

Research Concept on **C9 Alkylbenzenes**

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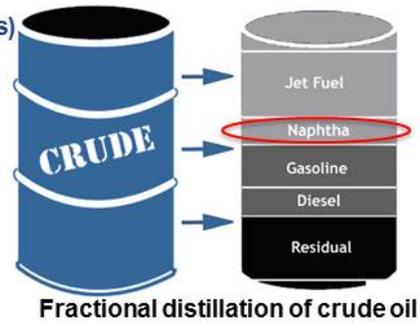
NTP Board of Scientific Counselors Meeting
June 17, 2014



C9 Alkylbenzenes (Aromatic Hydrocarbons: 9 carbons)

- **Production**

- Occur naturally in crude oil
- Produced from the naphtha fraction of crude oil
- U.S. annual production = **40 million tons**



- **Uses**

- Component of gasoline blending stream (*≈ 99% of production volume*)
- Solvents (*Worldwide production = 50,000-250,000 metric tons*)

- **Human Exposure**

- Inhalation & Oral
- Environmental and Occupational
- Occupational exposure levels: up to 3 ppm



C9 Alkylbenzenes

Compound	Weight % Crude Oil*	Weight % Solvent naphtha	
<i>9-carbon isomers (C9)</i>			
1,2,4-trimethylbenzene	0.343	20-45	} No chronic inhalation toxicity studies
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	← Carcinogen in rodents Inhalation NTP TR542
n-propylbenzene	0.066	0.5-5	
<i>8-carbon isomers (C8)</i>			
o-xylene (C8)	0.314	2-15	} ← No evidence for Carcinogenicity Oral gavage NTP TR327
m-xylene (C8)	0.632	1-5	
p-xylene (C8)	0.239	0.5-5	
ethylbenzene (C8)	0.149	0.1-0.5	← Carcinogen in rodents Inhalation NTP TR466
<i>10-carbon isomers (C10)</i>	0.546	1-3	
Total BTEX**	2.269	NA	

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

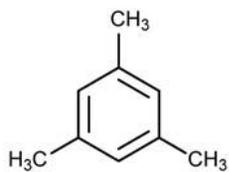
** - BTEX = benzene, toluene, ethylbenzene, xylene. Weight % in crude oil included for reference

C9 Aromatic Hydrocarbon Fraction

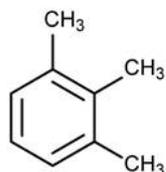
TSCA Section 4 Test Rule

- **Test substance** → commercial C9 fraction product
 - > 75% Ethyltoluene (ET)/ Trimethylbenzene (TMB) isomers
- **Genotoxicity:**
 - No evidence (*in vivo* or *in vitro*)
- **Inhalation Toxicity Studies**
 - Developmental and Reproductive Toxicity
 - Severe maternal and developmental toxicity (mice @ 1514 ppm)
 - Decreased fetal body weights in multi-gen study (rats @ 495 and 1480 ppm)
 - Neurotoxicity
 - Transient effects on motor activity observed in male rats (1320 ppm)
 - Neurobehavioral toxicity observed in pre-mating adult rats (1480 ppm)
 - Neurobehavioral toxicity in pregnant mice (1514 ppm)
 - Chronic Toxicity (12 month exposure; rats)
 - Dose-dependent macrophage infiltration and alveolar wall thickening in lung
 - Increased liver and kidney weights (373 ppm)

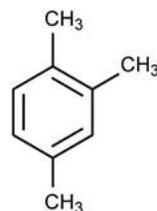
Trimethylbenzene (TMB) and Ethyltoluene (ET) isomers



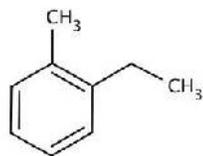
1,3,5 – trimethylbenzene
(mesitylene)



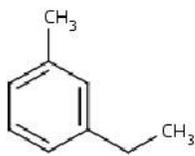
1,2,3 – trimethylbenzene
(hemimellitene)



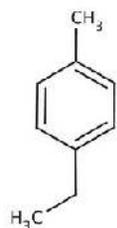
1,2,4 – trimethylbenzene
(pseudocumene)



2 – ethyltoluene

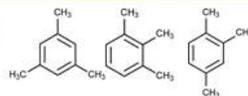


3 – ethyltoluene



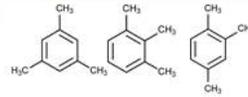
4 – ethyltoluene

Trimethylbenzenes (TMB) – EPA IRIS Assessment



- **External Review Draft : August 2013**
- **Meeting of Scientific Advisory Board (SAB) : June 17th – 19th, 2014**
- **Inhalation Toxicity Studies**
 - Short-term exposure (4 weeks)
 - Several studies identified
 - Respiratory irritation and neurological effects reported
 - Subchronic exposure (13 weeks)
 - Four studies identified (none identified for 1,3,5-TMB)
 - Effects observed in nervous, hematological and respiratory systems
 - Data used to derive Reference concentrations (RfC)

