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HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

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CRUDE OIL CATEGORY
CATEGORY ASSESSMENT DOCUMENT

Submitted to the US EPA

by

The American Petroleum Institute
Petroleum HPV Testing Group

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Gasoline Blending Steams Mammalian Toxicity Summary (separate document)

Crude Oil Robust Summaries (separate document)

EXECUTIVE SUMMARY

Only one substance, Petroleum (CAS # 8002-05-9) is in the HPV Crude Oil Category. Petroleum is defined as *"A complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen and sulfur compounds. This category encompasses light, medium, and heavy petroleums, as well as the oils extracted from tar sands. Hydrocarbonaceous materials requiring major chemical changes for their recovery or conversion to petroleum refinery feedstocks such as crude shale oils, upgraded shale oils and liquid coal fuels are not included in this definition*". Throughout this document the common name "crude oil" is used for Petroleum.

Crude oil is a naturally occurring substance derived from the decomposition over thousands of years of plant and animal organic matter under elevated temperature and pressure. In appearance, crude oils range from mobile, volatile, light colored liquids to dark, viscous tar-like materials with low vapor pressure. Although, crude oils are assigned a single CAS #, they are generally classified by their density, predominant type of hydrocarbon (paraffinic or naphthenic) present, and whether their sulfur content is high (sour) or low (sweet).

Crude oil is a complex combination of hydrocarbons consisting predominantly of paraffinic (straight and branched-chain alkanes), naphthenic (cycloalkanes or cycloparaffins), and aromatic hydrocarbons. The hydrocarbons in crude oil have carbon numbers that range from four (C_4 or butanes), to large molecules containing more than sixty carbons. Sulfur, oxygen and nitrogen compounds, organometallic complexes notably of nickel and vanadium, and dissolved gases, such as hydrogen sulfide, are also found in crude oil. An "average" crude oil contains 84% carbon, 14% hydrogen, 1-3% sulfur, and approximately 1.0% nitrogen, 1.0% oxygen and 0.1% minerals and salts. Analytical studies indicate that similar hydrocarbons, heterocyclics, metals and other constituents, e.g. hydrogen sulfide, are present in all crude oils but their proportions vary depending on the crude source. The composition of crude oils from different producing regions, and even from within a particular geological formation, can vary widely.

Environmental

Due to their complex compositions, crude oils vary widely in their physical/chemical properties. Despite these differing physical and chemical characteristics, some generalizations can be made regarding the environmental behavior of crude oil. When a release to the environment occurs, components of crude oil will partition into various environmental compartments. The lower molecular weight components may dissolve in water or volatilize to the atmosphere, intermediate fractions may float and spread out on water where the fractions may form emulsions and/or adsorb to soil and sediment, and the viscous, heavy or high molecular weight components may agglomerate and float or sink in water or adhere to soil and sediment. The rate at which partitioning occurs depends not only on the nature of the crude but also on the severity of the weathering processes encountered.

Chemical and physical transformations occur as components of crude oil disperse. Constituents that partition to the air interact with hydroxyl radicals in the atmosphere and have half-lives ranging from 0.4 days to 6.5 days; examples of compounds at either end of the half-life range include n-dodecane and benzene, respectively. Crude oils are subject to biodegradation, but biodegradation rates vary considerably, and crude oils are not considered “readily biodegradable” in standard tests. Low molecular weight components may readily biodegrade, but as molecular weight increases, hydrocarbons become increasingly insoluble in water, so that their bioavailability is limited. In general, hydrocarbons are regarded as being inherently biodegradable, although the degradation rates of the more complex, high molecular weight fractions may be very low.

As a result of crude oil spills and continuous long-term releases of crude oil components, a plethora of real-world data is available on the acute and chronic environmental effects of crude oil. Crude oil is, in general, harmful to aquatic organisms. In both marine and freshwater environments a spill event may cause extensive mortality to non-motile susceptible species such as phytoplankton, crustaceans and larvae or eggs of fish and invertebrates. In contrast, spills of crude oil may not acutely affect highly mobile species such as adult fish. Also, mollusks and polychaete worms have an apparent tolerance to oil contamination.

Because crude oil is extracted from world-wide sources and composed of many different types and molecular weights of hydrocarbons, it may be impractical to assign specific acute aquatic toxicity values that cover the full domain of crude types. In general, aquatic toxicity of crude oil is not likely to be any greater than that represented by the most toxic fraction. For concentrations presented as loading rates, acute toxicity could potentially fall within the range of 1 – 10 mg/L. Acute toxicity is attributed to those water-soluble hydrocarbon components that are either saturates (aliphatic and alicyclic) or mono- and di-aromatics. Polycyclic aromatic hydrocarbons (PAHs) in crude oil are not expected to contribute significantly to acute aquatic toxicity due to their limited bioavailability. However, their partition coefficients, i.e. log K_{ow} 3 to >6, indicate a potential to bioaccumulate, thus the chronic toxicity of PAHs may be a concern.

Other risks to other aquatic species including semi-aquatic birds, and sea mammals include physical fouling of plumage, fur, gills etc, by floating oil product. This results in loss of buoyancy, insulation and smothering of intertidal animals. Ingestion of oil resulting from attempts by animals to clean contaminated body parts may result in toxicity, including severe enteritis.

Spills in freshwater environments have been shown to adversely affect the aquatic macroinvertebrate community, with the observed effects associated with oil sorption and substrate coating. Recovery of such communities in some habitats may be rapid, e.g., riffle areas of streams/rivers, while impacts to backwater areas may persist for months. Ultimately, the type of crude oil and the local conditions and habitats will dictate the extent to which crude oil persists and the potential effects in the environment.

Human Health Effects

Crude oils have demonstrated little local irritation or acute dermal toxicity (LD_{50} >2.0 g/kg dermal). However, exploration, production, and transport of crude oil can result in significant levels of hydrogen sulfide and/or volatile organic compounds (VOCs) in some situations (i.e., enclosed spaces). The acute inhalation hazard is primarily from hydrogen sulfide. When the acute toxicity of hydrogen sulfide was assessed in rats, the calculated LC_{50} for a 4-hour inhalation exposure was 444 ppm. VOCs from crude oil are similar to the hydrocarbons found in gasoline and gasoline blending streams. The results of acute toxicity testing indicate that these materials are not acutely toxic by the inhalation exposure route, e.g., Rat LC_{50} >5g/m^3.

Repeat dose and developmental studies on inhaled hydrogen sulfide have determined NOAECs of 10 ppm and 80 ppm, respectively. The inhalation NOAECs for repeat dose and developmental effects of VOCs from crude oil are read-across data from studies on gasoline and gasoline blending streams. These values are: Repeat Dose NOAEC: 1507mg/m^3 to 10,153mg/m^3 (427 – 2880ppm^3); Developmental NOAEC: 5970mg/m^3 to 27750mg/m^3 (1694 – 7873ppm^3); *[Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]*

In the repeat dose inhalation studies with hydrogen sulfide and gasoline blending streams, there were no specific adverse effect on reproductive organs. In addition, two multi-generation reproduction studies on gasoline vapor in rats have determined NOAECs of over 20,000 mg/m^3. This data supports the conclusion that hydrogen sulfide and VOCs from crude oil have limited potential to be reproductive toxicants.

In situations involving repeated dermal exposure, the constituents with the greatest potential for toxicity are the polycyclic aromatic compounds (PACs). Analysis of 46 crude oils showed significant variation in the PAC profile between samples. Solvent extracts of crude oils which concentrate the PAC constituents have induced gene mutations in bacteria. In contrast, the injection of mice with whole crude oil did not produce activity in micronucleus assays but did induce an increase in sister chromatid exchanges. Several samples of crude oil have produced skin-tumors in mice following long-term skin application.

Studies of repeated exposure by the dermal route have demonstrated toxicity that was manifested by aberrant hematology, liver enlargement and thymic atrophy. Measured and modeled toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 55 and 544 mg/kg/day.

In developmental toxicity studies in rats, crude oils, primarily at maternally toxic doses, caused fetal death, decreased fetal weight, delayed skeletal ossification and parturition. Measured and modeled developmental toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled

data on 46 crude oil samples were between 53 and 2000 mg/kg/day. Crude oil is not expected to be a reproductive toxicant since repeated dermal exposures to crude oil for 13-weeks have not produced adverse effects in the reproductive organs of either male or female rats.

The Testing Group believes that the potential for mutagenicity and the systemic toxicity, developmental toxicity and/or carcinogenic effects from repeated dermal exposure is related to the PAC profile of the specific crude oil.

1. DESCRIPTION OF CRUDE OIL

1.1 Types and Composition:

Crude oils range from light colored oils to thick, black oil similar to melted tar. All crude oils contain carbon, hydrogen, sulfur, nitrogen, oxygen, minerals and salts in varying proportions depending on their source. Table 1 provides examples of the broad chemical composition of various crude oils.

Light/Heavy Crude Oils

The designation of "light" or "heavy" for crude oils is based on their density. API gravity is the common measure of crude oil density and is calculated as °API = 141.5/Sp. Gr. – 131.5; the higher the API gravity, the lower the specific gravity. Crude oils with lower densities and viscosities, and thus higher API gravities, usually contain higher levels of naphtha (gasoline-range hydrocarbons) with predominately volatile paraffinic hydrocarbons, which can be processed readily to produce gasoline and are considered "light" crude. Heavy crude oils are more viscous, have higher boiling ranges and higher densities, and thus have lower API gravities. Heavy crude oils are usually rich in aromatics and tend to contain more residual material, e.g. asphaltenes, and heterocyclics, e.g. sulfur, nitrogen, oxygen-containing hydrocarbon analogs.
For example, a Saudi Light Crude that is 63% paraffins, 37% naphthenes and aromatics and a Saudi Heavy crude that is 60% paraffins, 40% naphthenes and aromatics appear to differ only slightly in general hydrocarbon class content, but the compounds making up those classes vary significantly in molecular structure and size distribution, e.g. level of saturated hydrocarbons *vs*. unsaturated and naphtha *vs.* asphaltene content, respectively, so that their API gravities are sufficiently different to classify, within the same oil field, one crude as "light" with 34°API and the other as "heavy" with 28°API. The currently accepted API gravity values that differentiate between light and heavy crude oils are ≥33°API for "light" and ≤28°API for "heavy" (Platt's, 2003).

Paraffinic/Naphthenic Crude Oils

Crude oils are composed of paraffinic, naphthenic (cycloparaffinic) and aromatic hydrocarbons, and may be described as either paraffinic or naphthenic depending on the predominant proportion of hydrocarbon type present (The Petroleum Handbook, 1983). Paraffinic crude oils are rich in straight chain and branched saturated hydrocarbons while naphthenic or asphaltic crude oils contain mainly cycloparaffinic, saturated-ring hydrocarbons and aromatic, unsaturated ring hydrocarbons with at least one benzene ring (IARC, 1989). The aromatic fraction of crude oil contains higher molecular weight aromatic molecules including polycyclic aromatic hydrocarbons (PAH) which consist of only carbon and hydrogen and polycyclic aromatic compounds (PAC) in which some carbon atoms are substituted with heteroatoms, such as sulfur, oxygen and/or nitrogen. Most PAC and PAH in crude oil have one or more alkyl side-chains on the ring structure.

Sweet/Sour Crude Oils

A crude oil may also be described as sweet or sour depending on its sulfur content. As a general rule, crude oils with less than 1% sulfur are "sweet" and crude oils with over 1% are "sour". Strategic Petroleum Reserve (SPR) "sour" crude oils contain a maximum of 1.99 weight % total sulfur and "sweet" crude oils contain a maximum of 0.5 weight % total sulfur.

Table 1. Properties of Whole Crude Oils

Crude Source	Paraffins % vol	Naphthenes % vol	Aromatics % vol	Sulfur % wt.	API gravity (°API)
Light Crudes					
Saudi Light	63	18	19	2.0	34
South Louisiana	79	45	19	0.0	35
Beryl	47	34	19	0.4	37
North Sea Brent	50	34	16	0.4	37
Lost Hills Light	50% Aliphatics	-	50	0.9	>38
Mid range Crudes					
Venezuela Light	52	34	14	1.5	30
Kuwait	63	20	24	2.4	31
USA West Texas sour	46	32	22	1.9	32
Heavy Crudes					
Prudhoe Bay	27	36	28	0.9	28
Saudi Heavy	60	20	15	2.1	28
Venezuela Heavy	35	53	12	2.3	24
Belridge Heavy	Aliphatics 37%		63	1.1	14

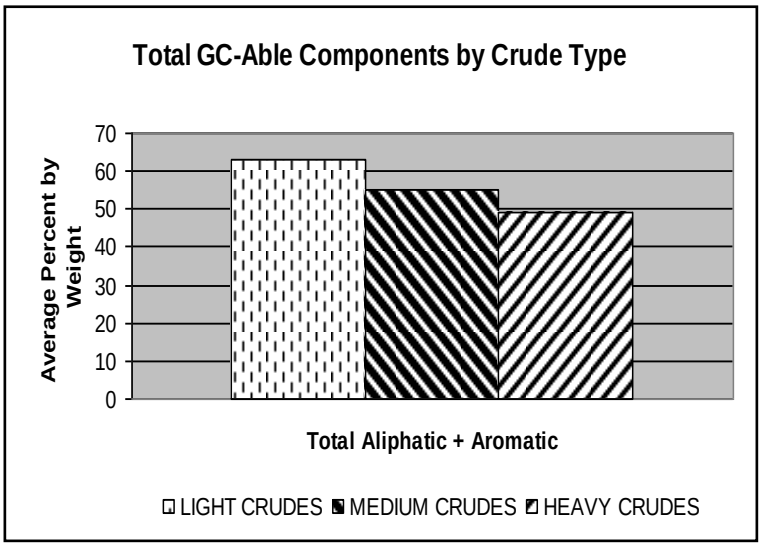
IARC, 1989; Mobil, 1997; OSHA, 1993 & International Crude Oil Market Handbook, 2004

1.2 Analytical Characterization

A comprehensive evaluation of gas chromatographic (GC)-analyzable fractions of 77 crude oils from worldwide oil fields (Figure 1) provides a profile of compositional similarities and differences among crude oils (US DOE/PERF, 2001). Total petroleum hydrocarbon (TPH) content was sorted as aliphatics up to C16, combining the saturated aliphatic paraffins and naphthenes (cycloparaffins), and as aromatic hydrocarbons, unsaturated rings from C6 to greater than C21. Content of benzene/toluene/ethylbenzene/xylene (BTEX), content of the 16 EPA standard marker polycyclic aromatic hydrocarbons, and distribution of 4-6 ring polycyclic aromatic hydrocarbons (PAH) were determined. The amount of material available for GC analysis is influenced by molecular structure and size. The heavier crude oils which have higher boiling ranges and higher densities contain a greater proportion of complex, high molecular weight hydrocarbons, e.g. asphaltenes and resins, in the non-distillable

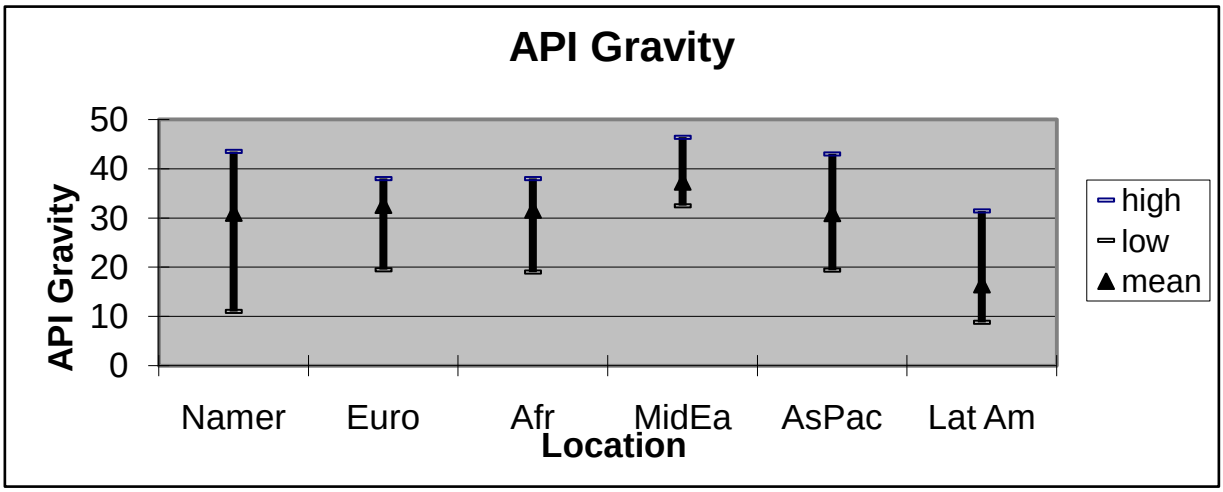
range, thus reducing the size of the fraction available for GC analysis as shown in Figure 1.

Figure 1



Results from testing 64 crude oils with identified API gravities are summarized in Figure 2 which demonstrates the range of API gravities of crude oils from various regions. In this study, North American (Namer) crudes show a wide range from very heavy, e.g. 11°API, to highly desirable light crudes, e.g. 43.5°API, with an average of 31°API. The European, Asian Pacific and African crude oils sampled averaged in the mid-range, i.e. >30° to <33° API. The Middle Eastern crude oils sampled tended to be lighter, e.g. 37°API, and more easily used for gasoline production. The Latin America crude oils sampled were heavier, i.e. average 16°API, and are therefore more useful as sources for lubricant base oils and heavier fuels, e.g. fuel oil #6.

Figure 2: API Gravity for Crude Oils



Light crude oils with lower boiling ranges and lower densities are richer in aliphatic hydrocarbons (Figure 3a) while heavy crudes contain a greater proportion of aromatic compounds with increasingly broad carbon ranges (Figure 3b).

Figure 3a

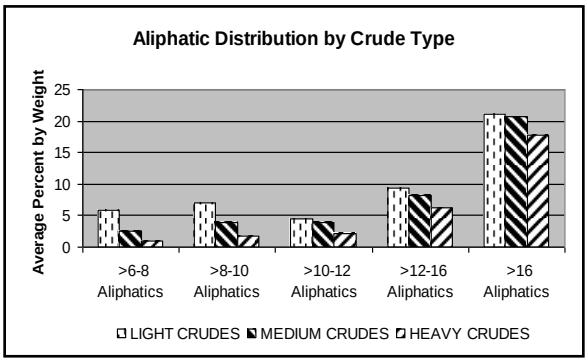
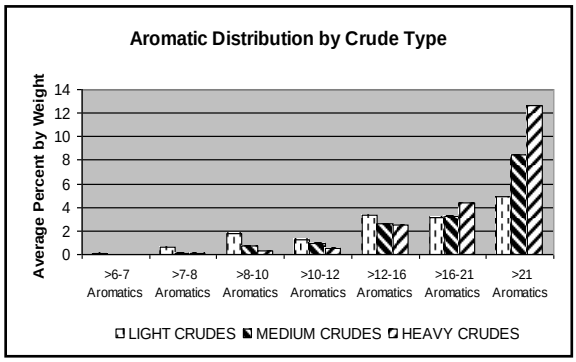


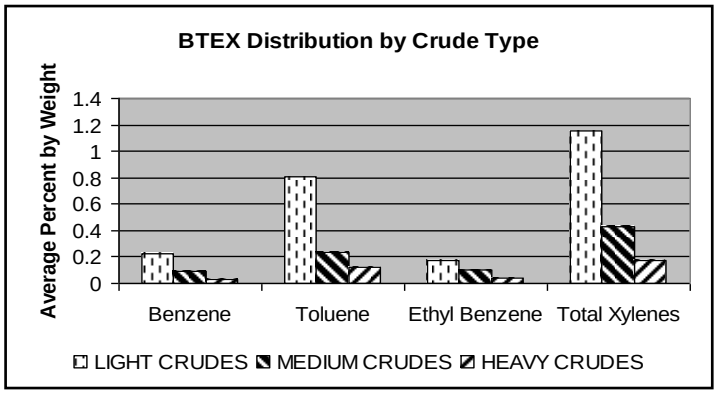
Figure 3b



Of the total petroleum hydrocarbons analyzed, the average weight % of fractions containing less than six carbons was greater in the light crudes, i.e. 6.6%, than in the medium crudes, i.e. 4.7%, and lowest in the heaviest crude oils, i.e. 1.2%. Conversely heavy crude oils contain larger percentages of molecules with carbon distributions greater than C44, i.e. 36%, compared to medium crudes, i.e. 16%, and light crudes, i.e. 8%.

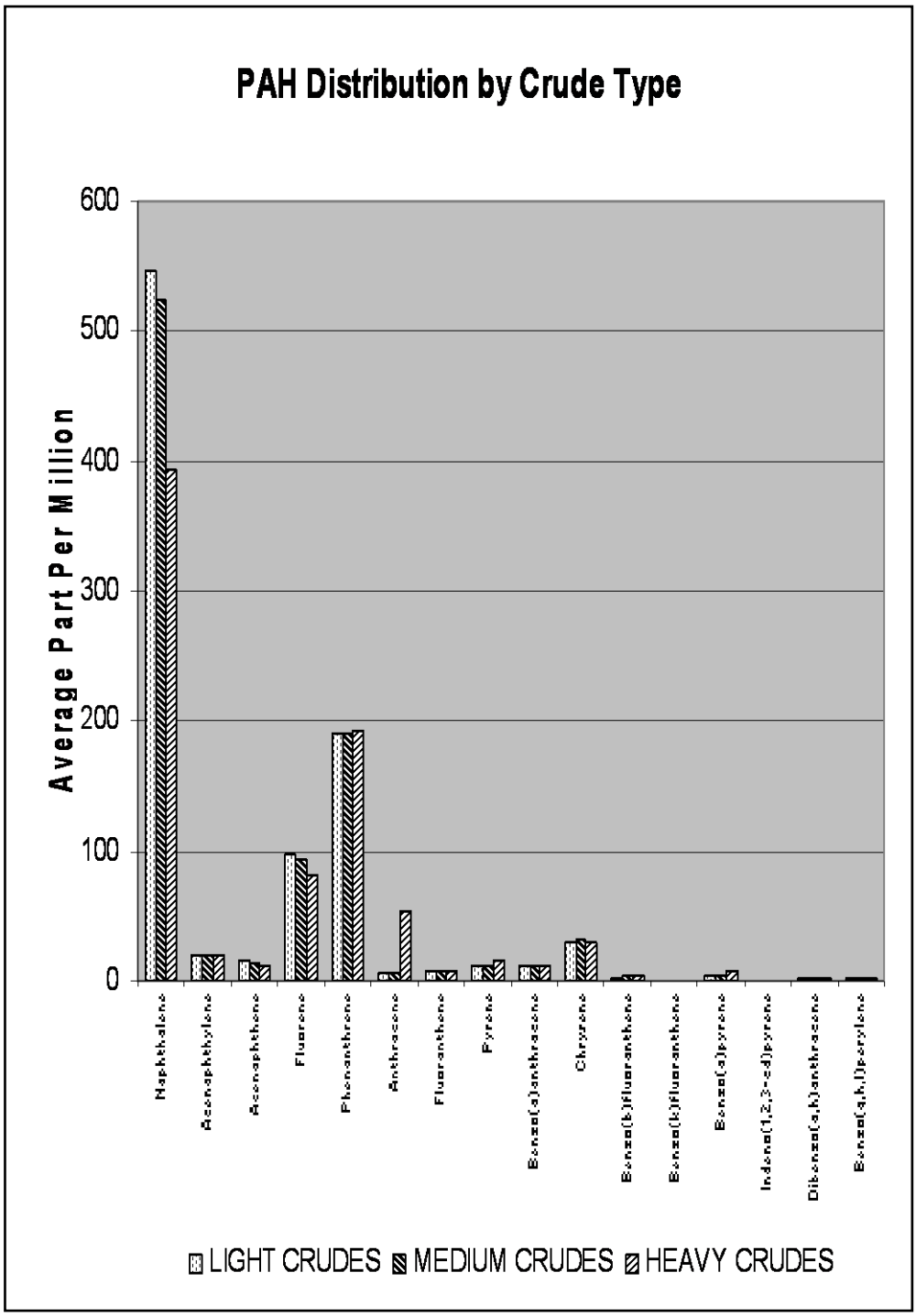
The distribution of BTEX is greater in the lower density light crudes as seen in Figure 4, although the weight % never exceeds 1.2%.

Figure 4



Analysis for the 16 EPA standard marker PAH compounds found that naphthalene was present at the highest concentration of >500 ppm in the light crude and at 400 ppm in the heavy crude while the known PAH carcinogens, benzo(a)pyrene and benzo(a)anthracene were present at very low levels in all the crude oil samples (Figure 5).

Figure 5.


PAH Distribution by Crude Type
Average Part Per Million
600
500
400
300
200
100
0
Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Indeno(1,2,3-cd)pyrene
Dibenzo(a,h)anthracene
Benzo(g,h,i)perylene
LIGHT CRUDES
MEDIUM CRUDES
HEAVY CRUDES

Crude oils contain polycyclic aromatic compounds (PACs). Although similar to polycyclic aromatic hydrocarbons (PAHs) that contain two or more fused-aromatic rings consisting only of carbon and hydrogen, PACs are a broader group of compounds that also includes heterocyclic compounds in which one or more of the carbon atoms in the PAH ring system is replaced by nitrogen, oxygen, or sulfur atoms. The PACs in Crude oil are formed when organic matter is converted into petroleum under elevated pressure and moderate temperatures (130 – 150°C). The nature of the processes which convert organic matter into petroleum involves semi-random chemical processes. The formation of crude oil produces hundreds to thousands of individual PACs. The types of PACs formed in petroleum include a complex variety of parent, i.e. un-substituted, and alkylated structures. The alkyl-substitutions are usually one to four carbons long and can include non-carbon compounds, such as sulfur. Multiple alkyl and cycloparaffinic substitutions of the parent structure are also common, especially in higher boiling fractions of petroleum. The relative abundance of the alkylated polycyclic aromatics (C_{1-4}) in petroleum far exceeds the abundance of the non-alkylated species (C_0) (Speight, 2007). The fact that the concentration of alkylated polycyclic aromatics is much greater than the non-alkylated polycyclic aromatics is the main feature of the petrogenic PACs found in petroleum.

