NTP Research Concept: C9 Alkylbenzenes

Project Leader

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Background and Rationale

Chemical Identification, Use and Exposure

C9 alkylbenzenes are simple monoaromatic hydrocarbons containing 3 carbon substitutions on a benzene ring. C9 alkylbenzenes include the trimethylbenzenes, ethyltoluenes and propylbenzenes (Appendix Fig. 1). C9 alkylbenzenes occur naturally in petroleum and other fossil fuel sources and are commercially isolated for various industrial uses. C9 alkylbenzenes are produced during petroleum refining as components of the C9 aromatic hydrocarbon fraction (C9 fraction) (Table 1). In addition to C9 aromatic hydrocarbons, the C9 fraction may contain C8 and C10 aromatic hydrocarbons typically in the range of 5-10%. Domestically, C9 fraction production in 1991 was estimated to be 40 million tons (*1*). The primary use of the C9 fraction (≈ 99% of production volume) is as an additive in the gasoline-blending stream (1). As such, vehicle emissions are a major anthropogenic source of C9 isomers (*1*). The remaining C9 fraction is isolated for solvent use (i.e., "Solvent naphtha, (petroleum), light aromatic" CAS# 64742-95-6) with the annual worldwide production of C9 fraction for solvent use is approximately 50,000-250,000 metric tons (*2*). In the U.S., solvent applications include industrial coatings/sealants, marine paint, wood deck sealer and automotive applications such as fuel injection cleaner and fuel additive diluent (*2*).

C9 alkylbenzenes occur in air, water, and soil. The environmental sources of C9 alkylbenzenes are anthropogenic and biogenic including automobile emissions, tobacco smoke, combustion processes (waste treatment, coal-fired power plants), and industrial releases. Occupational exposure may occur via inhalation and dermal contact where these compounds are produced and used. Workplace levels range from <1 to 3 ppm (<1 to 15 mg/m^3) for solvent-use industries and 0 to 1.3 ppm (0 to 6 mg/m^3) for chemical manufacturing industries (*2*). For the general population, exposures are possible via inhalation of ambient air, consumption of contaminated food and drinking water or direct contact with gasoline, solvents, or other consumer products

Toxicity

The carcinogenicity of benzene to humans is well established and carcinogenic activity has been observed in rats and mice following exposure to some C8, C9, and C10 substituted benzenes including cumene, ethylbenzene and naphthalene (*3-6*). Other than cumene, C9 alkylbenzenes generally have little toxicity data available.

Table 1. Concentrations of C8-C10 Alkylbenzenes in a crude oil and commercial solvent

Compound	Weight % Crude Oil*	Weight % Solvent naphtha
9-carbon isomers (C9)		
1,2,4-trimethylbenzene	0.343	20-45
1,3,5-trimethylbenzene	0.123	8-15
1,2,3-trimethylbenzene	0.112	2-8
3-ethyltoluene	0.182	5-20
4-ethyltoluene	0.069	2-20
2-ethyltoluene	0.087	2-8
cumene	0.036	0.5-5
n-propylbenzene	0.066	0.5-5
8-carbon isomers (C8)		
o-xylene (C8)	0.314	2-15
m-xylene (C8)	0.632	1-5
p-xylene (C8)	0.239	0.5-5
ethylbenzene (C8)	0.149	0.1-0.5
10-carbon isomers (C10)	0.546	1-3
*Total BTEX***	2.269	NA

** - Isomer weight percent as measured in MC-252 crude oil sample.*

*** - BTEX = benzene, toluene, ethylbenzene, xylene. Weight % in crude oil included for reference*

C9 fraction

Toxicity testing (mutagenicity, developmental and reproductive toxicity, subchronic neurotoxicity, and general inhalation toxicity) of the C9 fraction was mandated under Section 4(a) of the Toxic Substances Control Act (TSCA) in 1985. This test rule stipulated that testing be performed for a representative C9 fraction test article comprised of no less than 22% ethyltoluene (ET) isomers and 15% trimethylbenzene (TMB) isomers with a total ET-TMB content greater than 75% (*7*). The studies described below were conducted to meet the requirements of this test rule.

The mutagenic potential of the C9 fraction has been assessed *in vivo* and *in vitro* by mutation frequency in bacteria and Chinese hamster ovary (CHO) cells, sister chromatid exchange in CHO cells, and chromosomal aberrations in CHO cells and rat bone marrow cells. (*8*). No evidence of mutagenicity was observed *in vivo* or *in vitro* following C9 fraction exposure.

