201-15368

Petroleum HPV

June 17, 2004

The Honorable Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know
HPV CONSORTIUM
Heavy Fuel Oils Test Plan

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Heavy Fuel Oils Test Plan and Robust Summary. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format and as a IUCLID export file) are accompanying this letter via email to the EPA HPV electronic test plan submission address (chem.rtk@epa.gov). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee
Petroleum HPV Testing Group Technical Work Group

201-15368A

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

HEAVY FUEL OILS CATEGORY

Submitted to the US EPA

By

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration

June 17, 2004

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PLAIN LANGUAGE SUMMARY

The Heavy Fuel Oils (HFOs) category includes both finished products (residual fuels) and the primary refinery streams from which they are blended. Members of the heavy fuel oil category are a diverse group of substances encompassing hydrocarbons with a wide range of molecular weights, carbon numbers (C_7 to C_{50}) and boiling points (250 – 1112 °F (121 to 600 °C). In addition to petroleum hydrocarbons they contain heterocyclic compounds containing sulfur, nitrogen, and oxygen, and organo-metallic compounds. The residual fuels are low-grade fuels primarily used in industrial boilers and other direct source heating applications (e.g., blast furnaces) and as a principal fuel in marine applications. The finished heavy fuels (residual fuels) are products that consist primarily of the residuum of the refining process after virtually all of the higher-quality hydrocarbons have been distilled, cracked, or catalytically removed from crude oil feedstock. To produce a residual fuel of a specified viscosity, the high viscosity of the residual streams is reduced by adding a diluent (cutter stock) that is typically a lower quality distillate stream. The choice of the distillate cutter stock is itself variable and largely a function of availability at any given time within the refinery and the viscosity specifications of the product being manufactured. For instance, in refineries with catalytic cracking units, catalytically cracked cycle oils are common fuel diluents (CONCAWE, 1998). Because residual fuels are blended from a variety of different residual and distillate materials, the specifications for residual fuel oils are very general, with viscosity being the controlling specification. The exact blend used for a specific residual fuel is determined largely by the desired viscosity of the finished fuel and specifications set by ASTM (2002). As a result, the composition of residual fuel oils can vary widely and will depend on the refinery configuration, the crude oils being processed and the overall refinery demand.

The Testing Group is proposing to perform a repeat-dose/reproductive/developmental screening study (OECD 422) on a representative sample of a reformer residue. A representative sample of a residual fuel oil will be tested using the reproductive/developmental screening protocol (OECD 421). *In-vitro* genotoxicity assays (modified Ames & chromosomal aberration) will also be performed on samples of an atmospheric residue, atmospheric distillate, cracked distillate, reformer residue and a residual fuel oil.

The environmental fate characteristics of the materials in this category are due in large part to the physico-chemical parameters of the individual component hydrocarbons. Where physico-chemical data did not exist or were impractical to obtain, calculated physico-chemical and environmental data for selected constituents of category members has been developed using the EPIWIN© computer model.

When released to the environment, the dispersion of heavy fuel oil depends upon the severity of the weathering elements. High-energy environments will tend to disperse substances more quickly than low energy environments. During weathering, low molecular weight fractions will dissolve in the water column or volatilize to the air, whereas intermediate fractions will float and/or spread out on water or soil where they tend to form emulsions or adsorb to soil and sediment particles. The viscous high molecular weight components will agglomerate and float or sink depending upon the density of the substance. Volatile components are not expected to persist in the atmosphere due to their capacity to undergo direct and/or indirect photodegradation. Hydrocarbons are regarded as being inherently biodegradable, and the low molecular weight fraction that dissolves in water will rapidly biodegrade. The limited water solubility and bioavailability of the heavy fractions reduces their capacity for biodegradation. These substances will biodegrade, although at a much slower rate.

For the assessment of the ecotoxicity of heavy fuel oils, the category substances are considered as a single group, and the potential for any category member to elicit adverse effects in aquatic organisms is attributed to the solubility of the constituent hydrocarbons in that member. This is valid because petroleum hydrocarbons elicit effects through a common mode of toxicity termed nonpolar narcosis (van Wezel and Opperhuizen, 1995), which is produced by the soluble hydrocarbon fractions in aqueous preparations. Fuels having molecular weight hydrocarbons predominately higher than the solubility "cut-off" for acute toxicity show no measurable acute toxicity (CONCAWE 2001). The solubility cut-off varies with the hydrocarbon structure; thus, for paraffinic hydrocarbons the solubility cut-off occurs at about C10, while for alkylbenzenes it is about C14 (CONCAWE, 2001). Other individual components contribute to toxicity, but as molecular weight increases, both solubility and toxicity decline

Existing aquatic toxicity data show heavy fuel oil related refinery streams to have moderate to no aquatic toxicity depending upon species and test substance. Algae have exhibited a greater sensitivity than fish or invertebrates to heavy fuel oil exposure, and different substances have produced different toxicity in the same species. Because substances in the Heavy Fuel Oil category include a wide range of molecular weight hydrocarbons, existing data may not represent the ecotoxicity of some category members based on their distillation and carbon number ranges. However, the Testing Group believes the combination of existing residual fuel oil ecotoxicity data and data on kerosene and gas oil streams sufficiently covers what would be expected for the ecotoxicity of all members of the heavy fuel oil category. Kerosene and gas oils are composed of similar hydrocarbon constituents as in heavy fuel oils and have similar molecular weights as the light components of category members with low initial boiling points. Therefore, because the light-end components of the heavy fuel category members are expected to be the most toxic components, the ecotoxicity of the kerosene and gas oils represent a conservative estimate of the ecotoxicity for those heavy fuel oil category members. No additional ecotoxicity testing of heavy fuel oil is proposed.

DESCRIPTION OF THE HEAVY FUEL OILS CATEGORY

The Heavy Fuel Oils (HFOs) category includes both finished products (residual fuels) and the primary refinery streams from which they are blended. The residual fuels are low-grade fuels primarily used in industrial boilers and other direct source heating applications (e.g., blast furnaces) and as a principal fuel in marine applications. The residual fuels are products that consist primarily of the residuum of the refining process after virtually all of the higher-quality hydrocarbons have been distilled, cracked, or catalytically removed from crude oil feedstock. Historically, fuel oils were based on residues from the atmospheric column. However, the increasing demand for transportation fuels such as gasoline, kerosene and diesel has led to an increased value for the atmospheric residue as a feedstock for vacuum distillation and for cracking processes. As a consequence, most heavy fuel oils are currently based on vacuum residues and residues from thermal and catalytic cracking operations (CONCAWE, 1998). These high viscosity residual streams may in turn be “cut” with lower quality, lighter weight distillates to produce a finished residual fuel of a specified viscosity. The choice of the distillate cutter stock is itself variable and largely a function of availability at any given time within the refinery and the viscosity specifications of the fuel being manufactured. While the materials most frequently used as cutter stocks are included in this category, other streams may occasionally be used, e.g., kerosene and gas oils. These other materials are characterized in other API HPV Test Plans. The exact blend used for a specific residual fuel is determined largely by the desired viscosity of the finished fuel and specifications (ASTM, 2002). Some of the refinery streams in the category that have lower viscosities and lower polyaromatic compound (PAC) levels have low-volume, specialty applications such as cutter stock in cutback asphalt, synfuel binders and carbon electrode production.

Members of the heavy fuel oils category are a diverse group of substances that encompass hydrocarbons with a wide range of molecular weights, with carbon numbers ranging from C_7 to C_{50} and boiling points between 250 – 1112 °F (121 to 600 °C). However, “typical” heavy fuel oils are C_{20}-C_{50} with the low carbon numbers and boiling temperatures being associated with lighter weight “cutter” streams (CONCAWE, 1998). All the category members are complex mixtures, containing variable amounts of alkanes, cycloalkanes, aromatics, olefins, asphaltenes, and hetero-molecules containing sulfur, oxygen, nitrogen and organo-metals. Because they are complex mixtures composed of relatively high molecular weight compounds, the materials in this category are difficult to characterize in detail. Consequently, they are typically not defined by detailed compositional information but instead by process history, physical properties, and product use specifications (ASTM, 2003). Since viscosity is the controlling specification, and other limiting requirements - notably boiling point ranges - are unspecified, there is significant variation in the chemical composition of the resulting fuel products (Uhler, 2002; IARC, 1989).

Because the process history of a refinery stream determines its chemical composition, the Testing Group expects that streams that have undergone similar processing will have similar physical/chemical/biologic properties and environmental fate and transport characteristics. The refinery streams within the heavy fuels category can therefore be grouped into seven subcategories based on their process histories. As shown in Figure 1, the major processes used to produce the refinery streams included in the heavy fuel oils category are:

Atmospheric distillation

Heavy fuel oil related streams produced by atmospheric distillation comprise fractions of crude oil separated by heating (650-700 degrees °F) at atmospheric pressure. They include atmospheric distillates (heavy gas oils) and the heavier residual materials. The distillate HFO streams are similar to some of the refinery streams covered in the API HPV Gas Oils category, albeit of higher molecular weight. Some of these streams may be further hydrotreated or desulfurized to remove sulfur, nitrogen, and other impurities. Most atmospheric distillates undergo further processing in order to convert them into higher value fuels (diesel, kerosene). However, a portion of the heavier distillate streams may be used as blending stocks to reduce the viscosity of the residual streams. The residual refinery streams comprise a heterogeneous group of poorly defined, viscous, high boiling hydrocarbon streams that usually contain suspensions of resin/asphaltene complexes. These streams often have high levels of heterocyclic aromatic and naphthenic compounds. Varying percentages of sulfur, nitrogen, oxygen, and other elements are present as heterocyclic inclusions, primarily in the aromatics fraction. These residual streams often have a PAC content over 5%, but the content can be much higher depending on the processing the stream has undergone. As shown in

Figure 1, there are eight refinery streams in the heavy fuel oils category that are produced by atmospheric distillation (two distillate and six residual streams). See Appendix A for a more detailed description of each of these streams.

Vacuum distillation

The residuum from the atmospheric distillation unit is heated at higher temperatures to further separate heavier molecules. This is done under reduced pressure to prevent thermal cracking. In addition to producing lube oils, various vacuum distillates (vacuum gas oils) and vacuum residues are produced. Similar to the atmospheric distillates, some of the vacuum distillates may be hydrotreated or desulfurized to remove sulfur, nitrogen, and other impurities. Most vacuum distillates undergo further processing in order to convert them into higher value fuels (diesel, kerosene). However, a portion of the heavier vacuum distillate streams may be used as blending stocks to reduce the viscosity of the residual streams. The vacuum residual refinery streams comprise a heterogeneous group of poorly defined, viscous, high boiling hydrocarbon streams that usually contain suspensions of resin/asphaltene complexes. These streams often have high levels of heterocyclic aromatic and naphthenic compounds. Varying percentages of sulfur, nitrogen, oxygen, and other elements are present as heterocyclic inclusions, primarily in the aromatics fraction. These residual streams often have a PAC content over 5%, but the content can be much higher depending on the processing the stream has undergone. As shown in Figure 1, there are nine refinery streams in the heavy fuel oils category that are produced by vacuum distillation (seven distillate and two residual streams). See Appendix A for a more detailed description of each of these streams.

Cracking

Many of the distillate and residual streams used to blend heavy fuel oils are derived from cracking processes. Cracking is a process that breaks (“cracks”) the heavier, higher boiling petroleum streams produced by atmospheric or vacuum distillation into lighter molecular weight materials such as gasoline, diesel fuel, jet fuel and kerosene. Often, after cracking, streams may be hydrotreated (reduces nitrogen and aromatic content) or undergo desulfurization.

There are two basic types of cracking processes, those using heat and pressure (thermal cracking) to break molecular bonds, and those using a catalyst (catalytic cracking) to facilitate the cracking process. Both thermal and catalytic cracking are used to produce refinery streams that are used for blending heavy fuel oils.

Visbreaking, coking and steam cracking are types of thermal cracking. In visbreaking, the heavy feedstock is heated under pressure to crack the molecules in the stream. Coking is a severe method of thermal cracking. In steam cracking, the hydrocarbon stream is diluted with steam and then briefly heated (>900 °C) in a furnace. Light hydrocarbon feeds produce streams rich in the lighter alkenes, including ethylene, propylene and butadiene. Heavier hydrocarbon feeds give some of these, but also give products rich in aromatic hydrocarbons. Petroleum pitch, sold as a product for various applications, is a high aromatic residual material produced from either thermal cracking or catalytic cracking.

Catalytic cracking and hydrocracking are two types of catalytic cracking. Catalytic cracking is similar to thermal cracking except a catalyst facilitates conversion of the heavier to lighter products and requires less severe operating conditions than thermal cracking. Catalytic cracking converts heavy paraffins to light paraffins and olefins, heavy naphthenes to light naphthenes and olefins, and heavy aromatics to light aromatics, naphthenes and olefins. As noted above, petroleum pitch is a high aromatic residual material from either catalytic cracking or thermal cracking.

Hydrocracking is a combination of catalytic cracking and hydrogenation, using high pressure, high temperature, a catalyst, and hydrogen. It is typically used for feedstocks that are difficult to process by either catalytic cracking or reforming. When the feedstock has a high paraffin content, the primary function of hydrogen is to prevent formation of PACs. Hydrocracking converts sulfur and nitrogen compounds to hydrogen sulfide and ammonia.

The Testing Group thinks the refinery streams produced by the various cracking processes can be considered as a single subcategory since they represent a continuum in the severity of the cracking process.

All the cracking processes produce refinery streams that are similar from a physical-chemical perspective, being differentiated from each other primarily by the ratio of their unsaturated and saturated hydrocarbon content. While the ratio of the saturated and aromatic hydrocarbons may vary between subcategory members, the saturated and aromatic hydrocarbons species that make up the subcategory members are similar. For instance, refinery streams that are produced by catalytic cracking (most severe) have high levels of aromatics. In contrast, hydrocracked (least severe) streams have relatively low amounts of aromatics, since hydrocracking introduces hydrogen into the cracking process that results in saturation of aromatic compounds. As shown in Figure 1, there are eleven refinery streams in the heavy fuel oils category that are produced by cracking (five distillate and six residual streams). See Appendix A for a more detailed description of each of these streams.

Reforming

Catalytic reforming employs a catalyst to facilitate the structural rearrangement of hydrocarbon molecules in order to increase the aromatic content of a refinery stream, ultimately leading to a gasoline with a higher octane. During reforming, olefins are saturated to form paraffins, which are then converted to shorter paraffins, isoparaffins, and naphthenes. The naphthenes are converted to aromatics by dehydrogenation (Gary and Handwerk, 1994). As shown in Figure 1, there are two refinery streams in the heavy fuel oils category that are produced as residues of reforming. See Appendix A for a more detailed description of each of these streams.

Residual Fuel Oils

In addition to the process streams discussed above, the heavy fuel oil category also includes two blended residual fuel oils. These two fuel oils are most often produced by blending any combination of the distillate and residual streams contained in the seven process subcategories, provided the finished fuel meets the appropriate product specifications. Less frequently, the residual fuels can be blended using residual streams from this category, but petroleum distillates (cutter stocks) covered in other API HPV Test Plans. See Appendix A for a more detailed description of each of these two residual fuel oils.

Analytical data for representative materials in this category are shown in Table 1.

Table 1. Composition of Representative Samples of the HFO Category

Endpoint	Atmospheric Residue	Vacuum Distillate	Cracked Distillate	Cracked Residue	Residual Fuel Oil
CAS No.	64741-45-3	64741-57-7)	64741-81-7	64741-62-4	68476-33-5
Specific gravity	0.9698	0.9285	0.9383	1.0725	0.8491
Refractive index	1.5132	1.515	1.5259	Too dark	1.6306
Distillation (°F)					
IBP	531	548	411	395	340
End point	1041	1131	831	952	1239
Non-aromatics	32.18	40.03	49.6	41.7	55.8
Aromatics (wt %)	67.82	59.97	50.4	58.3	44.2

API, 1987;CONSCI 1992a,b; DataChem 1990

Composition of Heavy Fuel Oil Blending Streams

While detailed compositional information is limited, general compositional information can be inferred from a refinery stream's physical properties and the type of processing it has undergone. For instance, the higher the boiling temperature range of a stream, the higher the molecular weight of the oil's components, the higher the levels of PACs, polycycloparaffins and hetero-atoms (N, O, S, and metals) increase, and the lower the levels of paraffins (see Figure 2). Furthermore, since "cracking" raises the olefin and aromatic content of refinery streams, streams that have been "cracked" have higher olefin and aromatic hydrocarbon content than "straight run" streams that have undergone a limited amount of additional processing. For example, catalytically cracked clarified oil has been reported to contain 58% three to five ring aromatic hydrocarbons (IARC, 1989). Thus, a residual fuel blended using primarily catalytically cracked or steam cracked components will have a higher PAC content (may approach 20%) than a fuel blended primarily of a non-cracked stream, i.e. heavy vacuum gas oil (CONCAWE, 1998). If on the other hand, the blending stocks are predominantly atmospheric or vacuum distillates or residues, the concentration of three to seven ring aromatic hydrocarbons is likely to be of the order of 6-8% (IARC, 1989). The types and levels of PACs found in a specific fuel will depend on the processing the residual portion of the fuel has undergone, and the nature of the blending stream (“cutter” stock) that is used to adjust the viscosity of the finished fuel.

