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Trimethylbenzenes Listening Session

COMMENTS ON THE TOXICOLOGICAL REVIEW OF TRIMETHYLBENZENES

Hydrocarbon Solvents Panel





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



Thank you for the opportunity to present scientific and technical comments on the US EPA's draft TMB IRIS health assessment

The decision to consider the TMB isomers as equivalent is appropriate but not fully exploited

The literature strategy search and study selection is significantly flawed

- The process by which some studies were either not considered or not used in the assessment process was not transparent and not consistently applied
- There are other studies, which if used in the assessment address many of the uncertainties raised in the document
- The assessment should provide the specific criteria used to search for, include and exclude publications
- The assessment should include TSCA test rule data generated per FR50 20662 for TMB and should recognize all useful data submitted to and accepted by EPA under regulatory mandate and in the public domain
- The flawed literature strategy resulted in the selection of a key endpoint/study that is not scientifically sound and appropriate

Consideration of TMB Isomers as Equivalent is Appropriate but not Fully Utilized

RfC and RfD values were derived for 1,2,4-trimethylbenzene and then used for the other isomers

- In principle, this is reasonable and appropriate
- Data on any isomers or for any mix of isomers can be used to characterize the hazards of TMBs individually or collectively

We recommend that this approach be generalized to include all C9 aromatics

- Consistent with previous recommendations by EPA (FR, 1985)
- Basis for toxicology tests conducted on “C9 aromatic hydrocarbon fraction”
- Studies conducted under EPA guidelines and good laboratory practice regulations and submitted to the Agency
- Data peer-reviewed and published
- Conducted studies provide information addressing database uncertainties raised in the current TMB review

TSCA Section 4(a) Test Rule C9 Aromatic Fraction

EPA's 1985 TSCA section 4(a) test rule (FR50 20662) required testing of the C9 aromatic hydrocarbon fraction

- Endpoints: neurotoxicity, mutagenicity, developmental and reproductive toxicity, oncogenicity
- This data was largely omitted from the IRIS assessment without a clear explanation

EPA determined that because C9 aromatics are manufactured as complex substances and exposures are usually to mixed isomers, the C9 aromatic fraction could best be studied on a group basis

- Test material was comprised of approx 55% TMB isomers and 28% ethyl toluene (ET) isomers
- EPA agreed that a study of this substance would provide reasonable upper bound estimates for the toxicity of TMB and ET isomers

Studies followed EPA test guidelines and good laboratory practice regulations. Required tests included:

- Mutagenicity - a 5 test battery including both *in vitro* and *in vivo* tests
- Subchronic Neurotoxicity: motor activity, functional observations, neuropathology
- Reproductive Toxicity: 2 generation (extended to a 3rd generation) study
- Developmental Toxicity: mouse

Overall program addressed all toxicological endpoints that the EPA identified

- Strategy for carcinogenicity testing based on results of genotoxicity battery

Some testing was not required because sufficient data were available

- Developmental Toxicity: previously published rat studies on similar test materials
- Repeated exposure studies: 90 day and 12 month studies on similar test materials

Results of the 1985 Test Rule C9 Aromatic Fraction



Mutagenicity

- Tests included Ames, HGPRT forward mutation, in vitro chromosome aberration, in vitro sister chromatid exchange, in vivo chromosome aberration
- Data published (Schreiner et al. 1989)
- Results considered sufficient by EPA
- Further studies of carcinogenic potential judged unnecessary

Subchronic Neurotoxicity

- Rats exposed 6 hr/d, 5d/w for 90 days
- Exposure levels - 100, 500, 1500 ppm [500, 2500, 7500mg/mg3]
- Assessments included motor activity, functional observation battery, neuropathology
- There were no effects in the tests of motor activity or functional observations and no findings in the neuropathology examination
- Results published (Douglas et al. 1993)

Results of the 1985 Test Rule C9 Aromatic Fraction (Con't)

Developmental Toxicity

- Mice exposed by inhalation at levels of 100, 500, 1500 ppm [500, 2500, 7500mg/mg3]
- The highest dose (1500 ppm) resulted in ~ 50% mortalities
- Maternal and fetal body weights significantly reduced at 500 ppm
- There were no effects at 100 ppm
- Results published (McKee et al., 1990)
- Note that there are publications of developmental toxicity tests in rats involving both mixed C9s (Lehotzky et al., 1985; Ungvary et al., 1983) and individual isomers (Sallenfait et al. 2005).