The profile of PAC in some petroleum streams has been shown to be important for predicting the possible effects that can occur with repeated dermal exposures (TERA, 2008). For that reason, recently developed data on the percent of each Aromatic Ring Class (ARC) in 46 individual pentane de-asphalted crude oil samples are shown in Table 2. The Table is sorted by API Gravity, lowest to highest (heavy crude oil to lighter crude oil).

Table 2. PAC Profile of 46 Crude Oils [1,2]

Sample Number	Total DMSO extractable wt%	ARC 1 Wt%	ARC 2 Wt%	ARC 3 Wt%	ARC 4 Wt%	ARC 5 Wt%	ARC 6 Wt%	ARC 7 Wt%	API Gravity
70920	5.8	0.0	0.6	1.7	1.2	1.2	0.6	0.3	13.9
50905	3.3	0.0	1.0	1.6	0.3	0.2	0.1	0.0	19.4
10953	3.7	0.0	1.8	1.1	0.4	0.2	0.0	0.0	19.6
70918	3.9	0.1	1.6	1.6	0.4	0.4	0.1	0.0	20.8
30903	4.4	0.0	1.3	1.3	0.4	0.4	0.4	0.0	21.2
30913	3.7	0.0	1.9	1.5	0.2	0.1	0.1	0.0	21.4
70912	4.0	0.0	0.8	1.6	0.8	0.4	0.4	0.1	21.9
70917	5.3	0.1	1.6	1.6	1.1	0.5	0.5	0.1	23.3
30905	3.3	0.0	1.7	1.0	0.2	0.1	0.1	0.0	23.4
70916	4.2	0.1	1.2	1.2	0.8	0.4	0.3	0.1	23.4
70910	3.9	0.0	1.2	1.6	0.4	0.3	0.2	0.0	25.4
50910	4.3	0.0	0.9	2.1	0.9	0.3	0.2	0.0	26.6
10956	1.9	0.0	0.4	0.2	0.1	0.0	0.0	0.0	28.6
50907	2.5	0.0	0.5	1.0	0.5	0.3	0.2	0.1	28.9

Sample Number	Total DMSO extractable wt%	ARC 1 Wt%	ARC 2 Wt%	ARC 3 Wt%	ARC 4 Wt%	ARC 5 Wt%	ARC 6 Wt%	ARC 7 Wt%	API Gravity
70911	3.8	0.1	1.5	1.2	0.4	0.4	0.3	0.0	28.9
50908	2.7	0.1	1.3	0.8	0.2	0.1	0.1	0.0	29.3
10959	2.6	0.1	1.5	0.8	0.2	0.1	0.0	0.0	29.6
30965	3.9	0.1	2.3	1.2	0.2	0.2	0.1	0.0	29.6
10952	3.2	0.0	2.2	1.0	0.2	0.0	0.0	0.0	29.8
10954	3.3	0.0	1.6	1.0	0.3	0.1	0.0	0.0	30.4
50906	3.3	0.0	1.6	1.3	0.2	0.1	0.1	0.0	30.5
70913	4.7	0.1	1.9	1.4	0.5	0.3	0.2	0.0	30.5
50909	3.2	0.0	1.0	1.6	0.3	0.2	0.1	0.0	30.6
30902	4.5	0.2	2.2	1.3	0.4	0.3	0.2	0.0	30.7
70919	4.0	0.0	1.2	1.2	0.8	0.4	0.3	0.0	31.1
30906	5.0	0.2	2.5	1.5	0.4	0.3	0.1	0.0	31.5
30907	4.1	0.2	2.5	1.2	0.2	0.1	0.1	0.0	31.5
30909	5.0	0.1	3.5	1.0	0.1	0.0	0.0	0.0	32.4
30910	4.4	0.1	2.2	1.3	0.4	0.2	0.1	0.0	32.4
50904	3.6	0.0	1.4	1.4	0.3	0.2	0.1	0.0	32.4
10960	2.7	0.0	1.9	0.5	0.2	0.0	0.0	0.0	32.7
30904	4.6	0.1	2.8	1.4	0.1	0.0	0.0	0.0	33.1
10957	3.1	0.1	1.6	0.9	0.3	0.1	0.0	0.0	33.3
30964	4.9	0.1	2.0	2.0	0.4	0.2	0.2	0.0	33.3
30908	3.5	0.1	2.1	0.7	0.1	0.1	0.1	0.0	33.3
10951	3.8	0.0	2.3	1.1	0.4	0.1	0.0	0.0	33.4
50902	3.0	0.0	1.2	1.5	0.3	0.1	0.1	0.0	33.6
70914	2.5	0.0	1.2	0.7	0.2	0.1	0.1	0.0	36.5
10958	2.6	0.1	1.5	0.8	0.2	0.0	0.0	0.0	36.9
50901	3.7	0.1	1.9	1.5	0.2	0.1	0.1	0.0	38
30911	4.5	0.3	2.7	1.3	0.1	0.0	0.0	0.0	38.3
10955	3.0	0.1	1.8	0.9	0.2	0.0	0.0	0.0	39.1
30914	3.3	0.1	2.6	0.7	0.0	0.0	0.0	0.0	39.1
50903	4.2	0.0	2.1	1.7	0.2	0.1	0.0	0.0	39.4
30912	3.0	0.1	2.1	0.6	0.1	0.1	0.1	0.0	41.8
70915	3.2	0.2	1.9	0.6	0.2	0.1	0.1	0.0	46.2

1) As determined by PAC-2 Method as described by Roy *et al,* 1985 &1988.
2) ARC is “aromatic ring class”. “ARC 1 %” is the weight percent of PACs that have 1 aromatic ring within the total sample. “ARC 2 %” is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Crude oils also contain varying amounts of non-hydrocarbon sulfur, nitrogen, oxygen and trace metals. Sulfur is present as hydrogen sulfide (H_2S), thiols, mercaptans,

sulfides, benzothiophenes, polysufides, and/or as elemental sulfur. As a rule, the proportion, stability and complexity of sulfur compounds are greater in heavier crude oil fractions. H_2S is a primary contributor to corrosion in refinery process units and combustion of sulfur-containing petroleum products can produce undesirable byproducts such as sulfuric acid and sulfur dioxide. The total sulfur content of crude oils spans a range of <0.1% to ~5.0% by elemental analysis. In general, as API gravity decreases, sulfur content increases. For example, a light US crude from Rodessa, Louisiana, has an API gravity of 42.8 and a sulfur content of 0.28%, while an extremely heavy crude from Venezuela has an API gravity of 9.5 and contains 5.25% sulfur (Dickey, 1981; IARC, 1989).

Table 2 illustrates the range of sulfur content in the blended light/medium crude oils that comprise the US Strategic Petroleum Reserve [SPR] stored at 4 cavern sites – Bryan Mound, West Hackberry, Bayou Choctaw, and Big Hill (US DOE, 2002). In the SPR, sour crude oils contain a maximum of 1.99 weight % total sulfur and sweet crude oils contain a maximum of 0.5 weight % total sulfur. Sulfur is removed during refining by catalytic hydrogenation, by caustic wash or other sweetening processes.

Table 3. Properties of Crude Oil in the Strategic Petroleum Reserve

Crude Source	Paraffins % vol	Naphthenes % vol	Aromatics % vol	Sulfur % wt.	API gravity (^{0}API)
Bryan Mound Sweet	68	24	8	0.33	36
Bryan Mound Sour	71	18	11	1.39	35
West Hackberry Sweet	57	30	13	0.32	37
West Hackberry Sour	75	19	6	1.41	34
Bayou Choctaw Sweet	63	26	11	0.36	36
Bayou Choctaw Sour	68	21	11	1.38	32
Big Hill Sweet	61	30	9	0.48	36
Big Hill Sour	68	19	13	1.41	31

US DOE SRC, 2002

Heterocyclic PACs containing nitrogen include anilines, pyridines, quinolines, pyrols, carbazoles, benzonitriles and amides. Nitrogen is found in lighter fractions as basic compounds and in heavier fractions as non-basic compounds. Total nitrogen varies from <0.01% to 1.0% by elemental analysis. Oxygen-containing PACs in crude oils are generally in the phenol, ketone and carboxylic acid families.

The polar compounds in crude oils contain the heteroatoms of oxygen (O), nitrogen (N), and/or sulfur (S) and are known by a variety of names, including heterocyclics, resins, NSOs, polars, and asphaltenes (Prince, 2002, Tissot and White,1984). In crude oils, sulfur-containing heterocyclics exist in the greatest proportion with nitrogen heterocyclics occurring at much lower concentrations (Potter and Simmons, 1998). The majority of the sulfur and nitrogen-containing compounds of petroleum have high molecular masses and high boiling points, thus these materials become concentrated in the heavy fuel oil and tar fractions (Tissot and White, 1984). Although NSO-heteroatom

PACs are discussed here separately, these heteroatoms can occur together in the same highly complex, high molecular weight fractions. Heterocylic compounds also may contain metals in the form of salts of carboxylic acids or more typically as porphyrin chelates or organo-metal complexes (Tissot and White, 1984; Potter and Simmons, 1998; Prince, 2002).

Many of the non-hydrocarbon constituents in crude oil are entrained in non-distillable residues with high boiling ranges that can exceed 720°C (1328°F) and consist almost exclusively of heterocompounds. These residues, e.g. resins, maltenes, and asphaltenes, typically account for 30-50% total sulfur, 70-80% total nitrogen and 80-90% total vanadium and nickel in crude oil. Saturates and aromatics make up only a few weight percent of the non-distillable residue while 40-70% is pentane-insoluble asphaltenes. Resins and asphaltenes with molecular weights of 500-10,000 may constitute from 10% in light paraffinic crude oils to 20-60% in heavy crude oils. The viscosity of a crude oil is greatly affected by the presence of non-distillable residual fractions. The broad molecular range of heterocompounds in this fraction, low volatility and limited solubility severely impair analytical characterization (Algelt and Bodieszynski, 1994). These large, minimally soluble, highly polar molecular structures have very limited bioavailability and absorption, so that heterocompounds and small concentrations of metals entrained in these molecules cannot contribute significantly to possible biological activity induced by exposure to crude oil.

Small quantities of metals naturally occur in crude oils due to their presence in rock formations or salt water deposits from which the oils are drawn or introduced during processing (IARC, 1989). The metals are primarily in the form of stable molecular complexes that can be distilled at temperatures above 500 °C (IARC, 1989). Table 4 summarizes the concentration of metals in 26 representative crude oils from a PERF project where the mean concentrations detected were less than 1.5 ppm for all metals, i.e. total of 18 detectable metals, except nickel at 20 ppm, vanadium at 63 ppm and zinc at 3 ppm (API, 2001). Most metals are present in similar concentrations in all crude types with the exceptions of nickel and vanadium, which appear to increase in concentration as crude oils become heavier (API, 2001). Similar results (Table 5) were found in an API analysis of 46 crude oils that had been processed in the US where nickel and vanadium were found in the highest concentrations in the heaviest crude oils.

Metals are present in crude oils in such low levels that the potential for human health risks and ecological impact is unlikely to be a major risk management consideration at crude oil spill sites (Magaw *et al*., 2000). Inorganic salts such as magnesium chloride or calcium chloride are suspended as minute crystals in crude oil or dissolved in entrained water (brine). These salts are removed or neutralized prior to processing to prevent catalyst poisoning, equipment corrosion and fouling.

Table 4. PERF Survey of Metal Concentrations (ppm) in Crude Oils

Metal	LOD ppm	Light Crude Oils (≥33°API)			Medium Crude Oils (>28°- <33°API)			Heavy Crude Oils (≤28°API)		
		Mean (range)	**N**	**Dec Freq %**	**Mean (range)**	**N**	**Dec Freq %**	**Mean (range)**	**N**	**Dec Freq %**
Ag [silver]	0.01	0.15 (0.07-0.3)	11	100	*0.16 (0.14 - 0.23)*	7	100	0.13 (0.05 – 0.28	10	100
As [arsenic]	0.08	0.02	1	9	*0.13 (0.09 – 0.57)*	3	43	0.06 (0.17 – 0.19)	3	30
Ba [barium]	0.001	0.04 (0.01 - 0.37)	5	45	0.02 (0.003 - 0.04)	5	71	*0.08 (0.002 – 0.2)*	9	90
Be [beryllium]	0.005	-	ND	0	-	ND	0	-	ND	0
Cd [cadmium]	0.002	0.01 (0.003 – 0.03)	11	100	0.01 (0.003 – 0.11)	7	100	0.01 (0.005 - 0.02)	10	100
Co [cobalt]	0.01	0.07 (0.003 – 0.44)	4	36	0.10 (0.02 – 0.38)	3	43	*0.65 (0.11 – 1.33)*	10	100
Cr [chromium]	0.005	0.08 (0.02 – 0.37)	10	91	0.35 (0.07 – 0.87)	7	100	*0.46 (0.07 – 1.43)*	10	100
Cu [copper]	0.01	0.06 (0.01 – 0.13)	11	100	*0.09 (0.02 – 0.24)*	7	100	0.01 (0.03 – 0.23)	10	100
Hg [mercury]	0.01	*0.14*	1	9	-	ND	0	-	ND	0
Mo [molybdenum]	0.02	0.51 (0.31 – 0.87)	11	100	0.43 (0.31 – 0.53)	7	100	*1.23 (0.53 – 4.01)*	10	100
Ni [nickel]	0.02	2.48 (0.05 – 7.28)	11	100	8.45 (4.32-14.1)	7	100	*48.0 (6.87 – 93.0)*	10	100
Pb [lead]	0.001	*0.49 (0.005 – 0.10)*	11	100	0.03 (0.009 – 0.07)	7	100	0.04 (0.005 – 0.15)	10	100
Sb [antimony]	0.001	0.004 (0.001 – 0.02)	6	54	0.006 (0.003 – 0.01)	7	100	*0.02 (0.001 – 0.06)*	10	100
Se [selenium]	0.02	0.06 (0.02-0.27)	9	82	0.08 (0.03 – 0.13)	7	100	*0.33 (0.04 – 0.52)*	10	100
Sn [tin]	0.01	*1.91 (0.11 - 9.66)*	11	100	0.92 (0.04 – 2.3)	7	100	0.92 (0.04 – 3.26)	10	100
Tl [thallium]	0.002	*0.001*	2	18	0.0003	1	14	-	ND	0
V [vanadium]	0.02	3.42 (0.13 - 20.0)	11	100	16.72 (0.15 – 40.0)	7	100	*154.2 (1.4 – 370)*	10	100
Zn [zinc]	0.08	3.56 (2.04 – 8.42)	10	91	*4.09 (1.28 – 10.9)*	7	100	1.42 (0.58 – 3.70)	8	80

N = Number of samples
ND = None detected

Means and ranges for 26 crude oils from various sources throughout the world. *ITALICS* indicates highest mean metal concentration between grades of crude oil (Magaw, 1999 & API 2001)

Table 5. API Analysis of Certain Metal Concentrations in 46 Crude Oils

Metal	**LOD ppm**	**Light Crude Oils (N=15)** (≥33°API)		**Medium Crude Oils (N=19)** (>28°- <33°API)		**Heavy Crude Oils (N=12)** (≤28°API)	
		Mean (range) ppm	**Dec. Freq. %**	**Mean (range) ppm**	**Dec. Freq. %**	**Mean (range) ppm**	**Dec. Freq. %**
Fe [iron]	0.1	2.18 (0.2-15.9)	73	1.22 (0.2-6.1)	74	*8.1 (0.3-48.2)*	100
Ni [nickel]	0.1	2.10 (0.5-3.8)	100	8.8 (0.1-33.2)	95	*37.2 (4.3-71.5)*	100
V [vanadium]	0.1	4.04 (0.2-11.6)	93	14.03 (0.4-33.0)	95	*80.7 (7.6-175)*	100

Note: Means and ranges for metal concentrations (ppm) analyzed according to ASTM D5708 for 46 randomly chosen crude oils processed in the US in 2004 (Wilhelm et al, 2007).

Dec. Freq. = Detection frequency above the limit of detection
LOD = Limit of detection

2.0 CATEGORY DEFINITION and RATIONALE

Only one Chemical Abstract Service (CAS) number (Petroleum, 8002-05-9) represents all crude oils and constitutes the HPV Crude Oil Category. Crude oil is a Chemical Substance of Unknown, of Variable Composition, or of Biological Origin (UVCB) in the Toxic Substances Control Act (TSCA) Chemical Inventory and is defined as follows:

> *"A complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen and sulfur compounds. This category encompasses light, medium, and heavy petroleums, as well as the oils extracted from tar sands. Hydrocarbonaceous materials requiring major chemical changes for their recovery or conversion to petroleum refinery feedstocks such as crude shale oils, upgraded shale oils and liquid coal fuels are not included in this definition".*

Although the terms "crude oil" and "petroleum" refer to the same UVCB substance, to avoid confusion only the more commonly used term "crude oil" is used throughout this document.

Although crude oils are composed of a wide variety of constituents, analytical studies indicate that hydrocarbons, heterocyclics, metals and other constituents, e.g. hydrogen sulfide, are present in all crude oils with the diversity of crude oils originating from the proportional variability in these components depending on the source of the oil. Therefore, grouping all crude oils together into one category is appropriate to evaluate and predict potential screening level hazards.

The physical/chemical properties of crude oil are directly related to their carbon range and to a much lesser extent to their paraffinic, naphthenic and the aromatic character. The carbon range influences the volatility, water solubility, and viscosity of crude oils which in turn determines the environmental fate, ecotoxicity, and potential bioavailability of components. Hydrogen sulfide, volatile organic compounds (VOCs), and polycyclic aromatic compounds (PAC) found in crude oils are the most common constituents which present human health hazards.

3.0 TEST MATERIALS

Forty-six samples of uniquely identified crude oils were randomly chosen from a collection of 170 crude oil streams processed in the US in 2004 (Wilhelm et al, 2007). These samples were analyzed by the API for Metals by ICP-AES (ASTM D5708), Boiling Point Distribution of Samples with Residues by High Temperature GC (ASTM D7169), Density and Relative Density of Crude Oils (ASTM D5002). PAC-2 (Roy et al, 1985 &1988) analysis was done after the samples were pentane de-asphalted (modified ASTM D6560).

4.0 PHYSICAL-CHEMICAL PROPERTIES

In appearance, crude oils range from mobile, volatile, light colored liquids consisting to black, viscous tar-like materials. The chemical compositions of crude oils from different producing regions, and even from within a particular formation, can vary widely.

Although some physical-chemical property data exist for some crude oils, not all of the endpoints indicated below are defined and a consensus database for physical-chemical values for all crude oils does not exist. Further, because crude oils are complex substances with variable compositions, the measurement or calculation of a single numerical value for some of the physicochemical properties is not possible. For example, a substance that contains multiple constituents does not have a melting point, but rather a melting point range that reflects the constituents' properties. Where appropriate, values for physical-chemical properties are represented as a range of values according to crude oil's general composition of hydrocarbon compounds. Measured data have been provided when available. When measured values were not available, estimates of the values for physical-chemical endpoints were made for representative constituents covering a potential range of molecular weights and hydrocarbon isomeric structures using the EPI-Suite™ structure-activity estimation models (EPA, 2000).

4.1 Physical-Chemical Endpoints

The physicochemical endpoints for the EPA HPV chemical program include the following:

melting point,
boiling point,
vapor pressure,
octanol/water partition coefficient (K_{ow}), and
water solubility.

4.2 Melting Point

For complex substances like crude oil, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, including crude oil, the pour point is routinely used. The range of Figures quoted in the robust summary, -30°C to 30°C, is a typical range for the pour point as measured by a standard oil industry procedure, i.e. ASTM 1991b (ECB, 2000). Some low-wax crudes have pour points below -30°C.

Conclusion: The pour points of the majority of crude oils fall within an approximate range of -30°C to 30°C.

4.3 Boiling Point

Distillation temperatures for crude oil range from approximately -1°C to over 720°C (30 – 1328 °F) at 1013 Pa. This approximate range for crude oil is based on the boiling point of n-butane for the lower end and an upper estimate based on ASTM D7169 – 05, "Standard Test Method for Boiling Point Distribution of Samples with Residues Such as Crude Oils and Atmospheric and Vacuum Residues by High Temperature Gas Chromatography" (ASTM, 2005). The boiling points of 46 crude oils are presented in Table 6. The "percent recovered" in Table 6 indicates that some portion of the crude oil sample often boils above the limits of this analytical method (approximately 720°C or 1328°F). In practice, atmospheric distillation of crude oil at a refinery is not conducted above 275-300°C, to avoid thermal decomposition.

Table 6. Boiling Point Distribution of 46 Crude Oils[1]

Sample No.	API Gravity	Boiling Range (^{0}F)			
		Initial BP	T50[2]	Final BP	% Recovered
70920	13.9	171	853	1312	86
50905	19.4	86	852	1321	78
10953	19.6	90	827	1319	87
70918	20.8	92	866	1316	76
30903	21.2	91	916	1302	72
30913	21.4	86	831	1303	79
70912	21.9	111	928	1298	72
70917	23.3	124	744	1281	87
30905	23.4	93	817	1310	80
70916	23.4	54	791	1286	82
70910	25.4	158	737	1303	93
50910	26.6	82	754	1285	85
10956	28.6	169	700	1289	90
50907	28.9	124	687	1300	91
70911	28.9	79	598	1269	100
50908	29.3	84	737	1293	84
10959	29.6	80	738	1316	86
30965	29.6	84	732	1299	87
10952	29.8	77	691	1318	94
10954	30.4	112	626	1079	100
50906	30.5	95	732	1324	84
70913	30.5	48	581	1168	93
50909	30.6	86	739	1305	82
30902	30.7	75	720	1294	85
70919	31.1	112	698	1280	86
30906	31.5	78	605	1338	100
30907	31.5	82	713	1317	88
30909	32.4	79	598	1269	100
30910	32.4	77	671	1297	88

50904	32.4	82	662	1275	89
10960	32.7	87	571	1269	95
30904	33.1	97	577	1274	95
10957	33.3	86	674	1314	95
30908	33.3	44	928	1298	72
30964	33.3	75	649	1310	89
10951	33.4	81	642	1305	97
50902	33.6	86	583	1202	100
70914	36.5	46	610	1254	97
10958	36.9	79	618	1226	86
50901	38	74	540	1242	100
30911	38.3	80	583	1317	92
10955	39.1	78	557	1312	96
30914	39.1	96	502	1284	96
50903	39.4	66	499	1269	100
30912	41.8	92	618	1272	79
70915	46.2	103	478	1088	93

[1] ASTM D 7169
[2] Temperature at which 50% of the sample boils

Conclusion: The distillation range of crude oil is approximately -1°C to over 720°C (30°F to over 1328°F). In practice, the upper range of atmospheric distillation at a refinery is typically limited to 275-300°C to avoid thermal decomposition.

4.4 Vapor pressure

A range of vapor pressures of 6 to 45 kPa have been reported for different crude oils (Jokuty *et al*., 2002). Crude oil vapor pressure is a function of oil temperature and composition. The cited values represent vapor pressures of different crude oil types as reported in the Environment Canada database (Jokuty *et al*., 2002).

Conclusion: Vapor pressures of crude oils have been measured from 6 kPa to 45 kPa.

4.5 Partition Coefficient

The range of partition coefficients for constituent hydrocarbons in crude oil is 2 to > 6, based on the calculated log P_{ow} at 25°C (ECB, 2000). The calculation was done by the CLOGP Version 3.5 program (Calculation of LOG Partition coefficient octanol/water). The Figures represent the spread of calculated and/or measured values for typical hydrocarbon components of crude oil. Calculated values for higher molecular weight hydrocarbons are above 6, but such values are notional, since no correlation has been established between calculated and experimental values.

Conclusion: The range of partition coefficients of individual constituent hydrocarbons in crude oil covers an approximate range of 2 to >6.

4.6 Water Solubility

The aqueous solubilities of the main classes of hydrocarbons present in crude oil increase in the order of n-alkanes<isoalkanes<cycloalkanes< aromatics (McAuliffe, 1966; Tissot and Welte, 1984). The water solubilities of individual constituents also decrease with increasing molecular weight and size of alkyl substituents (McAuliffe, 1966: Tissot and Welte, 1984). Solubilities of crude oil components may extend up to one or two percent individually, however, total solubility of all components will be dictated by component composition and loading rates of oil to water (Shiu *et al*., 1990). Concentrations of dissolved hydrocarbons in the water-soluble fractions of twelve crude oils are reported in the robust summaries. Measurements were made in distilled and saltwater under different temperatures by purge-and-trap gas chromatography. Concentrations of hydrocarbons ranged from 10.42 mg/L to 58 mg/L in distilled water and from 7.75 mg/L to 25.5 mg/L in saltwater (Shiu *et al*., 1990).

Conclusion: Aqueous concentrations of petroleum hydrocarbons for 12 crude oils ranged from 10.42 to 58 mg/L in distilled water and from 7.75 to 25.5 mg/L in saltwater. At any particular loading rate, i.e. total nominal amount of substance per unit volume, the aqueous concentrations of each constituent represent a balance between the relative volumes of aqueous and petroleum phases, partition coefficient between phases, amount of component present and the maximum water solubility of each constituent.

4.7 Assessment Summary for Physical-Chemical Properties

Crude oil is comprised of many materials existing in varying proportions depending on the source of the crude oil. For this reason, specific physical-chemical properties also vary and may only be presented as wide ranges that reflect the underlying properties of the constituent compounds. The values reported above for pour point, boiling point, vapor pressure, partition coefficient, and water solubility as reported in reliable literature sources and databases, are provided for a range of different crude oils

5.0 ENVIRONMENTAL FATE

When a complex substance such as crude oil is released into the environment, the individual constituents separate and partition to the different environmental compartments in accordance with their own physical-chemical properties. The ultimate fates of the individual components in crude oil are influenced by both abiotic and biotic processes, and the relative importance of these processes will depend upon the environmental compartment to which the individual components partition. By understanding the environmental fate characteristics of these individual components, an overall assessment of the whole crude oil is possible. Therefore, the environmental fate attributes of various hydrocarbon constituents in crude oil are described in the following sections.

5.1 Environmental Fate Endpoints

The U.S. EPA has selected the following environmental fate endpoints by which these substances may be characterized:

photodegradation,
stability in water (hydrolysis),
environmental distribution (fugacity), and
biodegradation.

