HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

Aromatic Extracts

CATEGORY ANALYSIS AND HAZARD CHARACTERIZATION

Submitted to the US EPA

by

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration # 1100997

May 18, 2012

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EXECUTIVE SUMMARY

This document addresses the potential mammalian and environmental hazards of distillate and residual aromatic extracts. These substances are complex, highly viscous liquids that contain predominately aromatic hydrocarbons covering the carbon number range of C15 to C50. Aromatic extracts are produced as byproducts from the extraction of condensed ring polycyclic aromatic constituents during the production of lubricating oil basestocks and waxes. The aromatic extracts are used as blending components of heavy fuels, as feed stock for production of carbon black, petroleum pitches and resins, and in the manufacture of rubber and plastics. Aromatic extracts are referred to as either "distillate" aromatic extracts (DAE), or "residual" aromatic extracts (RAE), depending on whether they were produced from the extraction of distillate lubricating oil basestocks or residual lubricating oil basestocks.

Physical-Chemical Properties

Aromatic extracts are highly viscous liquids having component hydrocarbons that can have pour points from -6 to >20°C. Boiling ranges for samples of aromatic extracts were 288 to 584°C for DAEs and 344 to >734°C for RAEs. Vapor pressures for aromatic extracts are very low. Partition coefficients for constituent hydrocarbons may range from 5 to >20. Solubility in water is expected to be negligible.

Environmental Fate

The environmental fate of aromatic extracts is determined by the individual hydrocarbons present within the mixture. Because of their physical and chemical properties, these substances will tend to agglomerate rather than disperse if released to the environment. Although they have very low vapor pressures, individual hydrocarbon compounds at the lower molecular weight range (e.g., C15 compounds) may evaporate during weathering. Individual aromatic components that evaporate would be expected to undergo rapid indirect photodegradation. Aromatic extracts are not expected to partition to water, because their water solubility is very low. Modeled log Kow values of the low molecular weight hydrocarbons (e.g., C15 compounds) typically exceed 5, with the higher molecular weight hydrocarbons having partition coefficients >20. Environmental distribution modeling predicts that components would generally partition to soil. Once released to the environment, aromatic extracts are not likely to undergo rapid biodegradation. However, hydrocarbons in general are known to be inherently biodegradable over time.

Ecotoxicity

Some aromatic extracts may cause toxicity in freshwater invertebrates and algae. The lowest invertebrate EL50 was 35.9 mg/L and the lowest algal E_rL50 was 18.8 mg/L. Other tests of invertebrates found no effects at 1000 mg/L, and no effects on algae were observed when tested in 50% dilutions of 2000 mg/L WAFs. None of the acute studies in fish reported any adverse effects when aromatic extracts were tested using WAFs at 1000 mg/L. In reproduction tests with *Daphnia magna*, no effects on reproduction or survival of adult animals were observed when exposed for 21 days to 1000 mg/L WAF of DAE and RAE. Offspring produced during the tests also appeared healthy with no adverse effects noted.

Human Health Effects

DAEs have a low order of acute oral and dermal toxicity. DAE caused mutations *in vitro* in the optimized Ames test and the mouse lymphoma assay. Limited evidence of chromosomal aberrations in bone marrow was seen in rats exposed repeatedly to DAEs by oral or dermal routes; most DAEs are unlikely to produce chromosomal effects under *in vivo* conditions. In 13-week repeated dose toxicity studies, oral or dermal dosing of light or heavy paraffinic DAEs produced effects in the liver, thymus and blood. Little evidence of an effect on weights or histology of reproductive organs or on semen quality was observed in these studies. The same DAEs caused toxicity to the developing fetus when applied to the skin of pregnant rats during days 0-19 of gestation. However, treatment-related fetal effects occurred only in the presence of

effects in the mothers. Oral administration of heavy paraffinic DAE at 2000 mg/kg on a single day during the critical period of gestation resulted in increased fetal malformations and maternal toxicity in rats.

Although no acute toxicity studies were reported for RAEs, the acute toxicity of RAEs is expected to be less than DAEs due to the higher molecular weights and higher viscosities of the components in RAEs, which would be expected to limit bioavailability. Some samples of RAEs caused mutations *in vitro* in the optimized Ames test. Evaluation of chromosomes from bone marrow of rats dermally exposed repeatedly to RAEs did not show any evidence of chromosomal mutation. Four 13-week repeated dose dermal toxicity studies with RAEs also produced effects in the liver, thymus and blood but at higher doses than with the DAE discussed above. Microscopic examination of male and female reproductive organs in these four studies did not identify any effects which appeared to have been due to treatment. In a developmental toxicity study in rats by dermal application, RAE did not produce any adverse effects to dams or fetuses. NOAELs for reproductive toxicity of both DAEs and RAEs would not be expected to be below NOAELs for developmental effects.

Data from laboratory studies on a number of petroleum substances and modeled data based on statistical analyses show an association of the total amount and profile of polycyclic aromatic compounds to certain repeated-dose and developmental toxicity endpoints. Effects on these endpoints were shown to vary among samples in association with different polycyclic aromatic compound (PAC) content of those samples resulting from the crude source and refining conditions. Models were developed to predict quantitative estimates of effects on sensitive endpoints for untested samples based on the PAC content of those samples. Those predicted values were then used to aid in fulfilling the data requirements under HPV.

Untreated aromatic extracts can produce skin cancer in the mouse following dermal application. Numerous studies have shown that the mutation and cancer-causing potential of DAEs and compositionally related petroleum substances is directly related to the presence of PACs. As DAEs contain relatively high concentrations of PACs, they are commonly active in optimized Salmonella assays and, unless further refined to remove PACs, often produce squamous cell tumors when tested in chronic dermal application assays in mice. Additional processing can reduce potential mutagenicity. Similarly, some RAEs can contain relatively high concentrations of PACs, are active in optimized Ames tests, and would be expected to produce skin tumors in a chronic dermal assay; but others are neither mutagenic nor carcinogenic.

1. DESCRIPTION OF AROMATIC EXTRACTS

1.1. Definitions and Manufacture

As used in this document, "aromatic extracts" refers to solvent extracts of distillates or the residuum from a vacuum tower that have not been subjected to further processing such as hydrogenation, desulfurization, clay or acid treatment, additional distillation or solvent extraction.

Aromatic extracts are produced during the refining of lubricating oil basestocks and waxes. The residue (residuum) of atmospheric distillation of crude oil is distilled under vacuum to produce distillate and residual lubricating oil basestocks. The untreated lubricating oil basestocks contain undesirable components that negatively impact lubricant performances, i.e., color, odor, stability and/or viscosity, and therefore must be removed. These undesirable components include aromatic compounds containing sulfur, nitrogen, and oxygen as heteroatoms and polycyclic aromatic compounds (PACs). One way in which these undesirable components can be removed is to incorporate an extraction step, typically with

furfural, phenol or n-methyl pyrollidone to remove aromatic compounds from the lubricating oil feedstock, resulting in the production of aromatic extracts and lubricating oil basestocks.

The aromatic extracts can be grouped into two subcategories, distillate aromatic extracts (DAEs) and residual aromatic extracts (RAEs), according to the class of lubricating oil feedstock from which they are derived. Figure 1 is a refining diagram showing production of both lubricating oil basestocks and aromatic extracts. Both types of aromatic extracts are highly viscous to mobile liquids, which may be dark amber to black in color.

In the production of DAE, untreated distillates (lubricating oil feedstock) are extracted with a solvent such as furfural, phenol, N-methyl-2-pyrrolidone (NMP), or dimethyl sulfoxide (DMSO) to selectively remove the undesirable aromatic compounds, (especially 1-7 fused ring PACs). Other solvents can also be used. The solvent is then removed from the resulting extract, and the remaining aromatic concentrate (aromatic extract) is either sold as is or, if needed, further treated to lower the PAC content for specialty applications (treated DAE). Treated heavy paraffinic DAE has a separate CAS number (68783-04-0) that is not included in this HPV category. The viscosity of DAEs increases with increasing boiling range (Briggs and Mackerer, 1996; Roy et al., 1996).

In the production of RAE, the residuum from vacuum distillation is extracted with liquid propane to remove particulates, resins, and asphaltenes. In this process, the resins, asphaltenes, and particulates precipitate out and the propane/oil stream is then stripped of the propane. The very viscous stream that results is referred to as deasphalted oil (DAO). The DAO undergoes the same extraction process used for the vacuum distillate streams. Additional extraction steps can be used to reduce the level of PACs. As with DAE, RAE viscosity increases with increasing boiling range (Briggs and Mackerer, 1996; Roy et al., 1996).

Fig. 1. Simplified processing plan for a petroleum refinery

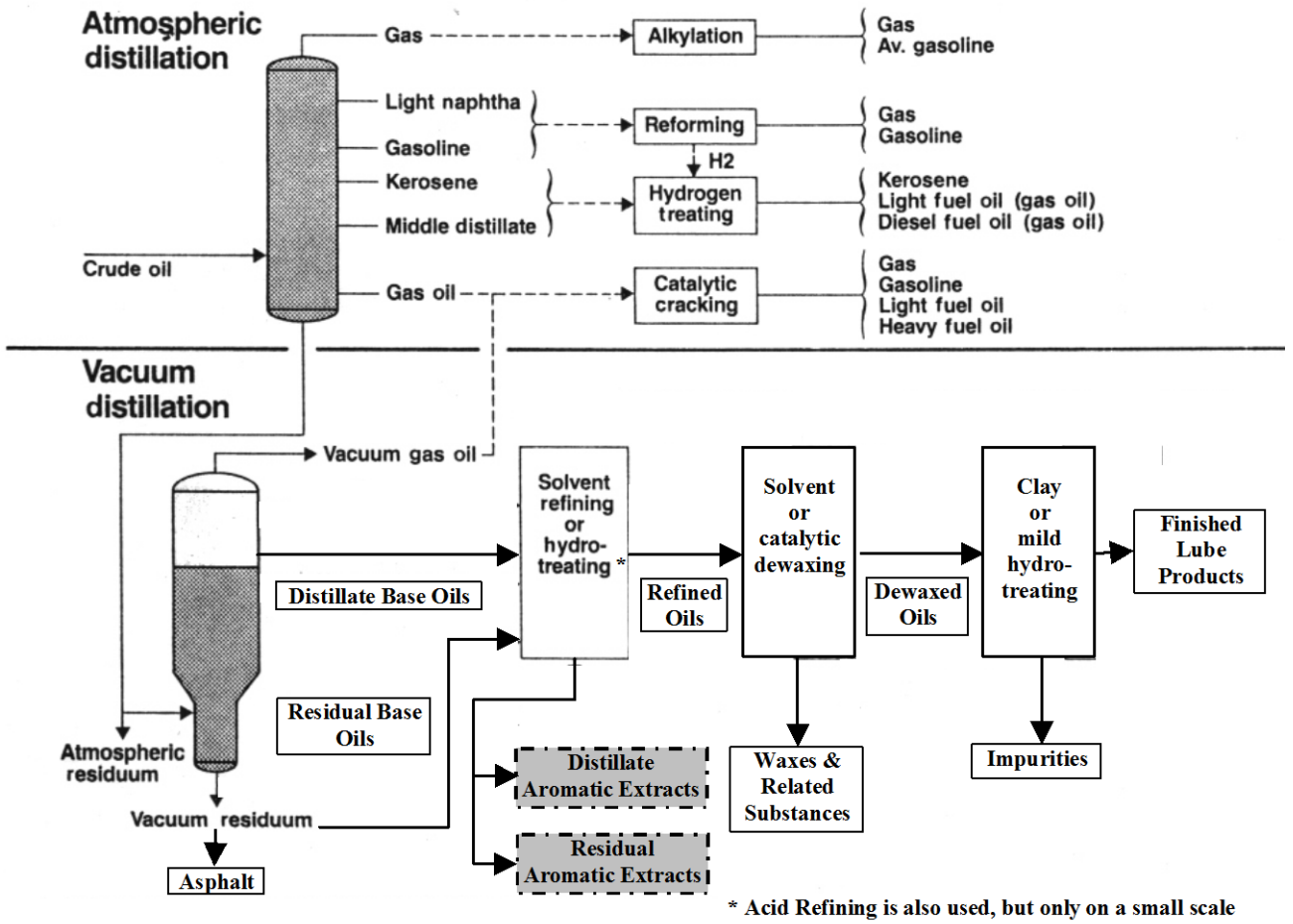

Figure adapted from Kane et al, 1984 and IARC, 1984.

1.2. Assignment of CAS Numbers

The names and definitions of refinery streams, including aromatic extracts, used by the Chemical Abstract Service (CAS) were developed in response to Section 8(b) of the Toxic Substances Control Act. This section of TSCA required identification and registration with the Environmental Protection Agency before July 1979 of each "chemical substance" being manufactured, processed, imported or distributed in commerce. Due to analytical limitations and known variability in refinery stream composition, identification of every specific individual molecular compound in every refinery process stream under all processing conditions was impossible. Recognizing these problems, the American Petroleum Institute (API) recommended to the EPA a list of generic names for refinery streams consistent with industry operations and covering all known processes used by refiners. The list, including generic names, CAS numbers and definition of each stream, was published by the EPA as "Addendum I, Generic Terms Covering Petroleum Refinery Process Streams."

Because of the variability inherent in the processing of petroleum materials, the definitions API developed for the CAS numbers are qualitative in nature, written in broad, general terms. The definitions often contain ranges of values, with little if any quantitative analytical information or concern for possible

compositional overlaps. Many of the definitions also include information on the material's process history. In fact, process history and not chemical composition was one of the primary criteria used by API to differentiate streams and assign CAS numbers.

The names and registry numbers from CAS for the five specific refinery substances in the aromatic extract category are listed in Table 1, including synonyms and the TSCA definitions of the substances (including hydrocarbon chain lengths)

With regard to the four DAEs, the basic differences among the CAS descriptions are carbon number (C15-C30 or C20-C50) and hydrocarbon type (paraffinic vs. naphthenic). These distinctions are a result of historical refinery naming practices and predominant nature of the crude oil or lubricating basestock run through the process unit. Furthermore, there are not large differences in straight and branched chain saturated hydrocarbons (paraffinic hydrocarbons) or polar compounds among the four DAEs. Since it has been observed that the constituents in these substances that cause mutagenic, carcinogenic, developmental, and subchronic effects are DMSO-extractable PACs found in the aromatic fraction (Simpson et al, 2008; Roy et al, 1988; Cruzan et al., 1986; Blackburn et al., 1986), these distinction in nomenclature do not indicate distinctions in toxicological properties. No such nomenclature differences exist for RAE.

1.3. Uses

Aromatic extracts have been used in applications where their solvency is valued, such as in the manufacture of rubber and plastic, where aromatic extracts are used as extenders, softeners and diluents that remain in the final product, contributing to both ease of processing and improved product performance. Some quantities of aromatic extracts have been used employed in tire manufacture and in specialty applications, such as asphalt blends, printing inks, wood preservatives, and seal coatings. Aromatic extracts are also used as components of heavy fuel blends (e.g., industrial fuel oil, bunker fuel) and as precursors of other hydrocarbon products (e.g., carbon black, petroleum resins, and petroleum pitch). Within a refinery, aromatic extracts can also be converted to other refinery products by processes such as cracking and coking to produce lighter hydrocarbon fuels or coke.

1.4. Typical Physical and Chemical Properties

Historical international values on various physical and chemical properties for aromatic extracts are in Table 2 to provide general background. These values were taken from three sources, namely CONCAWE (1992 and 2010) and tests performed on samples provided to API by US manufacturers as part of the HPV project. As such, these values represent samples taken at different times in Europe (CONCAWE 1992 and 2010) and in the US. Also slightly different analytical methods were employed in reporting some parameters (such as boiling range). Taken together, these data show the results that can be expected for major physical and chemical properties of AEs within the category.