The developmental and reproductive toxicity of C9 fraction inhalation exposure has been evaluated in mice and rats, respectively (*9*). Severe maternal toxicity was observed in pregnant mice as 44% of the dams died following C9 fraction inhalation exposure at 1514 ppm. The number of litters with viable fetuses and the number of live fetuses/litter were decreased following C9 fraction exposure at 1514 ppm as well. In a

multi-generational reproductive study in rats, decreased fetal body weights were observed in the F3 generation following C9 inhalation exposure at 495 and 1480 ppm.

No neurotoxicity was observed in male rats exposed to C9 fraction inhalation up to 1320 ppm in a 13-week study (*10*). However, gross neurobehavioral toxicity (abnormal gait, labored breathing, weakness, circling, and ataxia) was observed in pregnant mice following C9 inhalation exposure at 1514 ppm during gestation (10 days) (*9)*. In addition, neurotoxicity (ataxia, decreased motor activity) was observed in F1 male and female rats following C9 inhalation exposure (1480 ppm) in the same multigenerational study mentioned above (*9*).

Chronic inhalation toxicity has been evaluated in male and female rats (n=50/sex/group) exposed to C9 fraction inhalation at 0, 96, 198 or 373 ppm for 12 months (6hrs/day, 5 days/week) (*11*). At the end of the 12-month exposure, liver and kidney weights were significantly increased in male rats in the 373 ppm exposure group. In the lung, pulmonary macrophage infiltration and alveolar wall thickening with severity increasing with dose.In this study with no clear treatment related increase in tumors, one leiomyoma (left uterine horn, female, 373 ppm), one lymphoma (spleen, male, 373 ppm), one glioblastoma (cerebellum, male, 96 ppm) were observed.

Trimethylbenzenes

There are three TMB isomers: 1,2,3-TMB, 1,2,4-TMB and 1,3,5-TMB. The EPA is currently conducting an Integrated Risk Information System (IRIS) review of TMBs (*12*). In the IRIS toxicological review, no chronic inhalation studies were identified in the literature. Chronic reference concentrations (RfC) for 1,2,3-TMB (50 µg/m^3) and 1,2,4-TMB (50 µg/m^3) were derived from four subchronic inhalation studies (*13-16*). In these studies, exposure-response effects were observed in the nervous, hematological, and respiratory systems. No subchronic studies were found for derivation of an RfC for 1,3,5-TMB. Therefore, the RfC derived for 1,2,4-TMB (50 µg/m^3) was adopted for 1,3,5-TMB based on similarity between the two isomers regarding chemical properties, kinetics, and toxicity. For reference, the RfC established for benzene is 30 µg/m^3. Confidence in the dataset used to establish RfCs for the TMB isomers was considered low because: 1) the studies used for RfC derivation were all conducted at the same research facility; 2) the dataset lacks studies evaluating chronic toxicity as well as reproductive, developmental and neurotoxicity studies.

Ethyltoluenes

Ethylltoluene exists as three isomers: 2-ET, 3-ET and 4-ET. A hazard characterization assessment of 4-ET was generated in 2009 as part of EPA's High Production Volume (HPV) Challenge Program (*17*). No evidence of mutagenicity was observed *in vitro* following 4-ET exposure. In a 13-week, repeated dose oral gavage study, male and female rats were administered 4-ET at 0, 100, 300 or 900 mg/kg/day. Effects on mortality, body weight, organ weights clinical chemistry, and hematological parameters were reported at doses ≥ 300 mg/kg/day. In addition, testicular atrophy and decreased spermatogenesis were observed at 300 mg/kg/day. Inhalation toxicity of 4-ET was evaluated male and female rats at 0, 104, 305 and 979 ppm for 13 weeks (6 hrs/day, 5

days/week). Significantly reduced gonad weight and significantly increased liver weights were reported in the 979 ppm exposure group. No reproductive toxicity studies were available in this assessment. In developmental oral gavage toxicity studies in pregnant rats and rabbits, no toxicity (maternal or developmental) was reported in rats up to 200 mg/kg/day. In pregnant rabbits, mortality in dams was reported at 250 mg/kg/day. Developmental toxicity was observed at 125 mg/kg/day.

Cumene

As previously mentioned, carcinogenic activity has been observed in both rats and mice following inhalation exposure to cumene. Neoplasms in the lung and liver of mice and in the nose and kidney of rats occurred in a 2-year study (*3*). Based on the evidence for carcinogenicity in experimental animals, cumene is recommended for listing as *reasonably anticipated to be a human carcinogen* in the Report on Carcinogens (*17*).

n-Propylbenzene

No inhalation studies are available for n-propylbenzene. In a two week repeated dose oral gavage study, ototoxicity was observed in male rats. Increased liver weight, decreased body weight gain and increased liver enzyme activity were observed in male rats following intraperitoneal injection of *n*-propylbenzene for three days. In a six-month oral study in rabbits, hemosiderin deposition in the spleen was noted.