Figure 1. HFO Process Diagram

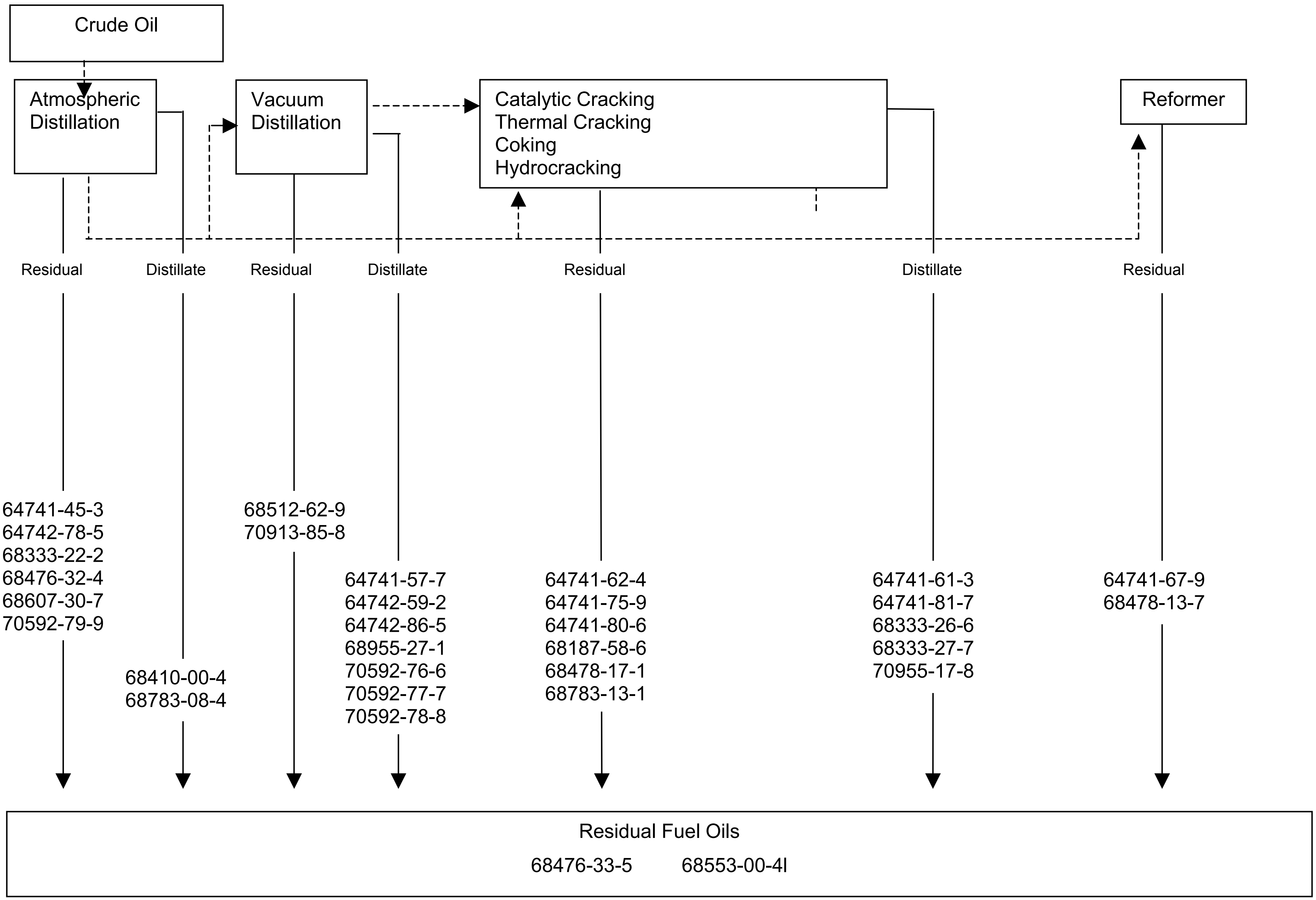
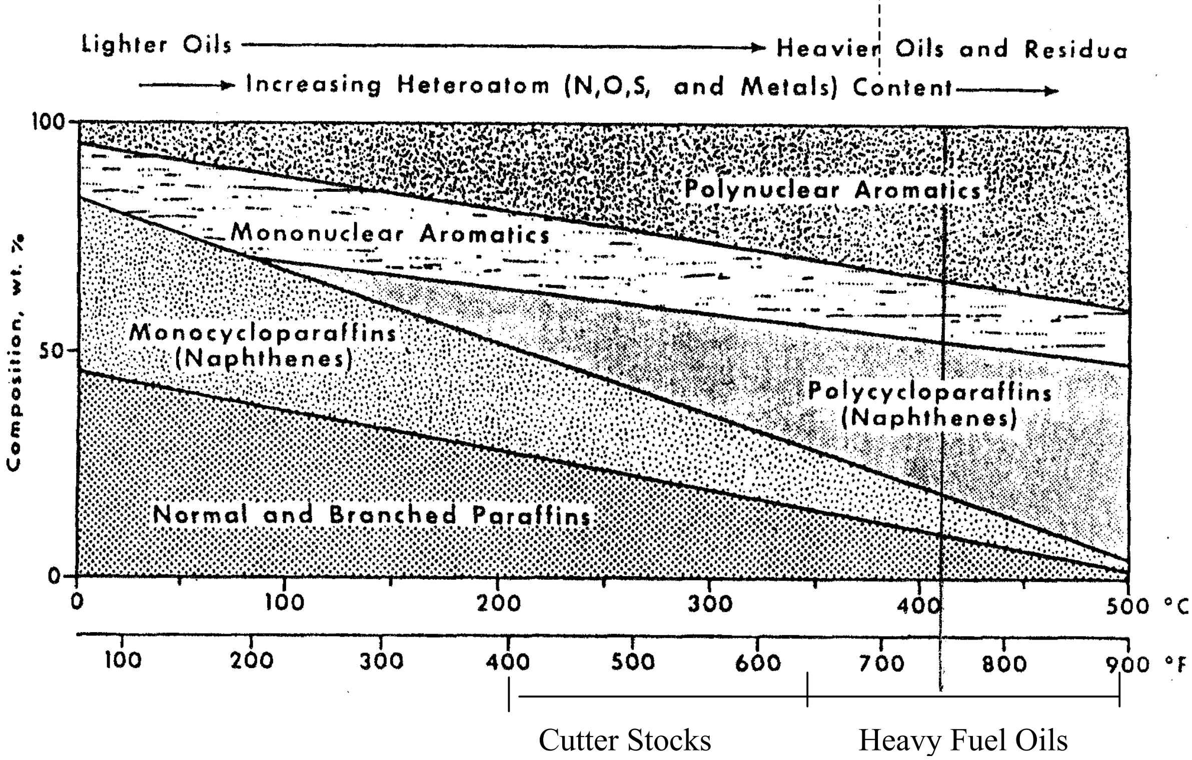


Figure 2. Refinery Stream Composition – Boiling Range vs. General Composition



Speight, 1998

Links to additional resources on refining processes and petroleum-related glossaries are presented in Appendix B.

CATEGORY RATIONALE AND TEST MATERIAL DESCRIPTION

With regard to mammalian toxicity, when analyzing the existing data, proposing testing and identifying potential test materials the Testing Group considered the following:

- The HFO category consists of finished products (residual fuels) and the primary refinery streams from which they are blended;
- The refinery streams in this category that are blended to produce finished heavy fuels can be grouped into seven subcategories according to their process history;
- Refinery streams within a process subcategory are similar from a process and physical/chemical perspective, and are expected to have similar biologic, environmental fate and transport characteristics;
- The range of toxicity of the materials in each process subcategory can be characterized by characterizing representative materials within each subcategory;
- The toxicity of the finished products (Residual Fuel Oils) will fall within the range of toxicities exhibited by the streams blended to produce the finished fuel;
- Because of compositional overlaps and similarities, other API HPV test programs involving gas oils, aromatic extracts, jet fuel/kerosene and asphalt will produce data that will assist in characterizing the materials in this category.

With regard to ecotoxicity, when analyzing the existing data, the Testing Group proposes that a material's solubility (i.e. carbon chain length) determines the degree of ecotoxicity. This is valid and consistent with the current theory on the mode of toxic action for non-polar organic molecules (such as petroleum hydrocarbons) to aquatic organisms (Peterson, 1994; van Wezel and Opperhuizen, 1995). Thus, the category substances are considered as a single group. Furthermore, the streams and products in the heavy fuel oil category are composed of constituent hydrocarbon compounds that also are represented in other petroleum product HPV categories that have substantial ecotoxicity data. Therefore, the Testing Group has employed data from other categories as read across to describe potential ecotoxicity for all streams and products in the heavy fuel oil category.

Because the compositions of the materials in this category vary over time, it is not possible to specify in this Test Plan the exact chemical makeup of the test samples that will be used in the mammalian testing. Specific analytical data on the test samples will be developed and made available when the samples are obtained. For a both the process subcategories and the residual fuel, the Testing Group will attempt to select test sample(s) that are thought to have the highest potential toxicities.

EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

General Evaluation

Reviews of this category of fuels have been published by two expert panels (CONCAWE, 1998; IARC, 1989). In preparing this Test Plan, the approach of the Testing Group has been to review the available toxicology studies and include in the robust summary a detailed description(s) of the one study or a small number of studies that best address each SIDS Level 1 endpoint. Other studies are cited in the appropriate "Remarks" section of the Robust Summary and are intended to supplement the readers' knowledge.

The Test Plan addresses the health effects endpoints of the category by:

- Evaluating the toxicology database for the heavy fuel oil related refinery streams and products,
- Using read-across information whenever possible among category members, and other API HPV categories, and
- Proposing selected testing on representative samples.

HEALTH EFFECTS

Acute Toxicity

The following acute toxicity results have been reported on representative samples of the seven heavy fuel oil subcategories:

Acute Oral Toxicity	LD_{50} value	Species	Observations
Residual Fuel Oils (4 samples)	5.13 - >25 ml/kg_{bw}	Rat	Lethargy; grease on fur
Process Subcategories			
Atmospheric residue	>5000 mg/kg_{bw}	Rat	Stained coats; dark red areas in lung lobes
Atmospheric distillate	No studies available	-	-
Vacuum residue	Data on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) are presented in the API HPV Asphalt Test Plan	-	-
Vacuum distillate (3 samples)	>5000 mg/kg_{bw}	Rat	↓ Activity; chromorhinorrhea, ↓ fecal output; urogenital staining; ↓ urine
Cracked residue	4320♀ 5270♂ mg/kg_{bw}	Rat	Mortalities; hypoactivity; piloerection; staining around mouth, nose,

			urogenital; hair loss; weight loss; intestinal mucosa damage
Cracked distillate	>5000 mg/kg_{bw}	Rat	Oral, nasal discharge; lethargy; abnormal stools; pale & mottled kidneys
Reformer residue	No studies available	-	-
UBTL, 1988,1990b; API, 1980a-d, 1982; Mobil, 1988b,c, 1992a			

Acute Dermal Toxicity	**LD_{50} value**	**Species**	**Observations**
Residual Fuel Oils (4 samples)	>5 ml/kg_{bw}	Rabbit	Erythema; slight congestion of liver
Process Subcategories			
Atmospheric residue	>2000 mg/kg_{bw}	Rabbit	Abnormal stool; dark red areas in lung
Atmospheric distillate	No studies available	-	-
Vacuum residue	Data on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) are presented in the API HPV Asphalt Test Plan	-	-
Vacuum distillate (4 samples)	>2000 mg/kg_{bw}	Rabbit	↓ Food consumption; soft stool; ↓ fecal output
Cracked residue	>2000 mg/kg_{bw}	Rabbit	No signs systemic toxicity; no gross findings
Cracked distillate	>2000 mg/kg_{bw}	Rabbit	Erythema & edema; mottled kidneys
Reformer residue	No studies available	-	-
API, 1980a-d, 1982; UBTL 1989a, 1992a,b			

Dermal Irritation	**Irritation Index**	**Species**	**Observations**
Residual Fuel Oils (4 samples)	0.27 – 1.54	Rabbit	Minimal – slight irritant
Process Subcategories			
Atmospheric residue	3.5	Rabbit	Moderately irritating
Atmospheric distillate	No studies available	-	-
Vacuum residue	0.18	Rabbit	Not irritating
Vacuum distillate (4 samples)	1.2 – 3.6	Rabbit	-
Cracked residue	0.2	Rabbit	-
Cracked distillate	5.6	Rabbit	Moderately irritating
Reformer residue	No studies available	-	-
UBTL, 1989d,e, 1992 d; API 1980a-d, 1982; Mobil, 1988a,b c, 1992a			

Eye Irritation	**Irritation Indices**	**Species**	**Observations**
	24 & 72 hr		
Residual Fuel Oils (4 samples)	2.67 – 7.67 & 0 – 1.33 rinsed 4.0 – 7.33 & 0.0 – 1.33 un-rinsed	Rabbit	Minimal – mild irritant
Process Subcategories			

Atmospheric residue	0.0 & 0.0 un-rinsed only	Rabbit	Not irritating
Atmospheric distillate	No studies available	-	-
Vacuum residue	5.0 & 4.7 un-rinsed 5.7 & 4.7 rinsed	Rabbit	Un-rinsed - not irritating – Rinsed - minimal irritant
Vacuum distillate (4 samples)	2.0 – 10.3 & 1.7* – 3.3*	Rabbit	Un-rinsed only * 48 hr score
Cracked residue	2.0 & 0 rinsed & un-rinsed	Rabbit	Minimal irritant
Cracked distillate	5.7 & 0.0 un-rinsed 5.3 & 0.0 rinsed	Rabbit	Un-rinsed - not irritating Rinsed – not irritating
Reformer residue	No studies available	-	-
UBTL, 1989f,g, 1991 API, 1980a-d, 1982; Mobil, 1988a,b, c, 1992a			

Sensitization	Challenge Response	Species	Observations
Residual Fuel Oils (4 samples)	-	Guinea Pig	3 samples non-sensitizer 1 sample mild sensitizer
Process Subcategories			
Atmospheric residue	0/10	Guinea Pig	Non-sensitizer
Atmospheric distillate	No studies available	-	-
Vacuum residue	0/10	Guinea Pig	Non-sensitizer
Vacuum distillate	1/10	Guinea Pig	Non-sensitizer
Cracked residue	0/10	Guinea Pig	Non-sensitizer
Cracked distillate	0/10	Guinea Pig	Non-sensitizer
Reformer residue	No studies available	-	-
API, 1980a-d, 1984; UBTL, 1986,1989b,c; 1990c, 1992c			

Summary: No additional testing is planned. Multiple acute toxicity studies have been reported on a number of representative samples of the heavy fuel oil refinery streams as well as on finished residual fuels. These data and data from the other 12 API HPV Test Plans show the acute toxicity of a wide-array of petroleum hydrocarbon streams is consistently low, with many oral LD50's greater than 5 g/kg, and dermal LD50s greater than 2 g/kg. While no data is presented for the vacuum residue process subcategory, the Testing Group thinks existing acute toxicity data on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) presented in the API HPV Asphalt Test Plan support the Group's position that this subcategory of materials would have low acute toxicities. Similarly, although no data is presented for the atmospheric distillate or reformer residue subcategories, materials produced by these processes have been considered in other API HPV Test Plans that have consistently shown the acute toxicities of the materials to be low. Consequently, the Testing Group thinks the acute toxicities of these materials can be characterized by "Read Across" from materials other API HPV Test Plans.

Repeat-Dose Toxicity

Studies of up to 13 weeks duration have been reported for representative streams in a number of the process subcategories within the heavy fuel oil category. In instances where there were multiple studies available for a subcategory, only the study of the longest duration was fully summarized in the attached Robust Summary (Appendix C).

Residual Fuel Oils

Dose levels of 0.5 (496 mg), 1.0 (992 mg), or 2.5 (2480 mg) ml/kg_{bw}/day of a heavy fuel oil (CAS no. 668476-33-5) were applied undiluted to the skin of male and female rats (UBTL, 1987). The test material was applied 5x/week for 4 weeks. The test material produced minimal reversible dermal irritation at all dose levels. Daily observations of the animals found no compound-related effects other than staining at the site of application. Similarly, there were no compound-related findings at necropsy other than the staining of the skin produced by the test article.

Eosinophil counts were significantly lower for the mid-dose and high-dose males. SGPT levels were significantly lower for the low- and high-dose females and the high-dose males. Glucose levels were significantly higher for the mid- and high-dose females and high-dose males. Total protein levels were significantly lower for the low-dose males. Hemoglobin levels were significantly lower for the high-dose males. Upon comparison and review of historic data, the study directors concluded the significant values obtained from the hematology or clinical chemistry assays were within normal limits and did not exhibit any clear dose-related trends.

Relative liver weights were significantly higher for the females in all dose groups and in the high-dose males. With the exception of the liver/brain weight ratios in the low-dose males, liver/body weight and liver/brain weight ratios were significantly higher for both sexes in all dose groups. The liver weight changes were thought by the study directors to be compound-related. Spleen/body weight ratios were significantly higher for the low and mid-dose females and the high-dose males. The spleen/brain weight ratios were significantly higher for the low-dose females and the high-dose males. The changes in relative spleen weights were not thought to be dose-related by the study directors.

Microscopic examination of selected tissues from the control and high dose groups found no compound-related effects other than hyperkeratosis (minimal severity) at the test compound application site.

Process Subcategories

Atmospheric Residue

Dose levels of 0.01 (9 mg), 0.25 (231 mg) or 1.0 (927.9 mg) ml/kg_{bw}/day of an atmospheric residue (CAS no. 64741-45-3) were applied undiluted to the skin of male and female rats (UBTL, 1990a). The test material was applied 5x/week for 4 weeks. There were no clinical observations made that were considered to be treatment-related. The only treatment-related finding at gross necropsy was a dark staining of the treated skin site. There were no compound-related effects on either hematology or clinical chemistry values. Nor were there any treatment-related differences in body weights or organ weights or organ/body weight ratios. The only treatment-related histopathological findings occurred in the skin and these consisted of trace to mild acanthosis and trace to moderate hyperkeratosis in the high dose animals. The study authors concluded that there were no systemic effects at the highest dose level tested.

Atmospheric Distillates

Dose levels of 30, 125 or 500 mg/kg_{bw}/day of a heavy atmospheric gas oil (CAS no. 68783-08-4) were applied undiluted to the skin of male and female rats (Mobil, 1992c). The test material was applied 5x/week for 13 weeks. To assess male reproductive health, a satellite group of 10 males was administered 500 mg/kg_{bw}/day of the gas oil. At the end of the study the epididymides and testes from the male rats in the control and 125 mg/kg_{bw}/day groups were given an in-depth histopathology examination, including spermatid (testes) and spermatozoa (epididymides) counts. In general, application of the test material produced only “slight” skin irritation. One of ten high dose males was sacrificed *in extremis*, the investigators considered the death to be treatment-related. There were treatment-related changes in a number of serum chemistry and hematological parameters in the rats in the mid- and high dose groups. At necropsy, treatment-related macroscopic findings in both sexes included increased liver size, decreased thymus size, thickening of the limiting ridge between the non-glandular and glandular sections of the stomach and enlarged and reddened lymph nodes. There were some organ weight (absolute and relative) differences in the 125 and 500 mg/kg_{bw}/day groups. The histopathology examination found treatment-related changes only in animals in the 500 mg/kg_{bw}/day groups. These changes included a severe reduction in hematopoiesis in the bone marrow; liver hypertrophy and connective tissue formation; increased areas of hematopoiesis, focal necrosis and individual cell death in the liver; and a reduction in the numbers of lymphocytes in the thymus glands. There were no treatment-related effects on any of the epididymal sperm or testicular spermatid parameters. The investigators concluded the no-observable-effect level (NOAEL) for the study was 30 mg/kg_{bw}/day.

Vacuum Residue

Data on repeat-dose toxicity studies in rabbits on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) are presented in the API HPV Asphalt Test Plan.

Vacuum Distillate

An undiluted heavy vacuum gas oil (CAS no. 64741-57-7) was applied to the skin of male and female rats 5 days each week for 13 weeks (Mobil, 1988d). Dose levels were 30, 125, 500 & 2000 mg/kg_{bw}/day. The unscheduled deaths of two of ten males in the high dose group were considered to be compound-related. Growth rates of males and females in the highest dose group were reduced compared to controls. At five and thirteen weeks, the 2000 mg/kg_{bw}/day dose group had reduced erythrocytes and platelets. Similar reductions were also found in the 500 mg/kg_{bw}/day females. Changes in several clinical chemistry values were also seen in the 2000 mg/kg_{bw}/day dose group. In addition in females dosed with 500 mg/kg_{bw}/day had reduced serum glucose levels, while in the 500 mg/kg_{bw}/day males, cholesterol was increased.

At gross necropsy, in the 500 and 2000 mg/kg_{bw}/day dose groups, relative thymus weights were reduced while relative liver weights were increased. Histological examination revealed decreased erythropoiesis and fibrosis of the bone marrow in the 2000 mg/kg_{bw}/day males. There was a reduction in thymic lymphocytes in both sexes in the 2000 mg/kg_{bw}/day group. Examination of the testes of animals in the 2000 mg/kg_{bw}/day dose group found no compound-related effect on sperm morphology. The investigators found the NOEL for both males and females was 125 mg/kg_{bw}/day.

Cracked Residue

Dose levels of 8, 30,125, 500 and 2000 mg/kg_{bw}/day of a clarified slurry oil (CAS no. 64741-62-4) were applied undiluted to the skin of male and female rats (Cruzan et al., 1986; Mobil, 1985b). The test material was applied 5x/week for 13 weeks. There was an adverse, dose-related effect on mortality, with none of the rats in the highest dose group (2000 mg/kg/day) surviving past the second week of the study. Compound-related effects were observed on body weights, and several hematology and clinical chemistry parameters. Based on these changes, and histopathology findings, the target organs of toxicity were judged by the study directors to be the liver, thymus and bone marrow. The investigators concluded that none of the dose levels used in the study represented a no-observable-adverse- effect level (NOAEL).

Cracked Distillate

Dose levels of 8, 30 and 125 mg/kg_{bw}/day of a visbreaker gas oil (CAS no. 68471-81-7) were applied undiluted to the skin of male and female Sprague-Dawley rats (Feuston et al., 1994; Mobil, 1992b). The test material was applied 5x/week for 13 weeks. There were no deaths during the study. No clinical signs of toxicity were observed, with the exception of dose-related skin irritation. There were no compound-related effects on body weights or hematology and clinical chemistry values. Urinalysis found no treatment-related effects. At necropsy, the only treatment-related findings were effects on the skin and enlarged lymph nodes (the latter predominantly in the higher dose groups). Microscopic examination of the skin revealed thickened epidermis with parakeratosis, chronic inflammation in the subcutis, ulcers and increased mitosis in the epidermal basal cells. The skin changes were more severe in females than the males. Lymph nodes were enlarged in the high dose animals and in most instances, microscopic examination revealed non-specific reactive hyperplasia. Epididymides and testes from the male rats in the control and 125 mg/kg_{bw}/day groups were given an in-depth histopathology examination, including spermatid (testes) and spermatozoa (epididymides) counts. Treatment with visbreaker gas oil did not cause any changes in testicular spermatid or epididymal spermatozoa count nor in sperm morphology. The investigators concluded the NOAEL in the study was > 125 mg/kg_{bw}.

Reformer Residue

No studies available.

Carcinogenicity

In addition to the repeat-dose studies discussed above, several dermal carcinogenicity studies on samples of cracked residual streams have been reported (API, 1989; McKee et al., 1990; Smith et al., 1951). Although carcinogenicity is not a required endpoint of the HPV program, the Testing Group believes the results may be useful in evaluating the repeated-dose endpoint. A tabulation of these studies can be found in section 5.7 of the attached Robust Summary (Appendix C).

