Provisional Reference Dose For The Aromatic Fraction Of Jet Fuel: Insight Into Complex Mixtures

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FOR THE DIRECTOR

//SIGNED//

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Deputy Chief, Biosciences and Protection Division
Air Force Research Laboratory
### Title and Subtitle
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### Abstract
Previous efforts to define toxicity criteria for human health risk assessment of complex petroleum mixtures use health effects information for only a subset of the chemicals in such mixtures. The Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) developed a practical alternative by defining all petroleum mixtures as thirteen carbon number range fractions based on expected transport characteristics following release to the environment. The TPHCWG developed toxicity criteria for each fraction using all available data, prioritizing mixture toxicity information. However, limited toxicity data were available to represent the fractions, including the EC>8 - EC16 aromatic fraction, which is believed to be one of the more toxic fractions. To address this data gap, a 90-day oral gavage toxicity study was conducted in female Sprague-Dawley rats and male C57BL/6 mice to characterize toxic effects of the EC>8 - EC16 aromatic fraction of Jet Fuel A. Animals were dosed at 0, 20, 100 and 500 mg/kg/day.
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PREFACE

This work was made possible through the professional collaboration of private and government scientists. The 90-day toxicity study was funded through a U.S. Air Force Small Business Technology Transfer Research project through contract No. F41624-97-C-9013. Menzie-Cura & Associates, Inc., Winchester, MA, acted as the agent of their primary client, the U.S. Air Force Air Force Research Laboratory's former Operational Toxicology Branch (AFRL/HEST, work transferred to the Applied Biotechnology Branch, AFRL/HEPB), Wright Patterson AFB, OH. Primary authorship of the report was performed by Operational Technologies Corporation (OpTech) under Contract Number F33601-02-F-A211. OpTech activities were conducted under the Project Management of Dr. Peter Lurker. Dr. David Mattie of AFRL/HEPB served as contract monitor for the Workunit 1710D408.

All housing and animal care conformed to the requirements stated in the “Guide for the Care and Use of Laboratory Animals” (National Academy of Sciences, 1996) and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Battelle’s Institutional Animal Care and Use Committee approved the study protocol.

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Additional thanks to the American Petroleum Institute and its Toxicology Task Force for funding the test material development and providing technical guidance. Thanks to Laura Young of BDM for her role in the test material isolation. Thanks to Ileana Rhodes and Julia Milazzo from Equillon Enterprises, LLC for their chemical analysis of the test material and consultative support. We would also like to thank Cheri Butler of Menzie-Cura and Associates, Inc. for her work in the development of the BBSL table and the pathology staff from the U.S. Air Force Research Laboratory, Operational Toxicology Branch; William Baker (now at Springborn Laboratories), William Brinkley, and Peggy Parish. We thank the following reviewers for their helpful suggestions and comments: Lorraine Twerdok, American Petroleum Institute; Suneeta Mahagaokar, Pennzoil-Quaker State Company, Michelle Andriot, DOW Chemical; and Robert Wilkenfeld, Chevron.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>ATSDR</td>
<td>Agency for Toxic Substance and Disease Registry</td>
</tr>
<tr>
<td>EC</td>
<td>effective carbon</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>HGB/HCT/RBC</td>
<td>hemoglobin/hematocrit/red blood cell count</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatograph</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>MA DEP</td>
<td>Massachusetts Department of Environmental Protection</td>
</tr>
<tr>
<td>m³</td>
<td>cubic meter</td>
</tr>
<tr>
<td>MCHC</td>
<td>mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NA</td>
<td>not applicable</td>
</tr>
<tr>
<td>NOAEL</td>
<td>no observed adverse effect level</td>
</tr>
<tr>
<td>psig</td>
<td>pounds per square inch, gauge</td>
</tr>
<tr>
<td>RBCA</td>
<td>Risk Based Corrective Action</td>
</tr>
<tr>
<td>RBSL</td>
<td>risk based screening level</td>
</tr>
<tr>
<td>RfC</td>
<td>reference concentration</td>
</tr>
<tr>
<td>RfD</td>
<td>reference dose</td>
</tr>
<tr>
<td>TPH</td>
<td>total petroleum hydrocarbon</td>
</tr>
<tr>
<td>TPHCWG</td>
<td>Total Petroleum Hydrocarbon Criteria Working Group</td>
</tr>
<tr>
<td>UF</td>
<td>uncertainty factor</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet light</td>
</tr>
<tr>
<td>wt%</td>
<td>weight percent</td>
</tr>
</tbody>
</table>
PROVISIONAL REFERENCE DOSE FOR THE AROMATIC FRACTION OF JET FUEL: INSIGHT INTO COMPLEX MIXTURES

INTRODUCTION

Petroleum release sites are difficult to evaluate because the composition and distribution of complex petroleum products change following release to the environment. Total petroleum hydrocarbon (TPH) standards are often applied to these sites, and the sites are remediated to these standards with an unknown reduction of human health risk at the site. Recognizing this dilemma, the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) developed a fraction-based approach to risk assessment at petroleum release sites (Weisman, 1998a). The results of this work have been published in five volumes that outline the scientific basis behind the approach and provide the data used by the group in making its decisions (Weisman (ed.), 1998b; Potter et al., 1998; Gustafson et al., 1997; Edwards et al., 1997; Vorhees et al., 2000). Figure 1 shows the function of these volumes in the overall approach.

Figure 1: Function of the TPHCWG Publications in the Overall Approach to Petroleum Assessment
The TPHCWG and Massachusetts Department of Environmental Protection (MA DEP) have developed provisional reference doses (RfDs) and reference concentrations (RfCs) for petroleum hydrocarbon fractions. Although there was some interaction between these organizations and a sharing of information, the RfDs were set independently. The MA DEP provisional RfDs and RfCs have recently undergone internal review (MA DEP, 2003).

