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August 10, 2004

Dear Interested Parties,

In 1994 the Massachusetts Department of Environmental Protection described a new approach for the evaluation of human health risks from ingestion exposures to complex petroleum hydrocarbon mixtures. The basis of the new approach was treating groups of compounds as if they had similar toxicities, in the absence of specific toxicity information on all the members of the group. The report included oral toxicity values for each of the designated petroleum hydrocarbon fractions. Combined with new analytical methods developed by the Department, this work became the basis for the successful VPH/EPH approach to assess petroleum hydrocarbon contamination at sites in Massachusetts.

Since those pioneering efforts, significant work has been done by DEP and others on this topic and additional information has become available to serve as a basis for updating the toxicity values. DEP is pleased to publish a revision to the original document, "*Updated Petroleum Hydrocarbon Fraction Toxicity Values for the VPH/EPH/APH Methodology*" (November, 2003). This document contains reviews of the more recent information, revisions to the oral toxicity values proposed in the 1994 report and new inhalation toxicity values.

DEP has incorporated this information into the upcoming revisions to the MCP Method 1 Standards for groundwater and soil. A public hearing draft of the standards is expected later this Fall. The Department would expect these updated values to be considered in Method 3 risk assessments submitted after January 1, 2005.

This document and further information on the development and application of the aliphatic/aromatic hydrocarbon evaluative technique employed by the Department, referred to as the "VPH/EPH" approach, may be obtained at http://www.state.ma.us/dep/bwsc/vph_eph.htm

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FINAL

UPDATED PETROLEUM HYDROCARBON FRACTION TOXICITY VALUES FOR THE VPH/EPH/APH METHODOLOGY

Prepared for:

Bureau of Waste Site Cleanup
Massachusetts Department of Environmental Protection
Boston, MA

Prepared by:

Office of Research and Standards
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November 2003

PREFACE

In 1994 the Massachusetts Department of Environmental Protection (MA DEP) described a new approach for the evaluation of human health risks from ingestion exposures to complex petroleum hydrocarbon mixtures (MA DEP, 1994). The basis of the new approach was treating groups of compounds as if they had similar toxicities, in the absence of specific toxicity information on all the members of the group. The report included oral toxicity values for each of the designated petroleum hydrocarbon fractions. Since that time, there have been more recent efforts by others on this topic and additional information has become available to serve as a basis for updating the toxicity values. This document contains reviews of the more recent information, revisions to the oral toxicity values proposed in the 1994 report and new inhalation toxicity values.

Readers will find different hydrocarbon compound size cutoffs for the hydrocarbon ranges identified here and in the 1994 report, compared to those in other related supporting documentation for this approach (i.e., the MA DEP VPH/EPH/APH analytical methods; guidance for Characterizing Risks Posed by Petroleum Contaminated Sites (MA DEP, 2002); risk spreadsheets for the MA DEP Bureau of Waste Site Cleanup). These differences relate primarily to subdivisions or truncation of ranges mandated by the analytical protocols employed for the analysis of the hydrocarbons (volatile (VPH), extractable (EPH) and air-phase(APH)) .

Correspondence tables relating the toxicologically-derived hydrocarbon fractions and their toxicity values to the analytically-defined reporting fractions are contained in Tables Preface-1 (for ingestion exposures) and Preface-2 (for inhalation exposures) on the following pages. The Interim Final report (MA DEP, 1994) identified upper end size cutoffs for both the alkanes and aromatics of compounds with 32 carbon atoms (C_{32}). The analytical methods that were subsequently developed for the volatile (VPH) and extractable (EPH) fractions of petroleum hydrocarbon mixtures identified slightly different cutoffs based upon the limitations of the methods. The limit for alkanes extended to 36 carbon atoms (C_{36}), and that for aromatics was reduced to 22 carbon atoms (C_{22}). Data presently being reported to the Department reflect these upper end cutoffs. In order to avoid confusion with the information contained in the Interim Report, this report has continued to refer to the original upper end cutoffs, although the Waste Site Cleanup program is now using the analytically defined limits.

Toxicologically Based-Fractions	VPH/EPH Analytical Reporting Fractions	Aliphatics				Aromatics		
		VPH		EPH		VPH		EPH
		C_5-C_8	C_9-C_{12}	C_9-C_{18}	C_{19}-C_{36}	BTEX	C_9-C_{10}	C_{11}-C_{22}
Aliphatics	C_5-C_8	0.04						
	C_9-C_{18}		0.1	0.1				
	C_{19}-C_{36}				2.0			
Aromatics	B,T,E,X					BTEX tox. values		
	C_9-C_{32}						0.03	0.03

Table Preface-1. Chronic Oral Reference Dose (mg/kg/d) Correspondence Table Between Toxicologically-Based Petroleum Hydrocarbon Fractions Described in This Report and Analytically-Defined Petroleum Hydrocarbon Fractions Reported by Laboratories.

Toxicologically Based-Fractions	VPH/EPH/APH Analytical Reporting Fractions	Aliphatics				Aromatics		
		VPH/APH		EPH		VPH/APH		EPH
		C_5-C_8	C_9-C_{12}	C_9-C_{18}	C_{19}-C_{36}	BTEX	C_9-C_{10}	C_{11}-C_{22}
Aliphatics	C_5-C_8	0.2						
	C_9-C_{18}		0.2	0.2				
	C_{19}-C_{36}				NA			
Aromatics	B,T,E,X					BTEX tox. values		
	C_9-C_{32}						NA 0.05*	NA NA
	C_9-C_{18}							

NA – not applicable for inhalation exposures

* Some of the smaller compounds (e.g., C_{11}, C_{12}) in the C_{11}-C_{22} range may have limited volatility. The toxicity value to be used for them should be 0.05 mg/m^3. Recovery of $\geq C_{10}$ compounds from air samples has been problematic with the APH methodology.

Table Preface-2. Chronic Inhalation Reference Concentration (mg/m^3) Correspondence Table Between Toxicologically-Based Hydrocarbon Fractions Described in This Report and Analytically-Defined Petroleum Hydrocarbon Fractions Reported by Laboratories.

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- p. 54, under #2. Aliphatic C_9-C_{18} Section. Last sentence of section. Corrected typographical error of 0.02 mg/m^3 to 0.2 mg/m^3.

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EXECUTIVE SUMMARY

The Massachusetts Department of Environmental Protection (MA DEP) introduced in 1994 the concept of petroleum hydrocarbon size-based fractions for use in evaluating the human health effects of exposures to complex mixtures of hydrocarbons, and provided oral toxicity values for each of the fractions. The Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) independently identified largely similar groupings of hydrocarbon fractions with somewhat different toxicity values in 1997. They also identified inhalation toxicity values.

The studies used by the TPHCWG to derive their toxicity values were not previously available for independent review. They were obtained by MA DEP for this review. These studies plus more recent published literature were reviewed in the context of MA DEP's original toxicological evaluation to identify the most current and appropriate toxicity values for the hydrocarbon fractions for both oral and inhalation exposures. MA DEP's currently proposed values are contained in Table 1.

The toxicity of the C_5 – C_8 aliphatic fraction should continue to be driven by considerations for the potential neurotoxicity (peripheral neuropathy) from exposures to commercial hexanes and potential diketone metabolites of n-alkanes.

These toxicity values will be the updated toxicity values used as the basis for medium-specific cleanup standards in the state's hazardous waste site characterization and cleanup program and will serve as the appropriate fraction toxicity values in human-health risk assessments conducted under that and other state programs.

Table 1. Oral and Inhalation Toxicity Values for Petroleum Hydrocarbon Fractions

Exposure Route	Carbon Range	1994 MA DEP mg/kg/d	2002 MA DEP Recommended Values (mg/kg/d)
Oral	**Aliphatic**		
	C_5 - C_8	0.06	0.04
	C_9-C_{18}	0.6	0.1
	C_{19} - C_{32}	6.0	2.0
	Aromatic		
	C_6 - C_8	Evaluate each chemical in the series separately	Evaluate each chemical in the series separately
	C_9-C_{32}	0.03	0.03
			(mg/m^3)
Inhalation	**Aliphatic**		
	C_5 - C_8	-	0.2
	C_9-C_{18}	-	0.2
	C_{19} - C_{32}	-	NA*
	Aromatic		
	C_6 - C_8	-	Use individual RfCs for compounds in this range
	C_9-C_{18}	-	0.05
	C_{19}-C_{32}	-	NA

NA – not applicable to inhalation exposures since compounds not volatile.

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1.0 INTRODUCTION

A key component of the evaluation of petroleum contaminated waste sites is the assessment of potential human health risks from exposures to petroleum hydrocarbon compounds, usually present as mixtures. An improved method for the evaluation of health hazards posed by oral exposures to these complex mixtures was developed and described by MA DEP in 1994 (MA DEP, 1994), in Hutcheson et al. (1996) and integrated into MA DEP's Bureau of Waste Site Cleanup (BWSC) site characterization program. The method involves segregating the petroleum hydrocarbon compounds present in mixtures into broad chemical classes (alkane/cycloalkane, alkene and aromatics) and further into subgroups or fractions based upon their size (defined by number of carbons atoms in the compounds). These designations were made upon consideration of the nature and degree of comparative toxicity of compounds and structure activity relationship (SAR) considerations.

For each subgroup of compounds, a "reference compound" was initially identified to represent the toxicity of all compounds in the range. It was usually chosen because its toxicity was relatively well characterized. For each reference compound, a US EPA-published oral reference dose value (RfD) was identified or, for those "reference compounds" without US EPA published values, an oral dose-response value was identified based on available toxicity information. A document describing how this method is to be used within the framework of the state's hazardous chemical waste site cleanup program has also been developed (MA DEP, 2002).

Subsequent to the completion of the first phase of this work in 1994, a national ad hoc workgroup known as the TPH (Total Petroleum Hydrocarbons Criteria Working Group (TPHCWG), composed of representatives from the military, the oil and gas industry, the consulting community, academia and some regulatory agencies introduced its version of an approach (TPHCWG, 1997a,b) for evaluating both oral and inhalation exposures and deriving toxicity values for petroleum hydrocarbons (PHCs).

The oral and inhalation toxicity values for the hydrocarbon groups identified by the TPHCWG were based on data from mixtures of chemicals of sizes generally falling within the hydrocarbon ranges. The original supporting studies for the TPHCWG hydrocarbon fractions were not available for outside review at the time of release of the TPHCWG work products. These original documents were primarily contract laboratory technical reports and have since been provided to MA DEP for this review by the sponsoring organization. Additional toxicological information has also come available since both groups completed their work. The availability of this new information warrants a reevaluation of the toxicity values for oral and inhalation exposures to petroleum hydrocarbons.

This report:

1. Evaluates the existing MA DEP oral toxicity values and new literature and recommends MA DEP's preferred oral toxicity values, and

2. reviews the various available studies on TPH fractional mixtures and individual compounds, and recommends fractional RfCs for the various petroleum hydrocarbon subgroups to be used by MA DEP.

2.0 ASSESSMENT OF ORAL AND INHALATION TOXICITY INFORMATION

2.1 ALIPHATIC FRACTIONS TOXICITY VALUES

Inhaled or ingested volatile hydrocarbons have both general and specific effects. Many organic solvents, including petroleum hydrocarbons, have the potential on acute high-level vapor exposure to cause central nervous system (CNS) disturbances like disorientation, euphoria, giddiness, and confusion; progressing to unconsciousness, paralysis, convulsion and death from respiratory or cardiac arrest (Browning, 1965). These effects have been observed with aliphatic and aromatic compounds found within the C_5 - C_9 (aliphatics) and C_6 - C_{10} (aromatics) carbon ranges.

The acute narcotic effects of the volatile hydrocarbons result from direct chemical action. The similarity of CNS disruption produced by hydrocarbons of diverse structures suggest that these effects result from a common process which is physical interaction of the solvents with the cells of the CNS (Andrews and Snyder, 1991). For example, interaction of the lipid-soluble hydrocarbons with the synaptosomal membranes causes CNS toxicities. The potency of the CNS effects depends on the structure of the individual hydrocarbon molecule.

Other nonspecific effects of hydrocarbons are exhibited after prolonged exposure to these agents. The nonspecific effects observed in animals and humans are neurobehavioral toxicities. The neurobehavioral effects are manifested as sensory, cognitive, affective and motor abnormalities. There is some evidence suggesting that the mechanism of the behavioral effects is alterations in the utilization and turnover of biogenic amines in the brain. These effects occur at lower hydrocarbon concentrations than those producing morphological changes. Recent animal studies indicate that both aromatic (Korsak and Rydzynski, 1996; Gralewicz et al., 1997) and aliphatic (Lund et al., 1995) volatile hydrocarbons may cause nonspecific neurobehavioral toxicities with differing intensities depending on the structure of the hydrocarbon.

Distinct from the general CNS effects of hydrocarbons are their associated specific organ toxicities. Examples of such effects include the hematopoietic toxicity of benzene and the neurodegenerative toxicity of n-hexane. The specific toxicities of hydrocarbons may be directly related to their metabolites as is the case with benzene and n-hexane (Andrews and Snyder, 1991).

2.1.1 C_5 - C_8 Aliphatic Fraction – Oral RfD

2.1.1.1 Basis for Existing Toxicity Values. In the MA DEP methodology, n-hexane was selected as a representative reference compound for the toxicity of aliphatic hydrocarbons containing 5 to 8 carbon atoms since its toxicity was well characterized. Other compounds which occur in this group (n-pentane, n-heptane and n-octane) were hypothesized to be structurally predisposed to cause peripheral neuropathy like that produced by n-hexane, but to a lesser extent. Both n-hexane and n-heptane are metabolized to γ-diketone metabolites with 2,5-gamma spacing. When a series of 2,5-hexanedione analogues were tested, only those with 2,5-gamma spacing caused peripheral neurotoxicity (St Clair et al., 1988). Representation of the potential toxicities of other compounds in this range by the toxicity of n-hexane has been criticized by some as overly conservative because they interpret the peripheral neuropathy seen with n-hexane to be unique to that compound. Faced with some uncertainty about the toxicities of these compounds, MA DEP chose in 1994 to adopt a more health protective approach and retain the RfD of 0.06 mg/kg/d derived from a gavage study of n-hexane as a toxicity surrogate for this fraction.

In order to update the 1994 MA DEP oral toxicity value for the $C_5 - C_8$ aliphatic fraction, API-sponsored studies on hexane isomer mixtures and current toxicologic studies identified in the open literature on mixtures or individual components of the fraction were reviewed. The available studies are discussed below.

1. Mixtures

Animal Studies

Animal studies were performed with commercial hexane (CH) which was composed of 53% n-hexane. The other constituents included 3-methylpentane, methylcyclopentane, 2-methylpentane, cyclohexane, 2,3-dimethylbutane, and <1% of several minor compounds. Subchronic, chronic, and developmental/reproductive inhalation studies of CH mixtures demonstrated no peripheral nerve damage, and no CNS, reproductive or developmental toxicities in rats and mice. The studies are discussed below and summarized in Table 2.

Subchronic Inhalation Neurotoxicity Study of Commercial Hexane in Rats (API, 1990a). Rats were exposed to CH vapors at 0, 900, 3,000 and 9,000 ppm, (0, 3,092, 10,307, 30,921 mg/m^3) for 6 hours/day, 5 days/week for 13 weeks. Treatment with commercial hexane at concentrations of up to 30,921 mg/m^3 (9,000 ppm) for 13 weeks had no effect on mortality, clinical condition, body weight, food consumption or gross pathology. There were no effects upon the behavioral parameters assessed as a functional observational battery and motor activity test. Neuropathological evaluations revealed no effects of treatment. The NOAEL in this study was reported to be 9,000 ppm (30,921 mg/m^3).

Subchronic Inhalation Toxicity of Commercial Hexane in Rats and Mice (API, 1990b). Fischer 344 rats and B6C3F1 mice were exposed to 0, 900, 3,000 or 9,000 ppm (0, 3,092, 10,307, 30,921 mg/m^3) commercial hexane vapor for 6 hours/day, 5 days/week for 13 weeks. A transient exposure-related excess lacrimation in both sexes of mice and female rats was observed, however no signs of exposure-related ocular diseases were observed. Clinical chemistry tests showed changes in the male rats in the high exposure group including increased platelets, creatinine, total protein and albumin, and a decrease in chloride levels. Absolute and relative liver weights were also increased in both species in the high concentration group except for the female rats. The kidney and the adrenal organ weight to body weight and organ weight to brain weight, ratios were significantly increased in the male and female rats exposed to 9,000 ppm. These results were not observed in mice. Hemorrhage in the liver (high level only) and acute/subacute inflammation in the liver (high level only) and kidney (mid and high levels) were observed in male rats. No microscopic effects were seen in the mice. Based on these data, the NOAEL for commercial hexane in both species was 3,000 ppm (10,307 mg/m^3).

Two Generation Reproduction Study of Inhaled Commercial Hexane in Rats (API, 1990c). Sprague-Dawley rats were exposed for 6 hours/day, 5 days/week to commercial hexane vapor at 0, 900, 3,000 or 9,000 ppm (0, 3,092, 10,307, 30,921 mg/m^3) for two generations, one litter per generation. A consistent pattern of adult toxicity at 9,000 ppm, evidenced by reduced body weights in F_1 males and females (but not F_0 males or females) was observed. Reproductive parameters were not affected in both the F_1 and F_2 generations. F_1 litters exhibited reduced body weight on lactational days 14 and 21 at 9,000 ppm. The F_2 generation of pups exhibited reduced body weights from lactational day 7 to weaning on day 28 at 9,000 ppm (30,921 mg/m^3). The NOAEL for general toxicity in adults and offspring in this study was 3,000 ppm (10,307 mg/m^3). The NOAEL for reproductive toxicity was at least 9,000 ppm (30,921 mg/m^3).

Developmental Toxicity of Commercial Hexane Vapor in Rats (API, 1989a). Sprague-Dawley rats were exposed to commercial hexane vapor for six hours per day on gestational days 6 through 15 at concentrations of 0, 900, 3,000 or 9,000 ppm (0, 3,092, 10,307, 30,921 mg/m^3). Maternal effects were observed at 3,000 and 9,000 ppm. Maternal toxicity at 9,000 ppm included significant weight reduction and treatment-related color changes in the lung at necropsy. At 3,000 ppm, body weight gain was reduced for gestation days 9 through 12. No developmental toxicity was observed at any of the concentrations. The NOAELs for maternal and developmental toxicity were 900 and 9,000 ppm respectively.