Table 1. CAS numbers and descriptions within the HPV aromatic extracts category

CAS No. and Name	TSCA Definition and Synonyms with Carbon Numbers Underlined
Distillate Aromatic Extracts	
64742-05-8	TSCA Definition: A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It

Extracts (petroleum), light paraffinic distillate solvent	consists predominantly of aromatic hydrocarbons having carbon numbers predominantly in the range of C15 through C30. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.
	Synonyms: light paraffinic distillate aromatic extracts, distillate aromatic extract, aromatic process oil, process oil, aromatic extract, rubber extender oil
64742-03-6 Extracts (petroleum), light naphthenic distillate solvent	TSCA Definition: A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It consists predominantly of aromatic hydrocarbons having carbon numbers predominantly in the range of C15 through C30. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.
	Synonyms: light naphthenic distillate aromatic extract, distillate aromatic extract, aromatic process oil, process oil, aromatic extract, rubber extender oil
64742-04-7 Extracts (petroleum), heavy paraffinic distillate solvent	TSCA Definition: A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It consists predominantly of aromatic hydrocarbons having carbon numbers predominantly in the range of C20 through C50. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons
	Synonyms: heavy paraffinic distillate aromatic extracts, distillate aromatic extract, aromatic process oil, process oil, aromatic extract, rubber extender oil
64742-11-6 Extracts (petroleum), heavy naphthenic distillate solvent	TSCA Definition: A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It consists predominantly of aromatic hydrocarbons having carbon numbers predominantly in the range of C20 through C50. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.
	Synonyms: heavy naphthenic distillate aromatic extract, distillate aromatic extract, aromatic process oil, process oil, aromatic extract, rubber extender oil
Residual Aromatic Extracts	
64742-10-5 Extracts (petroleum), residual oil solvent	TSCA Definition: A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It consists predominantly of aromatic hydrocarbons having carbon numbers predominantly higher than C25.
	Synonyms: residual aromatic extract, bright stock extract, aromatic process oil, process oil, aromatic extract, solvent extract

Table 2. Values of physical and chemical properties for samples of distillate and residual aromatic extracts		
Property	DAE	RAE
Boiling range (°C, initial - final)	250 - 680[1]	>380[2]
Boiling range (°C, initial - final) Boiling range (°F, initial – final)	288 - 584[3] 551 - 1083[3]	344 - >734[3] 652 - >1354[3]
Pour Point (°C)	-6[4] - 50[1]	> +20[4]
Vapor pressure, 20°C (kPa)	<0.1[1]	<0.01[4]
Water solubility, 20°C (mg/L)	1.4 – 5.8[4]	Sparingly[4]
Flash point closed cup (°C)	240 - 289[1]	298[2]
Autoignition temperature (°C)	>280 - 410[1]	>380[4]
Density at 15°C (kg/dm)	0.93 – 1.05[1]	0.96 – 1.02[2]
Viscosity, kinematic 100°C [5] (mm^2/s (cSt))	3.8 - 124[1]	92 - 100[2]
	4 - 50[3]	51 - 169[3]
Average Molecular Mass	300 - 580[4]	>400[4]
Carbon number range	C15 - C50[4]	>C25[4]
Aromatic Content (%m)	65 - 85[4]	60 - 80[4]
	66 - 86[3]	48 - 77[3]
DMSO extract (IP346)[6] (%m)	10 - 30[4]	NA[4]
	6 - 19[3]	NA[4]

1) Values were derived from CONCAWE (2010a) for one DAE (CAS 64742-04-7).
2) Values were derived from CONCAWE (2010a) for RAE (CAS 64742-10-5).
3) Values are for some of the samples in Table 3 that were submitted to API by manufacturers for the HPV project (2 light paraffinic distillate solvent extract, 6 heavy paraffinic distillate solvent extracts, 1 heavy naphthenic distillate solvent extract, and 3 residual oil solvent extracts).
4) Values were derived from CONCAWE, 1992
5) Viscosity measurements at 40°C may be subject to error due to non-Newtonian flow effects close to the pour point (CONCAWE, 1992). Therefore only data for viscosity at 100°C are shown here.
6) Material extractable in DMSO as measured by method IP 346 (IP, 1980, 1985). IP346 is not considered appropriate (NA) for RAEs (Petrolabs, 2010, CONCAWE, 1994).

1.5. Analytical Characterization

Aromatic concentrations in either DAE or RAE are largely dependent on the source and type of crude oil from which the extract is processed (Feuston et al., 1994). Apparently, the levels of the different types and classes of hydrocarbon compounds in DAE and RAE may not seem to be substantially different when one considers only overall concentrations of aromatic, polar and saturated hydrocarbons. However, DAEs generally contain much higher proportions of lower molecular weight aromatic hydrocarbons (Table 2) and significantly higher concentrations of 1-7 ring PACs (Table 3). RAEs have much greater average molecular weights, higher viscosities, significantly less solubility in aromatic solvents and markedly reduced concentrations of 1-7 ring PACs. In the RAEs, the naphthenes (relatively polar compounds) and aromatics have greater numbers of larger and longer side chains and there are substantial amounts of polycyclic naphthenes. As both the paraffinic and naphthenic side chains in the RAEs increase in size and number, the molecules become more paraffinic in nature.

Depending on the crude oil and refining conditions, some refinery substances contain 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 heteroatomic compounds in which one or more of the carbon atoms in the PAH ring system are replaced by nitrogen, oxygen, or sulfur atoms. Hundreds to thousands of individual PACs are produced during the formation of crude oil as organic matter is converted into petroleum under elevated pressure and moderate temperatures (130 – 150 °C). The resulting PACs include a complex variety of parent (i.e., unsubstituted) 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 (C1-C4) in petroleum far exceeds the abundance of the parent compound (C0) (Speight, 2007). The fact that the concentration of alkylated polycyclic aromatics is much greater than parent polycyclic aromatics is the main feature of the PACs found in petroleum (Altgelt and Boduszynski, 1994).

The PAC content (expressed as a "PAC profile", the weight percent in the sample of each ring-class of PACs, such as 1-ring, 2-ring, etc.) of some petroleum substances has been shown to be highly correlated with biological and toxicological effects in repeated-dose and developmental toxicity studies (Simpson, et al 2008). For that reason, available data on the profiles of PACs extracted with DMSO from samples of DAEs and RAEs are shown in Table 3. As can be seen, the distribution and concentration of 1-7 ring PACs of DAEs and RAEs are quite different among the samples available to API. Concentrations of DMSO-extractables ranged from 6.3 to 20.3% in the DAEs compared to 1.8 to 4.5% in the RAEs.

This correlation between PAC profiles and certain toxicological endpoints was used to develop models for the prediction of toxicological effects in repeated-dose and developmental toxicity studies based on the PAC profile. These models were used for samples of DAEs with data on PAC profile but without data from toxicity tests. The models were not applicable, however, to RAEs for reasons that are explained in section 7.

Table 3. PAC content of aromatic extract samples

Sample Identification	Type of Sample and CAS No.	DMSO wt % [1]	ARC 1[2] (%)	ARC 2 (%)	ARC 3 (%)	ARC 4 (%)	ARC 5 (%)	ARC 6 (%)	ARC 7 (%)
Site 7, Sample 23 (CRU 20906)	LP-DAE [3]	13.6	0.0	0.0	5.4	6.8	1.4	0.0	0.0
CRU 86187	HP-DAE[4]	20.3	0.0	0.0	4.1	8.1	6.1	2.0	0.4
Site 3, Sample 13 (CRU 100709)	LP-DAE	12	0.0	0.2	10.8	0.5	0.0	0.0	0.0
Site 4, Sample 3 (CRU 100711)	LP-DAE	12	0.0	0.0	4.8	4.8	2.4	0.4	0.0
CRU 86141	HP-DAE	19	0.0	0.2	7.6	7.6	1.9	0.2	0.0
CRU 86303	HP-DAE	14.6	0.0	0.0	0.3	2.9	5.8	4.4	1.0
CRU 89130	HP-DAE	13.9	0.0	0.1	5.6	8.3	0.0	0.6	0.0
Site 2, Sample 9 (CRU 100705)	HP-DAE	6.3	0.0	0.0	0.0	0.3	0.6	2.5	3.2
Site 3, Sample 10 (CRU 100706)	HP-DAE	7.7	0.0	0.0	2.3	4.6	1.5	0.0	0.0
Site 3, Sample 12 (CRU 100708)	HP-DAE	8	0.0	0.1	0.5	1.6	3.2	2.4	0.5
Site 3, Sample 14 (CRU 100710)	HP-DAE	7.4	0.0	0.0	0.5	0.7	1.5	3.0	2.2
Site 4, Sample 4 (CRU 100712)	HP-DAE	9.5	0.0	0.0	0.1	1.9	3.8	2.8	0.7
Site 5, Sample 1 (CRU 100713)	HP-DAE	8.3	0.0	0.0	0.1	0.8	3.3	3.3	0.7
950219	HP-DAE	13.5	0.0	0.1	8.1	4.0	0.4	0.0	0.0
	LN-DAE[5]								
Site 1, Sample 5 (CRU 100701)	HN-DAE[6]	19	0.0	0.0	1.5	5.7	5.7	5.7	0.4
Site 2, Sample 8 (CRU 100704)	RAE[7]	1.8	0.0	0.0	0.0	0.1	0.4	0.5	0.7
Site 3, Sample 11 (CRU 100707)	RAE	2.4	0.0	0.0	0.0	0.2	0.5	0.7	1.0
CRU 100714 – 100721 (Means from 8 nearly identical samples from same site)	RAE	2.3	0.0	0.0	0.0	0.1	0.2	0.7	1.3
Bright stock extract (CRU 87336)	RAE	4.5	0.0	0.9	1.4	0.4	0.4	0.9	0.4
CT-28 (CRU 87476)	RAE	2.6	0.0	0.0	0.0	0.1	0.3	0.5	1.6

1) Percent of DMSO-extractable PACs as determined by PAC-2 method as described in API (2008)
2) ARC is "aromatic ring class". "ARC 1 % is the weight of PACs within the total sample that have 1 aromatic ring; "ARC 2 %" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings as determined by the PAC-2 method,
3) LP-DAE is light paraffinic distillate aromatic extract (CAS 64742-05-8).
4) HP-DAE is heavy paraffinic distillate aromatic extract (CAS 64742-04-7).
5) LN-DAE is light naphthenic distillate aromatic extract (CAS 64742-03-6). No samples were available.
6) HN-DAE is heavy naphthenic distillate aromatic extract (CAS 64742-11-6).
7) RAE is residual aromatic extract (CAS 64742-10-5).

2. CATEGORY DEFINITION AND JUSTIFICATION

The rationale behind combining these five refinery substances into a category was based upon the similarity of production processes within the refinery; more specifically all are produced from vacuum distillates via solvent extraction processes, yielding substances having high aromatic content. There are three overlapping carbon ranges within the category i.e., a low of C15-C30, a middle of C20-C50 and a high range of greater than C25. The physical/chemical properties of the substances are directly related to

their carbon range and to a much lesser extent to the paraffinic or naphthenic character of the feedstocks from which they were extracted. The carbon number influences the volatility, water solubility, and viscosity of these substances which in turn determines their environmental fate, ecotoxicity, and potential bioavailability of toxic components. The mammalian toxicity of DAEs is directly related to the PAC profile of the sample. The mammalian toxicity of RAEs is related to both the PAC profile and the physical-chemical properties of the sample.

3. TEST MATERIALS

3.1 Previous Studies

Studies (acute toxicity, 28-day dermal and in-vitro mutagenicity) were conducted on a sample of a light paraffinic DAE (API 83-16, CAS 64742-05-8). Physical properties for this sample are in the Robust Summary for Aromatic Extracts. This test material appears to be slightly lighter than recently acquired samples of light paraffinic DAEs based on boiling point range (10-90%), viscosity (cSt @ 100 C) and aromatic content. (Data are not shown for other samples). An analysis of the PAC content of this sample was not conducted.

A sample of heavy paraffinic DAE (CAS 64742-04-7) was tested for acute toxicity and a second sample (318 Isthmus furfural extract, CRU 86187) was tested in 13-week repeated-dose, micronucleus, and developmental toxicity studies performed by the dermal route. Details are in subsequent sections of this document. The PAC profile of this second sample had generally high levels of PAC ring classes compared to recently acquired samples of heavy paraffinic DAE. (See Table 3.)

Four samples of RAE were tested in 13-week repeated-dose, *in vitro* mutagenicity, *in vivo* mutagenicity, and developmental toxicity studies. Details are in subsequent sections of this document. PAC-2 analysis of these test materials was not available at that time due to their extremely high boiling range, carbon number range, high viscosity and analytical complexity associated with speciation of 1-7 ring PACs. However, detailed analytical characterization of more recently obtained samples of RAEs has shown that RAEs contain lower concentrations of 1-7 ring PAC than the DAEs (Table 3).

3.2 New Studies

Since the original submission of the Aromatic Extracts Category Test Plan (December, 2003), the API has obtained additional compositional information on samples of DAEs and RAEs obtained from US refineries sponsoring this category. These data have helped to clarify and characterize compositional variability among category members. As explained above, the data on the PAC profiles have been used to predict effects in mammals for a set of samples of aromatic extracts, as described in subsequent sections. As requested by EPA, and to test the statistical model predictions, the Testing Group also conducted studies on a light paraffinic distillate aromatic extract (CRU 20906, CAS 64742-05-8). The studies were a 90-day repeat dose study, a developmental toxicity study, and an in vivo micronucleus study. No sample of a light naphthenic distillate aromatic extract could be obtained to conduct similar toxicology testing.

4. PHYSICAL-CHEMICAL PROPERTIES

4.1 Physical-Chemical Screening Information Data Set (SIDS)

The physical-chemical endpoints in the HPV chemicals program include the following:

- Melting Point
- Boiling Point
- Vapor Pressure
- Octanol/Water Partition Coefficient
- Water Solubility

Because the substances in this HPV category are complex and have variable composition, it is not possible to measure or calculate a single numerical value for most of the physicochemical properties. The range of individual hydrocarbon components in aromatic extracts defines these properties. For example, an aromatic extract does not have a defined melting point, but rather a melting point range. Therefore, melting point, boiling point, partition coefficient, and water solubility will be reported as ranges that reflect the properties of individual components within the aromatic extracts category. An exception is vapor pressure, which is a measure of the total partial pressure exerted by the sum of the components.

Although some data for substances in this category exist, values for all of these endpoints have not been determined and a consensus database for chemicals that represent substances in this category does not exist. Therefore, calculated and measured representative data have been identified and included in the robust summaries, where appropriate. The EPIWIN© computer model (US EPA, 2000), as discussed in the U.S. EPA document entitled "*The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*" was used to calculate physicochemical properties of representative constituents for selected aromatic extract substances. Because of the diversity of components/compounds making up aromatic extracts, it was not feasible to model the physicochemical endpoints for each potential component/compound. Instead, modeling efforts were directed toward representative hydrocarbon components/compounds in aromatic extracts most likely to partition to various environmental media yet still encompass the typical extremes in molecular weight.

4.1.1. Melting Point

To better describe the physical phase or flow characteristics of petroleum products, the pour point (ASTM D5985) is routinely used instead of melting point. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM, 2002a). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure forms and/or viscosity increases such that movement of the surface of the test specimen is impeded under the conditions of the test. Because not all petroleum products contain wax in their composition, the pour point determination encompasses both a change in physical state (i.e., wax crystal formation) and/or viscosity. The pour point measured for samples of distillate aromatic extracts ranged from –6°C to +50°C, whereas pour point for residual aromatic extracts is > +20°C (CONCAWE, 1992).

Conclusions:
Empirical data based on ASTM method D5985 for pour point showed that distillate aromatic extracts have typical points ranging from -6°C to +50°C, while pour points of residual aromatic extracts are > +20°C.

4.1.2. Boiling Point

As mixtures, aromatic extracts do not have a single numerical value for boiling point, but rather a boiling range that reflects the individual components. The production of aromatic extracts results in two

substances that have slightly differing components that affect the boiling range of those substances. As seen in Table 2, samples of aromatic extracts from the distillate fraction of the production process had a boiling range of 288°C to 584°C, while samples of aromatic extracts from the residuum fraction had a boiling range from 344°C to >734°C.

Conclusions:
Measured values for ranges of boiling points are 288°C to 584°C for distillate aromatic extracts and 344°C to >734°C for residual aromatic extracts.

4.1.3. Vapor Pressure

For complex substances such as petroleum products, the vapor pressure of the complex substance is the sum of the partial pressures of the individual components (Dalton's Law of Partial Pressures). Aromatic extracts are expected to have low vapor pressure due to their high viscosity, boiling range and molecular weights of the constituent hydrocarbons (C15 – C50 carbon atoms). Vapor pressures for distillate and residual aromatic extracts have been measured to be <0.1 kPa. (See Table 2.)

Conclusions:
Reported values for vapor pressures for distillate and residual aromatic extracts are <0.1 kPa.

4.1.4. Partition Coefficient

Aromatic extracts consist of hydrocarbons with carbon numbers in the range C15 to C50. The percent distribution of the hydrocarbon constituents (i.e., isoparaffins, naphthenes, and aromatics) and the carbon chain lengths determine in-part the partitioning characteristics of the complex substances. Generally, hydrocarbon chains with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). However, due to their complex compositions and low water solubility, measurements of the log Kow of these complex hydrocarbon substances typically cannot be made. For example, one study of the partitioning behavior of a DAE found that 85% of the components in the DAE had partition coefficients greater than the applicable range (log Kow of 0 to 6) for the method (Shell Research Ltd., 1984). The overall range of partition coefficients measured for the DAE was 4.4 to >6 (Shell Research Ltd., 1984). Modeling efforts also support this study. Partition coefficients of selected C15 and C50 chain-length hydrocarbon structures representing paraffinic, naphthenic, and aromatic constituents in aromatic extracts were modeled using EPIWIN©, KOWWIN V1.66 (US EPA, 2000). Modeling results showed log Kow values for C15 representative structures ranged from approximately 4.9 and to 7.6 for the C15 components, while values for C50 compounds ranged from 22 to 25.

Conclusions:
The complex compositions, high molecular weights, and low water solubility of the constituent hydrocarbons in these substances generally precludes the measurement of partition coefficient for the majority of the components. Based on empirical data, the range of log Kow values was 4.4 to >6, with 85% of the components greater than the upper limit of the method. Modeled log Kow values for individual constituent hydrocarbons that may exist in aromatic extracts supports this range.

4.1.5. Water Solubility

Molecular weight and chemical structure influence the ultimate degree of solubility. For example, water

solubility typically decreases with increasing molecular weight, while aromatic hydrocarbons show greater water solubility than saturated hydrocarbons for compounds of equal carbon numbers. Water solubility estimates obtained using WSKOW V1.40 (EPIWIN V3.10, EPA, 2000) for individual isomeric C15 structures range from <0.001 to 0.63 mg/L. Water solubility values for similar structures having 50 carbon atoms were all <0.001 mg/L. Since aromatic extracts are viscous, semi-solid to solid materials at ambient temperatures, water solubility is expected to be negligible for these materials.