The TPHCWG chose to delineate their fractions based on effective carbon (EC) number and fate and transport characteristics. Effective or equivalent carbon number is a unitless value representing the carbon atom equivalency to the n-alkanes based upon the target compounds retention time in a boiling point gas chromatograph column. EC is used by the petroleum industry for separating product streams and is used chemically for reporting results from boiling point gas chromatograph (GC) columns (Gustafson et al., 1997).

To develop the toxicity criteria for all TPHCWG fractions, the Working Group conducted a detailed search of published and unpublished toxicity studies for all components in these fractions with the goal of using mixture data whenever available to account for interactive effects among components. The literature search revealed only 95 components with toxicity data, despite the fact that petroleum products contain thousands of compounds. Of the 95 components, only 25 had sufficient toxicity information to develop provisional reference doses. Based on this review, the TPHCWG developed non-cancer toxicity criteria for each fraction based on individual component data or mixture toxicity criteria. Some fractions share the same RfDs because their toxicity is believed to be similar (Edwards et al., 1997). Table 1 provides the provisional RfDs and RfCs for TPHCWG fractions and the critical effects.

<table>
<thead>
<tr>
<th>Carbon Range</th>
<th>Aromatic RfD -- and -- RfC</th>
<th>Critical Effects</th>
<th>Aliphatic RfD -- and -- RfC</th>
<th>Critical Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC&lt;sub&gt;5&lt;/sub&gt; - EC&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.2*</td>
<td>Hepatotoxicity, Nephrotoxicity</td>
<td>5.0</td>
<td>Hepatotoxicity, Nephrotoxicity</td>
</tr>
<tr>
<td>EC&lt;sub&gt;6&lt;/sub&gt; - EC&lt;sub&gt;8&lt;/sub&gt;</td>
<td>0.4*</td>
<td>Hepatotoxicity, Nephrotoxicity</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>EC&lt;sub&gt;8&lt;/sub&gt; - EC&lt;sub&gt;10&lt;/sub&gt;</td>
<td>0.04</td>
<td>Decreased body weight</td>
<td>0.1</td>
<td>Hepatic and hematological changes</td>
</tr>
<tr>
<td>EC&lt;sub&gt;10&lt;/sub&gt; - EC&lt;sub&gt;12&lt;/sub&gt;</td>
<td>0.2</td>
<td>Decreased body weight</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>EC&lt;sub&gt;12&lt;/sub&gt; - EC&lt;sub&gt;16&lt;/sub&gt;</td>
<td>0.03</td>
<td>Nephrotoxicity</td>
<td>2.0</td>
<td>Hepatic granuloma</td>
</tr>
<tr>
<td>EC&lt;sub&gt;16&lt;/sub&gt; - EC&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0.03</td>
<td>Nephrotoxicity</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>EC&lt;sub&gt;21&lt;/sub&gt; - EC&lt;sub&gt;36&lt;/sub&gt;</td>
<td>NA</td>
<td>Hepatotoxicity, Nephrotoxicity</td>
<td>10.0</td>
<td>Hematological changes</td>
</tr>
</tbody>
</table>

Notes: Adapted from Vorhees et al. (2000). EC = effective carbon. NA = not applicable as fraction is not volatile under environmental conditions. *Excludes EC<sub>5</sub> - EC<sub>6</sub> as benzene noncancer toxicity was under review by U.S. EPA at the time of publication.

In using these TPHCWG provisional RfDs to estimate non-cancer hazard associated with exposure to petroleum, one must make several assumptions (Vorhees et al., 2000):
1. Fraction toxicity will not vary significantly from the single compound or mixture of components used to develop the toxicity criterion for the fraction. Toxicity criteria are designed to account for uncertainty in the underlying toxicity database by overestimating rather than underestimating fraction toxicity.

2. Application of each toxicity criterion is appropriate whether or not the specific compound or mixture from which the toxicity criterion was derived is present in the environmental samples, as long as compounds of similar equivalent carbon numbers and structure are present at the contaminated site.

3. The toxicity of a given fraction does not change with different petroleum product sources or due to weathering in the environment.

The reference values selected by each organization for the effective carbon (EC)_{8-16} aromatic fraction are similar, 0.04 and 0.03 mg/kg/day (TPHCWG and MA DEP, respectively). The TPHCWG established their provisional RfD using chiefly toxicity information for a mixture including naphthalene and methylnaphthalenes (mixture RfD of 0.03 mg/kg/day) plus RfDs for four fraction constituents set at 0.04 mg/kg/day. Some of these constituents were erroneously included, as their EC numbers exceed the definition of this fraction, while other constituents that should have been included based on EC numbers were left out (see Table 2). These errors were noted in the erratum prior to publication of the TPHCWG volumes. It was also noted that the errors did not alter the RfD chosen for this fraction (Edwards et al., 1997). The MA DEP C_{9-16} fraction legitimately includes these additional components as their fraction is based on simple carbon number. Since naphthalene is a target analyte under the Massachusetts Contingency Plan and is therefore evaluated separately from the fraction, the MA DEP fraction provisional RfD was set at the pyrene reference value of 0.03 mg/kg/day (MA DEP, 2003).