Table 2. API Sponsored Subchronic, Chronic, Reproductive and Developmental Inhalation Studies

Duration	Species	Study Design	Findings	NOAEL	Reference
Subchronic (Neurotoxicity)	Rat	0, 900, 3,000, or 9,000 ppm 6 hours/day/ 5 days/week for 13 weeks	No neurobehavioral or neuropathologic effects	9,000 ppm	API, 1990a
Subchronic (Systemic)	Mouse	0, 900, 3,000, or 9,000 ppm 6 hours/day, 5 days/week for 13 weeks	Transient exposure-related excess lacrimation, increases in absolute and relative liver weights in male and female mice at 9,000 ppm	3,000 ppm	API, 1990b
Subchronic (Systemic)	Rat	0, 900, 3,000, or 9,000 ppm 6 hours/day/ 5 days/week for 13 weeks	Changes in clinical chemistry, significant increases in relative kidney and adrenal weights in male rats at 9,000 ppm; significantly increased relative adrenal weights in female rats at 9,000 ppm; significantly increased liver weights at 9,000 ppm in male rats and an upward trend in female rats; hemorrhage and acute/subacute inflammation of the liver in male rats at 9,000 ppm; nephropathy in male rats at 3,000 and 9,000 ppm;	3,000 ppm	API, 1990b
Chronic (Oncogenicity)	Rat	0, 900, 3,000, or 9,000 ppm 6 hours/day, 5 days/week for 2 years	Histologic evidence of mucosal irritation in nasal turbinates and larynx No neoplastic effects	No NOAEL identified for nasoturbinal effects	API, 1995
Chronic (Oncogenicity)	Mouse	0, 900, 3,000, or 9,000 ppm 6 hours/day, 5 days/week for 2 years	Liver tumor in female mice at 9,000 ppm		API 1995
Reproductive.	Rat	0, 900, 3,000, or 9,000 ppm 6 hours/day, 5 days/week, for two generations	Reduced body weight in both F_1 and F_2 generations at 9,000 ppm.	3,000	API, 1990c
Developmental	Rat	0, 900, 3,000, 9,000 ppm 6 hours/day on days 6 – 15 of gestation	Maternal effects were noted at 3,000 and 9,000 ppm. Significant weight reduction and treatment-related changes in the lung at 9,000 ppm. Weight reduction at 3,000 ppm.	900 ppm (maternal) 9,000 ppm (develop.)	API, 1989a
Developmental	Mouse	0, 900, 3,000, 9,000 ppm 6 hr/day on days 6 – 15 of gestation	Slight maternal toxicity at 3,000 and 9,000 ppm. Significant increases in the incidence of color changes in the lungs and some increases in the frequency of dark brown foci in the lungs at 9,000 ppm. Some color changes and dark brown foci in the lungs at 3,000 ppm. Treatment-related increases in the incidence of skeletal variations at 9,000 ppm.	900 ppm (maternal) 3,000 ppm (develop.)	API, 1989b

Developmental Toxicity of Commercial Hexane Vapor in CD-1 Mice (API, 1989b). Mice were exposed to commercial hexane vapor for six hours/day on gestational days 6 through 15 at concentrations of 0, 900, 3,000 and 9,000 ppm. Slight maternal toxicity was observed at 3,000 and 9,000 ppm. There was a significant increase in the incidence of color change in the lungs as well as an increased (non-statistically significant) number of dams (4 of 9) exhibiting dark brown foci in the lungs at 9,000 ppm. At 3,000 ppm, characteristic color changes in the lungs were noted in two (of 25) dams; in three other dams dark brown foci were observed in the lungs. Treatment-related increases in bilateral bone islands at the first lumbar arch and all intermediate unossified phalanges were observed at 9,000 ppm. The NOAEL for maternal toxicity was 900 ppm and for developmental toxicity the NOAEL was 3,000 ppm.

Inhalation Oncogenicity Study of Commercial Hexane In Rats and Mice, Part I-Rats. (API, 1995). Rats were exposed to 0, 900, 3,000, 9,000 ppm (to 0, 3,092, 10,307, 30,921 mg/m^3) CH vapor for 6 hours/day, 5 days/week for 2 years. Excess lacrimation increased in the commercial hexane exposed males at 3,000 and 9,000 ppm. Body weight gain was significantly reduced in the 3,000 and 9,000 ppm exposure groups. Microscopic morphologic abnormalities that were considered to be related to commercial hexane exposure were found in the nasal turbinates and the larynx only. The incidences and/or severities of these findings in the exposure groups were increased when compared to the respective controls (Table 3).

Hypertrophy/hyperplasia of goblet cells in the nasoturbinal tissues, seen in numerous males and females, tended to occur more frequently in the exposure groups than in the controls. The severity showed a dose-related increase. Hyperplasia of the respiratory epithelium was seen more frequently and with greater severity in the exposure groups than in the controls. This response exhibited a positive dose-response relationship.

Intracytoplasmic eosinophilic material in the respiratory and submucosal glandular epithelium and in the sustentacular cells of the olfactory epithelium were seen more frequently and with greater severity in the exposure groups than in the controls. In the males, the severities in Groups 3 and 4 (3,000 and 9,016 ppm, respectively) were comparable and greater than those seen in Group 2 (900 ppm). In the females the severities in Groups 2, 3, and 4 were essentially similar.

Microscopic findings associated with inflammatory changes in the nasoturbinal tissues seen in a number of males and females from the exposure and control groups occurred most frequently as follows: a) subacute (chronic active)/chronic inflammation of the nasal mucosa in the males, followed by the females, from Group 4 (9,016) ppm; b) inflammatory cells/cell debris in the nasal lumen in

males from Group 4; c) edema of the nasal mucosa in males from Group 4. The authors concluded that a NOAEL was not identifiable from the data on the nasoturbinal tissues of the rats.

In the larynx, squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium was seen in a small number of animals from the control and exposure groups. In the males, the highest incidence was seen in Group 4 (9,016 ppm), followed by Group 3 (3,000 ppm). In the females, the incidence in Groups 3 and 4 was comparable, and greater than that seen in Group 1 (0 ppm). This finding was considered to be a localized response indicative of irritation. In all of the affected animals, the metaplastic epithelium was well differentiated and organized, with no evidence of atypia or dysplasia (Table 3).

An Inhalation Oncogenicity Study of Commercial Hexane In Rats and Mice, Part II-Mice. (API, 1995, Part II). Mice were exposed to 0, 900, 3,000, 9,000 ppm (0, 3,092, 10,307, 30,921 mg/m^3) CH for 6 hours/day, 5 days/week for 2 years. Mean body weights and body weight gains in the exposed animals were not statistically different from control values in the male mice. In the females however, at 9,000 ppm, body weight gain was significantly reduced.

Macroscopic examinations found an apparent treatment-related increase in liver masses and nodules among the females in the 9,000 ppm group but not among the males. Microscopic examinations found a treatment-related increase in hepatocellular neoplasms (adenoma and carcinoma) among females in the high exposure group. For females, the incidence of benign tumors was statistically significant for trend at 0.04 level. There were no significant pairwise differences. The incidence of malignant tumors was not significant for trend or pairwise comparisons. When benign and malignant tumors were combined there was a statistically significant trend at 0.01 level and a statistically significant difference between the high dose group and the control group. Liver tumors among males were not treatment-related. There was an increase in the incidence of pituitary proliferative changes (hyperplasia, adenoma and adenocarcinoma) among all treated groups of females but not among males. There was also a treatment-related decrease in the severity and a slight decrease in the incidence of cystic endometrial hyperplasia of the uterus among the females in 9,000 ppm group. The authors concluded that commercial hexane was an oncogen in female mice.

In the previously described API (1995, Part I) rat study, the nasoturbinal tissues were examined and were found to be severely affected by CH. These target tissues were not examined in mice in the API (1995, Part II) study.

Table 3. Microscopic Findings in Rat Respiratory Tract Tissues after Chronic Inhalation Exposure to Commercial Hexane (API, 1995 Part I)

			Number of animals affected							
			Male				Female			
		Group*:	1	2	3	4	1	2	3	4
Organ Examined	Tissue Examined	Number /group:	50	50	50	51	50	50	50	50
Nose/Turbinates		Number examined:	48	50	50	50	50	49	49	50
	• nasal mucosa (respiratory): goblet cell hypertrophy/hyperplasia		29	37	43	41	33	43	43	46
	• nasal mucosa (respiratory): epithelium-hyperplasia. intracytoplasmic eosinophilic material		2	19	36	43	6	34	38	42
	• nasal mucosa (respiratory/olfactory): epithelium-intracytoplasmic eosinophilic material		21	49	46	46	41	47	48	49
	• nasal mucosa (respiratory/olfactory): submucosal glands. epithelium-intracytoplasmic material		10	41	41	43	20	47	47	37
	• nasal mucosa (respiratory/olfactory): subacute (chronic active)/chronic inflammation		9	8	10	23	8	6	4	13
	• nasal lumen inflammatory cells/cell debris		13	16	13	23	9	5	10	6
Larynx		Number Examined:	49	19	18	50	48	10	14	48
	pseudostratified columnar epithelium: squamous/squamoid metaplasia (with hyperplasia)		4	0	2	11	1	0	2	7

* group 1 = control, and group 2, group 3, group 4, were exposed to 900, 3,000, and 9,016 ppm commercial hexane respectively.

While the above studies showed negative peripheral neurotoxicity results, there are other animal studies on commercial hexane and other C_5-C_8 mixtures that demonstrated peripheral neurotoxicity.

Chronic and continuous exposure of mice to commercial hexane containing 65 – 70 % n-hexane caused peripheral neurotoxicity (Miyagaki, 1967). Male mice (10 per test group) were exposed to 0, 100, 250, 500, 1,000, or 2,000 ppm (0, 353, 881, 1,762, 3,520 or 7,050 mg/m^3) of commercial hexane 24 hours/day, 6 days/week for one year. Monitored parameters included: electromyography,

strength duration curves, electrical reaction time and flexor/extensor chronaxy ratio, gait posture, and grade of muscular atrophy. Electromyographic analysis showed increased complexity in neuromuscular unit voltages in 0/6 control, 1/6 animals examined in the 100 ppm group, 3/6 animals examined in the 250 ppm group, 5/6 animals examined in the 500 ppm group, 3/3 animals examined in the 1,000 ppm group and 4/4 animals examined in the 2,000 ppm group.

Electromyography also showed a similar dose-related increase in both incidence and severity of reduced interference voltages from muscles in animals exposed to 500 ppm and higher but not in controls and the low exposure concentrations. Dose-related increases in abnormalities of strength-duration curves were also detected. Electromyographs also showed dose-related fibrillation. Abnormal posture and muscle atrophy were noted in a dose-related manner in animals exposed to 250 ppm and higher. The NOAEL identified in this study was 100 ppm. The flaw in the study was that only the data from 3 to 6 of the 10 animals were presented. Also, mice are reported to be less susceptible to n-hexane peripheral neurotoxicity than rats (see ATSDR, 1999). This observation suggests that more pronounced effects could have been detected if rats were used in this chronic study.

Rats were exposed to a mixture containing the n-hexane isomers 2-methylpentane, 3-methylpentane, cyclohexane, methylcyclohexane, methylcyclopentane, and 2,3-dimethylbutane with about 1% n-hexane (500 ppm), or n-hexane (99%) alone (100 ppm) or n-hexane plus mixed hexanes (1,000 ppm) 22 hours/day, 7 days week for approximately 6 months. Gait disturbances and peripheral nerve atrophy were observed in the n-hexane (500 ppm) alone group and in the groups treated with n-hexane plus hexane mixture (1,000 ppm).

The frequency of peripheral nerve atrophy was higher in the group receiving n-hexane alone than the group receiving the n-hexane plus hexane mixture. However it was not possible from the data to quantitate the difference in severity between mixture treated and pure n-hexane treated groups. This study demonstrated that hexane mixtures containing about 50% n-hexane caused peripheral nerve damage (IRDC, 1981). The difference between the IRDC (1981) and the API (1990a) studies appears to be the exposure duration. In the IRDC reported study exposure was continuous while in the API study it was intermittent suggesting that continuous exposure to lower concentration of commercial grade hexane (containing 50% n-hexane) may cause peripheral nerve damage. The neurotoxic exposure concentration in the IRDC reported study was 9 times lower than the exposure concentration reported in the API study that did not have any toxic effect. Interestingly, the IRDC reported study

was also sponsored by the API and it is not clear why the same exposure protocols were not used in the API (1990a) studies.

Krasavage et al. (1980) gavaged COBS rats with practical grade hexane (4,000 mg/kg/d), n-hexane (570, 1,140 or 4,000 mg/kg/d), 2-hexanol (675 mg/kg/d), 2,5-hexanedione (755 mg/kg/d), 2,5-hexanediol (780 mg/kg/d), 5-hydroxy-2-hexanone (765 mg/kg/d), or methyl n-butylketone (MnBK) (660 mg/kg/d) once daily, 5 days/week over a 90 - 120-day period. The practical grade hexane contained 40% n-hexane, 24% 3-methylpentane, 24% dimethylbutane, 9% cyclopentane, 2.5% cyclohexane and 1.2% 2-methylpentane. The highest dose of n-hexane, MnBK, and all hexane metabolites demonstrated clinical signs of polyneuropathy. No clinical signs of neuropathy were observed in rats treated with practical grade hexane. However, histologic examination of nerve tissues collected at termination revealed that all test compounds except the two lowest doses of n-hexane caused morphologic changes indicative of "giant axonal" neuropathy, which included multifocal axonal swellings, axonal myelin infolding and paranodal myelin retraction. The histologic anomaly occurred with equal frequency in rats treated with MnBK and n-hexane metabolites and with lowest frequency in rats treated with practical grade hexane. For n-hexane, it is difficult to conclude that 1140 mg/kg/day did not cause peripheral neuropathy as two out of the five animals died due to chemical pneumonitis immediately following intubation and were not included in the determination of the neurotoxic indices of this group.

Additionally, atrophy of testicular germinal epithelium occurred in animals treated with 2,5-hexanedione, 2,5-hexanediol, 5-hydroxy-2-hexanone, MnBK, 2-hexanol and n-hexane. Effects on body weight response paralleled the neurotoxic potency of each compound. n-Hexane, even at the two low doses which did not produce neuropathy, did affect body weight gain after 3 weeks of exposure.

For 2,5-hexanedione, 5-hydroxy-2-hexanol, 2,5-hexanediol, MnBK, 2-hexanol and n-hexane (4,000 mg/kg/d) the severity of the neurotoxic indices was directly related to the peak 2,5-hexanedione concentration which is a metabolic product of the above listed chemicals except 2,5-hexandione itself. However, n-hexane (570 or 1,140 mg/kg/d) that did not produce any clinical or histological signs of peripheral neurotoxicity had higher levels of peak serum 2,5-hexanedione (24 ± 1.6 and 44 ± 2.7 μg/l respectively) than practical grade hexane (14 ± 2.7 μg/l). Practical grade hexane demonstrated histological signs of peripheral neurotoxicity. This result suggests that the other mixtures (3-methylpentane, dimethylbutane, cyclopentane, cyclohexane and 2-methylpentane) or their metabolites may have contributed to the peripheral neurotoxicity of n-hexane in the mixture.

In the above study, the authors reported that 3 out of 5 rats treated with 4,000 mg/kg/d practical grade hexane, 1 out of 5 rats treated with 4,000 mg/kg/d n-hexane and 2 out of 5 rats treated with 1140 mg/kg/d n-hexane, died due to chemical pneumonitis immediately following intubation and these rats were not included in the determination of the neurotoxicity. Only two rats were evaluated for practical grade hexane neurotoxicity that would make a significant difference in the outcome and interpretation of the results.

Human Studies

The positive animal studies on hexane mixtures are also corroborated by human epidemiological data.

The occurrence of polyneuropathy in humans exposed repeatedly to commercial hexane has been well documented. Yamada (1972) investigated the cases of 17 workers who had reported symptoms of polyneuropathy (with subsequent development of muscular atrophy and paresthesia in the distal extremities) while exposed to hexane vapors for 2 years. Six of the employees were exposed to hexane levels ranging between 1,000 and 2,500 ppm. The hexane solvent used in the plants where the six subjects worked contained 16% methyl pentane, 20% methyl cyclopentane, and 64% n-hexane; a characteristic composition of commercial grade hexane. Eleven of the 17 employees worked in a different plant where the solvent used contained 95% n-hexane. Exposure levels ranged between 500 and 1,000 ppm. The n-hexane concentrations that failed to produce peripheral neurotoxicity in rats in the API (1990a) study were estimated to be 477, 1,590 and 4,770 ppm, based upon n-hexane being 53% of the exposure concentrations (900, 3,000, 9,000 ppm) that were used in the API studies.

Gaultier et al. (1973) also reported peripheral neurotoxicity in people occupationally exposed to solvent mixtures. The solvent used in the workplace contained only 5% n-hexane, 14% heptane and 80% pentane. Yamamura (1969) reported an outbreak of peripheral neurotoxicity resulting from exposure to hexane that was used as a glue solvent in a sandal factory in Japan. Inoue et al. (1970) reported that the hexane solvent in the glue used by the sandal makers who were studied by Yamamura (1969) contained 2-methylpentane, 3-methylpentane, methylcyclopentane, and n-hexane. Although the concentrations of the individual constituents were not given, the authors related that most commercial hexane solvents contained these four compounds with n-hexane constituting about 60% of the total.

A solvent that caused five cases of peripheral neurotoxicity in a workshop cleaning silk brocade sash contained $C_5 - C_9$ hydrocarbons and their isomers. n-

Hexane accounted for only 12.3 % of the total (Takeuchi et al, 1975). Peripheral neurotoxicity was also reported in Italian workers in shoe manufacturing plants. Analyses of the solvents and glues in the shoe factories in which the workers developed peripheral neurotoxicity indicated that the vapors contained alkanes including isopentane, n-pentane, 2-methylpentane, 3-methylpentane, n-hexane, isoheptane and n-heptane (Abbritti et al., 1976). After screening 654 employees in several shoe factories, 98 verified cases of peripheral neurotoxicity were detected. Analysis of the vaporized constituents of the glues and solvents demonstrated the presence of pentane, 2-methylpentane, 3-methylpentane, n-hexane, heptane, cyclohexane, and methy-cyclopentane. The individual solvent levels were not reported (Passero et al., 1983).

In summary, the epidemiological studies demonstrated that inhalation exposure to n-hexane, commercial grade hexane, or other mixtures in the group containing 12.3 – 60% n-hexane resulted in peripheral neurotoxicity. The data do not allow comparison of the severity of the peripheral neuropathy induced by pure n-hexane or the aliphatic mixtures in the series.

2. Individual Components of the $C_5 - C_8$ Aliphatic Fraction

Animal Studies

When rats were exposed via inhalation to 3,000 ppm n-hexane, n-pentane or n-heptane, 12 hours/day 7 days/week for 16 weeks, peripheral nerve damage occurred only with n-hexane (Takeuchi et. al., 1980; 1981). Rats exposed to 400 or 3,000 ppm n-heptane 6 hours/day, 5 days/week for 26 weeks showed no signs of peripheral neurotoxicity (API, 1980). n-Heptane appeared to produce no peripheral neurotoxicity directly in animals. However, a metabolite of n-heptane, 2,5-heptanedione, produced peripheral neurotoxicity similar to that of 2,5-hexanedione (metabolite of n-hexane) (Katz et al., 1980; Misumi and Nagano, 1984). In rats and humans, inhalation kinetics of n-hexane and n-heptane were compared with urinary excretion of 2,5-hexanedione (HDO) and 2,5-heptanedione (HPDO) respectively. Furthermore, the relative reactivities of HDO and HPDO with Nα-acetyl-L-lysine towards the formation pyrrole adducts were studied. Reaction of γ-diketone with primary amines of neurofilamental protein resulting in pyrrole adducts is regarded as the first step leading to peripheral neuropathy (Graham et al., 1995). The results indicated that about 0.5% was excreted as HDO in urine after exposure to 300 ppm n-hexane and about 0.01% was excreted as HPDO after exposure to 500 ppm n-heptane. *In vitro*, the rate of pyrrole formation from the reaction of HPDO with Nα-acetyl-L-lysine was half that obtained with HDO. These results indicate that the peripheral neurotoxic potency of n-heptane could be much lower than n-hexane.

Although n-heptane showed no peripheral neurotoxicity in the above studies reported by Frontali et al. (1981), Takeuchi et al. (1980, 1981) and API, 1980 above, Trauhaut et al. (1973) reported that rats exposed to 1,500 ppm technical grade heptane for five or six months demonstrated a reduced nerve conduction velocity, an increased refractory period, and decreased excitability of the sciatic and saphenous nerves as effectively as 2,000 ppm technical grade hexane. The heptane used in the experiment contained 52.4% of n-heptane, 16.2% of 3-methylhexane, 9.8% of other heptane isomers, and 21.5% of octane isomers, but did not contain n-hexane. The same level of pure n-heptane exposure (1,500 ppm, 9 hours/day, 5 days/week, for 7-14 weeks) did not cause peripheral neuropathy in rats (Frontali et al. 1981). The Trauhaut et al. (1973) and the Frontali et al. (1981) studies suggest that mixtures containing the heptane isomers may be more neurotoxic than pure n-heptane alone.