Conclusions:
The high molecular weights of constituent hydrocarbons in aromatic extracts limit the water solubility of these substances. While modeled solubility estimates show values ranging from <0.001 to 0.63 mg/L for individual hydrocarbon structures, water solubility is expected to be negligible for these complex substances.

4.2 Assessment Summary for Physical-Chemical Endpoints

The physical-chemical characteristics of the members of the aromatic extracts category reveal that these substances are dark solids or highly viscous liquids composed of individual hydrocarbons that can boil in the range of 288°C to 584°C C for DAEs and 344°C to >734°C for RAEs. Pour points were measured in the range of -6° C to +50°C for DAEs, while pour points of RAEs were >20°C. Measured vapor pressures of whole products were <0.1 kPa for both DAEs and RAEs, indicating a minimal tendency to volatilize. Modeling of partition coefficients for selected hydrocarbons in aromatic extracts showed log Kow values ranging from approximately 5 to greater than 7 for the C15 components, while values for C50 compounds were greater than 20. Based on water solubility estimates and the fact that aromatic extracts are viscous, semi-solid to solid materials at ambient temperatures, water solubility is expected to be negligible for these materials.

5. ENVIRONMENTAL FATE

5.1 Environmental Fate Endpoints

To assess the environmental fate properties for the HPV program, the U.S. EPA has selected important fate endpoints by which these substances may be characterized. The environmental fate endpoints include the following:

- Photodegradation
- Stability in water [Hydrolysis]
- Transport Between Environmental Compartments [Fugacity/Distribution]
- Biodegradation

5.1.1. Photodegradation

5.1.1.1. Direct

The direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation. Only light energy at wavelengths between 290 and 750 nm can potentially result in photochemical transformations, although absorption is not always sufficient for a chemical to undergo photochemical degradation (Harris, 1982a).

Saturated and one-ring aromatic hydrocarbons do not show absorbance in the 290 to 800 nm range and would not be expected to photodegrade. Polycyclic hydrocarbons have shown absorbance in this range of light energy and could potentially undergo photolysis reactions. The degrees and rates at which these compounds photodegrade depend on whether conditions allow penetration of light with sufficient energy to affect a change. For example, polycyclic aromatic compounds bound to sediments may persist due to limited energy absorption as a consequence of limited light penetration.

5.1.1.2. Indirect

Components in aromatic extracts that do not directly photodegrade (e.g., paraffins, naphthenes, and one-ring aromatic compounds) may be subject to indirect photodegradation. Indirect photodegradation is the reaction with photosensitized oxygen in the atmosphere in the form of hydroxyl radicals (OH^-). The potential to undergo indirect photodegradation can be estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (US EPA, 2000), which calculates a chemical half-life and an overall OH^- reaction rate constant based on a 12-hour day and a given OH^- concentration. Atmospheric oxidation rates and half-lives were calculated for the lowest molecular weight constituents of various components of aromatic extracts (e.g., C15 hydrocarbon structures), since these would have the most potential to volatilize to the atmosphere. AOP half-life estimates for these compounds ranged from 0.1 to 0.7 days and show a lack of persistence in the atmosphere.

Conclusions:

Since aromatic extracts are highly viscous materials with low volatility and low water solubility, direct and indirect photodegradation are not likely to be important fate processes. However, if conditions result in dispersion or volatilization such that sunlight and photosensitized oxygen compounds can interact with the constituents in aromatic extracts, half life estimates reflect a rapid degradation of those substances. Therefore, constituents in aromatic extracts will not persist in the atmosphere.

5.1.2. Stability in Water

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). Because aromatic extracts do not contain significant levels of these functional groups, these substances are not subject to hydrolysis.

Conclusions:

Aromatic extracts are stable to hydrolysis.

5.1.3. Transport between Environment Compartments (Fugacity/Distribution)

Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (e.g., air, water, soil, sediment, suspended sediment and biota). The US EPA has agreed that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay et al, 1996; Mackay et al, 1997). The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites the use of this model in its document "Determining the Adequacy of Existing Data" that was prepared as guidance for the HPV chemicals program (US EPA, 1999).

Results of Level 1 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. One drawback

of these and higher level models is their inability to predict the distribution of the entire set of constituents comprising complex petroleum substances. To gain an understanding of the potential environmental distribution for these complex mixtures, modeling was performed for different C15 and C50 compounds representing common isomeric structures in aromatic extracts (e.g., isoparaffins, naphthenes, and aromatics). Therefore, the partitioning data represent the low and high ends of the molecular weight spectrum for representative hydrocarbon compounds making up aromatic extracts. Other compounds having molecular weights between the low and high ends of the molecular weight spectrum are expected to have partitioning behaviors that fall within the ranges.

Upon an environmental release of aromatic extracts, the individual constituents will begin to partition in accordance with their own intrinsic physical-chemical characteristics. Because these substances are highly viscous semi-solids at ambient environmental temperatures, this physical state will govern the rate by which the individual constituents disperse. Modeling results show that aromatic extracts released to the environment would partition into soil with only negligible amounts dissolving in water. As the substance is exposed to weathering elements (e.g., processes related to the physical and chemical actions of air, water and organisms on the material after a release), some of the lowest molecular weight paraffins, iso-paraffins, naphthenes and 1-ring aromatic hydrocarbons may partition to the air over time. Based on the calculated half-lives for reaction with hydroxyl radicals, the fraction that partitions to the atmosphere is not expected to persist. The resulting values represent the potential ranges of distribution to environmental media for those hydrocarbon constituents found in these substances:

Table 4. Environmental distribution of representative hydrocarbon compounds determined by the EQC Level 1 fugacity model.

	Percent Distribution in Environmental Compartment					
	Air	Water	Soil	Sediment	Susp Sed	Biota
C15 compounds	0.3 – 68	<0.1 – 1	31 – 97	0.7 – 2	<0.1	<0.1
C50 compounds	<0.1	<0.1	98	2	<0.1	<0.1

Conclusions:
Aromatic extracts are complex substances comprised primarily of aromatic hydrocarbon compounds having high molecular weights and low water solubilities. When released to the environment, aromatic extract constituents will partition in accordance with their specific physical-chemical attributes. Fugacity modeling of individual constituents in aromatic extracts shows that these constituents will partition mostly to soil, with only the lowest molecular weight components distributing to the air. However, the physical state of these substances will limit the extent to which the distribution to air occurs.

5.1.4. Biodegradation

Aromatic extracts have not demonstrated a capacity to readily biodegrade in laboratory tests (Shell Research Ltd., 1994a). In two 28-day ready biodegradability studies on a distillate aromatic extract, no biodegradation was measured when using the modified Sturm and closed bottle test protocols. Similar results would be expected for residual aromatic extracts based upon the higher molecular weight components in those materials. Although these substances do not pass ready biodegradability criteria, hydrocarbons are considered inherently biodegradable (CONCAWE, 1992).

Conclusions:
Aromatic extracts are not readily biodegradable. However, hydrocarbons in general are known to be inherently biodegradable over time.

5.2 Assessment Summary for Environmental Fate

Aromatic extracts are complex and variable substances consisting of predominantly aromatic hydrocarbons. These substances are non-volatile, although some of the individual hydrocarbons at the low end of the molecular weight spectrum (e.g., C15) can have measurable vapor pressures in their pure state. Any aromatic extract constituents that partition to the atmosphere will interact with hydroxyl radicals and undergo photodegradation. The constituent hydrocarbons in aromatic extracts are not water soluble and are stable to hydrolysis. Releases to the environment will result in little mobility, but eventually these substances will be incorporated into soils/sediments followed by slow biodegradation.

6. ENVIRONMENTAL EFFECTS

6.1. Aquatic Endpoints - Acute Toxicity

The HPV Chemical Test Program includes acute toxicity to a freshwater fish, an invertebrate (*Daphnia magna*), and an alga. The substances in the aromatic extracts category are expected to have a similar degree of toxicity for all of these aquatic species when studies using similar solution preparation and

exposure techniques are compared. The endpoint values cited in the robust summaries and described below for the three trophic levels reflect the loading rates of the test substance added to exposure solutions. Termed water accommodated fractions (WAF), the WAF preparation is recommended as the appropriate procedure for testing complex substances comprised of constituents with low water solubility (OECD, 2000).

The studies reported here as key studies for the hazard characterization of aromatic extracts employed aqueous exposure solutions prepared as WAFs (Tables 5, 6, and 7). Fish tests were run as semi-static tests with WAF solutions being renewed daily. *Daphnia magna* acute and algal growth tests were run under static conditions, while the chronic tests were semi-static with renewals occurring three times per week. Test chambers used in the daphnid acute tests were covered to mitigate loss of volatile components. Fish test solutions were not covered, and the solutions were aerated to maintain proper levels of dissolved oxygen. Analytical verification of test solutions in the acute tests employed measurements of total organic carbon (TOC). TOC measurements in all studies were inconclusive and did not provide quantitative exposure estimates. Other studies cited in the discussion below are included because they are a part of the public dataset on aromatic extracts. However, due to deficiencies in methodology, those studies were not considered to provide a reliable characterization of the hazard of these substances to aquatic organisms.

The key acute toxicity tests on aquatic organisms described in the robust summaries consist of

- CAS No. 64742-10-5: Extracts (petroleum), residual oil solvent

  Acute toxicity to rainbow trout and *Daphnia magna*, and algal growth (BP Oil Europe, 1994a,b,c)
- CAS No. 64742-04-7: Extracts (petroleum), heavy paraffinic distillate solvent

  Acute toxicity to rainbow trout and *Daphnia magna* (BP Oil Europe, 1994d,e)

Exposure concentrations for these studies were reported as the test substance loading rates (mg/L) used to prepare the WAF solutions.

Other ecotoxicological reports described in the following sections were included in this review, although they were not considered key studies (Shell Research Limited, 1984, 1994b). However, the studies have been included in other regulatory chemical safety assessments of aromatic extracts. A brief review of those findings was considered relevant to the overall discussion of available data for aromatic extracts.

6.1.1 Acute Toxicity to Aquatic Vertebrates

The results of the studies described in detail in the robust summaries for acute toxicity tests to rainbow trout are provided in the following table.

Table 5. Acute toxicity of aromatic extracts to freshwater fish, *Oncorhynchus mykiss*

Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64742-10-5; Extracts (petroleum), residual oil solvent	96-h LL50 96-h LL0 NOELR	>1000 1000 1000	BP Oil Europe (1994a)

CAS No. 64742-04-7; Extracts (petroleum), heavy paraffinic distillate solvent	96-h LL50 96-h LL0 NOELR	>1000 1000 1000	BP Oil Europe (1994d)

Based on the available toxicity data for one distillate and one residual aromatic extract, no acute toxicity of these substances to rainbow trout was found for WAF solutions at the maximum loading rate of 1000 mg/L. No behavioral effects were noted.

Shell Research Limited (1984, 1994b) reported two acute toxicity studies with rainbow trout using a light naphthenic distillate (CAS No. 64742-03-6). No acute toxicity was reported in either study at a concentration of 1000 mg/L. Although these studies duplicated the results of the WAF studies in Table 5, deficiencies in testing methodology precluded their being considered as reliable studies. Deficiencies included not employing WAF preparations, the presence of excess test substance in the exposure solutions, use of aeration of the test medium, employing an adjuvant solvent in one of two tests, and no analyses were done of the dissolved components of the test substance in the exposure solutions.

Conclusions:
Hydrocarbon constituents in aromatic extracts are high molecular weight and low water solubility compounds. These characteristics limit the bioavailability of these substances to aquatic organisms. No acute toxicity or adverse effects were seen in fish exposed to WAF solutions at the 1000 mg/L loading rate.

6.1.2 Acute Toxicity to Aquatic Invertebrates

The results of the studies described in detail in the robust summaries for acute toxicity tests to *Daphnia magna* are provided in the following table.

Table 6. Acute toxicity of aromatic extracts to freshwater invertebrates, *Daphnia magna*			
Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64742-05-8; Extracts (petroleum), light paraffinic distillate solvent	48-h EL50 NOELR	35.9 1.0	CONCAWE (2010b)
CAS No. 64742-10-5; Extracts (petroleum), residual oil solvent	48-h EL50 48-h EL0 NOELR	>1000 1000 1000	BP Oil Europe (1994b)
CAS No. 64742-04-7; Extracts (petroleum), heavy paraffinic distillate solvent	48-h EL50 48-h EL0 NOELR	>1000 1000 1000	BP Oil Europe (1994e)

Based on the available toxicity data for freshwater invertebrates, the lowest EL50 value was 35.9 mg/L determined for a light paraffinic distillate aromatic extract (CONCAWE, 2010b). In other studies a heavy paraffinic distillate and a residual oil showed no effects in acute tests at a loading rate of 1000 mg/L (BP Oil Europe, 1994b, e). No adverse behavioral or sublethal effects were noted in those tests.

Shell Research Limited (1984, 1994b) reported an acute EC50 value of 1.4 mg/L for *D. magna* based on nominal concentrations of a light naphthenic distillate (CAS No. 64742-03-6). This study was not considered a reliable indicator of the hazard to *D. magna* because the study did not employ WAF preparations. Therefore, excess test substance was present in the test chambers. Additionally, the studies used an adjuvant solvent, and no analyses of the test solutions were done to confirm the concentrations of dissolved components of the test substance.

Conclusions:
Hydrocarbon constituents in aromatic extracts are high molecular weight and low water solubility compounds. While these characteristics limit the bioavailability of these substances to aquatic organisms, some aromatic extracts may elicit acute toxicity in aquatic invertebrates. The lowest acute EL50 was 35.9 mg/L.

6.1.3 Toxicity to Aquatic Plants (Algae)

The results of one study described in detail in the robust summaries for the toxicity of aromatic extracts to *Scenedesmus subspicatus* are provided in the following table. In this study, a single exposure level was prepared by diluting 50:50 a 2000 mg/L WAF solution.

Table 7. Growth inhibition of aromatic extracts to the freshwater alga, *Scenedesmus subspicatus*			
Test Substance	Toxicity Endpoint	Endpoint Value, % WAF	Reference
CAS No. 64742-05-8; Extracts (petroleum), light paraffinic distillate solvent	72-h E_rL50 NOELR (growth rate)	18.8 0.1	CONCAWE (2010c)
CAS No. 64742-10-5; Extracts (petroleum), residual oil solvent	72-h E_bL50 NOELR (biomass) 72-h E_rL50 NOELR (growth rate)	>50% WAF 50% WAF >50% WAF 50% WAF	BP Oil Europe (1994c)

Based on the available toxicity data for two category members, one distillate and one residual aromatic extract, the lowest EL50 value was 18.8 mg/L based on testing *Pseudokirchnerella subcapitata* with a light paraffinic distillate aromatic extract (CONCAWE, 2010c). In contrast, testing *Scendesmus subspicatus* against a residual oil aromatic extract resulted in no adverse effects when the organisms were exposed to a 50% WAF solution that had been prepared at a loading rate of 2000 mg/L. This was true whether the endpoints were based on cell growth (biomass) or population growth rate. There were no adverse sublethal effects noted.

Shell Research Limited (1984, 1994b) reported an EC50 value of 3.1 mg/L for *Selenastrum capricornutum* based on nominal concentrations of a light naphthenic distillate (CAS No. 64742-03-6). This study was not considered a reliable indicator of the hazard to *S. capricornutum* because the study did not employ WAF preparations. Therefore, excess test substance was present in the test chambers. Additionally, the studies used an adjuvant solvent, and no analyses of the test solutions were done to confirm the concentrations of dissolved components of the test substance.

Conclusions:
Hydrocarbon constituents in aromatic extracts are high molecular weight and low water solubility compounds. While these characteristics limit the bioavailability of these substances to aquatic organisms, some aromatic extracts may affect the growth rate of algae. The lowest E_rL50 was 18.8 mg/L.

6.2 Aquatic Endpoints - Chronic Toxicity

Chronic aquatic toxicity was measured for *Daphnia magna* (OECD, 1984) for two aromatic extracts (BP Oil Europe, 1995a, b). These included the following category members:

- CAS No. 64742-10-5: Extracts (petroleum), residual oil solvent
- CAS No. 64742-04-7: Extracts (petroleum), heavy paraffinic distillate solvent

As for the acute studies, the chronic tests employed WAF preparations and testing methods. All tests employed the semi-static design, with fresh WAF solutions being prepared three days per week. All tests were conducted in closed vessels to prevent loss of volatile components. Test endpoints were expressed

as WAF loading rates. Analytical verification of test solutions in these chronic tests employed measurements of TOC. Petroleum hydrocarbons in the WAF preparations also were measured using gas chromatography/mass spectrometry (GC/MS). As for the acute studies, TOC measurements were highly variable and inconclusive as evidence for quantitative exposures. The GC/MS analyses showed that the concentrations of dissolved hydrocarbons in the WAF solutions were no higher than the background concentrations in the control samples.

6.2.1 Chronic Toxicity to Aquatic Invertebrates

The results of the studies described in detail in the robust summaries for the chronic toxicity tests to *Daphnia magna* are provided in the following table.