In determining these fate characteristics for constituents in crude oil, the U.S. EPA's collection of physical-chemical and environmental fate models in EPI Suite™ (USEPA, 2000a) was used to estimate the properties of photodegradation, stability in water, and environmental distribution. Measured data, when available, were also included in the assessment. Biodegradation was examined for these substances in light of their physical-chemical properties and their capacities to undergo microbial oxidation/reduction reactions.

5.2 Photodegradation

5.2.1 Direct Photodegradation

A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 nm to 750 nm wavelength range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, while wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer (Harris, 1982a). However, to a limited extent, some degradation of polycyclic aromatic hydrocarbon (PAH) molecules in crude oil may occur as the result of photo-oxidative processes, although PAHs bound to sediments are reported to be less susceptible to photo-oxidation. The persistence of PAHs in sediments may in part be due to lack of light for photo-oxidation. Therefore, this fate process will not contribute to a measurable degradative removal of chemical components in this category from the environment.

5.2.2 Indirect Photodegradation

Fractions of crude oil that volatilize to air may undergo a gas-phase oxidation reaction with photochemically produced hydroxyl radicals (OH^{-}). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation (Schwarzenbach, 2003). The atmospheric oxidation potential (AOP) of the major constituents in crude oil was estimated using AOPWin (atmospheric oxidation program for Microsoft Windows), a subroutine in the EPI Suite™ (U.S. EPA, 2000) models and used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a reaction rate constant (cm^3/molec-sec) and a chemical half-life, i.e. hour or days, of a compound based upon average atmospheric concentrations of hydroxyl radicals (1.5×10^6 OH^{-}/cm^3) and a 12-h day at 25°C.

Atkinson (1990) gives data which enables half-lives to be calculated for the degradation of hydrocarbons in contact with hydroxyl radicals under sunlight conditions in the troposphere. Half-life values for typical hydrocarbon constituents in crude oils that volatilize to air are as follows:

Constituent	Half-life, days
benzene	6.5
n-butane	3.2
n-hexane	1.4
toluene	1.3
cyclohexane	1.1
n-decane	0.69
n-tetradecane	0.42
naphthalene	0.37

Hydrocarbons of carbon number greater than C20 will have little or no tendency to partition to air.

Conclusion: Direct photodegradation is not expected to play an important role in the environmental fate of crude oils. Indirect photodegradation via reaction with hydroxyl radicals may be important in the gas-phase degradation of hydrocarbons that volatilize to the troposphere. Atmospheric half-lives of 0.37 to 6.5 days have been calculated for representative components of crude oil.

5.3 Stability in Water (Hydrolysis)

Hydrolysis of an organic chemical is the transformation process by which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982b). The majority of chemical components in crude oils are hydrocarbons, which are not included in these chemical groups, and are not subject to hydrolysis reactions with water.

Conclusion: The substances in crude oil do not contain chemical moieties that undergo hydrolysis and, therefore, this process would not be expected to be an important fate pathway.

5.4 Transport Between Environmental Compartments

Equilibrium models can provide information on the way in which a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay *et al*., 1996, 1997). In its guidance document for HPV data development, the USEPA states that Level I fugacity

data is acceptable as an estimate of chemical distribution values. The EQC model is a Level I model that describes the equilibrium distribution of a fixed quantity of conserved, i.e., non-reacting, chemical at steady state within a closed environment with assumed volumes of air, water, soil and sediment. The model assumes the chemical becomes instantaneously distributed to an equilibrium condition using physical-chemical properties to quantify the chemical's behavior. The model does not include degrading reactions, advective processes or inter-media transport between compartments.

Results of Level I models are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition in the environment. Fugacity modeling for constituents in crude oil indicates that, at steady-state, the lower molecular weight components will mainly partition to air, with a maximum of about 1% of mono-aromatic hydrocarbons partitioning to water. As the molecular weights increase, less tendency exists for the hydrocarbons to partition to air with increasingly greater percentages distributing to soil. Collectively, the wide molecular weight range of the hydrocarbons in crude oil will mean that at equilibrium, distribution will be mainly to air and soil, with much less than 1.0% being present in water. These data are adequate to define environmental distribution of crude oil components.

Conclusion: When crude oil enters the environment, the constituent hydrocarbons will partition in accordance with their own physical-chemical characteristics. Because crude oil consists of a wide range of molecular weight and hydrocarbon types, fractions will partition mainly to air and soil.

5.5 Biodegradation

Most of the understanding on the biodegradability of petroleum hydrocarbons comes from biodegradation studies on crude oil, various streams from the fractional distillation of crude oil, and investigations of spill events, much of which have been reviewed by Bartha and Atlas (1977) and Connell and Miller (1980). Together with more recent reviews by Prince (2002), Prince *et al*. (2003) and Garrett, *et al*. (2003), a general consensus has developed regarding the biodegradability of petroleum hydrocarbons. First, virtually all kinds of crude oils are susceptible to microbial oxidation. The rate of oxidation is influenced by the microbial species and environmental factors such as available nutrients, oxygen, temperature and degree of dispersion. Second, molecular weight influences the rate at which microbial communities can utilize hydrocarbons. Low molecular weight components degrade relatively easy, while higher molecular weight components take longer to be consumed. Third, molecular structures of the hydrocarbons in the petroleum substance affect aerobic microbial biodegradation. Generally speaking, the structure-related pattern shows hydrocarbons in order of increasing difficulty of degradation: (1) n-alkanes, (2) isoalkanes, (3) alkenes, (4) one-ring alkylbenzenes (e.g., BTEX), (5) polycyclic hydrocarbons, and (6) high molecular weight cycloalkanes (Bartha and Atlas, 1977; Potter and Simmons, 1998). This order has been reported for spills in both temperate climates and arctic summer conditions (Garrett *et al*., 2003). The relative ease of biodegradability of these structures is a

generalization, and the body of scientific data points to various factors that might influence this pattern.

Other constituents of crude oil, such as those grouped in the general category of heterocyclic compounds because of their heteroatom content, i.e. oxygen, nitrogen, and sulfur may be more or less degraded according to their molecular weight distribution. However, many of these constituents exist as large molecular weight compounds that end up in the heaviest products resulting from crude oil refining. Current knowledge suggests these are not very biodegradable and will persist in the environment (Prince, 2002).

Empirical data indicate that crude oil can be -degraded equally well in sea water or fresh water, with the nutrient availability being a key factor in determining the rate of degradability. Adapted microorganisms are often found in ocean areas where crude oil spills are common. Zobell (1969) has calculated that when an adapted microbial population is available in well-aerated seawater at 20 to 30°C, the rate of crude oil oxidation ranges from 0.02 to 0.2 g of oil oxidized/m² ocean surface area/day. The same author found experimentally that complete oxidation of 1.0 mg of hydrocarbon requires between 3 and 4 mg of oxygen, i.e. has a biological oxygen demand (BOD) of 3 to 4 mg oxygen/mg. Since the oxygen content of sea-water is between 6 and 11 mg/L, depending on salinity and temperature, this means that the oxygen from about 320,000 liters of sea water is required to oxidize one liter of crude oil.

Five day respirometric tests run both in fresh water and in salt water at 30°C using a Kuwait crude oil resulted in 15% and 3% biodegradation, respectively (Bridie and Bos, 1971). Biodegradation rates for crude oils will vary considerably, but in standard 28-day studies, none would be expected to be readily biodegradable. However, the evidence from spillages and from natural seepages is that most of the non-volatile constituents of crude oil are inherently biodegradable, but that some of the highest molecular weight components are persistent in water (CONCAWE, 2001).

Conclusion: Whole crude oil would not be classified as readily biodegradable. However, the constituent hydrocarbons in crude oils are considered inherently biodegradable.

5.6 Assessment Summary for Environmental Fate

When crude oil enters the environment, the individual constituents partition to different environmental compartments and degrade in accordance with their own physical-chemical properties. In soils, crude oil will absorb into the soil matrix and volatile components will gradually partition to the atmosphere. Over time hydrocarbons available for microbial attack may be slowly degraded. In aquatic environments, crude oil will spread as a film on the surface of the water facilitating the loss of volatile components. Components that enter the troposphere will not likely persist as interactions with hydroxyl radicals leads to indirect photodegradation. Most components in crude oil are insoluble in water, but dissolved fractions become available for

biodegradation. Rates of mineralization are limited by available nutrients. As crude oil weathers, the fraction that does not biodegrade or volatilize can be physically isolated through incorporation into sediments and soils. Crude oil is not considered readily biodegradable, but the individual hydrocarbon constituents in general are regarded as inherently biodegradable.

6.0 ENVIRONMENTAL EFFECTS

The environmental effects endpoints in the HPV Challenge program include:

- Acute Toxicity to Fish,
- Acute toxicity to Aquatic Invertebrates, and
- Toxicity to Algae (Growth Inhibition).

For the assessment of ecotoxicity of poorly water-soluble mixtures such as crude oil, "loading rate" is now generally accepted as the way in which study results should be expressed (OECD, 2000). The "loading rate" is defined as the amount of the substance equilibrated with the aqueous test medium, and the aqueous phase at equilibrium is termed the water-accommodated fraction (WAF) for the loading rate (OECD, 2000). Toxicological endpoints such as the lethal loading rate (LL_{50}) or effective loading rate (EL_{50}) are used to express the amount of substance per unit volume that is lethal or produces a specific effect to 50% of the test organisms. Studies in which the results are expressed in terms of dilutions of a water-soluble fraction (WSF) do not allow the ecotoxicity of a substance to be expressed in terms of the amount of that product required to produce a particular effect. Therefore, such results are not comparable to results obtained by other exposure methods (Girling and Whale, 1994). Some studies have used oil/water dispersions (OWD) in which organisms are exposed to mixtures of oil and water resulting from high energy mixing of whole oil product in the dilution water. This method results in an expression of concentration of the applied substance (i.e., mg test substance/L), but the method does not prevent adverse effects due to physical entrapment or other adverse mechanical effects due to the insoluble oil. Besides endorsing the WAF method for preparation of exposure solutions, OECD (2000) also recommends the use of sealed test vessels with minimal or no headspace to minimize loss of volatile components in the exposure solutions. This procedure should be used in preparation of the WAF as well as test vessels during testing, as studies have clearly documented the loss of volatile hydrocarbons with open-air vessels or use of aeration during testing (Anderson et al., 1974; Lockhart et al., 1987; Tsvetnenko and Evans, 2002).

Although the WAF technique of preparing exposure solutions of sparingly soluble substances is currently preferred (OECD, 2000), to disregard other research using alternative exposure techniques would be to ignore a large and important body of work. For example, the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) was formed to create and evaluate an alternative exposure regime for assessing the ecotoxicity of spilled oils (Aurand and Coelho, 2005). The CROSERF test protocols were established on the basis that realistic environmental exposure to spilled oils is not constant, but begins with an exposure “spike” that subsides as the spilled oil

weathers (Aurand and Coelho, 2005). Testing in this manner is not consistent with regulatory ecotoxicological hazard assessment methods which attempt to maintain consistent exposure concentrations for the duration of the exposure period (OECD Guidelines for Testing of Chemicals). The CROSERF program included many test species and in some cases comparisons of spiked exposures to continuous exposures, and thus is valuable to the overall understanding of the toxicity of crude oil to aquatic organisms.

In this overview of the ecotoxicity hazard of crude oil, studies were selected such that toxicity comparisons could be made on the basis of similar exposure techniques. Whenever possible, the data review included studies of whole oil exposures as well as WAF and WSF exposures. For citing WAF and WSF studies, preference was given to those that included analytical measurements of the dissolved hydrocarbons in the exposure solutions. WAF and WSF preparation techniques have a large influence on the concentration and characterization of the dissolved components, and the analytical verification of the dissolved hydrocarbon concentrations provides some common means of comparing exposures. The following sections present selected acute aquatic toxicity studies of fish, aquatic invertebrates, and algae. Data are tabulated such that comparisons can be made on the basis of similar exposure techniques.

6.1 Acute Aquatic Toxicity

Crude oils would be expected to produce a similar range of acute toxicity for the three types of organisms (e.g., vertebrate, invertebrate, and plant) based on results of studies using comparable standardized test methods and exposure solution preparation procedures. This is expected because the majority of constituents in crude oil are neutral organic hydrocarbons that act in a common mode of action termed “non-polar narcosis”, which is brought about by disruption of biological membrane function (van Wezel and Opperhuizen 1995; Di Toro, et al., 2000). Any differences between toxicity endpoints (i.e., LC/LL50, EC/EL50) can be explained by the differences between the target tissue-partitioning behaviors of the individual chemicals (Verbruggen et al., 2000). For example, the existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of approximately 2-8 mmol/kg fish (wet weight) (McCarty and Mackay, 1993; McCarty et al., 1991). When normalized to lipid content the CBR is approximately 50 µmol/g of lipid for most organisms (Di Toro et al., 2000). Similarities in the range of toxic response elicited by exposure to complex petroleum substances may also be predicted based on physical-chemical properties and acute toxicities of the individual hydrocarbons (Peterson, 1994, CONCAWE, 1996a).

Whenever possible, aquatic toxicity test data with fish, invertebrates, and algae included studies that employed exposure solutions made by independent WAFs, dilutions of WAFs or WSFs, WAFs using spiked exposures, and whole oil:water dispersion techniques. When the data were available, endpoint results were based on measurements of dissolved hydrocarbons. Some of the studies included endpoints based on WAF loading rates as well as measured hydrocarbons. Techniques used to measure the dissolved fraction varied, but tended to include gas chromatography with

flame ionization detection (GC-FID) or GC coupled with a mass spectrometer (GC-MS). GC-FID was by far the more common technique, but differences in sample handling procedures such as purge-and-trap, solvent extraction, or a combination of both methods likely introduced some bias in the range of molecular weight hydrocarbons that defined the measurement. Thus, the endpoint basis column in the tables of toxicity data includes a clarification if a specific fraction of the total dissolved hydrocarbons was reported. The dissolved fraction may include C10 – C36 hydrocarbons or in some studies, a separate fraction of C6 – C9 may have been measured then added to the C10 – C36 fraction, which was expressed as the total hydrocarbon concentration (mg THC/L). The contribution of one or another hydrocarbon fraction to the total dissolved hydrocarbons is a variable that could have a bearing on the calculated toxicity value, and not all reports provided a detailed description of the analytical method.

6.1.1 Acute Toxicity to Aquatic Vertebrates

Table 7 presents aquatic toxicity data for aquatic vertebrates (fish) using independent WAFs, dilutions of a WAF or WSF, spiked exposures, and whole oil:water dispersions. Fuller and Bonner (2001) ran duplicate tests of Arabian Medium crude oil with inland silversides (*Menidia beryllina*) and sheepshead minnows (*Cyprinodon variegatus*). Using independent WAFs and measured dissolved hydrocarbons, the authors measured LC50 values of 4.9 mg/L and 5.5 mg/L, and 3.9 mg/L and 4.2 mg/L, respectively, for the two oils. The close proximity of the LC50 values for duplicate tests likely resulted from their preparation and testing methods. The use of sealed test vessels with no headspace combined with daily renewals of the test solutions helped maintain exposure concentrations.

Table 7. Acute Toxicity of Crude Oil to Fish

Crude Oil Type	Fish Species	Test/ Exposure Type	Endpoint Basis	Endpoint Value, mg/L	Reference
Independent WAF					
Arabian Medium crude oil	*Menidia beryllina* (inland silversides)	Independent WAFs; sealed vessels/zero headspace; daily static-renewal	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = 4.9 (test 1) 5.5 (test 2)	Fuller and Bonner (2001)
Arabian Medium crude oil	*Cyprinodon variegatus* (sheepshead minnow)	Independent WAFs; sealed vessels/zero headspace; daily static-renewal	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = 3.9 (test 1) 4.2 (test 2)	Fuller and Bonner (2001)
Alaska North Slope crude oil	*Menidia beryllina* (inland silversides)	Independent WAFs; covered vessels; daily static-renewal; aerated	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 15.59 96-h LL50 = 1,641	Rhoton et al. (2001)
Prudhoe Bay crude oil	*Menidia beryllina* (inland silversides)	Independent WAFs; covered vessels; daily static-renewal; aerated	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 14.81 96-h LL50 = 4,965	Rhoton et al. (2001)
WAF or WSF with Dilutions					
Norman Wells crude oil	*Oncorhychus mykiss* (rainbow trout)	WSF, 1:12.5 oil:water (v/v); dilutions; static; 3 tests (sealed, open, open aerated)	Headspace GC-FID (C1-C7) + solvent extraction (C8-C12) GC-FID measured mg TPH/L	48-h LC50 = 10.4 (sealed) 11.6 (open) N.D. (open/aerate)	Lockhart, et al. (1987)
Bass Strait crude oil	*Melanotaenia fluviatilis* (crimson-spotted rainbow fish)	WAF, 1:9 oil:water (v/v); dilutions; daily static-renewal	GC-FID mg TPH/L	96-h LC50 = 1.28	Pollino and Holdway (2002)
Spiked Exposure					
Louisiana Sweet crude oil	*Menidia beryllina* (inland silversides)	1:40 oil:water (v/v) WAF then diluted; static; open/aerated	GC-FID measured mg TPH/L	96-h LC50 = >2.9	Hemmer, et al. (2010)

Arabian Medium crude oil	*Menidia beryllina* (inland silversides)	Independent WAFs; CROSERF flow-through spiked exposure	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = >14.5 (test 1) >32.3 (test 2)	Fuller and Bonner (2001)
Arabian Medium crude oil	*Cyprinodon variegatus* (sheepshead minnow)	Independent WAFs; CROSERF flow-through spiked exposure	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = >6.1 (test 1) >5.7 (test 2)	Fuller and Bonner (2001)
Alaska North Slope crude oil	*Menidia beryllina* (inland silversides)	Independent WAF; CROSERF flow-through spiked exposure	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 26.36 96-h LL50 = 3520	Rhoton, et al. (2001)
Prudhoe Bay crude oil	*Menidia beryllina* (inland silversides)	Independent WAF; CROSERF flow-through spiked exposure	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = >19.86 96-h LL50 = >8152	Rhoton, et al. (2001)
Whole OWD					
Kuwait crude oil	*Cyprinodon variegatus* (sheepshead minnow)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = >80,000	Anderson, et al. (1974)
	Fundulus similis (longnose killifish)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = 14,800	Anderson, et al. (1974)
	Menidia beryllina (inland silverside)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = 9,400	Anderson, et al. (1974)
South Louisiana crude oil	*Cyprinodon variegatus* (sheepshead minnow)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = 29,000	Anderson, et al. (1974)
	Fundulus similis (longnose killifish)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = 6,000	Anderson, et al. (1974)
	Menidia beryllina (inland silverside)	OWD; open/mixed	Nominal whole oil loading, mg/L	96-h LC50 = 3,700	Anderson, et al. (1974)

Rhoton, et al. (2001) conducted acute toxicity tests of Alaska North Slope and Prudhoe Bay crude oils to inland silversides. Those tests revealed acute LC50 values of 15.59 mg/L and 14.81 mg/L, respectively, for the two crude oils. While the difference in toxicity may be due to the nature of the crude oils (for example, corresponding LL50 values were 1,641 and 4,965 mg/L), the authors acknowledged that despite daily renewals of the test solutions, aeration of the test solutions caused a drop in the concentration of dissolved hydrocarbons to near the detection limit after 12 hours (Rhoton et al., 2001).

Lockhart, et al. (1987) compared the toxicity of Norman Wells crude oil when tested using sealed, open, and open/aerated test vessels. Using dilutions of a 1:12.5 oil/water WSF and measuring the dissolved hydrocarbons by GC-FID, the authors measured the 48-hour LC50 to rainbow trout (*O. mykiss*) to be 10.4 mg/L using sealed vessels, 11.6 mg/L using open vessels, and no toxicity occurred in the vessels that were left open and were aerated. While the effect of aeration of the test solutions on toxicity was dramatic, the solutions left open to the air but not aerated appeared to retain hydrocarbons for a sufficient duration for toxicity to occur. Of all the fish studies cited here, the lowest LC50 was reported by Pollino and Holdway (2002). The authors calculated an LC50 of 1.28 mg/L based on measured dissolved hydrocarbons. Details of the experimental methods were not provided, and this precluded a full understanding what the analytical measurements represented with respect to range of carbon numbers.

As described in Section 6.0, the CROSERF protocols were designed to simulate what was believed to be an environmentally realistic exposure following an oil spill (Aurand and Coelho, 2005). Therefore, this design created an initial high concentration that gradually declined as the spilled crude oil weathered and the lighter fractions volatilized (Aurand and Coelho, 2005). The studies grouped as “Spiked Exposure” in Table 7 present acute toxicity values using this exposure scenario. For some tests, concentrations of dissolved hydrocarbons did not persist long enough in the exposure solutions to elicit toxicity (e.g., LC50 > initial measured concentration). Additionally, the LC50 values were generally greater than what was shown in static-renewal tests. This latter point can be seen by comparing the tests by Rhoton, et al. (2001) and Fuller and Bonner (2001) using static-renewal tests with independent WAFs versus the spiked exposure tests. In each comparison, the LC50 values for the spiked exposure tests were greater than the LC50 values for the static-renewal tests. These comparisons demonstrate that the exposure design can influence the toxicity endpoints for these tests.

For studies conducted using oil:water dispersions, the data produced by Anderson, et al. (1974) demonstrate that the source of the crude oil as well as differences in species sensitivity affect the test endpoints. Because the studies cited in Table 7 for OWDs were conducted in a similar manner at the same laboratory, inter-laboratory variability was controlled and the differences in toxicity were most likely due to the controlled variables of crude type and test species. Because dissolved hydrocarbons were not measured, the endpoints were defined by the total oil loading per unit volume of water. These tests were done in open/mixed vessels, and loss of volatile components of the oil would be expected. However, these nominal concentrations reveal that total oil loadings

necessary to elicit toxicity are quite high in comparison to what might be expected in the dissolved fraction. The range of LC50 values reported by Anderson, et al. (1974) was 3,700 mg/L to >80,000 mg/L. The static-renewal tests conducted by Rhoton, et al. (2001) reported LC50s and LL50s. The LL50 values for *Menidia* exposed to Alaska North Slope and Prudhoe Bay crude oils were 1,641 and 4,965 mg/L, respectively. These values were somewhat lower than those of Anderson, et al. (1974) and may have been due to daily renewal of the test solutions.

6.1.2 Acute Toxicity to Aquatic Invertebrates

Table 8 presents aquatic toxicity data for aquatic invertebrates using independent WAFs, dilutions of a WAF or WSF, spiked exposures, and whole oil:water dispersions. For studies conducted with independent WAFs and renewal of the test solutions, the EC50 values ranged from a low of 0.56 mg/L to 2.61 mg/L (Fuller and Bonner, 2001; Rhoton et al., 2001) when based on mean measured dissolved petroleum hydrocarbons (GC-FID or GC-MS). Rhoton et al. (2001) aerated the vessels and although test solutions were renewed, some loss of dissolved hydrocarbons was likely to have occurred. This may explain the higher LC50 values measured in their studies as compared to those of Fuller and Bonner, who employed sealed test vessels without aeration.

Table 8. Acute Toxicity of Crude Oil to Aquatic Invertebrates

Crude Oil Type	Invertebrate Species	Test/ Exposure Type	Endpoint Basis	Endpoint Value, mg/L	Reference
INDEPENDENT WAF					
Arabian Medium crude oil	*Americamysis bahia* (saltwater mysid) (formerly Mysidposis bahia)	Independent WAFs; sealed vessels; static-renewal	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = 0.56 (test 1) 0.67 (test 2)	Fuller and Bonner (2001)
Alaska North Slope crude oil	*Chionocetes bairdi* (Tanner crab)	Independent WAFs; static-renewal	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 2.54 96-h LL50 = 12.48	Rhoton (1999)
Alaska North Slope crude oil	*Americamysis bahia* (saltwater mysid)	Independent WAFs; static-renewal	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 2.61 96-h LL50 = 160	Rhoton (1999)
WAF or WSF with dilutions					
10 different crude oils	*Daphnia magna*	1:40 oil:water (v/v) WSF then diluted; sealed vessels/zero headspace; static	Purge and trap; GC-FID	48-h EC50 = 4.6 to 13.3 (2 oils not toxic)	Environment Canada (1994)
13 different crude oils	*Daphnia magna*	1:40 oil:water (v/v) WSF then diluted; sealed vessels/zero headspace; static	Headspace; GC-MSD	48-h EC50 = 4.8 to 28.7 (7 oils not toxic)	Environment Canada (1994)
WAF spiked exposure					
Arabian Medium crude oil	*Americamysis bahia* (saltwater mysid) (formerly Mysidposis bahia)	Independent WAFs; CROSERF flow-through	GC-MS measured mg TPH/L (C10-C36)	96-h LC50 = >14.3 (test 1) >11.6 (test 2)	Fuller and Bonner (2001)

Louisiana Sweet crude oil	*Americamysis bahia* (saltwater mysid)	1:40 oil:water (v/v) WAF then diluted; static; open/aerated	GC-FID measured mg TPH/L	48-h LC50 = 2.7	Hemmer, et al. (2010)
Alaska North Slope crude oil	*Chionocetes bairdi* (Tanner crab)	Independent WAFs; CROSERF flow-through	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 13.85 96-h LL50 = 285	Perkins, et al. (2003)
Alaska North Slope crude oil	*Americamysis bahia* (saltwater mysid)	Independent WAFs; CROSERF flow-through	GC-FID measured VOA (C6-9) + TPH (C10-36) = mg THC/L	96-h LC50 = 9.625 96-h LL50 = 654	Perkins, et al. (2003)
Whole OWD					
Kuwait crude oil	*Mysidopsis almyra*	OWD; open/mixed	Nominal whole oil loading, mg/L	48-h LC50 = 63	Anderson, et al. (1974)
Kuwait crude oil	*Paleomonetes pugio*	OWD; open/mixed	Nominal whole oil loading, mg/L	48-h LC50 = 6,000	Anderson, et al. (1974)
South Louisiana crude oil	*Mysidopsis almyra*	OWD; open/mixed	Nominal whole oil loading, mg/L	48-h LC50 = 37.5	Anderson, et al. (1974)
South Louisiana crude oil	*Paleomonetes pugio*	OWD; open/mixed	Nominal whole oil loading, mg/L	48-h LC50 = 200	Anderson, et al. (1974)
South Louisiana crude oil	*Penaeus aztecus*	OWD; open/mixed	Nominal whole oil loading, mg/L	48-h LC50 = >1000	Anderson, et al. (1974)
11 crude oils from North Sea origin	*Cragon cragon*	OWD, open	Nominal whole oil loading, mg/L	96-h LC50 = 27 to 110	Franklin and Lloyd (1982)
8 crude oils from Middle East origin	*Cragon cragon*	OWD, open	Nominal whole oil loading, mg/L	96-h LC50= 41 to 119	Franklin and Lloyd (1982)

Environment Canada (1994) tested 23 different crude oils from various parts of the world against *Daphnia magna* using dilutions of a WSF (1:40 oil/water ratio). Nine of the 23 crude oils produced insufficient toxicity to derive EC50 values, and for those crude oils that elicited toxicity, the EC50 values ranged from 4.6 mg/L to 28.7 mg/L.