Trimethylbenzenes (TMB) – EPA IRIS Assessment



- **Evidence of Carcinogenicity**

- Inadequate information to assess carcinogenic potential
- No chronic inhalation studies identified in the literature

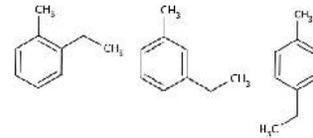
- **Dose Response Assessment**

- Reference values derived for two isomers from two inhalation studies
- Remaining reference values → route to route extrapolation & sufficient similarity

- **Confidence in the Database is “low to medium” because:**

- it lacks chronic, multi-generation reproductive/developmental, developmental toxicity, or developmental neurotoxicity studies
- the studies supporting the critical effect predominantly come from the same research institute

Ethyltoluenes (ET) Hazard Data



- **Genotoxicity:**
 - No evidence *in vitro*
- **Inhalation Toxicity Studies**
 - Subchronic Toxicity (rats)
 - Increased liver weights, decreased gonad weight (rats @ 979 ppm)
- **Oral Toxicity Studies**
 - Subchronic Toxicity (rats)
 - Mortality, effects on organ weights, body weights, clinical chemistry (\geq 300mg/kg/day)
 - Testicular atrophy & decreased spermatogenesis (300 mg/kg/day)
 - Developmental Toxicity (rabbits)
 - Maternal mortality (250 mg/kg/day)
 - Developmental toxicity (125 mg/kg/day)
- **No Reproductive or Chronic Toxicity Studies Available**

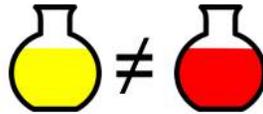
Rationale for Study

- High production volume → substantial human exposure (many sources, numerous scenarios)
- Several structurally related compounds are carcinogenic and neurotoxic to rodents and/or humans → cumene is the only C9 alkylbenzene tested for cancer
- TMB and ET isomers → most abundant C9 alkylbenzenes → little toxicity data exists
- Limitations in C9 alkylbenzene data sets for establishing safe exposure levels

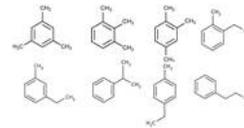
Key Issues

- **Test material selection**

- Entire C9 mixture
 - Proportion of isomers varies among products and media



- Individual isomers
 - Chronic testing each isomer is cost & resource prohibitive



- **Inhalation exposure testing**

- Require extensive resources
- Challenging for reproductive, developmental and neurotoxicity endpoints
- Study design efficiency
 - Inefficient to design multiple, separate studies for multiple relevant endpoints (reproductive, developmental, neurotoxicity)

Key Issues

- **Appropriate testing strategy**

- Understanding needs of regulatory & public health partner agencies

- Identify studies with highest value
- Determine appropriate balance of tradeoffs
 - Multiple subchronic studies **vs.** One chronic study



- Representative isomer **vs.** Prepared mixture of isomers



Specific Aims

1. Determine appropriate C9 alkylbenzene test agents for toxicity and carcinogenicity studies

- Test agent selection based on input from partner agencies

1. Evaluate the toxicity and carcinogenicity of up to two test agents

- Test agents selected as determined by Aim 1
- Prechronic and chronic whole body inhalation exposure
 - Including reproductive, developmental and neurotoxicity endpoints