Knowledge Gaps

The natural occurrence and commercial importance of C9 alkylbenzenes lead to substantial human exposure from many sources and in numerous scenarios. While several structurally related compounds are carcinogenic and neurotoxic to rodents and/or humans, cumene is the only C9 alkylbenzene tested for cancer. In addition, regulatory or safe exposure levels have been set for several alkylbenzenes (e.g., toluene, xylenes) but adequate data are not available to set safe exposure levels for many of the C9 alkylbenzenes.

Three of the isomers that constitute a small portion of the C9 fraction (cumene, ethylbenzene, naphthalene) have carcinogenic activity. The TMB and ET isomers are far more abundant constituents of the C9 fraction and yet little toxicity data exist for any TMB or ET isomers. The data gaps identified include the lack of chronic inhalation studies and adequate cancer studies. Based on these limitations, additional characterization of the toxicity and carcinogenicity of the C9 alkylbenzenes is warranted.

Key Issues

The relative proportion of the individual C9 compounds differs among products and media that represent sources of human exposure. Although some relevant mixtures have been tested, this information cannot be used to derive safe exposure levels for individual compounds. However, individually testing each isomer within the C9 fraction would be expensive and require extensive resources. A testing strategy must be developed that informs on relative hazard among different compounds and endpoints and builds on short term and alternative test systems when available. An understanding of the needs of regulatory and public health agency partners is necessary to identify

studies with the highest value and to determine an appropriate balance of tradeoffs within the testing program (e.g., multiple subchronic studies vs. one chronic study; thorough characterization of a mixed isomers test article vs. focused characterization of individual isomers).

The current dataset for the C9 alkylbenzenes indicates that, in addition to cancer, neurotoxicity, reproductive and developmental toxicity are relevant endpoints of concern. Inhalation is the primary route of exposure in humans to volatile chemicals such as C9 alkylbenzenes. However inhalation exposure studies require extensive resources and it would be inefficient to conduct multiple separate studies for individual C9 alkylbenzenes and for multiple, different endpoints. In addition, several C9 alkylbenzenes have been identified as drinking water contaminants, so oral exposure is also a route of concern. Bridging toxicokinetic studies between routes of exposure could be useful for routes of exposure comparisons and to improve the efficiency of the testing strategy.

Specific Aims

1. Determine appropriate C9 alkylbenzene test agents for toxicity and carcinogenicity studies, based on input from regulatory stakeholders.

2. Evaluate the toxicity and carcinogenicity of up to two test agents (as determined by Aim 1) following prechronic and chronic whole body inhalation exposure studies including reproductive, developmental, neurotoxicity endpoints.

3. Conduct short-term inhalation toxicity studies for additional C9 alkylbenzenes that are not included in Aim 2 designed to compare toxicity profiles between the various C9 isomers.

4. Explore the feasibility of using novel *in vitro* systems for volatile chemicals to generate a targeted *in vitro* assessment of the C9 alkylbenzenes.

Proposed Approach

In the initial phase of the testing strategy, appropriate C9 alkylbenzene test agents will be selected for a comprehensive evaluation of toxicity and carcinogenicity including neurological, reproductive and developmental toxicity endpoints in prechronic and chronic inhalation exposure studies. The C9 alkylbenzene test agents selected for this phase of testing will be determined based on input from appropriate regulatory stakeholders such as the EPA and/or NIOSH to optimize data generation for risk assessment. TMB and ET isomers are the predominant C9 alkylbenzenes in crude oil and commercial products. Therefore, two separate test agents, each individually representing TMB and ET isomers, are likely candidates for this comprehensive inhalation toxicity evaluation. Stakeholder input will be necessary to determine if each test agent should be an individual representative isomer of TMB or ET or a mixed preparation in which all three TMB or ET isomers are included in the test agent.

Following the test agent selection process in the initial phase of the testing strategy additional short-term inhalation toxicity studies will be conducted on C9 alkylbenzenes that are not selected for long-term studies. The test agents selected for the initial phase will be used to guide test agent selection and priority for this phase of testing. For example, if one representative isomer from both TMB and ET are individually selected for testing in a comprehensive toxicity evaluation, this phase of short-term testing will include the other individual TMB and ET isomers that are not included in phases one and two. Alternatively, if mixed TMB and ET isomer preparations are selected for testing in the initial testing, then short term toxicity testing on the individual TMB and ET isomers would be necessary in this phase for comparison of the toxicity profiles between the individual TMB and ET isomers.