Tests run with crude oil prepared as spiked concentrations with aquatic invertebrates showed results similar to the fish studies. EC50 values were higher than those obtained via independent WAF tests (with exposure solutions renewals) or those using dilutions of WSFs when attempts were made to maintain exposure concentrations (e.g., use of static/sealed test vessels). Toxicity values ranged from low of 2.7 mg/L (Hemmer, et al. 2010) to 13.85 mg/L (Perkins et al., 2003). Fuller and Bonner (2001) reported an LC50 greater than the highest concentration (>11.6 mg/L). Toxicity data for the spiked studies were based on measured concentrations at the beginning of the test and did not reflect exposures over the course of the testing period. The values cited by Hemmer, et al. (2010) appeared lower than what might be expected for a spiked exposure, but may be due to the use of open and aerated test vessels. Their report did not provide details of their analyses; therefore, the range of molecular weight hydrocarbons measured by their method was unknown.

The dispersion studies run by Anderson et al. (1974) and Franklin and Lloyd (1982) have value in that they permit comparisons to be made between test species and between different crude oils while maintaining standard testing procedures. Anderson et al. (1974) measured LC50 values ranging from 37.5 mg/L to 6000 mg/L. One crude oil was not toxic at the highest loading rate (LC50 > 1000 mg/L). These LC50s are considerably lower than LC50 values reported by the same authors for fish (Table 7) in tests of the same crude oils. Franklin and Lloyd (1982) used a dispersion technique similar to Anderson et al. (1974). For 11 crude oils from the North Sea region, LC50 values ranged from 27 to 110 mg/L, while those for eight Middle East crude oils ranged from 41 to 119 mg/L.

6.1.3 Toxicity to Algae

Although the effects of crude oil on algae have drawn less attention than those on fish and aquatic invertebrates, the data reviewed here suggest responses similar to those reported for aquatic invertebrates. Table 9 presents study results for tests of crude oil to algae. Gaur and Singh (1989) tested Assam crude oil in two ways, first using dilutions of a WSF, then in another test using direct addition of the crude oil to the algae medium via oil-soaked absorbent pads. The direct addition method resulted in lower EC50 values for both growth rate and cell yield than using dilutions of the WSF, but only by a factor of approximately 1.5. The authors used a 15-day exposure duration, and no data was reported for the standard 3-4 day time period commonly used in regulatory testing. Tsvetnenko and Evans (2002) conducted their tests in a manner more in line of standard regulatory guidelines (e.g., OECD, ASTM). They tested three crude oils having °API gravities of 21, 34, and 48. The crude oil having the greatest specific gravity (°API 21) resulted in the lowest EC50 values when based on cell biomass or growth rate (0.94 and 6.16 mg/L, respectively). Toxicity was somewhat less with the crude oils having higher °API gravities, but the endpoint values for the 34 and 48 °API crudes were not substantially different from each other.

Table 9. Acute Toxicity of Crude Oil to Algae

Crude Oil Type	Algal Species	Test/Exposure Type	Endpoint Basis	Endpoint Value, mg/L	Reference
Assam crude	*Anabaena doliolum*	Dilutions of 1:20 oil/water WSF Direct Addition	Dissolved hydrocarbons by spectrofluorometry	15-d EC50 = 9.06 (rate) 10.45 (cell yield) 5.73 (rate) 7.47 (cell yield)	Gaur and Singh, 1989
Western Australia crude °API=21	*Isochrysis sp.*	Dilutions of 1:10 oil/water WSF	GC-MS purge/trap (C6-C9) + solvent extraction and GC-FID (C10-C36)	96-h EC50 = 0.94 (biomass) 6.16 (rate)	Tsvetnenko and Evans, 2002
°API =34				5.51 (biomass) 8.38 (rate)	
°API=48				3.6 (biomass) 7.38 (rate)	

None of the cited studies were run using sealed test vessels to prevent the loss of volatile components, and Tsvetnenko and Evans (2002) measured a 50 – 70% drop in the dissolved hydrocarbons between the beginning and end of their tests. Gaur and Singh (1989) did not indicate the frequency of their hydrocarbon measurements, but the 15-d duration of their test suggests that loss of volatile fractions may have occurred.

6.1.3 Summary of Acute Aquatic Toxicity

The studies cited above and described more fully in the robust summaries show a wide range of organisms' responses to oil exposures. Some of this variability is due to using different methodologies, such as independent WAFs, dilutions of a WAF or WSF, spiked exposures, oil:water dispersions, and the use of open versus sealed test vessels. The choice of the analytical method also is important when the toxicity endpoints are based on measured concentrations of dissolved hydrocarbons. Analytical instruments used in the reviewed studies include GC-MS, GC-FID, and spectrofluorometry. These instruments together with the different sample preparation methods (e.g., solvent extraction, purge and trap, headspace analysis) may capture a wide range of molecular weight hydrocarbons or focus on a narrow range of carbon chain lengths. All these techniques were used in some of the cited reports, and this too presents a source of variability in the calculated endpoints.

Because crude oils are of such compositional complexity, to fully characterize all components that potentially might contribute to aquatic toxicity is not possible. However, crude oils and their refined products cause acute toxicity via nonpolar narcosis mode of action (van Wezel and Opperhuizen, 1995). Therefore, an understanding of the acute aquatic toxicity of crude oil relative to fractions of crude oil can be obtained from the ranges in ecotoxicity endpoint values for different distillation fractions. Presenting the acute toxicity of the different distillation fractions of crude oil provides an aquatic hazard of each fraction and collectively forms a set of ecotoxicity values within which crude oil falls. This is illustrated in Table 10, which shows the ranges of effect values (e.g. LL/EL50) for selected data cited in the crude oil robust summaries and the robust summaries of other petroleum HPV categories. Reports were selected based on similar testing methodology.

Table 10. Ranges for Ecotoxicity Endpoints for Crude Oil and Different Distillation Fractions of Crude Oil Based on WAF Studies

Petroleum HPV Category	Typical Carbon Range	Range of Effect Levels, LL/EL$_{50}$ (mg/L)			Source
		Fish	Invertebrate	Algae	
Crude Oil	C4 –C60+	1641 – 4965	12.48 - 160	No WAF Data	Rhoton (1999) Rhoton et al. (2001)
Gasoline	4 – 12	8.2 – 46	4.5 - 32	1.1 - 64	CONCAWE (1996b) Stonybrook Laboratories (1995a-d)
Kerosene	9 – 16	10 – 100	1.4 - 89	5 - 30	EBSI (1995a,b) Shell (1995a-c)
Gas Oil	9 – 30	3.2 – 65	2 - 300	1.9 - 78	Clark, *et al*. (2003) EBSI (1998a,b) EBSI (2001) Shell (1995d)
Heavy Fuel Oil	7 – 50	100 - 10,000	220 - 10,000	3 - 5,000	Mobil (1987a-c) Shell (1997a-c)
Lube Oils	15 - 50	>100 - >1,000	>1000 - >10,000	>50%[1]	BP International (1990a,b) EBSI (1995c)

					Shell (1988)
Aromatic Extracts	15 – 50	>1,000	>1,000	>1,000	BP Oil Europe (1994a-c)
Note: For the purpose of comparison, the above data are from summarized referenced sources that employed water-accommodated fractions (WAFs) as exposure solutions. Other ecotoxicity data in the public domain may be from oil-water dispersions (CONCAWE 2001) or water-soluble fractions as with some studies cited in the discussion herein and hence may not be directly comparable to studies using WAF solutions. [1] The endpoint value for algae represents a 50% dilution of a 1000 mg/L WAF, and was the highest level used in the test.					

The world's supplies of crude oils are represented by hydrocarbons covering a wide range of molecular weights. The point illustrated in Table 10 is that streams that are derived from crude oil that contain primarily relatively low molecular weight hydrocarbons such as gasoline (C4-C12), kerosene (C9-C16), and gas oil (C9-C30), typically show greater toxicity than those streams having predominantly higher molecular weight hydrocarbons (heavy fuels, lube oils, and aromatic extracts). Such a trend may be explained by the greater bioavailability of the low molecular weight and higher levels of more water soluble constituents. As petroleum streams become composed of higher molecular weight hydrocarbons, their limited water solubilities limit bioavailability, and hence toxicity. Regardless of the source of the crude oil, aquatic toxicity is not likely to be any greater than that represented by the most toxic distillation fraction. Thus, while the lowest acute toxicity endpoint for a crude oil was 12.48 mg/L, acute toxicity could potentially fall within the range of 1 – 10 mg/L, depending on the proportion of low molecular weight hydrocarbons in the crude's composition. This may be expected when testing is conducted using WAF loading rates using standard methods for difficult substances (OECD, 2000).

6.2 Aquatic Chronic Toxicity

Chronic toxicity to aquatic organisms would be expected based on the wide range of partition coefficients, i.e. log K_{ow} 2 to >6, of constituents in crude oils. Because thousands of hydrocarbon components in crude oils could potentially affect chronic toxicity, no reliable correlation exists between crude oil composition and chronic aquatic toxicity as determined by current, standardized test methods. However, chronic toxicity of crude oil fractions to the early life stages of fish is an area of ongoing research (Rhodes, *et al*., 2005). A review of data published in the scientific literature and summarized in the robust summaries indicates that chronic adverse effects to aquatic organisms are caused by exposure to crude oil, and the effects cover a range of chronic toxicity endpoints such as growth, embryo and larval survival, fecundity, gametophyte viability, developmental processes, cardiac arrhythmia, and osmoregulation (Pollino and Holdway, 2002; Perkins *et al*., 2003; Holdway, 2002; Din and Abu, 1992; Moffitt *et al*., 1992; Rhodes *et al*., 2005; Lockhart *et al*., 1996; Incardona *et al*., 2009). Chronic toxicity values vary with species and type of exposure, e.g., WAF, WSF, etc., but adverse effects have been reported at WAF loading rates of <1 mg/L.

6.3 Assessment Summary for Environmental Effects

Because crude oil is extracted from world-wide sources and composed of many different types and molecular weights of hydrocarbons, it may be impractical to assign specific acute aquatic toxicity values that cover the full domain of crude types. However, a generalization of the acute aquatic toxicity based on the data cited above indicates that when based on total crude oil loadings in water, either as WAFs or OWD, aquatic invertebrates are more sensitive than fish to crude oil exposure, and the lowest EL50 values may approach 10 mg/L. The lowest acute EL50 among the cited studies was 12.48 mg/L (Rhoton, 1999). When toxicity endpoints are based on measured concentrations of hydrocarbons in the dissolved phase of the exposure solutions, aquatic invertebrates still appeared to be more sensitive than fish or algae. The lowest EC50 value was 0.56 mg/L (Fuller and Bonner, 2001). All acute endpoints for fish were >1 mg/L when based on measured dissolved hydrocarbons. For algae, one test endpoint yielded an EC50 of 0.94 mg/L, but other data all fell within the range of 6 to 11 mg/L. In general, aquatic toxicity of crude oil is not likely to be any greater than that represented by the most toxic fraction. For concentrations presented as loading rates, acute toxicity could potentially fall within the range of 1 – 10 mg/L.

7.0 HUMAN HEALTH EFFECTS

Human health hazards associated with the exploration, production, and transportation of crude oil are most often from hydrogen sulfide, volatile organic compounds (VOCs) similar to gasoline, and polycyclic aromatic compounds (PAC). The inhalation exposure hazards of hydrogen sulfide and VOCs are described in the Petroleum HPV Category Assessment Documents for Refinery Gases and Gasoline Blending Streams respectively (Petroleum HPV, 2008 and 2009). The dermal exposure hazard of PAC is described in recent reports (API, 2008 and TERA, 2008) and also summarized in Appendix 3.

The analytical data in Table 2 is related to a series of statistical models that were developed to predict the potential repeated-dose and developmental toxicity of high-boiling petroleum substances by the dermal route of exposure in rats. The development of these models began with the observation that the more significant effects of several types of petroleum refinery streams in both developmental and repeated-dose studies appeared to be related to the total amount of 3-7 ring PACs (Feuston et al, 1994). The relationship was only qualitative and not predictive for individual samples. More recently statistical models were developed by API that quantitatively predict the doses at which potential effects occur on most sensitive endpoints in rats based on the profile of PACs in each sample. The models are empirically based on a number of toxicity studies on refinery streams for which analyses of PAC profile using a “PAC-2” method also existed. The PAC-2 analyses provided the percent of each aromatic ring class (ARC) that served as a basis for the models, i.e. ARC %, in Table 2. The endpoints used in the models were selected by an extensive analysis to determine the most sensitive endpoints among studies of developmental toxicity, repeated-dose toxicity, and optimized Ames tests.

These models have been applied to predict the potential toxicity of untested crude samples described later in this section.

Three types of quantitative values are used in this document.
1) Data from appropriate studies, such as No Observed Adverse Effect Level (NOAEL) on tested samples,
2) BMD_{10} (Bench Mark Dose) which is the dose that produces a 10% response relative to the control group that is calculated from data on tested samples (Crump, 1984),
3) PDR_{10} (Predicted Dose Response) which is the dose that produces a 10% change in the response relative to the control group predicted by statistical modeling using the PAC profile of untested samples (Appendix 3).

Appendix 5 contains summaries of several published studies that used unrealistic routes of administration and extremely high doses. Because of those deficiencies, the Petroleum HPV Testing Group recommends that they not be used for hazard or risk evaluation of crude oil. They are included in this document only for completeness.

7.1 Acute Toxicity

Crude oils have been tested for acute toxicity and were associated with low toxicity in all species *via* a variety of routes and endpoints. Data on the acute dermal toxicity and eye and skin irritation potential of five crude oils, i.e. four light crudes and one heavy crude, are summarized in Table 11. The results indicate that acute exposures to crude oils did not produce significant systemic toxicity by the dermal route and induced only minimal skin irritation. Only Lost Hills Light crude oil induced some conjunctival irritation at 24 hours.

Table 11. Acute Toxicity of Crude Oils[1]

Sample	**Dermal LD_{50} (Rabbit) g/kg**	**Skin Irritation (Rabbit)[2]**		**Eye Irritation (Rabbit 24hr) Conjunctival**
		Erythema	**Edema**	
Beryl [36.5°API]	>2.0	ND[c]	ND	1.7
Arab Lt [34.5 °API]	>2.0	0.9	0.1	1.3
Mid-Continent [40°API]	>2.0	ND	ND	0.3
Lost Hills Light [>38°API]	>2.0	1.6	1.3	3.7
Belridge Heavy [14°API]	>2.0	0.6	0.8	0.8

[1]Mobil, 1984a,b; 1985a,b; 1990a,b
[2] Mean scores on a scale of 0-4 of reactions at 24, 48, and 72 hrs. (Mobil 1985b)
ND = Not Determined

Lost Hills Light and Belridge Heavy crudes did not cause dermal sensitization in the guinea pig when tested using the Buehler method (Mobil, 1991a,b).

Exploration, production, and transport of crude oil can result in significant levels of hydrogen sulfide and/or VOCs in some situations (i.e., enclosed spaces). The acute inhalation hazard is primarily from hydrogen sulfide. When the inhalation acute toxicity of hydrogen sulfide was assessed in male and female Sprague-Dawley rats, the calculated LC_{50} was 444 ppm, with a confidence interval of 416 – 473 ppm (Tansy *et al*., 1981). VOCs from crude oil are expected to have low acute toxicity by the inhalation exposure route; e.g., Rat LC_{50} >5g/m^3 for 6-hour exposures, based on testing of various naphtha blending streams and gasoline (Petroleum HPV, 2008).

Conclusion: Crude oils have demonstrated little local irritation or systemic toxicity by dermal exposure. Neither of the two tested crude oils was a skin sensitizer. The acute inhalation hazard of crude oil is likely from the presence of hydrogen sulfide gas in some crude oils under specific conditions, i.e., enclosed spaces.

7.2 Repeat -Dose Toxicity

Inhalation Exposure:

Hydrogen sulfide toxicity, including nasal and pulmonary effects, was characterized in adult male and female Fischer-344 and Sprague–Dawley rats and $B_6C_3F_1$ mice (Dorman, 2004). Animals underwent whole-body exposure to 0, 10, 30, or 80 ppm H_2S for 6 h/day for at least 90 days. Exposure to 80 ppm H_2S was associated with reduced feed consumption during either the first exposure week (rats) or throughout the 90-day exposure (mice). Male Fischer-344 rats, female Sprague–Dawley rats, and female $B_6C_3F_1$ mice exposed to 80 ppm H_2S had depressed terminal body weights when compared with air-exposed controls. Subchronic H_2S inhalation did not result in toxicologically relevant alterations in hematological indices, serum chemistries, or gross pathology. Histologic evaluation of the nose showed an exposure-related increased incidence of olfactory neuronal loss (ONL) and rhinitis. ONL occurred following exposure to ≥30 ppm H_2S in both sexes of all experimental groups, with one exception, male Sprague–Dawley rats demonstrated ONL following exposure to 80 ppm H_2S only. A 100% incidence of rhinitis was found in the male and female B6C3F1 mice exposed to 80 ppm H_2S. In the lung, exposure to H_2S was associated with bronchiolar epithelial hypertrophy and hyperplasia in male and female Sprague–Dawley rats following exposure to ≥30 ppm H_2S and in male Fischer-344 rats exposed to 80 ppm H_2S. 10 ppm represented the NOAEC for hydrogen sulfide following subchronic inhalation.

The VOCs from crude oil are similar to those from gasoline and gasoline blending streams. Gasoline and gasoline blending streams have been evaluated for repeat dose hazard potential by inhalation (Petroleum HPV, 2008). Study details and references are found in Appendix 6. Subchronic studies demonstrate that the inhalation NOAECs and LOAECs were similar between the different hydrocarbon classes in the streams and the formulated product, gasoline, in rats. Since there were no appreciable differences between paraffinic, olefinic, naphthenic, and aromatic streams, a range of values derived from all of those repeated dose inhalation studies can be used to estimate the hazard of VOCs from crude oil. These values are:

LOAEC: 6572 mg/m^3 – 27,800 mg/m^3 (1864 – 7885ppm[a])
NOAEC: 1507mg/m^3 – 10,153 mg/m^3 (427 – 2880ppm[a])

[[a] - Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]

Dermal Exposure:
Lost Hills Light (>38°API, 0.86 wt% S) and Belridge Heavy (14°API, 1.05 wt% S) crude oils were applied dermally, without occlusion, to the clipped backs of male and female Sprague Dawley rats at dose levels of 0, 30, 125, and 500 mg/kg/day, 5 days/week for 13 weeks with accumulated material wiped off once weekly, one day after the last daily dose (Table 12). Following treatment, minimal skin irritation, i.e. flaking, was produced on the treated rats while hyperplasia and hyperkeratosis were evident in all the rats exposed to Lost Hills Light and almost all the rats treated with Belridge Heavy.

The effects of dermal exposure at 500 mg/kg of Belridge Heavy included reduced mean body weight gain and decreased platelet counts in male rats only and decreased hemoglobin, hematocrit and red blood cell counts in both sexes. Absolute liver weights and liver to body weight ratios, i.e. relative liver weights, were increased and absolute and relative thymus weights were decreased in both sexes at 500 mg/kg. Microscopically, the incidence of thymic atrophy was substantially increased in both sexes for most of the rats treated with 500 mg/kg of Belridge Heavy. Also, the incidence of hypertrophy and hyperplasia of the thyroid follicular epithelium was very apparent in males at the 500 mg/kg dose level of Belridge Heavy while a lower but still elevated incidence was observed at the two lower doses with no related thyroid effects observed in the female rats.

Dermal exposure to Lost Hills Light at 500 mg/kg did not affect body weight or body weight gain but did decrease hemoglobin, hematocrit and red blood cell counts in male rats only. Increased absolute and relative liver weights were found in both sexes at 500 mg/kg of Lost Hills Light but no significant thymus weight changes were seen at any dose. Microscopically, hyperplasia of treated skin was observed at all doses for Lost Hills Light and Belridge Heavy but was slightly more severe with Lost Hills Light than Belridge Heavy as would be expected with lower viscosity petroleum-related materials because of their greater potential to cause drying of the skin. With 500 mg/kg of Lost Hills Light treatment, thymic atrophy was observed in only ~16% of the rats while 500 mg/kg of Belridge Heavy produced this same effect in 65%. Hypertrophy and hyperplasia of the thyroid was observed in almost all of the Lost Hills Light treated males and a few of the females in the 30 mg/kg group were also affected while higher doses did not increase the incidence of the thyroid effects over control levels. No adverse effects were reported in other organ systems in either sex at any dose.

This study's Lowest Observable Adverse Effect Level (LOAEL) for all effects, without a NOAEL being established, for both crude oils was determined to be the lowest dose used, i.e. 30 mg/kg, which was based on the occurrence of skin irritation and marginal thyroid effects. Also, Belridge Heavy was richer in 3-5 ring polycyclic aromatic compounds (PAC) than Lost Hills Light demonstrated more severe toxicity as indicated by decreases in body weight gain, aberrant hematology, thymus atrophy and bone marrow histopathology (Feuston *et al*.,1997b). The refinery streams tested by Feuston *et al,* 1994 produced

similar toxicity with these endpoints, e.g. reduced body weight gain, aberrant hematology and thymus atrophy as well as also being associated with elevated PAC content.

Table 12. Dermal Repeat-Dose Toxicity Endpoints

Crude oil type	Study type	Doses	Results	Refer.
Lost Hills Light	Animal Rat Sex Male Route Skin Freq. Daily Duration 90 d.	30, 125 & 500 mg/kg	Body wt gain Reduced (500[2] mg/kg) Hematology Changes (500[1]- 125[2] mg/kg) Liver Enlargement (500[1]- 125[2]mg/kg) Thymus Wt Reduction (500[2] mg/kg) Histopathology Incidence: Skin - Inflammation (≤30[1] mg/kg) Thyroid – Hypertrophy/Hyperplasia of Follicular Epithelium (≤30[1] mg/kg) Thymus – Atrophy (500[1] – 125[2] mg/kg)	Feuston, 1997b & Mobil 1992a
Belridge Heavy	Animal Rat Sex Male Route Skin Freq. Daily Duration 90 d.	30, 125 & 500 mg/kg	Body wt gain Reduced (500[1]-125[2] mg/kg) Hematology Changes (500[1]- 125[2] mg/kg) Liver Enlargement (125[1]- 30[2] mg/kg) Thymus wt. Reduction (500[1]- 125[2] mg/kg) Histopathology Incidence: Skin - Inflammation (≤30[1] mg/kg) Thyroid – Hypertrophy/Hyperplasia of Follicular Epithelium (500[1] – 125[2] mg/kg) Thymus – Atrophy (500[1] – 125[2] mg/kg) Bone Marow – Increased Cellularity & Focal Necrosis (500[1] – 125[2] mg/kg)	Feuston, 1997b & Mobil 1992b

[1]LOAEL that is ≤ the lowest adverse effects dose when a NOAEL is not established.
[2]NOAEL

BMD_{10} values, as described by Crump (1984) and derived from the actual Feuston, *et al* 1997b studies are given in Table 13 for reductions in the following endpoints: absolute thymus weight, liver/body weight ratio, hemoglobin concentration and platelet count following dermal Lost Hills Light or Belridge Heavy treatment of rats. These calculated BMD_{10} values indicated that Belridge Heavy crude caused effects at lower doses for thymus weight, liver/body weight ratio and platelet counts. Exposure to Lost Hills Light had the most pronounced effect on hemoglobin concentrations.

Table 13. BMD_{10} Values for Sensitive Endpoints in Repeat Dose Studies

Repeat-dose endpoint	BMD_{10} (mg/kg/d)	
	LOST HILLS LIGHT	BELRIDGE HEAVY
Absolute Thymus weight		
Male	146	65
Female	491	287
Liver/body weight ratio		
Male	236	62
Female	449	82

Hemoglobin concentration		
Male	195	467
Female	307	631
Platelet count		
Male	855	619
Female	>2000	614

Using the statistical models described in Appendix 3, the PDR_{10} values, for 46 crude oil samples were calculated for the most sensitive endpoints that were previously selected for this type of study (Table 14). The lowest sample PDR_{10}s ranged from 55 to 544 mg/kg and the effects associated with the lowest endpoint PDR_{10}s were either a depression in platelet count or a reduced absolute thymus weight.