Reproductive Toxicity

- Rats exposed 6 hr/day, 7d/w for 10 weeks pre-exposure, 2 weeks of mating and then to GD20. Dams then removed from exposure until PND5. Exposure of offspring initiated on PND21
- There were no effects on fertility but there was evidence of toxicity among the very young offspring at 1500 ppm
- Results published (McKee et al., 1990)

Repeated Dose Toxicity

- Rats exposed 6 h/day, 5d/w for 90 days at levels of 450, 900 or 1800 mg/m3
- At termination samples were taken for hematological and clinical chemistry evaluations, and tissue samples were taken for pathological evaluation
- The principal effect was liver weight increase with a no adverse effect concentration of 1800 mg/m3.
- The results were published (Clark et al., 1989)

Literature Search Strategy & Study Selection is Significantly Flawed

The process by which some studies were either not identified or not used in the assessment was not transparent and not consistently applied

Several documents were excluded for various reasons, but the basis for exclusion is not clear or consistently applied

The assessment provides this vague explanation as to why the TMB test rule data was omitted from the assessment

- “These reports were not peer-reviewed and they either did not use appropriate durations of exposure that would support derivation of chronic health reference numbers (e.g., 14 days), reported minimal and difficult to interpret toxic effects, or investigated mixtures containing TMB isomers” (xxxix, lines 16-19)

Literature Search Strategy & Study Selection is Significantly Flawed (Con't)

As discussed in previous slides, exclusion of studies of mixtures of TMBs is not consistent with the principle of similarity used in the TMB assessment

- If all TMBs are equivalent, than any study on a mixture of TMBs should be as valid as a study of any individual isomer

The reason for the exclusion of the 90 day oral toxicity study of TMB (Koch Industries, 1995b) is not obvious

- This was a single isomer (1,3,5-TMB) study, oral exposures were for 90 days, the test followed EPA guidelines and the data were submitted to the EPA for review
- This study is more appropriate for RfD derivation than the inhalation study selected as route-to-route extrapolation would have been unnecessary and the levels of uncertainty reduced
- The statement in the report (page xxxiv) that [with respect to 1,3,5-TMB], “no chronic, subchronic or short-term oral exposure studies were found in the literature” is strictly true but misleading
- Subchronic oral toxicity data are available but were excluded from consideration

The decision to not use Saillenfait et al. (2005) is not transparent

- The apparent reason (page xxxi) is that the no effect level differed from that of other studies
- The other studies were not developmental toxicity studies so the comparison is not appropriate
- The relevant question is whether developmental toxicity or neurotoxicity would be the most appropriate endpoint on which to base an assessment

Decreased Pain Sensitivity was not Appropriate as the Key Study



The selection of the endpoint as the key indicator seems inconsistent with the cautionary notes in the neurotoxicity summary section

- “Most of the neurotoxicity tests in animals incorporated the application of footshock which, depending on the procedure can involve multiple contributing factors and can complicate interpretation regarding effects on discrete neurological function” (p 1-21, lines 8-10).

Pain Sensitivity, Test Description & Data



Basic Assessment was to expose animals and to then measure latency in response to a hot plate

- Korsak and Rydzynski (1996) reported that exposure to TMB isomers resulted in an increased latency to response when measured immediately after treatment but found no effects 2 weeks later
- Most likely explanation is that this is an acute, reversible response.
- Douglas et al. (1993) found no effects among animals exposed to C9 aromatic fraction at higher levels than used by Korsak and Rydzynski.