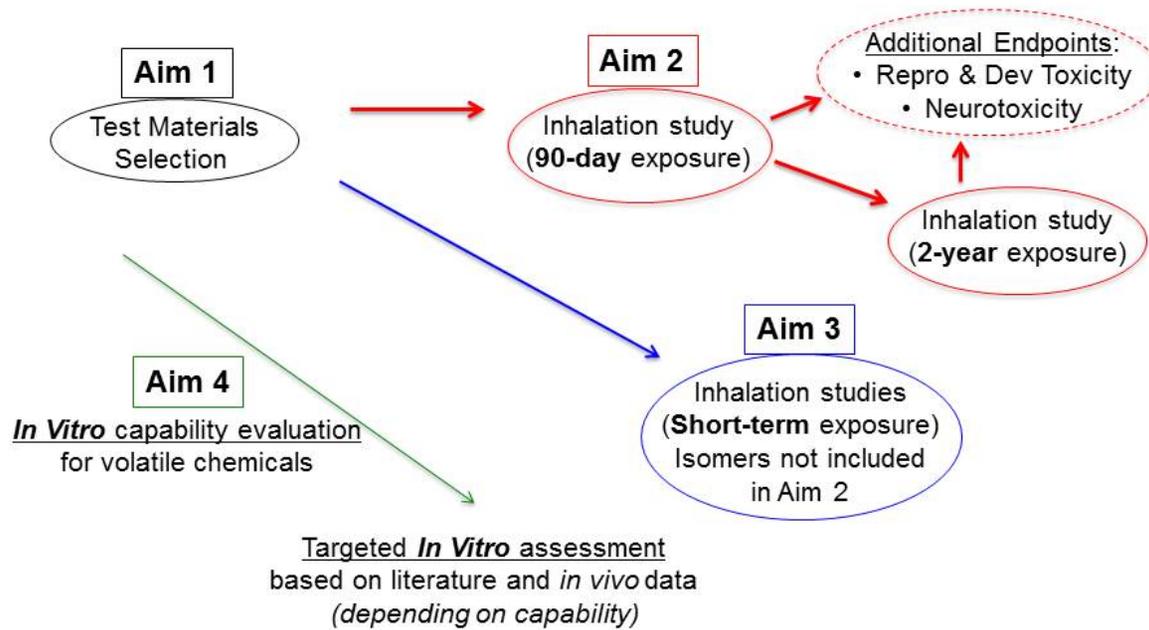
1. Conduct comparative short-term inhalation toxicity studies

- C9 alkylbenzenes not included in Aim 2

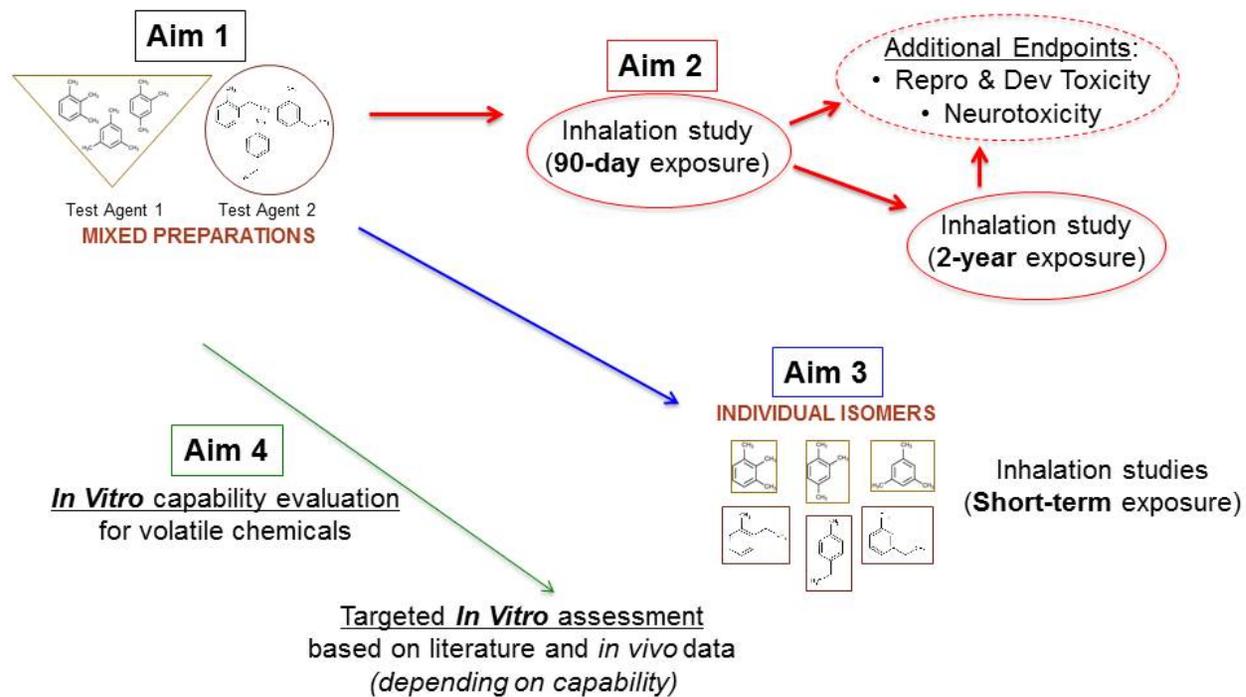
1. Assess *in vitro* screening of C9 alkylbenzenes

- Capability of screening volatile chemicals will be explored
- Target tissue/organs from *in vivo* testing data