Summary: The Testing Group proposes to test a representative sample of a reformer residue stream by the dermal route of administration using a 28-day repeated-dose/reproductive/developmental toxicity

screening protocol (OECD Test Guideline 422). No repeat dose information exists on the reformer residue streams. While no data is available for the vacuum residue process subcategory, the Testing Group thinks these materials can be characterized by "read across" from the data on the vacuum residue streams found in the API HPV Asphalt Test Plan.

***In-Vitro* (Mutagenicity)**

Subcategory	Assay	Results
Residual Fuel Oils	No studies to report	-
Process Subcategories		
Atmospheric residue	No studies to report	-
Atmospheric distillate	No studies to report	-
Vacuum residue	Mouse lymphoma - data on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) are presented in the API HPV Asphalt Test Plan	Weakly positive with activation
Vacuum distillate	Modified Ames–	Positive with activation
	Cytogenetic assay with Chinese Hamster Ovary cells	Negative with/without activation
Cracked residue	Modified Ames	Positive with/without activation
	Mouse lymphoma	Positive with/without activation
	Sister chromatid exchange	Positive with/without activation
	Cell transformation	Negative without activation Positive with activation
	Unscheduled DNA synthesis	Positive
	Bacterial forward mutation	Negative with/without activation
Cracked distillate	No studies to report	-
Reformer residue	No studies to report	-
		API, 1985a,b, e, f, 1986b,c; Mobil, 1985a, 1987e

Summary: The Testing Group is proposing to perform a modified Ames and chromosomal aberration (OECD 473) assays on representative samples of a residual fuel oil, atmospheric residue, atmospheric distillate, cracked distillate and reformer residue.

***In-Vivo* (Chromosomal Aberrations)**

Subcategory	Assay	Species	Results
Residual Fuel Oils	No studies to report	-	-
Process Subcategories			
Atmospheric residue	No studies to report	-	-
Atmospheric distillate	No studies to report	-	-
Vacuum residue	Cytogenetic Assay - data on two vacuum residuum samples (CAS no. 64741-56-6, API samples 81-13 and 81-14) are presented in the API HPV Asphalt Test Plan	Rat (♀, ♂)	Negative at up to 3 g/kg/day by gavage for 5 days
Vacuum distillate	Micronucleus	Rat (♀, ♂)	Negative at up to 2000 $mg/kg_{bw}/day$; 5x/week for 13 weeks via dermal route
Cracked residue	Cytogenetic Assay	Rat (♀, ♂)	Negative at up to 1 g/kg/day by

			gavage for 5 days
	Sister Chromatid Exchange	Mice (♀, ♂)	Single IP dose - positive at 4.0 (♀, ♂) & 2.0 (♂) g/kg_{bw}; negative at 0.4 g/kg_{bw}
	Unscheduled DNA Synthesis	Rat (♂)	Positive at 100 & 1000 mg/kg_{bw} by gavage; negative at 50 mg/kg_{bw}
Cracked distillate	No studies to report	-	-
Reformer residue	No studies to report	-	-
			API, 1985c,d, e; Mobil, 1987f

Summary. No additional *in-vivo* testing is planned. Adequate studies exist on the vacuum distillate and cracked residue subcategories. The Testing Group thinks results from the API HPV Gas Oils test plan can be "read across" to the atmospheric distillate subcategory given the similar process history. Similarly, the Testing Group thinks results on vacuum residue streams from the API HPV Asphalt test plan can be "read across" to characterize the vacuum residue subcategory. Given the high aromatic content of the cracked distillates and reformer residues, the Testing Group would expect these types of materials would produce positive results in the Ames assays. Other materials with high PAC content (aromatic extracts) have produced negative results in *in vivo* chromosomal aberration assays. If the *in vitro* assays are positive, the Testing Group will consider additional *in vivo* testing.

Reproductive/Developmental Toxicity

Residual Fuel Oils
No studies to report.

Process Subcategories

Atmospheric Residue
In a developmental screening study, dose levels of 50, 333, & 1000 mg/kg_{bw}/day of an atmospheric residue (CAS no. 64741-45-3) were applied to the skin of presumed-pregnant female rats on days 0-20 of gestation (UBTL, 1994). There were no deaths or treatment-related clinical effects amongst the dams. The study directors considered decreased body weight changes and the increase in gestation length at a dose of 1,000 mg/kg to be signs of compound-related maternal toxicity. Signs of developmental toxicity considered by the study directors to be compound-related included decreased pup body weights on Lactation Days 0 and 4 at a dose of 1,000 mg/kg. The report authors concluded that for maternal toxicity and signs of developmental toxicity the no-observable-adverse-effect level (NOAEL) was 333.0 mg/kg_{bw}/day.

Atmospheric Distillate
A developmental toxicity screening study has been reported on a heavy atmospheric gas oil (CAS no. 68783-08-4) (Mobil, 1991). Undiluted test material was applied daily on days 0 to 19 of gestation to the clipped skin of resumed-pregnant female rats. Dose levels included 8, 30, 125 and 500 mg/kg_{bw}/day. Signs of maternal toxicity considered by the study director to be related to administration of the test material included effects on body weights, body weight gain, food consumption, thymus weights (absolute & relative), liver weights (relative), and a number of clinical chemistry and hematological parameters. A red vaginal discharge (normally indicative of litter resorption) was observed in 7/11 animals in the 500 mg/kg_{bw}/day group and two females dosed with 125 mg/kg_{bw}/day. The investigators could not decide if the vaginal discharge was treatment-related since a similar observation had been noted in control animals. Evaluation of reproductive parameters in the 8 and 30 mg/kg_{bw} found no compound-related effects. Statistically insignificant differences in preimplantation losses were seen in both the 125 and 500 mg/kg_{bw}/day groups. There was a significant increase in the mean number/percent resorptions in the 500 mg/kg_{bw}/day group. Mean fetal body weights were significantly decreased for all viable fetuses in the 500 mg/kg_{bw}/day group and in the male pups of the 125 mg/kg group. There was a significant increase in incomplete ossification of a number of skeletal structures (nasal bones, thoracic centra, caudal centra, sternebrae, metatarsal and pubis) in the 125 and 500 mg/kg_{bw}/day groups. There were no treatment-related abnormalities found in the soft tissues. Exposure to gas oil did not adversely affect pup survival or development. The investigators

concluded the no-observable-adverse -effect levels (NOAELs) for both maternal toxicity and teratogenicity were 30 $mg/kg_{bw}/day$.

Vacuum Residue
No studies to report.

Vacuum Distillate
In a developmental screening study, dose levels of 30, 125, 500 and 1000 $mg/kg_{bw}/day$ of a heavy vacuum gas oil (HVGO, CAS no. 64741-57-7) were applied to the skin of presumed-pregnant female rats (Mobil, Undated). The test material was administered on days 0 to 19 of gestation. All animals were euthanized on day 20. Clinical signs of maternal toxicity considered by the study authors to be compound-related were seen primarily in the 1000 $mg/kg_{bw}/day$ dose group. In the dams, the only treatment-related findings seen at necropsy were lungs that had a pale appearance (500 and 1000 $mg/kg_{bw}/day$) and a reduction in thymus size. Organ weight data confirmed that the thymus weights in the highest dose group were reduced and furthermore, relative liver weights were increased in the 500 and 1000 mg/kg/day animals. A number of reproductive parameters were affected by treatment with HVGO. Fetuses of dams in both the 500 and 1000 $mg/kg_{bw}/day$ dose groups had treatment-related decreases in body weights. Upon external examination, one fetus in the 1000 $mg/kg_{bw}/day$ group was found to be edematous, pale in color, with both hind paws malformed. Malformations of the vertebral columns were observed in several fetuses, but only in the 500 $mg/kg_{bw}/day$ group. While a variety of skeletal malformations were observed in both treated and control groups, the degree of malformation was more severe in the HVGO-exposed groups. Visceral malformations were restricted to two fetuses in the 500 $mg/kg_{bw}/day$ group. Of the two fetuses, one had microphthalmia and the other had a diaphragmatic hernia that displaced the heart from the left- to right-hand side. The study authors concluded the no-observable-adverse-effect levels (NOAELs) for both maternal and fetal toxicity were 125 $mg/kg_{bw}/day$.

Cracked Residue
A developmental toxicity screening study has been reported on a clarified slurry oil (CSO, CAS no. 64741-62-4) (Hoberman et al., 1995). Undiluted test material was applied daily on days 0 to 19 of gestation to the clipped skin of resumed-pregnant female rats. Dose levels included 0.05, 1.0, 10, 50 or 250 $mg/kg_{bw}/day$. No deaths occurred among the dams and no dam aborted or prematurely delivered a litter. Neither maternal toxicity nor fetal developmental effects were seen at the 0.05 $mg/kg_{bw}/day$ dose level. Nor were any fetal malformations or variations observed in the 0.05 $mg/kg_{bw}/day$ group. Dose-related signs of maternal toxicity were seen at dose levels greater than 0.05 $mg/kg_{bw}/day$. The effects included decreased food consumption and decreased body and gravid uterine weights, and the occurrence of red vaginal exudates. Fetal developmental effects, as measured by number of live fetuses, total resorptions, early resorptions, % dead or resorbed conceptuses/litter and fetal body weights were seen at doses that were maternally toxic. There were no treatment-related incidences of fetal malformations. However, increased incidences of fetal variations that are generally interpreted as reversible delays in development associated with significant decreases in body weight were produced in fetuses in the 1.0, 10 and 50 $mg/kg_{bw}/day$ dose groups. These variations included moderate dilation of the renal pelvis, slight dilation of the lateral ventricles of the brain, bifid thoracic vertebral centrum and decreased average numbers of ossified caudal vertebrae, metacarpals and hindpaw phalanges. The study authors concluded the no-observable-adverse-effect levels (NOAELs) for both maternal and fetal toxicity were 0.05 $mg/kg_{bw}/day$.

To determine potential effects on gonadal function, reproductive organs and mating behavior, 0.1, 1, 10, 50 & 250 mg/kg/day of a clarified slurry oil (CAS no. 64741-62-4) were applied dermally to male rats for 70 days before a seven-day cohabitation period with untreated virgin female rats (Argus, 1992). Female rats were examined daily for viability and clinical observations, and body weights were recorded on days 0, 6 and 14 of presumed gestation. On day 14 of presumed gestation, the female rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic and abdominal viscera was performed. The uterus of each rat was examined for pregnancy, number and distribution of implantations, early resorptions and live and dead embryos. No deaths and no skin reactions were caused by the test material. All absolute and relative organ weights were comparable among the six dosage groups. There were compound-related effects on body weights, body weight gains, and food consumption. Mating and fertility parameters were unaffected at any of the dose levels. There were no compound-related effects on any testicular parameter.

Litter averages for corpora lutea, implantations, and live embryos and resorptions did not significantly differ among females mated with male animals from the six dosage groups. There were no dead embryos, and no dam resorbed all conceptuses. The study directors concluded that the paternal no-observable-adverse-effect-level (NOAEL) was 1 mg/kg/day. The reproductive NOAEL for the male rats was >250 mg/kg/day (no mating, fertility or testicular parameters in the male rats were affected by the highest dosage tested).

In a reproductive/developmental toxicity screen, female Sprague-Dawley rats were administered catalytically cracked clarified oil (CAS no. 64741-62-4) at dose levels of 0.05, 10, 250 mg/kg/day (UBTL, 1994). The test material was administered one week prior to the initiation of mating, throughout mating, and through Day 20 of gestation. Male rats to which the females were mated were not administered test compound. Food consumption and body weights were recorded throughout the premating, mating and gestation periods. On Day 4 of lactation, each female was sacrificed and the ovaries and uterine horns examined to determine the number of corpora lutea and implantation sites, respectively. Litters were observed during Days 0 –4 of lactation for signs of toxicity and mortality. Pups were examined daily for external abnormalities. On Days 0 and 4 of lactation, each pup was weighed and its sex was determined. On day 4 of lactation, all surviving pups were examined externally, sacrificed and discarded. No deaths occurred during the study. The study directors considered the following signs of maternal toxicity to be related to administration of the test material: a higher incidence of vaginal discharge at a dose of 250 mg/kg; decreased body weights, body weight changes, and food consumption at doses of 10 and 250 mg/kg; and decreased thymus size at a dose of 250 mg/kg. Signs of developmental toxicity considered to be compound-related were limited to the 250 mg/kg dose group; none of the females in this dose level delivered a litter. The study directors concluded the no-observable-adverse-effect levels (NOAEL) were 0.05 mg/kg for maternal toxicity and 10 mg/kg for signs of developmental toxicity.

Cracked Distillate

Doses of 8, 30, 125 and 250 mg/kg_{bw}/day of a heavy coker gas oil (CAS no. 64741-81-7) were applied daily to the skin of resumed-pregnant female rats in a developmental screening study (Mobil, 1987d). Animals were dosed on days 0-19 of gestation. All animals were euthanized on day 20. Treatment-related clinical observations in the dams consisted of erythema, flaking, scabbing, edema, eschar and fissuring and the occurrence of a red vaginal discharge. Eschar and fissuring occurred in the highest two dose groups only. Vaginal bleeding was seen in the groups receiving doses of 30 mg/kg_{bw}/day and higher. There was a dose-related decrease in mean body weight gains over the course of the experiment. At necropsy, the only treatment-related observation in the dams was an apparent reduction in thymus size, which was noted at all treatment levels. Absolute thymus weights were decreased, while absolute liver weights were increased. A number of clinical chemistry values were affected, but only at the highest dose of 250 mg/kg_{bw}/day. Select reproductive parameters were adversely affected, but only in the 125 and 250 mg/kg_{bw}/day groups. Viable and non-viable fetuses from these two dose levels were observed to have signs of abnormal external development, including reduced (shortened) lower jaws and edema. Visceral anomalies seen in these same two dose groups included displacement of esophagus from a left-sided to a right-sided position and distension of the ureters. Malformations of the vertebral column were restricted to fetuses of dams exposed to the test material. Although signs of aberrant development were observed in control animals, the degree of the observed effects was not as severe in the control groups as the groups exposed to test material. The authors concluded that the no-observable-adverse-effect-level for maternal and fetal toxicity was 30 mg/kg_{bw}/day.

Reformer Residue

No studies to report

Summary: The Testing Group proposes to test a representative sample of a residual fuel oil by the dermal route of administration using a reproductive/developmental toxicity screening protocol (OECD Testing Guideline 421). The Testing Group proposes to test a representative sample of a reformer residue by the dermal route of administration using a 28-day repeat-dose/reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). The Testing Group thinks the existing data on refinery streams in the process subcategories allows the developmental toxicity of these materials to be characterized. While no data exists for the vacuum residue subcategory, the Testing Group believes the physical nature of these materials (very viscous) will limit the bioavailability of any potentially biologically significant components. Furthermore, data is available for atmospheric residuum, a similar material, which because of its lower boiling point will contain higher levels of these potentially biologically significant components. Therefore, the Testing Group is not proposing any testing for this subcategory of materials. No reproductive or developmental data is available for the reformer residue streams. However, the Testing Group has already proposed a repeat-dose study on a reformer residue. The Testing Group is proposing to maximize the utility of the study by conducting a 28-day repeated-dose/reproductive/developmental toxicity screen (OECD Test Guideline 422). This will provide data not only on the repeat-dose toxicity of this subcategory of materials, but also on their reproductive/ developmental toxicity thereby adding to the extensive database that exists on these materials.

While none of the developmental studies summarized in this test plan provide data on pre-mating exposure of males or females, the existing data adequately characterize the reproductive endpoint for these materials. Repeat-dose studies on samples of atmospheric distillates, vacuum distillates, and cracked distillates have included an in-depth histopathological examination of reproductive organs. Furthermore, studies have been reported on catalytically cracked clarified oil (cracked residue) in which pre-mating exposures of males or females were conducted. Catalytically cracked clarified oil has produced the lowest maternal no-observed-adverse-effect-level (NOAEL) in the developmental screens that have been performed. The Testing Group expects the reproductive toxicity of the other materials in this category to be less than that of catalytically cracked clarified oil.

The lack of reproductive/developmental toxicity data on a finished residual fuel suggests the need for a reproductive/developmental screen on a representative sample of a residual fuel. The Testing Group is proposing a reproductive/developmental toxicity screen (OECD Test Guideline 421) of a residual fuel in order to develop a complete data set on a finished product and to test its hypothesis that the toxicity of the finished fuel will fall within the range of the toxicities of the streams from which the fuel is blended. To that end, the Testing Group will select a residual fuel test sample that is well characterized with respect to the components from which it was blended. The goal will also be to select a residual fuel oil sample that has a significant portion of cracked residues since these streams appear to show the highest potential for toxicity within this category.

EVALUATION OF EXISTING PHYSICO-CHEMICAL AND ENVIRONMENTAL FATE DATA AND PROPOSED TESTING

The physicochemical endpoints for the EPA HPV chemical program include melting point, boiling point, vapor pressure, octanol/water partition coefficient (log K_{ow}), and water solubility. Environmental fate endpoints include photodegradation, hydrolysis, environmental transport and distribution (fugacity), and biodegradation. Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured data have been identified and a technical discussion provided, where appropriate. The EPIWIN© (EPA, 2001) computer model, as discussed in the U.S. EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program" has been used to calculate physical-chemical properties of representative constituents of heavy fuel oils (EPA, 1999).

The substances covered under this HPV testing plan are mixtures of differing compositions. Because of the diversity of compounds encompassing heavy fuel oils, it is not feasible to model the physicochemical endpoints for each potential compound. Where modeling was necessary to fulfill an endpoint, such estimates were made for common hydrocarbon structures (e.g., saturated, aromatic, olefinic and heterocyclic hydrocarbons) and range of molecular weight hydrocarbons (i.e., number of carbon atoms) known to be represented in heavy fuel oil mixtures. Since molecular weight and structural conformation determine in large part many of the physico-chemical and fate processes, the modeled estimates for these isomeric structures are expected to represent potential ranges of values for all substances in the heavy fuel oil category.

Physico-Chemical Data

Melting Point

For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe the physical phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM, 1999).

Measured pour points ranged from –2 °C to 35 °C (CONSCI, 1992a,b; CONSCI, 1993a-d; Jokuty et al., 2002; NIPER, 1993a,b). Those values are consistent with the review by CONCAWE (1998), which stated pour points for these products are typically <30 °C. The differences in flow characteristics of heavy fuels are related to the composition of the crude oil from which they are produced, the variety of refining practices that result in products that go into heavy fuels, and the practice of adding a lighter "cutter stock" to heavy fuel oils to improve their flowable characteristics at low temperatures. Based on the cited data, heavy fuel oil streams and products will exist at ambient temperatures as dense, viscous oil. Those category members with the highest pour points will be characteristically solid to semi-solid substances, while those with the lowest pour points will have the greatest liquefaction.

Summary: No additional testing is proposed. The pour points of various heavy fuel oils have been adequately measured.

Boiling Point

For complex petroleum mixtures, boiling points are expressed as ranges (i.e., distillation ranges) that are governed by the individual constituent hydrocarbons within the mixtures. Constituent molecules have individual boiling points, and boiling points increase as molecular weight increases. Streams within the Heavy Fuel Oil HPV category are highly diverse mixtures, and are produced from a variety of refining processes; processes including both atmospheric and vacuum distillation; therefore, the boiling ranges of the streams also would be expected to vary widely. Based on CAS definitions for the refining streams in this category, boiling points can range from 121 to 600 °C (EPA, 2004). Actual boiling ranges for any of the heavy fuel oil streams will depend upon source of the feedstock and the refining process. Boiling ranges reported in various literature sources such as material safety data sheets (Sinclair Oil Corp; Total UK Ltd., 2003; and TFMM, 2001), government-sponsored databases (ECB, 2000; Jokuty et al., 2002), and the American Petroleum Institute (API, 1987) lend support to the CAS-defined boiling range.

Summary: No additional testing is proposed. Products within this category vary with respect to their distillation ranges and specific ranges depend upon the hydrocarbon composition of the feedstocks from which they are produced. Values cited provide typical ranges for the distillation of these materials.

Vapor Pressure

Few vapor pressure measurements are available for heavy fuel oils; therefore, the EPIWIN (EPA, 2001) computer model was used to estimate vapor pressures for representative components of these substances. For heavy fuel oil mixtures, the total vapor pressure is the sum of the partial pressures of the individual components (Dalton's Law of Partial Pressures), while the partial pressure of an individual constituent is a product of the vapor pressure of the pure material times its mole fraction in the mixture (Raoult's Law). Therefore, for complex mixtures such as heavy fuel oils, the partial pressures of individual constituents may be quite low.

