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Comments on Behalf of ACC's Hydrocarbon Solvents Panel Comments to CAAC Regarding EPA's Draft Assessment of TMBs

Presenter: M. David Adenuga, Ph.D
Senior Toxicologist, ExxonMobil Biomedical Sciences, Inc.
moyinoluwa.d.adenuga@exxonmobil.com

American Chemistry Council

Hydrocarbon Solvents Panel

- The Panel acts as a forum for manufacturers and distributors of hydrocarbon solvents to address domestic and international regulatory, legislative, safety, toxicological, and environmental initiatives
- The Panel promotes the use of sound science in regulatory initiatives for hydrocarbon solvents
- The current Panel members are Chevron Phillips Chemical Company LP, CITGO Petroleum Corporation, ExxonMobil Chemical Company and Sasol North America, Inc.

Overview

- **The Panel appreciates the opportunity to present scientific and technical comments on the US EPA's current draft TMB IRIS health assessment**

- **The Panel believes there are major deficiencies in the draft assessment that need to be urgently addressed:**
  - Lack of/Inconsistent application of a scientifically sound rationale for inclusion/exclusion of studies
  - Failure to utilize the best available science/all relevant information available

- **Briefly, the major deficiencies addressed in these comments include:**
  - Exclusion of US EPA-mandated TSCA Section 4(a) guideline studies where complex C9 aromatic fractions were tested
  - Flawed assessment of "pain sensitivity" as evidence for chronic neurological impairment
  - Unwarranted use of additional uncertainty factors
  - Rejection of the Koch 1995b oral toxicity study of mesitylene as the most appropriate study for the derivation of a reference dose

Manufacture, Use and Exposure Considerations for Trimethylbenzenes

Manufacture

- **According to the US EPA, TMB isomers are primarily "*produced during petroleum refining and production of aromatic hydrocarbons with nine carbons (i.e., C9 aromatic fraction)…*"**
  - Production process is known as catalytic reforming
    - Typical composition of commercial C9 aromatic fraction – 20-45% isomers of TMB, 25-40% isomers of ET, <4% isopropylbenzene and <5% mixed xylene isomers (Firth et al., 2008)
  - Additional refining steps may be used to produce products with narrower specifications
    - E.g. Solvents
    - Additional refining steps remove higher MW components of C9 aromatic fraction (minor C10-C11 aromatic constituents)
    - Eventual product – C9 aromatic fraction containing isomers of TMB, ET, propylbenzenes and xylenes

Uses

- **According to the EPA, primary uses for TMBs**
  - Blending agent in gasoline formulations (C9 aromatic fraction)
  - Solvents
  - Paint thinner

Executive Summary (occurrence and Health Effects) - Lines 2-5, Page xxxiv of Draft IRIS Assessment

Manufacture, Use and Exposure Considerations for Trimethylbenzenes

Exposure

- According to the US EPA, the "***vast majority*** *of the C9 fraction is used as a component of gasoline*" and hence, "***vehicle emissions*** *are expected to be the* ***major anthropogenic source*** *of trimethylbenzenes*"
- EPA cites TRI data for 124-TMB, however this data does not take into account source of the release which presumably occurs primarily in the context of a C9 aromatic fraction rather than as the release of a specific isomer

Conclusions

- TMBs are manufactured, utilized and released into the atmosphere **primarily** in the form of a C9 aromatic fraction
- C9 aromatic fraction comprises of alkylbenzenes (mostly C9) that are structurally, metabolically and toxicologically similar to TMB isomers – *Please see ACC Attachment II*
- If the above is true, then it stands to reason that toxicological studies of TMBs, in the context of C9 aromatic fractions, are relevant and should be considered in the IRIS Draft Assessment

Executive Summary (occurrence and Health Effects) - Lines 2-5, Page xxxiv of Draft IRIS Assessment

Existing Database for C9 Aromatic Fraction Studies

Studies were conducted according to EPA's 1985 TSCA Section 4(a) test rule (FR 50 20662)

- EPA agreed that data would represent reasonable upper bound estimates for toxicity of isolated TMB and ET isomers