Table 8. Chronic toxicity of aromatic extracts to the freshwater invertebrate, *Daphnia magna*

Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64742-10-5; Extracts (petroleum), residual oil solvent	21-d survival EL50 NOELR 21-d reproduction EL50 NOELR	>1000 1000 >1000 1000	BP Oil Europe (1995a)
CAS No. 64742-04-7; Extracts (petroleum), heavy paraffinic distillate solvent	21-d survival EL50 NOELR 21-d reproduction EL50 NOELR	>1000 1000 >1000 1000	BP Oil Europe (1995b)

For a distillate and residual aromatic extract, no toxicity to *Daphnia magna* was evident in a 21-day chronic life cycle toxicity test. WAF exposures at a loading rate of 1000 mg/L did not produce any adverse effect on survival or reproduction. Offspring appeared healthy and showed no adverse effects.

Due to the variable composition of substances in this category, the two chronic toxicity studies cited may not fully define the potential for chronic aquatic toxicity for all substances in this category. However, due to the high viscosity of these materials, which are semi-solid to solid at ambient temperatures, the potential for components such as PAHs to contribute to chronic effects in aquatic organisms would be expected to be extremely limited. This is due not only to the physical state of the materials but also to the high octanol/water partition coefficients of these components, which limits the partitioning into the aqueous phase. For these reasons, it is unlikely that water accommodated fractions of aromatic extracts prepared at the chronic exposure limit concentration of 1 mg/L would elicit effects of discernible significance in standard chronic toxicity assays. This is supported by the lack of chronic toxicity at 1000 mg/L found in the studies described in the robust summaries (BP Oil Europe, 1995a, b). Therefore, although it is not possible to accurately predict the extent of chronic aquatic effects of the water-equilibrated components for all aromatic extracts due to the variable composition, evaluation of chronic toxicity for both distillate and residual aromatic extracts did not identify any effects in aquatic organisms exposed to the water soluble components of those extracts.

Shell Research Limited (1987) reported a 19-day chronic NOEC for survival and reproduction of 0.05 mg/L and 0.02 mg/L, respectively, for *D. magna* based on nominal concentrations of a light naphthenic distillate (CAS No. 64742-03-6). This study was not considered a reliable indicator of the chronic hazard to *D. magna* because the study did not employ WAF preparations. Excess test substance was present in the test chambers at concentrations >0.05 mg/L. Additionally, the studies used an adjuvant solvent, and no analysis of the test solutions was done to confirm the concentrations of dissolved components of the test substance.

Conclusions:
Hydrocarbon constituents in aromatic extracts are high molecular weight (predominantly C15 to C50) and low water solubility compounds (<1ppm). These characteristics limit the bioavailability of these substances to aquatic organisms. No chronic toxicity or adverse behavioral effects were seen in freshwater invertebrates (daphnids) exposed to WAF solutions at the 1000 mg/L loading rate.

6.3. Assessment Summary for Environmental Effects

In summary, the constituents comprising aromatic extracts are high molecular weight materials that exist in a solid to semi-solid state at environmental temperatures. Their extremely low water solubilities limit their bioavailability. Despite these characteristics, some aromatic extracts may cause toxicity in freshwater invertebrates and freshwater algae. The lowest invertebrate EL50 was 35.9 mg/L and the lowest algal E_rL50 was 18.8 mg/L (CONCAWE, 2010a,b). In other tests of invertebrates, there were no effects at 1000 mg/L (BP Oil Europe, 1994b,e), and no effects on algae were observed when tested in 50% dilutions of 2000 mg/L WAFs (BP Oil Europe, 1994c). No studies in fish showed any adverse effects when aromatic extracts were tested in WAFs at 1000 mg/L (BP Oil Europe, 1994a, d).

Aromatic extracts were tested for chronic toxicity to aquatic invertebrates (*Daphnia magna*). In 21-day exposures to WAF preparations of a distillate aromatic extract and a residual aromatic extract, neither survival nor reproduction was impaired in the adult generation. Offspring produced during the test also appeared healthy with no adverse effects noted. For chronic exposures of DAE and RAE to aquatic invertebrates, the no-observed-effect level was 1000 mg/L WAF (BP Oil Europe 1995a, b).

7. HUMAN HEALTH ENDPOINTS

The DAEs and RAEs differ in toxicological properties. Accordingly, these substances are summarized separately in this section on health effects. The observed differences led to the exclusion of RAEs from use in models that were developed for prediction of effects on selected endpoints in subchronic and developmental toxicity tests based on compositional information. The rationale for those models and the exclusion of RAEs is discussed in the following paragraphs.

It has been known that biologically significant effects of several types of 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). Samples with high PAC content would be expected to have high mutagenicity indices in the optimized Ames test, produce tumors in skin-painting assays, and cause systemic effects in repeated-dose and developmental toxicity studies. Samples with low PAC content would not produce such effects. This relation between PACs and toxicity was qualitative and was not quantitatively predictive for individual samples.

More recently, statistically based models have become available to predict quantitatively the effect of individual samples of high-boiling petroleum substances on sensitive endpoints in developmental toxicity and subchronic toxicity studies performed via the dermal route. These statistical models, developed by the Petroleum High Production Volume Testing Group (HPVTG), quantitatively predict effects by individual samples on selected sensitive endpoints based on the profile of 1-7 ring PACs in each sample (Simpson, et al, 2008; API, 2008; TERA, 2008). The models are based on a number of toxicity studies on petroleum substances for which there are also analyses of PAC content using a "PAC-2" method. The PAC-2 analyses provided the weight percent of each ring class that served as a basis for the models (the percent ARC in Table 3). The endpoints used in the models were selected by an extensive analysis to determine the most sensitive endpoints among studies of both developmental toxicity and repeated-dose toxicity.

Although the numbers of samples used to develop the PAC models differed for repeated-dose and developmental toxicity studies, similar types of samples were used. These included crude oils, gas oils, heavy fuel oils, a heavy paraffinic DAE, a lubricating oil basestock, and a waste stream. The PAC models are expected to encompass those categories of refinery substances that are relatively high-boiling and contain PACs, such as those used to develop the models. The models may not be applicable to those refinery substances that have a significantly higher boiling range than the samples used to develop the models and correspondingly different physical properties. In general terms, such excluded substances include asphalts and residual aromatic extracts due to their typical T50 boiling point $\geq$ 538°C (1000°F) which makes them solids or semi-solids at ambient temperature.

Additional specific reasons for exclusion of the RAEs from the PAC models include the following.

1) The application of those models to RAEs has not been supported by empirical data.

2) Due to their high viscosity, RAEs tend to differ physically from the lighter samples used to develop the PAC models. The movement of PACs from the highly viscous RAEs into the skin may therefore differ from that of the less viscous refinery substances. This theoretical consideration necessitates caution in the application of the PAC models to RAEs.

3) The mutagenicity index (MI) of the optimized Ames test is widely used to predict results of skin-painting assays in mice for PAC-containing refinery substances. That prediction is based on correlations from a large number of studies on a variety of refinery substances. However, the correlation differs for RAEs such that a different cut-off is appropriate for RAEs compared to other,

less viscous refinery substances. RAEs are unique in this regard, again indicating the need for caution in the application of the PAC models to them.

Given this background and the uncertainty of application of the PAC models to RAEs, the exclusion of RAEs from the PAC models seems justified.

The current model domains encompass the majority, but not all, of the individual untested DAEs shown in Table 3. Therefore, for the most part, the potential toxicological effects of these substances can be predicted. Additional details are provided in subsequent sections. Appendix 1 contains a summary describing the association between toxicity and PAC content and also a brief description of the statistical models. More complete descriptions of the models are in other referenced documents (API, 2008; TERA, 2008).

As a result of modeling and statistical evaluation, four types of quantitative values are used in this document.

1) Data on tested samples from appropriate studies, such as NOAELs and LOAELs
2) BMD_{10} calculated from data on tested samples in appropriate studies
3) Predicted dose-responses (PDR_{10}s), i.e., the doses predicted to cause a difference of 10% from control value based on models using the PAC profile of untested samples and not necessarily indicative of an adverse effect
4) Read-across from tested or modeled samples to untested samples

7.1. Human Health Effects of Distillate Aromatic Extracts

7.1.1 Acute Toxicity

An oral LD_{50} study was conducted with an undiluted light paraffinic DAE (sample API 83-16, CAS No. 64742-05-8). Sprague-Dawley rats (5/sex) were given a single oral dose (5 g/kg based on fasted body weight) and observed for 14 days. There were no mortalities and animals gained weight during the study. Clinical signs included hypoactivity in all animals during the first 24 hours after dosing, ataxia in 2 males on day 2, and other incidental findings. No visible lesions were noted at necropsy. The oral LD_{50} was >5000 mg/kg (API, 1986a). Supporting information comes from an oral LD_{50} reported as >5000 mg/kg in male and female Wistar rats for a heavy paraffinic distillate aromatic extract (FDRL, 1974a).

A dermal LD_{50} study was conducted with an undiluted light paraffinic DAE (sample API 83-16, CAS No. 64742-05-8). New Zealand white rabbits (2/sex in each group) were given a single dose of either 2000 or 3000 mg/kg on the shorn dorsal skin. Groups with intact and with abraded skin were included, making a total of 4 groups of each sex. An occlusive dressing was then applied and later removed after 24 hours. The skin was wiped at that time. Body weights, dermal irritation, clinical signs, and mortality were recorded over 14 days. Diarrhea, dyspnea, hypoactivity, prostration, emaciation, and soft stool were observed in a few animals at 2000 mg/kg, but not with 3000 mg/kg. Dermal irritation ranged from slight to marked for atonia, desquamation, coriaceousness and fissuring. Other signs of dermal irritation included blanching, subcutaneous hemorrhaging, scab formation and eschar. However, no systemic abnormalities were observed at necropsy, and the dermal LD_{50} was >3000 mg/kg (API, 1986a). Supporting information comes from a dermal LD_{50} reported as >2000 mg/kg in rabbits for a heavy paraffinic DAE, CAS No. 64742-04-7 (FDRL, 1974b).

A light paraffinic DAE (sample API 83-16, CAS No. 64742-05-8) and a heavy paraffinic DAE (CAS No. 64742-04-7) produced skin irritation and transient eye irritation (API, 1986a; FDRL, 1974c and d). The same sample of light paraffinic DAE did not produce sensitization in a Buehler guinea pig skin sensitization assay (API, 1986a).

Conclusions:
Distillate aromatic extracts have low acute systemic toxicity. Samples of both light and heavy paraffinic DAE had oral LD_{50}s >5000 mg/kg and dermal LD_{50}s >2000 mg/kg. Based on a more limited data set, DAEs may also be skin irritants but do not appear to be irritating to the eye or to cause allergic contact dermatitis.

7.1.2. Repeated Dose Toxicity

7.1.2.1. Tested samples and NOAELs

In a 28-day study, groups of New Zealand white rabbits (5/sex) received dermal applications of 0, 250, 500 and 1000 mg/kg of neat light paraffinic DAE to shaved backs once/day, 3 times/week, for a total of 12 applications. The sample was API 83-16 (CAS No. 64742-05-8). The site was occluded for 6 hours after each dosing and then wiped. Endpoints recorded during the study included body weights, food consumption, and clinical signs. Additional endpoints at necropsy included hematology, clinical chemistry, weights of multiple organs, gross examination, and microscopic examination of selected tissues. The only observed treatment-related effects were increased relative liver weights among females at all dose levels, dose-related skin irritation in both sexes, and slight to severe proliferative changes observed

microscopically in skin of animals in the high dose group (API, 1986b). Based on the descriptions provided by the study director, the NOAEL for systemic effects was judged by the Petroleum HPV Testing Group to be 1000 mg/kg/day, the highest dose tested.

A light paraffinic DAE (CRU 20906, CAS No. 64742-05-8) in acetone was administered via dermal application (non-occluded) to the dorsoscapular area (covering approximately 10% of the body surface area) to 3 groups (Groups 3-5) of Crl:CD(SD) rats for a minimum of 90 days (WIL Research, 2012a). The test substance was applied for approximately 6 hours per day, 5 days per week, followed by a 2-day nondosing period. Dosage levels were 5, 50, and 150 mg/kg/day for Groups 3, 4, and 5, respectively. A concurrent vehicle control group (Group 2) received the vehicle on a comparable regimen. The dose volume was 1.5 mL/kg for Groups 2-5. A concurrent sham control group (Group 1) was subjected to the same procedures (i.e., shaving, collaring, sham dosing with glass rod, and removal of residual test substance) as the test substance-treated groups; however, no vehicle or test substance was applied to these animals. The scheduled necropsy occurred on study day 89 or 90. One 150 mg/kg/day group male was found dead on study day 40. All other animals survived to the scheduled necropsy. There were no test substance-related clinical observations, dermal observations, or effects on food consumption. There were no test substance-related ophthalmic, macroscopic, or microscopic findings. Test substance-related lower cumulative body weight gains were noted in the 150 mg/kg/day group females and resulted in slightly lower (8.1%) body weights when compared to the vehicle control group at study week 12′. There was no test substance-related dermal irritation. Test substance-related lower red blood cell (RBC) counts, hemoglobin, hematocrit, activated partial thromboplastin time (APTT), white blood cell (WBC) counts, lymphocyte counts, eosinophil counts, and platelet counts and higher red cell distribution widths (RDW) and hemoglobin distribution widths (HDW) were noted in the 50 and/or 150 mg/kg/day group. In addition, reticulocyte counts (absolute counts and percentages) were higher in the 150 mg/kg/day group males and the mean reticulocyte percentage was higher in the 150 mg/kg/day group females. Lower eosinophil counts were also noted in the 5 mg/kg/day group females. Test substance-related higher cholesterol, sorbitol dehydrogenase (SDH), and blood urea nitrogen (BUN), and lower alanine aminotransferase (ALT) and triglycerides values were noted in the 50 and/or 150 mg/kg/day groups. Test substance-related absolute and/or relative organ weight changes were noted for the spleen, liver, thymus, pituitary, heart, and thyroid/parathyroids in the 50 and/or 150 mg/kg/day groups. A no-observed-effect level (NOEL) could not be established due to the lower eosinophil counts noted in the 5, 50, and 150 mg/kg/day groups; however, the values were within the laboratories historical control reference range.

A heavy paraffinic DAE (318 Isthmus furfural extract, CRU 86187, CAS No. 64742-04-7) was administered dermally without occlusion to the shorn backs of Sprague-Dawley rats (10/sex), 5 days/week for 13 weeks at doses of 0, 30, 125, 500, and 1250 mg/kg/day (Mobil, 1990a; Feuston et al., 1996; Feuston et al., 1994). Rats wore Elizabethan collars to retard oral ingestion. The DAE contained ~78% aromatic compounds, of which approximately 23% was 3-5 ring PAC (the aromatic extract constituents which are believed to be the most biologically active). Evidence of toxicity included mortality, decreased body weight, aberrant serum chemistry and hematology parameters, altered organ weight, and histopathological changes in several organs. All comparisons were made relative to controls. Four animals died spontaneously at the high dose and two at 500 mg/kg/day before termination. All rats receiving 1250 mg/kg as well as all 10 males and 3 of 10 females receiving 500 mg/kg/day were terminated prior to schedule. Pallor and decreased body temperature were noted in these groups. In addition, gain in body weight was significantly decreased in male rats exposed to $\geq$500 mg/kg/day and female rats exposed to $\geq$30 mg/kg/day. Red blood cell counts, hemoglobin, hematocrit, and platelet counts were significantly decreased in both sexes at 125 mg/kg/day with trends for decreases at 30 mg/kg. White blood cell counts were significantly increased in females at 30 and 125 mg/kg, but were significantly decreased at 500 mg/kg. Several serum chemistry values were affected by treatment at $\geq$125 mg/kg/day. Parameters in urinalysis were not affected. Absolute liver weight increased and thymus weight decreased at $\geq$125 mg/kg/day in both sexes and thymus weight was decreased in females at 30

mg/kg. Among the histological changes, slight to moderate fibrosis and decreased cellularity of bone marrow was observed at ≥125 mg/kg/day. In liver, dilatation of centrilobular sinusoids, single cell necrosis in a few animals and increased hepatic vacuolation occurred at ≥125 mg/kg/day. Thymic atrophy occurred at ≥125 mg/kg/day. Slight to moderate diffuse cortical vacuolation of adrenals as well as cortical necrosis at a lower incidence and lesser severity were noted in males at all dose levels (including 30 mg/kg/day). Skin irritation at the site of application was also noted at all doses. Small focal hemorrhages were seen in several organs including the brain, spinal cord, heart, lung, testes and bone marrow. Testes, epididymides, prostates, and seminal vesicles were characterized as "small" in males at 500 and 1250 mg/kg/day, but no histopathological changes were noted at the highest non-fatal dose (125 mg/kg/day). The left epididymides from 5 rats exposed to test material at 125 mg/kg/day and 5 controls were examined separately for number and morphology of sperm. The measured sperm parameters were not affected by dermal dosing. The NOAEL for systemic effects with dermal dosing was judged by the study director to be <30 mg/kg/day.

As part of the study just described on a heavy paraffinic DAE (318 Isthmus furfural extract, CAS No. 64742-04-7), two additional groups of 10 male rats received oral doses of 125 and 500 mg/kg/day for 13 weeks (Mobil, 1990a). Clinical signs were similar to those observed in animals exposed dermally. Two animals given 500 mg/kg/day died and two were sacrificed *in extremis*, making a total of 4 pre-termination deaths among the 10 animals in this group. Weight gain was reduced with 500 mg/kg/day; mean final body weight was 80% of control weight. The numbers of red blood cells were decreased at both doses and platelet counts were decreased at 500 mg/kg/day. With the exception of an 80% increase in sorbitol dehydrogenase activity with 500 mg/kg/day, serum chemistry values were unaffected. Findings at necropsy for animals exposed to the test substance were similar to those for the animals exposed by the dermal route. In addition, focal areas of red discoloration and/or generalized reddening were observed in the brain, spinal cord, stomach and testes of many of the rats at both dose levels. Increased liver weight and decreased weights of thymus and prostate were noted at 125 mg/kg/day. Additional organs were affected at 500 mg/kg/day, including seminal vesicles and epididymides.