Table 14. PDR_{10} Values[1] for Sensitive Endpoints in Repeat Dose Studies

Sample Number	API Gravity	Thymus Weight[2]		Platelet Count[2]		Hemoglobin Concentration[2]		Liver/body weight ratio[2]		Lowest PDR_{10}
		Male	Female	Male	Female	Male	Female	Male	Female	
	Degree	PDR_{10}	PDR_{10}	PDR_{10}	PDR_{10}	PDR_{10}	PDR_{10}	PDR_{10}	PDR_{10}	
70920	13.9	63	55	194	197	669	669	255	254	55
50905	19.4	599	529	2000	2000	2000	2000	840	834	529
10953	19.6	268	236	233	236	>1000	>1000	>1000	>1000	233
70918	20.8	408	360	534	542	>1000	>1000	>1000	>1000	360
30903	21.2	246	217	353	358	983	983	367	365	217
30913	21.4	388	342	394	399	2000	2000	636	632	342
70912	21.9	143	126	266	270	551	551	229	228	126
70917	23.3	106	93	123	125	485	485	228	227	93
30905	23.4	876	772	202	205	2000	2000	1638	1638	202
70916	23.4	147	130	177	179	739	739	352	350	130
70910	25.4	346	305	733	744	2000	2000	633	630	305
50910	26.6	250	221	453	460	1108	1107	416	410	221
10956	28.6	617	544	545	553	>1000	>1000	>1000	>1000	544
50907	28.9	196	173	441	447	1003	1002	422	420	173
70911	28.9	327	289	305	309	1630	1629	637	633	289
50908	29.3	864	762	337	342	2000	2000	1350	1342	337
10959	29.6	907	800	341	347	>1000	>1000	>1000	>1000	341
30965	29.6	407	359	204	207	>1000	>1000	>1000	>1000	204
10952	29.8	367	324	213	216	2000	2000	2000	2000	213
10954	30.4	363	320	275	280	>1000	>1000	>1000	>1000	275
50906	30.5	443	391	391	397	2000	2000	925	920	391
70913	30.5	288	254	224	227	2000	2000	943	937	224
50909	30.6	599	529	2000	2000	2000	2000	840	834	529
30902	30.7	410	364	203	205	2000	2000	1125	1118	203
70919	31.1	176	156	201	204	1267	1267	603	599	156
30906	31.5	333	294	205	208	2000	2000	2000	2000	205
30907	31.5	552	487	218	221	2000	2000	2000	2000	218
30909	32.4	>1000	>1000	221	225	2000	2000	2000	2000	221

30910	32.4	525	463	205	209	2000	2000	2000	2000	205
50904	32.4	408	360	558	567	2000	2000	1273	1266	360
10960	32.7	358	316	193	196	2000	2000	>1000	>1000	193
30904	33.1	393	347	299	303	2000	2000	1266	1258	299
10957	33.3	649	572	282	286	>1000	>1000	>1000	>1000	282
30908	33.3	439	387	131	133	2000	2000	>1000	>1000	131
30964	33.3	476	420	212	215	2000	2000	1605	1595	212
10951	33.4	255	225	177	179	>1000	>1000	>1000	>1000	177
50902	33.6	565	498	816	828	1985	1984	642	638	498
70914	36.5	813	717	418	424	2000	2000	2000	2000	418
10958	36.9	1034	912	287	292	2000	2000	2000	2000	287
50901	38	842	743	396	401	2000	2000	847	842	396
30911	38.3	378	334	429	435	2000	2000	1638	1638	334
10955	39.1	877	774	265	269	2000	2000	2000	2000	265
30914	39.1	429	379	239	243	2000	2000	2000	2000	239
50903	39.4	377	332	343	348	2000	2000	2000	2000	332
30912	41.8	601	530	187	190	>1000	>1000	>1000	>1000	187
70915	46.2	1137	1003	207	210	2000	2000	2000	2000	207

[1]mg/kg/day dose
[2]reduction

Conclusions: Repeat dose inhalation studies have not been done with crude oil but numerous studies on gasoline and gasoline blending streams (similar to the VOCs from crude oil) in rats have determined a range of NOAECs that can be use for read-across to crude oil; NOAEC: 1507mg/m^3 to 10,153mg/m^3 (427 – 2880ppm[a]) *[[a] - Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]* Repeat dose studies with hydrogen sulfide have established a NOAEC of 10 ppm in rats and mice based on injury to the nasal olfactory epithelium.

Studies of repeated exposure by the dermal route have demonstrated toxicity that was indicated by changes in hematology values, liver enlargement and thymic atrophy. Measured and modeled toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 samples of crude oil were between 55 and 544 mg/kg/day.

7.3 Genetic Toxicity: *In Vitro*

Standard gene mutation assays performed with *S. typhimurium,* with and without metabolic activation from rodent liver homogenate, did not produce mutagenic activity with the crude oils, Arab Light, 34.5°API, light crude (Petrilli *et al*., 1980) or Wilmington, 18°API, heavy crude (Lockard *et al*., 1982). This lack of response in the *in vitro* systems is thought to be due to limited solubility of the oils in aqueous medium and possible competition of non-biologically active components for available metabolic sites (Hermann *et al*., 1980; Cragg *et al*., 1985; Vandermeulen *et al*., 1985).

Testing of Kuwait, 31°API a medium crude, and Saran Gachs crude oil and their water-soluble fractions (WSF) with 5 strains of *S. typhimurium* also gave no significant indication of mutagenicity in spot tests or plate tests, i.e. top agar + test material with or without metabolic activation mixture poured on agar plate (Vandermeulen *et al*., 1985). Four chromatographic fractions of Kuwait crude and its water soluble fraction were obtained by elution with 2 bed volumes each of hexane, followed by 10% benzene/hexane, 50% benzene-hexane and acetone. Significant mutagenic activity was obtained in the F4 fraction which contained unresolved peaks of presumably polar DMSO-soluble high molecular weight components of Kuwait crude oil. Mutagenic activity was not enhanced by metabolic activation. Less mutagenic activity was also seen in F2 which contained 3-4 ring material. Testing of fractionated Kuwait-WSF produced variable results not clearly indicative of mutagenesis. The higher mutagenic response seen by F4 compared to F2 indicated that considerable mutagenic activity resides in the DMSO-soluble large molecular weight components of 4 rings or higher identified by HPLC/UV analysis.

The optimized Ames test, which employs DMSO extraction, hamster liver S-9 and increased metabolic activation mixture (see Appendix 2 for details) identifies mutagenic potential and predicts potential dermal carcinogenicity of petroleum-based complex mixtures and is correlated with the level of 3-7 ring PAC (ASTM, 2002; Mackerer *et al*., 2003). In 1995, the optimized Ames test was standardized as an ASTM method [ASTM E1687-95]. When several crude oils were tested in the optimized Ames test, Arab Light (Mobil, 1984), Beryl (Mobil, 1984), Mid-Continent (Mobil 1984) and Belridge Heavy (Mobil, 1990c) all showed significant mutagenic activity. However, Lost Hills Light crude oil did not produce a mutagenic response when tested with the optimized Ames test (Mobil, 1984; 1990c).

Studies by Roy *et al*. (1985; 1988) have demonstrated a strong correlation between PAC content and mutagenicity index in the optimized Ames test for petroleum-derived substances which produce dermal tumors when tested in mice. The utility of this relationship for read-across to untested substances has been expanded by statistical modeling (see Appendix 2 for details). The outcome of optimized *Salmonella* tests can be predicted from PAC compositional information with an accuracy that seems comparable to that associated with variability inherent with either the experimental methods or the methods used to calculate mutagenicity index from the experimental data. The mutagenicity index (MI) results for 46 samples of crude oil were predicted using the statistical model. The MI is the slope of the initial portion of the dose response curve expressed in units of revertants per microliter. The mutagenicity index was highly correlated with dermal carcinogenic potential, suggesting that oils with MI values < 1 were unlikely to be dermally carcinogenic, oils with MI values $\geq$ 1 but < 2 were indeterminate, and oils with MI values $\geq$ 2 would likely produce skin tumors if tested in mice.

When predictive modeling was done on 46 samples of crude oil for MI, only 2 of the 46 samples gave predicted MIs of less than 1(samples 10960 and 10956 in Table 2). These results demonstrate that most crude oils are expected to be in vitro mutagens and potential dermal carcinogens.

When tested in *In vitro* studies crude oils did not induce cytotoxicity or chromosome damage in Chinese Hamster ovary cells (Mobil, 1991c,d). Wilmington crude did not induce sister chromatid exchange in human lymphocytes *in vitro* (Lockard *et al*., 1982).

Conclusions: *In vitro* gene mutation has been demonstrated in bacterial assays for extracts of a variety of crude oils. Predictive modeling based on analytical determination of their PAC profile also demonstrates that crude oil is typically expected to be an in vitro mutagen and potential dermal carcinogen. Generally, standard *in vitro* tests performed without extraction or optimization of test conditions with crude oils in bacterial or mammalian cells did not demonstrate genetic toxicity.

7.4 Genetic Toxicology: *In Vivo*

Results of micronucleus assays in Sprague Dawley rats treated dermally for 13-weeks with Lost Hills Light or Belridge Heavy crude oils at doses of 0, 30, 125 or 500 mg/kg for 13 weeks demonstrated that these crude oils did not induce cytogenetic damage in the bone marrow of the treated rats after repeated exposures (Mobil, 1990c; 1991e). Also, a single intraperitoneal (ip) injection of Wilmington heavy crude oil at a dose of 6.1 g/kg to ICR mice did not induce an increase in micronuclei (Lockard *et al*., 1982). However, intraperitoneal injection of 7.2 g/kg did induce a slight statistically significant increase in sister chromatid exchanges (Lockard *et al*., 1982). Sister chromatid exchange is indicative of DNA perturbation expressed as a direct transfer of similar labeled genetic material between chromatids with no loss or gain of chromatin. The biological significance is unknown.

Conclusions: The "in vivo" micronucleus assay does not demonstrate cytogenetic activity from crude oil exposure either by the dermal route, the most relevant to man, or by the more extreme intraperitoneal route. The results of micronucleus tests on a range of petroleum HPV category substances in addition to crude oil support the conclusion that clastogenic effects are unlikely to be induced by crude oils. (McKee *et al*, 2010).

7.5 Developmental Toxicity

<u>Inhalation Exposure:</u>

Hydrogen sulfide has been studied to determine if it had an adverse impact on pregnancy outcomes, offspring prenatal and postnatal development, or offspring behavior (Dorman et. al., 2000). Virgin male and female Sprague–Dawley rats (12 rats/sex/concentration) were exposed (0, 10, 30, or 80 ppm H_2S; 6 h/day, 7 days/week) for 2 weeks prior to breeding. Exposures continued during a 2-week mating period (evidence of copulation = gestation day 0 = GD 0) and then from GD 0 through GD 19. Exposure of dams and their pups (eight rats/litter after culling) resumed between postnatal day (PND) 5 and 18. Adult male rats were exposed for 70 consecutive days. Offspring were evaluated using motor activity (PND 13, 17, 21, and 60 ± 2), passive avoidance (PND 22 ± 1 and 62 ± 3), functional observation battery (PND 60 ± 2), acoustic startle response (PND 21 and 62 ± 3), and neuropathology (PND 23 ± 2 and 61 ± 2). There were no deaths and no adverse physical signs observed in F_0 male or female rats during the study. A statistically significant decrease in feed consumption was observed in F_0 male rats from the 80-ppm hydrogen

sulfide exposure group during the first week of exposure. There were no statistically significant effects on the reproductive performance of the F_0 rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. Exposure to hydrogen sulfide did not affect pup growth, development, or performance on any of the behavioral tests.

The VOCs from crude oil are similar to those from gasoline and gasoline blending streams. Numerous developmental toxicity studies have been done on gasoline and gasoline blending streams (Petroleum HPV, 2008). Study details and references can be found in Appendix 6. Developmental toxicity has not observed in inhalation in rats for samples in any gasoline blending stream with the exception of one 40% olefinic sample [chamber vapor content 41% olefins] developmental study in which increased resorptions were reported at the highest dose [2128ppm; (7660mg/m^3)]. Of note is that the authors were not sure of the biological significance of this occurrence. Another sample of the same substance with higher olefin content [chamber vapor content 61% olefins] run at higher exposure concentrations did not show any developmental toxicity. In addition, no developmental effects were seen with wholly vaporized gasoline [NOAEC = 1600ppm (5970mg/m^3], a 10% distillate sample of unleaded gasoline [NOAEC = 8993ppm (23881mg/m^3)], or a gasoline vapor condensate [NOAEC = 20,638 mg/m^3]. No increases in resorptions were reported in any of these studies. NOAEC values for developmental effects reflect the maximum doses tested. Parental systemic LOAEC and NOAEC values over all studies reflect primarily decreases in body weights at maximum doses. The read-across ranges that can be used for VOCs from crude oil are:

Developmental NOAEL = 5970mg/m^3 to 27750mg/m^3,

Parental systemic toxicity LOAEL = 13650 mg/m^3 to 27750 mg/m^3:
NOAEL = 2275 mg/m^3 to 25000 mg/m^3

<u>Dermal Exposure:</u>
Lost Hills Light and Belridge Heavy crude oils were evaluated for pre- and post-natal developmental toxicity by the dermal route (Feuston *et al*., 1997a; Mobil, 1991f,g). “Prenatal rats” were sacrificed on GD20; “postnatal rats” delivered naturally and remained, untreated, with their litters until sacrifice at 3-4 weeks postpartum. Lost Hills Light was applied to clipped backs of pregnant rats at doses of 0, 125, 500 and 1000 for the postnatal group and 2000 for the prenatal group mg/kg/day on GD 0-19. Belridge Heavy was applied with the same regimen at doses of 0, 30, 125, and 500 mg/kg/day. Application sites were not occluded but rats were fitted with Elizabethan collars to inhibit possible oral ingestion of test material. The studies are summarized in Table 15.

Both crude oils produced maternal and developmental toxicity. Maternal effects included slight (Lost Hills Light) to moderate (Belridge Heavy) skin irritation. For prenatal treatment with Lost Hills Light or Belridge Heavy the NOAEL for maternal toxicity was 125 mg/kg while the LOAEL was 500 mg/kg for decreases in body weight gains and increases in relative liver weights. Also, maternal treatment with 2000 mg/kg of Lost Hills Light produced decreases in absolute and relative thymus weights. For postnatal treatment the NOAEL for maternal toxicity was 500 mg/kg for Lost Hills Light and 125 mg/kg for Belridge

Heavy. The LOAEL for Lost Hills Light was 1000 mg/kg and for Belridge Heavy was 500 mg/kg for decreases in body weight gains during gestation.

In the prenatal rats treated with Lost Hills Light, the NOAEL was not established but the LOAEL for developmental toxicity was ≤125 mg/kg for delayed ossifications. Delayed ossifications occurred with all doses of Lost Hills Light while decreases in fetal body weights and live fetuses and increases in resorptions with concomitant decrease in litter size occurred at 2000 mg/kg. Further, a visceral malformation described as a "right-sided esophagus" was found at a low (4.1%) incidence with a 2000 mg/kg Lost Hills Light dose. For Belridge Heavy, the NOAEL for developmental toxicity was 125 mg/kg and the LOAEL was 500 mg/kg for decreases in fetal body weights and live fetuses, increases in resorptions and delayed ossifications.

In the postnatal rats treated with Lost Hills Light, the NOAEL for developmental toxicity was 500 mg/kg and the LOAEL was 1000 mg/kg for decreases in pup weights on Days 21 and 28. The NOAEL for developmental toxicity with Belridge Heavy was not established but the LOAEL was ≤30 mg/kg for the reduction in the "day four pup viability index". The pup viability index was reduced with all doses of Belridge Heavy while parturition delays occurred at 500 mg/kg. (Feuston *et al*., 1997a; Mobil, 1991f,g). In addition, although Lost Hills Light treatment induced delayed ossification at 125 mg/kg and the higher doses, Belridge Heavy produced much more serious toxicity at doses lower than those where Lost Hills Light produced the same adverse effects, e.g. increased resorptions and decreased fetal weights or produced adverse effects that did not occur even with the highest Lost Hills Light dose, e.g. decreased pup viability index and delayed parturition. Generally, the greater severity of effects was seen in animals from groups exposed to Belridge Heavy.

The developmental toxicity results with crude oil are consistent with results of studies performed with petroleum refinery streams and products that have significant concentrations of PACs. Feuston *et al*,. 1994 demonstrated following the dermal application of a series of refinery steams, a correlation between the incidence of resorptions as well as a decrease in fetal weights and increasing 3-5 ring PAHs content with a LOAEL range for an increased incidence of resorptions of 8 to 1000 mg/kg(See Appendix 3).

Table 15. Developmental Toxicity Studies with Crude Oil

CRUDE OIL	**STUDY** ANIMAL/ ROUTE/EXPOSURE DAY (s)	**DOSE RANGE**	**RESULTS** {Exposure Day(s)}	**REFER.**
LOST HILLS LIGHT	Rat/Dermal / GD 0-19/ (GD 20 Necropsy)	125, 500 or 2000 mg/kg/ day (Prenatal)	Maternal NOAEL= 125 mg/kg LOAEL= 500 mg/kg Body Wt. Gain Decreases & Increases Relative Liver Wts. Offspring LOAEL ≤125 mg/kg for Delayed Ossifications	Feuston, 1997a & Mobil 1991f

LOST HILLS LIGHT	Rat/Dermal / GD 0-19/ (Parturition Day: 21 for Maternal & 28 for Pup Necropsy)	125,500 or 1000mg/kg/ day (Postnatal-Not dosed)	Maternal NOAEL= 500 mg/Kg LOAEL= 1000 mg/kg for Body Wt. Gain Decreases on GD 20 PUPS NOAEL= 500 mg/kg LOAEL= 1000 mg/kg for Decreases in Body Wts. on Parturition Day 21 & 28.	Feuston, 1997a & Mobil 1991f
BELRIDGE HEAVY	Rat/Dermal / GD 0-19/ (GD 20 Necropsy)	30,125 or 500 mg/kg/ day (Prenatal)	Maternal NOAEL= 125 mg/kg LOAEL= 500 mg/Kg for Body Wt. Gain Decreases & Increase Relative Liver Wts. Offspring NOAEL= 125 mg/kg LOAEL = 500 mg/kg for Increased Resorptions, and Decreased Fetal Wts., Live Fetuses & Delayed Ossifications	Feuston, 1997a & Mobil 1991g
BELRIDGE HEAVY	Rat/Dermal / GD 0-19/ (Parturition Day: 21 for Maternal & 28 for Pup Necropsy)	30,125 or 500 mg/kg/ day (Postnatal-Not dosed)	Maternal NOAEL= 125 mg/kg LOAEL= 500 mg/Kg for Body Wt. Gain Decreases Offspring LOAEL <30 mg/kg for Decreased Viability Indices	Feuston, 1997a & Mobil 1991g

GD = Gestation Day(s)

BMD_{10} values, as described by Crump (1984) and derived from the actual Feuston, *et al* 1997a studies are given in Table 16 for reductions in the following endpoints: fetal body weight, live fetuses per litter, and percent resorptions following dermal treatment of rats with Lost Hills Light or Belridge Heavy crude oil. For BMD_{10} values the Percent Resorptions were the most sensitive indicator of developmental toxicity.

Table 16. BMD_{10} Values for Sensitive Endpoints in Pre-Natal Developmental Toxicity Studies

Developmental endpoint	**BMD_{10} (mg/kg/d)**	
	LOST HILLS LIGHT	**BELRIDGE HEAVY**
Fetal Body Weight	1870	370
Live Fetuses per Litter	424	122

Percent Resorptions	91	106

Using the statistical models described in Appendix 3, the predicted dose response (PDR_{10}) values for 46 crude oil samples with PAC analytical data were calculated (Table 17). The statistical models generate estimates for sensitive pre-natal developmental toxicity endpoints. The lowest sample PDR_{10}s ranged from 53 to 2000 mg/kg and the consistent indicator of the lowest endpoint PDR_{10}s was the number of live fetuses per liter.

Table 17. PDR_{10} Values[1] for Pre-Natal Developmental Toxicity Endpoints[2]

Sample No.	API Gravity	Fetal Body weight[3]	Live Fetuses/Litter[3]	% Resorption[4]	Lowest PDR_{10}
	Degree	PDR_{10}	PDR_{10}	PDR_{10}	
70920	13.9	202	61	100	61
50905	19.4	2000	666	1494	666
10953	19.6				
70918	20.8	>1000			>1000
30903	21.2	431	89	157	89
30913	21.4	1036	221	399	221
70912	21.9	282	68	119	68
70917	23.3	214	53	90	53
30905	23.4	1504	431	672	431
70916	23.4	346	96	160	96
70910	25.4	1037	233	426	233
50910	26.6	679	169	304	169
10956	28.6				
50907	28.9	515	151	258	151
70911	28.9	653	138	237	138
50908	29.3	1507	381	634	381
10959	29.6				
30965	29.6	2000	>1000	2000	>1000
10952	29.8	2000			2000
10954	30.4	2000			2000
50906	30.5	1528	517	942	517
70913	30.5	819	227	371	227
50909	30.6	2000	666	1494	666
30902	30.7	926	224	364	224
70919	31.1	478	129	213	129
30906	31.5	1581	1151	1391	1391
30907	31.5	1571	662	938	662
30909	32.4				
30910	32.4	1525	698	947	698
50904	32.4	2000	1115	2000	1115
10960	32.7	2000			2000
30904	33.1	1403	588	967	588

10957	33.3	2000			2000
30908	33.3				
30964	33.3	1722	840	1289	840
10951	33.4	2000			2000
50902	33.6	1467	349	679	349
70914	36.5	2000	913	1336	913
10958	36.9	2000	>1000	>1000	>1000
50901	38	1703	390	716	390
30911	38.3	2000	2000	2000	2000
10955	39.1	2000	>1000		>1000
30914	39.1	2000	2000	2000	2000
50903	39.4	>1000			>1000
30912	41.8	2000			
70915	46.2	1409	387	579	387

[1] mg/kg/day dose
[2] blank cells indicate the model results were considered unreliable
[3] reduction
[4] increase

Conclusions: Inhalation exposure to the volatile constituents of crude oil, e.g., hydrogen sulfide or gasoline-like VOCs, are not expected to be a significant developmental toxicity hazard. A developmental neurotoxicity study on inhaled hydrogen sulfide determined a NOAEC of 80 ppm. VOCs from crude oil can be evaluated by using read-across data from studies on gasoline and gasoline blending streams. The developmental NOAEC values from various gasoline and gasoline blending streams studies are 5970mg/m^3 to 27750mg/m^3 (1694 – 7873ppm[a]). *[[a] - Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]*

Studies of a “light” and a “heavy” crude oil by the dermal route have demonstrated developmental toxicity that was indicated by changes including , (1) decreases in fetal body weights, (2) decreases in the Pup Viability Indices, (3) increases in resorption incidences. Measured and modeled developmental toxicity endpoints show a wide range of responses from different samples of crude oil via dermal exposure. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 53 and 2000 mg/kg/day.

Adverse developmental effects have been observed in animals treated dermally with individual PAC or petroleum-related materials with substantial concentrations of PAC. The results with crude oil are consistent with data from similar studies conducted with petroleum refinery streams that revealed a relationship between endpoints of developmental toxicity and increasing levels of 3-7 ring PAC (Feuston *et al*., 1994).

7.6 Reproductive Toxicity

Inhalation Exposure:

Hydrogen sulfide has been studied to determine if it had an adverse impact on pregnancy outcomes, offspring prenatal and postnatal development, or offspring behavior (Dorman et. al., 2000). Virgin male and female Sprague–Dawley rats (12 rats/sex/concentration) were

exposed (0, 10, 30, or 80 ppm H_2S; 6 h/day, 7 days/week) for 2 weeks prior to breeding. Exposures continued during a 2-week mating period (evidence of copulation = gestation day 0 = GD 0) and then from GD 0 through GD 19. Adult male rats were exposed for 70 consecutive days. There were no deaths and no adverse physical signs observed in F_0 male or female rats during the study. A statistically significant decrease in feed consumption was observed in F_0 male rats from the 80-ppm hydrogen sulfide exposure group during the first week of exposure. There were no statistically significant effects on the reproductive performance of the F_0 rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. In this study, as well as the repeat dose inhalation studies with hydrogen sulfide (Dorman et al., 2004), there were no specific adverse effects on male or female reproductive organs.

The VOCs from crude oil are similar to those from gasoline and gasoline blending streams. Several developmental/ reproduction screening studies have been done in rats with gasoline blending streams and two multi-generation reproductions studies have been done in rats with gasoline vapor (Petroleum HPV, 2008). Study details and references can be found in Appendix 6. The range of NOAEC for reproductive effects from all the available studies was 13650 mg/m^3 to 27750 mg/m^3. These results can be used to read-across to the reproductive toxicity of crude oils. In addition, in the repeat dose inhalation studies with gasoline and gasoline blending streams, there were no specific adverse effect on reproductive organs.

<u>Dermal Exposure:</u>
Guideline compliant reproductive toxicity studies were not found for crude oils. However, no changes in weight or histopathological effects were found in the reproductive organs of male and female rats exposed dermally for thirteen-weeks to two crude oils, i.e. Lost Hills Light and Belridge Heavy, at doses up to 500 mg/kg (Feuston *et al*., 1997b; Mobil, 1992a,b).The same studies reported no effects on epididymal spermatozoa morphology and count or testicular spermatid counts but a significant decrease in sperm motility was observed at 500 mg/kg/day with one crude oil, but not the other. Data from the repeat dose and developmental studies on Lost Hills Light and Belridge Heavy crudes oils are sufficient for evaluating reproductive toxicity under the EPA guidance for the HPV Challenge Program.

Reproductive toxicity screening studies in male and female rats of clarified slurry oil (CSO), a product of refinery processing of crude oil, utilizing dermal administration has been reported (Hoberman et al., 1995a). CSO typically contains very high levels of PAC constituents and is considered to be the most mutagenic and carcinogenic substances produced by petroleum refining with a high degree of developmental toxicity, i.e. dermal developmental toxicity LOAEL of 1 mg/kg (Hoberman, et al., 1995b). Reproductive endpoints, e.g., sperm production and male and female fertility, were unaffected at 250 mg/kg/day of CSO, a dose at which fetal survival was severely compromised in a developmental toxicity study that extended to postnatal day (PND) 4. Assuming that the reproductive toxicity of clarified slurry oil is representative of other PAC-containing petroleum substances, a reasonably assumption could be made that reproductive effects,

such as fertility and sperm production, would not be sensitive effects of PAC-containing materials compared to developmental toxicity effects.

In addition, the potential for a variety of PAC-containing petroleum substances, including crude oils, to affect reproductive organs was assessed via a series of 13-week repeat-dose studies in which the testes, accessory sex organs, and epididymides were weighed in males, and the potential for pathological changes was evaluated with microscopic examinations. Little evidence of reproductive organ effects was found in the repeated-dose studies of crude oil or the other petroleum streams evaluated. Accordingly, a conclusion could be reached that effects on reproductive organs are not a likely consequence of exposure to PAC-containing petroleum substances.