1. **Genetic toxicity – Schreiner et al., 1989**

- Tests included – Ames assay, CHO HGPRT forward mutation assay, CHO chromosomal aberration, *in vivo* rat chromosome aberration assay and CHO-SCE
- All results were negative – similar to results with single isomers of TMB, ET, propylbenzene and xylenes

2. **Subchronic neurotoxicity – Douglas et al., 1993**

- Rats exposed to 500, 2500, 7500 mg/m^3, 6h/day, 5 days/week for 90 days
- Rats tested 48-hrs after last exposure to avoid confounding acute effects
- No effect on motor activity or FOB
- Extensive histopathological examination of neural tissues – no effects noted

3. **Reproductive toxicity – Mckee et al., 1990**

- Rats exposed to 500, 2500 or 7500 mg/m^3 6h/day, 5 days/week for 10 weeks pre-mating, 2 weeks mating period and up to GD20 (females)
- No effects on fertility

4. **Developmental toxicity – Mckee et al., 1990**

- Mice exposed to 500, 2500 or 7500 mg/m^3 6h/day on GD 6-15
- No teratogenic effects
- Fetal effects on body weight at 7500 mg/m^3 was associated with a decrease in maternal body weight
  - Unvgary et al., 1983 – 600, 1000, 2000 mg/m^3 for 24 h/day from GD 7-15 – transient skeletal/organ retardations absent at PND90
  - Lehotzky et al., 1985 - 600, 1000, 2000 mg/m^3 for 24 h/day from GD 7-15 – no evidence for developmental neurotox effects
  - Saillentfait et al., 2005 – similar results with individual TMB isomers

5. **Subchronic toxicity – Clark et al., 1989**

- Rats exposed to 450, 900 or 1800 mg/m^3, 6h/day, 5 days/week for 12 months
- Sporadic hematological changes at 6 months, none at study termination
- Key effect at study termination – increased liver weights

Exclusion of US EPA-mandated TSCA Section 4(a) studies is not Justified

1. **Figure LS-1 – Literature search and study selection strategy for TMBs - Types of studies not considered further for inclusion in toxicological review (Figure LS-1)**

- Studies of complex solvent mixtures – all C9 aromatic fraction studies
- Not available in English – Unvgary et al., 1983, Lehotzky et al., 1985

ACC Comment

- No logical rationale for these exclusion criteria is provided in the main text of the draft assessment
- These criteria was applied inconsistently
  - Examples – Battig et al., 1956 (German) and Hissink et al., 2007 (complex solvent)

2. **Sufficient toxicokinetic/toxicological similarity cannot be made for constituents of C9 aromatic fraction as some constituents (i.e., the C10 compounds) are not specifically identified**

ACC Comment

- Capillary gas chromatographic analysis of C9 aromatic fraction was provided in study reports made available to the EPA
- C10 components are clearly identified as structurally similar alkylbenzenes e.g., butylbenzene isomers, dimethyl-ethylbenzene isomers, diethylbenzenes (**Please see ACC Attachment II**).
- Ungvary et al., 1983 and Lehotzky et al., 1985 studies show no effect on toxicity endpoints with/without ≥ C10 constituents

3. **US EPA indicated Douglas et al., 1993 not reliable since it did not show neurotoxic effects at 7500 mg/m^3 compared to effects in pregnant/non pregnant mice in Mckee et al., 1990**

ACC Comment

- US EPA failed to take into consideration clear differences in timing of effects –
  - effects noted in Mckee et al., 1990 (abnormal gait, slight ataxia etc.,) reproductive study were observed **immediately** after exposure
  - Neurotoxicity endpoints were measured in Douglas et al., 1993 **48 hrs after last exposure** to avoid confounding acute effects
- Despite similar exposure concentration, exposure/unit mass is 14-fold greater in the mouse than the rat
  - Rat standard BW – 350 g, mouse standard BW – 25 g

4. **US EPA suggested that "negative" results of C9 aromatic fraction studies could be due to interactive effects of other constituents altering the ADME of TMB**