Histopathological changes were similar to those seen in the rats exposed dermally, but without the skin lesions. The left epididymides from 5 rats exposed to test material at 500 mg/kg/day and 5 controls were examined separately for number and morphology of sperm. A slight increase in the number of sperm with abnormal heads was noted with males dosed at 500 mg/kg/day (mean of 16.2% versus 9.4% in controls). There relevance of this change is uncertain given the other significant effects at this dose and the fact that 4 of 10 males died or were sacrificed *in extremis*. Overall the study director concluded that the test substance appeared to be more toxic with dermal administration compared to oral administration based primarily on mortality and clinical signs. Dermal administration appeared to have a greater effect on liver weights, whereas oral exposure seemed to have a greater effect on weights of the secondary sex glands.

No repeated dose toxicity studies have been reported for either light or heavy naphthenic DAE.

7.1.2.2. BMD_{10}s calculated from data on tested samples

The BMD_{10} is a value calculated using methods described by Crump (1984) and is derived from data from actual studies. Actual data were available from the 90-day repeated-dose dermal study on heavy paraffinic DAE (CRU 86187) and a light paraffinic DAE (CRU20906). Both predicted PDR_{10} and determined BMD_{10} values are shown in Table 9 for four repeated-dose endpoints. The BMD_{10s} shown in Table 9 are the lowest of the BMD_{10}s for the individual endpoints.

7.1.2.3. PDR_{10}s modeled using the PAC profile of untested samples

It is possible to characterize the repeated-dose toxicity of untested samples using PDR_{10}s, modeled predictions based on PAC profiles (See Appendix 1). The PDR_{10} is a value predicted to cause a change of 10% from control value. The calculated PDR_{10}s are shown in Table 9. Note that a PDR_{10} is not necessarily an indicator of an adverse effect. As well as the PDR_{10} values that are given for each sensitive endpoint, the lowest of the values for each sample has been identified and is referred to as the sample PDR_{10}. PDR_{10}s were calculated for both light and heavy paraffinic DAEs. No PAC profiles were available to calculate PDR_{10}s for light naphthenic DAE. Valid PDR_{10}s could not be calculated for the one sample of heavy naphthenic DAE because the PAC profile was outside the domains of the current models. (See Appendix 1.)

Note that the available BMD_{10s} were similar to the lowest PDR_{10} for that sample (13&15 versus 42&58 mg/kg/day, respectively). This general agreement between BMD_{10} and PDR_{10}s lends additional support to the use of both approaches.

Apparent differences between control and treated groups can sometimes occur with samples that have very low levels of PACs. In particular, slightly lower body weights and elevated relative liver weights have been observed in groups treated with lubricating oil basestocks, as discussed in API's Category Analysis and Hazard Characterization Document on Lubricating Oil Basestocks. Therefore the interpretation of results from the PAC models should be done with the knowledge that such variation in responses is possible with dermal application of high doses of substances that contain low levels of PACs.

7.1.2.4. Read-across from tested or modeled samples to untested samples

Blank cells in Table 9 indicate samples for which there was no information on measured toxicological endpoints and PAC profiles were outside the model domains, making predictions of PDR10s unreliable. The toxicological potential of these samples can be judged via read-across from the actual or modeled data.

7.1.2.5. Conclusions for Repeated-Dose Toxicity

Several dose-related effects involving multiple organs were reported in 13-week dermal studies in rats with light and heavy paraffinic DAEs, resulting in a NOAEL less than 5 and 30 mg/kg/day respectively, (the lowest doses tested). As the NOAEL was not obtained experimentally, BMD_{10s} of approximately 13 and 21 mg/kg/day were calculated for light and heavy DAE respectively.

Based on the available information and the lowest PDR_{10} among the four endpoints modeled for each sample, the following estimates of toxicity may be made for the four different DAE types as follows:

Light paraffinic DAE CASRN 64742-05-8	PDR_{10}s ranged from 41 to 72 mg/kg/day based on the lowest PDR_{10} for each of the four modeled endpoints. The lowest BMD_{10} for the tested sample was 13 mg/kg/day and the NOAEL was < 5 mg/kg/day.
Heavy paraffinic DAE CASRN 64742-04-7	PDR_{10}s ranged from 25 to 78 mg/kg/day based on the lowest PDR_{10} for each of the four modeled endpoints. The lowest BMD_{10} for the tested sample was 21 mg/kg/day and the NOAEL was < 30 mg/kg/day.

Table 9. Repeated-dose PDR_{10}s and BMD_{10}s (mg/kg/day) for DAEs by endpoint, sex, and test substance

Sample	Type of Sample and CAS No.	Relative Liver Weight PDR_{10}		Absolute Thymus Weight PDR_{10}		Platelet Count PDR_{10}		Hemoglobin Concentration PDR_{10}		Sample PDR10 (lowest value)	Basis for Sample PDR_{10}	Study BMD_{10} (lowest value)[1]
		Male	Female	Male	Female	Male	Female	Male	Female			
Site 7, Sample 23 (CRU 20906)	LP-DAE[2]	79	78	71	63	42	43	80	80	42	Platelet count	
		36[6]	360[6]	24[6]	13[6]	31[6]	14[6]	77[6]	188[6]			13
CRU 86187	HP-DAE[3]	58	58	67	59	72	73	173	172	58	Relative liver weight	
		24[6]	27[6]	29[6]	21[6]	27[6]	66[6]	102[6]	170[6]			21
Site 3, Sample 13 (CRU 100709)	LP-DAE	72	72					793	792	72[7]	Relative liver weight	
Site 4, Sample 3 (CRU 100711)	LP-DAE	73	73	69	61	103	105	183	182	61	Thymus weight	
CRU 86141	HP-DAE	64	63	93	82	50	50	67	66	50	Platelet count	
CRU 86303	HP-DAE	48	47	32	28	593	603			28[7]	Thymus weight	
CRU 89130	HP-DAE	93	92	37	33	25	26	105	104	25	Platelet count	
Site 2, Sample 9 (100705)	HP-DAE											
Site 3, Sample 10 (CRU 100706)	HP-DAE	101	100	47	41	45	46	228	227	41	Thymus weight	
Site 3, Sample 12 (100708)	HP-DAE	76	75	70	62	1221	1242	>2000	>2000	62	Thymus weight	
Site 3, Sample 14 (100710)	HP-DAE											
Site 4, Sample 4 (100712)	HP-DAE	68	68	60	53	608	619			53[7]	Thymus weight	
Site 5, Sample 1 (100713)	HP-DAE	79	78	222	196			735	734	78[7]	Relative liver weight	
950219	HP-DAE	78	78	87	77	636	647	211	211	77	Thymus weight	
Site 1, Sample 5 (100701)	HN-DAE[5]											

1) A BMD_{10} could be calculated only if data from repeated-dose study were available.
2) LP-DAE is light paraffinic distillate aromatic extract (CAS 64742-05-8).
3) HP-DAE is heavy paraffinic distillate aromatic extract (CAS 64742-04-7).
5) HN-DAE is heavy naphthenic distillate aromatic extract (CAS 64742-11-6).
6) Values are BMD_{10} for each endpoint and sex.
7) A value for PRD_{10} was not available for all endpoints.

Light naphthenic DAE CASRN 64742-03-6	Empirical (NOAEL), predicted (PDR_{10}) and calculated (BMD_{10}) toxicity values were not available for this CASRN.
Heavy naphthenic DAE CASRN 64742-11-6	Empirical (NOAEL), predicted (PDR_{10}) and calculated (BMD_{10}) toxicity values were not available for this CASRN.

No repeated dose toxicity studies have been reported for either light or heavy naphthenic DAE. Therefore, the available experimental data are limited for the characterization of the group of all four DAE CASRNs. However, as stated previously, the basic differences among the CAS descriptions of DAEs are carbon number and hydrocarbon type (paraffinic vs. naphthenic). These distinctions are a result of historical refinery naming practices and the predominant nature of the crude oil or lubricating basestock run through the process unit. There are not large differences in straight and branched chain saturated hydrocarbons (paraffinic hydrocarbons) or polar compounds among the four DAEs. In addition, the PACs in individual samples of DAEs are considered responsible for mutagenic, carcinogenic, developmental, and subchronic effects. Therefore the distinctions in nomenclature do not indicate distinctions in toxicological properties. Given these facts, it is expected that existing data, data from ongoing testing, and models can be used to indicate potential toxicity for untested samples of DAEs.

7.1.3. Genetic Toxicity *In Vitro* and *In Vivo*

7.1.3.1. *In Vitro* (Mutagenicity)

DAEs are expected to be mutagenic unless they are further processed, yielding treated DAEs with lower PACs. In assays of mutagenicity *in vitro*, samples of DAEs have been mutagenic in the optimized Ames test in bacteria. The mutagenicity index (MI) for 5 samples of paraffinic DAEs ranged from 4.6 to 17 (Roy et al., 1988; Mobil, 1985a,b,c,d,e). Samples were not differentiated into light or heavy paraffinic DAEs. A number of distillate streams derived from crude oil have been shown to be mutagenic in this assay, with a strong correlation between mutagenicity and 3-7 ring PAC content (Blackburn, et al., 1986, Roy, et al., 1988; Mackerer et al, 2003). Note that the results of the optimized Ames assay can be predicted for DAEs from the PAC profile (Appendix 2; McKee et al, 2011). This prediction is pass/fail; a quantitative MI is not estimated.

In an assay of gene mutations *in vitro* in mammalian cells, a mouse lymphoma assay was performed for light paraffinic DAE (sample API 83-16, CAS No. 64742-05-8). The DAE, dissolved in ethanol, was found to be mutagenic with and without activation, and a dose-response was observed with activation (API, 1986c).

7.1.3.2. *In Vivo* (Chromosomal Aberrations)

A sample of light paraffinic DAE (CRU 20906, CAS No. 64742-05-8) in acetone was administered dermally once daily, 5 days a week for a minimum of 90 days to male and female Crl:CD(SD) rats. The positive control, cyclophosphamide, was administered orally via gavage as a single dose. Bone marrow was harvested approximately 24 hours after the final dose. CRU 20906 produced a statistically significant ($p \leq 0.05$) increase in the mean percentage of micronucleated polychromatic erythrocytes (%MN-PCEs) at the highest dose level, 150 mg/kg/day, however, only 2 of 5 males were elevated over control. A statistically significant increase in the mean %MN-PCEs was also observed at the middle dose level, 50 mg/kg/day, in female rats. However, a dose response was not observed as the highest dose level, 150 mg/kg/day, did not exhibit a statistically significant increase in %MNPCEs. Thus, the results in female rats were equivocal. CRU 20906 did not produce a statistically significant increase in the mean percentage of micronucleated normochromatic erythrocytes (%MNNCEs) compared to the vehicle

control. In addition, CRU 20906 did not produce a statistically significant change in the mean ratio of polychromatic to total erythrocytes (PCE:TE ratio) relative to the vehicle control. The group mean values for both %MN-PCEs and PCE:TE ratios for the vehicle and positive control groups were within the values previously obtained by the laboratory. CRU 20906 met the criteria for a positive response in male rats and an equivocal response in female rats for the induction of bone marrow micronuclei in polychromatic erythrocytes (WIL Research, 2012a).

In vivo micronucleus evaluations were performed on bone marrow harvested at termination of the 13-week oral and dermal assay of a heavy paraffinic DAE (CAS 64742-04-7) described in section 7.1.2. No treatment-related increase in micronuclei was observed (Mobil, 1987). Micronucleus tests with other petroleum streams that contain higher amounts of PACs have also been negative, leading to the conclusion that PAC-containing petroleum substances are unlikely to produce chromosomal effects when tested in SIDS-level assays under *in vivo* conditions (McKee et al, 2010).

7.1.3.3. Conclusions for Genetic Toxicity

The mutagenic potency in the optimized Ames test described above has been found to be correlated with the concentration of the PAC-enriched fraction obtained by extraction with DMSO. Because the composition of DAEs can vary with crude oil and refining conditions, the levels of 1-7 ring PACs can differ among samples. DAEs are expected to be mutagenic unless they are further processed, yielding treated DAEs with lower PACs. Results for one light paraffinic DAE were positive in a mouse lymphoma assay. Therefore, sufficient data have been provided to provide a screening level characterization of the potential for *in vitro* mutagenicity of DAEs.

In vivo micronucleus tests on a heavy paraffinic DAE and a range of other petroleum substances have been negative. The exception to this general trend was a recent micronucleus test in which positive responses occurred in 2 of 5 males at the highest tested dose of a light paraffinic DAE WIL Research, 2012a). Given the limited number of affected animals in this test, the weight of evidence from tests on several petroleum streams, and read-across from the heavy paraffinic DAE, it is concluded that most DAEs are unlikely to produce chromosomal effects under *in vivo* conditions.

7.1.4 Developmental Toxicity

7.1.4.1. Tested samples and NOAELs

A light paraffinic DAE (CRU 20906, CAS no. 64742-05-8), in the vehicle (acetone) was administered by dermal application to the dorsal scapular area (approximately 10% of total body surface area) of 4 groups (Groups 3-6) of 25 bred female Crl:CD(SD) rats once daily from gestation days 0 through 19; animals were exposed to the test substance for 6 hours each day. Exposure levels were 5, 25, 150, and 450 mg/kg/day administered at a dosage volume of 1.5 mL/kg. A concurrent vehicle control group (Group 2) composed of 25 bred females received the vehicle on a comparable regimen. A concurrent sham control group (Group 1) was subjected to the same procedures (*i.e.* shaving, collaring, sham dosing with a glass rod, and removal of residual test substance) as Groups 2-6; however, no vehicle or test substance was applied to these animals. The females were approximately 13 weeks of age at the initiation of test substance exposure. Test substance-related moribundity was noted at 150 and 450 mg/kg/day. In the 150 mg/kg/day group, 1 female was euthanized *in extremis* on gestation day 15. In the 450 mg/kg/day group, 3 females were euthanized *in extremis* on gestation days 14, 16, and 18. Maternal toxicity was evidenced in the 25, 150, and 450 mg/kg/day groups by adverse clinical and/or macroscopic findings and a higher incidence of dermal observations at these exposure levels. Moribundity was noted at 150 and 450 mg/kg/day as a result of lower mean food consumption with corresponding mean body weight losses

and/or lower mean body weight gains noted in these groups generally throughout the treatment period. Mean thymus weights (absolute and relative to brain) were noted in the 25, 150, and 450 mg/kg/day groups. No evidence of maternal toxicity was noted at 5 mg/kg/day. Developmental effects were noted in the 150 and 450 mg/kg/day groups as evidenced by increased mean litter proportions of postimplantation loss (primarily early resorptions) with a corresponding decrease in the mean numbers and litter proportions of viable fetuses. In addition, lower mean male, female, and combined fetal weights were noted in the 25 and 150 mg/kg/day groups. Lower fetal weights were also noted for the single surviving litter in the 450 mg/kg/day group. Test substance-related fetal developmental variations (sternebra(e) nos. 5 and/or 6 unossified, reduced ossification of the skull, reduced ossification of the vertebral arches, sternebra(e) nos. 1, 2, 3, and/or 4 unossified, and cervical centrum no.1 ossified) were noted in the 150 mg/kg/day group and for surviving fetuses in the 450 mg/kg/day group and were indicators of developmental delay and correlated to lower fetal weights. Intrauterine growth and survival at 5 mg/kg/day and skeletal fetal morphology at 5 and 25 mg/kg/day were unaffected by test substance administration. Based on these results, an exposure level of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity and embryo/fetal development when extract, light paraffinic distillate solvent was administered by dermal application to bred Crl:CD(SD) rats (WIL Research, 2012b).

Undiluted heavy paraffinic DAE (318 Isthmus furfural extract, CRU 86187, CAS No. 64742-04-7) was applied dermally in a prenatal developmental toxicity study in Sprague Dawley rats (Mobil, 1990c). This sample is identified as 86187 in Table 2. Doses were 0, 8, 30, and 125 mg/kg/day on gestation days (GD) 0-19, 500 mg/kg/day on GD 0-16, and 1,000 mg/kg/day on GD 10-12. The application site was not occluded and the site was not wiped after dosing. Rats wore Elizabethan collars to retard oral ingestion. Endpoints of maternal toxicity included body weight, food consumption, hematology, serum chemistry, liver weight, and thymus weight. Uterine weight, implantations, resorptions, and number of corpora lutea were also recorded. Fetal endpoints were evaluated, including fetal body weight and development (external, skeletal and visceral exam) (Mobil, 1990c; Feuston et al., 1996).

With doses given on GD 0-16, statistically significant effects occurred at doses of 125 mg DAE/kg/day or greater for both maternal and developmental endpoints. Maternal effects at 125 and 500 mg/kg/day included decreased body weight, decreased body weight gain, decreased food consumption, reduced gravid uterine weight, decreased thymus weight (absolute and relative), increased liver weight (relative), increased white blood cell count, decreased platelet count (500 mg/kg/day only), and dose-related changes in serum chemistry.