The heavy fuel oil category is composed of many different refining streams, and the vapor pressures of such substances will vary with the source of the crude and the refining method used. Measured values of <100 Pa (Total UK Ltd, 2003) and >500 Pa (ECB, 2000) for two heavy fuel oil streams reflected this variability. Vapor pressure estimates made using EPIWIN© version 3.10 (EPA, 2001) for constituent hydrocarbons representing paraffinic, olefinic, naphthenic, aromatic, and polar/heterocyclic components in heavy fuel streams bounded the measured data. Vapor pressure estimates of low molecular weight hydrocarbons (C7 – C11) of varying isomeric structures ranged from 7 Pa to 9×10^{3} Pa. As molecular weights increased, vapor pressure estimates decreased. Estimates for the high molecular weight constituents (C38 – C50) ranged from 2×10^{-7} Pa to 5×10^{-17} Pa. The modeled data represent a potential vapor pressure range for the hydrocarbon constituents expected to occur in all subcategories of heavy fuel oils.

Summary: No additional testing is proposed. The vapor pressures of representative hydrocarbon constituents of heavy fuel oils have been adequately characterized. Measured data reflect the variability expected for substances in this category.

Partition Coefficient

In mixtures such as the heavy fuel oils, the percent distribution of the hydrocarbon groups (i.e., paraffins, naphthenes, olefins, aromatics, polar/heterocyclics) and the carbon chain lengths determines in-part the partitioning characteristics of the mixture. Generally, hydrocarbons with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). However, due to their complex composition, unequivocal determination of the log K_{ow} of the hydrocarbon mixtures cannot be made. Rather, partition coefficients of representative hydrocarbon types covering the range of carbon atoms found in substances in this category were modeled using the EPIWIN©, version 3.10 computer model (U.S. EPA, 2001).

The isomeric structures and numbers of carbon atoms were selected because they are representative of the types and molecular weights of hydrocarbon compounds found in heavy fuel oils. Therefore, the estimated values represent the potential range of values for partition coefficient for hydrocarbon constituents in this category. The estimates show log K_{ow} values to range from 1.7 to 25. Standardized methods for measuring partition coefficient are analytically limited to substance up to log Kow ~4, or occasionally 5 (OECD, 1995). An estimation method is available for log Kow up to 6 (OECD, 1989). Based on the estimated values determined by EPIWIN©, available analytical methods begin to fail for hydrocarbon compounds that contain roughly 15 to 20 carbon atoms. The modeled data represent a potential log Kow range for the hydrocarbon constituents expected to occur in all subcategories of heavy fuel oils.

Summary: No additional modeling is proposed. Partition coefficients (K_{ow}) of 1.7 to 25 were calculated for representative hydrocarbon components of heavy fuel oils.

Water Solubility

When released to water, dissolution of the water-soluble constituents in heavy fuel oils will depend upon environmental factors affecting the mixing and weathering of the substance. Lower molecular weight components that have the highest solubilities also have appreciable vapor pressures and thus would tend to both dissolve and volatilize from the surface. Under controlled conditions with minimal opportunity for volatilization, the water solubility of heavy fuel oil no. 6 was measured to be 6.26 mg/l (Shiu et al., 1990). Additional supporting data gave a range of water solubilities from 0.4 to 6.3 mg/l (Suntio et al., 1986; Anderson et al., 1974; MacLean and Doe, 1989). Because blended fuels are composed of the refining streams from which they are produced, these solubility values are expected to approximate the water solubility for all substances within the heavy fuel oil subcategories. Specific solubility values for heavy fuels oils and the refining streams are dependent upon the composition of the crude oil and the refining process that it undergoes. Calculated and measured water solubilities differ for individual components of complex petroleum substances. At any particular loading rate, aqueous concentrations of each component are a function of relative volume of aqueous and petroleum phases, partition coefficient between phases, amount of component present and the maximum water solubility of each component.

Summary: No testing modeling is proposed. Reported water solubility values for heavy fuel oils ranged from 0.4 to 6.3 mg/l.

Environmental Fate Data

Photodegradation

The direct aqueous 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 result in photochemical transformations in the environment, although absorption is not always sufficient for a chemical to undergo photochemical degradation. Saturated and one-ring aromatic hydrocarbons do not show absorbance in the 290 to 800 nm range and would not be expected to be directly photo-degraded. Polyaromatic hydrocarbons (PAHs), on the other hand, have shown absorbance of the 290 to 800 nm range of light energy and are capable of being degraded by ultraviolet radiation (Fasnacht and Blough, 2002). The degree and rate at which these compounds photo-degrade depends upon whether conditions allow penetration of light with sufficient energy to effect a change. In laboratory experiments, ultraviolet illumination of thin films of heavy fuel oil led to disappearance of polyaromatic compounds (Garrett et al., 1998). The loss was more pronounced for larger polycyclic species and the more alkylated forms of the parent hydrocarbon. This pattern of loss also was demonstrated *in situ*, where disappearance of PAHs on painted granite panels placed in an intertidal marine location was greatest for larger and more alkylated species (Jezequel, et al. 2003).

Hydrocarbon components in heavy fuel oils are subject to indirect photodegradation within the tropospheric layer of the atmosphere. Indirect photodegradation is the reaction with photosensitized oxygen in the atmosphere in the form of hydroxyl radicals (OH) and other oxygen containing radicals (e.g., NO_3). The potential to undergo indirect photodegradation estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (EPA, 2001), 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 half-lives were calculated for the various molecular weight and isomeric structures representing constituent hydrocarbons (paraffins, naphthenes, olefins, aromatics, and polar/heterocyclics) in heavy fuel oils. Structures and molecular weights of selected constituents were chosen on the basis of carbon number as identified in the category substances and known hydrocarbon composition of heavy fuel oils. Therefore, the estimated values identify a potential AOP range for substances in the heavy fuel oil category. AOP half-lives for heavy fuel oil hydrocarbon constituents ranged from <0.1 day to 5.2 days. The modeled data represent a potential range of photodegradation half-lives for the hydrocarbon constituents expected to occur in all substances in the heavy fuel oil category.

Summary: No additional modeling is proposed. Atmospheric half-lives of <0.1 to 5.2 days have been calculated for representative hydrocarbon components of heavy fuel oils.

<u>Stability in Water</u>

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, 1982). Because heavy fuel oils do not contain significant levels of these functional groups, materials in the heavy fuel oils category are not subject to hydrolysis.

Summary: Computer modeling will not be conducted for materials in the heavy fuel oils category because they do not undergo hydrolysis.

<u>Chemical Transport and Distribution in the Environment (Fugacity Modeling)</u>

Fugacity-based multimedia modeling provides 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, not measured endpoint). A widely used fugacity model is the EQC (Equilibrium Criterion) model (Trent University, 1999). 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 (U.S. EPA, 1999).

Based on the physical-chemical characteristics of component hydrocarbons in heavy fuel oils, the lower molecular weight components are expected to have the highest vapor pressures and water solubilities, and the lowest partition coefficients. These factors enhance the potential for distribution in the environment. To gain an understanding of the potential transport and distribution of heavy fuel oil components, the EQC model was used to characterize the environmental distribution of hydrocarbons having different molecular weight and isomeric structures. Structures and molecular weights of selected constituents were chosen on the basis of carbon number as identified in the category substances and known and estimated hydrocarbon composition of heavy fuel oils (Potter and Simmons, 1998; Quann and Jaffe, 1992; Saeger and Jaffe, 2002). Therefore, the estimated values identify the potential environmental distribution for component hydrocarbons in the Heavy Fuel Oil Category.

Partitioning behavior of constituent hydrocarbons of substances in the Heavy Fuel Oil Category depends largely on molecular weight, with smaller compounds (e.g., 7 to 12 carbon atoms) partitioning to the air or water according to their vapor pressure or water solubility properties. In the atmosphere they are expected to degrade rapidly via indirect photodegradation processes, while water soluble components are subject to biodegradation. Once hydrocarbons attain a size of approximately 20 carbon atoms, their low vapor pressure and water solubility prevent their partitioning to air or dissolving in water. Much real-world information has been gained from studies on heavy fuel oil spills (Fuel oil #6 or Bunker C) since this oil is carried by all cargo ships and is the most frequently spilled oil (Jezequel et al. 2003). When spilled on water, heavy fuel oil usually spreads into thick, dark colored slicks that will often breakup into discrete patches and tar balls (NOAA, 2004). Only the lowest molecular weight fractions would be expected to disperse into the water column, and only 5-10% of the material is expected to evaporate within the first few hours of a spill. The specific gravity of a particular fuel oil may vary from 0.95 to 1.03; thus, spilled oil can float, suspend, or sink (NOAA, 2004). Small changes in water density may dictate whether the oil will sink or float. With time and the effects of weathering, heavy fuel oil attains a tar-like consistency, and these fractions will become incorporated into soil or bottom sediments where they will undergo slow to moderate biodegradation. Overall, the principle routes of weathering of spilled heavy fuel include physical removal, dissolution, photo-oxidation, and biodegradation (Jezequel, et al. 2003).

Summary: No further modeling is proposed. Fugacity-based computer modeling has been done for representative constituent hydrocarbon components of heavy fuel oils.

<u>Biodegradation</u>

Extensive research on oil degradation in marine environments indicates that virtually all kinds of oil are susceptible to microbial oxidation. When a heavy fuel oil is spilled, shoreline microbial communities respond quickly to the oiling, with numbers of hydrocarbon-degrading microbes and mineralization potentials increasing after exposure (Leahy and Colwell, 1990). The rate of mineralization is influenced by microbial characteristics (e.g., species), and environmental factors such as available nutrients, oxygen, temperature and degree of dispersion (Mulkins-Phillips and Stewart, 1974; Rashid, 1974; Prince, 2002; Garrett, et al., 2003). In marine systems, degradation is most enhanced by the addition of nutrients (nitrogen and phosphorus), as these are considered most limiting in seawater (Richmond et al., 2001). In general, due to the high viscosity of heavy fuels, their tendency to slowly weather into discrete tar balls, and the eventual incorporation of those substances into soil/sediment can physically isolate and prevent dispersion and microbial attack (Richmond et al., 2001; Prince et al., 2003). However, over time, component hydrocarbons are depleted through selective biodegradation (Lee, et al. 2003). This structure-related trend shows hydrocarbons in order of increasing difficulty for biodegradation to be: 1) n-alkanes, 2) isoalkanes, 3) alkenes, 4) monoaromatic alkylbenzenes, 5) polyaromatic compounds, and 6) high molecular weight cycloalkanes (Bartha and Atlas, 1977; Potter and Simmons, 1998). Less is known about the biodegradability of polar components in oils, which are collectively known as asphaltenes and resins. Many of these have molecular weights of 2000 to 5000 and show little degradation; hence they can persistent in the environment for a long time (Prince, 2002; Prince et al., 2003). In standard 28-day studies, heavy fuel oils would not be expected to be readily biodegradable. In one study, Walker et al. (1975) measured an 11% biodegradation of Bunker C fuel oil over 28 days when incubated with a mixed culture of estuarine bacteria in a nutrient medium. Based on the current understanding of hydrocarbon biodegradability, constituents in heavy fuel oils would be expected to biodegrade over time.

Summary: **No additional testing is proposed.** A technical discussion of hydrocarbon biodegradation based on reviews of available literature was incorporated in a robust summary format.

EVALUATION OF EXISTING ECOTOXICITY DATA AND PROPOSED TESTING

Various ecotoxicity studies on heavy fuel oils have been reported using different means of test substance exposures. These have been reviewed and reported by CONCAWE (1998). For the assessment of ecotoxicity of poorly water soluble mixtures of hydrocarbons as found in petroleum products, the generally accepted procedure is to report results expressed in terms of the "loading rate" (OECD, 2000). The loading rate is defined as the amount of the product that is equilibrated with the aqueous test medium, and the aqueous phase at equilibrium is termed the water-accommodated fraction (WAF) for the specific loading rate. Toxicological endpoints such as the LL50 or EL50 are used to express the loading rate of the product lethal to or produce a specific effect in 50% of the test organisms. Exposures may be prepared as oil-water dispersions (OWDs), where the insoluble petroleum fractions remain in the exposure solutions. This method also results in an expression of the concentration of the applied product (i.e., mg test substance/l), but the methodology does not prevent potential adverse effects to the organisms due to physical entrapment. Small species appear to be more susceptible than large species. Water-soluble fractions (WSFs) and their dilutions also may be reported in ecotoxicity studies. Ecotoxicity endpoints based on water-soluble fractions have been reported to range from 0.9 mg/l to >4.5 milligrams total petroleum hydrocarbons (TPH)/l (MacLean and Doe, 1989; Jokuty et al., 2002). These preparations are expressed in terms of the measured concentrations of hydrocarbons in solution but do not allow the ecotoxicity of the product to be expressed in terms of the amount of that product required to produce a particular effect. Such results are not comparable to results obtained under WAF or OWD preparation methods.

For the assessment of the ecotoxicity of heavy fuel oils, the category substances are considered as a single group, and the potential for any category member to elicit adverse effects in aquatic organisms is attributed to the solubility of the constituent hydrocarbons in that member. This is valid because petroleum hydrocarbons elicit effects through a common mode of toxicity termed non-polar narcosis (Peterson, 1994; van Wezel and Opperhuizen, 1995), which is produced by the soluble hydrocarbon fractions in aqueous preparations. Fuels having molecular weight hydrocarbons predominately higher than the solubility "cut-off" for acute toxicity show no measurable acute toxicity (CONCAWE, 2001). The solubility cut-off varies with the hydrocarbon structure; thus, for paraffinic hydrocarbons the solubility cut-off occurs at about C10, while for alkylbenzenes it is about C14 (CONCAWE, 2001). Other individual components contribute to toxicity, but as molecular weight increases, solubility declines, as does toxicity.

Petroleum streams in the heavy fuel oil category generally consist of hydrocarbon molecules having 20 to 50 carbon atoms, although some streams in this category have low-end carbon atoms from 7 to 15. Heavy fuel oils also may be blended with gas oils or similar low viscosity fuels to meet market specifications. This makes a highly heterogeneous group of petroleum substances that spans a wide range of molecular weight fractions. Based on distillation and carbon number ranges of the category members, existing data on heavy fuel oil ecotoxicity may not represent the ecotoxicity of category members that contain a relatively high proportion of hydrocarbons below the solubility cut-off for acute toxicity (i.e., those having hydrocarbons with low-end carbon numbers of 7 to 15 and low initial boiling points). In spite of the heterogeneity of the heavy fuel oil category, the constituents in heavy fuels are generic hydrocarbon structures (e.g., saturates, aromatics, etc.) represented in other petroleum HPV categories. Therefore, the Testing Group proposes to use ecotoxicity data from the following HPV categories to read-across to cover the lightest heavy fuel oil streams:

- Referenced data cited in the Kerosene/Jet Fuel Test Plan and robust summaries (API, 2003a),
- Referenced data cited in the Gas Oils Test Plan and robust summaries (API, 2003b), and
- Data generated from the proposed testing of select gas oils streams high in saturated and aromatic hydrocarbons.

Kerosenes and jet fuels are composed of saturated and aromatic hydrocarbon compounds having structures and molecular weights similar to the light-end components in heavy fuel oils. Hence, the ecotoxicity of those substances provides conservative estimates of the ecotoxicity of the lightest heavy fuel oils. Blended heavy fuel oils also contain a fraction of heterocyclic compounds that show solubility and molecular weight relationships similar to other hydrocarbons (i.e., solubility decreases with increasing molecular weight). These compounds may contribute to aquatic toxicity, and their contribution is integrated in the current heavy fuel oil test data. When all ecotoxicity data sets are combined, the ranges of endpoint values are expected to cover the potential ecotoxicity of all category members of the heavy fuel oil category that contain various fractions of saturated, aromatic, and heterocyclic compounds.

Robust summaries for kerosene and gas oils ecotoxicity data cited below may be found in the HPV submissions for Kerosenes/Jet Fuel Test Plan (API, 2003a) and Gas Oils Test Plan (API, 2003b).

Acute Toxicity to Fish

Data for heavy fuel oils showed slight or no acute toxicity to fish when tested as either WAFs or OWDs. Shell (1997a,b) tested WAFs of a "light" and a "heavy" residual fuel oil (CAS No. 68476-33-5) and found the 96-hour LL50s for exposures to rainbow trout (Oncorhynchus mykiss) to be between 100 mg/l and 1000 mg/l for the "heavy" residual fuel oil and >1000 mg/l for the "light" residual fuel oil. Mobil (1987a) obtained a 96-hour LL50 of >10,000 mg/l when bluegill (Lepomis macrochirus) were exposed to OWDs of No. 6 fuel oil.

Fish LL50 values for kerosene/jet fuel tested as WAFs were between 10 mg/l and 100 mg/l (API, 2003a). LL50 values for distillate fuels cited in the gas oil HPV test plan ranged from 3.2 to 65 mg/l (API, 2003b).

Acute Toxicity to Aquatic Invertebrates

Data for invertebrates (Daphnia magna) showed a similar range of sensitivities as fish. In testing WAFs of the "light" and "heavy" residual fuels, Shell (1997c,d) found the EL50 of the "light" to be >1000 mg/l while that of the "heavy" to between 220 mg/l and 460 mg/l. Mobil (1987b) exposed daphnids to solutions in which oil was coated on the inside surface of the test vessels. Exposures done in this manner resulted in no immobilized daphnids at the maximum loading rate of 10,000 mg/l.

Invertebrate EL50 values for kerosene/jet fuel tested as WAFs were ranged 1.4 mg/l to <89 mg/l (API, 2003a). EL50 values for distillate fuels cited in the gas oil HPV test plan ranged from 2.0 to <300 mg/l (API, 2003b).

Toxicity to Algae

Shell (1997e,f) reported the EL50 values based on growth rate and biomass for 72-hour exposures of Raphidocelis subcapitata to a "light" and a "heavy" residual fuel oil. Tests were run in sealed vessels without headspace. For the "light" material, the ELr50 (rate-based) was concluded to lie between 100 mg/l and 300 mg/l while the ELb50 (biomass-based) was between 3 mg/l and 10 mg/l. This contrasted somewhat with toxicity endpoints for the "heavy" residual fuel oil. Those tests gave ELr50 and ELb50 values of between 30 mg/l and 100 mg/l. Mobil (1987c) coated the surface of test flasks with No. 6 fuel oil and measured algal (R. subcapitata) biomass over 96 hours. Tests vessels were plugged with cotton, but otherwise allowed air exchange. Growth inhibition at the maximum loading rate of 10,000 mg/l was 47.5%, but the authors concluded that the ELb50 was >5,000 mg/l. Inhibition of 22% to 27% occurred in the lowest three test levels, and the authors suggested physical obstruction of light penetration may have affected cell growth.

In 96-hour exposures using WAFs of kerosene, EL50 values ranged from 5.0 to 6.2 mg/l when based on inhibition of growth rate, and ranged from 5.9 mg/l to 11 mg/l when based on biomass (API, 2003a). In 72-hour WAF exposures, EL50 values based on growth rate and biomass fell within the range of 10 mg/l to 30 mg/l (API, 2003a).

Algae exposed to WAFs of distillate fuels produced 72-hour EL50 values based on growth rate that ranged from 2.2 to <46 mg/l, while values based on algal biomass ranged from 1.8 to 25 mg/l (API, 2003b).

The heavy fuel oil, kerosene, and gas oil ecotoxicity data described above are listed in the following Table 2. Together, these data encompass values that represent the potential toxicity of all members in the Heavy Fuel Oil HPV category.

Table 2. Ecotoxicity Data for Heavy Fuel Oil, Kerosene/Jet Fuel Oil, and Gas Oil.