Table 2. RfDs for Aromatics from Original TPHCWG Publications

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>Effective Carbon Number(^a)</th>
<th>Compound</th>
<th>IRIS RfD in 1997(^b) (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8.5</td>
<td>ethylbenzene</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>8.6 - 8.81</td>
<td>xylenes</td>
<td>2.0</td>
</tr>
<tr>
<td>8</td>
<td>8.83</td>
<td>styrene</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>9.13</td>
<td>isopropylbenzene</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>9.13</td>
<td>naphthalene</td>
<td>0.04(^c)</td>
</tr>
<tr>
<td>10 - 11</td>
<td>11.69 - 12.99</td>
<td>naphthalene/methyl naphthalenes mixture</td>
<td>0.03(^d)</td>
</tr>
<tr>
<td>12</td>
<td>14.26</td>
<td>biphenyl</td>
<td>0.05</td>
</tr>
<tr>
<td>13</td>
<td>16.55</td>
<td>fluorene</td>
<td>0.04</td>
</tr>
<tr>
<td>14</td>
<td>19.43</td>
<td>anthracene</td>
<td>0.3</td>
</tr>
<tr>
<td>16</td>
<td>20.8</td>
<td>pyrene</td>
<td>0.03</td>
</tr>
<tr>
<td>16</td>
<td>21.85</td>
<td>fluoranthene</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Notes: Compounds within the large box belong in the TPHCWG EC\(_{8-16}\) aromatic fraction according to their effective carbon number. RfDs included within the smaller dark box were used in the original evaluation of the RfD for this fraction (Edwards et al., 1997).\(^a\)Gustafson et al. (1997). \(^b\)Edwards et al. (1997). \(^c\)This value was a provisional RfD calculated by MA DEP (Edwards et al., 1997). \(^d\)This value is a provisional RfD calculated by the TPHCWG (Edwards et al., 1997).
The TPHCWG recommended that provisional RfDs assigned to the aromatic EC$_{8\text{-}16}$ and EC$_{16\text{-}34}$ fractions were more uncertain than those assigned to other fractions given the limited available toxicity data (Vorhees et al., 2000). Similarly, the Agency for Toxic Substances and Disease Registry (ATSDR) indicated in their Toxicological Profile for Total Petroleum Hydrocarbons (TPH) that the "database for the aromatic fraction EC$_{8\text{-}16}$ lacks information on a mixture or mixtures that could represent the entire combined fraction [...] Health effects data from these mixtures and from potential representative chemicals, including naphthalene, suggest some commonality of effect among constituents of the fraction" (ATSDR, 1999).

For these reasons, a 90-day oral gavage study was conducted to assess toxicity of the EC$_{8\text{-}16}$ aromatic fraction (Smith et al., 1999a). This study involved isolation of a large quantity of test material and performance of a 7-day range finding study (Smith et al., 1999b). The objective of this report is to briefly describe this toxicity study conducted to reduce uncertainty in the oral reference value for the EC$_{8\text{-}16}$ aromatic fraction and to propose an updated provisional RfD for the fraction.

**ISOLATION OF TEST MATERIAL**

The EC$_{8\text{-}16}$ aromatic fraction (boiling range 151-287°C) was prepared from Jet-A jet fuel by BDM Petroleum Technologies, Bartlesville, OK, using a large-scale silica liquid chromatographic column. The Jet-A fuel, blended from different geographical locations, was formulated and maintained by the U.S. Air Force Research Laboratory, Propulsion Directorate, Fuels Branch, Wright Patterson AFB, OH. The U.S. Air Force (Department of Defense Directive 4140.25) and NATO (North Atlantic Treaty Organization) (Standard Agreement 4632) have selected JP-8 as the single battlefield fuel. JP-8 complies with specifications that are almost the same as those of the civilian aviation fuel Jet-A except for additives required by the U.S. Department of Defense. These additives were not present in the Jet A used to generate the test fraction.

Liquid chromatographic separation of saturates and aromatics on silica is a commonly employed procedure and serves as the basis for several standardized methods (e.g., ASTM D2007) for determination of petroleum compounds. Initial purity of the isolate was determined both by BDM and independently by Equilon Enterprises, L.L.C., Houston, TX. Equilon also provided analysis of the neat material at the end of the study and found insignificant evidence of degradation (Smith et al., 1999b).

Figure 2 is a schematic of the separation column that was used to isolate the EC$_{8\text{-}16}$ aromatic fraction. Briefly, a rack was constructed to hold three 55 gallon drums of solvent at a height of seven feet. Silica was placed in a fourth drum, three feet above ground level and connected to the solvent with ½ inch stainless tubing. A head pressure (2 psig N$_2$) was applied to start the siphon and eliminate headspace. A 400-mesh screen on the bottom of the collection drum prevented loss of silica. The solvent and jet fuel eluent was recovered from the lower drum via a ¼ inch stainless tube exiting the collection pan.
The silica in the lower drum was preconditioned with petroleum ether (10 - 15 gallons). Pre-weighted aliquots of 20 kg total Jet-A were dissolved in equal volumes of petroleum ether and siphoned into the silica drum at a rate of 5 gallons/hour. After charging the column, the flow rate was increased to 10 gallons/hour. Eluent was collected in 5-gallon containers and monitored for ultraviolet light (UV) absorbance. At the earliest point where initial elution of aromatics was possible, UV spectra were recorded at 1-gallon intervals. Once absorbance was >0.1, ethyl ether was used as the eluting solvent and eluent was collected for aromatic recovery. UV absorbance indicated appreciable levels of aromatics after elution with 50 gallons of ethyl ether, so the silica drum was charged with five gallons neat methanol, followed by ethyl ether. Elution
was complete after 115 gallons had been collected. Mobile solvent removal was by rotary evaporators at <40°C, 300 - 400 torr, to obtain the final aromatic product.

The aromatic content of each eluent batch was estimated by comparing its UV absorbance to that of the original product. The UV ratio of the eluent to whole fuel multiplied by 0.14 (defined aromatic content of Jet-A) was used as an approximate value for aromatics. Seven eluent batches with significant aromatic content based on UV absorbance but with appreciable saturate carryover were again separated using fresh silica as described above. Following solvent removal, the resulting mixture of purified aromatic eluent batches was combined with 15 other batches from the initial separation to obtain 3,571 g of raw aromatic concentrate. This was distilled over an all-glass packed-helice column (estimated to provide 5 theoretical plate efficiency). This distillation removed residual solvent and provided 2,856g of a 151-287°C boiling range material that was filtered through predried celite 521 and blanketed with nitrogen.