In subsequent studies animals were held for longer periods of time and foot shock was introduced

Key Studies/Endpoint Used in the Assessment are not Appropriate



The Draft utilizes a “Pain Sensitivity” endpoint to generate the RfC

Results are not consistent.

- Results not consistent across isomers
 - No effects in 1,2,3-TMB studies
- Effects not directionally consistent
 - Latency increased in some studies, reduced in others
- Dose-response was not demonstrated
 - Effects often more pronounced in 100 ppm groups than 250 ppm groups
- Inconsistencies in temporality
 - Significant effects reported at different time intervals after foot-shock

Statistically significant findings in non-acute studies only found after foot-shock administration.

- No agreed guidelines for study conduct and no rationale for administering foot shock was provided
- Incorporation of foot shock complicates the interpretation of the studies (page 1-21, lines 8-10)

The Quality of the Data Used in the Assessment is Problematic



Several studies used in the assessment significantly lack an appropriate protocol questioning their use in the assessment

Gralewicz et al. (1997)

- Rats exposed to 1,2,4-TMB for 4 weeks
- Held for 35 days without treatment
- Tested for pain sensitivity
- Foot shock administered
- Tested for pain sensitivity immediately after foot-shock
- Tested for pain sensitivity 24 hours after foot shock

Results

- No differences in latency to response in initial tests
 - Replicated findings of Korsak and Rydzynski
 - Support conclusion that this is an acute, reversible effect
- No differences in latency to response immediately after foot shock
- Significant increase in time to response 24 hours after foot shock
 - Results not dose responsive, more profound effects found in animals exposed to 100 ppm than to 250 ppm

The Quality of the Data Used in the Assessment is Problematic (Con't)

Gralewicz and Wiaderna (2001)

- Rats exposed to 1,2,3-, 1,2,4- and 1,3,5 TMB at 100 ppm for 4 weeks
- Held for 39 days without treatment
- Tested for pain sensitivity
- Foot shock administered
- Tested for pain sensitivity immediately after foot-shock
- Tested for pain sensitivity 24, 72 and 120 hours after foot shock

Results

- No differences in latency to response in initial tests
 - Replicated findings of Korsak and Rydzynski and Gralewicz et al. (1997)
 - Support conclusion that this is an acute, reversible effect
- No differences in latency to response immediately after foot shock
- No significant effects on response 24 hours after foot shock
 - Not consistent with Gralewicz et al. 1997 who reported significant effects with TMB 24 hours after foot shock
- Significant reduction in time to latency response with 1,3,5-TMB 72 hours after treatment
 - Not consistent with Gralewicz et al., 1997 who reported a significant increase in time to latency response with 1,3,5-TMB
- Significant reduction in time to latency to response with 1,2,4- and 1,3,5-TMB 120 hours after exposure but no effects with 1,2,3-TMB
 - Does not support theory of “equivalence” as one isomer apparently produced no effects

The Quality of the Data Used in the Assessment is Problematic (Con't)

Wiaderma et al. (2002)

- Rats exposed to 1,3,5-TMB for 4 weeks; 25, 100, 250 ppm
- Held for 35 days without treatment
- Tested for pain sensitivity
- Foot shock administered
- Tested for pain sensitivity immediately after foot-shock
- Tested for pain sensitivity 24, 48, 72, 96, 120, and 240 hours after foot shock

Results

- No differences in latency to response in initial tests
 - Replicated findings of Korsak and Rydzynski
 - Support conclusion that this is an acute, reversible effect
- No differences in latency to response immediately after foot shock
- Significant reduction in time to latency found only in the 240 hours post-foot shock group
 - Results similar across treatment groups (i.e., no apparent dose response)
 - Did not replicate findings of significant differences 72 and 120 hours post foot shock (Gralewicz et al. 2001).