When male rats were exposed to n-heptane vapor (100, 500 or 1,000 ppm) for up to two weeks, reduced RNA concentration, and increased NADPH-diaphorase activity were observed in the brain at the lowest exposure level. Increased proteolysis was detected in the cerebral samples in the second week at all exposure concentrations. All biochemical effects were abolished after two weeks of withdrawal from exposure with the exception of reduced amount of glutathione at the lowest dose. None of the rats demonstrated clinical signs of peripheral neuropathy (Savolainen and Pfaffli, 1980) after two weeks of exposure.

Rats were orally treated with 1,251 mg/kg/d n-hexane, 2-methylpentane, 3-methylpentane, or methylcyclopentane for eight weeks. Body weight change, motor nerve conduction velocity, motor distal latency, and mixed nerve conduction velocity were measured in the tail before treatment, and after two, four, six and eight weeks of treatment with individual solvents. The n-hexane group showed a distinct impairment of the functional states of the peripheral nerve. The other solvents, 2-methylpentane, 3-methylpentane, or methylcyclopentane caused some significant differences in comparison with controls although these differences were not as distinct as those in the n-hexane group (Ono et al., 1981). These results suggest that the hexane isomers cause neurotoxicity but to a lesser extent than n-hexane.

Human Studies

Humans exposed for an extended period of time to the petroleum fraction with the boiling range 70°C to 100°C developed peripheral neuropathy (Cavigneaux, 1972). This fraction would normally contain various isomers of heptane as major ingredients. Eighteen individuals who had been exposed to 95% n-heptane for periods ranging 1 to 9 years were investigated for peripheral neurotoxicity.

Electrophysiological examinations were performed on 12 of the test subjects. Mild peripheral neurotoxicity was demonstrated in the tested people (Crespi et al. 1979). This study did not specify the exposure concentrations of n-heptane in the workplace. Data on exposure of humans to technical grade heptane were unavailable. The data suggest that n-heptane which failed to produce peripheral neuropathy in animals may cause this disease in humans but to a lesser extent than n-hexane, practical grade hexane or practical grade heptane.

In Vitro Studies

In vitro studies were conducted to investigate the effects of various neurotoxic compounds and n-heptane on primary neural cell cultures from fetal rats. The responses of the neural cells to the neurotoxic compounds were evaluated three and seven days after the first dosing by determining cell viability, and amounts of glial fibrillary acid protein (GFAP), and neuron-specific enolases (NSE) and neurofilaments in primary cortical cell cultures from rats. GFAP is an indicator of astrocyte proliferation (gliosis) that results from toxic or mechanical injury to neurons, and NSE is a cellular marker of neurons (Schmuck and Schluter, 1996).

n-Heptane demonstrated both acute and delayed cytotoxicity while 2,5-hexanedione, the metabolite of n-hexane, demonstrated only delayed cytotoxicity. n-Hexane did not cause cytotoxicity, and the authors attributed the lack of effect of n-hexane on cell viability to its more rapid evaporation rate from the culture dishes when compared to n-heptane. However, n-hexane caused other toxicities in the neural cell cultures suggesting that evaporation may not be a factor in n-hexane's lack of cytotoxicity. The no effect concentrations (NOECs) of n-heptane for GFAP, NSE and neurofilament at day 7 were lower than the NOEC for cytotoxicity at the same time point indicating that n-heptane's effect on these parameters started to occur prior to cell death. The concentrations of n-heptane required to produce effects in all the described parameters are lower than those for n-hexane and 2,5-hexanedione (see Table 4). However, n-heptane should be studied at much lower concentrations that are not cytotoxic in order to accurately determine its effect on neuronal cell cultures.

Table 4. Determination of the Cell (a) Viability, by Calcein-AM, (b) Intracellular Amount of Glial Fibrillary Acid Protein (GFAP), (c) Neuron-Specific Enolase (NSE) and (d) Neurofilaments in Primary Cortical Cell Cultures From Rat.

	(a) Viability				(b) GFAP			
Compound	Day 3 NOEC (µmol/L)	EC50 (µmol/L)	Day 7 NOEC (µmol/)	EC50 (µmol/L)	Day 3 NOEC (µmol/)	EC50 (µmol/L)	Day 7 NOEC (µmol/)	EC50 (µmol/)

n-Hexane	>1.2	>1.2	>1.2	>1.2	>1.2	>1.2	0.12**	>1.2
2,5-hexanedione	>0.88	>0.88	0.09**	>0.88	>0.88	>0.88	0.44**	>0.88
n-Heptane	0.1**	0.49**	0.10**	0.27**	0.1**	1**	0.01**	0.22**

	(c) NSE				(d) Neurofilament			
Compound	Day 3 NOEC (µmol/)	EC50 (µmol/)	Day 7 NOEC (µmol/)	EC50 (µmol/)	Day 3 NOEC (µmol/L)	EC50 (µmol/)	Day 7 NOEC (µmol/)	EC50 (µmol/)
n-Hexane	0.58**	>1.2	0.58**	>100	0.12**	>1.2	5**	100**
2,5-hexanedione	>0.88	>0.88	>0.88	>100	>0.88	>0.88	<1**	75**
n-Heptane	0.10**	>1	0.01**	0.40**	0.05**	1*	0.01*	0.24**

Note: evaluations were made three and seven days after first application and, for comparison, the no effect concentrations (NOEC) and effective concentrations (EC50) were documented. Statistical evaluations of between compound differences were made by ANOVA followed by a t-test (** = $p<0.001$). Table is modified and adopted from Schmuck and Schluter, (1996).

Putative Metabolite Studies for Various $C_5 - C_8$ Components

With regards to n-hexane, the neurotoxic agent has been identified as a γ-diketone metabolite, 2,5-hexanedione. The knowledge that a γ-diketone metabolite of n-hexane is responsible for peripheral neuropathy led to structure activity relationship studies of other short and long chain diketones including 2,4-pentanedione and 2,5-heptanedione. The 2 carbon spacing between the carbonyl groups is essential for the induction of peripheral neurotoxicity. The metabolism of only n-hexane and n-heptane was extensively studied and γ-diketone metabolites had been identified for these compounds (Filser et al., 1996). However, there are several studies on the toxicities of other aliphatic diketones containing 5, 6, 7, and 8 carbon atoms that could be possible metabolites of aliphatic solvents containing the respective carbon atoms.

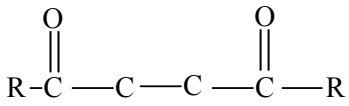

Figure 1. A γ-Diketone Structure

O'Donoghue and Krasavage (1979) have tested a series of diketones (2,3-, 2,4-, and 2,5-hexanedione, 2,5-heptanedione, and 3,6-octanedione) in the rat, and demonstrated that only γ-diketones (2,5-hexanedione, 2,5-heptanedione, and 3,6-octanedione) caused peripheral neuropathy. A study using various ketones including the diketones 2,4-pentanedione and 2,5-hexanedione was performed in rats (Misumi and Nagano, 1984). The diketone 2,5-hexandione showed disturbances in gait and severe paralysis in the hind limbs of the treated animals. In the 2,4-pentanedione group, increased salivation was observed on the 15^{th} day of treatment and those animals demonstrated disturbances in gait on the 45^{th} day. Thereafter, all the animals developed a spastic paralysis of the hind limbs but were not flaccid as were the animals receiving 2,5-hexanedione.

The authors reported that it was difficult to compare the neurotoxic potency of 2,4-pentanedione with that of 2,5-hexanedione, but considering only the peripheral nerves, the neurotoxicity of 2,4-pentanedione seemed to be less than that of 2,5-hexanedione. However, its neurotoxic activity in the central nervous system was greater than that of 2,5-hexanedione. Moreover, animals treated with daily doses of 200 mg/kg of 2,4-pentanedione exhibited clinical and neurophysiological evidence of central nervous system toxicity, but repeated subcutaneous injections of 400 mg/kg/d of the compound caused increased salivation, convulsions, and ataxia followed by death in all tested animals. No animals died as a result of repeated injections of equivalent amounts of 2,5-hexanedione. The study suggests that 2,4-pentanedione may be more toxic to the central nervous system and to the whole animal while 2,5-hexanedione may be more toxic to the peripheral nerves.

Several γ-diketones and related compounds that produce peripheral nerve degeneration characterized by multifocal axonal swellings, often referred to as "giant axonal neuropathy, are presented in Table 5. All the C_5, C_6, C_7, and C_8 diketones caused peripheral neurotoxicity. The 2,4-pentanedione, unlike the other toxic diketones which induce mainly peripheral nerve damage, caused severe central nervous system toxicity (Topping et al., 1994). The γ-diketone, 3,4-dimethyl-2,5-hexanedione, is 30 times more potent than the prototype 2,5-hexanedione (Anthony et al., 1983). A potential precursor of this toxic diketone, 3,4-dimethylhexane has been identified in various petroleum fractions (TPHCWG, 1997b). The data support MA DEP's view that, while lacking direct evidence for peripheral neuropathy in humans associated with exposures to n-alkanes other than n-hexane and n-heptane, numerous compounds which could be metabolites of these n-alkanes have the same structural features which have been associated with peripheral neuropathy.

The toxicological information on the C_5 - C_8 fraction can be summarized as:

- Commercial hexane and practical grade heptane may cause peripheral nerve damage in humans similar to that observed for n-hexane; Commercial grade-induced peripheral nerve damage may be less than that caused by pure n-hexane. However there are no data to quantitatively compare their potencies;

- The peripheral neurotoxicities of mixtures containing various components of the $C_5 - C_8$ aliphatic fraction with very little n-hexane or no n-hexane content at all suggest that aliphatic mixtures containing very little or no n-hexane content may cause peripheral nerve damage;

- Other aliphatic hydrocarbons containing 5, 7, 8, and 9 carbon atoms may be metabolized to ketones and γ-diketones that may cause central and peripheral nerve damage;

- The target organ for n-pentane may be the central nervous system based on the data on 2,4-pentanedione. The data do not permit a calculation of an RfD for this compound based on central nervous system effects. This diketone could be a potential metabolite of n-pentane and may affect both the central and peripheral nervous systems;

- There may be more toxic compounds in the series other than n-hexane because some γ-diketones like 3,4-dimethyl-2,5-hexanedione are reported to be 30 times more potent than 2,5-hexanedione. The parent compound that could possibly be metabolized to 3,4-dimethyl-2,5-hexanedione is 3,4-dimethylhexane.

Table 5. Observations on Peripheral Neuropathies of Ketones and Related Substances (from Topping et al., 1994)

# Carbon Atoms in Compound	Chemical	Structure	Peripheral Neuropathy Observed
5	2,4-pentanedione	$CH_3COCH_2COCH_2$	+[a]
6	n-hexane	$CH_3(CH_2)_4CH_3$	+
	practical grade hexanes	Mixed hexanes	+
	methyl n-butyl ketone	$CH_3CO(CH_2)_3CH_3$	+
	5-hydroxy-2-hexanone	$CH_3CO(CH_2)_2CHOHCH_3$	+
	2,5-hexanedione	$CH_3CO(CH_2)_2COCH_3$	+
7	ethyl n-butyl ketone	$CH_3CH_2CO(CH_2)_3CH_3$	+
	2,5-heptanedione	$CH_3CO(CH_2)_3CH_2CH_3$	+
	3-methyl-2,5-hexanedione	$CH_3COCHCH_3CH_2COCH_3$	+
8	3,6-octanedione	$CH_3CH_2CO(CH_2)_2COCH_2CH_3$	+
	5-methyl-3-heptanone	$CH_3CH_2COCH_2CHCH_3CH_2CH_3$	+
	3,4-dimethyl-2,5-hexanedione	$CH_3COCHCH_3CHCH_3COCH_3$	+

[a] 2,4-pentanedione produces CNS damage that is clinically, anatomically, and morphologically different from "giant" axonal neuropathy

Derivation of Oral Toxicity Value

After reviewing the available studies on $C_5 - C_8$ aliphatic fraction mixtures or individual components of the fraction, no adequate data were identified to derive fractional RfD or RfDs for individual compounds in the $C_5 - C_8$ carbon range except for n-hexane. To be health protective, MA DEP still chooses to retain the n-hexane toxicity value as a representative toxicity number for the fraction. This decision is based on the fact that other compounds in the range are not well studied and the various diketone metabolite studies suggest that some of the components of the fraction may be more toxic to the peripheral nerves than n-hexane. Moreover, studies in humans with mixtures containing very low levels of hexane (5%) but high levels of n-pentane (80%) and heptane (14%) caused peripheral nerve damage suggesting that compounds like n-pentane require more investigation. As discussed previously, the putative diketone metabolite of n-pentane caused both central and peripheral nerve damage unlike n-hexane, that to a large extent, affects peripheral nerves only.

The n-hexane subchronic gavage study in rats conducted by Krasavage et al. (1980), that was discussed previously, was used to derive a representative toxicity value for the $C_5 - C_8$ aliphatic subgroup. The LOAEL identified in this study was 570 mg/kg/d. The duration adjusted LOAEL is 407 mg/kg/d (570 mg/kg/d x 5 days/7 days = 407 mg/kg/d). An uncertainty factor of 10,000 was applied to the duration adjusted LOAEL to derive an oral RfD of 0.04 mg/kg/d. **This oral RfD of 0.04 mg/kg/d is recommended for MA DEP use for the $C_5 - C_8$ aliphatic fraction.** This value is different from the 1994 MA DEP oral toxicity number because the US EPA (1989) LOAEL (570 mg/kg/d) had not previously been adjusted for the less than continuous duration of exposure**.** The derivation process is presented in Table 6.

Table 6. Oral Toxicity Value for the C_5 - C_8 Aliphatic Fraction

Species	Endpoint	Duration. Adj. $LOAEL_{adj}$* (mg/kg/d)	UF Applied	RfD mg/kg/d	Source
Rat	Reduced body weight and neurotoxicity at higher doses	407	10,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic 10 – for LOAEL to NOAEL	0.04	Krasavage et al., 1980

*Duration adjusted by MA DEP. The toxicity value derived by the (US EPA, 1989) was not adjusted for duration of exposure. An uncertainty factor of 10,000 was applied by them to the LOAEL (570 mg/kg/d) to obtain an RfD of 0.06 mg/kg/d. This RfD was used by MA DEP in 1994.

For comparison, the TPHCWG used the API-sponsored chronic inhalation studies on commercial hexane in mice and rats to derive a representative oral RfD for the C_5 - C_8 aliphatic fraction by first deriving an inhalation RfC. From all of the studies, the most appropriate NOAEL identified for either the rat or mice chronic bioassay was 3,000 ppm (10,307 mg/m^3). The exposure duration-adjusted NOAEL (NOAEL x 6/24 x 5/7) was estimated to be 1,841 mg/m^3. By applying an uncertainty factor of 100 (10 for human variability, 10 for animal to human extrapolation), a chronic inhalation RfC of 18.4 mg/m^3 was estimated for the C_5 - C_8 aliphatic hydrocarbon fraction. An oral RfD of 5 mg/kg/d was then calculated from the inhalation RfC by assuming that the inhalation rate for a 70 kg human is 20 m^3/day and absorption is 100%. Given the level of this RfD compared to that of pure n-hexane, the TPHCWG concluded that n-hexane toxicity can be influenced by the presence of other petroleum components.

2.1.2 C_5 – C_8 Aliphatic Fraction - Inhalation RfC

Of the chemicals within this subgroup, inhalation toxicity values exist only for n-hexane (2.0 mg/m^3). Adequate data were not identified to develop RfCs for any of the other individual compounds in this carbon range. However, various American Petroleum Institute-sponsored chronic exposure studies on commercial hexane exist. These studies were discussed in Section 2.1.1.1.

As discussed previously, neurotoxicity was not observed in the API (1990a)-sponsored studies in rats that were exposed to up to 9,000 ppm commercial hexane. However, commercial hexane produced microscopic morphologic abnormalities that were considered to be treatment-related in the nasal turbinates and the larynx of rats exposed to up to 9,000 ppm commercial hexane. These effects were not reported in mice treated chronically with commercial hexane (API, 1995, Part I). However, inhalation of n-hexane resulted in morphologic alterations in mice (Dunnick et al., 1989). The authors of the rat study (API, 1995, Part I) acknowledged that no NOAEL could be identified for the effects in the nasal turbinates.

Sandmeyer (1981), and Von Oettingen (1940) have summarized the available toxicological information on paraffins showing that one of the chief effects of alkane vapor inhalation is irritation of the respiratory passages. Pentane, hexane, and heptane were at one time investigated for use as anaesthetics (Fuhner, 1921) but they produced undesirable side effects such as respiratory irritation and central nervous system inhibition leading to respiratory arrest (Fuhner, 1921).

An RfC can be derived using the noted respiratory effects. Use of this type of endpoint in developing an RfC is not without precedent. The U S. EPA has considered such severe respiratory tract effects in the derivation of inhalation RfCs for other compounds such as acetaldehyde, acrylonitrile, 1,2-dichloropropanol, epichlorohydrin, ammonia and many

others (see the compounds in the respective IRIS database listings). The TPHCWG did not consider this frank upper respiratory toxicity observed in chronically treated rats (Table 3) in the derivation of an RfC for commercial hexane. The LOAEL for respiratory effects was 900 ppm (3,092 mg/m^3) while the NOAEL for other systemic effects in the same study was reported to be 3,000 ppm (10,307 mg/m^3). Since the study demonstrated respiratory effects at a much lower exposure concentration than any other effects observed, the inhalation RfC should be estimated for this endpoint.

For gases and vapors that are very reactive and that have their toxic effect in the respiratory tract, the US EPA has an approach for deriving human equivalent concentrations (US EPA, 1990) as outlined below. This methodology will be used to estimate a toxicity value for commercial hexane based on nasoturbinal effects. The RfC based on the respiratory endpoint is estimated for gas: respiratory effect in the extrathoracic region as follows:

The LOAEL identified for nasoturbinal effects is 3,092 mg/m^3.

LOAEL adj. = E (mg/m^3) x (exposure hours/day/24 hours) x (exposure day/week/7 days)

$LOAEL_{HEC}$ = $LOAEL_{adj}$ (mg/$m^{3)}$ x RDGRET

RDGRET = $[VR/SR_{et}]/[VH/SH_{et}]$

where,

E	=	Exposure concentrations
$LOAEL_{adj}$	=	LOAEL adjusted for duration
$LOAEL_{HEC}$	=	LOAEL human equivalent concentration
RDGRET	=	Regionally deposited gas ratio, extrathoracic region
VR	=	Rat ventilation rate(0.33 m^3/day) (i)
SR_{et}	=	Surface area of the extrathoracic region for rat (11.6 cm^2)
VH	=	Human ventilation rate 20 m^3/day
SH_{et}	=	Surface area of the extrathoracic region in man (177 cm^2)
RDGRET	=	0.33 m^3/day/11.6 cm^2 /20 m^3/day /177 cm^2 = 0.25

$LOAEL_{adj}$ = 3,092 x 6/24 x 5/7 = 552 mg/m^3

$LOAEL_{HEC}$ = 552 mg/m^3 x 0.25 = 138mg/m^3

RfC = $LOAEL_{HEC}$ = 138 mg/m^3/300* ≈ **0.5 mg/m^3**

An uncertainty factor of 300 was applied (10 for human variability, 10 for LOAEL to NOAEL extrapolation, 3 for animal to human extrapolation). 3 instead of 10 was used for animal to human exposure since dosimetric adjustment was made using the regionally deposited gas ratio for the appropriate respiratory region.

Based on the nasoturbinal effects, an RfC of 0.5 mg/m^3 was estimated. It should be noted that this RfC is close to the US EPA RfC of 0.2 mg/m^3 derived for n-hexane based on neurotoxicity. As discussed previously, the human data suggest that the more serious health effect observed in people occupationally exposed to n-hexane or commercial hexane is

peripheral neuropathy as opposed to respiratory toxicity.