The developmental effects at 125 and 500 mg/kg/day included reduced number of dams with viable fetuses, increased number of dams with resorptions, reduced litter size of viable fetuses, increased percent resorptions, and decreased fetal body weight. On gross examination, one fetus in the 125 mg/kg/day group appeared edematous. No treatment-related increases in skeletal or visceral abnormalities were noted.

At 30 mg/kg/day, there was a 2-fold increase in the percent resorptions which was not statistically significant, but was considered by the investigators to be of possible biological significance. Marginal (non-significant) changes in maternal body weight, thymus weight, liver weight, and gravid uterine weight (which may have been a reflection of the increase in percent resorptions) also occurred at this dose. No significant adverse maternal or fetal effects were seen at 8 mg/kg.

The dose of 1000 mg/kg/day was given only on GD 10-12. Dams in this group had lower food consumption and gained less weight than controls during and following the period of dosing, but their overall net gain in body weight was not lower than that of control. Absolute and relative weights were lower for thymus and higher for liver. The authors reported 25% decrease in fetal body weight, and a

statistically significant increase in the incidences of external and skeletal anomalies compared to controls. These anomalies were not observed at lower doses. It is important to note that the increased incidence of external anomalies was statistically significant among fetuses, but not among litters. Also, the skeletal anomalies reported at an increased incidence were primarily anomalies of incomplete ossification, which are often interpreted as evidence of delayed development.

Overall, significant fetal effects occurred only at doses that also caused maternal effects. The study director concluded that maternal toxicity was seen with doses as low as 30 mg/kg and that a decrease in viable fetuses was seen at 125 mg/kg/day (statistically significant) and at 30 mg/kg/day (biologically significant). The Petroleum HPV Testing Group concluded from these statements that (1) the LOAEL for statistically significant effects on fetuses was 125 mg/kg/day, with a corresponding NOAEL of 30 mg/kg/day, and (2) the LOAEL for possibly biologically significant effects on fetuses was 30 mg/kg/day, with a corresponding NOAEL of 8 mg/kg/day.

In order to further explore the suspected teratogenic potential of a heavy paraffinic DAE and other refinery streams, Feuston and Mackerer (1996) conducted a developmental toxicity study in which the test materials were given by gavage on a single day of gestation. This study design was chosen in order to limit the embryolethal effects of these compounds and to maximize the chances of detecting any teratogenic potential. While the oral route of exposure is not considered a relevant route of exposure for human hazard assessment of petroleum streams, the results of this study are included here for the sake of completeness. Feuston and Mackerer (1996) administered a single oral dose (2000 mg/kg) of heavy paraffinic DAE (CAS 64742-04-7), clarified slurry oil (CSO), or syntower bottoms (STB) on gestation day (GD) 11, 12, 13, 14, or 15 (DAE and STB only on GD 15). Additional rats were given oral doses of 125, 500, or 2000 mg/kg of DAE, CSO, or STB on GD 12. The controls were administered tap water by gavage on GD 11-15 (DAE and STB) or GD 11-14 (CSO). The samples were selected because they were the most systemically and developmentally toxic petroleum substances among those tested by the dermal route. Dams were sacrificed and necropsied on GD 20 and fetuses were examined. For each refinery stream tested, evidence of maternal toxicity (i.e., decreased body weight; decreased thymus weight) was observed at doses of 500 mg/kg and greater. Statistically significant increases in resorptions were observed at 2000 mg/kg of CSO or STB, but not DAE. According to the study authors, a common pattern of fetal malformations (including cleft palate, diaphragmatic hernia, and paw and tail defects) was observed for all petroleum substances tested. The increases in fetal malformations were statistically significant at doses of 500 mg/kg or greater for CSO and STB and 2000 mg/kg for DAE. The investigators noted that the ability to produce adverse effects on development was greatest for CSO (containing high levels of PACs) and least for DAE. Developmental toxicity was not observed in the absence of maternal toxicity with any of these petroleum streams. Although the oral route of administration has little relevance to human occupational exposure, DAE, CSO, and STB were shown to have teratogenic potential when a large dose was given by gavage to pregnant rats on a single day during the critical period of gestation.

7.1.4.2. BMD_{10}s calculated from data on tested samples

The lowest BMD_{10} calculated from the study on light paraffinic DAE (CRU 2006, CAS No. 64742-05-8) was 42 mg/kg/day (for living fetuses per litter). There is no PDR_{10} value for this endpoint because the model prediction is an extrapolation (see Appendix 1). Where there was a PDR_{10} prediction (e.g., reduced fetal body weight) it was not consistent with the BMD_{10}, 420 versus 117 mg/kg/d, e.g., the model predicted the sample to be less toxic than it actually was.

The lowest BMD_{10} calculated from the study on heavy paraffinic DAE (318 Isthmus furfural extract, CRU 86187, CAS No. 64742-04-7) was 15 mg/kg/day. This value agreed well with the lowest modeled

PDR_{10} of 15 mg/kg/day, and both values were based on the mean number of living fetuses per litter. (See Table 10.)

7.1.4.3. PDR_{10}s modeled using the PAC profile of untested samples

PDR_{10}s for sensitive endpoints were calculated based on PAC profiles (See Appendix 1) and are shown in Table 10. Note that a PDR_{10} is not necessarily an indicator of an adverse effect. The lowest PDR_{10} is identified as the sample PDR_{10}.

7.1.4.4. Read-across from tested or modeled samples to untested samples

Blank cells in Table 10 indicate samples that have not direct data on toxicity and that have PAC profiles such that predicted results using the statistical models (PDR_{10}s) would be extrapolations and therefore would not be reliable. Read-across from the actual or modeled data may be appropriate for these samples. The PDR10 for maternal thymus weight is included for reference regarding possible effects on sensitive endpoints in the dams.

Table 10. Maternal and developmental PDR_{10}s and BMD_{10}s (mg/kg/day) for DAEs by endpoint and sample

Sample	Type of Sample	Maternal Thymus Weight PDR_{10}		Fetal Body Weight PDR_{10}	Live Fetuses per Litter PDR_{10}	Percent Resorptions PDR_{10}	Sample PDR_{10} (lowest value)	Basis for Sample PDR_{10}	Study BMD_{10}[1] (lowest value)
Site 7, Sample 23 (CRU 20906)	LP-DAE [3]			420			420	Fetal BW	
				117[2]	42[2]	47[2]			42
CRU 86187	HP-DAE[4]	34		82	15	26	15	Live fetuses per litter	
		16[2]		85[2]	15[2]	17[2]			15
Site 3, Sample 13 (CRU 100709)	LP-DAE	474		586	53	133	53	Live fetuses per litter	
Site 4, Sample 3 (CRU 100711)	LP-DAE			2000			2000[7]	Fetal BW[7]	
CRU 86141	HP-DAE			1728			1728[7]	Fetal BW[7]	
CRU 86303	HP-DAE	7		45	7	13	7	Live fetuses per litter	
CRU 89130	HP-DAE	38		70	24	40	24	Live fetuses per litter	
Site 2, Sample 9 (100705)	HP-DAE								
Site 3, Sample 10 (CRU 100706)	HP-DAE			>2000			>2000[7]	Fetal BW[7]	
Site 3, Sample 12 (100708)	HP-DAE	13		119	18	35	18	Live fetuses per litter	
Site 3, Sample 14 (100710)	HP-DAE								
Site 4, Sample 4 (100712)	HP-DAE	10		93	14	27	14	Live fetuses per litter	
Site 5, Sample 1 (100713)	HP-DAE	9		83	12	24	12	Live fetuses per litter	
950219	HP-DAE	2000		255	133	219	133	Live fetuses per litter	
Site 1, Sample 5 (100701)	HN-DAE[6]								

1) A BMD_{10} could be calculated only if data from developmental toxicity study was available.
2) Values are BMD_{10} for each endpoint. The basis for the study BMD_{10} was live fetuses per litter.
3) LP-DAE is light paraffinic distillate aromatic extract (CAS 64742-05-8).
4) HP-DAE is heavy paraffinic distillate aromatic extract (CAS 64742-04-7).
5)
6) HN-DAE is heavy naphthenic distillate aromatic extract (CAS 64742-11-6). PDR_{10}s were not calculated because they would have been extrapolations.
7) A value for PRD_{10} was not available for all endpoints.

Conclusions:
In the study on developmental toxicity of a heavy paraffinic DAE (sample 86187), significant fetal effects occurred only at dermal doses that also caused significant maternal effects. The overall NOAEL was judged to be 8 mg/kg/day for biologically significant changes and 30 mg/kg/day for statistically significant effects.

The developmental toxicity of the remaining DAEs was predicted using their PAC profiles and the models described in Appendix 1. Based on the available information and the lowest PDR_{10} among the three modeled developmental endpoints in Table 10, the following estimates of developmental toxicity can be made for the four different types of DAEs as follows:

Light paraffinic DAE CASRN 64742-05-8	PDR_{10}s ranged from 53 to 2,000 mg/kg/day. Sample 20906 had a PDR_{10} of 420 mg/kg and a BMD10 of 42 mg/kg/d, compared to the study NOAEL of 5 mg/kg/d.
Heavy paraffinic DAE CASRN 64742-04-7	PDR_{10}s ranged from 7 to >2,000 mg/kg/day. Sample 86187 had a PDR_{10} of 15 mg/kg and a BMD10 of 15 mg/kg/day, both intermediate between the two NOAELs described previously (8 and 30 mg/kg/day).
Light naphthenic DAE CASRN 64742-03-6	Empirical (NOAEL), predicted (PDR_{10}) and calculated (BMD_{10}) toxicity values were not available for this CASRN.
Heavy naphthenic DAE CASRN 64742-11-6	Empirical (NOAEL), predicted (PDR_{10}) and calculated (BMD_{10}) toxicity values were not available for this CASRN.

No developmental toxicity studies have been reported for either light or heavy naphthenic DAE. However, as stated previously, the distinctions in nomenclature among the four CAS numbers for DAEs do not indicate distinctions in toxicological properties. Therefore it is expected that existing data and models can be used to indicate potential toxicity for untested samples of DAEs.

Although the oral route of administration has little relevance to human occupational exposure, increases in fetal malformations and maternal toxicity were observed in rats with one oral dose of 2000 mg/kg of heavy paraffinic DAE on a single day during the critical period of gestation.

7.1.5 Reproductive Toxicity

No reproductive toxicity studies were identified for any of the DAEs. However, under the High Production Volume (HPV) Challenge Program, U.S. EPA has provided guidance on the requirements for evaluating reproductive toxicity. The EPA guidance indicates a reproductive toxicity study may not be required for certain petroleum substances if there is (1) a 90-day repeat-dose study in which the potential for effects on reproductive organs was assessed and (2) a developmental toxicity study (US EPA, 2010). Such data are available for samples of a light and a heavy paraffinic DAE.

The results of the 90-day repeated-dose study on a light paraffinic DAE (CAS# 64742-05-8) were summarized in section 7.1.2.1, but further details are provided here on endpoints for reproductive organs. Ten rats (both sexes) were dosed dermally with 0, 5, 50, and 150 mg/kg/day. One male animal receiving 150 mg/kg died on test. No effects were seen on the weights of epididymides, prostate, testes, ovaries, or uterus. Mean weights are shown in Table 11. Also, no significant treatment-related changes were seen in histopathology of epididymides, ovaries, prostate, seminal vesicles, or testes.

Table 11. Mean body weight and reproductive organ weights, light paraffinic DAE					
	Sham	0 mg/kg	5 mg/kg	50 mg/kg	150 mg/kg
Organ Weights					
Males					
Final body weight	490	450	460	450	447
Epididymides (g)	1.37	1.30	1.24	1.36	1.29
Prostate (g)	1.09	1.03	1.09	0.98	1.15
Testes (g)	3.69	3.45	3.48	3.72	3.50
Females					
Final body weight (g)	279	270	274	258	248
Ovaries & oviduct weight (g)	0.134	0.125	0.123	0.112	0.127
Uterus weight (g)	0.79	0.59	0.73	0.73	0.59

The results of the 90-day repeated-dose study on a heavy paraffinic DAE (CAS# 64742-04-7) were summarized in section 7.1.2.1, but further details are provided here on endpoints for reproductive organs. Rats (both sexes) were dosed dermally with 0, 30, 125, 500, and 1250 mg/kg/day. All animals receiving 1250 mg/kg as well as all males and 3 females receiving 500 mg/kg died or, due to clinical signs, were terminated prior to the scheduled sacrifice. At the highest nonlethal dose (125 mg/kg), no effects were seen on the weights of epididymides, prostate, testes, ovaries, or uterus. Mean weights are shown in Table 11. Also, no significant treatment-related changes were seen in histopathology of epididymides, ovaries, prostate, seminal vesicles, or testes. Lastly, morphology of epididymal spermatozoa was evaluated in 5 controls and 5 males dermally treated with 125 mg/kg. As shown in Table 12, the measured parameters were not affected by treatment. Overall, these data showed no observed effects on the tested reproductive parameters at the highest nonlethal dermal dose.

As explained in section 7.1.2.1, two additional groups of 10 males were dosed orally with 125 or 500 mg/kg/day. Four of the males dosed with 500 mg/kg died or were sacrificed *in extremis*. Given this mortality, other evidence of systemic toxicity, and mean body weights that were 80.5% of controls, the relevance of the significantly lower weights for epididymides, prostate, and seminal vesicles shown in Table 12 is questionable. The weight of the prostate with oral dosing at 125 mg/kg was significantly less than the weight in controls, but no significant treatment-related effects were seen in histopathology of epididymides, ovaries, prostate, seminal vesicles, or testes. In the morphological evaluation of epididymal spermatozoa, the number of abnormal sperm was increased with 500 mg/kg. Similar data were not available for the nonlethal oral dose of 125 mg/kg.

Table 12. Mean body weight, organ weights and parameters of sperm morphology, heavy paraffinic DAE						
	Dermal Route				Oral Route	
	0 mg/kg	30 mg/kg	125 mg/kg	500 mg/kg	125 mg/kg	500 mg/kg
Organ Weights						
Males						
Final body weight	435.1	434.0	424.6		407.1	350.3[1]
Epididymides (g)	1.296	1.269	1.267		1.229	0.956[1]
Prostate (g)	1.117	1.021	0.988		0.792[1]	0.405[1]
Seminal vesicles (g)	0.848	0.833	0.858		0.735	0.437[1]
Testes (g)	3.456	3.465	3.413		3.401	3.073
Females						

Final body weight (g)	277.2	256.3[1]	250.1[1]			
Ovaries weight (g)	0.090	0.089	0.091			
Uterus weight (g)	0.374	0.471	0.465			
Sperm Morphology						
No. normal sperm	490.6		488.4			483.8
No. abnormal sperm	9.4		11.6			16.2[1]
% normal sperm	98.1		97.7			96.8
% abnormal sperm	1.9		2.3			3.2

1) Significantly different from untreated controls ($p < 0.05$)

A study on developmental toxicity of a heavy paraffinic DAE was summarized in section 7.1.4.1. Significant fetal effects occurred only at doses that also caused significant maternal effects. The overall NOAEL was judged to be 8 mg/kg/day for biologically significant changes and 30 mg/kg/day for statistically significant effects.

In addition, the potential for a variety of PAC-containing petroleum substances, including DAEs, to affect reproductive organs was assessed via a series of 13-week repeated-dose studies in which the testes, accessory sex organs, and epididymides were weighed in males and the potential for pathological changes was evaluated in microscopic examinations. There was little evidence of reproductive organ effects in the repeated-dose studies of heavy paraffinic DAE or the other petroleum streams evaluated even at the highest non-lethal dose (Appendices 2 and 4 of API, 2008).

The most sensitive endpoints of reproductive toxicity of PAC-containing petroleum substances are likely to be the sensitive endpoints observed in the developmental toxicity studies (i.e., decreased fetal survival and growth). Developmental toxicity was evaluated in a large number of developmental toxicity studies on high-boiling, PAC-containing petroleum substances, including DAE, in which embryonal and fetal development were examined among the offspring of pregnant rats given the test material dermally. When developmental effects occurred, the most common and statistically significant effects at the lowest levels were decreases in fetal/pup survival and body weight (Feuston et al, 1994; API, 2008). There was little evidence of teratogenicity (i.e. malformations) in any of these dermal 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. Developmental toxicity was observed in these studies at dose levels that did not produce effects on weights or histology of reproductive organs or on semen quality in 13-week repeat-dose studies.

Lastly, screening-level fertility studies in males and females of a petroleum substance with high PAC content, i.e., CAS No. 64741-62-4, catalytically cracked clarified oil, by Hoberman et al (1995a) indicated that reproductive endpoints (e.g., sperm production and male and female fertility) were unaffected at 250 mg/kg/day applied dermally, a dose at which no litters survived in a dermal developmental toxicity study (Hoberman et al, 1995b). In the female screening reproductive toxicity study, females were exposed during the mating period and were not dosed during pregnancy. The lack of exposure during pregnancy may explain why no effect on litter size was observed. The HPV Testing Group believes that catalytically cracked clarified oil has the highest PAC levels of all petroleum substances and therefore can be considered as "worst case" for other petroleum-derived substances with lower PAC levels. On this basis it can be reasonably assumed that reproductive effects, such as fertility and sperm production, would not be sensitive effects of PAC-containing materials compared to developmental effects.