TEST MATERIAL VERIFICATION

GC results showed that 97% of the final product boiled at the prescribed range and therefore fell within the EC_{28} - EC_{16} fraction. Group-type mass spectrometric (MS) analysis (Teeter, 1985) of the product indicated an aromatic (plus sulfur compounds) content of 80.6 wt%, with the balance largely comprised of 2- and 3-ring non-aromatic naphthenes. However, MS analysis likely overestimated the proportion of naphthenes present because of assumptions based on normal petroleum composition built into the calculation procedure. Saturated fragment ions originating via elimination of alkyl- and cycloalkyl-groups connected to aromatic rings normally make a minimal contribution to the overall saturated ion intensity used to calculate saturate content. However, for this highly aromatic concentrate, their contribution was significant, and may have accounted for the majority of the apparent saturate content. Similarly, the UV assay based on the whole fuel absorbance is not reliable because some highly absorptive species were removed during distillation to the 287°C endpoint. For example, the relative UV absorbance of the aromatic concentrate dropped about 30% after distillation. Ironically, determination of low levels of aromatics in a predominately saturated matrix such as jet fuel is relatively simple, yet accurate determination of a small proportion of saturates in an aromatic matrix is a difficult analytical problem. Table 3 summarizes chemicals in the test material fraction that were present at a concentration of one percent or greater. The data were obtained from the GC/MS analysis of the top 100 (by percent) identified chemicals in the test material. The concentrations of chemicals identified ranged from 0.21% to 3.9% with 57% of the material described by the 33 most abundant compounds.
Table 3. List of Chemicals in EC$_{28}$ - EC$_{16}$ Aromatic Fraction Present at Greater Than or Equal to 1% Concentration

- ethyl benzene
- xylenes
- styrene
- isopropylbenzene
- n-propylbenzene
- methyl benzenes
- trimethyl benzenes
- butyl benzenes
- diethyl benzenes
- ethyl naphthalenes
- indans
- triethyl benzenes
- dimethyl naphthalenes
- biphenyl
- acenaphthylene
- acenaphthene

Note: Adapted from Smith et al. (1999a), Appendix G.

90-DAY ORAL GAVAGE STUDY OF EC$_{28}$ - EC$_{16}$ AROMATIC FRACTION

The objective of this study was to characterize the potential toxic effects elicited by the daily oral administration of EC$_{28}$ - EC$_{16}$ aromatic fraction of Jet A in female rats and male mice for 90 days. The complete findings were reported by Smith et al. (1999a) and presented in part as a poster (Smith et al., 2000).

The study was conducted with female Sprague-Dawley CD Rats and male C57BL/6 mice. The female rat was chosen to avoid the known hypersensitivity of male rats exposed to hydrocarbons, resulting in α-2 microglobulin nephropathy (Alden, 1986). This nephropathy is not considered to be relevant to human health effects (Flamm and Lehman-McKeeman, 1991). The male mouse was chosen to extend the study to a second species and also screened for sex-specific interactions with the test material. The Sprague-Dawley and C57BL/6 strains were selected because of the extensive experience with petroleum fuel studies completed by the U.S. Air Force Research Laboratory (currently AFRL/HEPB, Wright Patterson Air Force Base, OH) from 1973 to present.

Materials and Methods

Dosing concentrations were designated after a 7-day oral range-finding study (Smith et al., 1999b). EC$_{28}$ - EC$_{16}$ aromatic fraction of Jet A was formulated for daily oral gavage administration at concentrations of 0, 2, 10 and 50 mg/mL in corn oil (Mazola, Ltd., Cordova, TN) for mice and 0, 8, 40 and 200 mg/mL in corn oil for rats. Formulated doses and carrier control dose were stored at -5 to 4°C, and used within 15 days of preparation. The EC$_{28}$ - EC$_{16}$ aromatic fraction of Jet A concentration of the dosing solutions was determined by a gas chromatographic method.

Sixty male C57BL/6 mice and 60 female Sprague-Dawley (CD) rats from Charles River Laboratories, Portage, MI, were used for this study. All rats were approximately five weeks of age at receipt and approximately seven weeks of age at the initiation of dosing. All mice were approximately seven weeks at receipt and approximately nine weeks of age at the start of dosing. Body weights at the first dosing ranged from 140.5 to 179.2 g for the rats and from 22.0 to 25.8 g for the mice. Routine quarantine and serological testing procedures were observed upon receipt of the animals.
The rats were individually housed in polycarbonate cages while mice were housed two to a cage during quarantine and then individually during the acclimation and study period. The environmental conditions of the animal room provided 12 hour light/12 hour dark cycles, room temperature and relative humidity from 64 to 79°F and 30 to 70 percent, respectively, and fresh air at a minimal rate of ten changes per hour. All animals were identified by cage card throughout the quarantine period and by tattoo following randomization and assignment of a unique study number. Animals were assigned to four treatment groups per species, each comprised of fifteen animals. The animals were randomized to treatment groups using the Xybion PATH/TOX System, Cedar Knolls, NJ, assuring homogeneity of mean body weights across all groups. Dose volumes were based on the most recent body weights, which were recorded weekly.

Each animal was allowed ad libitum access to Certified Rodent Lab Diet® 5002 (PMI Feeds, Inc.) during quarantine and study periods. Water was provided ad libitum via an automatic watering system using a water source which conformed to EPA drinking water standards.

Each animal was observed approximately one to two hours following dosing and a second time at least six hours after dosing each day for overt signs of toxic or pharmacologic effect and change in general behavior and appearance during the study. Body weights were recorded at time of randomized group assignment, prior to initiation of dosing (Day 1), weekly during the study and prior to necropsy (Day 91). Weekly total food consumption measurements were determined for all animals by measuring full and empty feeder weights.