Species	Material	Exposure Type	Effect / concentration (mg/l)	Reference
FISH				
Heavy Fuel Oil				

Rainbow trout (Oncorhynchus mykiss)	CAS No. 68476-33-5 (light) residual fuel oil	WAF	96-h LL50 = >1000	Shell, 1997a
Rainbow trout (O. mykiss)	CAS No. 68476-33-5 (heavy) residual fuel oil	WAF	96-h LL50 = 100 – 1000	Shell, 1997b
Bluegill (Lepomis macrochirus)	No. 6 fuel oil	OWD	96-h LL50 = >10,000	Mobil, 1987a
Kerosene/Jet Fuel				
Various species	Various	WAF	96-h LL50 = 18 20 10 – 100 25	API, 2003a
Gas Oil				
Various species	Distillate fuels	WAF	96-h LL50 = 57 3.2 6.6 57 21 65	API, 2003b
INVERTEBRATES				
Heavy Fuel Oil				
Daphnia magna	CAS No. 68476-33-5 (light) residual fuel oil	WAF	48-h EL50 = >1000	Shell, 1997c
D. magna	CAS No. 68476-33-5 (heavy) residual fuel oil	WAF	48-h EL50 = 220 – 460	Shell, 1997d
D. magna	No. 6 fuel oil	OWD	48-h EL50 = >10,000	Mobil, 1987b
Kerosene/Jet Fuel				
D. magna	Various	WAF	48-h EL50 = 21 1.4 40 – 89 1.9	API, 2003a
Gas Oil				

D. magna	Distillate fuels	WAF	48-h EL50 = 7.8 5.3 14 42 2.0 210 68 13 100 – 300 13 6.4 36 9.6	API, 2003b
ALGAE				
Heavy Fuel Oil				
Raphidocelis subcapitata	CAS No. 68476-33-5 (light) residual fuel oil	WAF	96-h ELr50 = 100 – 300 96-h ELb50 = 3 – 10	Shell, 1997e
R. subcapitata	CAS No. 68476-33-5 (heavy) residual fuel oil	WAF	96-h ELr50 = 30 – 100 96-h ELb50 = 30 – 100	Shell, 1997f
R. subcapitata	No. 6 fuel oil	OWD	96-h ELb50 = >5,000	Mobil, 1987c
Kerosene/Jet Fuel				
R. subcapitata	Various	WAF	96-h ELr50 = 6.2 5.0 96-h ELb50 = 11 5.9 72-h ELr50 = 10 – 30 72-h ELb50 = 10 – 30	API, 2003a

Gas Oil				
R. subcapitata	Distillate fuels	WAF	72-h ELr50 = 2.9 2.2 78 22 22 – 46 72-h ELb50 = 1.8 2.2 25 10 10 – 22	API, 2003b

WAF = Water Accommodated Fraction

OWD = Oil-Water Dispersion

Summary. No further testing is proposed. The ecotoxicity data for heavy fuel oils combined with data for kerosene/jet fuel and gas oils HPV categories provides a range of ecotoxicity endpoints expected to encompass the ecotoxicity of all members of the heavy fuel oils HPV category.

MATRIX OF AVAILABLE DATA AND PROPOSED TESTING

Table 3. Matrix of Available Data and Proposed Testing

	REFINERY Streams Subcategories							Finished Residual Fuels
TEST	Atmospheric Residue	Atmospheric Distillate	Vacuum Residue	Vacuum Distillate	Cracked Residue	Cracked Distillate	Reformer Residue	Residual Fuel Oil
Physical/Chemical Properties[7]								
Melting Point	Ad							
Boiling Point	Ad							
Vapor Pressure	Ad							
Water Solubility	Ad							
Partition coefficient (log Kow)	Ad							
Ecotoxicity[7]								
Algae Growth Inhibition	RA[9]							
Acute Freshwater Invertebrate	RA[9]							
Acute Freshwater Fish	RA[9]							
Environmental Fate[7]								
Biodegradation	Ad							
Stability in Water	NA							
Photodegradation (estimate)	Ad							
Transport and Distribution	Ad							
Mammalian Toxicity								
Acute	Ad	RA[1]	RA[2]	Ad	Ad	Ad	RA[3]	Ad
Repeat-dose	Ad	Ad	Ad[2]	Ad	Ad	Ad	Test[5]	Ad
Reprod/Develop	Ad	Ad	Ad	Ad	Ad	Ad	Test[5]	Test[6]
Genotoxicity, in vitro	Test[8]	Test[8]	Ad	Ad	Ad	Test[8]	Test[8]	Test[8]
Genotoxicity, in-vivo	RA[4]	RA[1]	RA[2]	Ad	Ad	RA[4]	RA[4]	RA[4]

Ad = Adequate, indicates adequate existing data.
NA = Not Applicable, t*he endpoint is not applicable" due to inherent physical and/or chemical properties. A technical discussion was developed to address such endpoints.*
[1] read across from API HPV Gas Oils Test Plan
[2] read across from API HPV Asphalt Test Plan
[3] read across from API HPV Aromatic Extracts Test Plan Test Plan
[4] read across from *in-vitro* genotoxicity assay
[5] OECD 422 repeat dose/reproductive/developmental screen
[6] OECD 421 reproductive/developmental screen
[7] For the assessment of the physical/chemical properties, ecotoxicity, and environmental fate, the category substances are considered as a single group, and the potential for any category member to elicit adverse effects in aquatic organisms is attributed to the solubility of the constituent hydrocarbons in that member
[8] Modified Ames & in vitro chromosomal aberration (OECD 473).

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APPENDIX A.

CAS Numbers and Definitions of Category Members

The CAS numbers and definitions of refinery streams, including those in the heavy fuel oils category, 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 only ranges of values for carbon numbers, with little if any quantitative analytical information or concern for possible compositional overlaps. As a result, the CAS descriptions are not useful in determining the exact composition of any specific refinery stream.

<u>Residual Fuel Oils</u>

68476-33-5
Fuel oil, residual
The liquid product from various refinery streams, usually residues. The composition is complex and varies with the source of the crude oil.

68553-00-4
Fuel oil, no. 6
A distillate oil having a minimum viscosity of 900 SUS at 37.7°C (100°F) to a maximum of 9000 SUS at 37.7°C (100°F).

<u>Process Subcategories</u>

Atmospheric Residual

64741-45-3
Residues (petroleum), atm. Tower
A complex residuum from the atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350 °C (662°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

64742-78-5
Residues (petroleum), hydrodesulfurized atmospheric
A complex combination of hydrocarbons obtained by treating an atmospheric tower residuum with hydrogen in the presence of a catalyst under conditions primarily to remove organic sulfur compounds. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68333-22-2
Residues (petroleum), atmospheric
A complex residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having

carbon numbers predominantly greater than C11 and boiling above approximately 200°C (392°F). This stream is likely to contain 5 wt.% or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68607-30-7
Residues (petroleum), topping plant, low-sulfur
A low-sulfur complex combination of hydrocarbons produced as the residual fraction from the topping plant distillation of crude oil. It is the residuum after the straight-run gasoline cut, kerosene cut and gas oil cut have been removed.

70592-79-9
Residues (petroleum), atm. tower, light
A complex residuum from the atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly greater than C11 and boiling above approximately 200°C (392°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68476-32-4
Fuel oil, residues-straight-run gas oils, high-sulfur
NONE

Atmospheric Distillate

68410-00-4
Distillates (petroleum), crude oil
A complex combination of hydrocarbons produced by distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C50 and boiling in the range of approximately 205°C to greater than 495°C (401°F to above 923°F).

68783-08-4
Gas oils (petroleum), heavy atmospheric
A complex combination of hydrocarbons obtained by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C7 through C35 and boiling in the range of approximately 121°C to 510°C (250°F to 950°F),
Residual

Vacuum Residual

68512-62-9
Residues (petroleum), light vacuum
A complex residuum from the vacuum distillation of the residuum from the atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly greater than C13 and boiling above approximately 230°C.

70913-85-8
Residues (petroleum), solvent-extd. vacuum distilled atm residuum
A complex residuum produced by the solvent extraction of the vacuum distillate of the complex residuum from the atmospheric distillation of crude oil.

Vacuum Distillate

64741-57-7
Gas oils (petroleum), heavy vacuum
A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers

predominantly in the range of C20 through C50 and boiling in the range of approximately 350°C to 600°C (662°F to 1112°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

64742-59-2
Gas oils (petroleum), hydrotreated vacuum
A complex combination of hydrocarbons obtained by treating a petroleum fraction with hydrogen in the presence of a catalyst. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C50 and boiling in the range of approximately 230°C to 600°C (446°F to 1112°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

64742-86-5
Gas oils (petroleum), hydrodesulfurized heavy vacuum
A complex combination of hydrocarbons obtained from a catalytic hydrodesulfurization process. It consists of hydrocarbons having carbon numbers predominantly in the range of C20 through C50 and boiling in the range of approximately 350°C to 600°C (662°F to 1112°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68955-27-1
Distillates (petroleum), petroleum residues vacuum
A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from the atmospheric distillation of crude oil.

70592-76-6
Distillates (petroleum), intermediate vacuum
A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C14 through C42 and boiling in the range of approximately 250°C to 545°C (482°F to 1013°F). This stream is likely to contain 5 wt.% or more of 4- to 6-membered condensed ring aromatic

70592-77-7
Distillates (petroleum), light vacuum
A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C35 and boiling in the range of approximately 250°C to 545°C (482°F to 1013°F).

70592-78-8
Distillates (petroleum), vacuum
A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C15 through C50 and boiling in the range of approximately 270°C to 600°C (518°F to 1112°F). This stream is likely to contain 5 wt.% or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

Cracked Residual

64741-62-4
Clarified oils (petroleum), catalytic cracked (also listed in Olefins Panel Fuel Oils Category)
A complex combination of hydrocarbons produced as the residual fraction from distillation of the products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

64741-75-9
Residues (petroleum), hydrocracked
A complex combination of hydrocarbons produced as the residual fraction from distillation of the products of a hydrocracking process. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F).

64741-80-6
Residues (petroleum), thermal cracked
A complex combination of hydrocarbons produced as the residual fraction from distillation of the product from a thermal cracking process. It consists predominantly of unsaturated hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68187-58-6
Pitch, petroleum, arom
The residue from the distillation of thermal cracked or steam-cracked residuum and/or catalytic cracked clarified oil with a softening point from 40 degree C to 180 degree C (104 degree F to 356 degree.F). Composed primarily of a complex combination of three or more membered condensed ring aromatic hydrocarbons.

68478-17-1
Residues (petroleum), heavy coker gas oil and vacuum gas oil
A complex combination of hydrocarbons produced as the residual fraction from the distillation of heavy coker gas oil and vacuum gas oil. It predominantly consists of hydrocarbons having carbon numbers predominantly greater than C13 and boiling above approximately 230°C (446°F).

68783-13-1
Residues (petroleum) coker scrubber condensed-ring-aromatic-containing
A very complex combination of hydrocarbons produced as the residual fraction from the distillation of vacuum residuum and the products from a thermal cracking process. It consists predominantly of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring

Cracked Distillate

64741-61-3
Distillates (petroleum), heavy catalytic cracked
A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C15 through C35 and boiling in the range of approximately 260 °C to 500°C (500°F to 932°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

64741-81-7
Distillates (petroleum), heavy thermal cracked
A complex combination of hydrocarbons from the distillation of the products from a thermal cracking process. It consists predominantly of unsaturated hydrocarbons having carbon numbers predominantly in the range of C15 through C36 and boiling in the range of approximately 260°C to 480°C (500°F to 896°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68333-26-6
Clarified oils (petroleum), hydrodesulfurized catalytic cracked
A complex combination of hydrocarbons obtained by treating catalytic cracked clarified oil with

hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350°C (662°F). This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

68333-27-7
Distillates (petroleum), hydrodesulfurized intermediate catalytic cracked
A complex combination of hydrocarbons obtained by treating intermediate catalytic cracked distillates with hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C30 and boiling in the range of approximately 205°C to 450°C (401°F to 842°F). It contains a relatively large proportion of tricyclic aromatic hydrocarbons.

70955-17-8
Aromatic hydrocarbons, C12-20
A complex combination of hydrocarbons obtained from the distillation of biphenyl and naphthalene feedstocks. It consists predominantly of hydrocarbons having carbon numbers predominantly in the range of C12 through C20, such as alkylbenzenes, alkylnaphthalenes, indans, fluorenes, acenaphthalenes, phenanthrenes and anthracenes, and boiling in the range of approximately 282.degree.C to 427.degree.C (540.degree.F to 800.degree.F).

Reformer Residual

64741-67-9
Residues (petroleum), catalytic reformer fractionator
A complex combination of hydrocarbons produced as the residual fraction from distillation of the product from a catalytic reforming process. It consists of predominantly aromatic hydrocarbons having carbon numbers predominantly in the range of C10 through C25 and boiling in the range of approximately 160 °C to 400°C (320°F to 725°F). This stream is likely to contain 5 wt. % or more of 4- or 6-membered condensed ring aromatic hydrocarbons.

68478-13-7
Residues (petroleum), catalytic reformer fractionator residue distn.
A complex residuum from the distillation of catalytic reformer fractionator residue. It boils approximately above 399°C (750°F).

APPENDIX B.

Links to Additional Resources

Refining Processes: General Descriptions

http://www.chevron.com/about/learning_center/refinery
http://www.lubrizol.com/lubetheory/default.htm
http://www.orionrefining.com/flow.htm
http://www.osha-slc.gov/dts/osta/otm/otm_toc.html
http://www.shellglobalsolutions.com/base_oils/library/library.htm
http://www.shell-lubricants.com/learningcenter/aboutoil.html
http://www.shellus.com/welcome/history/hist_oil_main.html
http://www.epa.gov/compliance/resources/publications/assistance/sectors/notebooks/petrefsnpt1.pdf
http://www.mts.net/~dbrad1/base_oil.htm

Petroleum Related Glossaries

http://www.caltex.com.au/products_glo.asp
http://www.citgo.com/CommunityInvolvement/Classroom/Glossary.jsp
http://www.epplp.com/gloss.html
http://www.prod.exxon.com/exxon_productdata/lube_encyclopedia/
http://www.hellenic-petroleum.gr/english/glossary/gl_main.htm
http://www.prod.exxon.com/exxon_productdata/lube_encyclopedia/
http://www.oilanalysis.com/dictionary
http://www.orionrefining.com/glossary.htm
http://www.gedolbear.com/glossary.htm
http://www.shellglobalsolutions.com/base_oils/glossary/a_g.htm
http://www.ursa-texaco.com/English/glossary_a.html
http://www.eia.doe.gov/pub/oil_gas/petroleum/data_publications/petroleum_marketing_annual/current/pdf/glossary.pdf

APPENDIX C.

Robust Summary

(Separate document)

201-15368A

ROBUST SUMMARY
OF INFORMATION ON

Substance Group **Heavy Fuel Oils**

Summary prepared by American Petroleum Institute

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Number of pages: 114

NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, H. J., Andreae, M. and Tillman, U, (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : Heavy fuels are blends of the residues and distillates that are derived from various refinery distillation, cracking and reforming processes. These heavy fuels are complex mixtures which may boil in the range from 121 to 600 °C.
They consist of aromatic, aliphatic and naphthenic hydrocarbons, generally having carbon numbers in the range of C7 to C50, together with asphaltenes and smaller amounts of heterocyclic compounds containing sulfur, nitrogen and oxygen.

The individual streams in this category may be:

Atmospheric distillates
    Distillates from atmospheric distillation of crude oil
Atmospheric residues
    Residues from atmospheric distillation of crude oil
Vacuum distillates
    Distillates from vacuum distillation of atmospheric residue
Vacuum residues
    Residues from vacuum distillation of atmospheric residue
Cracked distillates
    Distillates of streams derived from cracking processes
Cracked residues
    Residues of streams derived from cracking processes
Reformer residues
    Residues of streams derived from distillation of reformer processes

Toxicological information applicable to some of the above subcategories have been summarized in test plans and robust summaries for either gas oils or asphalt (vacuum residue) and this is indicated in the appropriate sections below. Otherwise data are included below on streams from the subcategories outlined above.

Data from acute toxicity studies are also included in this robust summary on four samples of blended heavy fuel oil. These samples of fuel oil differ in gravity and sulfur content as shown:

	API sample number			
Parameter	**78-6**	**78-7**	**78-8**	**79-2**
API gravity	11.7	17.1	23.1	5.2
Specific gravity	0.99	0.95	0.92	1.04
Sulfur content	2.7%	0.8%	0.2%	1.2%

Analytical data on heavy fuel oil streams are scarce, since they are blended into heavy fuels normally because they have no commercial value in any other use and consequently have not been fully characterized.

The limited data available for some of the samples for which toxicological information is available are shown below.

Parameter	Atmospheric Residue F-132	Cracked Residue 81-15	Cracked Distillate 97-01
CAS No.	64741-45-3	64741-62-4	64741-81-7
Gravity (°API)		0.3	
Specific gravity	0.9279	1.0725	0.9383
Molecular weight	347	276	
Refractive index	1.5132	Too dark	1.5259
Viscosity (cST @40°C)		379	
Bromine NO.		17	
Flash point (°F)		396	
Ash (wt %)		0.05	
Total sulfur (wt %)	1.23	1.18	
Total nitrogen (wt. %)	1617 ppm		0.52
Total oxygen (wt %)	0.19	0.85	
Pour point (°F)	+88	35	
Distillation (°F)			
IBP	531	395	411
End point	1041	952	831
Asphaltenes (%)			4.2
Carbon residues (wt %)			4.6
Saturates (wt %)		8.0	41.7
Aromatics (wt %)	67.82	58.3	50.4
Polar compounds (wt %)		9.0	7.9
Pentane insolubles (wt%)		24.7	
PNAs %wt in DMSO fraction			4.67

Information on other materials for which there are toxicology data are given with the relevant robust summary below.

1.13 REVIEWS

Memo : CONCAWE

Remark : CONCAWE compiled the available mammalian and ecotoxicity data available into a product dossier on heavy fuel oils.
(29)

Memo : IARC

Remark : IARC reviewed the available information on the carcinogenicity of fuel oils and the review was published in the IARC monograph series.

The conclusions of the evaluation were:
There is sufficient evidence for the carcinogenicity in experimental animals of residual (heavy) fuel oils.

The overall evaluation was:
Residual (heavy) fuel oils are possibly carcinogenic to humans (Group 2B).
(51)

Memo : Bingham et al

Remark : Bingham et al (1980) published a review of the carcinogenic potential of petroleum hydrocarbons. The review included information on two blended heavy fuel oils.
(28)

2.1 MELTING POINT

Method : ASTM D97 (ASTM, 1999)
GLP : No data
Test substance : Heavy fuel oils

Remark : Heavy fuel oils do not have sharply-defined melting points because they are highly heterogeneous mixtures of petroleum hydrocarbons of varying molecular weights. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 1999). The test for pour point measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase 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 change in physical state (i.e., crystal formation) and/or viscosity property.

Values given represent a range of measured pour point determinations for various distillate and residual heavy fuel oil related refining streams and products. Measured values are highly variable and can differ significantly even within a CAS-defined refining process. This is due to variability in the hydrocarbon make-up of crude oils and the refining process applied to the raw materials. Adding to the variability in pour point values is the practice of blending heavy petroleum fractions with lighter "cutter stock" for the purpose of enhancing the flow properties of heavy fuel oils. However, the measurements shown are generally consistent with the review by CONCAWE (1998) who stated that typical pour point values for heavy fuel oils are <30 °C.

Result :

Heavy Fuel Oils	Pour Point (°C)	Ref./ cert. of analysis
Distillates, heavy thermal cracked		
(CAS No. 64741-81-7)	16	(Niper, 1993)
	35	(30330008)
	16	(30330013)
Distillates, vacuum		
(CAS No. 70592-78-8)	27	(2102010)
Residues, atmospheric tower bottoms		
(CAS No. 64741-45-3)	18	(21020141)
Gas oils, heavy vacuum		
(CAS No. 64741-57-7)	31	(30330004)
	35	(30330016)
Gas oils, hydrodesulfurized heavy vacuum		
(CAS No. 64742-86-5)	13	(Niper, 1993)
Clarified oils, catalytic cracked		
(CAS No. 64741-62-4)	1.7	(API,1987)
Bunker C fuel oil	15	(Jokuty, 2002)
Bunker C light fuel oil	6	(Jokuty, 2002)
Bunker C (Alaska) fuel oil	-2	(Jokuty, 2002)
Heavy fuel oil no. 6	-1	(Jokuty, 2002)

Reliability : (2) valid with restrictions

(1) (20) (25) (29) (32) (33) (34) (35) (36) (37) (53) (83)

2.2 BOILING POINT

Test substance : Heavy fuel oils

Remark : The values shown under "results" refer to CAS number definitions cited by EPA (2004). The following information is provided as supporting data for the CAS definitions. They represent distillation ranges for commercial heavy fuel oil products cited in reference databases and material safety data sheet sources. Distillation ranges will vary depending on factors such as the source of the crude oil and in the refining process used.

	Boiling Range, °C	Ref
Residual Fuel Oil (CAS No. 68512-62-9):	427 - 760	1
Residual Fuel Oil (CAS No. 68476-33-5):	160 - 500	2
Heavy Fuel Oil (CAS No. 68476-33-5):	160 - 600	3
Catalytically Cracked Clarified Oil (CAS No. 64741-62-4)	150 - 600	4
Catalytically Cracked Clarified Oil (CAS No. 64741-62-4)	202 - 511	5
Bunker C Light Fuel Oil	241 - 712	6
Bunker C (Alaska) Fuel Oil	160 - 719	6
Bunker C Fuel Oil	247 - 723	6

Result : For the following petroleum streams in the Heavy Fuels HPV category, boiling ranges were obtained from the CAS number definitions (EPA, 2004).