Across a number of developmental toxicity studies that examined embryonic and fetal development, the effects most commonly observed, and statistically significant at the LOAELs, were related to fetal/pup survival and body weight (Feuston et al, 1994; API, 2008). Little evidence of teratogenicity, i.e. malformations, was found in any of the conventional developmental toxicity studies. As expected, increased incidences of skeletal variations, i.e., delayed ossification, were often observed at dose levels producing decreased fetal/pup body weight. Based on the results of a large number of repeat-dose studies and developmental toxicity studies, as well as the two reproductive toxicity screening studies of CSO, the most sensitive endpoints related to reproductive and developmental toxicity appear to be those associated with the survival and growth of fetuses and offspring; effects on fertility, sperm production and reproductive organ effects do not appear to be sensitive endpoints for assessment of the potential hazards of PAC-containing petroleum substances.

Conclusions:
Inhalation exposure to volatile constituents of crude oils are not expected to produce reproductive toxicity since they did not produced adverse effects in the reproductive organs of male and female rats exposed for 13 weeks. In those studies with hydrogen sulfide or gasoline blending streams, no changes in weight or histopathological effects were found in the reproductive organs. Developmental studies on hydrogen sulfide or gasoline blending streams show they are also unlikely to produce reproductive toxicity at relevant exposure concentrations. In addition, two multi-generation reproductions studies have been done in rats with gasoline vapor (see Appendix 6). The NOAECs for reproductive effects in both those studies were greater than 20,000 mg/m^3.

Dermal exposure to crude oils are not expected to produce reproductive toxicity since they did not produce adverse effects in the reproductive organs of male and female rats exposed for 13 weeks to light and heavy crude oils. In those studies, no changes in weight or histopathological effects were found in the reproductive organs and epididymal spermatozoa morphology and count and testicular spermatid counts were unaffected. (Feuston *et al*., 1997b; Mobil, 1992a,b). Although a definite number cannot be provided for a NOAEL for reproductive effects, available data from three sources provide sufficient information to conclude that the NOAEL for reproductive effects would be greater than the NOAEL for developmental toxicity. First, little evidence of changes in weight or histological

appearance of reproductive organs of male and female rats via the dermal routes of exposure in the 13-week subchronic studies of crude oils. Second, developmental toxicity endpoints in studies of crude oils, including both *in utero* and postnatal development, were more sensitive than effects on the reproductive organs and semen in 90-day repeat-dose toxicity studies. Third, the published NOAEL in a pair of screening-level fertility studies with a refinery stream containing high amounts of PACs, was >250 mg/kg (Hoberman et al., 1995a). Given the hypothesis that reproductive toxicity would also be correlated with PAC profile, it can be concluded that the NOAEL for reproductive toxicity is expected to be greater than the NOAEL for developmental toxicity.

7.7 Other Health Effects

A number of crude oil samples, representing a range of compositions, have been investigated for their potential to cause skin cancer in mouse skin-painting studies of 104-110 week duration. All four crude oils including some distillation fractions of API Crude C and D (See below), produced skin tumors in 33-100% of mice with latency periods of 40-76 weeks, and were considered dermal carcinogens. Tumor incidence and latency depended on crude oil source and dose (Table 18). Numerous studies have shown that the mutagenic and carcinogenic potential of complex petroleum-related substances, all of which are derived from crude oil, correlates with the presence of 3-7 ring PAC (Roy *et al*, 1988; Blackburn *et al*., 1984; Cruzan *et al*., 1986; Blackburn *et al*., 1986). Further studies have shown these PAC can be absorbed through the skin and enter the general circulation (Roy *et al*., 1996; Roy *et al*., 1998)

Table 18. Summary of Mouse Skin Carcinogenesis Studies with Crude Oil

Crude Oil	Dosing Regimen	% of Animals with Tumors	Mean Latency	Reference
Naphthenic (Gulf Coast). API Crude C	50mg, 2x/week for 110 weeks	33%	76 weeks	Lewis et al, 1984
Paraffinic (high Sulfur) API Crude D	50mg, 2x/week for 110 weeks	56%	64 weeks	Lewis et al, 1984
San Joaquin Valley, 21^0 API gravity	17mg, 3x/week for 105 weeks	84%	62 weeks	Clark et al, 1988
Wilmington, 18^0 API gravity	0.17mg, 3x/week for 104 weeks 1.7mg, 3x/week for 104 weeks 16.8mg, 3x/week for 104 weeks	0 46% 100%	- 40 weeks 67 weeks	Renne et al, 1981

Conclusions: Crude oil applied to mice has caused statistically significant increases in skin tumors.

7.8 Assessment Summary for Health Effects

Crude oils have demonstrated little local irritation or acute systemic toxicity (LD_{50} >2.0 g/kg dermal). However, transport and storage of crude oil can result in significant levels of hydrogen sulfide and/or volatile organic compounds (VOCs) in some situation (i.e., enclosed spaces). The acute inhalation hazard is primarily from hydrogen sulfide. When the acute toxicity of hydrogen sulfide was assessed in rats, the calculated LC_{50} for a 4-hour inhalation exposure was 444 ppm. VOCs from crude oil are similar to the hydrocarbons found in gasoline and gasoline blending streams. The results of acute toxicity testing indicate that these materials are not acutely toxic by the inhalation exposure route, e.g., Rat LC_{50} >5g/m^3.

Repeat dose and developmental studies on inhaled hydrogen sulfide have determined NOAECs of 10 ppm and 80 ppm, respectively. The inhalation NOAECs for repeat dose and developmental effects of VOCs from crude oil are read-across data from studies on gasoline and gasoline blending streams. These values are:

Repeat Dose NOAEC:1507mg/m^3 to 10,153mg/m^3 (427 – 2880ppm^a)
Developmental NOAEC: 5970mg/m^3 to 27750mg/m^3 (1694 – 7873ppm^a)
[^{a} - Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]

In the repeat dose inhalation studies with hydrogen sulfide and gasoline blending streams, there were no specific adverse effect on reproductive organs. In addition, two multi-

generation reproduction studies on gasoline vapor in rats have determined NOAECs of over 20,000 mg/m^3. This data supports the conclusion that hydrogen sulfide and VOCs from crude oil have limited potential to be reproductive toxicants.

In situations involving repeated dermal exposure, the constituents with the greatest potential for toxicity are the polycyclic aromatic compounds (PACs). Solvent extracts of crude oils which concentrate the PAC constituents have induced gene mutations in bacteria. In contrast, the injection of mice with crude oil did not produce activity in micronucleus assays but did induce an increase in sister chromatid exchanges. Several samples of crude oil have produced skin-tumors in mice following long-term skin application.

Studies of repeated exposure by the dermal route have demonstrated toxicity that was indicated by changes in hematology values, liver enlargement and thymic atrophy. Measured and modeled toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 55 and 544 mg/kg/day.

In developmental toxicity studies in rats, crude oils, primarily at maternally toxic doses, caused fetal death, decreased fetal weight, delayed skeletal ossification and parturition. Measured and modeled developmental toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 53 and 2000 mg/kg/day. Crude oil is not expected to be a reproductive toxicant since repeated dermal exposures to crude oil for 13- weeks have not produced adverse effects in the reproductive organs of either male or female rats.

The Testing Group believes that the potential for mutagenicity and systemic toxicity, developmental toxicity and/or carcinogenic effects from repeated dermal exposure is related to the PAC profile of the specific crude oil.

8.0 HUMAN EXPOSURE SUMMARY

Crude oil is not a consumer product and general population exposure including children is not expected. However, inhalation and dermal exposure of workers to crude oil may take place during the drilling and completion of a well (exploration and production), and the transport, storage, and refining of crude oil.

8.1 Occupational Exposure

The individual constituents of crude oil volatilize accordance with their own individual physical-chemical properties. The two primary inhalation hazards are from hydrogen sulfide and from the VOCs similar to gasoline which can readily volatilize from crude oil. There are enforceable (OSHA Permissible Exposure Limits) and recommended (ACGIH Threshold Limit Values) occupational exposure standards for numerous volatile constituents typically found in crude oil. Examples of these standards are shown in Table 19. These standards are one means by which human exposures to individual crude oil constituents are controlled.

Table 19. OSHA and ACGIH Occupational Exposure Standards for Some Volatile Constituents of Crude Oil (8-hour Time Weighted Averages)

Category	Crude Oil						
Carbon Number	C4	C5	C6	C7	C8	C9	Others
Component OSHA and/or ACGIH Occupational Exposure Standard	**Butane** **ACGIH** 1000 ppm (C1-C4 alkanes)	**Pentane** **OSHA** 500 ppm (n-pentane) **ACGIH** 600 ppm (all isomers)	**Benzene** **OSHA** 1 ppm **ACGIH** 1 ppm	**Toluene** **OSHA** 200 ppm **ACGIH** 20 ppm	**Ethyl Benzene** **OSHA** 100 ppm **ACGIH** 100 ppm	**Cumene** **OSHA** 50 ppm **ACGIH** 50 ppm	**Hydrogen sulfide** **OSHA** 2 mg/m^3 **ACGIH** 1 ppm
	Propane, 2-methyl **ACGIH** 1000 ppm (C1-C4 alkanes)		**Hexane** **OSHA** 500 ppm **ACGIH** 50 ppm		**Xylenes** **OSHA** 100 ppm **ACGIH** 100 ppm	**Trimethyl Benzene** **ACGIH** 25 ppm (all isomers)	**Methyl mercaptan** **ACGIH** 0.5 ppm
			Cyclohexane **OSHA** 300 ppm **ACGIH** 100 ppm				**Gasoline** **ACGIH** 300 ppm

Specific laws and regulations are in place to limit occupational exposure during exploration and production activities and transportation of crude oil. These include;

1. Occupational Safety and Health Act
   a. 29 CFR 1910.106 Flammable and combustible liquids
   b. 29 CFR 1910.110 Storage and handling of liquefied petroleum gases
   c. 29 CFR 1910.119 Process safety management of highly hazardous chemicals
   d. 29 CFR 1910.132-1910.138 Personal protective equipment
   e. 29 CFR 1910.146 Permit-required confined spaces
   f. 29 CFR 1910.147 The control of hazardous energy (lockout/tagout)
   g. 29 CFR 1910.307 Hazardous (classified) locations
   h. 29 CFR 1910.1000 Air contaminants
   i. 29 CFR 1910.1003 Access to employee exposure and medical records.
   j. 29 CFR 1910.1028 Benzene
   k. 29 CFR 1910.1051 1,3-Butadiene.
   l. 29 CFR 1910.1200 Hazard communication
   m. 29 CFR 1910.1201 Retention of DOT markings, placards and labels.
   n. 29 CFR 1910.1450 Occupational exposure to hazardous chemicals in laboratories
2. Marine Occupational Safety and Health Standards
   a. 46 CFR 197.501 Applicability
   b. 46 CFR 197.505 Definitions
   c. 46 CFR 197.510 Incorporation by reference
   d. 46 CFR 197.515 Permissible exposure limits
   e. 46 CFR 197.520 Performance standard
   f. 46 CFR 197.525 Responsibility of the person in charge
   g. 46 CFR 197.530 Persons other than employees
   h. 46 CFR 197.535 Regulated areas
   i. 46 CFR 197.540 Determination of personal exposure
   j. 46 CFR 197.545 Program to reduce personal exposure
   k. 46 CFR 197.550 Respiratory protection
   l. 46 CFR 197.555 Personal protective clothing and equipment
   m. 46 CFR 197.560 Medical surveillance
   n. 46 CFR 197.565 Notifying personnel of benzene hazards
   o. 46 CFR 197.570 Recordkeeping
   p. 46 CFR 197.575 Observation of monitoring
   q. 46 CFR 197.580 Appendices
3. International Convention for the Safety of Life at Sea
   a. 74 Fed. Reg. 30,612 (June 26, 2009)
4. Hazardous Materials Transportation Act
   a. 49 CFR 105 Hazardous Materials Program Definitions and General Procedures
   b. 49 CFR 106 Rulemaking Procedures
   c. 49 CFR 107 Hazardous Materials Program Procedures
   d. 49 CFR 110 Hazardous Materials Public Sector Training and Planning Grants
   e. 49 CFR 130 Oil Spill Prevention and Response Plans
   f. 49 CFR 171 General Information, Regulations, and Definitions
   g. 49 CFR 172 Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, Training Requirements, and Security Plans
   h. 49 CFR 173 Shippers General Requirements for Shipments and Packaging
   i. 49 CFR 176 Carriage by Vessel
   j. 49 CFR 178 Specifications for Packaging
   k. 49 CFR 180 Continuing Qualifications and Maintenance of Packaging

8.2 Environmental Releases

Exploration and production activities and transportation of crude oil are controlled under a number of laws and regulations to limit release of crude oil into the environment. These include:

1. Clean Water Act
   a. National Pollutant Discharge Elimination System: Onshore Wells, Stripper Wells, Stormwater Discharges*
      i. Exemption. The 1987 Water Quality Act (WQA) amended the CWA to specify that EPA and states shall not require NPDES permits for uncontaminated storm water discharges from oil and gas exploration, production, processing or treatment operations, or transmission facilities.
      ii. 40 CFR Part 122, Subpart A Definitions and General Program Requirements
      iii. 40 CFR 122.21 Application for A Permit
      iv. 40 CFR 122.22 Signatories to Permit Application
      v. 40 CFR 122.26 Storm Water Discharges
      vi. 40 CFR 122.28 General Permits
      vii. 40 CFR 122.29 New Sources and New Dischargers
      viii. 40 CFR Part 122, Subpart C Permit Conditions
      ix. 40 CFR Part 122, Subpart D Transfer, Modification, Revocation and Reissuance, and Termination of Permits
      x. 40 CFR Part 125, Subpart A Criteria and Standards for Imposing Technology-Based Treatment Requirements under Sections 301(b) and 402 of the [CWA]
      xi. 40 CFR Part 125, Subpart D Criteria and Standards for Determining Fundamentally Different Factors under Sections 301(b)(1)(A), 301(b)(2)(A) and (E) of the [CWA]
      xii. 40 CFR Part 125, Subpart G Criteria for Modifying the Secondary Treatment Requirements under Sections 301(h) of the [CWA]
      xiii. 40 CFR Part 125, Subpart H Criteria for Determining Alternative Effluent Limitations under Section 316(a) of the [CWA]
      xiv. 40 CFR Part 125, Subpart I Requirements Applicable to Cooling Water Intake Structures for New Facilities under Sections 316(b) of the [CWA]
      xv. 40 CFR Part 403 General Pretreatment Regulations for Existing and New Sources of Pollution
      xvi. 40 CFR Part 435 Oil and Gas Extraction Point Source Category
   b. Oil Spill Prevention, Notification and Cleanup
      i. 30 CFR 250.203, 250.204, 254 Oil Spill Contingency Plan
      ii. 33 CFR 133 Oil Spill Liability Trust Fund; State Access
      iii. 33 CFR 135 Offshore Oil Pollution Compensation Fund
      iv. 33 CFR 136 Oil Spill Liability Trust Fund; Claims Procedures; Designation of Source; and Advertisement
      v. 33 CFR 137 Oil Spill Liability: Standards for Conducting All Appropriate Inquiries under the Innocent Land-Owner Defense
      vi. 33 CFR Part 153 Control of Pollution by Oil and Hazardous Substances, Discharge Removal
      vii. 33 CFR Part 154 Facilities Transferring Oil or Hazardous Material in Bulk
      viii. 33 CFR Part 156 Oil and Hazardous Material Transfer Operations
      ix. 40 CFR 110 Discharge of Oil
      x. 40 CFR 112 Oil Pollution Prevention
      xi. 40 CFR 116 Designation of Hazardous Substances
      xii. 40 CFR 117 Determination of Reportable Quantities for Hazardous Substances
   c. Wetlands
      i. 33 CFR Part 320-330 Procedures and Criteria for the Issuance of Permits

ii. 40 CFR Part 230 Guidelines for Specification of Disposal Sites
iii. 40 CFR Part 231 Discharge of Dredged or Fill Material
iv. 40 CFR Part 232 Program Definitions and Exemptions

2. Safe Drinking Water Act*

a. Exemption. The SDWA, under 42 U.S.C. § 300h, currently requires states to regulate, and imposes minimum regulatory requirements on, "subsurface emplacement of fluids by well injection" in order to protect drinking water supplies. The Act currently exempts from such requirements the underground injection of fluids or propping agents (other than diesel fuels) pursuant to hydraulic fracturing operations related to oil, gas, or geothermal production activities.
b. 40 CFR 144-148 Underground Injection Control (UIC) program

3. Outer Continental Shelf Lands Act

a. 30 CFR 250 Oil and Gas and Sulphur Operations in the Outer Continental Shelf
b. 30 CFR 251 Geological and Geophysical (G&G) Explorations of the Outer Continental Shelf
c. 30 CFR 252 Outer Continental Shelf (OCS) Oil and Gas Information Program
d. 30 CFR 253 Oil Spill Financial Responsibility for Offshore Facilities
e. 30 CFR 254 Oil Spill Response Requirements for Facilities Located Seaward of the Coastline
f. 30 CFR 256 Leasing of Sulphur or Oil and Gas in the Outer Continental Shelf
g. 30 CFR 259 Mineral Leasing Definitions
h. 30 CFR 260 Outer Continental Shelf Oil and Gas Leasing
i. 30 CFR 270 Nondiscrimination in the Outer Continental Shelf

4. Clean Air Act

a. National Emission Standards for Hazardous Air Pollutants
i. 40 CFR Part 63, Subpart Y National Emission Standards for Marine Tank Vessel Loading Operations

9.0 CATEGORY ANALYSIS CONCLUSIONS

Only one substance, Petroleum (CAS # 8002-05-9), is in the Crude Oil Category. Crude oils are a naturally-occurring substance that formed over millions of years, Crude oil is a complex combination of hydrocarbons consisting predominantly of paraffinic (straight and branched-chain alkanes), naphthenic (cycloalkanes) and aromatic hydrocarbons covering the carbon number range from C_4 to C_{60}+. Also included are low concentrations of heterocyclics, e.g. sulfur, nitrogen, oxygen-containing hydrocarbon analogs and metals, e.g. nickel and vanadium. An "average" crude contains 84% carbon, 14% hydrogen, 1-3% sulfur, and approximately 1.0% nitrogen, 1.0% oxygen and 0.1% minerals and salts.

Analytical studies indicate that similar hydrocarbons, heterocyclics, metals and other constituents, e.g. hydrogen sulfide, are present in all crude oils with the diversity of crude oils originating from the proportional variability in these components that depends on the source of the oil. This means certain generalities in the physicochemical and environmental fate attributes can be inferred given the predominant types of structures making up the crude oil. First, regardless of the structure type, low molecular weight constituents tend to have higher vapor pressures, lower partition coefficients and higher water solubilities than higher molecular weight components and second, given similar molecular weights, saturated hydrocarbons tend to have greater vapor pressures, higher partition coefficients, and lower water solubilities than aromatic constituents.

From an environmental fate perspective, constituent hydrocarbons in crude oil follow specific pathways. Overall, members of this category with relatively lower molecular weights and higher volatilities will partition to the air more than higher molecular weight components. Once in air, these more volatile constituents will not persist, but will be removed by reaction with hydroxyl radicals or direct photolytic reactions. Less volatile constituents will partition to soils and/or sediments. Hydrocarbons dissolved in water are resistant to hydrolytic reactions, but the dissolved fraction may partition to suspended matter, volatilize, or biodegrade.

Because crude oil is extracted from world-wide sources and composed of many different types and molecular weights of hydrocarbons, it may be impractical to assign specific acute aquatic toxicity values that cover the full domain of crude types. However, a generalization of the acute aquatic toxicity based on the data cited above indicates that when based on total crude oil loadings in water, either as WAFs or OWD, aquatic invertebrates are more sensitive than fish to crude oil exposure, and the lowest EL50 values may approach 10 mg/L. When toxicity endpoints are based on measured concentrations of hydrocarbons in the dissolved phase of the exposure solutions, aquatic invertebrates still appeared to be more sensitive than fish or algae. All acute endpoints for fish were >1 mg/L when based on measured dissolved hydrocarbons. For algae, one test endpoint yielded an EC50 of 0.94 mg/L, but other data all fell within the range of 6 to 11 mg/L. In general, aquatic toxicity of crude oil is not likely to be any greater than that represented by the most toxic fraction. For concentrations presented as loading rates, acute toxicity could potentially fall within the range of 1 – 10 mg/L.

There are numerous existing regulations on the exploration, production, and transportation of crude oil to limit release into the environment. These include the Clean Water Act, the Outer Continental Shelf Lands Act, and the Clean Air Act.

The human health effects of crude oil can be from both inhalation and/or dermal exposure. Because crude oils vary in their composition, the worst case hazard is assumed until sufficient data is available on a specific crude oil or situation (like enclosed spaces) to make an informed judgment about the presence or severity of the hazard.

The dermal hazard of crude oil from a single exposure is low (LC_{50} >2g/kg). The acute inhalation hazard of crude oil is most likely from hydrogen sulfide. When the acute toxicity of hydrogen sulfide was assessed in rats, the calculated LC_{50} for a 4-hour inhalation exposure was 444 ppm. VOCs from crude oil are similar to the hydrocarbons found in gasoline and gasoline blending streams. The results of acute toxicity testing indicate that these materials are not acutely toxic by the inhalation exposure route with the rat LC_{50} >5g/m^3. Repeat dose and developmental studies on inhaled hydrogen sulfide have determined NOAECs of 10 ppm and 80 ppm, respectively. The inhalation NOAECs for repeat dose and developmental effects of VOCs from crude oil are read-across data from studies on gasoline and gasoline blending streams. These values are: Repeat Dose NOAEC:1507mg/m^3 to 10,153mg/m^3 (427 – 2880ppm^a) and Developmental NOAEC: 5970mg/m^3 to 27750mg/m^3 (1694 – 7873ppm^a) *[^{a} - Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]*

In the repeat dose inhalation studies with hydrogen sulfide and gasoline blending streams, there were no specific adverse effect on reproductive organs. In addition, two multi-generation reproduction studies on gasoline vapor in rats have determined NOAECs of over 20,000 mg/m^3. This data supports the conclusion that hydrogen sulfide and VOCs from crude oil have limited potential to be reproductive toxicants.

There are occupational exposure standards established for many volatile crude oil constituents that limit worker exposure to acceptable concentrations. These standards include the Marine Occupational Safety & Health Standards, the ACGIH® TLVs, and the International Convention for the Safety of Life at Sea.

In situations involving dermal exposure, the constituents with the greatest potential for toxicity are the polycyclic aromatic compounds (PACs). Data on 46 crude oils show that the PAC profile (the 1 to 7 aromatic ring classes) is highly variable from sample to sample. Solvent extracts of crude oils which concentrate the PAC constituents have induced gene mutations in bacteria. The injection of mice with whole crude oil did not produce activity in micronucleus assays but did induce an increase in sister chromatid exchanges. Several samples of crude oil have produced skin-tumors in mice following long-term skin application.

Studies of repeated exposure by the dermal route have demonstrated toxicity that was indicated by changes in hematology values, liver enlargement and thymic atrophy. Measured and modeled toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 55 and 544 mg/kg/day.

In developmental toxicity studies in rats, crude oils, primarily at maternally toxic doses, caused fetal death, decreased fetal weight, delayed skeletal ossification and parturition. Measured and modeled toxicity endpoints show a wide range of responses from different samples of crude oil. The benchmark dose (BMD_{10}) for measured data on two crude oils and the predicted dose response (PDR_{10}) for modeled data on 46 crude oil samples were between 53 and 2000 mg/kg/day. Crude oil is not expected to be a reproductive toxicant since repeated dermal exposures to crude oil for 13-weeks have not produced adverse effects in the reproductive organs of either male or female rats.
The Testing Group believes that the potential for mutagenicity, systemic toxicity, developmental toxicity and/or carcinogenic effects from repeated dermal exposure is related to the PAC profile of the specific crude oil. The data described above for crude oil are sufficient to fully characterize the HPV Program screening level endpoints for Physical/Chemical Properties, Environmental Fate, Environmental Effects, and Human Health Effects.