Based on the results of a large number of repeat-dose studies and developmental toxicity studies of DAE and other PAC-containing petroleum substances (Appendices 2 and 4 of API, 2008), as well as the two reproductive toxicity screening studies of carbon black oil, 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 do not appear to be sensitive endpoints for

assessment of the potential hazards of PAC-containing petroleum substances. Accordingly, it can be concluded that the most sensitive endpoints of reproductive toxicity for DAE and other high-boiling, PAC-containing petroleum streams are likely to be the developmental toxicity effects (i.e., decreased fetal survival and growth) observed in developmental toxicity studies. Consequently, reproductive toxicity studies of DAEs appear to be unnecessary since they would not be expected to identify reproductive endpoints more sensitive than those detected as a result of *in utero* exposure in developmental toxicity studies.

Conclusions:
No reproductive toxicity studies of DAEs were identified; thus, a NOAEL from reproductive toxicity studies is not available. However, available data from three sources indicate that the NOAEL from reproductive toxicity studies of DAEs would not be lower than the NOAEL from developmental toxicity studies of DAEs.

1) Little evidence of an effect on weights or histology of reproductive organs or on semen quality was observed in 13-week repeated-dose studies of light or heavy paraffinic DAE at dermal doses of up to 150 mg/kg/day, respectively. In comparison, in a developmental toxicity study of the same samples, dermal administration on GD 0-19 produced significant effects on fetal survival and growth with BMD_{10s} of 42 and 15 mg/kg/d (light and heavy DAE respectively).
2) In a large number of studies of developmental and repeated-dose toxicity studies of other PAC-containing, high-boiling petroleum substances, clear developmental toxicity was observed at doses that produced little evidence of effects on male and female reproductive organs (i.e., weight and histopathology) and on semen quality in repeated-dose toxicity studies (API, 2008).
3) The published NOAEL in a pair of dermal screening-level fertility studies of catalytically cracked clarified oil, a refinery stream containing high amounts of PACs, was ≥ 250 mg/kg/day, a dose associated with a significant decrease in fetal survival in a dermal developmental toxicity study.

Thus, it can be concluded that the NOAEL for reproductive toxicity of DAEs is unlikely to be lower than the NOAEL for developmental toxicity because the most sensitive endpoints in either developmental or reproductive toxicity studies are believed to be effects on fetal survival and growth resulting from *in utero* exposure. Based on EPA's guidance, this compilation of information from subchronic and developmental toxicity studies is judged to fulfill the need for data on the potential reproductive toxicity of DAEs.

7.2 Human Health Effects of Residual Aromatic Extracts

7.2.1 Acute Toxicity

No acute toxicity studies have been reported for RAEs. However, acute toxicity from RAEs would be expected to be less than that for DAEs based on the higher molecular weights and higher viscosities of the components, which in theory could reduce bioavailability. Based on read-across from acute toxicity data for DAEs to RAEs, the LD_{50}s for RAE would be >5000 mg/kg and >2000 mg/kg for oral (rat) and dermal (rabbit) routes of exposures, respectively.

7.2.2 Repeated Dose Toxicity

7.2.2.1. Tested samples and NOAELs

Four undiluted samples of RAE were administered dermally in rats for 13 weeks (Mobil, 1990b). Samples were identified as BSE Australia (CRU 86525), BSE Statfjord (CRU 87293), BSE Ninian (CRU 87058), and Mobilsol 40 (CRU 87476; CAS No. 64742-10-5). (BSE stands for bright stock extract, another name for RAE.) Viscosity

of Mobilsol 40 was ~58 cSt at 100°C. Mobilsol 40 was a treated RAE. The same procedures were used as in the study with heavy paraffinic DAE (Mobil, 1990a) with the exception that doses of Mobilsol 40 were 500 and 2000 mg/kg/day while the only dose for the other three samples was 2000 mg/kg/day. Also, only females were treated with BSE Statfjord and only males with BSE Ninian. Body weight, clinical signs, urinalysis, and gross observations at necropsy were not affected by treatment. Several small changes in serum chemistry and hematology parameters were noted, including slightly decreased RBC count in females only. Spleen weight increased at 2000 mg/kg with one sample and liver weight increased with two samples. No treatment-related effects were seen with microscopic examination of organs and tissues, including those of the male and female reproductive organs. Epididymal spermatozoa morphology and count and testicular spermatid counts were unaffected by treatment with Mobilsol 40 or BSE Australia at 2000 mg/kg. The study director concluded that the NOAEL was 500 mg/kg/day for Mobilsol 40 and <2,000 mg/kg/day for the other three RAEs. The NOAEL for the later three RAEs could not be clearly established since 2,000 mg/kg/day was the only dose evaluated. These four RAEs were observed to be substantially less toxic than the DAEs, possibly due to the relatively lower concentrations and different profiles of DMSO-extractable PACs as well to the high viscosity of RAEs that substantially reduces absorption (Potter et al., 1999).

7.2.2.2. BMD_{10}s calculated from data on tested samples

BMD_{10}s were not calculated due to the lack of dose-response data in the available studies.

7.2.2.3. PDR_{10}s modeled using the PAC profile of untested samples

No calculations of PDR_{10}s were made for RAEs for the reasons explained in section 7.

7.2.2.4. Read-across from tested samples to untested samples

Read-across can be used from the tested sample of Mobilsol 40 to untested samples. Part of the rationale for this statement is that, although PDR_{10}s are not shown for RAEs, the PAC profile of that sample of Mobilsol 40 (identified as CT-28, CRU 87476, in Table 3) is generally similar to those of the untested RAEs in Table 3. Given this similarity and an expected similarity in viscosity among the samples, read-across can be used.

Conclusions:
Effects observed with four RAE samples included slight effects on serum chemistry, RBC number, and the weights of spleen and liver with some samples. The NOAEL for one sample was 500 mg/kg and <2,000 mg/kg/day for the other three samples. Read-across can be used to untested RAEs. Sufficient data have been provided to show the potential repeated-dose toxicity of RAEs.

7.2.3. Genetic Toxicity *In Vitro* and *In Vivo*

7.2.3.1. *In Vitro* (Mutagenicity)

Results from optimized Ames tests on 8 samples of RAE were reported in Blackburn, et al. (1996). The MI values in the optimized Ames tests ranged from 0.2 to 3.4, indicating a range of biological activity. Therefore individual samples could be assumed to be positive for mutagenicity *in vitro* unless data indicate otherwise. Although the number of samples of RAEs was limited, they appeared to be similar to other petroleum substances in that the results in the optimized Ames tests were related to the presence and level of DMSO-extractable PACs. Additional MIs for individual samples of RAEs are provided in McKee et al (2011). As discussed previously, RAEs are unique in terms of the application of PAC models to them. Therefore the model for prediction of results from the optimized Ames assay (described in Appendix 2) has not been used for RAEs.

Conclusions:

Because the composition of RAEs can vary with crude oils and refining conditions, the levels of PACs can differ among various samples. As a result, the mutagenicity for various samples ranged from negative to positive. Sufficient data have been provided to characterize the potential *in vitro* mutagenicity of RAEs.

7.2.3.2. *In Vivo* (Chromosomal Aberrations)

In vivo micronucleus evaluations were performed on bone marrow harvested at termination of the four subchronic dermal assays of RAEs described in the repeat-dose toxicity section. No treatment-related increases in micronuclei were observed (Mobil, 1988). The systemic effects observed with dermal application of these RAEs indicated that components in the RAEs were systemically available.

Conclusions:
Data indicate RAEs did not cause chromosomal aberrations in an *in vivo* genotoxicity assay. Accordingly, sufficient data have been provided to characterize the in vivo genotoxic potential of RAEs.

7.2.4. Developmental Toxicity

7.2.4.1. Tested samples and NOAELs

In a developmental toxicity study similar to that described for heavy paraffinic DAE, a RAE (sample Mobilsol 40, CRU 87476, CAS No. 64742-10-5) was applied dermally to pregnant Sprague Dawley rats at doses of 0, 500, and 2000 mg/kg/day on days 0-19 of gestation. In a separate group, the same RAE was administered at 2000 mg/kg/day on GD 0-19 and all females were sacrificed and examined grossly on postpartum day 4. No maternal toxicity was seen aside from slight skin irritation in the dams and a slight decrease in body weight and body weight gain of the group of dams treated with 2000 mg/kg/day and sacrificed on GD 20. Mean body weight on GD20 was 94% of mean control weight. No such difference was seen in the group sacrificed on postpartum day 4; therefore the observed reduction was not considered biologically significant. Evidence of developmental toxicity was not observed in any group treated with the test substance. Thus, the study director concluded that the NOAEL for maternal and developmental toxicity was 2000 mg/kg/day, the highest dose evaluated (Mobil, 1989).

7.2.4.2. BMD_{10}s calculated from data on tested samples

A BMD_{10} was not calculated for this study because no toxicity was observed at the highest dose tested.

7.2.4.3. PDR_{10}s modeled using the PAC profile of untested samples

No calculations of PDR_{10}s were made for RAEs for the reasons explained in section 7.

7.2.4.4. Read-across from tested samples to untested samples

Read-across can be used from the tested sample of Mobilsol 40 to untested samples. Part of the rationale for this statement is that, although PDR10s are not shown for RAEs, the PAC profile of that sample of Mobilsol 40 (identified as CT-28, CRU 87476, in Table 3) is generally similar to those of the untested RAEs in Table 3. Given this similarity and an expected similarity in viscosity among the samples, read-across can be used.

Conclusions:
A sample of RAE had a NOAEL of 2,000 mg/kg/day for both maternal and developmental effects. Sufficient data have been provided to characterize the potential developmental toxicity of RAEs.

7.2.5. Reproductive Toxicity

No reproductive toxicity studies were identified for RAEs. However, under the High Production Volume (HPV) Challenge Program, U.S. EPA has provided guidance on the requirements for evaluating reproductive toxicity. The EPA guidance indicates a reproductive toxicity study may not be required for certain petroleum substances if there is (1) a 90-day repeat-dose study in which the reproductive organs were examined and (2) a developmental toxicity study.

The results of the 90-day repeat-dose studies of RAEs were summarized earlier in Section 7.2.2. As with DAEs, no adverse effects on reproductive organs were observed even at the highest doses tested. More specifically, no treatment-related effects were seen in the following endpoints with dermal dosing with Mobilsol 40 or BSE-Australia.

1) Weights of testes, prostate, epididymides, ovaries, and uterus
2) Histopathology of testes and ovaries
3) Weight of testicular parenchyma and cauda epididymis
4) Number of testicular sperm and number/g testis
5) Number and morphology of epididymal sperm, number/g cauda

No treatment-related effects were seen in weights of testes, prostate, and epididymides or in the histopathology of testes following dermal dosing of males with BSE-Ninian. No treatment-related effects were seen in weights of ovaries and uterus or in the histopathology of ovaries following dermal dosing of females with BSE-Statfjord.

Also, a developmental toxicity study on a RAE was described in section 7.2.4.1. The NOAEL for maternal and developmental effects with dermal dosing in rats was 2000 mg/kg.

In addition to these data on RAEs, the rationale derived from data on syntower bottoms and applied to DAEs in section 7.1.5 of this document can also be applied to RAEs. Also, the body of information from a number of developmental toxicity studies on petroleum substances described in section 7.1.5 and applied to DAEs can also be applied to RAEs.

It can be concluded that the most sensitive endpoints of reproductive toxicity for RAE and other high-boiling, PAC-containing petroleum streams are likely to be the developmental toxicity effects (i.e., decreased fetal survival and growth) that have been observed in developmental toxicity studies. Consequently, reproductive toxicity studies of RAEs appear to be unnecessary since they would not be expected to identify reproductive endpoints more sensitive than those detected as a result of *in utero* exposure in developmental toxicity studies. Based on EPA's guidance, this compilation of information from subchronic and developmental toxicity studies is judged to fulfill the need for data on the potential reproductive toxicity of DAEs.

Conclusion

Although a definite number cannot be provided for a NOAEL for reproductive effect, available data from three sources provide sufficient information to conclude that this value would be higher than the NOAEL for developmental toxicity for RAEs.

1) No changes were observed in weight or histological appearance of reproductive organs of male and female rats via the oral or dermal routes of exposure in four13-week subchronic studies.
2) Developmental toxicity endpoints, including both *in utero* and postnatal development, were more sensitive than effects on the reproductive organs in a developmental toxicity study.
3) The published NOAEL in a reproductive study with clarified slurry oil, a refinery stream containing high amounts of PACs, was >250 mg/kg.

Overall, the NOAEL for reproductive toxicity for RAEs is expected to be greater than the NOAEL for developmental toxicity for RAEs.

7.3. Health Effects Other

7.3.1. Carcinogenicity - Dermal

Carcinogenicity testing is beyond the scope of HPV, but it should be noted for information purposes that many studies have been performed to evaluate the dermal carcinogenicity of aromatic extracts in the mouse. Numerous studies have shown that the mutagenic and carcinogenic potential of aromatic extracts and other compositionally related heavy petroleum streams correlates with the presence of PACs (Roy et al, 1988; Cruzan et al., 1986; Blackburn et al., 1986). Further studies have shown these PACs can be absorbed through the skin and enter the general circulation (Roy et al., 1996; Roy et al., 1998). Some samples of DAEs have been shown to be potent dermal carcinogens in a number of mouse skin painting bioassays (IARC, 1984). The dermal carcinogenicity of samples of RAE has ranged from non-carcinogenic to positive findings in some tests (Blackburn et al, 1996; Reddy et al., 1997; Kane et al., 1984; Doak et al., 1985; Gradiski et al., 1983; Bingham et al., 1980; King, D.J., 1991; Shell Research Ltd., 1991).

7.4. Assessment Summary for Health Effects

Distillate Aromatic Extracts (DAEs)

DAEs have a low order of acute oral and dermal toxicity. Several dose-related effects involving multiple organs (including liver, thymus, and blood) were reported with 13-week dermal or oral dosing of heavy paraffinic DAE to rats, resulting in a NOAEL <30 mg/kg/day with dermal applications. Marginal effects on adrenals and blood cells were observed at this low dose. No evidence of treatment-related effects was found in microscopic examination of male and female reproductive organs in the 13-week studies. PDR_{10}s based on the PAC content of additional DAEs ranged from 25 to 78 mg/kg.

In a study on developmental toxicity of a dermally administered heavy paraffinic DAE, significant fetal effects occurred only at doses that also caused significant maternal effects. The developmental NOAEL was judged to be 8 mg/kg/day for biologically significant effects and 30 mg/kg/day for statistically significant effects. PDR_{10}s for developmental effects based on the PAC content of additional DAEs ranged from 7 to >2,000 mg/kg/day, indicating that effects of may vary among individual DAEs due to PAC content. Oral administration of heavy paraffinic DAE at 2000 mg/kg on a single day during the critical period of gestation resulted in increased fetal malformations and maternal toxicity in rats. Although DAEs have not been tested for reproductive toxicity, data from (1) developmental testing of DAE, (2) subchronic studies with DAEs, and (3) studies on clarified slurry oil (a high boiling, high-PAC substance) suggest that reproductive toxicity would be a less sensitive endpoint than developmental effects.

DAEs are expected to be mutagenic unless further processed to lower PACs. Results for one DAE were positive in a mouse lymphoma assay and negative for chromosomal aberrations in an *in vivo* genotoxicity assay with a heavy paraffinic DAE. There is a large body of toxicity data relating to carcinogenicity and mutagenicity of aromatic extracts and other compositionally related heavy petroleum streams that dates back over 50 years. Some untreated aromatic extracts produce skin cancer in the mouse. Numerous studies have shown that the mutation and cancer-causing potential of aromatic extracts is directly related to the presence of PACs.

Residual Aromatic Extracts (RAEs)

Although, no acute toxicity studies were reported for RAEs, acute toxicity for RAEs would be expected to be less than that of DAEs due to the higher molecular weights and higher viscosities of the components in RAEs, theoretically reducing bioavailability. Effects with repeated doses observed with four samples of RAEs were

similar but more limited than those obtained with a sample of DAE. NOAELs for the RAEs were 500 mg/kg for one sample and <2,000 mg/kg for the other three.

When tested for developmental toxicity, a sample of RAE had a NOAEL of 2,000 mg/kg/day for both maternal and developmental effects. Based on these results and the same rationale as for DAEs, reproductive toxicity is not expected to represent a sensitive endpoint for RAEs.

Because the composition of RAEs can vary with crude oil and refining conditions, the levels of PACs can differ among various samples. As a result, the results of some optimized Salmonella tests were positive whereas others were negative. However, results were negative for chromosomal aberrations in an *in vivo* genotoxicity assay with a RAE.

8. HUMAN EXPOSURE SUMMARY

Aromatic extracts are dark solids to highly viscous liquids with relatively low vapor pressures. Dermal contact is deemed to be the primary route of exposure to workers. Because aromatic extracts are maintained in closed systems within the refinery, the potential dermal exposure is expected to be low except for accidental release or incidental contact during routine maintenance and repair. AEs are blended into heavy fuel, used as precursors in the synthesis of carbon black, petroleum pitches and resins, and used in the manufacture of rubber and plastics. In rubber and plastic manufacture, aromatic extracts are physically incorporated into the final solid product, resulting in little or no exposure to the end use consumer. Little or no exposure information is available about exposure to aromatic extracts during the manufacture of carbon black, tires or plastics.

9. MATRIX OF AROMATIC EXTRACTS CATEGORY DATA

The data matrix for aromatic extracts is divided into the following four tables.