CAS No.	Substance	Boiling Range °C
64741-45-3	Residues, atmospheric tower	>350
64741-57-7	Gas oils, heavy vacuum	350 - 600
64741-61-3	Distillates, heavy catalytic cracked	260 - 500
64741-62-4	Clarified oils, catalytic cracked	>350
64741-67-9	Residues, catalytic reformer fractionator	160 - 400
64741-75-9	Residues, hydrocracked	>350
64741-80-6	Residues, thermal cracked	>350
64741-81-7	Distillates, heavy thermal cracked	260 - 480
64742-59-2	Gas oils, hydrotreated vacuum	230 - 600
64742-78-5	Residues, hydrodesulfurized atmospheric tower	>350
64742-86-5	Gas oils, hydrodesulfurized heavy vacuum	350 - 600
68333-22-2	Residues, atmospheric	>200
68333-26-6	Clarified oils, hydrodesulfurized catalytic cracked	>350

68333-27-7	Distillates, hydrodesulfurized intermediate catalytic cracked	205 - 450
68410-00-4	Distillates, crude oil	205 - >495
68478-13-7	Residues, catalytic reformer fractionator residue	>399
68478-17-1	Residues, heavy coker gas oil and vacuum gas oil	>230
68512-62-9	Residues, light vacuum	>230
68783-08-4	Gas oils, heavy atmospheric	121 - 510
68783-13-1	Residues, coker scrubber condensed-ring aromatic-containing	>350
70592-76-6	Distillates, intermediate vacuum	250 - 545
70592-77-7	Distillates, light vacuum	250 - 545
70592-78-8	Distillates, vacuum	270 - 600
70592-79-9	Residues, atmospheric tower, light	>200
70955-17-8	Aromatic hydrocarbons, C12-20	282 - 427

Reliability : (2) valid with restrictions
The values given are for standard definitions established for these refining processes by EPA (2004) or data supplied in Material Safety Data Sheets for commercial products. Actual boiling ranges vary depending on the charge stock used in the refining and the source of the crude from which they originated.
(20) (43) (44) (53) (100) (103) (104)

2.4 VAPOUR PRESSURE

Decomposition :
Method : Calculated: MPBPWIN V1.40 in EPIWIN V3.10 (U.S. EPA, 2000)
GLP : No
Test substance : Heavy fuel oils

Remark : Complex mixtures of petroleum products exert vapor pressures according to the sum of the partial pressures of the individual components (Dalton's Law of Partial Pressures), and the pressures of the individual components are a product of their mole fractions in the mixture times their vapor pressure in the pure form (Raoult's Law). Refining streams in the Heavy Fuel Oils Category consist of highly heterogenous mixtures of hydrocarbons generally having 20 to 50 carbon atoms, although some streams in this category have low-end carbon numbers of 7 to 15. Given the wide range of carbon atoms possible, and the variety of paraffinic, naphthenic, olefinic, aromatic and heterocyclic hydrocarbons, the potential number of unique isomeric structures is very large. Therefore, partial pressures of individual constituents would be quite small. Heavy fuel streams having the greatest proportion of low molecular weight constituents would be expected to have the highest vapor pressures.

The chemicals selected to calculate vapor pressures represent molecular weights and different isomeric structures (paraffinic, naphthenic, olefinic, aromatic, and heterocyclic hydrocarbon compounds) known to exist in heavy fuel oils. Structures were chosen based on known hydrocarbon composition and compositional modeling (Potter and Simmons, 1998; Quann and Jaffe, 1992; Saeger and Jaffe, 2002). Therefore, the data listed identify potential vapor pressures for constituent hydrocarbons in the Heavy Fuel Oil HPV Category. The modeled values are expected to cover all streams and products in the heavy fuel oil HPV category. Actual vapor pressures of substances in this category will vary dependent on their composition. Vapor pressure data reported in product MSDS information and electronic databases provide supporting evidence for the estimates. They reflect the varied nature of these substances. Examples include the following:

	Reference
CAS No. 68476-33-5 (Residual fuel oil)	
Reid Vapor Pressure @ 37.8 C<100 Pa	Total UK Ltd., 2003
CAS No. 64741-62-4 (Catalytically cracked clarified oil)	
Reid Vapor Pressure @ 20 C >500 Pa	ECB, 2000

Result :

Chemical	**No. Carbon Atoms**	**Calculated Vapor Pressure, Pa @ 25 °C**
n-alkanes	7	$6x10^{3}$
	11	$5x10^{1}$
	20	$6x10^{-4}$
	50	$2x10^{-7}$
iso-alkanes	7	$9x10^{3}$
	11	$8x10^{1}$
	20	$6x10^{-4}$
	50	$2x10^{-7}$
cyclo-alkanes		
1-ring	7	$6x10^{3}$
	11	$5x10^{1}$
	20	$2x10^{-2}$
	50	$2x10^{-13}$
2-ring	11	$9x10^{1}$
	20	$2x10^{-2}$
	50	$2x10^{-13}$
3-ring	12	$3x10^{1}$
	20	$2x10^{-2}$
	50	$2x10^{-13}$
Olefins		
	7	$8x10^{3}$
	11	$1x10^{2}$
	20	$4X10^{-1}$
	50	$3X10^{-13}$
aromatics		
1-ring	7	$4x10^{3}$
	11	$6x10^{1}$
	20	$3x10^{-3}$
	50	$2x10^{-14}$
2-ring	11	7
	20	$7x10^{-4}$
	50	$3x10^{-15}$
3-ring	14	$4x10^{-4}$
	20	$1x10^{-4}$
	50	$5x10^{-16}$
polar/heterocyclic compounds		
Quinolines		
quinoline	9	8
C5-quinoline	14	$2x10^{-2}$
C11-quinoline	20	$1x10^{-4}$

C41-quinoline	50	$9x10^{-16}$
Pyridines		
C2-pyridine	7	$3x10^{2}$
C9-pyridine	14	$2x10^{-1}$
C15-pyridine	20	$8x10^{-4}$
C45-pyridine	50	$2x10^{-16}$
Carboxy Acids		
C1-1-ring	7	8
C1-2ring	11	$8x10^{-2}$
C2-3-ring	16	$8x10^{-5}$
C6-3-ring	20	$4x10^{-5}$
C32-4-ring	50	$3x10^{-16}$
Thiophenes/Benzothiophenes		
C3 thiophene	7	$1x10^{2}$
dibenzothiophene	12	$3x10^{-2}$
C-8 dibenzothiophene	20	$1x10^{-5}$
C38 dibenzothiophene	50	$5x10^{-17}$

Reliability : (2) valid with restrictions
Vapor pressures for representative molecular structures in heavy fuel oils were estimated using a validated computer model.
(44) (86) (89) (92) (104) (105)

2.5 PARTITION COEFFICIENT

Method : Calculated): EPIWIN V3.10 (U.S. EPA, 2000)
GLP : No
Test substance : Heavy fuel oils

Remark : Substances in the heavy fuel oil category have a carbon number distribution primarily between C20 and C50, although some individual refining streams in this category have low end carbon numbers of 7 to 15. The predominant hydrocarbon structures include saturated alkanes (e.g., straight and branched chain), cyclic alkanes, aromatics (e.g., one to multi-ring compounds), and to a lesser extent olefinic compounds and heterocyclic compounds that contain sulfur, oxygen and nitrogen atoms. The constituent hydrocarbons used to estimate partition coefficients are representative of compounds known to occur in heavy fuel oil mixtures. Structures were chosen based on known hydrocarbon composition and compositional modeling (Potter and Simmons, 1998; Quann and Jaffe, 1992; Saeger and Jaffe, 2002). Therefore, the data given cover the principal isomeric structures contained in heavy fuel oil and represent a potential range of partition coefficients for the substances in this category. The modeled values are expected to cover all streams and products in the heavy fuel oil HPV category. Actual partition coefficients of substances in this category will vary dependent on their composition.

Standardized methods for partition coefficient determinations are analytically limited to substances up to Log Kow ~4 (and occasionally 5) (OECD, 1995), and an estimation method is available for log P values up to 6 (OECD, 1989). Hence, analytical methods begin to fail for hydrocarbon compounds that contain roughly 15 to 20 carbon atoms.

Result :

Chemical	No. Carbon Atoms	Log Kow @ 25 °C
n-alkanes	7	4.7
	11	5.7
	20	10

		50	25
iso-alkanes		7	3.7
		11	5.7
		20	10
		50	25
cyclo-alkanes			
	C1,1-ring	7	3.6
	C5	11	5.6
	C14	20	10
	C44	50	25
	C1, 2-ring	11	4.6
	C10	20	9
	C40	50	24
	3-ring	12	4.2
	C6	20	8.1
	C36	50	23
Olefins			
		7	4.0
		11	5.6
		20	10
		50	25
aromatics			
	C1,1-ring	7	2.7
	C5	11	4.9
	C14	20	8.9
	C44	50	24
	C1, 2-ring	11	3.9
	C10	20	8.1
	C40	50	23
	3-ring	14	4.1
	C6	20	7.4
	C36	50	22
polar/heterocyclic compounds			
Quinolines			
	quinoline	9	2.0
	C5-quinoline	14	4.7
	C11-quinoline	20	7.6
	C41-quinoline	50	22
Pyridines			
	C2-pyridine	7	1.7
	C9-pyridine	14	5.3
	C15-pyridine	20	8.2
	C45-pyridine	50	25
Carboxylic Acids			
	C1-1-ring	7	2.0
	C1-2-ring	11	3.4
	C2-3-ring	16	4.4
	C6-3-ring	20	6.8
	C32-4-ring	50	22

Thiophenes/Benzothiophenes			
	C3 thiophene	7	3.3
	dibenzothiophene	12	4.4
	C8 dibenzothiophene	20	8.2
	C38 dibenzothiophene	50	23

Reliability : (2) valid with restrictions

(84) (85) (86) (89) (92) (105)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 6.26 mg/l at 22 °C
GLP : No data
Test substance : Fuel oil No. 6 (CAS 68553-00-4 - assumed by reviewer)

Method : Saturated oil solutions were prepared by adding approximately 10 ml of oil to 50 - 100 ml of double-distilled water in a 125-ml separatory funnel. The funnel was gently shaken with a wrist-action shaker or gently stirred with a magnetic stirrer for at least 24 hours, then placed in a temperature bath at the desired temperature (20 ± 2 °C) for at least 48 hours prior to analysis. Care was taken to ensure that no oil-in-water emulsion formed by maintaining the turbulence level below that necessary to separate oil particles from the oil layer.

Purge-and-trap (vapor) extraction followed by capillary gas chromatographic analysis was used to measure water soluble fractions of the fuel oil. A Hewlett-Packard model 5840 GC equipped with a flame ionization detector and a 7675A purge-and-trap sampler was used for the analysis. Approximately 1-2 ml of the saturated aqueous solutions was bubbled with the GC carrier gas (N_2) and the dissolved volatile hydrocarbons were purged and subsequently sorbed onto a Tenax-GC trap. By thermodesorption, the hydrocarbons were then directly swept onto the GC column for analysis. The analytical column was a 0.5 mm x 50 m glass capillary column coated with SE-30. Operating GC conditions were:
initial oven temperature: 40 °C for 10 minutes
temperature increase: 5 °C/min
final oven temperature: 200 °C for 20 min
carrier gas flow rate: 5 ml/min
detector temperature: 300 °C
Peak areas were integrated by an HP-5840 GC terminal.

Remark : Test substance was a Fuel Oil No. 6 having a density of 0.925 g/cm^3 and a viscosity of 22.7 cp at 20 °C.

Additional supporting data are provided in section 2.14.

Limited detail is provided for the exact amounts of fuel oil used for preparing the aqueous solutions, nor is there any information regarding the composition of the tested fuel, either as hydrocarbon type or inorganic components (such as sulfur). Also, no information on the GC calibration standard composition used to identify and quantify soluble components in the equilibrated aqueous -oil solutions is provided. Individual components of complex petroleum substances have specific and differing solubilities. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.

Reliability : (2) valid with restrictions
The water solubility study meets basic scientific principles, but lacked some details on the preparation of the soluble fractions.

(99)

2.14 ADDITIONAL REMARKS

Memo : Water solubility of Bunker C heavy fuel oil

Remark : The following values are provided as supporting data for the water solubility endpoint. The data were cited in a government reference database (Jokuty et al., 2000). The original source of the data is given as cited in the database.

Heavy Fuel Oil	Water Type	Temp (°C)	Solubility (mg/l)	Ref.
Bunker C	distilled	22	0.4	Suntio, 1986

Reliability : (4) not assignable
Data was presented in a reference database without specific details on measurement methods

(53) (102)

Memo : Water solubility of Bunker C light residual fuel oil

Remark : The following values are provided as supporting data for the water solubility endpoint. Water soluble fractions of hydrocarbons were prepared by combining in Erlenmeyer flasks reconstituted fresh or salt water and Bunker C light fuel oil using a ratio of 40:1 by volume. Flasks were fitted with a stopcock near the bottom to remove the water soluble fractions, covered to exclude light, and capped to prohibit loss of volatile components. Flasks were stirred for 3 days using a teflon-coated stir bar and a magnetic stirrer set at the slowest speed to prevent emulsification of the oil. After stirring, the water soluble fractions with overlying excess whole oil were stored tightly capped in the dark for up to 5 days before analysis. Water soluble fractions were extracted with hexane and measured for total petroleum hydrocarbons by fluorescence spectroscopy using a Perkin Elmer MPF-3 Fluorescence Spectrophotometer. The fluorescence intensity of the water soluble fractions were compared to a calibration curve for the oil. Calibration curves were prepared by analyzing varying concentrations of each test material made up with hexane. Standard solutions and extracts were scanned to determine the optimum excitation and emission wavelengths.

Heavy Fuel Oil	Water Type	Temp (°C)	Solubility (mg/l)
Bunker C light	Fresh	20	4.5
	Salt		2.3

Reliability : (2) valid with restrictions
Details of the composition of the test sample were not provided.

(58)

Memo : Water solubility of Bunker C residual fuel oil

Remark : The following values are provided as supporting data for the water solubility endpoint. Water soluble fractions of hydrocarbons were prepared from a Venezuelan Bunker C residual oil by placing 1 part oil over 9 parts seawater (10% oil fractions) in a glass bottle. The bottle was capped to prevent loss of volatile components and the solution was slowly stirred for a period of 20 hours at room temperature (20 ± 2 °C). The stirring speed was adjusted to give a vortex that extended no further than 25% of the distance to the bottom of the container. After mixing, the oil/water mixture was rested for 1 - 6 hours then the water phase was siphoned from below the oil/water surface through a nylon filter prior to analysis. Total petroleum hydrocarbons in the water samples were determined by the American Petroleum Institute method no. 733-58 by infrared analysis of the carbon tetrachloride extractable oil.

Heavy Fuel Oil	Water Type	Temp (°C)	Solubility (mg/l)
Bunker C residual	salt	20	6.3

Reliability : (2) valid with restrictions
Details of the composition of the test sample and analytical methodology were not reported.

(2)

Memo : Water solubility of catalytically cracked clarified oil (CAS No. 64741 62 4)

Remark : The following value is provided as supporting data for the water solubility endpoint. The data was cited in the European Chemicals Bureau IUCLID dataset (ECB, 2000). The original source of the data is given as cited in the dataset.

Water solubility: <100 mg/l Ref: Mobil, 1993

Reliability : (4) not assignable
Data was presented in a reference database without specific details on measurement methods.

(41) (81)

3.1.1 PHOTODEGRADATION

Method : Calculated): by subroutine AOPWIN V1.90 in EPIWIN V3.10 (u.s. EPA 2000)

GLP : No

Test substance : Heavy fuel oils

Remark : Chemicals having the potential to photolyze have UV/visible absorption maxima in the range of 290 to 800 nm. Saturated alkanes and single-ring alkylated aromatic hydrocarbon constituents in heavy fuel oils are not recognized as absorbing light energy within this spectrum. Hence they are not expected to undergo direct photodegradation. Direct photolysis of polyaromatic hydrocarbons by reaction with sunlight in the presence of oxygen is known to occur (Fasnacht and Blough, 2002), and may be a significant removal process where such substances are present in, or near the surface of water (CONCAWE 2001).

Petroleum hydrocarbons have the capability to react with photosensitized OH radicals in the troposphere, resulting in degradation of the parent compound (Atkinson, 1990). These reactions are termed indirect photodegradation, with saturated as well as single and multi-ring aromatic hydrocarbons taking part to some extent. The potential to undergo indirect photodegradation was estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (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 half-lives were calculated for the various molecular weight and isomeric structures representing constituent hydrocarbons in heavy fuel oils. The estimates shown indicate that if volatile components of heavy fuel oils enter the troposphere, these compounds will undergo moderate to rapid indirect photodegradation and will not persist in the air.

Result :

Concentration of substance: N/A
Temperature C: 25 °C

Direct Photolysis:

Half-life T1/2 N/A
Degradation % N/A
Quantum Yield N/A

Indirect Photolysis:
Sensitizer Type: Hudroxyl radicals (OH-)
Concentration of Sensitizer: 1.5×10^{6} OH^{-}/cm^{3}
Rate Constant: Various
Half-life T1/2, days: See table of half-lives below
Breakdown Products: N/A

Chemical	No. Carbon Atoms	Calculated AOP Half-life, days
n-alkanes	7	1.6
	11	0.9
	20	0.4
	50	0.2
iso-alkanes	7	1.6
	11	0.9

		20	0.4
		50	0.2
cyclo-alkanes			
	1-ring	7	1.1
		11	0.7
		20	0.4
		50	0.2
	2-ring	11	0.5
		20	0.3
		50	0.1
	3-ring	12	0.6
		20	0.3
		50	0.1
olefins			
		7	0.3
		11	0.3
		20	0.2
		50	0.1
aromatics			
	1-ring	7	2.0
		11	1.1
		20	0.5
		50	0.2
	2-ring	11	0.2
		20	0.2
		50	0.1
	3-ring	14	0.3
		20	0.3
		50	<0.1
polar/heterocyclics			
Quinolines			
	quinoline	9	0.9
	C5-quinoline	14	0.4
	C11-quinoline	20	0.3
	C41-quinoline	50	<0.1
Pyridines			
	C2-pyridine	7	5.2
	C9-pyridine	14	0.9
	C15-pyridine	20	0.5
	C45-pyridine	50	0.2
Carboxy Acids			
	C1-1-ring	7	1.1
	C1-2ring	11	0.5
	C2-3-ring	16	0.2
	C6-3-ring	20	0.3
	C32-4-ring	50	0.1
Thiophenes/Benzothiophenes			
	C3 thiophene	7	0.4
	dibenzothiophene	12	0.4
	C-8 dibenzothiophene	20	0.1
	C38 dibenzothiophene	50	<0.1

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(26) (30) (42) (45)

3.1.2 STABILITY IN WATER

Test substance : Heavy fuel oils

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the heavy fuel oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

Reliability : (1) valid without restriction
(49)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Method : Calculations by fugacity-based Environmental Equilibrium Partitioning Model (EQC model) (Mackay, 1991)

Year :

Remark : Substances in the heavy fuel oil category have a carbon number distribution primarily between C20 and C50, although some individual refining streams in this category have low end carbon numbers of 7 to 15. The predominant hydrocarbon structures include saturated alkanes (e.g., straight and branched chain), cyclic alkanes, aromatics (e.g., one to multi-ring compounds), and to a lesser extent olefinic compounds and heterocyclic compounds that contain sulfur, oxygen and nitrogen atoms. The constituent hydrocarbons used to estimate environmental distribution are representative of compounds known to occur in heavy fuel oils. They were chosen based on known hydrocarbon compositional analysis and compositional modeling (Potter and Simmons, 1998; Quann and Jaffe, 1992; Saeger and Jaffe, 2002). Therefore, the data represent a potential range of partitioning behaviors for constituent hydrocarbons in all members of the Heavy Fuel Oil category.