Clinical pathology evaluations were performed for each animal. Rats were fasted overnight (water remained available) for scheduled clinical pathology evaluations conducted on Day 91. Animals were anesthetized using a mixture of carbon dioxide/oxygen during the blood collection procedure. Blood samples were collected in tubes both with and without anticoagulant (EDTA) for hematology and serum chemistry analyses, respectively. Blood for hematology evaluation was collected via cardiac puncture. Coagulation parameters (prothrombin time and activated partial thromboplastin time) and methemoglobin were evaluated only for the rats, due to the limited blood volume available from mice. Blood collected for coagulation parameters were collected into tubes containing sodium citrate.

Complete necropsies were performed on all rats and mice. Selected organs were weighed and preserved at the time of necropsy. Histopathological evaluations of tissues from rodents were performed at the Air Force Research Laboratory.

All appropriate quantitative in-life, clinical pathology and postmortem data were analyzed for test substance effects by analysis of variance. Statistical significance for each comparison was reported at the 0.05 level. For data whose variances were considered homogeneous across test groups, as determined by Bartlett’s test for homogeneity at the p<0.05 level, tests for differences between the control and comparison groups were made using Dunnett’s test. For nonhomogeneous data, as determined by Bartlett’s test for homogeneity at the 0.05 level, tests for pairwise differences between the control and each of the comparison groups were made using Cochran and Cox’s modified two-sample t-test.
Results

The doses administered to each animal were within 10% of the EC$_{20}$ - EC$_{16}$ aromatic fraction of Jet A target concentrations: 20, 100 and 500 mg/kg/day, based on the individual animal's most recent bodyweight. The test substance was verified to be stable for the duration of the study (Smith et al., 1999a).

One mouse was euthanized in a moribund condition and one rat died prior to scheduled necropsy from the high dose groups (500 mg/kg) on Study Days 8 and 41, respectively; both are believed to be a result of gavage error. All remaining rodents survived until scheduled termination.

Clinical observations of mice included hunched posture and lethargy. The correlation between dose level and incidence of these effects suggests a test substance effect. Rough coat was observed in all control and treated mice, suggesting it was due to a vehicle effect. Clinical observations of the rats included lethargy. The reduced activity corresponds to the finding in the mice and is considered a test substance effect. Irritation from the test substance also caused short-lived (10 to 20 minutes after dosing) shoveling behavior (where the animal pushed bedding material with its nose) and excessive salivation after dosing.

No biological or statistical differences in group mean body weight values were identified for any treated groups in either the rats or mice. There was a general trend, with some statistically significant points, for the groups of rats treated with test substance to have greater food consumption than their control group. For the mice, the high dose group had a trend of decreased food consumption, again with some weeks being significantly diminished, compared with their controls. All other food consumption values for the treated groups of mice were similar to concurrent controls.

Mean hemoglobin, hematocrit and red blood cell counts (HGB/HCT/RBC) were minimally decreased in the mid- and high-dose female rats. Based on the dose-dependency and frequent statistical significance of these decreases, they were interpreted to be treatment-related. HGB/HCT/RBC results of the low-dose female rats and all three treated groups of male mice were similar to controls. Other alterations of hematologic parameters, sometimes statistically significant, were noted in treated groups, but were interpreted to be unrelated to treatment because they involved small (acceptable relative to expected variation) differences from control values, such as increased mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCHC) in high-dose male mice, and decreased MCHC in high-dose female rats. Other alterations involved small but noticeable changes from control in all dose groups, but the absence of a dose-dependent pattern led to the conclusion that they were not treatment-related, for example the decreased platelet counts in all treated groups of male mice.

There was a treatment-related pattern of statistically significantly reduced activated partial thromboplastin times for all test article-treated rats, but these values all fell within the normal range seen in historical control data. Further, decreased clotting time is not normally considered a toxic change (Smith et al., 1999a). Therefore the decreases in the activated partial thromboplastin times are not considered toxicologically significant.

None of the clinical chemistry results were interpreted to indicate any treatment effects. At necropsy, the livers from 13/15 of the 500 mg/kg/day rats were visibly enlarged. Organ weight measurements revealed highly significant liver weight increases (measured as absolute
weights, as liver relative to brain weight, and liver relative to body weight) in the 500 mg/kg/day rats compared with their controls. The 500 mg/kg/day mice had increased liver weights, but this increase was not statistically significantly different from their control group.

The 500 mg/kg/day rats had statistically larger kidney-to-body weight and kidney-to-brain weight ratios. The absolute kidney weights were larger than the controls, but not significantly different. Since there are no corresponding kidney lesions nor significant changes in clinical pathology parameters normally related to changes in renal function, the elevated relative kidney weights do not seem to have any toxicologic relevance. There were no other organ weight differences between treated and control groups of either rats or mice.

Several microscopic lesions in the various tissues of animals examined were revealed by histopathological examination. These lesions were not attributed to a test substance effect. Although livers among the high dose rats were visibly enlarged, corresponding lesions were not observed by microscopic examination.

**Discussion and Conclusions**

The data generated following daily oral gavage administration of EC$_8$ - EC$_{16}$ aromatic fraction of Jet A at dosages of 20, 100 and 500 mg/kg/day to female Sprague-Dawley (CD) rats and male C57BL/6 mice can be summarized into key points. The EC$_8$ - EC$_{16}$ aromatic fraction of Jet A administration caused significantly increased mean food consumption in the 500 mg/kg/day rat group. There were also sporadic decreased mean food consumption values for the 500 mg/kg/day male mice, which were occasionally statistically significant. It is not clear why treatment with the test substance would increase the food consumed by rats, but decrease the food consumed by mice, especially since there were no substantial changes in body weights over the course of the study for either species.