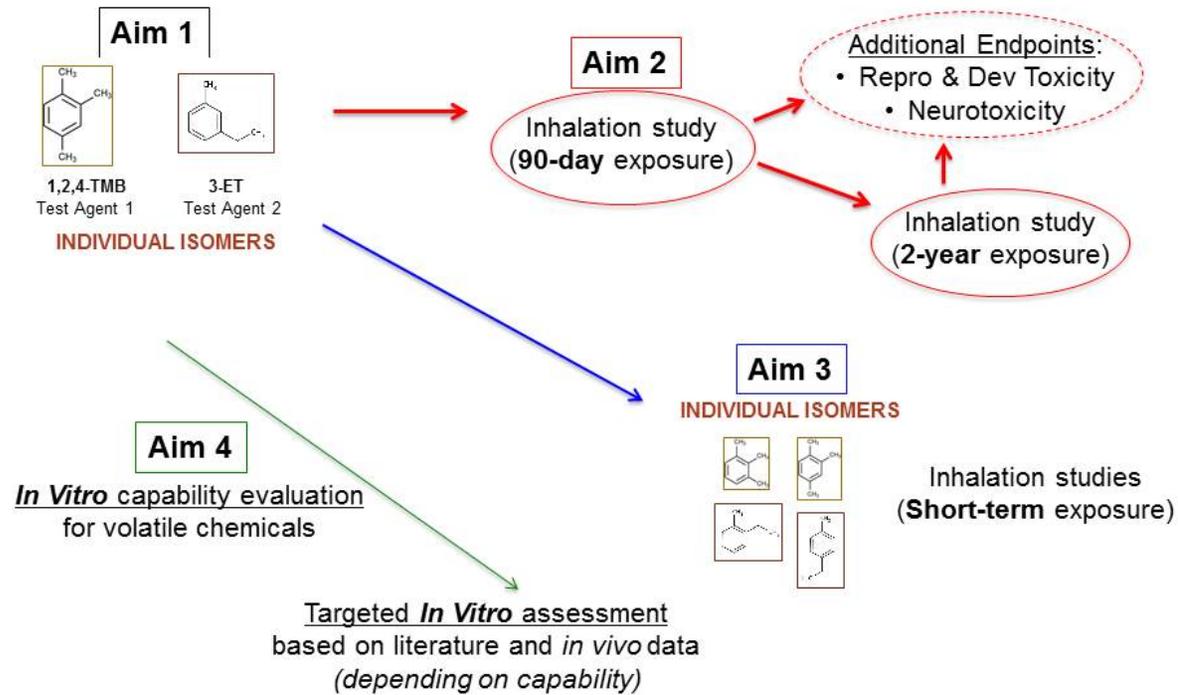
Proposed Approach



Proposed Approach MIXED PREPARATIONS



Proposed Approach INDIVIDUAL ISOMERS





Significance & Expected Outcome

- Produce useful toxicity and carcinogenicity information to complement data on other aromatic hydrocarbons
- Address limitations in current dataset to aid in risk characterization
- Generate robust comparisons between different C9 alkylbenzenes → useful information in future hazard evaluations of similar compounds
- Provide the opportunity to evaluate the utility of alternative testing systems for volatile chemicals



Thank you!

Review Questions

1. Comment on the merit of the proposed project relative to the mission and goals of the NTP. *The NTP's stated goals are to: Provide information on potentially hazardous substances to all stakeholders; Develop and validate improved testing methods; Strengthen the science base in toxicology; Coordinate toxicology testing programs across DHHS (<http://ntp.niehs.nih.gov/go/about>).*
 2. Comment on the clarity and validity of the rationale for the proposed project. Has the scope of the problem been adequately defined? Are the relevant knowledge gaps identified and clearly articulated?
 3. Comment on the strategy and approach proposed to meet the stated objectives of the project. Are specific aims reasonable and clearly articulated? Is the scope of work proposed appropriate relative to the public health importance of the issue(s) under consideration? If not, what modifications do you recommend? Where steps to further refine the strategy and/or approach are proposed, are they appropriate?
 4. There are challenges inherent to achieving the aims of any proposed project. Are the relevant challenges and/or key scientific issues identified and clearly articulated? Where approaches to overcome challenges are proposed, are they appropriate? Are you aware of other scientific issues that need to be considered?
 5. Rate the overall significance and public health impact of this project as low, moderate, or high. Identify any elements of the proposed project that you feel are more important than others, and/or that have a higher likelihood of success at meeting pre-defined specific aims.
1. Provide any other comments you feel NTP staff should consider in developing this project.