In conclusion, the available data suggest that there may be compounds in the C_5-C_8 hydrocarbon fraction in addition to n-hexane that may cause peripheral or central nervous system effects. However, the data do not permit estimation of toxicity values for the individual compounds or mixtures. Until appropriate human or animal data are available on the C_5-C_8 mixtures or on the individual components of the fraction, **MA DEP recommends the U.S. EPA (1993a) derived RfC of 0.2 mg/m^3 for n-hexane, which is based on a neurotoxic endpoint, as a representative surrogate for the C_5-C_8 fraction. This RfC would be protective of the respiratory effects also.** The critical study and uncertainty factors used to derive the n-hexane RfC are presented in Table 7.

The TPHCWG used the API-sponsored chronic inhalation studies on commercial hexane in mice and rats to derive a representative oral RfC for the C_5 - C_8 aliphatic fraction. From all of the studies, the most appropriate NOAEL identified for either the rat or mice chronic bioassay was 3,000 ppm (10,307 mg/m^3). The exposure duration-adjusted NOAEL (NOAEL x 6/24 x 5/7) was estimated to be 1,841 mg/m^3. By applying an uncertainty factor of 100 (10 for human variability, 10 for animal to human extrapolation), a chronic inhalation RfC of 18.4 mg/m^3 was calculated.

Table 7. Inhalation Toxicity Value for the C_5 - C_8 Aliphatic Fraction

Species	Endpoint	Dur. Adj. LOAEL (mg/m^3)	UF Applied	RfC mg/m^3
Human (Epi. Study) (US EPA, 1993a)	Neurotoxicity	73	300 10 - LOAEL to NOAEL, 10 - Human Variability 3 - database deficiency	0.2

2.1.3 C_9 - C_{18} (MA DEP)) Aliphatic Fractions Oral RfD

2.1.3.1 Basis for Existing Toxicity Values. The MA DEP previously assigned the toxicity value estimated for n-nonane to all C_9 through C_{18} hydrocarbons. This RfD (0.6 mg/kg/d) is ten times that developed for n-hexane. The n-nonane RfD was derived by MA DEP based on the relative potencies of n-hexane and n-nonane described below:

- Subchronic inhalation studies using n-nonane (Carpenter et al., 1978) showed that n-hexane (Dunnick et al., 1989**)** is ten times more potent than n-nonane;
- Review of threshold limit values (TLVs) and recommended exposure limits

(RELs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH) and the National Institute for Occupational Safety and Health (NIOSH) respectively indicated that the exposure limits for n-nonane are approximately an order of magnitude greater than those for n-hexane.

Since various rodent studies on dearomatized streams which together cover the entire range of the $C_9 – C_{16}$ aliphatic fraction are now available, these studies were reviewed to derive a more appropriate toxicity value for the fraction. The available studies are discussed below.

2.1.3.2 Summaries of Petroleum Stream Toxicity Studies. The studies on petroleum streams are unpublished but were provided to MA DEP for review. The data are briefly summarized below.

C_9 - C_{12} Isoparaffins/n-Alkanes/Naphthenes: Typical Aromatic Content 0.1% (Anon., 1991a)

Rats were orally dosed with 0, 500, 2,500 or 5,000 mg/kg/d of C_9 - C_{12} aliphatic petroleum hydrocarbon fraction for 90 days. A high dose recovery group was also included. The mean body weights decreased in the male rats in the mid and high dose group when compared to controls. Hematological studies revealed dose-related significant increases in platelet counts in both male and female animals. Other hematological changes observed in male rats included increases in white blood cell, hematocrit and hemoglobin counts.

Significant increases in serum chemistry values (urea nitrogen, gamma glutamyl transpeptidase (males), cholesterol (males and females), and triglycerides (females) were observed in the mid and high dose groups. Significant increases in alanine aminotransferase were observed in the mid and high dose male rats. High and low dose groups of both sexes showed decreased serum glucose levels. Other significant alterations in the serum included increases in bilirubin, creatinine, chloride and triglyceride levels.

Significant increases in liver weights were observed in the mid and high dose males and in all dose groups in female rats. Kidney weights were significantly increased in all treated males. Adrenal weights were also significantly increased in both males (high dose) and females (mid and high dose) groups. Treatment-related microscopic changes were observed in the kidney of male rats in all dose groups; the liver of male/female rats in all dose groups and stomach and/or anus of males/females in the mid and high

dose group.

No NOAEL could be determined in this study. The LOAEL was estimated to be 500 mg/kg/d. An uncertainty factor of 5,000 (10 for animal to human extrapolation, 10 to account for human variability, 10 for subchronic to chronic and 5 for LOAEL to NOAEL extrapolation) was applied to convert the LOAEL to an RfD. A value of 5 was chosen for conversion of the LOAEL to a NOAEL, since NOAELs in the other fractional studies described below (100 mg/kg/d are only 5 times lower than the LOAEL identified for this fraction. An RfD of 0.1 mg/kg/d was estimated for the C_9 - C_{12} hydrocarbon fraction.

C_{10} - C_{13} Isoparaffins/Naphthenes/n-Alkanes: Typical Aromatic Content 0.1% (Anon., 1991b)

Rats were orally treated with 0, 100, 500, or 1,000 mg/kg/d with a C_{10}-C_{13} aliphatic petroleum hydrocarbon fraction for 13 weeks. Hematological studies revealed a significant increase in platelet count in the high dose male rats. Serum chemistry results demonstrated a significant decrease in aspartate aminotransferase in the high dose females. Other serum chemistry changes included a significant decrease in glucose (males/females), dose-related increase in male creatinine, male phosphorous, male alanine aminotransferase, and female cholesterol with the respective high dose groups being significantly increased compared to controls. Linear dose-related increases in male kidney weights were observed in the mid and high dose groups. Liver weights were significantly increased in the high dose females. Microscopic examination showed treatment-related changes in male kidneys which are characteristic of kidney changes produced in male rats. This effect is known as α_{2u}-globulin nephropathy. This nephropathy is considered to be a male rat specific phenomenon without human significance. A NOAEL of 100 mg/kg/d was identified in this study based on the observed liver effects. An uncertainty factor of 1,000 (10 to account for sensitive individuals, 10 for animal to human extrapolation, and 10 for subchronic to chronic adjustment) was applied to the NOAEL. The fractional RfD derived from these data was 0.1 mg/kg/d.

C_{11}- C_{17} Isoparaffinic Solvent; Typical Aromatic Content: <0.05% (Anon., 1990)

Rats were orally treated with 0, 100, 500, or 1,000 mg/kg/d of the C_{11} - C_{17} aliphatic petroleum hydrocarbon fraction for 13 weeks. A high dose recovery group was also included. In male rats, hematological studies showed significant increases in hemoglobin and corpuscular hemoglobin

levels following the 28 day recovery period. Serum chemistry analysis showed dose-related decreases in the male triglyceride levels in both the high and mid dose groups differing significantly from controls. Increased liver weights were observed in both mid and high dose male and female rats. No histopathological alterations were observed.

The NOAEL identified in this study is 100 mg/kg/d. An uncertainty factor of 1,000 (10 to account for sensitive individuals, 10 for animal to human extrapolation, and 10 for subchronic to chronic adjustment) was applied to the NOAEL and an RfD of 0.1 mg/kg/d was derived for the fraction.

Equivalent RfDs (0.1 mg/kg/d) were also derived from the dearomatized petroleum stream studies representing the C_9 - C_{12}, and C_{10} - C_{13} fractions. An oral RfD of 0.1 mg/kg/d was therefore selected as a surrogate for the C_9 - C_{16} fraction based on the results of the oral toxicity studies covering overlapping fractions of the total C_9 - C_{16} carbon range.

JP-8 Jet Fuel (Matti et al., 1995)

Male rats were orally gavaged with 0, 750, 1,500 and 3,000 mg/kg/d of JP-8 for 90 days. Body weights were significantly reduced in both mid and high dose groups. Glucose, total bilirubin, AST, and ALT were significantly altered in the treated groups. Dose dependent irritation of the GI tract was also noted. Neutrophil (elevation) and lymphocyte (depression) counts were significantly different in all treated groups from controls. In the high dose group, organ/body weight ratios were significantly different for brain, liver, kidneys, spleen and testes. However, individual organ weights were not significantly altered in the treated group. The LOAEL identified in this study is 750 mg/kg/d based on altered liver enzyme levels and serum chemistry changes. The NOAEL was adjusted by an uncertainty factor of 10,000 (10 for sensitive individuals, 10 for animal to human extrapolation, 10 subchronic to chronic extrapolation and 10 for LOAEL to NOAEL). The estimated RfD was 0.1 mg/kg/d. The confidence in this RfD is low because of the high aromatic content of the JP-8 fraction and the high magnitude of the uncertainty factor applied. All the RfDs derived from the various studies and the uncertainty factors applied are shown in Table 8.

2.1.3.3 Discussion and Recommendation.

After reviewing the data on C_9 - C_{12}, C_{10} - C_{13}, C_{11}- C_{17} aliphatic fractions, MA DEP has chosen these studies as the more appropriate ones on which to base an RfD for this fraction.. A study on JP-8 was also evaluated (Matti et al., 1995), but was not used as a basis for

this toxicity value because JP-8 has up to 20% aromatic content versus the petroleum streams that have maximally 1.5%, and in most cases less than 0.1% aromatics. The data on the other petroleum streams were used for the derivation of an oral RfD for the C_9 – C_{18} fraction because of their low aromatic content. **The MA DEP recommended RfD for the C_9 – C_{18} aliphatic fraction is 0.1 mg/kg/d.** This value is recommended over that previously supported because the exposures were oral rather than inhalation, they were with mixtures of compounds within the size range of carbon compounds of interest, they were more recent, and the three studies gave consistent results.

The TPHCWG also used results from the same studies to derive a representative RfD of **0.1 mg/kg/d** for the $C_{>8}$ - C_{16} fraction.

Table 8. Oral Studies and RfDs for the C_9 - C_{18} Aliphatic Fraction

Fraction	Critical Effects	NOAEL (mg/kg/d)	Uncertainty Factors	Oral RfD (mg/kg/d)	Source
C_9 – C_{12}	Changes in serum chemistry and liver weight	500 (LOAEL)	5,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic 5 – for LOAEL to NOAEL	0.1	Anon, 1991a
C_{10} – C_{13}	Changes in serum chemistry and liver weight	100	1,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic	0.1	Anon, 1991b
C_{11} – C_{17}	Changes in serum chemistry and liver weight	100	1,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic	0.1	Anon, 1990

2.1.4 C_9 - C_{18} (MA DEP) Aliphatic Fractions Inhalation RfC

2.1.4.1 Summaries of Toxicity Studies. Various toxicity studies that were identified by the TPHCWG and the MA DEP are summarized in the following paragraphs.

Isoparaffinic Hydrocarbons (IPH): C_{10} - C_{11}. Male and female rats were exposed to 0, 1,910, 5,620 mg/m^3 (0, 300, 900 ppm) isoparaffinic hydrocarbon (IPH) vapors (typical aromatic content 0.1%) for 6 hours/day, 5 days/week for 12 weeks (Phillips and Egan, 1984). Study animals were examined at 4, 8 and 12 weeks of exposure. Significant weight reduction was observed in male rats exposed to IPH at low and high exposure concentrations. In male rats exposed to IPH there was a significant decrease in erythrocytes after 12 weeks of exposure in both low and high exposure groups. No such effects were observed in female rats. Relative kidney weights were significantly increased in male rats exposed to 1,910 and 5,620 mg/m^3 IPH. In female rats transient increases in absolute and relative kidney weights were observed at 5,620 mg/m^3 at 8 weeks of exposure. At 5,226 mg/m^3, relative liver weights were significantly increased at 12 weeks of exposure and absolute and relative liver weights were increased at 4 weeks of exposure in male rats. According to the authors, the only treatment-related effects were the tubular nephrotoxicity in male rats. It was stated in the paper that the observed effects in the kidney are consistent with a mechanism that appears to be unique to male rats and not relevant to humans. However, no data were presented to prove whether the kidney effect is truly α_{2u}-globulin nephropathy.

The LOAEL identified in this study is 5,226 mg/m^3 based on changes in blood chemistry, body and liver weight changes. The LOAELwas adjusted for continuous exposure (5,226 mg/m^3 x 6 hours/24 hours x 5 days /7 days to give 933 mg/m^3). An uncertainty factor of 3,000 (10 for human sensitivity, 10 for animal to human extrapolation, 10 for subchronic to chronic adjustment and 3 for LOAEL to NOAEL extrapolation) was applied to the duration adjusted LOAEL to give an RfC of 0.3 mg/m^3. An uncertainty factor of 3 instead of 10 was applied to extrapolate from LOAEL to NOAEL because the effects were not considered to be serious.

Dearomatized White Spirit (DAWS): C_7 - C_{11}. Male and female rats were exposed to 0, 1,970 and 5,610 mg/m^3 (0, 300, or 900 ppm) DAWS vapors (typical aromatic content 0.1%) for 6 hours/day, 5 days/week for 12 weeks (Phillips and Egan, 1984). Mean body weights in male rats were significantly reduced in the high exposure group. Significant reductions in erythrocyte counts were observed in male and female rats in the low exposure group. This result was difficult to interpret since no such effects were observed in the high exposure group. Relative kidney and liver weights were significantly increased in male rats in the high exposure group. In female rats, significant increases in

relative liver weights were observed at 5,610 mg/m^3.

The LOAEL identified in this study is 5,610 mg/m^3 based on changes in body and liver weights. The LOAEL was adjusted for continuous exposure (5,610 mg/m^3 x 6 hours/24 hours x 5 days/7 days = 1,002 mg/m^3). An uncertainty factor of 3,000 (10 for sensitive individuals, 10 for animal to human extrapolation and 10 for subchronic to chronic adjustment, and 3 for LOAEL to NOAEL adjustment) was applied to derive an RfC of 0.3 mg/m^3. An uncertainty factor of 3 instead of 10 was applied to extrapolate from LOAEL to NOAEL because the effects were not considered to be serious.

In another study identified by MA DEP, rats were exposed to 0, 2,620, 5,253 mg/m^3 (0, 400 or 800 ppm) DAWS for 6 hours/day, 5 days/ week for 6 months (Lund et al., 1995). After an exposure-free period of 2-6 months duration, neurophysiological, neurobehavioral, and microscopic pathologic examinations were performed. The study demonstrated exposure-related changes in sensory evoked potentials, and a decrease in motor activity during dark periods. No changes in learning and memory functions were observed. The measurements of the flash evoked potential (FEP), somatosensory evoked potential (SEP), and auditory brain stem responses (ABR) all revealed changes in the later latency peaks, which reflect the more associative aspects of sensory processing. According to the authors, the results demonstrated that 6 months of exposure to DAWS induced long-lasting and possibly irreversible effects in the nervous system of the rat. No NOAEL was observed in this study. The LOAEL is determined by MA DEP to be 2,620 mg/m^3 (400 ppm). Adjusting this LOAEL to continuous exposure (2,620 mg/m^3 x 6 hours/24 hours x 5 days/7 days = 468 mg/m^3) and applying an uncertainty factor of 3,000 (10 for human variability, 10 for animal to human extrapolation, 10 for adjusting for LOAEL to NOAEL, and 3 to adjust for less than lifetime exposure) resulted in an RfC of 0.2 mg/m^3. An uncertainty factor of 3 instead of 10 was applied for subchronic to chronic extrapolation since the exposure was for six months and caused irreversible effects.

In acute exposure animal studies, white spirit with low aromatic content produced significant response reductions of learned performances (Kulig, 1990). Increased levels in brain noradrenaline, dopamine, and 5-hydroxytryptamine were observed in rats exposed to various levels of white spirit (Lam et al., 1992). Changes in indices of oxidative stress were reported in animals exposed to this compound for 3 weeks (Lam et al., 1994).

2.1.4.2. Discussion and Recommendation

The neurotoxicity study of Lund et al. (1995) revealed that exposure of rats to DAWS for six months induced long-lasting and possibly irreversible effects in the nervous system. The test system used in that study was an improvement over the subjective studies normally used to measure neurobehavioral effects of toxicants. The tests reflect the functions of the nervous system directly. The measures have been shown to be highly reproducible both within and between individuals and almost equivalent among different species. Long lasting functional impairments of the nervous system are generally found at lower exposure concentrations than those causing morphological changes. For instance, exposure of painters to white spirit caused neuropsychological disorders which led to early disability work status. In most of the studies, workers were exposed to mixtures of organic solvents with the principal component being white spirit. The effects were mainly functional disturbances in the central nervous system including memory and learning impairments (see Lund et al. (1995) and references therein). Functional impairments of the nervous system are suggested as a criterion for neurotoxicity (Lund et al., 1995). These results suggest that neurotoxicity may be a more sensitive endpoint than other effects observed in animals exposed to DAWS and IPH. **MA DEP has therefore derived an RfC of 0.2 mg/m^3 based on neurotoxicity** for the $C_9 - C_{18}$ aliphatic fraction described in the Lund et al. (1995) study. This number is similar to the value obtained (0.3 mg/m^3) when other systemic effects are used as a basis for an RfC.

For comparison, the TPHCWG derived an inhalation RfC of 1.0 mg/m^3 using the Phillips and Egan (1984) study on $C_{10} - C_{11}$ and $C_7 - C_{11}$ hydrocarbons and a study on JP-8 by Mattie et al. (1991).

2.1.5 C_{19} - C_{32} Aliphatic Fraction. Oral RfD

2.1.5.1 Basis for Existing Toxicity Values. The MA DEP grouped together alkanes C_{19} and longer and used eicosane as a reference compound for the range. The toxicity value was derived from a lifetime dietary feeding study (API, 1992) of white mineral oil, a complex mixture of C_{15} - C_{50} saturated hydrocarbons with low toxicity. A NOAEL of up to 6,000 mg/kg/d was reported in that study. By applying an uncertainty factor of 1,000 to this NOAEL (10 for subchronic exposure, 10 to account for animal to human extrapolation, and 10 to protect sensitive individuals), an RfD of 6.0 mg/kg/d was derived by MA DEP.

2.1.5.2 Summaries of Toxicity Studies on White Mineral Oils.

Numerous subchronic and chronic feeding studies have been conducted on a wide range of food grade white oils and waxes (see review by Miller et al., 1996). One fairly extensive rat study with mineral oils by Smith et al. (1996) is the only significant study that has been identified after the API (1992) study used for MADEP's original toxicity value. The TPHCWG identified this study as the basis for its toxicity value for this

fraction. Having reviewed their evaluation, MA DEP is in agreement with the basis for the derivation of their oral RfD.

White mineral oils are complex mixtures of highly refined mineral hydrocarbons consisting primarily of saturated paraffinic hydrocarbons (predominantly branched chain alkanes) and naphthenic hydrocarbons (alkanes containing one or more saturated cyclic structures). These oils are pure aliphatic hydrocarbons with no aromatic components and other contaminants. They are approved by the US Food and Drug Administration as direct food additives and also widely used in cosmetics and pharmaceutical products.

The Smith et al. (1996) study was a subchronic feeding study of seven white mineral oils representing different molecular weight fractions (Table 10). Male and female Fisher 344 rats were administered a range of white mineral oils mixed in the diet at concentrations of 20, 200, 2,000 and 20,000 ppm for 13 weeks. The daily intake of white mineral oils was

Table 9. Inhalation Studies and RfCs for the C_9 - C_{18} Fraction

Fraction	Critical Effects	Duration-Adjusted LOAEL (mg/m^3)	Uncertainty Factors	Inhalation RfC mg/m^3	Source
$C_{10} - C_{11}$	Changes in blood chemistry, body and liver weight	933	3,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic 3 – for LOAEL to NOAEL	0.3	Philips and Egan, 1984
$C_7 - C_{11}$	Changes in body & liver weight	1,002	3,000 10 – for animal to human 10 – for human variability 10 – for subchronic to chronic	0.3	Philips and Egan, 1984
DWS C_8- C_{12}	**Neurotoxicity**	**468**	**3,000** **10 – for animal to human** **10 – for human variability** **3 – for subchronic to chronic** **10 – for LOAEL to NOAEL**	**0.2**	**Lund et al., 1995**

Footnote: bolded study basis for fractional RfC.

approximated to be equal to 2, 20, 200 and 2,000 mg/kg/d. Histopathologic effects consistently occurred in the liver and mesenteric lymph nodes. The effects were dose related, were of greater magnitude in females than in males, and were greater with lower molecular weight test materials than with the higher molecular weight oils.