ACC Comment

- Mckee et al., 2010 compared acute effects of 124-TMB and C9 aromatic fraction in rats
  - Effects on operant visual discrimination, FOB and motor activity were transient and similar in both exposure conditions
  - Slightly more profound effects in C9 aromatic fraction – justifying US EPA's original conclusion from 1985 that studies on complex substances represent upper bound of toxicity for individual isomers
- Jarnberg et al., 1998 – 8-hr human volunteer exposure to 124-TMB and 124-TMB in white spirits
  - Disposition in humans was similar

Individual TMB Isomers and C9 Aromatic Fraction are Toxicologically Similar

Comprehensive toxicity comparison tables provided in "ACC Attachment II"

Neurotoxicity

- Transient acute effects with exposure to TMB, xylene, isopropylbenzene or C9 aromatic fraction
  - Similar effects – lethargy, slight ataxia, effects on motor activity, hunched gait within minutes of exposure, lasting up to 1 hr.
  - No effects 24-48 hrs after exposure

Repeat dose toxicity

- Data provided for TMB, ET, isopropylbenzene, xylene and C9 aromatic fraction
- Effects limited to body weight decreases at high concentration, liver weight increase, hematological changes consistent with mild anemia, respiratory tract irritation

Developmental toxicity

- Data provided for TMB, ET, isopropylbenzene, xylene and C9 aromatic fraction
- Fetotoxicity at maternally toxic concentrations
- No evidence for teratogenicity

Reproductive toxicity

- Data provided for n-butylbenzene, xylenes, 1,4-diethylbenzene and C9 aromatic fraction
- No effects on reproductive and fertility indices

Overall, there is no justification for concluding that studies of C9 aromatic fractions are dissimilar to those from individual TMB isomers

Thus, a risk management need to regulate TMB alone does not imply that relevant scientific literature on a complex substance primarily containing TMB isomers should be ignored – especially when exposure to TMB occurs in the context of the complex substance

“Pain Sensitivity” is not Indicative of Chronic Neurological Impairment

Basic procedure for “pain sensitivity” assessment through hotplate method - evaluation of response to an unconditioned noxious stimuli

- **Korsak & Rydzynski et al., 1996**
  - Rats exposed to 25, 100 or 250 ppm 123- or 124-TMB 6h/day, 5 days/week for 3 months
  - Hot plate behavior was tested **immediately** after termination of exposure
  - Rat placed on hot plate within enclosure for up to 60 sec or until response (licking of foot)
  - Statistically significant increase in paw lick latency with 100 and 250 ppm 124-TMB
  - Statistically significant increase in paw lick latency only with 250 ppm 123-TMB (25 ppm group was within physiological range)
  - **No effects** in pain sensitivity tests conducted **2 weeks after** termination of exposure

- **Effects are similar to those observed with Douglas et al., 1993 and L1 trials in Gralewicz et al., 1997, Wiaderna et al., 1998, Gralewicz and Wiaderna 2001 etc.**

Subsequent procedures employing footshock are models of “conditioned analgesia”

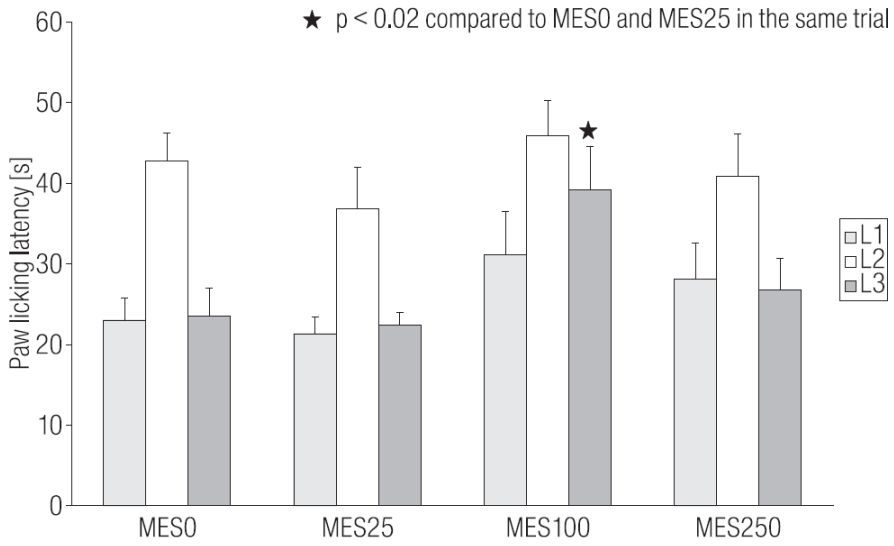
- **Three (3) trials conducted - L1, L2, L3**