Liver weights (absolute and relative to body and brain weights) were significantly increased in the high dose group (500 mg/kg/day) of the rats. The liver weights of the high dose (500 mg/kg/day) mice were also larger than controls, but this difference was not statistically significant. Thirteen of the fourteen high dose (500 mg/kg/day) rats that survived to study completion were observed to have enlarged livers. Enlargement of the livers was likely due to increased metabolic enzymes to process the increased body burden of hydrocarbons. Similarly, the increase in relative kidney weights was probably due to increased handling of hydrocarbons by the kidneys. Administration of EC$_8$ - EC$_{16}$ aromatic fraction of Jet A did not induce any other macroscopic changes in any tissue examined during necropsy at Day 91. There were no microscopic lesions attributed to test substance effect in collected tissues from the 500 mg/kg/day mice and rats. Tissues from the lower dose groups were not examined due to the absence of lesions in the high dose group.

Clinical observations included lethargy in the high dose (500 mg/kg/day) groups of both species as well as the low (20 mg/kg/day) and mid (100 mg/kg/day) groups of mice. Hunched posture was observed in all the treated groups of mice. In addition, the high dose (500 mg/kg/day) and mid dose (100 mg/kg/day) rats were observed to shovel their bedding around the cage and salivate excessively after dosing. These last two observations were likely due to oral irritation caused by the test substance.

Mean hemoglobin, hematocrit and red blood cell counts (HGB/HCT/RBC) were minimally decreased in the mid- and high-dose female rats (100 and 500 mg/kg/day, respectively). Based
on the dose-dependency and frequent statistical significance of these decreases, they were interpreted to be treatment-related.

In conclusion, daily oral administration of up to 20 mg/kg/day EC₈ - EC₁₆ aromatic fraction of Jet A was well-tolerated by female Sprague-Dawley (CD) rats and male C57BL/6 mice during a ninety day period. Doses of 500 mg/kg/day produced increased liver and kidney weights in the rats; hemoglobin, hematocrit and red blood cell counts were decreased in the rats; clinical signs of lethargy in both species; hunched posture in the mice; and shoveling and salivation in the rats. Doses of 100 mg/kg resulted in decreased hemoglobin, hematocrit and red blood cell counts in the rats, hunched posture and lethargy in the mice and shoveling and salivation in the rats. Based on these findings, the no-observed adverse effect level (NOAEL) of EC₈ - EC₁₆ aromatic fraction of Jet A at dosages of 0, 20, 100 and 500 mg/kg/day was 20 mg/kg/day.

DEVELOPMENT OF A PROVISIONAL REFERENCE DOSE

The TPHCWG identified 55 chemicals in the EC₈ - EC₁₆ aromatic range. Available current toxicity information for components of this fraction is summarized in Table 4. One study of a naphthalene/methylnaphthalene mixture was used in part for the original provisional RfD by the TPHCWG in 1997 (Edwards et al., 1997). This study is unpublished and the conditions of the study and details of the observations could not be verified during this review. However, the resulting reference value from the study (0.03 mg/kg/day) is similar to the naphthalene RfD (0.02 mg/kg/day) released by U.S. EPA in 1998.

Table 4. Summary of Critical Effects and IRIS RfDs for Components of the EC₈ - EC₁₆ Aromatic Fraction for which Toxicity Data are Available

<table>
<thead>
<tr>
<th>Compound</th>
<th>Critical Effect</th>
<th>POD (mg/kg/day)</th>
<th>UF</th>
<th>Current IRIS RfD (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylbenzene</td>
<td>liver &amp; kidney toxicity</td>
<td>97.1</td>
<td>1000</td>
<td>0.1</td>
</tr>
<tr>
<td>xylenes</td>
<td>↓ BW, ↑ mortality</td>
<td>179</td>
<td>1000</td>
<td>0.2</td>
</tr>
<tr>
<td>styrene</td>
<td>RBC &amp; liver effects</td>
<td>200</td>
<td>1000</td>
<td>0.2</td>
</tr>
<tr>
<td>isopropylbenzene</td>
<td>↑ kidney weight</td>
<td>110</td>
<td>1000</td>
<td>0.1</td>
</tr>
<tr>
<td>naphthalene</td>
<td>↓ BW</td>
<td>71</td>
<td>3000</td>
<td>0.02</td>
</tr>
<tr>
<td>naphthalene/methyl naphthalenes mixture</td>
<td>liver &amp; thyroid toxicity</td>
<td>300</td>
<td>10000</td>
<td>0.03</td>
</tr>
<tr>
<td>biphenyl</td>
<td>kidney toxicity</td>
<td>50</td>
<td>1000</td>
<td>0.05</td>
</tr>
<tr>
<td>acenaphthene</td>
<td>liver toxicity</td>
<td>175</td>
<td>3000</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Notes: BW = body weight. POD = point of departure = NOAEL or LOAEL, conversions (% diet) or adjustments (5 days to 7 days/week) included. RBC = red blood cell. UF = total uncertainty factor, including modifying factors when applied. aU.S. EPA (2004). bThis unpublished study was used to estimate the original EC₈ - EC₁₆ fraction provisional RfD by the TPHCWG (Edwards et al., 1997).

An updated provisional RfD using the 90-day fraction study was derived using an approach similar to that described in TPHCWG Volume 4 to allow for a comparison with the TPHCWG results. The approach applied uncertainty factors to NOAELs or LOAELs from the critical
studies. The TPHCWG primarily accepted default values of 10 for assignment of uncertainty factors (UFs) (Edwards et al., 1997). The U.S. EPA states that a value of 3 may be used for a half-power uncertainty factor, with the exact value of the UF chosen dependent on the quality of the studies available, the extent of the database and scientific judgment (U.S. EPA, 2002a).

Using the 90-day study, the point of departure was the NOAEL of 20 mg/kg/day, with the critical effect being hematological changes. Application of several UFs is appropriate: a 10-fold factor to account for sensitive members of the human population and a 10-fold factor for extrapolating from experimental animals to humans.