Histopathologic effects in the liver, considered the critical effect for RfD development, were classified by size as granulomas or microgranulomas. Granulomas consisted of focal collections of macrophages surrounded by inflammatory cells, and variable degrees of fibrosis. Microgranulomas were small collections of macrophages with few lymphocytes at the periphery. The incidence of liver granuloma/microgranuloma was significantly increased in female rats treated with 2,000 mg/kg/d with the lower molecular weight (C_{17} - C_{34}) mineral oils, (average MW 240-280). The higher MW white mineral oils ($C_{>34}$, average MW >480) were without effect.

The histopathological effects seen in the mesenteric lymph nodes consisted of focal collection macrophages often in the cortical region of the lymph nodes in female and male mice treated with the lower molecular weight oils. As in the liver, the high molecular weight oils were without effect. The focal collections of macrophages were classified as histiocytosis. These responses occurred at lower doses than those at which liver granulomas occurred. However, the lymph node responses have not been considered to be an adverse effect because they are a normal adaptive response to the ingestion of foreign material (Shuurman et al., 1994).

Table 10. Test Materials and Physical Properties

Sample	Crude type	Refining Method	Viscosity (cSt)		Average Mol. weight	Average Carbon # distribution
			40°C	100°C		
N10A	naphthenic	Acid treatment	13.3	3.1	320	**C_{15-30}**
N15H	naphthenic	Hydrogenation	16.6	3.4	330	**C_{17-30}**
P15H	paraffinic	Hydrogenation	15	3.5	350	**C_{18-30}**
N70A	naphthenic	Acid treatment	76.4	7.9	410	**C_{21-35}**
N70H	naphthenic	Hydrogenation	68	7.6	420	**C_{22-37}**
P70H	paraffinic	Hydrogenation	69.5	8.6	485	**C_{27-43}**
P100H	paraffinic	Hydrogenation	99.8	11	510	**C_{28-45}**

Sample abbreviation: N = naphthenic; P = paraffinic; A = acid-treated; H = hydrogenated;
number (10, 15, 70, 100) approximate viscosity at 40°C . Data adopted from Smith et al. (1996)

Other observed effects at the high dose (2,000 mg/kg/d) included significantly increased lymph node, liver, spleen and kidney weights, and markedly decreased red blood cell, and significantly increased white blood cell counts with one or more of the oils tested. The increased white blood cell counts were accompanied by increased reticulocyte, lymphocyte and eosinophil.

The NOAEL for the low molecular weight oils (C_{17} - C_{34}) was identified as 200 mg/kg/d. A factor of 100 was applied to the NOAEL to derive an RfD of 2 mg/kg/d (Table 11). The value of 100 represents uncertainity factors of 3 for animal to human extrapolation, 10 for human variability, and 3 for subchronic to chronic extrapolation.

The NOAEL identified for the high molecular weight oils containing paraffinic hydrocarbons with carbon numbers beyond those considered with this policy ($C_{>34}$) was 2,000 mg/kg/d. An RfD of 20 mg/kg/d was derived by the TPHCWG by applying an uncertainty factor of 100 (3 for animal to human extrapolation, 10 for human variability, and 3 for subchronic to chronic extrapolation) to the NOAEL. The trend of increases in oral RfD magnitudes (i.e., representing decreasing toxicity) between hydrocarbon fractions of increasing molecular size is consistent with the trend first identified in MA DEP's (1994) original document on the hydrocarbon fractions.

The justification for using uncertainty factors less than 10 for animal to human and subchronic to chronic extrapolations was that exposures of humans to both natural dietary oils and white mineral hydrocarbons (MHC) have not been associated with any known clinical effects. MHC-induced lipid granulomas found in human tissues are characterized as being benign, circumscribed lesions containing mineral oils in the center (Wanless and Geddie, 1985), as opposed to the lesions detected in F/344 rats which are reactive and associated with inflammation and occasional parenchymal cell necrosis. F/344 rats are more sensitive than many other species (Shubik et al., 1962; McKee et al., 1987; Firriolo et al., 1995; Smith et al., 1995) for the observed inflammatory effects of mineral oils. These effects did not appear to progress to tumors when rats were fed chlorinated paraffin with molecular size similar to low molecular weight mineral oils (NTP, 1986).

Table 11. Oral Studies and RfDs for the C_{19} - C_{32} fraction

Fraction	Critical Effects	NOAEL (mg/kg/d)	Uncertainty Factors	Oral RfD (mg/kg/d)
$C_9 - C_{32}$	Liver granuloma	200	100 3 – for animal to human 10 – for human variability 3 – for subchronic to chronic	2
$C_{>34}$	Changes in body and liver weight	2,000	100 3 – for animal to human 10 – for human variability 3 – for subchronic to chronic	20

2.1.5.3 Discussion and Recommendation.

MA DEP recommends a new RfD of 2.0 mg/kg/d for the C_{19} - C_{32} aliphatic fraction, which is lower than the previous MA DEP value of 6 mg/kg/d. The differences between the two values stem from the data sets used to derive the numbers.

The original MA DEP RfD value for this fraction was based on a lifetime feeding study in rats reported in an API (1992) document which identified NOAELs ranging from 1,200 to 6,000 mg/kg/d. The types and purities of the oils used were not described, although the oils contained compounds which were larger than the upper end size cutoff for the fraction being considered here. The presence of these less toxic compounds (see identification of RfD for $C_{>34}$ of 20 mg/kg/d above) probably influenced the overall toxicity of the mixture that was tested, resulting in an RfD that may have been an overestimate of the appropriate RfD for the C_{19}-C_{32} aliphatic fraction.

The study selected to derive an RfD of 2.0 mg/kg/d for the C_{19}-C_{32} aliphatic fraction appears to be a reasonable, preferable choice over the study originally selected by MA DEP because:

(i) the well-designed study used seven highly refined mineral oils representing a full range of these types of products (C_{15}-C_{45});

(ii) the effects of the mineral oils appeared to be inversely related to molecular weight. The lower molecular weight (C_{17}-C_{34}) mineral oils demonstrated effects in the liver and mesenteric lymph nodes. Only minimal effects such as increased liver and spleen weights, elevated alanine aminotransferase levels (P70H), and increased aspartate aminotransferase levels (P100H) were observed with these higher molecular weight ($C_{>34}$) mineral oils at the highest dose tested.

(iii) the RfD based on these studies is more appropriate than that previously used because effects were seen at lower doses in these experiments than in the earlier studies;

(iv) the minimal effect with the higher molecular weight mineral oils is consistent with studies showing no absorption for alkanes above C_{32} (Albro and Fishbein, 1970).

In closing the discussion of this section, it is important to note that emerging studies suggest that petroleum distillate exposures may be associated with the occurrence of autoimmune diseases in humans and animals. In humans, exposures to petroleum distillates appear to increase the risk of undifferentiated connective tissue diseases (UCTD) (Lacey et al., 1999). This case control epidemiological study of connective tissue disorders in occupationally exposed people reported odds ratios (1.81 and 2.73 for "mineral spirits" and "paint thinners or removers" respectively) for UCTD. The study at best seems to suggest a relationship between solvent exposure and connective tissue disease. Exposures were poorly characterized or not reported, and no compositional characterization of materials that the individuals were exposed to was provided. Mineral spirits/paint thinners are broadly defined classes of hydrocarbons typically containing hydrocarbons between C_9 and C_{11} with 10-30% aromatic content and the residual being paraffinic (Irwin, 1997). The

direct relevance of effects data from exposures to these compounds to probable effects in the C_{19}-C_{32} aliphatics range would be limited because of the involvement of smaller compounds outside of the size range being considered here and the contribution of toxicity from the usually more toxic aromatic compounds (c.f. oral RfD for C_{19}-C_{32} aliphatics of 2 mg/kg/d versus that of 0.03 mg/kg/d for aromatics encompassing the size range (C_9-C_{32})).

Rodent studies with mineral oils and one particular compound from this fraction (pristane) provide more specific, supporting evidence for a relationship between autoimmune disease induction and exposures to petroleum hydrocarbons, particularly this fraction. Shaheen et al. (1999) and Richards et al. (1999, 2001) investigated the ability of pristane (2,6,10,14-tetramethylpentadecane, a low molecular weight component of white mineral oil), to induce lupus-like syndrome in various nonautoimmune mice. A single intaperitoneal (IP) (0.5 ml) injection of pristane to mice induced lipogranuloma formation consisting of phygocytic cells that engulfed the oil and lymphocytes. The animals also developed antibodies characteristic of systemic lupus erythmatosus (SLE), accompanied by severe glomerulonephritis with immune complex deposition, mesangial or mesangiocapillary proliferation and protein urea. The authors stated that although the precise mechanism of the induction of SLE by pristane remained unclear, they speculated that the injection of pristane and other oils might stimulate immunologic pathways mimicking those involved in the idiopathic form of SLE, and that such studies might lead to information concerning possible human health risks associated with ingestion and inhalation of mineral oils and exposure to hydrocarbons in the environment (Shaheen et al., 1999). Other authors have also been able to consistently induce the same set of clinical and histopathological symptoms after IP pristane injection in other mouse strains.

Neither the human nor the mouse studies relating petroleum distillates to connective tissue diseases are appropriate for deriving a reference dose for the C_{19}- C_{32} aliphatic fraction. The limitations of the human studies were previously noted and those of the mouse studies are that the routes of exposure were intraperitoneal and there were no dose-response data. Literature searches have not identified experiments with compounds other than pristane in the fraction of interest, or with mixtures of compounds in the $C_{19} - C_{32}$ aliphatic carbon range linking exposure to these compounds with connective tissue disease.

Also, in a long-term study recently completed in Fischer 344 rats, the lymph node histiocytosis did not appear to result in complications and toxicity in the lymph nodes, and a study conducted by the industry to assess the ability of mineral oils to modify immune function found very little effect if any (pers. comm. Dr. David Hattan, U.S. Food and Drug Administration, Office of Food and Additive Safety). MA DEP is trying to procure these studies for further analysis.

A recent assessment of the state of the science on environmental agent exposures and autoimmune disease characterizes it as being in the early stages of hazard identification (Cooper et al., 1999). There are a number of issues which will need to be addressed in order to justify extrapolation of the toxicology of SLE in rodents to humans. Some of

them are:

- the role of genetic and hormonal factors in determining responses to hydrocarbon exposures;
- dose-response dynamics for autoimmune responses;
- the broader applicability of results with pristane to the remainder of this aliphatic fraction.

The responses being documented in rodents after exposure to these compounds suggest that more severe effects than liver granuloma formation or mesenteric lymph node histocytosis are being elicited.

Given the nascent stage of the science on this issue at the present time, MA DEP will retain the oral RfD recommended above for this fraction. A more in-depth assessment of the literature dealing with petroleum hydrocarbon exposures and the pathogenesis of SLE will be conducted in the near future and the oral RfD for this fraction could be updated if the science supports it.

2.1.6 C_{19} - C_{32} Aliphatic Fraction Inhalation RfC

No appropriate inhalation toxicity data were identified for individual components or fractions in the C_{19}-C_{32} aliphatic carbon range. This may be because hydrocarbon constituents in this fraction are not volatile and inhalation is not a likely exposure pathway. However, as in the high molecular weight aromatic hydrocarbons, aliphatic compounds in C_{17}-C_{32} carbon range can bind to soil particles. Inhalation exposure to respirable particulates containing high molecular weight PHCs is possible; but there are no data to estimate inhalation toxicity to particulate-bound hydrocarbons.

2.2 AROMATIC FRACTION TOXICITY VALUES

2.2.1 C_6 - C_8 Aromatic Compounds Oral RfDs.

In the MA DEP fractions approach (MA DEP, 1994), aromatic hydrocarbons with fewer than nine carbon atoms (benzene, toluene, ethylbenzene, styrene, xylenes) are evaluated on a compound-specific basis. This is because each of the aromatic hydrocarbons in this carbon range has extensive databases and most have toxicity values. US EPA derived RfDs were available for styrene (0.2 mg/kg/d), ethylbenzene (0.1 mg/kg/d,), toluene, (0.2 mg/kg/d), and o-, m-, and p-xylene (2 mg/kg/d). The toluene RfD is under review by the US EPA and the proposed RfD is 0.04 mg/kg/d (US EPA, 2002). No toxicity data were identified on mixtures in the carbon range specified above.

MA DEP continues to recommend not including these compounds with less than nine carbons in the carbon range approach, but rather evaluating them individually.

The approach selected by the TPHCWG was to use a representative RfD of 0.2 mg/kg/d for the fraction and this value is included for comparison.

2.2.2 C_6 - C_8 Aromatic Compounds Inhalation RfC.

US EPA derived RfCs are available for all of the compounds in this fraction except for xylenes and benzene. ATSDR has also developed inhalation minimal risk levels (MRLs) for toluene and xylenes. A fraction-specific inhalation RfC has been assigned to the group representing carbon ranges $C_{>7}$ - C_8 by the TPHCWG (Table 12).

2.2.2.1 Summaries of Toxicity Studies. The rationales for the development of the toxicity values by the different groups are briefly discussed below.

Ethylbenzene. The ATSDR (1990a) and US EPA (1995) have summarized the inhalation toxicity of ethylbenzene. It, like many other organic solvents, affects the central nervous system (CNS) and it is a mucous membrane irritant upon acute high level exposures.

One of the main human health concerns for inhaled ethylbenzene is its suspected developmental and reproductive effects (ATSDR, 1990a). This end point was the basis of the U. S. EPA's inhalation RfC for this compound (US EPA, 1995). They evaluated two inhalation studies conducted with rats and rabbits exposed for 6-7 hours/day, 7 days/week during days 1-19 and 1-24 of gestation to 434 or 4,342 mg/m^3 (100 or 1,000 ppm) of ethylbenzene respectively. A separate group of rats was also exposed pregestationally for 3 weeks prior to mating and exposure was continued into the gestational period (US EPA, 1995).

In rabbits, the only adverse effect noted was that the number of live kits per litter was significantly reduced at the high exposure concentration. The NOAEL for this study was 434 mg/m^3 (US EPA, 1995).

In rats exposed during gestation, a significantly increased incidence of abnormal ribs was observed in the high exposure group and an elevated incidence of extra ribs occurred in both the high and low exposure groups. Both absolute and relative liver, kidney, and spleen weights were significantly increased in the high exposure group pregnant rats. In the rats exposed for 3 weeks pregestationally, there was an increased incidence of extra ribs in the high exposure group. Only relative kidney weights were significantly increased in this group. In the rat study, as in the rabbit study, 434 mg/m^3 (100 ppm) was considered a NOAEL (US EPA, 1995).

Table 12 Aromatic TPH Components and Fractions with Inhalation Toxicity Values.

Carbon Range	Compounds	US EPA RfC mg/m^3	ATSDR MRL mg/m^3	TPHCWG Fractional RfC mg/m^3
C_6 - C_8 (MA DEP) $C_{>7}$-C_8 (TPHCWG)	Benzene	NA	NA	0.4
	Toluene	0.4*****	1.0 (0.3)*	
	Ethylbenzene	1.0	NA	
	Styrene	1.0	NA	
	Xylene (o-, p-, m-)	NA	0.4 (0.1)*	
$C_9 - C_{18}$ (MA DEP) $C_{>8} - C_{16}$ (TPHCWG)	Isopropylbenzene	0.4	NA	NA
	Naphthalene	0.003	0.01	NA
	Acenaphthene	NA	NA	NA
	Biphenyl	NA	NA	NA
	Fluorene	NA	NA	NA
	Anthracene	NA	NA	NA
	Fluoranthene	NA	NA	NA
	Pyrene	NA	NA	NA
	C_9 aromatic mixtures**	NA	NA	0.2***
	C_9 aromatic mixtures**	NA	NA	1.3
$C_9 - C_{32}$ (MA DEP) $C_{>16} - C_{35}$ (TPHCWG)	NA	NA	NA	NA

* adjusted for continuous exposure by MA DEP
** based on C_9 hydrocarbon mixture studies
*** also selected as the surrogate RfC for the $C_{>8}$ - C_{16} aromatic fraction by MA DEP
***** The RfC is under review and the proposed RfC by the US EPA (2002) is 4 mg/m^3

The US EPA (1995) derived an RfC of 1 mg/m^3 based on these NOAELs using an uncertainty factor of 300 (10 to account for sensitive individuals, 10 to adjust for the absence of multigenerational reproductive and chronic studies and 3 for animal to human extrapolation).

Styrene. Styrene causes CNS effects and mucous membrane irritation at high exposure concentrations in people who breathe large amounts of styrene for a short time. The CNS effects include depression, concentration problems, muscle weakness, tiredness, and nausea. Styrene is also a suspected human and animal carcinogen (ATSDR, 1990b).

The US EPA (1993b) used an occupational study which examined neuropsychological functions in 50 workers whose mean duration of styrene exposure was 8.6 years (SD of 4.5). The air concentration of styrene was estimated to be 43 – 1,282 mg/m^3 (10-300 ppm). Workers with absence of

metabolic and neurologic disorders, smoking habits of 20 cigarettes/day and alcohol intake of 80 ml ethanol/day were chosen. These same eligibility criteria were used to select a control group of 50 workers that were matched for age, sex, and educational level. The exposed workers were further segregated with 4 subgroups (n=9-14) according to increasing styrene exposure.

The critical endpoints considered were neuropsychological effects such as visuo-motor speed, memory and intellectual function. Correlation analysis of the test results and styrene exposure levels showed clear concentration response correspondence in at least three of the eight tests (memory, intellectual function, and visuo-motor speed). When the results were analyzed using duration of exposure as a covariate, increases in reaction times and decreases in memory and concentration were apparent. A NOAEL of 94 mg/m^3 (22 ppm) was determined from this study and an RfC of 1 mg/m^3 was estimated by adjusting the occupational exposure to continuous exposure and by applying an uncertainty factor of 30 (3 for data inadequacy, 3 to account for sensitive individuals and 3 to account for lack of chronic study).

Toluene. Toluene inhalation primarily affects the CNS and mucous membranes. Acute CNS effects include CNS depression, neurological dysfunction, and narcosis. Chronic exposures have resulted in permanent effects such as ataxia, tremors, and impaired speech, vision and hearing. Cardiac arrhythmia and hepatic effects have been reported. Developmental and reproductive effects were produced in toluene exposed animals. *In vitro* and *in vivo* tests demonstrated that toluene is not genotoxic (ATSDR, 1994).

Neurologic disorders are the main human health concerns from chronic exposure to toluene. The USEPA (1997b) has derived an inhalation RfC of 0.4 mg/m^3 based on a chronic occupational study of Foo et al. (1990). The exposed workers scored lower in 6 of 8 of the tests administered when compared to controls from the same workplace who were not exposed to toluene. The toluene exposure concentrations were 49 mg/m^3 (13 ppm) in controls and 332 mg/m^3 (88 ppm) in exposed workers. The occupational LOAEL used to derive the RfC was 332 mg/m^3. The duration-adjusted LOAEL was estimated to be 119 mg/m^3. An uncertainty factor of 300 (10 for human variability, 10 for use of a LOAEL and 3 for database deficiencies) was applied to the LOAEL to estimate an RfC of 0.4 mg/m^3. The toluene RfC is under review by the US EPA and the proposed RfC is 4 mg/m^3 (US EPA, 2002).

