as relates to the selection of an appropriate NOAEL. All three manuscript peer reviewers agreed that the effects (clinical chemistry and tissue weights) were accidental and not toxicologically relevant.

[3] Neurological and respiratory endpoints are not appropriate endpoints on which to judge the validity of the 90-day oral toxicity study of 1,3,5-trimethylbenzene

As stated in above, the EPA's major criticism of the reliability of the Adenuga et al (2014) study was that it did not include an evaluation of a neurotoxicity endpoint. The EPA, citing one of the external peer reviewers of the original Koch Industries study report, indicated that a lower NOAEL would have been identified had the study investigated endpoints "*more pertinent to human health*" (e.g., behavioral, respiratory or electrophysiological endpoints). This is conjecture and not consistent with the study design and the rationale for the study, which was to develop a reference value for drinking water contamination.

Firstly, it is hard to understand how evaluating a respiratory endpoint could have been considered "pertinent to human health" based on an oral study in this case. Inhalation toxicity studies of individual isomers of trimethylbenzene, ethyltoluene, isopropylbenzene etc. indicate that the respiratory effects seen are largely local portal of entry effects and hence would not be expected in an oral toxicity study. In the 3-month inhalation studies of 1,2,3- and 1,2,4-trimethylbenzene for example, the respiratory effects were limited to irritation of the respiratory tract, observed as increased inflammatory cells in bronchoalveolar (BAL) fluid and goblet cell hyperplasia (Korsak et al., 1997; Korsak et al., 2000). Certainly these effects would not be expected via oral exposure.

Secondly, the EPA cites two studies of acute oral exposure to 1,3,5-trimethylbenzene that evaluated both electrophysiological and locomotor activity in rats. In the first study, acute exposures to 250, 1000 or 4000 mg/kg 1,3,5-trimethylbenzene resulted in slight dose-dependent **increases** in animal locomotor activity (Tomas et al., 1999), certainly not evidence of CNS depression. In the second study, gavage administration of 250, 1000 or 4000 mg/kg 1,3,5-trimethylbenzene resulted in changes in electrocortical activity (Tomas et al., 2000). However, the changes were observed within 60 minutes of solvent administration which would be indicative of an acute CNS effect but not a persistent neurological effect. In fact, a similar acute CNS effect was noted in an oral 90-day subchronic toxicity study of m-xylene. In this study, oral administration of 2000 mg/kg-day consistently resulted in abnormal gait, tremors and ataxia in rats within 5 minutes of administration. These effects wore off within 1-hour of exposure and no long-term neurological effects were noted at study termination (NTP, 1986). The highest administered dose in the Adenuga et al (2014) study was 600 mg/kg-day. No clinical evidence of acute CNS depression was reported in this study and the 600 mg/kg-day dose level is several fold lower than doses where oral

administration of 1,3,5-trimethylbenzene (albeit in 2 acute studies) and xylene caused acute effects in rodents. In other words, the weight of the evidence does not support the potential for a neurological effect at the doses tested in the Adenuga et al. (2014) study and the use of this endpoint as a rationale for excluding this study in the development of an RfD for trimethylbenzenes is not justified.

In summary, the 90-day oral toxicity study of 1,3,5-trimethylbenzene was conducted to fulfill the goal of developing a reference value to regulate drinking water exposure to trimethylbenzenes. It was conducted according to EPA guidelines and identifies a point of departure (POD) for oral exposures in rats. This POD of departure takes into account all possible endpoints appropriate for an oral exposure and is thus appropriate for the derivation of an RfD. In addition, the use of this study eliminates the increased uncertainty that comes with extrapolating from an inhalation study as has been done in the Draft Assessment.

References

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