Partitioning behavior depends largely on molecular weight, with smaller compounds (e.g., 7 to 12 carbon atoms) partitioning to the air due to relatively high vapor pressures. In the atmosphere they are expected to degrade rapidly via indirect photodegradation processes. Once hydrocarbons attain C20, they partition to the terrestrial environment where they are expected to undergo slow to moderate biodegradation. Mobility of the heavier fractions in the aquatic environment is low due to low water solubility, while the hydrocarbons that are soluble also have substantial vapor pressures as well as ability to biodegrade. Much real-world information has been gained from studies on heavy fuel oil spills (Fuel oil #6 or Bunker C) since this oil is carried by all cargo ships and is the most frequently spilled oil (Jezequel et al. 2003). When spilled on water, heavy fuel oil usually spreads into thick, dark colored slicks that will often breakup into discrete patches and tarballs (NOAA, 2004). Only the lowest molecular weight fractions would be expected to disperse into the water column, and only 5-10% of the material is expected to evaporate within the first few hours of a spill. The specific gravity of a particular fuel oil may vary from 0.95 to 1.03; thus, spilled oil can float, suspend, or sink (NOAA, 2004). Small changes in water density may dictate whether the oil will sink or float. With time and the effects of weathering, heavy fuel oil attains a tar-like consistency, and these fractions will become incorporated into soil or bottom sediments where they will undergo slow to moderate biodegradation. Overall, the principle routes of weathering of spilled heavy fuel include physical removal, dissolution, photooxidation, and biodegradation (Jezequel, et al. 2003).

Result : Air, Water, Soil, Sediment, Suspended Sediment, Fish.

PERCENT DISTRIBUTION

Hydrocarbon Constituent (Carbon No.)	Air	Water	Soil	Sed	Susp. Sed	Fish
n-alkanes						
(C7)	100	<0.1	<0.1	<0.1	<0.1	<0.1
(C11)	93	<0.1	7	<0.1	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
Iso-alkanes						
(C7)	100	<0.1	<0.1	<0.1	<0.1	<0.1
(C11)	95	<0.1	5	<0.1	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
1-ring cycloalkanes						
(C7)	100	<0.1	<0.1	<0.1	<0.1	<0.1
(C11)	99	<0.1	0.9	<0.1	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
2-ring cycloalkanes						
(C11)	97	0.1	3	0.1	<0.1	<0.1
(C20)	2	<0.1	96	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
3-ring cycloalkanes						
(C12)	94	0.4	5	0.1	<0.1	<0.1
(C20)	2	<0.1	96	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
olefins						
(C7)	100	<0.1	0.1	<0.1	<0.1	<0.1
(C11)	96	<0.1	4	<0.1	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
1-ring aromatics						
(C7)	99	0.8	0.4	<0.1	<0.1	<0.1
(C11)	88	0.4	11	0.2	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
2-ring aromatics						
(C11)	53	6	40	0.9	<0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1
3-ring aromatics						
(C14)	1	4	93	2	0.1	<0.1
(C20)	<0.1	<0.1	98	2	<0.1	<0.1
(C50)	<0.1	<0.1	98	2	<0.1	<0.1

Polar/heterocyclics						
quinoline (C9)	3	89	8	0.2	<0.1	<0.1
C5-quinoline (C14)	5	2	91	2	<0.1	<0.1
C11-quinoline (C20)	<0.1	<0.1	98	2	<0.1	<0.1
C41-quinoline (C50)	<0.1	<0.1	98	2	<0.1	<0.1
C2-pyridine (C7)	8	88	4	<0.1	<0.1	<0.1
C9-pyridine (C14)	0.2	0.5	97	2	<0.1	<0.1
C15-pyridine (C20)	<0.1	<0.1	98	2	<0.1	<0.1
C45-pyridine (C50)	<0.1	<0.1	98	2	<0.1	<0.1
C1-carboxylic acid, 1-ring (C7)	4	88	8	0.2	<0.1	<0.1
C1-carboxylic acid, 2-ring (C11)	0.5	30	68	1.5	<0.1	<0.1
C2-carboxylic acid, 3-ring (C16)	<0.1	4	94	2	<0.1	<0.1
C6-carboxylic acid, 3-ring (C20)	<0.1	<0.1	98	2	<0.1	<0.1
C32-carboxylic acid, 4-ring (C50)	<0.1	<0.1	98	2	<0.1	<0.1
C3-thiophene (C7)	90	4	6	0.1	<0.1	<0.1
dibenzothiophene (C12)	3	4	91	2	<0.1	<0.1
C8-dibenzothiophene (C20)	<0.1	<0.1	98	2	<0.1	<0.1
C38-dibenzothiophene (C50)	<0.1	<0.1	98	2	<0.1	<0.1

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(57) (86) (89) (92)

3.5 BIODEGRADATION

Remark : See Section 3.8

3.8 ADDITIONAL REMARKS

Memo : Biodegradability of heavy fuel oils

Remark : Few studies are available on the biodegradation of heavy fuel oils under laboratory conditions using standardized guideline testing methods. Most of the understanding on the biodegradability of petroleum hydrocarbons comes from biodegradation studies on crude oil, various streams from the fractional distillation of crude oil, and investigations of spill events, all of which have been reviewed by Bartha and Atlas (1977) and Connell and Miller (1980). Based on such reviews, a general consensus has developed on the biodegradability of petroleum hydrocarbons. First, virtually all kinds of oil are susceptible to microbial oxidation. The rate of oxidation is influenced by microbial characteristics, and environmental factors such as available nutrients, oxygen, temperature and degree of dispersion. Second, the molecular weight influences the rates at which microbial communities can utilize those hydrocarbons, with low molecular weight components being relatively easy to metabolize, while higher molecular weight components take longer to be consumed. Third, the ease of aerobic microbial biodegradation is affected by the structure of the hydrocarbon constituents in the petroleum substance. Such structure-related trend shows hydrocarbons in order of increasing difficulty to be degraded: (1) n-alkanes, (2) isoalkanes, (3) alkenes, (4) one-ring alkylbenzenes (e.g., BTEX), (5) polyaromatic hydrocarbons, and (6) high molecular weight cycloalkanes (Bartha and Atlas, 1977; Potter and Simmons, 1998).

Prince (2002), Prince et al. (2003) and Garrett, et al. (2003) reviewed the findings of many laboratory and field biodegradation studies under temperate or summer arctic conditions. They summarize that the majority of compounds in crude and refined oil products are biodegradable, but their disappearance from the environment following a spill follows a well-defined order. This order holds for spills in temperate climates and arctic summer conditions alike (Garrett et al., 2003). When biodegradation begins, the smaller linear alkanes and one and two-ring aromatic molecules are initially degraded followed by branched alkanes and polynuclear aromatic compounds. Three-ring aromatics such as fluorene, phenanthrene, and dibenzothiophene are degraded at similar rates and in preference to four-ring compounds. Another general rule for biodegradation of PAHs is that parent compounds tend to degrade faster than alkylated analogs. Less is known about the biodegradability of resins and asphaltenes, but the current knowledge suggests these are not very biodegradable and will persist in the environment for a long time.

For heavy fuel oils, none would be expected to be readily biodegradable based on the molecular weights of constituent hydrocarbons. However, studies have shown that these materials follow the general understanding for biodegradation of the individual components. For example, Walker et al. (1975) found that while only 11% of a Bunker C fuel oil was biodegraded by a mixed culture of estuarine bacteria, 25% of the saturated fraction and 10% of the aromatic fraction were degraded. Inoculum originated from an estuarine creek known to be exposed to low levels of oil contamination. Culture flasks containing nutrient medium supplemented with nitrogen and phosphorus were inoculated with the creek water, spiked with Bunker C (0.1% v/v), then incubated on a shaker (60 strokes/min) for 28 days at 15 ° C. After 28 days, the cultures were extracted with chloroform, fractionated, and analyzed by mass spectrometry.

The 1970 spill of 108,000 barrels of Bunker C fuel oil in Chedabucto Bay, Nova Scotia afforded an opportunity to study the natural fate of such substances. Over the course of several years, high energy areas of shoreline intertidal and sublittoral locations showed a greater loss of n-alkane and aromatic components than in isolated protected areas (Rashid, 1974; Keizer et al., 1978). Although the loss was not specifically identified as being due to biodegradation, Rashid (1974) suggested that the hydrocarbon constituents remaining in the environmental samples were indicative of what would be expected from a combination of biodegradation and physical weathering processes.

A 1973 spill of heavy fuel oil near Vancouver Island, British Columbia also provided opportunities to study the fate of heavy fuel oil. Cretney et al. (1978) studies the chemical characteristics of the spilled fuel over a four-year period. They showed initial loss of the lower molecular weight components by dissolution and evaporation, with almost complete removal within the first year of the spill of n-alkanes by biodegradation. High molecular weight saturates were more resistant, followed by the non-alkane components in the C28+ range. After four years, an unresolved complex consisting of high molecular weight cycloalkanes remained.

Mulkins-Phillips and Stewart (1974) studied the ability of mixed cultures of bacteria to degrade Bunker C fuel oil. Beach and water samples were taken from different locations from Chedabucto Bay, Nova Scotia, one year following the spill. These samples were enriched by growing the indigenous bacteria in minimal medium containing 0.125% Bunker C fuel oil. Flasks were incubated for 14 days in the laboratory and the resulting enriched culture was used as inoculum for the different experiments. Biodegradation experiments were carried out in culture flasks holding 50 ml of minimal medium containing 0.125% by volume of Bunker C. Periodically, the entire contents of a flask was extracted with benzene. The extracts were placed in a pre-weighed bottle and evaporated at 80 °C, and the weight of the bottle and contents was recorded. The weight of the test flasks were corrected for the weight of control flasks and biodegradation was calculated as a percent of the weight loss. Such experiments were carried out at various temperatures (5, 10 and 15 °C). Results showed comparable degradation rates at 10 and 15 °C but considerably slower rates at 5 °C. Bunker C was degraded as high as 88% in these experiments. These rates are likely overstated because the gravimetric method did not account for high molecular weight resins and asphaltenes. Isolated pure cultures of Nocardia sp. from the environmental samples were enriched and used to measure the effect of additions of nitrogen and phosphorus on the generation time and size of the microbial populations. Additions of phosphorus were found to shorten the generation time and increase the population size of Nocardia. Additions of nitrogen had a positive effect on population size, but no effect on generation time. The authors concluded that the rate of natural biodegradation would be limited by temperature and phosphorus but likely not by open sea nitrogen concentrations.
In summary, when a heavy fuel oil is spilled, microbial communities respond quickly to the oiling, with numbers of hydrocarbon-degrading bacteria and mineralization potentials increasing after exposure (Leahy and Colwell, 1990). The rate of mineralization is limited by the high viscosity of these substances and available nutrients (Richmond et al., 2001), while over time, the weathering of the material into discrete tar balls can physically isolate and prevent dispersion and microbial attack. Given time, component hydrocarbons are depleted from spilled heavy fuels through selective biodegradation (Lee et al., 2003; Bartha and Atlas, 1977).

Reliability : (2) valid with restrictions
The technical discussion was prepared from a review of recent and past research and field investigations covering the current accepted scientific understanding on the biodegradability of petroleum hydrocarbons.
(27) (31) (38) (48) (54) (55) (56) (82) (86) (87) (88) (90) (91) (127)

Memo : Photodegradation of polyaromatic hydrocarbons

Remark : Saturated hydrocarbon components of crude oil and refined products do not undergo photodegradation because they do not absorb light energy in the range of 290 to 800 nm. For those components, indirect photodegradation by reaction with sensitized oxygen radicals is the major photochemical degradation pathway (Atkinson, 1990). In contrast, polyaromatic hydrocarbons (PAHs) may be degraded by either direct or indirect photochemical reactions (Fasnacht and Blough, 2002). Most PAHs can absorb surface solar radiation, and if sufficient energy is absorbed, degradation of the parent material may occur(Garrett et al, 1998). Dutta and Harayama (2000) found that photooxidation affected mainly aromatic hydrocarbons and concluded that an oil's susceptibility to biodegradation is increased by the photooxidation of the PAH components. Recent studies by Prince et al. (2003) and Jezequel et al (2003) on the photodegradation of crude and heavy fuel oils have shown that photodegradation follows a clear pattern, with alkylated PAH derivatives being more affected than the parent compound. This has been demonstrated for homologous series of chrysenes, dibenzothiophenes, and phenanthrenes as well as whole product materials such as crude and heavy fuel oils (Bunker C).

The vast majority of the hydrocarbon components of the substances in the heavy fuel oils category, and particularly those with carbon numbers of 20 or more, will have little or no tendency to partition to air. However any hydrocarbons that do partition to air will be exposed to the combination of direct and indirect photodegradation.

Reliability : (2) valid with restrictions
The technical discussion was prepared from a review of recent and past research covering the current accepted scientific understanding of photodegradation of polyaromatic hydrocarbons.

(26) (40) (45) (47) (52) (88)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Semistatic
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1994
GLP	:	Yes
Test substance	:	Fuel oil, residual CAS 68476-33-5

Method : Statistical method: Visual inspection

Result : No fish exposed to WAF of light fuel oil died during the test. 96-hr LL_0 = 1000 mg/l based on nominal loading rates. After 96 h, 1 of the 7 control fish died. All fish in the 100 mg/l treatment exhibited no toxic symptoms. All fish in the 1000 mg/l WAF showed abnormal swimming.
Total peak area of the dissolved components of each batch of freshly prepared WAFs was similar. Peak area values ranged from 19-21 x 10^8 at loading rate of 1000 mg/l and 9-11 x 10^8 at 100 mg/l. Peak profile was different at different loading rates but peak profile for new and old media was similar. Mean reduction in total peak area was 27% during the test (range 5 - 47%). Peak profiles for the WAFs differed significantly from profile of light fuel oil in dichloromethane. Only two loading rates were tested which is less than a minimum of five concentrations stated in the guidelines. Water hardness was higher than targeted range of 50 - 250 mg/l as $CaCO_3$. Hardness range of 286 - 292 mg/l as CaCO3 was normal for this laboratory and did not adversely affect the health of the fish.

Test condition : Individual treatment concentrations were prepared as water accomodated fractions (WAF). Nominal loading rates in the definitive test were 0, 100, and 1000 mg/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 252 mg/l as $CaCO_3$, hardness 277 mg/l as $CaCO_3$, conductivity 520 S/cm, pH 7.4). Test substance was mixed in dilution water for 70 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MSD. Mixtures were allowed to settle ~1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 11-liter glass aspirators which were completely filled with WAF and contained 7 fish per vessel. Test fish had a mean length of 4.7 cm (range 4.0 to 5.2 cm) and a mean weight of 1.0 g (range 0.67 to 1.3 g). Fingerlings were obtained from Zeals Trout Farm, Zeals, Wiltshire, U.K. One replicate per treatment and control were used. Test solutions were renewed daily with surviving fish transferred to the freshly prepared WAFs. Dissolved oxygen and pH were measured in the fresh and old media at 24-h intervals. Temperature of water in a vessel adjacent to test vessels was determined at hourly intervals throughout the test. Total hardness and residual chlorine were determined in each batch of fresh control media. Test temperature was 15 - 16 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen ranged from 8.8 to 9.1 mg/l in the fresh media and 8.1 to 9.2 mg/l in the old solutions. pH was 7.2 - 7.7. A gas chromatographic method with mass selective detection was used to quantify the total peak area of dissolved components of light fuel oil in the test media. Samples were collected from each freshly prepared WAF and control and each batch of old media except at 96 h. 500 ml samples were extracted with dichloromethane and then analyzed.

Reliability : (1) valid without restriction

(96)

Type	:	Semistatic
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1994
GLP	:	Yes
Test substance	:	Fuel oil, residual CAS 68476-33-5

Method : Statistical method: Visual inspection

Result : 96-h LL_{50} lie within the range of 100-1000 mg/l loading rates. The highest $NOEL_R$ (loading rate in which 1 fish died per test vessel) was 100 mg/l. After 96 h, there was 100% survival in the control and 10 mg/l WAF. All fish survived in the 100 mg/l but two fish showed abnormal swimming. Four of the seven fish died in the 1000 mg/l WAF and the other 3 were immobilized.
Amount of heavy fuel oil in the test solutions varied between the four batches of media prepared to give RIC values of 1.9 x 10^5 to 2.7 x 10^5 at 10 mg/l loading rate, 6.8 x 10^5 to 27 x 10^5 at 100 mg/l, and 31 x 10^5 to 53 x 10^5 at 1000 mg/l. Mean reduction in peak area over the 24-h period was 20% (range 0 - 57%).
Water hardness was higher than targeted range of 50 - 250 mg/l as $CaCO_3$. Hardness range of 262 - 285 mg/l as $CaCO_3$ was normal for this laboratory and did not adversely affect the health of the fish.
Use of loading rates, which differed by a factor of 10, was necessary because of logistical difficulties of daily renewal of WAFs which required ~72 h of stirring.

Test condition : Individual treatment concentrations were prepared as water accomodated fractions (WAF). Nominal loading rates in the definitive test were 0, 10, 100, and 1000 mg/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 255 mg/l as $CaCO_3$, hardness 287 mg/l as $CaCO_3$, conductivity 536 S/cm, pH 7.4). Test substance was mixed in dilution water for 68-70 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MSD. Mixtures were allowed to settle ~1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 11-liter glass aspirators which were completely filled with WAF and contained 7 fish per vessel. Test fish had a mean length of 4.4 cm (range 4.3 to 4.7 cm) and a mean weight of 0.76 g (range 0.56 to 0.89 g). Fingerlings were obtained from Exmoor Trout Farm, North Molton, Devon, U.K. One replicate per treatment and control were used. Test solutions were renewed daily with surviving fish transferred to the freshly prepared WAFs. Dissolved oxygen and pH were measured in the fresh and old media at 24-h intervals. Temperature of water in a vessel adjacent to test vessels was determined at hourly intervals throughout the test. Total hardness and residual chlorine were determined in each batch of fresh control media.
Test temperature was 15 - 16 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen ranged from 8.8 to 9.5 mg/l in the fresh media and 8.5 to 9.3 mg/l in the old solutions. pH was 7.1 - 7.8.
A gas chromatographic method with mass selective detection was used to quantify the areas of two representative reconstructed ion chromatographic (RIC) peaks of dissolved components of heavy fuel oil in the test media. Samples were collected from each freshly prepared WAF and control and each batch of old media. 500 ml samples were extracted with dichloromethane and then analyzed.

Reliability : (1) valid without restriction

(98)

Type	:	Static
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	No
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1987
GLP	:	No
Test substance	:	No. 6 Fuel oil, vacuum residual oil

Method : Binomial Probability Analysis (not used)

Remark : Only four concentrations were tested which is less than a minimum of five concentrations stated in the guidelines.

Result : A 96-hr LC_{50} value was not determined due to insufficient mortality at the maximum treatment of 10,000 mg/l. Therefore no statistical analysis was performed. Mortality at 96hr: no mortality in the control treatment; 5% for 500, 1000, and 5000 mg/l treatments and 25% for the 10,000 mg/l treatment.

Test condition : Individual treatment concentrations were prepared as oil-water dispersions (OWD). Nominal loading rates in the definitive test were 0, 500, 1000, 5000, and 10,000 mg/l. Control and dilution water were site well water. Report characteristic alkalinity of 150 mg/l as $CaCO_3$, hardness 262 mg/l as $CaCO_3$, and pH 7.7 for well water.

Test fish had a mean length of 27 mm and a mean weight of 0.41 g. Fish were obtained from ARO Inc, Hampton, N.H, and acclimated at least 14 days prior to testing. Twenty fish per treatment and control were used. The semi-solid test substance was heated in a 60 °C oven prior to dispensing and then added volumetrically to glass petri dishes, and which were then reheated to provide uniform distribution of the oil on the petri dish. The density of the process oil of 1.00 g/ml was used to calculate the mass of test material added. The glass petri dishes were then transferred to 10 gallon glass aquaria (test systems) containing 30 liters of well water within one hour after the transfer of the fish test organisms. The control chamber consisted of the same dilution water, petri dish, and test organisms. Test systems were held in a recirculating water bath maintained at a mean temperature of 21.5 °C (20.3-22). Generation of the oil-water dispersion was based on a modification of the procedure used by the Ministry of Agriculture, Fisheries and Food (MAFF), England. The test chambers were fitted with a removable PVC cylinder that housed a stainless steel shaft and a 3 bladed propeller. The propeller was rotated in order to produce flow in the cylinder by drawing small quantities of water and soluble oil components into the top of the cylinder and expelling them through apertures near the bottom of the cylinder. The motor speed settings were adjusted so that the vortex extended 0.25 to 0.50 inches below the water surface. Test solutions were not renewed during the study.

Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (7.5 to 9.4 mg/l) and pH was 8.11 - 8.26. Ammonia levels were noted as being below detectable limits in the study chambers at study termination.