The ATSDR (1994) has also developed a minimal risk level (MRL) of 0.4 ppm ($\approx$ 1.0 mg/m^3) based on a chronic duration occupational study of

Orbaek and Nise (1989). Exposed workers had more neurasthenic complaints than control subjects. Workers with many neurasthenic complaints did not perform as well. The occupational exposure LOAEL used to estimate the MRL was 43 mg/m^3 (11.6 ppm). An uncertainty factor of 30 (3 for using minimally adverse LOAEL and 10 for human variability) was applied to the occupational LOAEL to derive the MRL.

Both the US EPA and the ATSDR used chronic occupational exposure studies to derive inhalation toxicity values. Although the occupational LOAEL used by the US EPA (332 mg/m^3) was higher than the occupational LOAEL used by the ATSDR (43 mg/m^3), the inhalation toxicity value derived by ASTDR was equivalent to that estimated by the U. S. EPA. This is mainly because the ATSDR did not adjust the occupational LOAEL for a continuous exposure scenario. It is not clear why the above adjustment was not made. If such an adjustment were made to the occupational LOAEL, the adjusted MRL would be about 0.3 mg/m^3.

Xylenes. The major effects of xylenes are on the central nervous system. High vapor exposures cause CNS effects including headache, nausea, mental confusion, dizziness, tremors, unconsciousness and coma. Other reported adverse effects include hepatic, renal, cardiac, respiratory and developmental abnormalities. Genotoxicity tests for xylenes have been negative (ATSDR, 1995a).

The ATSDR (1995a) derived a chronic duration MRL of 0.6 mg/m^3 using an occupational study. Workers (175) were exposed to time weighted average (TWA) concentration of xylenes of 61 mg/m^3 (14 ppm) for an average of 7 years. Mixed xylene exposures accounted for 70% or more of the total exposure. No hematological, hepatic or renal effects were observed. The occupational NOAEL was 61 mg/m^3 and was used to derive the chronic duration MRL. The ATSDR did not adjust the occupational LOAEL for continuous exposure. If such an adjustment were made to the NOAEL, the adjusted MRL becomes about 0.1 mg/m^3.

2.2.2.2 Discussion and Recommendation. MA DEP defines this fraction to include benzene and continues to recommend evaluating the potential toxicities of the chemicals in this group individually because of the availability of good compound-specific toxicity information.

In contrast, the TPHCWG selected the fractional approach and picked an RfC of 0.4 mg/m^3 as a representative value for $C_{>7} - C_8$ aromatic subset from the existing US EPA derived RfCs for toluene, ethylbenzene, styrene and xylene isomers presented in Table 11.

2.2.3 C_9 - C_{32} Aromatic Fraction Oral RfD

The entire range of C_9 through C_{32} aromatic hydrocarbon compounds have been grouped as a single fraction. Alkenes with the same carbon range were also evaluated similarly to aromatics in this fraction (MA DEP, 1994).

US EPA-derived RfDs were available only for 8 (acenaphthene, anthracene, biphenyl, fluorene, fluoranthene, isopropylbenzene (cumene), naphthalene, and pyrene) of the compounds in this carbon range (Table 13). The RfDs ranged from 0.02 to 0.3 mg/kg/d. Other data for naphthalene, 1-methylnaphthalene and fluoranthene not used as a basis for these RfDs shed additional light on target organs and relative potencies. An oral study of 1-methylnaphthalene suggested that the target site for ingested 1-methylnaphthalene might be the pulmonary tissues. Chronic oral administration of 1-methylnaphthalene was associated with significantly increased nodular alveolar proteinosis in male and female mice. A significant increase in pulmonary adenoma was observed in males (Murata et al., 1993). The ATSDR identified 71.6 mg/kg/d as the LOAEL for alveolar proteinosis and derived an MRL of 0.07 mg/kg/d by applying an uncertainty factor of 1,000 (10 for LOAEL to NOAEL extrapolation, 10 for animal to human extrapolation and 10 to account for sensitive individuals) to the LOAEL.

Table 13. US EPA-Derived Oral Toxicity Values for Compounds in the C_9 - C_{32} Aromatic Fraction

Carbon number	Compounds	RfD mg/kg/d
C_9	isopropylbenzene	0.1
C_{10}	naphthalene	0.02
C_{12}	acenaphthene	0.06
C_{12}	biphenyl	0.05
C_{13}	fluorene	0.04
C_{14}	anthracene	0.3
C_{16}	fluoranthene	0.04
C_{16}	pyrene	0.03

The US EPA derived a chronic RfD of 0.02 mg/kg/d for naphthalene based on a subchronic gavage study in rats (US EPA, 1998). The duration-adjusted NOAEL identified in the study was 71 mg/kg/d based on decreased mean terminal body weight in male rats. The RfD was estimated by applying an uncertainty factor of 3,000 (10 to extrapolate from rats to humans, 10 to protect sensitive humans, 10 to extrapolate from subchronic to chronic exposure and 3 for database deficiencies including lack of chronic oral exposure studies and 2-generation reproductive studies).

After consideration of this information, MA DEP continues to recommend the pyrene RfD of 0.03 mg/kg/d for the $C_9 - C_{32}$ aromatic fraction. Since naphthalene and 2-methylnaphthalene are target analytes in the Massachusetts Contingency Plan (MCP) risk characterization process, their toxicities are evaluated separately and not as a part of the

$C_9 - C_{32}$ fraction. Individuals who use the TPH methodology for non-MCP purposes should consider also evaluating the risks posed by naphthalene and 2-methylnaphthalene exposures separately and not as part of the $C_9 - C_{32}$ fraction.

1-methylnaphthalene, a structural analog of naphthalene and 2-methylnaphthalene, has not had an oral RfD until recently and has not been evaluated as a target analyte under the MCP. The ATSDR-derived chronic oral MRL of 0.07 mg/kg/d is higher than the recommended fractional RfD of 0.03 mg/kg/d for the $C_9 - C_{32}$ aromatic subgroup. Since it is structurally similar to naphthalene and 2-methylnaphthalene, and has an oral toxicity value, it would be justifiable to treat 1-methylnaphthalene as a target analyte. However, a policy decision has been made to address the toxicity of 1-methylnaphthalene by integrating it into the aromatic C_9-C_{32} fraction mass, and then using the fractional RfD of 0.03 mg/kg/d for risk evaluation purposes. This treatment for this compound will tend to overestimate the potential toxicity of this particular compound; however, it is present in such low concentrations (<1.5 weight percent) in mixed fuels (jet fuels, diesel and #2 fuel oils) (TPHCWG, 1997a) that the numerical overestimate of toxicity should not be of much toxicological significance.

In comparison to the MADEP value, the TPHCWG recommended an RfD of 0.04 mg/kg/d for the fractions in the $C_9 - C_{16}$ carbon range, and 0.03 mg/kg/d for $C_{>16}$- C_{35} carbon range.

Table 14. Basis for Oral Toxicity Values for the $C_9 - C_{32}$ Aromatic Fraction

Species	Fractions Tested	Endpoint	$NOAEL_{adj}$	UF Applied	RfD mg/kg/d
Rat	$C_9 - C_{32}$	Kidney effects	75	3,000 10 – animal to humans 10 – human variability 10 – subchronic to chronic 3 – data deficiency	0.03 (USEPA, 2003)

2.2.4 $C_9 - C_{18}$ Aromatic Fraction Inhalation RfCs

The fate and transport section of the TPHCWG report series identified 77 individual hydrocarbons within the C_9 - C_{16} carbon range (TPHCWG, 1997a). Of these compounds, a US EPA derived RfC was identified for only isopropylbenzene (C_9) (0.4 mg/m^3), commonly known as cumene (US EPA, 1997a). Naphthalene (C_{10}) is another compound in the C_9 - C_{16} carbon range that has since had a chronic inhalation toxicity value of 0.003 mg/m^3 derived by the US EPA (Table 12).

2.2.4.1 Summaries of Toxicity Studies. Recent inhalation studies on trimethylbenzene isomers were identified. No other data on any of the other individual hydrocarbon components of the C_9 - C_{16} fraction were found. Three published inhalation studies on C_9

aromatic mixtures were discussed by the TPHCWG. The data on isopropylbenzene, naphthalene, trimethylbenzenes and the C_9 aromatic mixtures are briefly summarized below:

Isopropylbenzene. Isopropylbenzene (cumene) is a potent narcotic, skin and mucous membrane irritant. It is absorbed through the intact skin more rapidly than toluene, xylene or ethylbenzene. In some short term, high dose experiments, animals exhibited damage to the spleen and fatty changes to the liver, but no renal or pulmonary irritancy (Sandmeyer, 1981). Two successive subchronic inhalation toxicity studies on isopropylbenzene were evaluated by the US EPA for the RfC derivation (EPA, 1997a). In the first study, groups of rats were exposed to 0, 492, 2,438 or 5,909 mg/m^3 (0, 199, 496, or 1,202 ppm) isopropylbenzene vapor for 6 hours/day, 5 days/week for 13 weeks. In the second study, the group size was reduced and an additional group (246 mg/m^3) was added to incorporate a 4-week post exposure group.

The critical treatment-related effects were increased relative and absolute kidney weights in female rats, and increased relative and absolute adrenal weights in both sexes at the highest concentrations (5,909 mg/m^3) tested. A NOAEL of 2,438 mg/m^3 was selected to derive the RfC of 0.4 mg/m^3. The NOAEL was adjusted for continuous exposure (2,438 x 6 hours/24 hours x 5 days/7 days = 435 mg/m^3) and an uncertainty factor of 1,000 (10 for subchronic to chronic extrapolation, 10 for animal to human extrapolation, 3 for sensitive individuals and 3 for database deficiency in reproductive effects) was applied to the NOAEL to derive the inhalation RfC.

Naphthalene. Naphthalene is a hematopoietic and pulmonary toxicant. Male and female mice were exposed to 0, 52, or 157 mg/m^3 (0, 10, or 30 ppm) of naphthalene, for 6 hours/day, 5 days/week for two years (NTP, 1992). Both sex groups demonstrated chronic inflammation and metaplasia of the olfactory epithelium, hyperplasia of the respiratory epithelium and dose-related increases in inflammatory lesions of the lungs. The US EPA (1998) selected 52 mg/m^3 as LOAEL. The duration-adjusted and the human equivalent LOAEL that was calculated using dosimetric adjustment was 9.3 mg/m^3. An uncertainty factor of 3,000 (10 to extrapolate from mice to humans, 10 to protect sensitive humans, 10 to extrapolate from LOAEL to NOAEL, and 3 for database deficiencies including the lack of a 2-generation study and chronic inhalation studies for other animal species) was applied to derive an RfC of 0.003 mg/m^3. ATSDR used 52 mg/m^3 as a LOAEL and derived a chronic inhalation minimal risk level (MRL) of 0.01 mg/m^3 (ATSDR, 1995b). The difference between the US EPA derived RfC and the MRL derived by ATSDR is the additional uncertainty factor that was appropriately applied for database deficiency by the US EPA.

Trimethylbenzene Isomers. Rats were exposed to 0, 123, 491, and 1,227

mg/m^3 (0, 25, 100, or 200 ppm) 1,2,4-trimethylbenzene (TMB) 6 hours/day, 5 days/week for 4 weeks (Gralewicz et al., 1997). Behavioral tests such as radial maze performance, open field activity, passive avoidance and shock-induced changes in pain sensitivity were conducted between days 14 and 54 after exposure.

No change in body weight gain was observed in any of the treated groups. Significant changes in CNS function as demonstrated by the behavioral tests were observed in the groups treated with 491 and 1,227 mg/m^3 of TMB. The NOAEL in this study was 123 mg/m^3. The authors concluded that exposure to 1,2,4-trimethylbenzene might lead to long-lasting changes in the functional state of the CNS. They further discussed that behavioral effects observed between days 21 and 54 after the last exposure may not be due to the presence of TMB or its metabolites in the rat CNS since TMB is metabolized and eliminated quickly.

Another observation discussed in the paper was that the lower concentration (492 mg/m^3) was more potent in the neurobehavioral effects assessment than the higher concentration (1,227 mg/m^3). This phenomenon was not an experimental artifact since it was also observed in another study conducted in the same laboratory using 1,2,3-trimethylbenzene. Although no mechanism was proposed for the pronounced toxicity at the lower exposure concentrations, further studies were recommended to elucidate the mechanism. Understanding the mechanism of the low exposure level toxicities to these chemicals is important since it is relevant to environmental exposure.

The suggested mechanism for the neurobehavioral toxicity is alterations in the utilization and turnover of biogenic amines in the brain. This hypothesis is inferred from such effects observed in animals exposed to other methylated benzenes (toluene and xylenes). The dopaminergic system is particularly vulnerable to methylbenzenes.

In another experiment, subchronic exposure of rats to 1,2,4-TMB or 1,2,3-TMB at concentrations of 0, 123, 491, and 1,227 mg/m^3 (0, 25, 100, and 200 ppm) caused the same concentration-dependent behavioral effects. The neurotoxic effects of 1,2,3-TMB were more pronounced than the effects observed with 1,2,4-TMB (Korsak and Rydzynski, 1996). The NOAEL in this study was also 123 mg/m^3.

An RfC 0.02 mg/m^3 can be derived from the above identified NOAEL of 123 mg/m^3 by adjusting for continuous (123 x 6 hours/24 hours x 5 days/7 days = 22 mg/m^3) exposure and by applying an uncertainty factor of 1,000 (10 for subchronic to chronic extrapolation, 10 for animal to human extrapolation, and 10 to account for sensitive individuals).

Aromatic Mixtures. Naphthenes are catalytically converted to aromatic compounds to make high-octane gasoline blending components. A portion of this wide-boiling point range hydrocarbon stream can be separated by distillation and used for other purposes. One such distillate is a mixture composed primarily of 9-carbon aromatic compounds usually consisting of isomers of ethyltoluene (28%) and trimethylbenzene (40 - 55%). Other C_9 minor components include isopropylbenzene (3%), n-propylbenzene (4%), and other aromatics containing more than 10 carbon atoms (6%). The percentages of the components may differ slightly from one distillate to another. These C_9 aromatic mixtures are commonly known as high flash aromatic naphtha (HFAN) and are used mainly as solvents (Douglas et al., 1993). The various studies on HFAN are summarized below:

Neurotoxicity of C_9 Mixtures. Male rats were exposed by inhalation to HFAN for 90 days at concentrations of 0, 490, 2,544 or 7,362 mg/m^3 (0, 100, 500 or 1,500 ppm) for 6 hours/day, 5 days/week to investigate the neurotoxicity of the solvent (Douglas et al., 1993). During the testing period, animals were examined monthly for motor activity and a functional observation battery of tests was applied which consisted of tests for hind limb grip strength, audio startle response, thermal response, and hind foot splay. Selected nervous system tissues were examined histopathologically.

Significant weight reduction was observed in animals exposed to the highest concentrations (7,362 mg/m^3). No histopathologic effects on the nervous tissues and no neurobehavioral abnormalities were observed in any of the treated groups.

The LOAEL identified in this study was 7,362 mg/m^3 based on significant weight reduction in animals. The same level of exposure caused high mortality rates and CNS effects in pregnant mice (McKee et al., 1990). By adjusting the LOAEL for continuous exposure (7,362 mg/m^3 x 6 hours/24 hours x 5days/7days = 1,315 mg/m^3) and using an uncertainty factor of 10,000 (10 to account for human variability, 10 for subchronic to chronic extrapolation, 10 for animal to human extrapolation, 3 for LOAEL to NOAEL adjustment and 3 for database, an RfC of 0.1 mg/m^3 can be derived. A factor of 3 and not 10 was considered for LOAEL to NOAEL extrapolation because the effect was not considered to be serious. The rationale for an additional factor of 3 for database deficiency is discussed in Section 2.2.4.2.

Systemic Toxicity. Clark et. al. (1989) exposed male rats to high flash aromatic naphtha vapors at 0, 450, 900 or 1,800 mg/m^3, 6 hours/day, 5 days/week for 12 months. Transient reduction in body weight gain was

observed in male and female rats that did not last through the duration of the study. Hematological and clinical chemistry tests did not show any consistent dose-related effects. A possible increase in male "aggression" at the highest concentration was believed to be related to treatment. There was also a significant increase in male liver and kidney weights in the high exposure group.

The NOAEL identified in this study based on hepatic effects and CNS effects in male rats is 900 mg/m^3. By adjusting the NOAEL for continuous exposure (900 mg/m^3 x 6 hours/24 hours x 5 days/7 days = 160) and by applying an uncertainty factor of 3,000 (10 for animal to human extrapolation, 10 for human variability and 10 for subchronic to chronic extrapolation and 3 for database deficiency) (rationale for the need of uncertainty factor for database deficiency is presented in the discussion Section 2.2.4.2), an RfC of 0.05 mg/m^3 was estimated.

Developmental/Reproductive Toxicity. Mice were exposed by inhalation to high flash aromatic naphtha (HFAN) vapors at 0, 100, 500 or 1,500 ppm (0, 491, 2,544, or 7,362 mg/m^3) for 6 hours/day during gestational days 6-15 (McKee et al., 1990). A three generation reproductive study was also conducted in rats exposed to 0, 100, 500, 1,500 ppm (0, 491, 2,544 or 7,362 mg/m^3) of high flash aromatic naphtha.

The highest exposure concentration (7,362 mg/m^3) caused 44% mortality in pregnant mice.

Other clinical observations in pregnant mice included significantly reduced weight gain, ataxia, labored breathing, hunched posture, weakness, inadequate grooming, and circling. Maternal body weight gain was also significantly reduced at the medium exposure concentration (2,544 mg/m^3). At the lowest exposure concentration (491 mg/m^3), maternal body weight was reduced, but not significantly. A marked decrease in hematocrit was observed in the dams exposed to the highest concentrations.

There was also evidence of developmental toxicity at the highest exposure concentration. The number of live fetuses per litter and the mean fetal body weight were significantly reduced. Post implantation loss was markedly elevated, ossification was delayed and the number of fetuses with cleft palate was increased. At the medium (2,544 mg/m^3) exposure level, maternal and fetal body weights were significantly reduced. Minimal maternal weight reduction was observed at the low (491 mg/m^3) exposure level. This concentration was determined to be a NOAEL for maternal and developmental toxicity. In a three-generation reproductive study, rats were exposed to 0, 490, 2,544 or 7,362 mg/m^3 (0, 100, 500 or 1,500 ppm) HFAN (McKee et al., 1990).

All the F0 males survived to the scheduled sacrifice with significant weight reduction in both the 2,544 and 7,362 mg/m^3 exposure groups. Within the highest exposure group there was 23% mortality in female rats (about 12% prior to mating and about 12% during gestation and lactation). Body weight gain was also significantly reduced in both the medium and high exposure groups. No other significant effects were observed.

In the F1 generation, birth weights were not significantly different from controls at any exposure level. However, mean body weights were significantly reduced in pups in the high exposure group when maternal exposure was continued through the lactational period. No other reproductive effects were observed.

In the F2 generation, CNS effects manifested as ataxia and reduced motor activity were observed in the high exposure group. Also, 20% of the exposed females died (10% during gestation, 5% during delivery and 5% during lactation). The fraction of live-born offspring was slightly but significantly reduced in the high exposure group.

In the F3 generation, the F2 pups used to produce the F3 generation were exposed at an earlier age than pups exposed in the previous studies and most of the animals (36/40 males and 34/40 females) exposed to the highest concentration died in the first week of exposure. Body weights of pups from surviving dams exposed to the high exposure concentration were significantly reduced at birth, but were not significantly reduced at lactation day 4. As in the previous generations, once maternal exposure was initiated, body weight gain of the pups in the high dose group was significantly less than controls. The concentration that did not produce adverse effects in the three generation reproductive study is 100 ppm (490 mg/m^3).

The developmental and the reproductive studies suggest that mice are the more sensitive species to the developmental effects of HFAN. At the highest concentration (7,362 mg/m^3), there was 44% mortality, clinical signs of toxicity including CNS effects in pregnant mice, and severe developmental effects in the pups. This concentration caused only 23% mortality in female rats with only significant weight reduction in survivors.