Capabilities for *in vitro* assessment of the C9 fraction isomers will be evaluated in parallel to *in vivo* testing described above. *In vitro* assessment of C9 alkylbenzenes would require specialized systems and equipment designed to generate atmospheres for exposing cells *in vitro* to volatile chemicals. These *in vitro* methods for screening volatile chemicals exist and will be explored. Target tissue/organ toxicity will be determined based on the literature that currently exists, along with the data generated during the current *in vivo* testing program to create a targeted approach to *in vitro* assessment.

Significance and Expected Outcome

C9 alkylbenzenes are high production volume chemicals with the potential for widespread exposure. Furthermore, the toxicity and carcinogenicity associated with closely related aromatic hydrocarbons such as benzene, ethylbenzene and cumene suggests that a better understanding of the potential hazards of C9 fraction compounds is necessary. A toxicological evaluation of the C9 alkylbenzenes that incorporates a prioritization based on individual isomer prevalence in mixtures of commercial importance would aid in characterizing risk of the C9 alkylbenzenes. Robust and sensitive comparisons between different C9 alkylbenzenes may provide useful information in future hazard evaluations for similar compounds. Furthermore, this testing strategy would provide the opportunity to evaluate the utility of alternative testing systems for volatile chemicals. Chronic inhalation studies of the most prevalent C9 fraction isomers with the highest suspicion of toxicity based on a suite of short-term studies would provide useful carcinogenicity information to complement existing data on other aromatic hydrocarbons.

References

1. U.S. EPA (U.S. Environmental Protection Agency). (1994a). Chemical summary for 1,2,4-trimethylbenzene [EPA Report]. (EPA/749/F-94/022A). Washington, DC. http://www.epa.gov/chemfact/s_trimet.txt. Last accessed on April 18, 2014.

2. U.S. ICCA (U.S. International Council of Chemical Associations). (2012). Initial Assessment Profile for C9 Aromatic Hydrocarbon Solvents Category (Screening

Initial Data Set [SIDS]) Organisation for Economic Cooperation and Development Cooperative Chemicals Assessment Meeting (OECD CoCAM). http://webnet.oecd.org/hpv/ui/handler.axd?id=bd162768-5b30-44e0-bfc0-0f639b113191. Last accessed on April 18, 2014.

3. National Toxicology Program (NTP), 1999. Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Series No. 466, NIH Publication No. 99-3956. National Institutes of Health, Public Health Service, US Department of Health and Human Services, Research Triangle Park. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr466.pdf. Last accessed on April 18, 2014.

4. National Toxicology Program (NTP), 2009. Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Series No. 542, NIH Publication No. 09-5885. National Institutes of Health, Public Health Service, US Department of Health and Human Services, Research Triangle Park. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr542.pdf. Last accessed on April 18, 2014.

5. National Toxicology Program (NTP), 1992. Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F1 Mice (Inhalation Studies). Technical Report Series No. 410, NIH Publication No. 92-3141. National Institutes of Health, Public Health Service, US Department of Health and Human Services, Research Triangle Park. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr410.pdf. Last accessed on April 18, 2014.

6. National Toxicology Program (NTP), 2000. Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies). Technical Report Series No. 500, NIH Publication No. 01-4434. National Institutes of Health, Public Health Service, US Department of Health and Human Services, Research Triangle Park. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr500.pdf. Last accessed on April 18, 2014.

7. U.S. EPA (U.S. Environmental Protection Agency). (1985). Identification of specific chemical substance and mixture testing requirements; Ethyltoluenes. trimethylbenzenes, and the C9 aromatic hydrocarbon fraction. Fed Reg 50: 20662-20677. http://hero.epa.gov/index.cfm?action=reference.details&reference_id=1419446. Last accessed on April 18, 2014.

8. Schreiner, CA; Edwards, DA; Mckee, RH; Swanson, M; Wong, ZA; Schmitt, S; Beatty, P. (1989). The mutagenic potential of high flash aromatic naphtha. Cell Biol Toxicol 5: 169-188.

9. Mckee, RH; Wong, ZA; Schmitt, S; Beatty, P; Swanson, M; Schreiner, CA; Schardein, JL. (1990). The reproductive and developmental toxicity of High Flash Aromatic Naphtha. Toxicol Ind Health 6: 441- 460.