To extrapolate from subchronic to chronic exposure, uncertainty factors of 3 or 10 have been proposed for other chemicals in the fraction. The 1998 Naphthalene Toxicological Review reports that for the critical 90-day study, a UF of 10 is appropriate (U.S. EPA, 1998). The 1997 Cumene Toxicological Review reports a UF of 3 for the six-month critical study (U.S. EPA, 1997). Clark et al. (1989) evaluated a high flash point aromatic naphtha mixture in a 12-month inhalation study. Results were similar to those from the Jet A aromatic fraction; increased liver weights without accompanying histopathological changes were observed, along with significant but transient hematological changes. The authors selected the highest exposure level, 1800 mg/m$^3$, as the NOAEL. The test material was almost 54% trimethyl benzenes, while the material for this study was 31% alkylated benzenes, indicating some similarity of the test materials. However the material in the Clark study and the fraction used in this study, even though similar in compound classes present, are still different mixtures.

Of the complex mixtures used as jet fuels, JP-4 has the highest aromatic content and therefore is the most similar fuel to the fraction in this study. JP-4 contains 21 to 25% aromatics, including benzene (Chubb et al., 1995; Kinkead et al., 1995). JP-4 has been studied in a 12-month intermittent (6 hours/day, 5 days/week) inhalation study to determine carcinogenic potential. In addition to no conclusive neoplastic effects, non-tumorigenic effects were limited to dose dependent decreased body weights following a 12-month recovery period in male and female F344 rats from both exposure groups (1000 and 5000 mg/m$^3$). Hematological and blood chemistry changes (decreased leukocytes and blood glucose) also occurred immediately following the 12-month exposure period (Bruner et al., 1993). Significantly decreased bodyweights occurred in F344 rats after 90-days continuous exposure to 1000 mg/m$^3$ but hematological parameters remained normal over this time period at exposures of 500 and 1000 mg/m$^3$ (Kinkead et al., 1995). F344 rats exposed dermally to 100 μL JP-4 5 days/week for 6 months resulted in decreased bodyweight and leukocyte counts (Chubb et al., 1995). JP-4 represents a broader mixture than the material used in this study. However, results from longer studies with JP-4 indicate a likelihood of toxic effects from the aromatic fraction remaining similar over chronic exposures. Based on weight of evidence for longer term studies of similar mixtures, an uncertainty factor of 3 may be appropriate for extrapolating from subchronic to chronic doses for the EC$_{8}$ - EC$_{16}$ aromatic fraction as the lack of chronic study is not considered likely to reduce the NOAEL by more than a factor of 3.

Therefore, a comparable provisional RfD update using the fraction study would include a total uncertainty factor of 300. When applied to the NOAEL of 20 mg/kg/day, the provisional RfD would be 0.07 mg/kg/day. This is similar to the previously calculated reference value for a naphthalene/methyl naphthalenes mixture (Edwards et al., 1997). The MA DEP provisional RfD for their similar fraction (C$_{9}$ - C$_{16}$ aromatic) is also close, 0.03 mg/kg/day (MA DEP, 2003).

However, a fourth UF should also be considered for database deficiencies, although this factor was not applied when the fraction provisional RfDs were first calculated in 1997. This is the first
study for this mixture and not all toxic impacts were evaluated. Both the cumene and naphthalene reviews include a UF of 3 to account for database deficiencies (U.S. EPA, 1997; 1998). Weighing these factors, an uncertainty factor of 3 is appropriate for the neurological and reproductive-developmental effects database deficiency. The total uncertainty for the study would therefore be 1000 and, when applied to the 20 mg/kg/day NOAEL, results in a reference value of 0.02. The provisional RfD from the fraction study is equal to the current IRIS RfD for naphthalene (U.S. EPA, 1998); this is the value is being recommended by the U.S. EPA for TPH contaminated Superfund sites (U.S. EPA, 2002b).

Among the numerous additional uncertainties that exist, the aliphatic portion of the test material can be considered both a strength and a weakness of the study. The presence of saturated (aliphatic) in the test article may have impacted the observed toxicity from the possible effects of a "purely aromatic" material. Mixture effects (synergism or antagonism) may have occurred that would not have been present in a purely aromatic faction. However, the presence of these non-aromatic compounds is due to the complex composition of petroleum products and represents a realistic strength of the study. The toxicity of the fraction that people could potentially be exposed to in the environment has been quantified, rather than assessing the fraction toxicity based on only individual aromatic compounds.

### Table 5. Consensus for EC>8 - EC<16 Aromatic Fraction Provisional RfD

<table>
<thead>
<tr>
<th>Compound</th>
<th>Critical Effect</th>
<th>POD (mg/kg/day)</th>
<th>UF</th>
<th>RfD (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPHCWG EC&gt;8 - EC&lt;16 aromatic fraction provisional RfD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>multiple RfDs for fraction constituents at 0.03 and 0.04 mg/kg/day</td>
<td>NA</td>
<td>NA</td>
<td>0.04</td>
</tr>
<tr>
<td>MA DEP C&lt;sub&gt;9&lt;/sub&gt; - C&lt;sub&gt;16&lt;/sub&gt; aromatic fraction provisional RfD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>based on IRIS pyrene RfD: kidney effects</td>
<td>75</td>
<td>3000</td>
<td>0.03</td>
</tr>
<tr>
<td>naphthalene/methyl naphthalenes mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>liver &amp; thyroid toxicity</td>
<td>300</td>
<td>10000</td>
<td>0.03</td>
</tr>
<tr>
<td>U.S. EPA PPRTV for EC&gt;9 - EC&lt;16 aromatic fraction&lt;sup&gt;d&lt;/sup&gt;</td>
<td>based on IRIS naphthalene RfD: ↓ bodyweight</td>
<td>71</td>
<td>3000</td>
<td>0.02</td>
</tr>
<tr>
<td>aromatic fraction study updated provisional RfD</td>
<td>hematological changes</td>
<td>20</td>
<td>1000</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Notes: POD = point of departure = NOAEL or LOAEL, conversions (% diet) or adjustments (5 days to 7 days/week) included. PPRTV = Provisional Peer Reviewed Toxicity Value, approved by U.S. EPA for use at Superfund sites when IRIS values are not available. UF = total uncertainty factor, including modifying factors when applied. <sup>a</sup>Edwards <i>et al.</i> (1997). <sup>b</sup>MA DEP (2003). <sup>c</sup>This unpublished study was used to estimate the original EC>8 - EC<16 fraction provisional RfD by the TPHCWG (Edwards <i>et al.</i>, 1997). <sup>d</sup>U.S. EPA (2002b).