Table 12. Physical and chemical properties of aromatic extracts

Table 13. Environmental fate of aromatic extracts

Table 14. Environmental effects of aromatic extracts

Table 15. Data on mammalian toxicity for aromatic extracts

Table 13. Physical and chemical properties of aromatic extracts

Endpoint	**Light Paraffinic DAE** CAS # 64742-05-8	**Light Naphthenic DAE** CAS # 64742-03-6	**Heavy Paraffinic DAE** CAS # 64742-04-7	**Heavy Naphthenic DAE** CAS # 64742-11-6	**Read-Across Range to Untested Category Members**	**RAE** CAS # 64742-10-5
Pour Point	-6 to +50°C	-6 to +50°C	-6 to +50°C	-6 to +50°C	-6 to >20°C	>20°C
Boiling Range	288 to 534°C		289 to 579°C	326 to 584°C	288 to 584°C	344 to >734°C
Vapor Pressure	<0.1 kPa	<0.1 kPa	<0.1 kPa	<0.1 kPa	<0.1 kPa	<0.1 hPa (<0.01 kPa)
Partition Coefficient		4.4 to > 6			5 to >20	5 to >20
Water Solubility	Based on individual hydrocarbon solubility estimates using WSKOW (US EPA, 2000), the low molecular weight components (e.g., C15 hydrocarbons) in aromatic extracts have water solubility values ranging from <0.001 to 0.63 mg/L. As molecular weight increases, solubility decreases for these types of hydrocarbons. Water solubility of the complex mixtures would be negligible as these substances exist in a solid to semi-solid state at environmental temperatures.					

Table 14. Environmental fate of aromatic extracts						
Endpoint	**Light Paraffinic DAE** CAS # 64742-05-8	**Light Naphthenic DAE** CAS # 64742-03-6	**Heavy Paraffinic DAE** CAS # 64742-04-7	**Heavy Naphthenic DAE** CAS # 64742-11-6	**RAE** CAS # 64742-10-5	**Read-Across Range to Untested Category Members**
Photodegradation	The low vapor pressures and the physical state of aromatic extracts indicate that volatilization to the atmosphere will not be a significant fate process. However, the individual hydrocarbon constituents in aromatic extracts have the capability to undergo photodegradation through interaction with atmospheric hydroxyl radicals. Should conditions exist where constituents in aromatic extracts end up in the atmosphere, half-lives are estimated to be 0.7 days or less.					
Stability in Water	The majority of chemical constituents in aromatic extracts are hydrocarbons which lack the chemical linkages known to undergo hydrolysis. Therefore, aromatic extracts are stable in water.					
Environ. Transport	Fugacity modeling of individual C15 and C50 hydrocarbon constituents in aromatic extracts indicates that these substances will tend to become incorporated into the soils/sediments of the compartment to which they are released. Although some of the lowest molecular weight constituents in aromatic extracts show a potential to partition to the atmosphere, the physical state of these substances indicates that such partitioning would occur at a slow rate.					
Biodegradation		not readily biodegradable				not readily biodegradable

Table 15. Environmental effects of aromatic extracts

Endpoint	**Light Paraffinic DAE** CAS # 64742-05-8	**Light Naphthenic DAE** CAS # 64742-03-6	**Heavy Paraffinic DAE** CAS # 64742-04-7	**Heavy Naphthenic DAE** CAS # 64742-11-6	**RAE** CAS # 64742-10-5	**Read-Across Range to Untested Category Members**
Acute Fish						
96-h LL50			>1000 mg/L		>1000 mg/L	>1000 mg/L
96-h LL0			1000 mg/L		1000 mg/L	1000 mg/L
NOELR			1000 mg/L		1000 mg/L	1000 mg/L
Acute Daphnia						
48-h EL50	35.9 mg/L		>1000 mg/L		>1000 mg/L	35.9 mg/L
48-h EL0			1000 mg/L		1000 mg/L	
NOELR	1.0 mg/L		1000 mg/L		1000 mg/L	1.0 mg/L
Algae						
72-h E_bL50					>50% WAF [1]	
72-h E_rL50	18.8				>50% WAF	18.8
$NOELR_{biomass}$					50% WAF	
$NOELR_{growth\ rate}$	0.1				50% WAF	0.1
Chronic Daphnia						
21-d survival EL50			>1000 mg/L		>1000 mg/L	>1000 mg/L
21-d survival NOELR			1000 mg/L		1000 mg/L	1000 mg/L
21-d reproduction EL50			>1000 mg/L		>1000 mg/L	>1000 mg/L
21-d reproduction NOELR			1000 mg/L		1000 mg/L	1000 mg/L

[1] The algal endpoints were based on results of testing a 50% dilution of a 2000 mg/L loading rate WAF.

Table 16. Data on mammalian toxicity for aromatic extracts

Endpoint	Qualifier	Light Paraffinic DAE CAS # 64742-05-8	Light Naphthenic DAE CAS # 64742-03-6	Heavy Paraffinic DAE CAS # 64742-04-7	Heavy Naphthenic DAE CAS # 64742-11-6	Read-Across Range to Untested DAE Category Members	RAE CAS # 64742-10-5
Acute (Oral)	LD50	>5 g/kg		>5 g/kg		>5 g/kg	
Acute (Dermal)	LD50	>3 g/kg		>2 g/kg		>2 g/kg	
Repeated-Dose (Dermal)	NOAEL	<5 mg/kg/day		<30 mg/kg/day			<2000 / >500 mg/kg/day
	BMD_{10}	13 mg/kg/day		21 mg/kg/day			
	PDR_{10}	42 -72 mg/kg/day[2]		25 - 78 mg/kg/day[2]		25 – 78 mg/kg/day	NA
Genotoxicity, in vitro, bacterial	Optimized Ames					Positive or negative, varies with sample[3]	Positive or negative, varies with sample
Genotoxicity, in vitro, non-bacterial		Positive				Positive[3]	
Genotoxicity, in vivo	Micronucleus	Positive[4]		Negative		Expected negative	Negative
Reproductive toxicity (Dermal)	NOAEL						
Developmental toxicity (Dermal)	NOAEL	5 mg/kg/day		8 - 30 mg/kg/day[5]			2000 mg/kg/ day, fetal and maternal[6]
	BMD_{10}	42 mg/kg/day		15 mg/kg/day			
	PDR_{10}	53 – 2,000 mg/kg/day		7 - >2,000 mg/kg/day		7 - >2,000 mg/kg/day	NA

1) BMD_{10}
2) Values shown are the lowest modeled PDR_{10} among the modeled samples.
3) Individual samples can be assumed to be positive unless data for each sample indicate that it is not mutagenic.
4) Most aromatic extracts are expected not to produce chromosomal effects *in vivo*.

5) The LOAEL for statistically significant effects on fetuses was 125 mg/kg/day and the corresponding NOAEL was 30 mg/kg/day. The LOAEL for possibly biologically significant effects on fetuses was 30 mg/kg/day and the corresponding NOAEL was 8 mg/kg/day.
6) NOAELs for RAEs with higher PAC content may be lower than 2,000 mg/kg/day.

10. CATEGORY ANALYSIS CONCLUSIONS

Aromatic extracts are dark solids or highly viscous liquids with a boiling range of 288 to 584°C for DAEs and 344 to >734°C for RAEs. Pour points are near ambient temperatures (-6°C to +50°C for distillate aromatic extracts and >+20°C for residual aromatic extracts) and vapor pressures are very low. Water solubility is expected to be negligible for these materials, thereby limiting their bioavailability. Despite these characteristics, some aromatic extracts may cause toxicity in freshwater invertebrates and freshwater algae. The lowest acute EL50 was 35.9 mg/L for *Daphnia magna*. For algae, the lowest E_rL50 was 18.8 mg/L. None of the fish studies reviewed showed any adverse effects at 1000 mg/L. In tests for chronic toxicity in aquatic invertebrates, neither survival nor reproduction was impaired in the adult generation. Offspring produced during the test also appeared healthy with no adverse effects noted; the NOELR was 1000 mg/L WAF.

Because aromatic extracts are substances with variable composition, the total amount and profile of PACs and other classes of constituents can differ among different samples. As a result, mammalian toxicity with repeated exposures, mutagenicity, and carcinogenicity has been shown to vary with the PACs in the samples. Also, DAEs and RAEs differed in their observed toxicological properties in mammals. Accordingly, these substances were summarized separately in the health effects section for clarity of presentation.

DAEs have low acute systemic toxicity in mammals dosed orally and dermally. Rats treated dermally with light or heavy paraffinic DAE over 13 weeks had several dose-related effects involving multiple organs, resulting in a NOAEL less than 30 mg/kg/day. PDR_{10}s based on PAC profile (see Appendix 1) for untested samples of light and heavy paraffinic DAEs were similar, ranging from 25 to 78 mg/kg/day.

DAEs are expected to be mutagenic unless further processed to reduce PACs. One sample of a light paraffinic DAE was positive in a mouse lymphoma assay *in vitro*. There are sufficient data to estimate the *in vitro* mutagenicity of DAEs for purposes of the HPV program. On a practical basis, individual samples of DAEs should be assumed to potentially mutagenic under *in vitro* conditions unless there are sufficient data to indicate otherwise (e.g., optimized Ames test, modeling based on PAC profile, refining history). *In vivo* micronucleus tests on a heavy paraffinic DAE and a range of other petroleum substances have been negative. The exception to this general trend was a positive response in 2 of 5 males at the highest tested dose of a light paraffinic DAE. Given the limited number of affected animals in this test, the weight of evidence from tests on several petroleum streams, and read-across from the heavy paraffinic DAE, it is concluded that most DAEs are unlikely to produce chromosomal effects under *in vivo* conditions.

The NOAEL for developmental toxicity for a light paraffinic DAE was 5 mg/kg/day. The NOAEL for developmental toxicity with a heavy paraffinic DAE was 8 mg/kg/day for biologically, but not statistically, significant effects. Statistically significant developmental effects at higher doses were associated with significant maternal effects. Modeled PDR_{10}s based on PAC profile for light and heavy paraffinic DAEs ranged from 7 to >2,000 mg/kg/day. Oral administration of heavy paraffinic DAE at 2000 mg/kg on a single day during the critical period of gestation resulted in increased fetal malformations and maternal toxicity in rats. Reproductive toxicity is not considered to be a sensitive endpoint for DAEs based on results from subchronic and developmental toxicity tests with DAE and other PAC-containing substances. Reproductive toxicity NOAELs for DAEs would not be expected to be below developmental NOAELs.

Due to the compositional similarity among the four types of DAEs, a combination of existing data, data from ongoing testing, and models based on the content off PACs can be used to indicate potential subchronic and developmental toxicity for untested samples of DAEs.

Acute toxicity from RAEs would be expected to be less than that for DAEs based on the higher molecular weights and higher viscosities of the components. In repeated-dose studies, the NOAELs for four RAEs were 500 mg/kg/day for one sample and <2,000 mg/kg/day for the other three samples.

The mutagenicity index for various samples ranged from no effect to positive for RAEs due to variation in PAC content. As with DAEs, individual samples of RAEs should be assumed to be potentially mutagenic under *in vitro* conditions unless there are sufficient data to indicate otherwise (e.g., optimized Ames test, modeling based on PAC profile, refining history). Four samples of RAE did not cause chromosomal aberrations in *in vivo* micronucleus assays. Based on these results and the lack of positive findings in this assay with other compositionally similar petroleum substances, it is unlikely that other RAEs would cause chromosomal effects in *in vivo* micronucleus assays.

A sample of RAE had a NOAEL of 2,000 mg/kg/day for both maternal and developmental effects. These data provide a limited basis to evaluate the developmental toxicity of RAE, with the understanding that toxicity of individual RAEs is expected to vary with PAC profile. In addition, there was sufficient read-across information to predict the reproductive toxicity of RAE and, as with DAEs, the reproductive toxicity NOAELs would not be expected to be below developmental NOAELs.

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12. LIST OF APPREVIATIONS AND ACRONYMS

API – American Petroleum Institute
BOD – biological oxygen demand
AUGC – area under the growth curve
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
DAE – Distillate aromatic extract
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
EPA/US EPA – United States Environmental Protection Agency
g/cm^3 – grams per cubic centimeter
h - hour
HLS – Huntingdon Life Sciences
HPV – High Production Volume
HSDB – Hazardous Substances Data Bank
IRDC – International Research and Development Corporation
^{o}K – degrees Kelvin
kPa - kilopascal
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
Loading Rate – total amount of test substance added to dilution water to prepare water accommodated fractions (WAFs) for ecotoxicity testing
LOAEL – lowest observable adverse effect level
mg/kg – milligrams per kilogram
mg/L – milligrams per liter
mg/m^3 – milligrams per cubic meter
mL - milliliter
mm - millimeter
nm - nanometer
NOAEL – no observable adverse effect level
NOEC – no observable effect concentration
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
PAC - polycyclic aromatic compound
PAH – polycyclic aromatic hydrocarbon
PNA – polynuclear aromatic
ppm – part per million
RAE – Residual aromatic extract
SIDS – Screening Information Data Set
UNEP – United Nations Environment Program

US EPA – United States Environmental Protection Agency
UV - ultraviolet
WAF – water accommodated fraction
wt% - weight percent
μg - microgram
μg/L – microgram/liter
> greater than
< less than

13. 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.

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.

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.

ARC: Aromatic ring class that reflects the weight percent of PACs that have a given number of aromatic rings (1 through 7) within the total analyzed sample.

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 environmental which is available for uptake by organisms. (AIHA, 2000)

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.

BMD: The Benchmark Dose is the dose producing a predetermined change in response and is calculated from a dose-response model statistically fitted to experimental data. (Gephart, et al, 2001)

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. (US EPA, 2007)

***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.

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. (US NLM, 2007)

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).

Ecological Effects – all endpoints (OECD definitions)

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. (US EPA, 1999)

Environmental Fate Effects – all endpoints (OECD definitions)

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. (US EPA, 1996)

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.

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.

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.

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

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).

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

Lowest-Observed-Adverse-Effect Level (LOAEL): The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group (US EPA, 2002).

Modified Ames Test: A modification of the Ames test used for petroleum materials and designed to facilitate physical contact between the test substance and the bacteria as well as enhance the reactions among the bacteria. Also referred to as the optimized Ames test.

Mutagenicity Index: The primary endpoint in the modified Ames test indicating the slope for the linear portion of the dose-response curve (number of revertant colonies vs dose of test substance per plate.

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

Optimized Ames Test: See modified Ames test.

PAC Profile: The listing of the weight percent of each of the DMSO-extractable 1- through 7-ring polycyclic aromatic compounds from a test material. (API, 2008)

PAC 2: An analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008)

PDR_{10}: The Predicted Dose for a Response that is a 10% change from control. The prediction is based on models developed from a series of exposure-response studies. (API, 2008)

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.

Polycyclic aromatic compounds (PAC): compounds of two or more fused-aromatic rings consisting only of carbon and hydrogen (PAH) or which may also contain one or more atoms of nitrogen, oxygen or sulfur (a heteroatom) that replace one or more of the carbon atoms in a fused ring system (API, 2008)

Polycyclic aromatic hydrocarbons (PAH): compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only (API, 2008).

Portal-of- Entry Effect: A local effect produced at the tissue or organ of first contact between the biological system and the toxicant (US EPA, 1994).

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. (OECD, 2007)

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. (US EPA, 1996)

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.

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).

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.

Appendix 1. Correlation between PAC Profile and Selected Endpoints of Mammalian Toxicity

As explained in the section on category definition and justification, the mammalian toxicity of the substances found in aromatic extracts is expected to be related to their PAC profile; 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 each of seven separate ring classes.

The initial indication that PAC content could be used to predict the toxicity of untested substances in aromatic extracts was based on the publication by Feuston et al. (1994). Their research, based on thirteen 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 Petroleum High Production Volume Testing Group (HPVTG) 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 both the repeated-dose and developmental toxicities of high-boiling petroleum substances, i.e. those with initial boiling points greater than approximately 300 °F (API, 2008). Predictive models for 7 selected repeated-dose and developmental dermal toxicity endpoints in the rat were developed and discussed (API, 2008). The report was reviewed in a peer consultation process and the report and results 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 repeated-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 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 repeated-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 7

[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)**.

biological endpoints that were selected for final statistical characterization were 4 repeat-dose measures (thymus weight, liver to body weight ratio, platelet count and, hemoglobin concentration), and 3 developmental measures (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 substances prepared and 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 they were used for 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 developed by Crump (Crump, 1984). Calculation of the BMD model was done with either a linear or quadratic model; the choice was based on the available data and modeling results criteria similar to the recommendations suggested by the EPA in their BMD computer program. More specifically, BMD10s were calculated using a SAS program to mimic the EPA program in many, but not all, ways. The BMD10s were based on a specific algorithm with the following criteria:

1) The BMD10 must be a positive value.
2) The linear model needs at least three data points to be considered, the quadratic model needs at least four.
3) The slope of the curve must be in the deleterious direction to be considered.
4) If the quadratic model's R-sq is at least 10% better than the linear model's R-sq, the quadratic model gets one point.
5) If the quadratic model has an error variance at least 5% smaller than the linear model, the quadratic model gets one point. (This is very similar to the AIC criterion).

If the quadratic model has at least two points it is chosen. (The AIC-like criterion will be the driver, but the process is open for other criteria.) The default is the linear model (the simpler model which is the preferred model), unless the quadratic is shown to be superior.

Comparison of the PDR_{10} and BMD_{10} from a series of samples shows close agreement; this indicates the usefulness of the PDR_{10} when there is no biological endpoint testing data 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 2.

Appendix 2. Optimized Ames Test 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, 2002b). 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 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°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 $\geq$ 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.