10.0 Data Matrix for Crude Oil

Endpoint	Hydrogen Sulfide	VOCs (Gasoline and Gasoline Blending Streams)	Measured Data on Crude Oil	Predicted Results for Crude Oil	Read-Across to Untested Crude Oils
Pour Point			-30°C to 30°C		-30°C to 30°C
Boiling Range			-1°C to over 720°C		-1°C to over 720°C
Vapor Pressure			6 kPa to 45 kPa.		6 kPa to 45 kPa.
Partition Coefficient				2 to > 6	2 to >6
Water Solubility			10.42 to 58 mg/L in distilled water and from 7.75 to 25.5 mg/L in saltwater		7.75 to 58 mg/L
Photodegradation				Atmospheric half-lives of 0.37 to 6.5 days have been calculated for representative components of crude oil.	0.37 to 6.5 days
Stability in Water				Stable. No hydrolysis expected	Stable
Environ. Transport				Because crude oil consists of a wide range of molecular weight and hydrocarbon types, fractions will partition mainly to air and soil.	Mainly to air and soil
Biodegradation				Whole crude oil would not be classified as readily biodegradable. However, the constituent hydrocarbons in crude oils are considered inherently biodegradable.	Not readily biodegradable
Acute Fish				The acute toxicity could potentially fall within the range of 1 – 10 mg/L WAF, depending on the proportion of low molecular weight hydrocarbons in the crude's	1 – 10 mg/L WAF

				composition.	
Acute Daphnia				The acute toxicity could potentially fall within the range of 1 – 10 mg/L WAF, depending on the proportion of low molecular weight hydrocarbons in the crude's composition.	1 – 10 mg/L WAF
Algae				The acute toxicity could potentially fall within the range of 1 – 10 mg/L WAF, depending on the proportion of low molecular weight hydrocarbons in the crude's composition.	1 – 10 mg/L WAF
LD_{50} Dermal			>2 g/kg		>2 g/kg
LC_{50}	444 ppm (4-hr)	>5 mg/m^3 (6-hr)			In the absence of H2S, >5 mg/m^3
Repeat Dose (inhalation)	NOAEC = 10 ppm	NOAECs = 1507 mg/m^3 to 10,153 mg/m^3 (427 – 2880 ppm)			In the absence of H2S, NOAEC >1507 mg/m3 (427 ppm)
Repeat Dose (dermal)			Benchmark Dose (BMD_{10}) = 62 to 146 mg/kg/d	Predictive Dose Response (PDR_{10}) = 55 to 544 mg/kg/day	55 to 544 mg/kg/day
In vitro Mutagenicity			Optimized Ames test positive for several crude oils	Mutagenicity Index (MI) predicted to be >1 for most crude oils	Positive
In vivo Mutagencity			Negative in micronuclei assay		Negative
Developmental Toxicity (inhalation)	NOAEC = 80 ppm	NOAEC = 5970 mg/m^3 to 27750 mg/m^3			In the absence of H2S, NOAEC >5970 mg/m^3
Developmental Toxicity (dermal)			Benchmark Dose (BMD_{10}) = 91 to 106 mg/kg/d	Predictive Dose Response (PDR_{10}) = 53 to 2000 mg/kg/day	53 to 2000 mg/kg/day
Reproductive Toxicity (inhalation)	NOAEC = 80 ppm	The range of NOAECs for reproductive effects from			In the absence of H2S, NOAEC >13650 mg/m3

		all available studies was 13650 mg/m^3 to 27750 mg/m^3			
Reproductive Toxicity (dermal)			>91 mg/kg/d (Developmental BMD_{10})	> 53 to mg/kg/day (Developmental PDR_{10})	Greater than the metrics for Developmental Toxicity

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12. LIST OF APPREVIATIONS AND ACRONYMS

API – American Petroleum Institute
°API – API gravity
ARC – Aromatic Ring Class
ASTM – American Society for Testing and Materials.
ATSDR– Agency for Toxic Substances and Disease Registry.
AUGC – Area Under the Growth Curve
BMD10 – Benchmark Dose for estimated dose that produces a 10% response relative to the control group.
BMD_{PAC10} – Benchmark Dose for the estimated PAC dose that produces a 10% response relative to the control group.
BOD – Biological Oxygen Demand

CAD – Category Assessment Document
CAS RN/CAS #/CAS No. - Chemical Abstract Service Registry Number
^{o}C – Degrees Celsius
CIR – Cosmetics Ingredients Review Panel
CONCAWE – CONservation of Clean Air and Water in Europe.
d – Day(s)
DMSO – Dimethyl Sulfoxide
EINECS – European Inventory of Existing Commercial Chemical Substances
EL_{50} – Effective loading rate lethal to 50% of the test population
E_bL_{50} – Effective loading rate that causes 50% reduction in algal cell biomass
E_rL_{50} – Effective loading rate that causes 50% reduction in algal growth rate
EMBSI – ExxonMobil Biomedical Sciences Inc.
EPA/US EPA – United States Environmental Protection Agency
FWPCA – Federal Water Pollution Control Act.
g/cm^3 – Grams per cubic centimeter
h - Hour
HLS – Huntingdon Life Sciences
hPa – Hectopascal or 10^2 pascals; standard metric system unit for pressure
HPV – High Production Volume Challenge Program (EPA)
HSDB – Hazardous Substances Data Bank
IARC – International Agency for Research on Cancer (WHO)
ip – intraperitoneal route of administration
IP – Institute of Petroleum.
IPCS – International Programme on Chemical Safety (WHO)
IRDC – International Research and Development Corporation
°K – Degrees Kelvin
K_{ow} – Octanol-Water Partition Coefficient
kPa – Kilopascal or 10^3 pascals; standard metric system unit for pressure
LC_{50} – Lethal concentration for 50% of the test population
LC_{50} – Lethal dose level for 50% of the test population
LL_{50} – Lethal loading rate for 50% of the test population
LOAEL – Lowest observable adverse effect level
MCHC – Mean Corpuscular Hemoglobin Concentration
mg/kg – Milligrams per kilogram
mg/L – Milligrams per liter
mg/m^3 – Milligrams per cubic meter
mL – Milliliter
mm – Millimeter
ND – Not Determined or None Detected
nm – Nanometer
NOAEL – No Observable Adverse Effect Level
NOEC – No Observable Effect Concentration
NOEL – No Observable Effect Level
NOELR – No Observable Effect Loading Rate
NTP – National Toxicology Program
OECD – Organization for Economic Cooperation and Development

OPPTS – US EPA Office of Prevention, Pesticides and Toxic Substances
OSHA – Occupational Safety and Health Administration.
PAC – Polycyclic Aromatic Compound
PAH – Polycyclic Aromatic Hydrocarbon
PDR_{10} – Predicted Dose Response estimate developed from the regression model for the dose of
PCA that produces a 10% change in the response relative to the control group.
PERF – Petroleum Environmental Research Forum.
PNA – Polynuclear Aromatic
P_{ow} – n-Octanol/water Partition coefficient
ppm – Part per million
S – Sulfur
SETAC – Society of Environmental Toxicology and Chemistry.
SIDS – Screening Information Data Set
TSCA – Toxic Substances Control Act.
UNEP – United Nations Environment Program
U.S. DOE – U.S. Dept. of Energy.
US EPA – United States Environmental Protection Agency
UV - Ultraviolet
UVCB – Chemical Substance of Unknown or Variable Composition
WAF – Water Accommodated Fraction
wt% - Weight percent
μg - Microgram
μg/L – Microgram/Liter
> Greater than

13.0 GLOSSARY

NOTE: The following terms are used in this document. To the extent possible definitions were taken from relevant authoritative sources such as EPA, OECD, ASTM and IUPAC.

Alicyclic: A class of organic compounds containing only carbon and hydrogen atoms joined to form one or more rings but is not aromatic.

Aliphatic: A group of organic chemical compounds in which the carbon atoms are linked in open chains**.**

°API: API gravity is a measure of density, not an indicator of paraffinic or aromatic content of crude oil and is calculated as °API = 141.5/Sp. Gr. – 131.5. The currently accepted API gravity values to differentiate between light and heavy crude oils are ≥33°API equals “light” and ≤28°API equals “heavy” (Platt’s, 2003).

Asphalt: A very complex combination of high molecular weight organic compounds containing a relatively high proportion of hydrocarbons having carbon numbers

predominantly greater than C25 with high carbon-to-hydrogen ratios. It also contains small amounts of various metals such as nickel, iron, or vanadium. It is obtained as the non-volatile residue from distillation of crude oil or by separation as the raffinate from a residual oil in a deasphalting or decarbonization process. (**US EPA**; http://iaspub.epa.gov/sor_internet/registry/substreg/home/overview/home.do)

Asphaltenes: A group of complex aromatic hydrocarbons that are found in the heavier fractions of crude oil, e.g. asphalt (bitumen) and are soluble in carbon disulfide and insoluble in petroleum naphthas.

Atmospheric oxidation potential (AOP) program (AOPWIN™): Estimates the gas-phase reaction rate for the reaction between the most prevalent atmospheric oxidant, hydroxyl radicals, and a chemical. (http://www.epa.gov/oppt/exposure/pubs/episuite.htm)

Atrophy: A wasting of tissues, organs or the entire body.

Bioavailability: The state of being capable of being absorbed and available to interact with the metabolic processes of an organism. Typically a function of chemical properties, physical state of the material to which an organism is exposed, and the ability of the individual organism to physiologically take up the chemical. Also, the term used for the fraction of the total chemical in the environment which is available for uptake by organisms.

Bitumen: Asphalt

Category Member: The individual chemical or substance entities that constitute a chemical category.

Category: A chemical category, for the purposes of the HPV Challenge Program, is a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. These structural similarities may create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and/or human health effects.

Corpora lutea: A temporary endocrine structure in mammals formed in the ovary at the site of a ruptured ovarian follicle.

Cracking: The breaking up of heavy molecular weight hydrocarbons into lighter hydrocarbon molecules by the application of heat and pressure, with or without the use of catalysts. **(US OSHA**
Technical Manual SECTION IV: CHAPTER 2 PETROLEUM REFINING PROCESSES
http://www.osha.gov/dts/osta/otm/otm_iv/otm_iv_2.html)

Crude Oil (Petroleum): A complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small

amounts of nitrogen, oxygen and sulfur compounds. (**US EPA;** http://iaspub.epa.gov/sor_internet/registry/substreg/home/overview/home.do)

Dose: The amount of a substance available for interactions with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The **potential dose** is the amount ingested, inhaled, or applied to the skin. The **applied dose** is the amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). The **absorbed dose** is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of the skin, lung, and digestive tract) through uptake processes. **Internal dose** is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by an particular organ or cell is termed the delivered or **biologically effective dose** for that organ or cell **(US EPA, 2002)*.***

Dose-Response Relationship: The relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biological changes in incidence or in degree of change (response) **(US EPA, 2002).**

Dystocia: Difficult childbirth.

Ecological Effects – All endpoints (OECD definitions)

Fish, Acute Toxicity Test: In a four-day exposure, acute toxicity is defined by the LC_{50}, the concentration of test substance in water which kills 50% of the test population of fish. Test methodology is described in OECD Guideline 203, in OECD Guidelines for the Testing of Chemicals.

***Daphnia* sp., Acute Immobilization Test:** In a one or two-day exposure, acute toxicity is defined by the EC_{50}, the concentration of test substance in water which causes immobilization to 50% of the test population of invertebrates. Test methodology is described in OECD Guideline 202, Part 1, in OECD Guidelines for the Testing of Chemicals.

Alga, Growth Inhibition Test: In a three-day exposure, growth inhibition is defined by the EC_{50}, the concentration of test substance in growth medium which results in a 50% reduction in either alga cell growth or growth rate relative to a control group. Test methodology is described in OECD Guideline 201, in OECD Guidelines for the Testing of Chemicals.

Endpoint: In the context of the EPA High Production Volume Challenge Program, an endpoint is a physical-chemical, environmental fate, ecotoxicity, and human health attribute measurable by following an approved test methodology (e.g., OECD Guidelines for Testing of Chemicals). Melting point, biodegradation, fish acute toxicity, and genetic toxicity are examples of endpoints that are measured by an approved test method.

Environmental Fate Effects – All endpoints (OECD definitions)

Photodegradation: The photochemical transformation of a molecule into lower molecular weight fragments, usually in an oxidation process. This process may be

measured by Draft OECD Guideline, "*Phototransformation of Chemicals in Water – Direct and Indirect Photolysis*". This process also may be estimated using a variety of computer models.

Stability in Water: This environmental fate endpoint is achieved by measuring the hydrolysis of the test substance. Hydrolysis is defined as a reaction of a chemical RX with water, with the net exchange of the group X with OH at the reaction center. Test methodology for hydrolysis is described in OECD Guideline 111, in OECD Guidelines for the Testing of Chemicals.

Transport Between Environmental Compartments: This endpoint describes the distribution of a chemical between environmental compartments using fugacity-based computer models. The results of the model algorithms provide an estimate of the amount of the chemical within a specific compartment. The environmental compartments included in many models are air, water, soil, sediment, suspended sediment, and aquatic biota.

Biodegradation: Breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. As an endpoint in EPA's HPV program, biodegradation is measured by one of six methodologies described in OECD Guidelines 301A-F, in OECD Guidelines for the Testing of Chemicals.

Exposure: Contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure is quantified as the amount of an agent available at the exchange boundaries of the organism (e.g., skin, lungs, gut). **(US EPA, 2002).**

Feedstock: A refinery product that is used as the raw material for another process; the term is also generally applied to raw materials used in other industrial processes. **(Speight, 2007).**

Female Mating Index: Number of females with confirmed mating (sperm and/or vaginal plug)/number of females placed with males. (**OECD**)

Fugacity: Estimate of the "escaping" tendency of a chemical species from a particular environmental compartment.

Gas Oil: Middle-distillate petroleum fraction with a boiling range of about 350°-750° F, usually includes diesel fuel, kerosene, heating oil, and light fuel oil. **(US OSHA Technical Manual SECTION IV: CHAPTER 2 PETROLEUM REFINING PROCESSES http://www.osha.gov/dts/osta/otm/otm_iv/otm_iv_2.html)**

Hazard Assessment: The process of determining whether exposure to an agent can cause an increase in the incidence of a particular adverse health effect (e.g., cancer, birth defect) and whether the adverse health effect is likely to occur in humans **(US EPA, 2002)**.

Hazard Characterization: A description of the potential adverse health effects attributable to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the associated dose, route, duration, and timing of exposure **(US EPA, 2002).**

Hazard: A potential source of harm **(US EPA, 2002**).

Health Effects – All endpoints (OECD definitions, unless otherwise specified)

Acute Toxicity: The adverse effects occurring within a short time-frame of administration of a single dose of a substance, multiple doses given within 24 hours, or uninterrupted exposure over a period of 24 hours or less. Exposure may be via oral, dermal or inhalation routes as described in OECD Guidelines 401, 402, 403, and 420 in OECD Guidelines for the Testing of Chemicals.

Developmental Toxicity: Adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally until the time of sexual maturation. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency.

Genetic Toxicity *in vivo* (Chromosomal Aberrations): The assessment of the potential of a chemical to exert adverse effects through interaction with the genetic material of cells in the whole animal. Genotoxicity may be studies in the whole animal using methods described in OECD Guideline 475, in OECD Guidelines for the Testing of Chemicals.

Genetic Toxicity *in vitro* (Gene Mutations): The assessment of the potential of a chemical to exert adverse effects through interaction with the genetic material of cells in cultured mammalian cells. Genotoxicity may be studies in cultured cells using methods described in OECD Guideline 476, in OECD Guidelines for the Testing of Chemicals.

Repeated Dose Toxicity: The adverse effects occurring due to repeated doses that may not produce immediate toxic effects, but due to accumulation of the chemical in tissues or other mechanisms, produces delayed effects. Repeated dose toxicity may be studied following methods described in OECD Guidelines 407, 410, or 412 in OECD Guidelines for the Testing of Chemicals.

Reproductive Toxicity: The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

Hematocrit: The proportion of the blood that consists of packed red blood cells and is expressed as a percentage by volume.

Hyperkeratosis: Hypertrophy of the horny layer of the epidermis.

Hyperplasia: A condition in which an increase in the number of normal cells exists in a tissue or organ.

Hypertrophy: Enlargement or overgrowth of an organ or tissue due to the increased size of its constituent cells

Loading Rate: The amount of the test material that is equilibrated with the aqueous test medium or the total amount of test substance added to dilution water to prepare water accommodated fractions (WAFs) for ecotoxicity testing (OECD, 2000).

Lowest-Observed-Adverse-Effect Level (LOAEL): The lowest exposure level at which a statistically or biologically significant increase exists in the frequency or severity of adverse effects between the exposed population and its appropriate control group **(US EPA 2002).** Note: In studies with an absence of a NOAEL the LOAEL is considered ≤ the lowest adverse effects dose

Maltenes: The fraction of asphalt which is soluble in n-alkane solvent; such as, pentane or heptane.

Mean corpuscular hemoglobin concentration (MCHC): The average concentration of hemoglobin in a given volume of packed red blood cells.

Naphtha: A general term used for low boiling hydrocarbon fractions that are a major component of gasoline. Aliphatic naphtha refers to those naphthas containing less than 0.1% benzene and with carbon numbers from C3 through C16. Aromatic naphthas have carbon numbers from C6 through C16 and contain significant quantities of aromatic hydrocarbons such as benzene (>0.1%), toluene, and xylene. (US OSHA Technical Manual SECTION IV: CHAPTER 2 PETROLEUM REFINING PROCESSES http://www.osha.gov/dts/osta/otm/otm_iv/otm_iv_2.html)

No-Observed-Adverse-Effect Level (NOAEL): The highest exposure level at which no biologically significant increase exists in the frequency or severity of adverse effects between the exposed population and its appropriate control group; some effects may be produced at this level, but these effects are not considered adverse or precursors to adverse effects **(US EPA 2002)**.

Pathosis: A state of disease.

Portal-of- Entry Effect: A local effect produced at the tissue or organ of first contact between the biological system and the toxicant

Pour point: The lowest temperature in °F at which an oil will flow (ASTM D97)

Resin (petroleum): A complex combination of organic compounds, predominantly hydrocarbons, obtained as a fraction of the extract from solvent extraction of residuum. It consists predominantly of high molecular weight compounds with high carbon-to-hydrogen ratios.

Read Across: Read-across can be regarded as using data available for some members of a category to estimate values (qualitatively or quantitatively) for category members for which no such data exist.

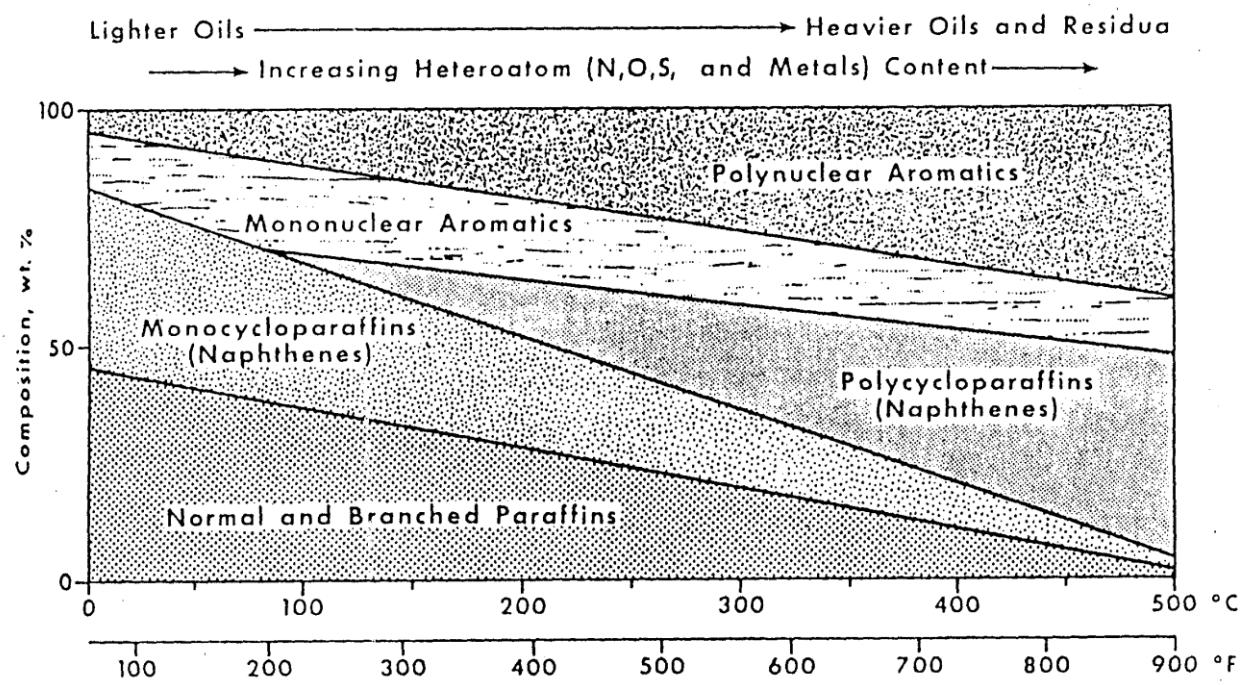
Systemic Effects or Systemic Toxicity: Toxic effects as a result of absorption and distribution of a toxicant to a site distant from its entry point **(US EPA 2002)**.

Target Organ: The biological organ(s) most adversely affected by exposure to a chemical or physical agent **(US EPA 2002).**

Tar: Viscous, dark-brown to black substances obtained by the destructive distillation of coal.

APPENDIX 1; Crude Oil Chemistry and Composition

The hydrocarbons in crude oil – paraffins, naphthenes (cycloparaffins) and aromatics – share some structural features but differ in the ratio of hydrogen to carbon atoms and how those atoms are arranged. Olefins are not present in crude oils and are formed from rearrangement of atoms during the cracking process to produce gasoline-blending streams. Paraffins occur in higher concentrations in lower boiling fractions of crude oil while the concentration of naphthenes (cycloparaffins) and aromatics increase in the higher boiling range fractions (Figure A1-1)



Feedstock composition represented by the distribution of chemical types.

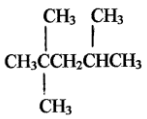
Mobil, 1997

Hydrocarbon molecules in crude oil may include from 1 to more than 50 carbon atoms at room temperature. When isolated, hydrocarbons with 1-4 carbon atoms are gases, those with 5-19 carbon atoms are usually liquid, and those with 40 or more carbon atoms are solids.

Paraffins: C_nH_{2n+2} where n= number of carbon atoms.

Carbons are joined by single bonds (e.g. butane, $CH_3CH_2CH_2CH_3$). Paraffins with 4 or more C atoms may have 2 or more structural arrangements or structural isomers, for example:

normal octane, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or isooctane

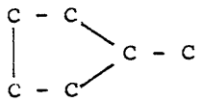


Normal paraffins occur in most crude oils but vary in total concentration (King, 1983). As a rule, crude oils of older geological age contain higher quantities of n-paraffins. Occurrences of paraffins relative to other hydrocarbon classes decreases as the boiling point range of fractions distilled from crude oil increases

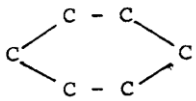
Branched or isoparaffins are found throughout the crude oil boiling range but do diminish with increasing boiling point. Certain lower molecular weight branched paraffins are capable of producing kidney damage, i.e. light hydrocarbon nephropathy through a mechanism that is specific to male rats and not relevant to humans (EPA, 1991).

Naphthenes: Cycloparaffins in gasoline have 5 or 6 carbon atoms arranged in a ring and belong to either a cyclopentane or cyclohexane series, for example:

C_6H_{12} Methylcyclopentane



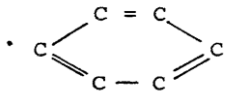
C_6H_{12} Cyclohexane



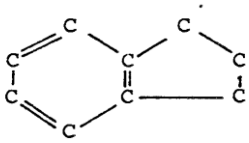
Cycloparaffins constitute a substantial proportion of petroleum with 5- to 6-membered ring structure being the predominant type. Most individual cycloparaffins that have been isolated are in the boiling range of gasoline and jet fuel. The cycloparaffin portion of the lubricant oil fractions of crude oil are a complex mixture of non-condensed and condensed 5- and 6-member rings. Polycycloparaffins may act as inhibitors in skin carcinogenesis (King, 1988)

Aromatics: Some carbon atoms are arranged in a ring joined by aromatic bonds, e.g.

benzene, C_6H_6 .



In polycyclic aromatic hydrocarbons (PAHs), some carbons are shared by 2 or more rings, e.g. indane, C_9H_{10}



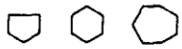
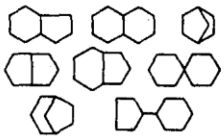
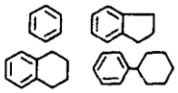
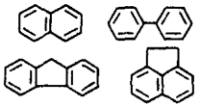
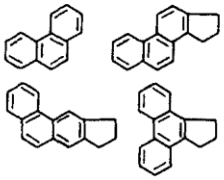
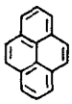
Aromatic hydrocarbon types are typically present in the same relative proportion in different crude oils. Where several possibilities for alkyl substitution exist, the predominant isomers are generally those containing substituents with the lowest number of carbon

atoms. In heavier, lubricant-type fractions, mixed aromatic-cycloparaffin hydrocarbons predominate, as mono-, di-, or tricyclic aromatic-cycloparaffin hydrocarbons. Certain polycyclic aromatics (PACs) are associated with mutagenicity, systemic toxicity and skin cancer. Heterocycles are closely related compounds in which an atom of nitrogen, oxygen or sulfur replaces one of the carbon atoms in the ring and are commonly found with PAHs (API, 2002).

Resins and asphaltenes are high molecular wt fractions (500-10,000) containing N, S, and oxygen found in the residuum/bottoms of crude oils. These classes of hydrocarbons have high polarity, low solubility and limited bioavailability and toxicity and typically constitute 10% of light paraffinic oils and up to 60% of heavy crude oils.

Much of the compositional information described above was derived from the extensive analysis of a Ponca Oklahoma crude, performed under the sponsorship of the American Petroleum Institute and is summarized in Table 1.1 (King, 1983).

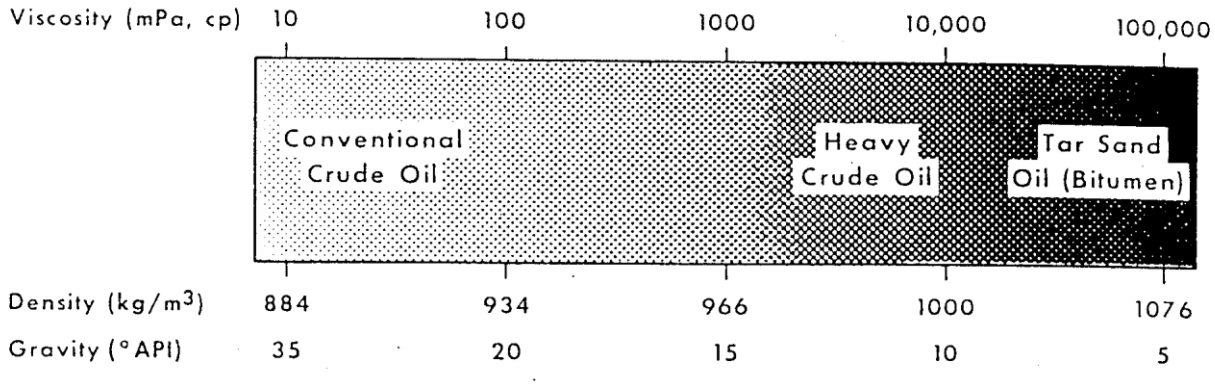
TABLE 1.1: Types of Hydrocarbons Isolated from Ponca Crude

HYDROCARBON TYPE	BASIC STRUCTURES	NUMBER ISOLATED	FRACTIONS WHERE FOUND
Paraffins			
n-Paraffins	C-C-C-C-C-C-	33	All Distillates
Branched Paraffins	C C C-C-C-C-C-C-	41	Gasoline, Light Gas Oil
Cycloparaffins			
Monocycloparaffins		39	Gasoline, Kerosine
Bicycloparaffins		29	Gasoline, Kerosine
Tricycloparaffins		3	Gasoline, Kerosine
Aromatics			
Mononuclear Aromatics		48	Gasoline, Kerosine, Light Gas Oil
Dinuclear Aromatics		60	Kerosine, Light Gas Oil
Trinuclear Aromatics		10	Heavy Gas Oil-Light Lubricant
Tetranuclear Aromatics		3	Heavy Gas Oil-Light Lubricant

APPENDIX 2: Crude Product Potential

Crude oils are classified by viscosity, density and API gravity. API gravity was developed as a means to identify the gasoline production potential of a crude oil; the higher the API gravity, the more valuable the crude. Figure A2-1 illustrated classification of crude oil by this density-gravity method.