Characteristics of an Appropriate Key Study/Report for an IRIS Assessment

Study

- Follows testing guidelines and good laboratory practices
 - Or if not, at least the objectives are clear and the procedures sufficiently detailed
- Results are unambiguous
 - It should not be necessary to use statistical techniques to identify differences
- There should be evidence of dose-response
- Effects are toxicologically important and can be related to established biological processes
 - If underlying mechanisms or modes of action are known, results can be put in proper perspective

Results

- Evaluated according to standard practices
 - Statistical procedures should be appropriate for the analysis
- Data can be replicated
 - It should be possible to reproduce findings in different laboratories
- Results are consistent across studies
 - Or at least similar studies should give similar results
- Complete data sets are available for review
 - If data are questionable it should be possible to independently verify results

More Appropriate Studies that were not Considered for the Assessment

Most useful studies for RfC derivation are Clark et al. (1989) and Douglas et al. (1993)

Clark et al (1989)

- Rats exposed by inhalation for 12 months at levels of 450, 900, 1800 mg/m³.
- Study consistent with regulatory guidelines
- Results accepted by the EPA for use in assessing hazards of TMB isomers
- Outcomes consistent with subchronic inhalation toxicity study of 1,2,3-TMB (Korsak et al. 2000), oral toxicity study of 1,3,5-TMB (Koch Industries, 1995b) and supported by a rich data base
 - Subchronic neurotoxicity, developmental toxicity, reproductive toxicity, mutagenicity

Douglas et al (1993)

- Rats exposed by inhalation for 90 days at levels of 100, 500, 1500ppm [500, 2500, 7500mg/m³]
- Study consistent with regulatory guidelines
- Results accepted by the EPA for use in assessing hazards of TMB isomers
- Study evaluates standard neurotoxicity endpoints: Motor activity, functional observation battery including hot plate latency response.

More Appropriate Studies that were not Considered for the Assessment (Con't)

Most useful study for RfD derivation is Koch Industries, (1995b) instead of extrapolation from inhalation data

- 1,3,5-TMB administered by gavage at doses of 50, 200 and 600 mg/kg/day
- Study consistent with regulatory guidelines
- Results accepted by the EPA for use in characterizing the hazards of 1,3,5-TMB following oral administration
- Results consistent with studies of shorter duration on other TMB isomers (reviewed by Firth, 2008)

Reliance on these studies is more straight forward and reduces uncertainties identified in current investigation

- Reliance on these studies obviates the need for pharmacokinetic analysis or route-to-route extrapolation
- Satisfies issues related to confidence in the database
 - Available data include repeated dose studies by both oral and inhalation routes, subchronic neurotoxicity, developmental toxicity in rats and mice, developmental neurotoxicity, reproductive toxicity, and mutagenicity.
 - Results are supported by studies of individual isomers
- Avoids reliance on studies with interpretational difficulties

Recommendations and Calculations for RfD and RfC values for TMBs More Appropriate Studies

Toxicology data for TMB isomers and in aggregate reviewed for utility in RfC and RfD derivations (Firth et al., 2008).

Procedures for RfC and RfD derivation followed USEPA recommendations

- RfC calculations considered 3 inhalation studies and resulted in a recommended value of 3 mg/m³
 - Clark et al. (1989), subchronic toxicity study - 3 mg/m³
 - Douglas et al. (1993), neurotoxicity study - 4 mg/m³
 - McKee et al. (1990), reproductive toxicity study - 4 mg/m³
 - Recommended value - 3 mg/m³
- RfD calculations based on 90 day oral study from Koch Industries (1995)
 - 0.4 mg/kg/day

Overall Recommendations for the Draft TMB Assessment



Revise the literature search and study selection to use all useful data submitted to and accepted by EPA under regulatory mandate and in the public domain in a transparent and consistent manner

- EPA should reconsider the selection of the definitive study to establish the inhalation RfC based on standard criteria, consistency and reproducibility of results
- Consider the TMB isomers as equivalent and fully apply the data set accordingly
- The oral 90 day study with 1,3,5 TMB should be employed to calculate the oral RfD rather than extrapolation from inhalation data