The female animals are also more sensitive to HFAN toxicity. No mortalities were observed in male rats exposed to concentrations that were lethal to female rats under the same exposure conditions.

The reproductive effects in the three-generation rat study are manifested as reduced weight gain in the offspring at the highest exposure concentrations. No other major reproductive effects were observed. Since mice appear to be

the most sensitive species for the developmental toxicity of HFAN, reproductive studies using these species are recommended for any definitive conclusions about the reproductive toxicity of HFAN.

In another developmental/reproductive study, rats were exposed to Armatol (a branched chain product conforming to the specifications of high flash aromatic naphtha) vapor at 0, 589, 982 or 1,963 mg/m^3 for 24 hours/day from day 7 to 15 of gestation (Ungvary et al., 1983). Maternal weight gain during gestation was slightly but significantly reduced at all exposure levels. Exposure of rats to 982 or 1,963 mg/m^3 resulted in developmental delays. The developmental NOAEL in this study was 589 mg/m^3. Maternal body weight was significantly but slightly reduced at these exposure levels. No maternal NOAEL was identified in this study.

2.2.4.2 Discussion and Recommendation. The developmental and maternal NOAEL from the McKee et al. (1990) mouse study was 491 mg/m^3 and the developmental NOAEL from the Ungvary et al. (1983) rat study was 589 mg/m^3. No maternal NOAEL was identified in the Ungvary et al. study. The studies reviewed have demonstrated that pregnant animals are more sensitive than their non-pregnant counterparts and the male animals tested. In male rats, reduced weight gain was the only anomaly observed at the highest exposure concentration (7,362 mg/m^3) (API, 1990c), while this concentration was lethal to 22% of pregnant and non- pregnant female rats and 44% of pregnant female mice.

Although the developmental and reproductive NOAELs are lower than the NOAEL identified for systemic effects for HFAN, it is not health protective to derive a chronic toxicity value from the developmental data using the US EPA methodology because: the intermittent exposure concentrations are not adjusted to reflect continuous exposure, and (2) the exposures are considered acute. The NOAEL based on systemic effects (900 mg/m^3) is twice as high as the developmental NOAEL (491 mg/m^3) and the reproductive NOAEL (490 mg/m^3). The reproductive studies were conducted in rats which are less sensitive species than mice. Thus the reproductive NOAEL based on rat studies may not be appropriate to derive a protective health number for the $C_9 - C_{16}$ fraction. More reproductive studies in sensitive species are recommended, especially since studies in humans indicate that exposure to low levels of organic solvents disrupt the brain neurotransmitter levels and the endocrine system, ultimately affecting fertility (Reutman et al., 2002).

The available RfCs for the individual compounds (isopropylbenzene, naphthalene and trimethylbenzenes) in the $C_9 - C_{16}$ aromatic range and the RfCs for the C_9 aromatic mixtures are presented in Table 15.

Data on mixtures are an appropriate choice for deriving fractional RfCs. However, as reflected by the magnitude of the uncertainty factors applied to the identified NOAELs and LOAELs, confidence in the RfCs based on the HFAN (C_9 mixtures) studies is low. Uncertainty factors for database deficiency were applied because of lack of appropriate

reproductive data in most sensitive species. Moreover, another question regarding a surrogate RfC derived using C_9 aromatic mixtures is whether it is representative of all the components in the C_9 - C_{16} petroleum hydrocarbon aromatic subgroup. While an RfC derived based on C_9 mixtures may be a representative value for the volatile alkyl benzenes, the volatile aromatics possessing more than one ring in their structures may not be represented. This assumption is based on metabolic and toxicity considerations.

Polycyclic aromatic hydrocarbons (PAHs) having two to three rings (naphthalene, acenaphthene, anthracene, fluorene, pheneanthrene) are present in air predominantly in the vapor phase (ATSDR, 1995c). PAHs that have four rings (fluoranthene, and pyrene) exist both in the vapor and particulate phase (ATSDR, 1995c). Exposure to PAHs that exist in both the particulate and vapor phase via inhalation is possible. However, it is not within the scope of this report to address toxicity from particulate inhalation. The data indicate that inhalation could be a pathway for exposure to the volatile PAHs such as acenaphthene, anthracene, fluorene, fluoranthene, naphthalene, 2-methylnaphthalene and phenanthrene and the semi-volatiles such as fluoranthene and pyrene. All these PAHs fall within the C_9 - C_{16} aromatic subgroup.

Those individuals using this approach subject to the requirements of the MCP guidance should note that these PAHs are targeted analytes, having their own toxicity values. They are not evaluated as part of the C_9-C_{16} aromatic subgroup, but individually for this and other programmatic reasons.

The most investigated alkylated benzenes such as toluene and xylenes are primarily metabolized through side chain oxidation (Philpot and Smith, 1984). The principal identified cytochrome P450 isozymes that metabolize these compounds are the phenobarbital inducible cytochrome *P*4502B family which oxidize substrates in conformationally unhindered positions giving products that are easily conjugated and eliminated (Philpot and Smith, 1984; Ionnides and Park, 1987). It is reasonable to assume that side chain oxidation of other alkylated benzenes may be mediated by the same enzyme system.

The polycyclic aromatic hydrocarbons on the other hand are mainly metabolized by the 3-methylcholanthrene-inducible cytochrome *P*4501A family (Ionnides and Park, 1987). These isozymes metabolize chemical carcinogens at conformationally hindered positions resulting in reactive metabolites that are poor substrates for subsequent conjugation and detoxication. While these PAH metabolizing enzymes have been characterized using the carcinogenic PAHs like benzo(a)pyrene, such studies are lacking for the lower molecular weight PAHs. Recently, an enzyme inhibition study using naphthalene showed that one of the enzymes that may metabolize naphthalene was cytochrome *P*-4501A; the other being cytochrome *P*-4503A (Tingle et al., 1993). Phenanthrene is also metabolized by cytochrome *P*-4501A (Shou et al., 1994).

Thus the methylbenzenes and the PAHs included in the C_9 - C_{16} carbon range may not

compete for primary oxidation pathways in their metabolism and they also may have different toxicities. This differential toxicity is exemplified by the pulmonary cytotoxicity of naphthalene and the CNS toxicities of alkylbenzenes.

It is therefore health protective to apply an uncertainty factor ranging between 3 and 10 to the C_9 aromatic mixture data to account for a database deficiency. Prior to the availability of an RfC for naphthalene it might have been justifiable to use an uncertainty factor of 10, but with the information conveyed by the naphthalene RfC, a UF of 3 seems justified because previous uncertainty about these compounds' toxicities has now been addressed by the toxicity information for them and by evaluating their toxicity separately as target analytes. The uncertainty factor for database deficiency was reduced to 3 to address the lack of toxicity information on non-PAH compounds in the $C_9 - C_{16}$ aromatic fraction range thus giving an RfC of 0.05 mg/m^3. The available RfCs that can be derived from the various studies for this fraction are presented in Table 15.

Although naphthalene's RfC is lower than the others presented in Table 15, it is not selected as a representative RfC for the fraction since naphthalene is: (1) a target analyte in the MCP; and (2) basing the fractional RfC on naphthalene toxicity data would be unduly conservative. Its structural analog, 2-methylnaphthalene, is also a target analyte. Individuals who use the TPH methodology should also evaluate naphthalene and 2-methylnaphthalene separately. As discussed previously, the MCP guideline does not treat 1-methylnaphthalene as a target analyte and its toxicity is evaluated based on the fractional RfC.

Thus, MA DEP recommends an RfC value of 0.05 mg/m^3 as a surrogate toxicity number for the C_9 - C_{18} aromatic TPH fraction which is based on mixture studies.

In comparison, the TPHCWG derived an RfC of 0.2 mg/m^3 for the fraction.

Table 15. Inhalation Toxicity Values for Individual C_9 - C_{18} Fraction Components or Mixtures

Species	Compound/ Fractions Tested	Endpoint	NOAEL/ $LOAEL_{adj}$ (mg/m^3)*	UF Applied	RfC mg/m^3
Rat	Isopropyl benzene	Change in kidney and liver weight	435 ($NOAEL_{adj}$)	1,000 10 – animal to human. 3 – human variability 10 – subchronic to chronic 3 – data deficiency	0.4 (US EPA, 1997a)
Mice	Naphthalene	Respiratory tract toxicity	9.3 ($LOAEL_{adj}$)	3,000 10 – animal to human. 10 – human variability 10 – LOAEL to NOAEL 3 – database deficiency	0.003 (US EPA, 1998)
Rats	**Trimethylbenzene isomers**	**Neurotoxicity**	**22 ($NOAEL_{adj}$)**	**1,000** **10 – animal to human.** **10 – human variability** **10 – subchronic to chronic**	**0.02**
Rats	High flash aromatic naphtha (HFAN) or C_9 mixtures	Weight reduction	1,315 ($LOAEL_{adj}$)	10,000 10 – animal to human. 10 – human variability 3 – LOAEL to NOAEL 3 – database deficiency 10 – subchronic to chronic	0.1
Rats	High flash aromatic naphtha	CNS effects and change in organ weight	160 ($NOAEL_{adj}$)	3,000 10 – animal to human. 10 – human variability 3 – database deficiency 10 – subchronic to chronic	0.05

* Duration adjusted NOAEL/LOAEL

Bolded information is basis for fractional RfC

2.2.5 C_{19} - C_{32} Aromatic Fraction – Inhalation RfC

No appropriate data were identified to support development of inhalation RfCs for the individual components or mixtures in this carbon range, although some of the PAHs in this group (chrysene and benzo(a)anthracene) can partially exist in the vapor phase in the ambient air. The high molecular weight PAHs like benzo(a)pyrene and dibenz(g,h,i)perylene exist primarily in the particulate phase in air (ATSDR, 1995c). The compounds in this carbon range are not very volatile and inhalation of gaseous compounds is not a likely route of exposure. However, it should be noted that the high molecular weight aromatic hydrocarbons can bind to soil particles because of their high K_{oc}, and inhalation exposure to these chemicals may depend on inhaled particulate matter. No data exist to estimate toxicity value for soil-bound and inhaled C_{17} - C_{35} PAHs.

3.0 CONCLUSIONS AND RECOMMENDATIONS

MA DEP in 1994 developed the first fractional approach to evaluate human health risks from oral exposures to mixtures of petroleum hydrocarbon compounds (PHC) and developed oral reference doses (RfDs) for various PHCs fractions. However, fraction-specific toxicity values for inhalation exposures were not derived. Subsequent to this effort, the national ad hoc workgroup known as the TPH Criteria Working Group (TPHCWG) introduced a modified version of the fractional approach and derived fraction-specific oral RfDs and inhalation reference concentrations (RfCs).

MA DEP has used data available after its 1994 work to update its oral toxicity values and identify inhalation RfCs for the volatile petroleum hydrocarbon fractions specified in 1994.

MA DEP recommends the following oral (Table 16) and inhalation toxicity values (Table 17) for the fractions.

Table 16. MA DEP Oral Toxicity Values

Carbon Range*	2002 MA DEP Recommended Values (mg/kg/d)	Critical Effect
Aliphatic		
C_5 - C_8	0.04	Neurotoxicity
C_9-C_{18}	0.1	Hepatic and hematological
C_{19} - C_{32}	2.0	Liver granuloma
Aromatic		
C_6 - C_8	Evaluate each chemical in the series separately	
C9 – C32	0.03	Nephrotoxicity

Table 17. MA DEP Inhalation Toxicity Values

Carbon Range*	2002 Recommended Values (mg/m^3)	Critical Effect
Aliphatic		
C_5 - C_8	0.2	Neurotoxicity
C_9-C_{18}	0.2	Neurotoxicity
C_{19} - C_{32}	NA*	NA
Aromatic		
C_6 - C_8	Use individual RfCs for compounds in this range	
C_9-C_{18}	0.05	Body weight reduction, hepatic, renal, and developmental effects
C_{19}-C_{32}	NA	

* NA = Not applicable. Compounds in this size range not volatile.

1. Aliphatics C_5 - C_8: The chronic commercial hexane studies demonstrated that an inhalation exposure to a hexane mixture containing 53% n-hexane produced no neurotoxicity in rodents. However, and most importantly, other chronic human and animal studies showed that commercial hexane causes peripheral neuropathy when given orally or by inhalation. In addition, many potential diketone metabolites of n-alkanes produce peripheral neurotoxicity. **Until appropriate data on individual components of the fraction or mixtures are found, MA DEP will use an oral toxicity surrogate value of 0.04 mg/kg/d for this fraction, based on a well-designed oral toxicity study on n-hexane.**

Although a chronic intermittent commercial hexane (51%) exposure up to 9,000 ppm

(API, 1990a) showed no peripheral neurotoxicity, there are studies demonstrating that continuous commercial hexane exposure up to 1,000 ppm (IRDC, 1981) caused peripheral neurotoxicity. Moreover, other studies demonstrated that commercial hexane mixtures containing only 12% n-hexane or other aliphatic mixtures in the series caused neurotoxicity in humans and animals. **Until data on mixtures or individual compounds are found, MA DEP recommends using the US EPA derived RfC for n-hexane (0.2 mg/m^3) as a surrogate toxicity value for this fraction. The endpoint in the study was neurotoxicity.**

2. Aliphatic C_9 - C_{18}: New oral gavage studies on various petroleum streams covering C_9 - C_{17} carbon ranges were used to derive an oral RfD of 0.1 mg/kg/d. The observed adverse effects in the treated animals included body weight, organ weight, and blood chemistry changes. These newer, well designed and executed studies are a better and more defensible choice for use in derivation of the RfD for this fraction than that which was previously presented by MA DEP in the 1994 Interim report**. MA DEP therefore recommends adoption of the toxicity value of 0.1 mg/kg/d for this fraction.**

As previously discussed, a recent neurotoxicity study revealed that exposure of rats to dearomatized white spirit for six months induced long-lasting and possibly irreversible effects in the nervous system. The RfC based on this study was 0.2 mg/m^3. Emerging neurotoxicity data on white petroleum spirits supports use of an RfC based on neurotoxicity. Exposure of painters to white spirit resulted in neuropsychological disorders which led to early disability. In most of the studies, workers were exposed to mixtures of organic solvents, the principal component being white spirit. The effects were mainly functional disturbances in the central nervous system including memory and learning impairments.

In acute animal studies, white spirit with low aromatic content produced significant reductions in animal response to learned performances. Increased levels in brain noradrenaline, dopamine, and 5-hydroxytryptamine were observed in rats exposed to various levels of white spirit. Changes in indices of oxidative stress in the synaptosomes were also reported in animals exposed to white spirit for 3 weeks.

Since the rat study on which the RfC is based is a well-conducted study, it was chosen as the basis for **MA DEP's recommended inhalation toxicity value of 0.2 mg/m^3 for the C_9 - C_{18} aliphatic fraction.**

3. Aliphatics C_{19}-C_{32}: The oral RfD determined for this fraction based on a subchronic feeding study of several different highly refined white mineral oil samples representing various mineral hydrocarbon (MHC) sizes is 2.0 mg/kg/d. The low molecular weight (average molecular weight 320-420) MHC caused liver granulomas and mesenteric lymph node histiocytosis, while the high molecular weight MHC demonstrated minimal effect. This study was more recent and therefore a more defensible choice for derivation of the RfD for this fraction than that presented by MA DEP in 1994. **MA DEP therefore recommends an RfD of 2.0 mg/kg/d for the aliphatic fractions containing 19 through 32 carbon atoms.**

4. Aromatics C_6 - C_8: MA DEP recommends that chemical specific RfDs and RfCs

continue to be used for each of the compounds in the C_6 - C_8 aromatic range. This is because the toxicity values for each are well supported and these compounds have a wide range of toxicity. Thus, to use one surrogate value that is based on an average toxicity of the group would not allow for recognition of the potential toxicological contributions of the most toxic compounds in the group. Alternatively, to designate the surrogate based on the toxicity of the most toxic compound would over-estimate the risks associated with the less toxic compounds in the group.

5. Aromatics C_9 - C_{32}: The TPHCWG identified 77 individual compounds in this carbon range. US EPA derived RfDs were available only for 8 (acenaphthene, biphenyl, fluorene, anthracene, fluoranthene, naphthalene, and pyrene) of these compounds. The RfDs range from 0.03 to 3 mg/kg/d. The TPHCWG also derived an additional oral RfD of 0.03 mg/kg/d based on a naphthalene/methylnaphthalene mixture study. **MA DEP recommends that an RfD of 0.03 mg/kg/d for the aromatic hydrocarbons containing 9 through 32 carbons continue to be used to represent the toxicities of all compounds in this fraction**. As previously concluded by MA DEP (1994), use of other RfDs for subdivisions of this fraction may convey more certainty in the data than is warranted.

6. C_9 - C_{18} Aromatic Fraction. Although the C_9 - C_{32} compounds are grouped together and an oral surrogate value is assigned representing all compounds in the fraction, the more volatile compounds containing 9 to 16 carbons are subdivided for inhalation toxicity evaluation. Compounds containing carbon atoms ranging between 17 and 32 were considered to be non-volatile and toxicity values were not estimated for this subgroup. **MA DEP recommends an RfC of 0.05 mg/m^3 for this subgroup based on the evaluation of RfCs identified for individual components and fractional mixtures**.

4.0 REFERENCES

Abbritti, G., Siracusa, A., Cinanchetti, C., Coli, C.A., Curradi F., Perticoni, G.F., De Rosa, F.(1976). Shoe makers' polyneuropathy in Italy—The aetiological problem. Br. J. Ind. Med. 33:92-99.

Albro, P.W., and L. Fishbein. 1970. Absorption of aliphatic hydrocarbons by rats. Biochem. And Biophys. Acta 219:437-446.

Andrews, L. S. and Snyder, R. 1991. Toxic effects of solvents and vapors. In: Casarett and Doull's Toxicology: The Basic Science of Poisons (M.O. Amdur, J. Doull and C.D., Klaassen, Eds). pp 681-722. Pergamon Press, New York

Anonymous, 1990. Aliphatic petroleum hydrocarbon fluid aromatic content <0.05%, carbon range C11-C17. Completion Date: December 20, 1990. Study provided by American Petroleum Institute Washington, DC.

Anonymous, 1991a. 90 day subchronic oral toxicity study in rats. Aliphatic petroleum hydrocarbon fluid (less than 0.5% aromatics), boiling point range 180-210^{o}C, Carbon range C_9-C_{12}. Completion Date: October 24, 1991 under Guideline 82-1. Study provided by American Petroleum Institute, Washington, DC.

Anonymous, 1991b. 90 day oral toxicity study in the rat. Aliphatic petroleum hydrocarbon fluid. Carbon range C_{10}-C_{13}, aromatic content 0.1%. Completion date: October 15, 1991. Study provided by American Petroleum Institute, Washington, DC.

Anthony, D.C., Boekelheide, K., and Graham, D.G. 1983. The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione.Toxicol. Appl. Pharmacol. 71:362-371.

API, 1980. 26 Week Inhalation Toxicity Study of Heptane in the rat. Conducted by BioDynamics Inc. for the American Petroleum Institute, BioDynamics Project No. 78-7233

API 1989a. Developmental toxicity study of commercial hexane vapor in CD (Sprague-Dawley) rats. HESD Publ. N0. 36-33319. American Petroleum Institute. Washington, DC.

API 1989b. Developmental toxicity study of commercial hexane vapor in CD-1 mice. HESD Publ. No. 36-33318. American Petroleum Institute. Washington, DC.

API 1990a. A thirteen week inhalation toxicity study of commercial hexane on behavior and neuropharmacology in rats. HESD Publ. No. 37-31154. American Petroleum Institute. Washington, DC.

API 1990b. A thirteen week inhalation toxicity study of commercial hexane in the rat and mouse. Bio/Dynamics Inc. Project No. 89:81-91. American Petroleum Institute. Washington, DC.

API 1990c. Two generation study of inhaled commercial hexane in CD (Sprague-Dawely) rats. HESD Publ. No. 38-31216. American Petroleum Industry, Washington, DC.