Figure A2-1: Classification of crude oil by density-gravity method.



Type of Crude	Characteristics
1. Conventional or "light" crude	Density-gravity range less that 934kg/m^3 (>33^{0}API)
2. "Heavy" crude oil	Density-gravity range from 1000kg/m^3 to more than 934kg/m^3 (10^{0}API to <28^{0}API) Maximum viscosity of 10,000mPa.s(cp)
3. "Extra-heavy" crude oil; may also include atmospheric residua. (b.p.>340^{0}C; >650^{0}F)	Density-gravity greater than 1000kg/m^3 (<10^{0}API) Maximum viscosity of 10,000mPa.s(cp)
4. Tar sand bitumen [before upgrade] or natural asphalt; may also include vacuum residua. (b.p.>510^{0}C; >950^{0}F)	Density-gravity greater than 1000kg/m^3 (<10^{0}API) Viscosity greater than 10,000mPa.s(cp)

Mackerer and Biggs, AIHCE, 1996; Platts, 2003

Heavier crude oils have higher density-gravity values and higher viscosity, with lower API gravity, making them less suitable for gasoline stocks but better candidates for lubricant and heavy fuel production. Figure A2-2 shows yield comparisons for 4 typical crude oils.

Figure A2-2: Yield comparison of crude oils

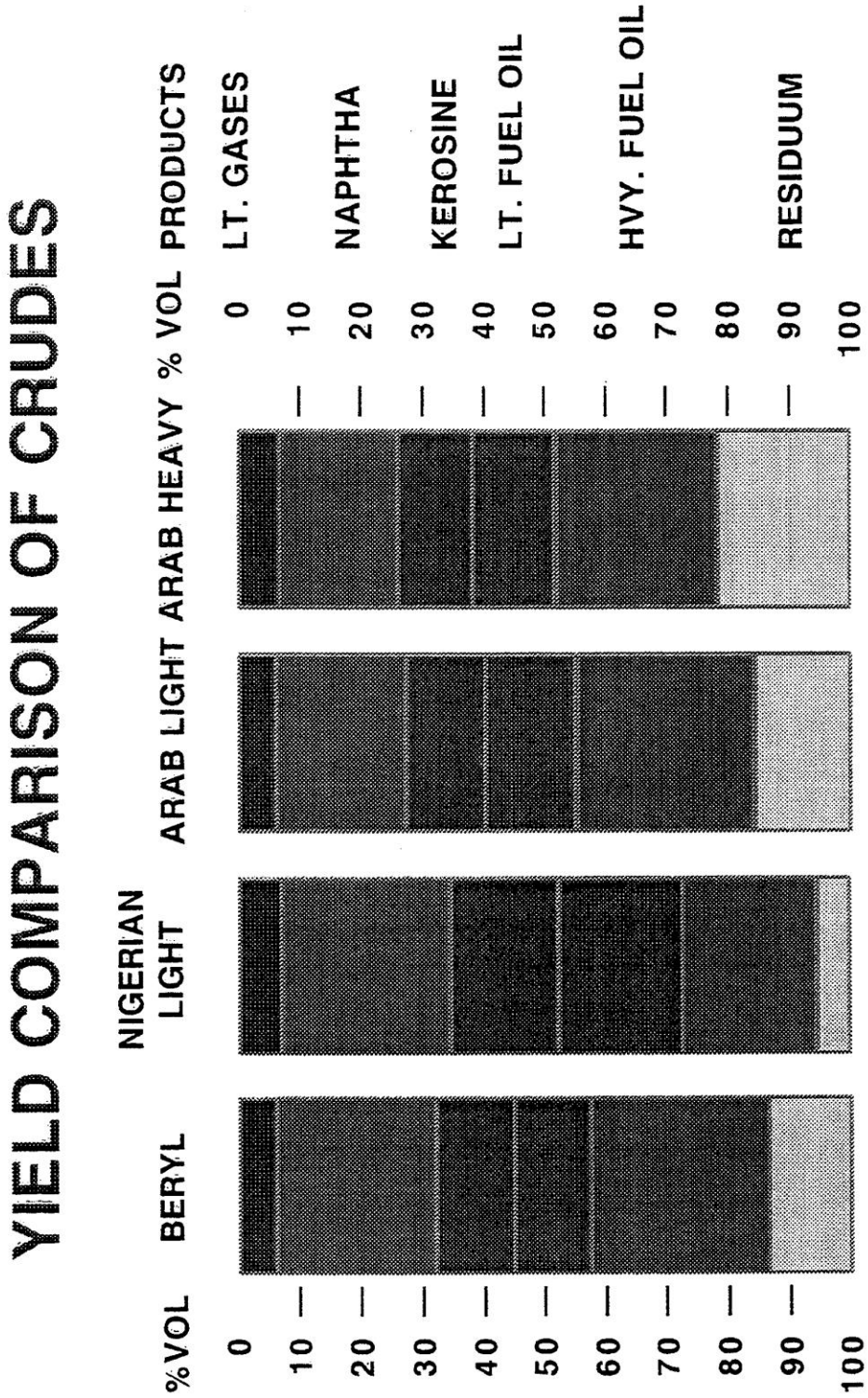


Table 2.1 BRIEF SUMMARY OF THE HISTORY OF REFINING PROCESSING

YEAR	PROCESS NAME	PROCESS PURPOSE	BYPRODUCTS, ETC.
1862	Atmospheric Distillation	Produce Kerosine	Naphtha, Tar, etc.
1870	Vacuum Distillation	Lubricants (original) Cracking feedstocks (1930's)	Asphalt, Residual Coker Feedstocks
1913	Thermal Cracking	Increase Gasoline	Residual, Bunker Fuel
1916	Sweetening	Reduce Sulfur	
1930	Thermal Reformation	Improve Octane Number	Residual
1932	Hydrogenation	Remove Sulfur	Sulfur
1932	Coking	Produce Light Products	Coke
1933	Solvent Extraction	Improve Lubricant Viscosity Index	Aromatics
1935	Solvent Dewaxing	Improve Pour Point	Waxes
1935	Cat. Polymerization	Improve Gasoline Yield & Octane No.	Petrochem Feedstocks
1937	Catalytic Cracking	Higher Octane Gasoline	Petrochem Feedstocks
1939	Visbreaking	Reduce Viscosity	Increased Distillate, Tar
1940	Alkylation	Increase Gasoline Octane & Yield	High Octane Aviation Gasoline
1940	Isomerization	Produce Alkylation Feedstock	Naphtha
1950	Deasphalting	Increase Cracking Feedstock	Asphalt
1952	Catalytic Reforming	Convert Low Quality Naphtha	Aromatics
1954	Hydrodesulfurization	Remove Sulfur	Sulfur
1956	Inhibitor Sweetening	Remove Mercaptans	
1957	Cat. Isomerization	Convert to Molecules w/High Oct. No.	Alkylation Feedstocks
1960	Hydrocracking	Improve Quality & Reduce Sulfur	Alkylation Feedstocks
1961	Fluid Cat. Cracking	Increase Gasoline Yield	Petrochem Feedstocks
1974	Catalytic Dewaxing	Improve Pour Point	
1975	Resid. Hydrocracking	Increase Gasoline Yield from Residual	

Table A2-1 summarizes the history of petroleum refining. Since the first refinery was established in 1862, processes have been developed and continually improved to maximize the yield and efficiency of production of high quality fuels, lubricants and petrochemicals from petroleum crude, and concomitantly to minimize or eliminate undesirable components.

APPENDIX 3: Correlation between PAC Profile and Selected Endpoints of Mammalian Toxicity

As indicated in the Crude Oil Test Plan submitted to the EPA in 2003, the mammalian toxicity of crude oils is expected to be related to their PAC profiles; particularly the toxicity measured in repeat-dose, developmental, and *in vitro* mutagenicity studies. The PAC[1] profile is the weight percent of DMSO-extractable, aromatic compounds contained in the 1 to 7 aromatic ring classes.

The initial indication that PAC content could be used to predict the toxicity of untested petroleum-related materials including crude oils was based on the publication by Feuston et al. (1994). Their research, based on thirteen petroleum-derived refinery streams, examined the correlations between the weight percentage of several chemical classes of compounds and the magnitude of various effects produced in rats treated dermally with these substances in repeat-dose and developmental toxicity studies. In general, Feuston et al. found that the toxicity of the streams was correlated with the concentrations of the 3 to 7 ring PACs. The analyses were based on the ranks of several measures of toxicity and the individual PAC concentrations.

In 2004, the API Testing Group recognized the need to further evaluate the observations made by Feuston et al. (1994) and commissioned a Task Group (PAC Analysis Task Group, or TG) comprised of experts in the fields of petroleum chemistry, toxicology, and biostatistics. The TG issued a report describing the relationships between PAC profile and the repeat-dose and developmental toxicities of high-boiling petroleum-related substances, i.e. those with initial boiling points greater than approximately 300 °F (API, 2008). Predictive models for seven selected repeat-dose and developmental dermal toxicity endpoints in the rat were reported (API, 2008). The report was reviewed in a peer consultation process and/are publicly available (TERA, 2008). Reports are in preparation on the relationship between PACs and reproductive and genetic toxicities of high-boiling petroleum substances.

Four potential sources of information were reviewed for the project: the publication by Feuston et al (1994); other published literature on the toxicity of individual PAH and PAC containing materials; studies sponsored by the American Petroleum Institute (API); and unpublished company laboratory reports. The unpublished laboratory reports consisted of: (1) reports of repeat-dose toxicity studies, (2) reports of developmental toxicity studies, (3) two reproductive toxicity screening studies, one each with treated males and females, on a single substance containing a high concentration of PAC, (4) an exploratory dose range-finding study in non-pregnant female rats, (5) reports of mutagenesis tests, primarily results of optimized Ames tests, and (6) reports of compositional data on the tested substances. All unpublished company laboratory reports (repeat-dose, developmental

[1] Note that "polycyclic aromatic hydrocarbons" (PAH) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic aromatic compounds (PAC) is a more inclusive term than PAH since, in addition to the PAHs, PAC also includes compounds in which one or more atoms of nitrogen, oxygen or sulfur (a heteroatom) replaces one or more of the carbon atoms in a fused ring system and perhaps more importantly includes alkylated (methyl, ethyl, etc.) rings **(API, 2008)**.

toxicity, and analytical) were judged to be either “reliable without restrictions” or “reliable with restrictions, i.e. reliability scores of 1 or 2 (Klimsch, et al. 1997).

The relationship between acute toxicity and PAC was not investigated statistically since the reported oral LD_{50} values for high-boiling petroleum substances are generally greater than the maximum doses tested, typically 5 g/kg and 2 g/kg for oral and dermal exposures, respectively (API 2001, 2002, 2003a, b, c & d, 2004). These data demonstrate that the respective petroleum-derived streams are not toxic, at least within the operational definitions of the regulatory testing guidelines.

To model the outcomes of repeat-dose and developmental studies, sets of matched data of PAC composition and biological effects were selected. Each biological endpoint had an average of about 80 data points. The seven biological endpoints that were selected for final statistical characterization were four repeat-dose measures, i.e. thymus weight, liver to body weight ratio, platelet count and, hemoglobin concentration, and three developmental measures, i.e. fetal weight, live fetal count, and percent resorptions. The endpoints selected for modeling are consistent with effects reported for both individual PACs and PAC containing substances (SCF, 2002, ATSDR, 1995; IPCS, 1998; IRIS 2007; RAIS, 2007). The endpoints selected are also supported by other studies on PAC-containing petroleum-related substances submitted by the Petroleum HPV Testing Group as robust study summaries to satisfy the USEPA HPV Challenge Program requirements for the Aromatic Extracts, Crude Oil, Gas Oils, Heavy Fuel Oils, Lubricating Oil Basestocks, and Waxes and Related Materials.

The PAC compositional data was developed using an analytical technique referred to as the “PAC-2 Method,” or ‘Mobil Oil PAC Method” or, simply “Method II” (Feuston et al., 1994; Roy et al., 1985; Roy et al., 1988), a variation of the Institute of Petroleum IP 346 method (IP, 1980). In the PAC-2 Method, the percent of sample mass is determined for each PAC ring class (1 through 7) contained in PAC-concentrated dimethyl sulfoxide (DMSO) extracts of the test material. The analysis was performed by gas chromatography with flame ionization detection (GC/FID) or mass spectrometry (GC/MS).

The dose-response relationships between the “PAC profile” and specific biologic effects were successfully predicted using linear regression models. The correlations between observed and model-predicted data were very high ($r > 0.90$). The predictive ability of the models was rigorously tested and the models were found to be accurate predictors when used with interpolated data. A test material that has its PAC profile and dose within the range of the PAC profiles and doses used to develop the model gives rise to an interpolated model prediction. Predictions from samples that do not meet this requirement are considered extrapolated predictions. Extrapolated predictions might not be accurate and are considered unreliable by the Testing Group.

Interpolated model results can be used to estimate the dose that would cause a 10% change in the response relative to the control group (PDR_{10}). The concept is similar to the Benchmark Dose (BMD) for continuous endpoints (Crump, 1984). Comparison of the PDR_{10} and BMD_{10} from a series of samples has shown a close agreement indicating the

usefulness of the PDR_{10} when no biological endpoint testing data exists and only the PAC profile is available to assess toxicity.

While similar to the BMD, the PDR_{10} has several advantages:

- The PDR_{10} is based on one validated model, whereas the BMD can be developed from several competing models, making the BMD strongly dependent on the selected model (Gephart et al, 2001).
- The PDR_{10} can be applied to untested materials for which there are compositional data (ie, PAC profiles) but no response data, whereas the BMD cannot be used for untested materials.
- The PDR_{10} is based on the large amount of data accumulated over multiple studies, whereas the BMD is based on a single study, usually with only 3 to 5 data points.

A copy of the full report detailing the development and testing of the predictive models developed by the Testing Group can be obtained through either API or TERA (API, 2008; TERA, 2008).

The genetic toxicity endpoints, *in vitro* gene mutation and in vivo chromosomal aberrations, assessed principally in micronucleus tests, are addressed in Appendix 4.

APPENDIX 4: Optimized AmesTest and Statistical Modeling

The optimized Ames test was developed to improve the performance of the reverse mutation *Salmonella* assay for detecting mutagenic and potentially carcinogenic lubricant base stocks and related refinery streams (ASTM, 2002). The method involves concentration of polycyclic aromatic compounds (PAC) by extraction, employing the most consistently PAC- sensitive strain of *Salmonella* [TA98] and increasing the metabolic activation system to maximize metabolism of the streams being evaluated. These modifications allowed detection of positive bacterial gene mutation response identified as an increase of mutant colonies in treated groups at least 2-fold that of negative controls as in the Standard Ames Assay and allowed prediction of potential dermal carcinogenesis by calculation of a mutagenicity index (MI).

The mutagenicity index (MI) is the slope of the initial portion of the dose response curve expressed in units of revertants per microliter. The mutagenicity index was highly correlated with dermal carcinogenic potential, suggesting that oils with MI values < 1 were unlikely to be dermally carcinogenic, oils with MI values $\geq$ 1 but < 2 were indeterminate, and oils with MI values $\geq$ 2 would likely produce skin tumors if tested in mice. The test method was refined to provide the greatest predictive value of gene mutagenicity and potential carcinogenicity for the widest range of high boiling [>300^{0}C] PAC-containing streams and thus provides a more sensitive general *Salmonella* protocol for this class of petroleum substances. In 1995, the optimized Ames test was standardized as an ASTM method [ASTM E1687-95].

Correlation of Mutagenic Activity with PAC Profile

The relationship of the MI with the PAC profile of refinery streams with known dermal carcinogenic potential has been established. The method of quantifying PAC constituents in which the condensed ring aromatics are removed by DMSO extraction and analyzed for 3-7 ring PAC by gas chromatography (GC) was developed by Roy *et al*. (1985; 1988). Having demonstrated a strong correlation between analytical distribution of PAC and mutagenicity in the optimized Ames test for petroleum-derived substances which produce dermal tumors when tested in mice, the utility of this relationship for read-across to untested substances has been expanded by statistical modeling.

Statistical Modeling of Analytical Data with the Optimized Salmonella Assay (Ames Test)

A statistical model has been developed to predict MI scores for untested substances encompassing precision in the critical 0-2 range (McKee, et al., 2010). This model employs the 1-7 ring PAC profile for each sample to predict MI scores. This model separated the data from 193 samples of a range of PAC-rich petroleum streams into those with mutagenicity index values equal to or greater than 1.0 and those with MI values less than 1.0. This model was not designed to quantify mutagenic potency but to identify whether or not a substance had an MI value less than 1 or not; this result can be used as an indication of whether the material has the potential to induce gene mutations in the optimized *Salmonella* assay and thus, to potentially be active in dermal carcinogenesis assays as well.

The statistical model is based on a series of three steps each predicting if the test substance was above or below an MI cut-point using a binary logistic general additive model. Step 1 predictes the probability that the substance has an MI of 5 or larger. The second step used only the substances predicted to have an MI below 5 and tested for a split at an MI of 2 or larger (the samples from the first step that are predicted to be above 5 were set at 5 and were no longer in the model process). The third step uses only the substances predicted to have an MI below 2 and tested for a split at an MI of 1 or larger (again with the substances from the second step that were predicted to be greater than 2 were set to 2 and were no longer in the modeling process). At each step the probability for a decision is based on a value of 0.50. For example, in the first step, if the probability of the substance having an MI less then 5 was greater than 0.50 the substance was assigned a predicted MI of 'less than 5.' The final result was the combination of the results from the 3 steps with each substance predicted as being either < 1 or ≥ 1.

The model predictions agreed with the experimentally determined results 98% of the time, with the majority of the incorrect predictions being at MI values that were close to 1.0. When the model was tested with 49 hold out samples, 94% of the predictions were in agreement with the experimentally determined values.

From this information it is apparent that the outcome of optimized Ames tests can be predicted from compositional information with an accuracy that seems comparable to that associated with variability inherent with either the experimental methods or the methods used to calculate mutagenicity index from the experimental data.

APPENDIX 5. Other Routes of Exposure

This Appendix contains summaries of several published studies that used unrealistic routes of administration and extremely high doses. Because of those deficiencies, the Petroleum HPV Testing Group recommends that they not be used for hazard or risk evaluation of crude oil. They are included in this document only for completeness.

Acute Toxicity

Data on the acute oral toxicity of five crude oils, i.e. four light crudes and one heavy crude oil, are summarized in the Table below.

Sample[1]	Oral LD_{50} (Rat) g/kg
Beryl [36.5°API]	>5.0
Arab Lt [34.5 °API]	>5.0
Mid-Continent [40°API]	>5.0
Lost Hills Light [>38°API]	>5.0
Belridge Heavy [14°API]	>5.0

[1]Mobil, 1984a,b; 1985a,b; 1990a,b

In a study of three crude oil samples, Smith (1981) reported acute oral LD_{50} values in the mouse ranging from >10.0g/kg for mixed crude oils to >16.0g/kg for Wilmington heavy crude (18°API) and Recluse crude.

Repeat Dose

Three crude oils (Arab Light, 34.5°API, light; Prudhoe Bay, 28°API, heavy; and South Louisiana, 35°API, light) were administered orally to male CD-1 mice once daily for five days. Prudhoe Bay crude oil was administered at doses of 0, 2, 4, 8, 10, 12, or 16 ml/kg/day; Arab Light and South Louisiana crude oils were given at 10 ml/kg/day only. All three crude oils induced small hematologic changes, i.e. decreases in packed cell volume and mean corpuscular hemoglobin concentration (MCHC) that was consistent with hemolysis. At all doses tested these oils also produced liver enlargement and thymic and splenic atrophy without concurrent pathological effects on tissue structure. However, liver enlargement was considered likely an adaptive, physiological response and thymic atrophy, a non-specific, stress-related secondary effect (Leighton, 1990).

Developmental

Prudhoe Bay heavy crude oil (PBO) (28°API) was administered orally to pregnant Sprague Dawley rats either as a single dose of 5 ml/kg on selected days of gestation, i.e. 3, 6, 11, 15 or 17 , as a single dose at levels up to 10 ml/kg on Day 6 of gestation or as repeated daily doses of 1 or 2 ml/kg/day on gestation days (GD) 6-17 (Khan *et al*., 1987). With all three treatment regimes, at maternally toxic doses, increased rates of resorptions, increased fetal deaths and decreased fetal weights were observed. Administration of 5 ml/kg PBO, on Day 6 of gestation vs. the other gestation days, produced the maximum

effect on the increase in resorption rates and decrease in fetal weights as well as the decrease in maternal weight gains. When a series of doses of PBO were administered on Day 6 of gestation, even the lowest dose of 2 ml/kg was able to produce a significant increase in resorption rates and a decrease in maternal body weight gains. A NOAEL was not established, but a Lowest Observable Adverse Effect Level (LOAEL) for maternal and developmental toxicity was determined to be ≤1 ml/kg when PBO was administered daily on days 6-17 of gestation.

Reproduction

Sperm morphology in mice was also examined after five days of daily intraperitoneal injections of 18°API Wilmington heavy crude at levels up to 2.1 g/kg/day. Evaluation of tissue samples did not indicate any significant increase in the incidence of abnormal sperm (Lockard *et al*., 1982).

A Nigerian Bonny Light crude oil (NBL) dissolved in a nonionic surfactant (Tween 80) and water mixture and administered orally by gavage to male rats at doses of 200, 400, and 800 mg/kg daily for 7 days provided suggestive evidence of dose-dependent testicular and epididymal toxicity (Orisakwe *et al*., 2004). Histopathological changes occurred at all doses in the testes and included thickening of the connective tissue lining, distortion of the basement membrane at 200 mg/kg of NBL and degeneration of seminiferous tubules, coagulation of spermatocytes and cellular necrosis at the higher doses. However, testes weights both absolute and relative to body weight were significantly lower only in the 800 mg/kg group, i.e. 36% and 26% relative to the control animals, respectively. In contrast, relative testes weight was significantly increased compared to controls at 200 mg/kg.

Also, at all doses, final body weights were significantly lower relative to the control group but a dose-response relationship was not apparent. Epididymal sperm number was reduced in the 400 and 800 mg/kg groups, i.e. by 64% and 81%, relative to the control group, respectively. A NOAEL was not established in this study, but a LOAEL of ≤200 mg/kg existed for a decrease in body weight gain and the histopathological changes in the testes (Orisakwe *et al*., 2004).

The Orisakwe *et al* (2004) study had a number of significant limitations. First, the group size was small, consisting of only 5 rats per group. Second, the results were poorly reported. For example, entirely different dose levels of the test material were reported in Tables 1 and 2 of this publication. Third, the rats weighed 80%, 92%, and 85% of the control value at the low, middle, and high dose level, respectively, after only one week of exposure. Weight changes of this magnitude suggest that dose levels were selected without regard for the maximum tolerated dose. In fact, in another publication, the same authors reported that Nigerian Bonny light crude oil produced hematologic effects at the low dose and liver toxicity and “severe pathologic changes” at all doses (Orisakwe *et al*., 2005). The possibility that testicular changes may be secondary effects of severe systemic toxicity was not discussed. And fourth, in this study, the presence of the surfactant changed the physical characteristics of the crude oil through emulsification and may have altered the absorption of the PACs or other crude oil components in the gastrointestinal tract in the test animals and may thereby have affected the toxicity and the potency of this

crude oil relative to treatment with this same crude oil without surfactant or other tested oils (Orisakwe et al., 2004).

In another study, Obidike et al. (2007) reported changes in testicular morphology and cauda epididymal sperm reserves of male rats exposed to Nigerian Qua Iboe Brent (NQIB) light crude oil (36°API). In this study, male rats were administered 0.1, 0.2 or 0.4 ml/rat of NQIB crude oil orally by gavage every other day for 4 weeks. Treatment with NQIB produced a dose-dependent reduction in the cauda epididymal sperm reserves and histopathological changes including interstitial exudates, degeneration, and necrosis of spermatogenic and interstitial (Leydig) cells. These adverse changes led to a marked reduction in the number of spermatocytes, spermatids and spermatozoa but with a relative increase in the number of spermatogonia suggesting that crude oil exposure disrupts the maturation process of spermatogonia (Obidike *et al*., 2007).

This study had a number of serious deficiencies. First, there was no appropriate control group. The authors reported that the control animals “received no crude oil;” apparently, the controls received no gavage treatment of any sort. Second, the doses of NQIB were given as absolute amounts and not relative to body weights that could result in a change in the dosage with each dose. Third, the authors reported a statistically significant increase in relative testes weight at the low and high dose levels. However, the data are inconsistent with a statistically significant increase at any dose. For example, the control and high dose values were 1.25±0.06 and 1.26±0.02, respectively. Fourth, since final body weights were not reported, a determination of whether the maximum tolerated dose was exceeded could not be made. Food or water consumption, clinical symptoms, or histological changes in any organ other than the testes were not reported. The selected dose levels in this study may have been chosen without regard for the maximum tolerated dose. Whether the induced testicular changes may be secondary effects of severe systemic toxicity was not discussed.

In a follow-up study by the same authors, male rats were allowed to recover for 8 weeks after exposure to 165, 330, or 660 mg/kg of NQIB crude oil given orally by gavage every other day for 4 weeks (Igwebuike *et al*., 2010). All the treated rats had similar testicular pathology and a dose-related reduced spermatogenic activity in the seminiferous tubules, oligospermia in the cauda epididymides as well as hyperemia and edema in the interstices. The authors reported that there was “evidence of recovery and the restoration of active spermatogenesis.” Unfortunately, this study suffered from many of the same limitations as the initial study (Obidike *et al*., 2007). Further, there were significant discrepancies between the results of the two studies. Since final body weights were not reported for the two studies of NQIB mentioned above a determination of whether the maximum tolerated dose was exceeded could not be made. Food or water consumption, clinical symptoms, or histological changes in any organ other than the testes were not reported. The selected dose levels in these studies may have been chosen without regard for the maximum tolerated dose. Whether the induced testicular changes may be secondary effects of severe systemic toxicity was not discussed. Consequently, the studies are of limited value and need to be replicated