- **L1 is equivalent to Korsak and Rydzynski, 1996 - no shock applied before hotplate exposure**
  - No effects when L1 was conducted more than a few hours after termination of exposure

- **L2 and L3 trials involve exposure to footshock prior to hotplate treatment - unconditioned stimuli (shock) used to induce analgesia before or during exposure to noxious stimuli (hotplate)**
  - Rats received footshock (100 ms at 0.5 Hz for 2 mins) immediately after L1. L2 performed 2-3s after footshock
  - L3 performed 24 h after L2
  - Effects were inconsistent and mostly not significantly different

“Pain Sensitivity” is not Indicative of Chronic Neurological Impairment

- Example – Wiaderna et al., 2002 (135-TMB)
  - Rats exposed to 25, 100 or 250 ppm 6h/day, 5 days/week for 4 weeks
  - Pain sensitivity and conditioned analgesia conducted 50-51 days after exposure



- Note
  - No effects on pain sensitivity in trial 1 (L1) at any dose level similar to Korsak & Rydzynski, 1996 and Douglas et al., 1993
  - Latency in trial 2 is increased compared to L1 in all conditions (including control) – no statistical significance
  - Authors state in results section - “*in none of the groups did the reaction latencies in trial 3 differ significantly from those determined in trial 1*” and “*no significant differences were detected between the groups in the values of the proportions L2/L1 and L3/L1*”
  - Table 1-1 – The US EPA draft assessment records this as “statistically significant”

“Pain Sensitivity” is not Indicative of Chronic Neurological Impairment

US EPA failed to acknowledge several limitations that severely undermine the objective assessment of the “pain sensitivity”/”conditioned analgesia” studies

- Results could have been affected by passive avoidance test just 2 days prior
  - Natural tendency for animals to step off platform suppressed using 10s footshock
- Increase in L3 could reflect improved memory of conditioned stimuli (hotplate) associated with a footshock received 24 hrs prior
- Wiaderna et al., 1998 observed that in the 124-TMB study, paw lick was usually preceded by attempts to get out of the enclosure. The more persistent the attempts, the longer was the paw lick latency
  - Is paw lick latency related to treatment or other confounding effects?
  - Or are animals reacting to pain, not by licking the paws (footshock memory), but by trying to escape?
- Historical control data shows wide range of “normal” latency to paw lick
  - L1 values (for 4-week studies) range from 9 – 22 seconds

Overall, the “pain sensitivity” (all L1 trials) convincingly show that this effect is acute in nature. Comparing the acute, 4-week and 3-month studies, effects were only observed **immediately** after exposure and **did not progressively worsen** with prolonged exposure.

The Use of Additional Uncertainty Factors (U_F) is not Warranted

- An additional subchronic to chronic U_F of 3x was included to account for increasing severity of pain latency effects
  - This is not applicable since the "pain latency" and "conditioned analgesia" effects are quite clearly transient, acute responses
  - Korsak and Rydzyński, 1996; Gralewicz et al. 1997; Gralewicz and Wiaderna 2001; Wiaderna et al. 1998, 2002 and Douglas et al., 1993
  - C9 aromatic fraction study (Douglas et al., 1993) included a thorough histopathological evaluation of peripheral nerves, spinal cord and teased nerve fibers - No pathological changes were noted