API 1992. Mineral Oil Review. Departmental Report No. DR 21. Washington, DC.

API 1995. An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice. Part I and Part II. Toxicology Report No. 40:CAIS No. 41-33.231 and 41-33.232. American Petroleum Institute, Washington, DC.

ATSDR 1990a. Toxicological Profile for Ethylbenzene. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA

ATSDR 1990b. Toxicological Profile for Styrene. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA

ATSDR 1994. Toxicological Profile for Toluene. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

ATSDR. 1995a. Toxicological Profile for Xylenes Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA

ATSDR. 1995b. Toxicological Profile for Naphthalene, 1-Methylnaphthylene, 2-Methylnaphthalene, Update"; Draft for Public Comment. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

ATSDR 1995c. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA

.

ATSDR 1999. Toxicological Profile for Hexane. Department of Health and Human Services, US Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Browning, E. 1965. Toxicity and Metabolism of Industrial Solvents. Elsevier Publishing Co. New York. pp. 739.

Carpenter, C.P. et al. 1978. Petroleum Hydrocarbons toxicity studies XVII. Animal response to n-nonane. Vapor. Toxicol. Appl. Pharmacol. 44:53-61.

Cavigneaux, A. (1972). Polyneuritis caused by n-hexane. Cah. Notes Document. 67:199-202

Clark, D.G., Butterworth, S.T., Martin, J.G., Roderick, H.R., and Bird, M.G. 1989. Inhalation toxicity of high flash aromatic naphtha. Toxic Ind. Health. 5:415-428.

Cooper, G. S.; Germolec, D.; Heindel, J., and Selgrade, M. 1999. Linking environmental agents and autoimmune diseases. Environ Health Perspect. 107 Suppl 5:659-60.

Crespi, V., Costanzo, M.D., Ferrario, F., et al. 1979. Electrophysiological findings in workers exposed to n-heptane fumes. J. Neurol. 222:135-138.

Douglas, J.F., McKee, R.H., Cagen, S.Z., Schmitt, S.L., Beatty, P.W., Swanson, M.S., Schreiner, C.A., Ulrich, C.E., and Cockrell, B.Y. 1993. A neurotoxicity study assessment of high flash aromatic naphtha. Toxic. Ind. Health. 9:1047-1058.

Drinker, P., Yaglou, C.P., Warren, M.F. 1943. The threshold toxicity of gasoline vapor. J. Ind. Hyg. Toxicol. 57(2):163-172.

Dunnick, J.K., D.G. Graham, R.S. Yang, S.B. Haber, and H.R. Brown, 1989. Thirteen-week Toxicity Study of n-hexane in B6C3F1 Mice after Inhalation Exposure. Toxicology 57(2):163-172.

Firriolo, J.M., Morris, C.F., Trimmer, G.W., Twitty, L.D., Smith, J.H., and Freeman, J.J. 1995. Comparative 90-day feeding study with low viscosity mineral oil in Fischer 344 and Sprague-Dawley derived CRL:CD rats. Toxicol. Pathol. 23:26-33.

Filser, J.G., Csanady, Gy. A., Dietz, W., et al. 1996. Comparative estimation of the neurotoxic risks of n-hexane and n-heptane in rats and humans based on the formation of the metabolites, 2,5-hexanedione and 2,5-heptanedione. (Adv. Exp. Med. Biol. 387:411-427.

Foo, S.C., J. Jeyaratnam and D. Koh. 1990. Chronic neurobehavioral effects of toluene. Br. J. Ind. Med. 47(7): 480-484.

Frontali, N., Amantini, M.C., Spagnolo, A.M., and Saltari, M.C. 1981. Experimental neurotoxicity and urinary metabolites of the C_5 - C_7 aliphatic hydrocarbons used as glue solvents in shoe manufacture. Clin. Toxicol. 18:1357-1367.

Fuhner, H. 1921. The narcotic effect of gasoline and its components - pentane, hexane, heptane and octane. Biochem. Z. 115:235-261.

Gaultier, M., Rancurel, G., Piva, C., Efthymioc, M.L. 1973. Polyneuritis and aliphatic hydrocarbons. J. Eur. Toxicol. 6:294-296.

Gralewicz, S., Wiaderna, D., Tomas and Rydzynski, K. 1997. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in rat. Neurotox. Teratol. 19:327-333.

Graham, D.G., Amarnath, V., Valentine, W.M., Pyle, S.J., and Anthony, D.C. (1995). Pathogenic studies of hexane, and carbon disulfide neurotoxicity, Crit. Rev. Toxicol. 47:293-304.

Hutcheson, M.S., Pedersen, D., Anastas, N.D., Fitzgerald, J., and Silverman, D. 1996. Beyond TPH: Health-Based Evaluation of Petroleum Hydrocarbon Exposures. Regulatory Toxicology and Pharmacology 24:85-101.

Inoue, T., Takeuchi, Y., Takeuchi, S., Yamada, S., Suzuki, H., Matsushita, T., Miyagaki, H., Maeda, K., Matsmumoto, T. 1970. Industrial health survey of high incidence of n-hexane intoxication among vinyl sandal manufacturers. Jpn. J. Ind. Health. 12:73-84.

Ionnides, C., Park, D.V. 1987. The cytochrome P448-a unique family of enzymes involved in chemical toxicity and carcinogenesis. Biochem. Pharmacol. 36:4197-4207.

IRDC. 1981, Six-month continuous inhalation exposure of rats to hexane mixtures-Phase II. Unpublished study by International Research and Development Corporation, Mattawan MI. Prepared for the American Petroleum Institute, Washington DC. EPA-FYI-AX-0282-0166.

Irwin, R.J. 1997. Environmental contaminants encyclopedia. Mineral spirits entry. Report by National Park Service, Water Resources Division. Fort Collins, Colorado. 22 pp. (htto://www.nature.nps.gov/toxic/minspiri.pdf)

Katz, G.V., O'Donoghue, J.L., DiVincenzo, G.D., and Terhaar, D.J. 1980. The relative neurotoxicity of methyl-n-butyl ketone, n-hexane, and their metabolites. Toxicol. Appl. Pharmacol. 52:433-441.

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.

Krasavage, W.J., O'Donoghue, J.L., Divencenzo, G.D. and Erhaar, T. 1980. Relative neurotoxicity of MBK, n-hexane and their metabolites. Toxicol. Appl. Pharmacol. 52(3):433-441.

Kulig, B.M. (1990). Neurobehavioral effects of white spirit during acute and chronic exposure. Toxicologist 10:308 (Abstract).

Lacey, J.V., Jr., Garabrant, D.H., Laing, T., Gillespie, B.W., Mayes, M.D., Cooper, B.C., and Schottenfeld, D. 1999. Petroleum distillate solvents as risk factors for undifferentiated connective tissue disease (UCTD). Am. J. Epidemiol. 149:761-770.

Lam, H.R, Lof, A., Ladefoged, O. 1992. Brain concentration of white spirit components and neurotransmitters following a three week inhalation exposure of rats. Pharmacol. Toxicol. 70:394-396.

Lam, H.R., Ostergaard, G., Guo, S.X., Ladefoged, O., and Bondy, S.C. 1994. Three weeks' exposure of rats to dearomatized white spirit modifies indices of oxidative stress in brain, kidney and liver. Biochem. Pharmacol. 47:651-657.

Lund, S.P., Simonsen, L., Hass, U., Ladefoged, O., Lam, H.R., and Ostergaard, G. 1995. Dearomatized white spirit inhalation exposure causes long-lasting neurophysiological changes in rats. Neurotox. Terat.18:67-76.

MA DEP, 1994. Interim Final Petroleum Report: Development of Health-Based Alternative to the Total Petroleum Hydrocarbon (TPH) Parameter. Office of Research and Standards, Boston, MA. August, 1994.

MA DEP, 2002. Characterizing Risks Posed by Petroleum Contaminated Sites: Implementation of the MADEP VPH/EPH Approach. Final Policy #WSC-02-411. Massachusetts Department of Environmental Protection, Boston, MA October, 2002.

Matti, D.R., Alden, C.L., Newell, T.K., Gaworski, C.L. and Flemming, C.D. 1991. A 90-day continuous vapor inhalation study of JP-8 jet fuel followed by 20 or 21 months of recovery in Fischer 344 rats and C57bl/6 mice. Toxic. Pathol. 19:77-87.

Matti, D.R., Marit, G.B., Flemming, C.D. and Cooper, J.R. 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. Tox. Ind. Health 11:423-435.

McKee, R.H., Plutnick, R.T., and Traul, K.A. 1987. Assessment of potential reproductive and subchronic toxicity of EDS coal liquids. Toxicology 46:267-280.

McKee, R.H., Wong, Z.A., Schmitt, S.L., Beatty, P., Swanson, M., Schreiner, C.A., and Schardein, J.L. 1990. The reproductive and developmental toxicity of high flash aromatic naphtha. Toxic. Ind. Health. 6:441-460.

Miller, M.J., Lonardo, E.C., Greer, R.D., Bevan, C., Edwards, D.A., Smith, J.H., and Freeman, J.J. 1996. Variable responses of species and strains to white mineral oils and paraffin waxes. Reg. Toxicol. Pharmacol. 23:55-68.

Millner, G.C., James, R.C. and Nye, A.C. 1992. Human health-based soil cleanup guidelines for diesel fuel no. 2. Journal of Soil Contamination. 1(2):103-157.

Misumi, J., and Nagano, M. 1984. Neurophysiological studies on the relationship between structural properties and neurotoxicity of aliphatic hydrocarbon compounds in rats. Brit. J. Indus. Med. 41:526-532.

Miyagaki, H. 1967. Electrophysiological studies on the peripheral neurotoxicity of n-hexane. Jap. J. Ind. Health. 9:660-671.

Murata, T., Denda, A., Maruyama, H., et al., 1993. Chronic toxicity and carcinogenicity Studies of 1-methylnaphthalene in B6C3F1 mice. Fundam Appl Toxicol 21:44-51

NTP 1986. National Toxicology Program. Toxicology and carcinogenesis studies of chlorinated paraffins (C_{23}, 43% chlorine, CAS No. 63449-39-8) in F344IN rats and B6C3F1 mice (gavage studies). NTP Toxicity Report No. 86-2561:202.

NTP 1992. National Toxicology Program. Technical report series No. 410. Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in $B6C3F_1$ mice (inhalation studies). Research Triangle Park, NC: U. S. Department of Health and Human Services, US Public Health Service, National Institutes of Health. NIH Publication No. 92-3141.

O'Donoghue, J.L, and Krasavage, W.K. 1979. The structural-activity relationship of aliphatic diketones and their potential neurotoxicity. Toxicol. Appl. Pharmacol. 48:A55.

Ono, Y., Takeuchi, Y., Hisanga, N. 1981. A comparative study on the toxicity of n-hexane and its isomers on the peripheral nerve. Int. Arch. Occup. Environ. Health 48:289-294.

Orbaek, P., Nise, G. 1989. Neurasthenic complaints and psychometric function of toluene-exposed rotogravure printers. Am. J. Ind. Med. 16:67-77.

Passero, S., Battistini, N., Cioni, R., et al. 1983. Toxic polyneuropathy of shoe workers in Italy. A clinical, neurophysiological and follow-up study. Ital. J. Neurol. Sci. 4:463-472.

Phillips, R.D., and Egan, G.F. 1984. Subchronic inhalation exposure of dearomatized white spirit and C_{10} - C_{11} isoparaffinic hydrocarbon in Sprague-Dawley rats. Fundam. Appl. Tox. 4:808-818.

Philpot, R.M.and Smith, B.R. (1984). Role of Cytochrome *P*-450 and related enzymes in the pulmonary metabolism of xenobiotics. Environ. Health Perspect. 55:359-367.

Reutman, S.R., LeMasters, G.K., Knecht, E.A., Shukla, R., Lockey, J.E., Burroughs, G.E., Kesner, J. 2002. Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. Environ. Health Persp. 110:805-811.

Richards, H. B.; Satoh, M.; Jennette, J. C.; Okano, T.; Kanwar, Y. S., and Reeves, W. H. 1999. Disparate T cell requirements of two subsets of lupus-specific autoantibodies in pristane-treated mice. Clin Exp Immunol. 115(3):547-53.

Richards, H. B.; Satoh, M.; Jennette, J. C.; Croker, B. P.; Yoshida, H., and Reeves, W. H. 2001. Interferon-gamma is required for lupus nephritis in mice treated with the hydrocarbon oil pristane. Kidney Int. 60(6):2173-80.

Sandmeyer, E.E. 1981. Aliphatic hydrocarbons, In: Patty's Industrial Hygiene and Toxicology, 3rd Rev. Ed., Vol. 2B.Toxicology, G.D. Clayton and F.E., Clayton, Eds, pp. 3175-3194. John Wiley and Sons, New York..

Savolainen, H., Pfaffli, P. 1980. Neurochemical effects on rats of n-heptane inhalation exposure. Arch. Environ. Contam. Toxicol. 9:727-732.

Schmuck, G and Schluter, G.1996. An *in vitro* model for toxicological investigations for environmental neurotoxins in primary neuronal cell cultures. Toxicol. Ind. Health 12:683-696.

Schuurman, H.J., C.F. Kuper, and J.G. Vos. 1994. Histopathology of the immune system as a tool to assess immunotoxicity. Toxicology 86:187-212.

Shaheen, V. M., Satoh, M., Richards, H. B., Yoshida, H., Shaw, M., Jennette, J. C., and Reeves, W. H. 1999. Immunopathogenesis of environmentally induced lupus in mice. Environ Health Perspect. 107 Suppl 5:723-727.

Shou, M., Korzekwa, K.R., Krausz, K.W., Crespi, C.L., Gonzalea, F.J. 1994. Regio- and sterio-selective metabolism of phenanthrene by twelve cDNA-expressed human, rodent and rabbit cytochromes P-450. Cancer Lett. 83:305-313.

Shubik, P., Saffotti, U., Lijinsky, W., Pietra, G., Rappaport, H., Toth, B., Raha, C.R., Tomatis, L. Feldman, R., Ramhai, H. 1962. Studies of the toxicities of petroleum waxes. Toxicol. Appl. Pharmacol. 4(Suppl.):1-62.

Smith, J.H., Bird, M.G., Lewis, S.C., Freeman, J.J., Hogan, G.K., and Scala, R.A. 1995. Subchronic feeding study of four mineral oils in rats and dogs. Drug Chem. Toxicol. 18:83-103.

Smith, J.H., Mallett, A.k, Priston, R.A.J., Brantom, P.G., Worell, N.R., Sexsmith, C, and Simpson, B.J. 1996. Ninety-day feeding study in Fischer 344 rats of highly refined petroleum-derived food grade white oils and waxes. Toxicol. Pathol. 24:214-230.

Smith, D. A. and Germolec, D. R. 1999. Introduction to immunology and autoimmunity. Environ Health Perspect. 107 Suppl 5:661-5.

St. Clair, M.B., Amaranth, V., Moody, M.A., et al. 1988. Pyrrole oxidation and protein cross-linking as necessary steps in the development of gamma-diketone neuropathy. Chem. Res. Toxicol. 1:179-185.

Takeuchi, Y., Mabuci, C., Takagi, S. 1975. Polyneuropathy caused by petroleum benzine. Int. Arch. Arbeitsmed 34:185-197.

Takeuchi, Y., Ono, Y., Hisanga, N., Kitoh, J., and Sugiura, Y. 1980. Comparative study of n-pentane and n-heptane in the rat. Brit. J. Ind. Med. 37:241-247.

Takeuchi, Y., Ono, Y., Hisanga, N., Kitoh, J., and Sugiura, Y. 1981. A Comparative Study of n-pentane, n-hexane and n-heptane to the peripheral nerve of the rat. Clin. Toxicol.. 18:1395-1402.

Tingle, M D., Pirmohamed, M., Templeton, E., Wilson, A.S., Madden, S., Kitteringham, N.R., and Park, B.K. 1993. An investigation of the formation of cytotoxic, genotoxic, protein-reactive and stable metabolites from naphthalene by human liver microsomes. Biochem. Pharmacol. 9:1529-1538.

Topping, D.C., Morgott, D.A., David, R.M., and O'Donoghue, J.L. 1994. Ketones. In: Patty's Industrial Hygiene and Toxicology" 4th ed. G.D.Clayton and F.E Clayton, Eds PP 1739-1878. Volume IIC. Wiley and Sons Inc. New York.

TPHCWG. 1997a. Selection of representative TPH fractions based on fate and transport considerations. Vol 3. Total Petroleum Hydrocarbon Criteria Working Group. Amherst Scientific Publishers. Amherst, MA.

TPHCWG. 1997b. Development of Fraction-Specific Reference Doses (RfDs) and Reference Concentrations (RfCs) for Total Petroleum Hydrocarbons (TPH) Vol.4. Total Petroleum Hydrocarbon Criteria Working Group. Amherst Scientific Publishers. Amherst, MA.

Trauhaut, R., Laget, P., Piat, G., Nguyen Phu-Lich, Dutertre-Catella, H., Vu-Ngoc-Huyen. 1973. Preliminary electrophysiologic results following experimental poisoning with technical hexane and heptane in white rats. Arch. Mal. Prof. Med. Trav. Secur. Soc. 34:491-500.

Ungvary, G., Tatrai, E., Lorincz, M., Fittler, Z., Barcza, G. 1983. Investigation of the embryotoxic effects of armatol, a new C_9 aromatic mixture. Egeszsegtudomany 29:138-148.

US EPA. 1989. Health and environmental effects document for n-hexane. Prepared by the Office of Health and Environmental Assessment. Environmental Criteria and Assessment Office. Cincinnati, OH for the Office of Solid Waste and Emergency Response. Washington, DC.

US EPA. 1990. Interim Methods For Development of Inhalation Reference Concentrations. Environmental Criteria and Assessment Office. Office of Research and Development. US Environmental Protection Agency, Research Triangle Park, NC. 27711.

US EPA. 1993a. Integrated Risk Information System. Reference concentration for chronic inhalation exposure (RfC) for n-hexane. U.S Environmental Protection Agency. Washington, DC.

US EPA. 1993b. Integrated Risk Information System. Reference concentration for chronic inhalation exposure (RfC) for styrene. U.S Environmental Protection Agency. Washington, DC.

US EPA. 1995. Integrated Risk Information System. Reference concentration for chronic inhalation exposure (RfC) for ethylbenzene. US Environmental Protection Agency. Washington, DC.

US EPA. 1997a. Integrated Risk Information System. Reference concentration for chronic inhalation exposure (RfC) for cumene. U.S Environmental Protection Agency. Washington, DC.

US EPA. 1997b. Integrated Risk Information System. Reference concentration for chronic inhalation Exposure (RfC) for acrylonitrile. US Environmental Protection Agency, Washington, DC.

US EPA 1998. Integrated Risk Information System. Reference dose for chronic oral exposure for naphthalene. US Environmental Protection Agency. Washington, DC.

US EPA. 2002 Toxicological Review of Toluene (Draft). US Environmental Protection Agency. Washington, DC.

US EPA 2003. Integrated Risk Information System. Reference dose for chronic oral exposure for pyrene. US Environmental Protection Agency. Washington, DC.

Von Oettingen, W.F. 1940. Toxicity and potential dangers of aliphatic and aromatic hydrocarbons. US Public Health Bull. No. 225.

Wang, J.S., and Busby, W.F. 1993. Induction of lung and liver tumors by fluoranthene in preweanling CD-1 mouse bioassay. Carcinogenesis, 14:1871-1874.

Wanless, I.R., Geddie, W.R. 1985. Mineral oil lipogranulomata in liver and spleen. Arch. Pathol. Lab. Med. 109:283-286.

Yamada, S. 1972. Polyneuritis in workers exposed to n-hexane, its cause and symptoms. Jpn. J. Ind. Health. 9:651-659.

Yamamura, Y. 1969. N-Hexane polyneuropathy. Folia. Psychiatr. Neurol. Jpn. 23:45-57.