10. Douglas, JF; Mckee, RH; Cagen, SZ; Schmitt, SL; Beatty, PW; Swanson, MS; Schreiner, CA; Ulrich, CE; Cockrell, BY. (1993). A neurotoxicity assessment of high flash aromatic naphtha. Toxicol Ind Health 9: 1047-1058.

11. Clark, DG; Butterworth, ST; Martin, JG; Roderick, HR; Bird, MG. (1989). Inhalation toxicity of high flash aromatic naphtha. Toxicol Ind Health 5: 415-428.

12. U.S. Environmental Protection Agency (EPA). 2013. IRIS Toxicological Review of Trimethylbenzenes (Revised External Review Draft). Internet address: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=254525. Last accessed on April 18, 2014.

13. Korsak, Z; Stetkiewicz, J; Majcherek, W; Stetkiewicz, I; Jajte, J; Rydzynski, K. (2000). Sub-chronic inhalation toxicity of 1,2,4-trimethylbenzene (pseudo cumene) in rats. Int J Occup Med Environ Health 13: 155-164.

14. Korsak, Z; Stetkiewicz, J; Majcherek, W; Stetkiewicz, I; Jajte, J; Rydzynski, K. (2000). Subchronic inhalation toxicity of 1,2,3-trimethylbenzene (hemimellitene) in rats. Int J Occup Med Environ Health 13: 223- 232.

15. Korsak, Z; Rydzynski, K; Jajte, J. (1997). Respiratory irritative effects of trimethylbenzenes: An experimental animal study. Int J Occup Med Environ Health 10: 303-311.

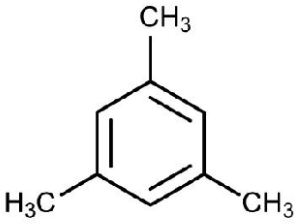
16. Korsak, Z; Rydzynski, K. (1996). Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int J Occup Med Environ Health 9: 341-349.

17. U.S. Environmental Protection Agency (EPA). 2009. Screening-level hazard characterization; *p*-Ethyltoluene (CASRN 622-96-8). Internet address: http://www.epa.gov/hpvis/hazchar/622-96-8_p-Ethyltoluene_Sept2009.pdf. Last accessed on April 18, 2014.

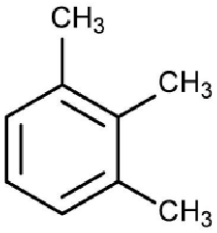
18. National Toxicology Program (NTP). 2013. Report on Carcinogens (RoC) Monograph on Cumene. Internet address: http://ntp.niehs.nih.gov/ntp/roc/thirteenth/monographs_final/cumene_508.pdf. Last accessed on April 18, 2014.

Appendix

Figure 1. Structures of 9-carbon isomers comprising the C9 aromatic hydrocarbon fraction



1,3,5 – trimethylbenzene
(mesitylene)
CASRN: 108-67-8



1,2,3 – trimethylbenzene
(hemimellitene)
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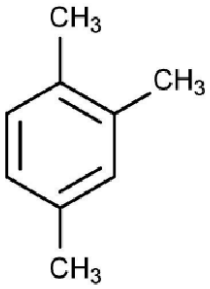
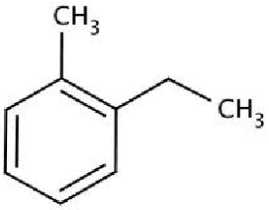


1,2,4 – trimethylbenzene
(pseudocumene)
CASRN: 95-63-6



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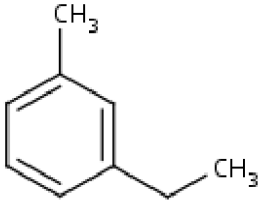

3 – ethyltoluene
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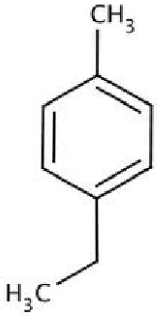
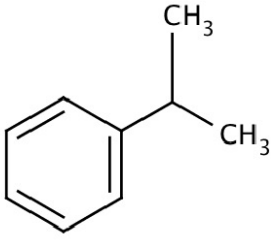
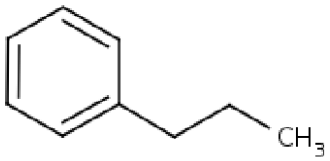


4 – ethyltoluene
CASRN: 622-96-8



cumene
CASRN: 98-82-8



n-propylbenzene
CASRN: 103-65-1