- US EPA included a U_F of 3x to account for database insufficiency
  - US EPA cites lack of a multigenerational reproductive/developmental toxicity study
  - These studies are available for C9 aromatic fraction (Mckee et al., 1990, Unvgary et al., 1983, Lehotzky et al., 1985)

Koch, 1995b Oral Study is Most Appropriate for an RfD

- Study was conducted in response to TSCA Section 4(a) test rule (FR 58 59667, 1993)
  - To support EPA's development of HAs for unregulated drinking water contaminants under the SDWA
  - Conducted as a gavage study – most appropriate for development of an RfD
  - Avoids unnecessary route-to-route extrapolation with inherent with utilizing inhalation studies
  - Rats administered 50, 200 or 600 mg/kg-bw 135-TMB, 5 days/week for 90 days
  - Treatment-related changes – liver enlargement and increased serum phosphorus in high dose males/females
  - Liver effects are adaptive – no histopathological evidence of liver injury (hypertrophy only in 14-day study)
  - Increased phosphorus not toxicologically relevant
  - Effects are similar to those reported in inhalation studies (except respiratory irritation)
  - NOAEL – 600 mg/kg-bw [Calculated RfD of 1.2 mg/kg-bw/day]
  - Published as Adenuga et al., 2014

- US EPA cited "several deficiencies" as a rationale for excluding the study [pg 2-48]
  - These "deficiencies" are not discussed in the main body of the text
  - All 3 of EPA's independent peer review panel agree that study quality was high… "*all the elements required by the EPA 798.2650 guidelines were included*"
  - Conclusions of "unsuitability" from 2/3 peer reviewers made in the context of neurotoxicity endpoints – leading charge questions from the US EPA
    - Objective evaluation should have focused on study quality alone

Koch, 1995b Oral Study is Most Appropriate for an RfD

- US EPA contended that NOAEL would have been lower if study had investigated endpoints "*more pertinent to human health*" (e.g., behavioral and respiratory)
  - Respiratory effects
    - Irritation of respiratory tract is a **portal of entry effect** seen only in inhalation studies of C9 aromatic fraction and individual constituents
    - Would not be expected in oral study
  - Behavioral effects
    - Acute effects with oral exposure to alkylbenzenes and TMB isomers occur at doses several fold higher than the highest dose in the Koch, 1995b study
      - m-xylene oral 90-day study – **No effect at 1000 mg/kg**. Abnormal gait, tremors and ataxia in rats only within 1 hour of administration at 2000 mg/kg (NTP, 1986).
      - 135-TMB oral gavage study – exposure to 4000 mg/kg resulted in increased locomotor activity – no evidence of CNS depression (Tomas et al., 1999)
      - 135-TMB oral gavage study – exposure to 4000 mg/kg resulted in changes in electrocortical activity. **No effect at 1000 mg/kg** (Tomas et al., 2000).
      - Changes in electrocortical activity observed within 60 minutes of exposure – similar to m-xylene effects which are transient, acute effects only
      - Overall, no neurotoxicity effects (including transient, acute effects) were observed at 1000 mg/kg – 1.7-fold higher than the 600 mg/kg high dose in the Koch, 1995b study
- No evidence to support potential neurological effect at NOAEL (600 mg/kg-bw)
- NOAEL of 600 mg/kg-bw is a conservative estimate of safe dose by the oral route

Overall Recommendations for Draft TMB Assessment

- US EPA should incorporate all available relevant data
  - Data on C9 aromatic fraction (currently summarized in appendix E) needs to be incorporated into the main document
    - Weight of evidence and database sufficiency
- EPA needs to reconsider the flawed assumption of "pain sensitivity" as evidence for chronic neurological effects
  - Separate discussion on pain sensitivity and conditioned analgesia
  - Need to clearly and transparently identify study limitations that undermine biological relevance of endpoints
- EPA needs to reconsider the use of unjustified uncertainty factors
  - Subchronic – chronic
  - Database insufficiency
- Oral 90-day study on 135-TMB should be used to calculate the oral RfD
  - More appropriate than currently used repeated inhalation study
  - Avoids necessity of route-to-route extrapolation