In developing this updated provisional RfD, we began with a NOAEL and applied typical uncertainty factors. Our approach is comparable to that used previously for TPH fractions (Edwards <i>et al.</i>, 1997; MA DEP, 2003). Using the consensus of reference values (Table 5) applicable to the mixture that is the EC>8 - EC<16 aromatic fraction, we recommend the provisional RfD for this fraction be lowered from 0.04 mg/kg/day (Edwards <i>et al.</i>, 1997) to 0.02 mg/kg/day. As seen in Table 5, this is also in accord with the U.S. EPA's 2002 recommendations for TPH contamination at Superfund sites. Provisional Peer Reviewed
Toxicity Values (PPRTVs) are applied for cleanup level derivation at Superfund hazardous waste sites when IRIS values are not available (U.S. EPA, 2003). The U.S. EPA recommends using the naphthalene RfD as the PPRTV for the \( EC_{9} - EC_{16} \) aromatic fraction (U.S. EPA, 2002b).

**IMPACT OF PROVISIONAL RfD ON JP-8 CONTAMINATED SOIL RISK BASED SCREENING LEVELS**

Table 6 lists the TPH risk based screening levels (RBSLs) for fresh JP-8 jet fuel, assuming residential exposure scenarios. The RBSLs were calculated using exposure pathway specific equations for determining soil cleanup levels, as described in TPHCWG Volume 5 (Vorhees et al., 2000). These equations are based on the American Society for Testing and Materials (ASTM) Risk Based Corrective Action (RBCA) standard (ASTM, 1995). The same calculations were completed for JP-8 using the original TPHCWG provisional Rfd of 0.04 for the \( EC_{8} - EC_{16} \) fraction, the current MA DEP \( C_{9} - C_{16} \) provisional Rfd and the updated provisional Rfd value of 0.02 for the \( EC_{8} - EC_{16} \) fraction based on the aromatic fraction study. Since these Rfds are all the same magnitude and very similar in value, TPH RBSLs do not change over common residential exposure pathways. Pathway specific RBSLs, and therefore potential site cleanup levels, remain the same regardless of whether the original TPHCWG provisional Rfd or the updated provisional Rfd is used.

Table 6. Comparison of Residential Pathway-Specific Soil TPH RBSLs using Current and Proposed RfDs for the \( EC_{8} - EC_{16} \) Aromatic Fraction

<table>
<thead>
<tr>
<th>RBSL Based On...</th>
<th>RBSL for Residential Exposure Pathway...</th>
<th>Direct Contact with Surface Soil*</th>
<th>Leaching to Groundwater</th>
</tr>
</thead>
<tbody>
<tr>
<td>original TPHCWG ( EC_{8} - EC_{16} ) aromatic provisional Rfd: 0.04 mg/kg/day</td>
<td>5000 mg/kg</td>
<td>600 mg/kg</td>
<td></td>
</tr>
<tr>
<td>current MA DEP ( C_{9} - C_{16} ) aromatic provisional Rfd: 0.03 mg/kg/day</td>
<td>5000 mg/kg</td>
<td>600 mg/kg</td>
<td></td>
</tr>
<tr>
<td>current U.S. EPA PPRTV ( EC_{9} - EC_{16} ) aromatic reference value: 0.02 mg/kg/day</td>
<td>5000 mg/kg</td>
<td>600 mg/kg</td>
<td></td>
</tr>
<tr>
<td>aromatic fraction study updated provisional Rfd: 0.02 mg/kg/day</td>
<td>5000 mg/kg</td>
<td>600 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

Note: *The "direct contact with surface soil" exposure pathway combines four exposure pathways: soil ingestion, dermal exposure to soil and inhalation of soil vapor and fugitive dust.

**CONCLUSIONS AND ADDITIONAL WORK NEEDED**

This study fills an important data gap in our understanding of petroleum toxicity. It supports the overall TPHCWG approach of assigning toxicity criteria to the fractions and reduces the uncertainty (in one fraction) of evaluating a complex mixture through the toxicity of individual chemical compounds. However, additional research is warranted to further reduce uncertainty associated with the provisional Rfd for this fraction and other fractions with few data to support toxicity values. Specifically, a study similar to that presented in this paper with the \( EC_{16} - EC_{35} \)
aromatic fraction should be a priority. Reproductive/developmental studies on both of these aromatic fractions would further reduce the uncertainty of their provisional RfDs.

Aside from additional studies, risk-based evaluations of petroleum fractions could be further improved with a more rigorous quantitative analysis of UF s and possibly benchmark dose derivation utilizing dose-response data from all fraction mixtures and constituents. A preliminary database has been assembled (Baird et al., 2002), but the project would require additional funding to complete the data derived UF.

REFERENCES


MA DEP. 2003. *Updated petroleum hydrocarbon fraction toxicity values for the VPH/EPH/APH methodology.* Massachusetts Department of Environmental Protection, Office of Research and Standards, Boston, MA.