Reliability : (1) valid without restriction

(64)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1994
GLP : Yes
Test substance : Fuel oil, residual CAS 68476-33-5

Result : There was no immobilization of D. magna in the control and 1000 mg/l WAF during the test. 48-hr EL_0 = 1000 mg/l based on nominal loading rates.
Total peak area of the dissolved components in the 0 hr new and 48 hr old 1000 mg/l WAF solutions was 27 x 10^8 and 5 x 10^8 representing a reduction in total peak area of 81%. Peak profile for the WAF differed significantly from profile of light fuel oil in dichloromethane.
Only one loading rate was tested. Test temperature was higher than targeted.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0 and 1000 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis filtered water following EPA guidelines (hardness 196 mg/l as $CaCO_3$). Test substance was mixed in dilution water for 69 hrs (mixing time of 24 hr would have been sufficient to attain equilibrium) in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MSD. Mixtures were allowed to settle ~1hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 150-ml Erlenmeyer flasks which were completely filled with WAF and contained 10 daphnids per vessel. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 14 and 28 days. Two replicates per treatment and control were used. Dissolved oxygen and pH were measured at the beginning and end of the test. Temperature of water in a vessel adjacent to test vessels was determined at hourly intervals throughout the test. Total hardness of the control medium was determined at the start of the test.
Test temperature was 21 - 23 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen ranged from 8.4 to 8.7 mg/l. pH was 7.9 - 8.2.
A gas chromatographic method with mass selective detection was used to quantify the total peak area of dissolved components of light fuel oil in the test media. Samples, collected at the beginning and end of the test, were extracted with dichloromethane and analyzed.

Reliability : (1) valid without restriction

(95)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1994
GLP : Yes
Test substance : Fuel oil, residual CAS 68476-33-5

Result : 48-h EL_{50} lie within the range of 220-460 mg/l loading rates. The highest $NOEL_R$ (loading rate which caused 10% immobilization) was 100 mg/l. There was no immobilization of D. magna in the control and 46 and 100 mg/l WAF after 48-h. There were 5, 13, and 20 daphnids immobilized in the 220, 460, and 1000 mg/l WAFs, respectively.
RIC peak areas for the 0-h samples were 3.6, 10, 9.1, 17, and 29 x 10^5 for the 46, 100, 220, 460, and 1000 mg/l WAFs. The corresponding RIC peak areas for the 48-h samples were 3.9, 7.8, 8.7, 14, and 17 x 10^5. Mean reduction in peak area over the 48-h period was 17% (range 0-41%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 46, 100, 220, 460, and 1000 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis filtered water following EPA guidelines (hardness 180 mg/l as $CaCO_3$).
Test substance was mixed in dilution water for 44 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MSD. Mixtures were allowed to settle ~1hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 150-ml Erlenmeyer flasks which were completely filled with WAF and contained 10 daphnids per vessel. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 14 and 28 days. Two replicates per treatment and control were used. Dissolved oxygen and pH were measured at the beginning and end of the test. Temperature of water in a vessel adjacent to test vessels was determined at hourly intervals throughout the test. Total hardness of the control medium was determined at the start of the test.
Test temperature was 19 - 21 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen ranged from 8.7 to 8.9 mg/l. pH was 8.1 - 8.2.
A gas chromatographic method with mass selective detection was used to quantify the areas of two representative reconstructed ion chromatographic (RIC) peaks of dissolved components of heavy fuel oil in the test media. Samples (250 ml), collected at the beginning and end of the test, were extracted with dichloromethane and analyzed.

Reliability : (1) valid without restriction

(93)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : No
Method : OECD Guide-line 202
Year : 1987
GLP : No
Test substance : No. 6 Fuel oil, vacuum residual oil

Method : Binomial Probability Analysis (not used)
Result : A 48-hr EC_{50} value was not determined due to insufficient mortality at the maximum treatment of 10,000 mg/l. Therefore, no statistical analysis was performed. Number of immobilized daphnids after 48 hrs were 1, 0, 0, 1, 0, and 0 in the 0, 100, 500, 1000, 5000, and 10,000 mg/l treatments.

Test condition : Nominal loading rates in the definitive test were 0, 100, 500, 1000, 5000, and 10,000 mg/l. Control and dilution water were site well water. Report characteristic alkalinity of 150 mg/l as $CaCO_3$, hardness 262 mg/l as $CaCO_3$, and pH 7.7 for well water.
The semi-solid test substance was heated in a 60 °C oven prior to dispensing and then added volumetrically to 250 ml glass beakers, which were then reheated to provide uniform distribution of the oil. The density of the process oil of 1.00 g/ml was used to calculate the mass of test material added. Two hundred ml of well water (control and dilution) was added after test material distribution, with subsequent addition of test organisms. Test

solutions were not renewed during the study. Test systems were held in a water bath maintained at a mean temperature of 22.5 °C (±2 °C).
Test daphnids were obtained from the third brood onwards of cultures maintained by the testing laboratory that have been aged <28 days. The primary culture originated from Analytical Bio-Chemistry Laboratories Inc., Columbia, MO. Triplicate replicates per treatment and control were used, with 10 organisms per replicate.
Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was 8.3 to 9.1 mg/l. pH was 7.71 to 8.29.

Reliability : (1) valid without restriction

(63)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1994
GLP : Yes
Test substance : Fuel oil, residual CAS 68476-33-5

Method : Williams test used to determine NOELs

Result : Based on nominal loading rates, ranges within which lie 72-hr EL_{50} (biomass) and 72-hr EL_{50} (growth rate) were 3-10 mg/l and 100-300 mg/l, respectively. 72-hr NOEL (biomass) = <1 mg/l; 72-hr NOEL (growth rate) = <1 mg/l.

Nominal Conc. (mg/l)	72 h % Inhibition	72 h Mean Cell Conc. ($x10^6$cells/ml)
Control	n/a	0.12
1.0	22	0.093
3.0	19	0.097
10	46	0.065
30	58	0.05
100	44	0.067
300	77	0.027
1000	72	0.033

n/a - Not applicable

Difference between EbL_{50} and ErL_{50} was due to an initial lag followed by recovery at loading rates between 3 and 100 mg/l. The initial lag affected the 72-hr EbL_{50} and not the 72-hr ErL_{50}.
Total peak area of the dissolved components ranged from <1 x 10^8 at loading rate of 1mg/l to 16-20 x 10^8 at 1000 mg/l. Peak profile was different at different loading rates but peak profile for new and old media was similar. Mean reduction in total peak area was 44% during the test (range 20 -67%). Peak profiles for the WAFs differed significantly from profile of light fuel oil in dichloromethane.
There was a maximum pH change of 1.1 which was greater than the target of <1. This was a result of the growth of the cultures and could not be avoided.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 1.0, 3.0, 10, 30, 100, 300, and 1000 mg/l. Control and dilution water was algal nutrient medium prepared according to EPA guidelines except that boric acid was present at 105 g/l and sodium bicarbonate at 50 mg/l. Test substance was mixed with dilution water for 22 hrs, and the mixture was allowed to settle for approximately 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 300 ml Erlenmeyer flasks completely filled with test solution. There were four flasks for each

treatment and seven control flasks. Three of the four treatment and six of the seven control flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Uninoculated flasks were used to determine particle counts without algal cells using a Coulter Multisizer. Two marbles were placed in each flask to ensure good mixing during incubation. Flasks were incubated in a cooled orbital (100 cycles/min) incubator under constant illumination. Loading rates causing a 50% reduction in growth were calculated on the basis of areas under the growth curves (EbL_{50}) and average specific growth rates (ErL_{50}). Percent reduction in growth at each loading rate compared to controls was used to estimate EL_{50} values. Cell counts were made on samples from each flask at 24-hr intervals. pH was measured at the start and end of the test. Air temperature in the test incubator was monitored at hourly intervals throughout the test.
Test temperature was 24 - 25 °C. The pH ranged from 7.5 - 8.0 at test initiation and 8.5 - 8.7 at test termination.
A gas chromatographic method with mass selective detection was used to quantify the total peak area of dissolved components of light fuel oil in the test media. 500 ml samples, collected at the beginning and end of the test, were extracted with dichloromethane and analyzed.

Reliability : (1) valid without restriction

(97)

Species : Selenastrum capricornutum (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1994
GLP : Yes
Test substance : Fuel oil, residual CAS 68476-33-5

Method : Williams test used to determine NOELs
Result : 72-h EL_{50} for biomass and growth rate both lie within the range of 30-100 mg/l loading rates. 72-hr NOEL (biomass) = 1 mg/l; 72-hr NOEL (growth rate) = 3 mg/l.

Nominal Conc. (mg/l)	72 h % Inhibition	72 h Mean Cell Conc. ($x10^6$cells/ml)
Control	n/a	0.13
1.0	8	0.12
3.0	15	0.11
10	36	0.083
30	38	0.08
100	82	0.023
300	93	0.009
1000	92	0.01

n/a - Not applicable

RIC peak areas for the 0-h samples were 0.07, 0.24, 1.2, 3.0, 14, 18, 27 x 10^5 for the 1, 3, 10, 30, 100, 300, and 1000 mg/l WAFs. The corresponding RIC peak areas for the 72-h samples were 0.05, 0.2, 0.89, 2.2, 10, 12, and 20 x 10^5. Mean reduction in peak area over the 72-h period was 27% (range 17-33%).
There was a maximum pH change of 1.8 which was greater than the target of <1. This was a result of the growth of the cultures and could not be avoided.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 1.0, 3.0, 10, 30, 100, 300, and 1000 mg/l. Control and dilution water was algal nutrient medium prepared according to EPA guidelines except that boric acid was present at 105 g/l and sodium bicarbonate at 50 mg/l. Test substance was mixed with dilution water for 47 hrs, and the

mixture was allowed to settle for approximately 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 300 ml Erlenmeyer flasks completely filled with test solution. There were four flasks for each treatment and seven control flasks. Three of the four treatment and six of the seven control flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Uninoculated flasks were used to determine particle counts without algal cells using a Coulter Counter. Two marbles were placed in each flask to ensure good mixing during incubation. Flasks were incubated in a cooled orbital (100 cycles/min) incubator under constant illumination (~5000 lux). Loading rates causing a 50% reduction in growth were calculated on the basis of areas under the growth curves (EbL50) and average specific growth rates (ErL_{50}). Percent reduction in growth at each loading rate compared to controls was used to estimate EL_{50} values. Cell counts were made on samples from each flask at 24-hr intervals. pH was measured at the start and end of the test. Air temperature in the test incubator was monitored at hourly intervals throughout the test. Test temperature was 24 - 25 °C. The pH ranged from 7.7 - 7.9 at test initiation and 8.6 - 9.7 at test termination.
A gas chromatographic method with mass selective detection was used to quantify the areas of two representative reconstructed ion chromatographic (RIC) peaks of dissolved components of heavy fuel oil in the test media. Samples (250 ml), collected at the beginning and end of the test, were extracted with dichloromethane and analyzed.

Reliability : (1) valid without restriction

(94)

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : No
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1987
GLP : No
Test substance : No. 6 Fuel oil, vacuum residual oil

Method : Binomial Probability Analysis (not used)
Remark : Since test material was coated on the flasks during administration, there may have been some physical obstruction of light transmittance which may have affected cell growth. The report does not clarify whether only the flask bottoms or bottom and sides were coated with the test material.
Result : The reported 96-hr EC_{50} was greater than 5000 ppm. The reported NOEC was less than 100 ppm. No additional data analysis for algal effects are reported. Cell growth and percent inhibition for each treatment relative to the control are reported at 96 hr:

Nominal Conc. (mg/l)	96 hr % Inhibition	96 hr Cell Conc. (cells/ml)
Control	n/a	$1.2E^6$
100	27.5	$8.7E^5$
500	22.5	$9.3E^5$
1000	24.5	$9.1E^5$
5000	39.2	$7.3E^5$
10,000	47.5	$6.3E^5$

Test condition : Nominal loading rates in the definitive test were 0, 100, 500, 1000, 5000, and 10,000 mg/l.

The semi-solid test substance was heated in a 60 °C oven prior to dispensing and then added volumetrically to 250 ml glass Erlenmeyer flasks, which were then reheated to provide uniform distribution of the oil. The density of the process oil of 1.00 g/ml was used to calculate the mass of test material added. Control and dilution water was algal nutrient medium prepared with distilled, autoclaved site well water.

Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Cells were incubated in algal media contained in 250 ml flasks which were maintained in an orbital (100 cycles/min) incubator at 24 ± 2 °C. Cell density was determined prior to study initiation by microscopic cell count. Nutrient medium was inoculated with algal cells (in log phase growth) to yield an initial concentration of 10,000 cells/ml. One hundred milliliters of inoculated nutrient medium was then added to each 250 ml Erlenmeyer flask previously dosed with process oil. Control systems containing only algal inoculated medium were also prepared. There were three flasks for each of the dose treatments and control test systems. After media addition, the flasks were fitted with cotton plugs and maintained in an orbital (100 cycles/min) incubator at 24 ± 2 °C. After 96 hrs, the cell density was determined microscopically for each flask. The 96-hour EC_{50} value was calculated on the basis of percent cell number increase or reduction relative to growth in controls.
Lighting was continuous at ~4304 lumens. The pH of all test treatment solutions ranged from 7.95 - 8.75.

Reliability : (2) valid with restrictions

(65)

4.9 ADDITIONAL REMARKS

Memo : Aquatic toxicity of Bunker C Fuel Oils

Remark : Aquatic toxicity values determined as percent water soluble fraction tests. Data cited in Jokuty, et al. (2002; Environment Canada database).

Species	Endpoint	Value, mg/l
Neanthes arenaceodentata	96H LC_{50}	3.6
Capitaella capitata	96H LC_{50}	0.9
Mysidopsis almyra	48H LC_{50}	0.9
Palaemonetes pugio	96H LC_{50}	2.6
Penaeus aztecus	96H LC_{50}	1.9
Menidia beryllina	96H LC_{50}	1.9
Fundulus similes	96H LC_{50}	1.7
Cyprinodon variegates	96H LC_{50}	3.1

Reliability : (4) not assignable
Endpoint values given in government database lacked details of exposure information and explanation of concentration measurements.

(53)

Memo : Aquatic toxicity of Kerosene/Jet fuel and Gas Oil HPV Category members.

Remark : Individual petroleum streams in the heavy fuel oil category generally have hydrocarbon constituents consisting of 20 to 50 carbon atoms, although some streams in this category have low-end carbon atoms from 7 to 15. Heavy fuel oils also may be blended with gas oils or similar low viscosity oils to meet market specifications. Therefore, existing ecotoxicity data for heavy fuels may not represent toxicity values for all process streams defined in the HPV category. However, constituents in heavy fuels are generic hydrocarbon structures (e.g., saturates, aromatics, etc.) represented in other petroleum HPV category groups. For this reason, data from other petroleum categories were used to bridge existing ecotoxicity data for heavy fuels such that all members in the heavy fuel oil category are covered.

The following data for kerosene and gas oils are included because they provide potential ecotoxicity endpoints for heavy fuel oil streams with low initial boiling points and low-end hydrocarbon constituents of C7 to C15. Data from the kerosene and gas oils categories were selected because these substances contain similar hydrocarbon structures with molecular weights covering the low-end carbon numbers of heavy fuel oil category members. Therefore, the ecotoxicity data for those petroleum streams were used to read across to the heavy fuel oil category. The combination of 1) existing heavy fuel oil data, 2) current data cited in the kerosene and gas oils HPV categories, and 3) data from proposed testing of specific gas oil streams are expected to provide ecotoxicity endpoint values that span expected ecotoxicity of all substances in the heavy fuel oil HPV category. Complete robust summaries of the cited studies were included in the robust summary files submitted to EPA under their respective HPV category (API, 2003a,b).

Test Substance	Exposure Type	Endpoint	Results (mg/l)	Ref.
Fish				
Kerosene	WAF	96-h LL_{50}	18	API, 2003a
	"	" "	20	API, 2003a
	"	"	10 - 100	API, 2003a
	"	" "	25	API, 2003a
Gas Oil	"	"	57	API, 2003b
	"	" "	3.2	API, 2003b
	"	" "	6.6	API, 2003b
	"	" "	57	API, 2003b
	"	" "	21	API, 2003b
	"	" "	65	API, 2003b
Invertebrate				
Kerosene	"	48-h EL_{50}	21	API, 2003a
	"	" "	1.4	API, 2003a
	"	" "	40 – 89	API, 2003a
	"	" "	1.9	API, 2003a
Gas Oil	"	" "	"7.8	API, 2003b
	"	" "	5.3	API, 2003b
	"	" "	14	API, 2003b
	"	" "	42	API, 2003b
	"	" "	2.0	API, 2003b
	"	" "	210	API, 2003b
	"	" "	68	API, 2003b
	"	" "	13	API, 2003b
	"	" "	100 - 300	API, 2003b
	"	" "	13	API, 2003b
	"	" "	6.4	API, 2003b
	"	" "	36	API, 2003b
	"	" "	9.6	API, 2003b
Algae				
Kerosene	"	96-h ELr_{50}	6.2	API, 2003a
	"	96-h ELb_{50}	11	API, 2003a
	"	72-h ELr_{50}	10 - 30	API, 2003a
	"	72-h ELb_{50}	10 - 30	API, 2003a
	"	96-h ELr_{50}	5.0	API, 2003a
	"	96-h ELb_{50}	5.9	API, 2003a
Gas Oil	"	72-h ELr_{50}	2.9	API, 2003b
	"	72-h ELb_{50}	1.8	API, 2003b
	"	72-h ELr_{50}	2.2	API, 2003b
	"	72-h ELb_{50}	2.2	API, 2003b
	"	72-h ELr_{50}	78	API, 2003b
	"	72-h ELb_{50}	25	API, 2003b
	"	72-h ELr_{50}	22	API, 2003b
	"	72-h ELb_{50}	10	API, 2003b
	"	72-h ELr_{50}	22 - 46	API, 2003b

"	72-h ELb_{50}	10 - 22	API, 2003b

WAF = water accommodated fraction

Reliability : (1) valid without restriction

(22) (23)

5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}
Value : > 5000 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : Undiluted
Doses : Single dose of 5 g/kg bw
Year : 1990
GLP : Yes
Test substance : Atmospheric residue, sample F-132. (See section 1.1.1.)

Method : Undiluted test material was administered orally by gavage to groups of 5 male and 5 female, fasted young adult, Sprague-Dawley rats.
Following administration of test material, each animal was observed hourly for the first four hours and twice daily thereafter for 14 days.
Body weights were recorded the day before dosing, immediately before test material administration and again seven and 14 days after dosing.
At study termination surviving animals were euthanized and subjected to a gross necropsy examination. Any abnormalities were recorded.
Result : There were no mortalities during the study.
Clinical signs consisted of an oral discharge occurring in one animal within an hour of dosing and stained coat of eight animals on day 1. A swollen penis was also observed in one animal on day 2. There were no other clinical observations and growth was normal throughout the study. At necropsy, lesions consisting of dark red areas 1-2 mm in diameter in some lung lobes of 3 males and 2 females.
No other adverse effects observed.
Reliability : (1) valid without restriction

(117)

Type : LD_{50}
Test substance : Atmospheric distillates

Remark : There are no data available on heavy atmospheric distillates. However, data on the lighter atmospheric distillates would represent a worst case since the molecules are smaller and thus more likely to be absorbed. Data on such materials have been reviewed in the Robust summaries for gas oils.

(23)

Type : LD_{50}
Test substance : other TS: Vacuum residues

Remark : No data available.

Type : LD_{50}
Value : > 5000 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : Undiluted
Doses : Single dose of 5 g/kg
Year : 1988
GLP : No data

Test substance	:	Vacuum distillates
Method	:	A single oral dose of undiluted test material was administered to groups of 5 male and 5 female Sprague Dawley rats that had been fasted overnight prior to dosing. The animals were observed for signs of toxicity 30 minutes after dosing and again at 1 and 4 hours and daily thereafter for 14 days. Body weights were recorded prior to dosing and again on days 0, 7 and 14 after dosing. All animals were necropsied on day 14 of the study.
Remark	:	LD_{50} values determined according to the same protocol have been reported for two other samples of vacuum distillate with the following results. Visbreaker HGO >5000 mg/kg Mobil 62496-99 VB Mittelol >5000 mg/kg Mobil 64635-38
Result	:	There were no deaths and all animals gained weight throughout the study. Clinical signs of toxicity included decreased activity of all animals at 30 minutes and in 8/10 animals 1 hour after dosing. On day 1, observations in up to half the animals included: chromorhinorrhea, decreased fecal output and urogenital staining, and decreased urine output. The incidence of these observations was smaller on day 2. There were no clinical observations after day 8. There were no findings at gross necropsy. The LD_{50} was, therefore, greater than 5 g/kg. Visbreaker HGO >5000 mg/kg Mobil 62496-99 Vis gas oil VIBRA >5000 mg/kg Mobil 62500-03 VB Mittelol >5000 mg/kg Mobil 64635-38
Test substance	:	Data are available on four samples of vacuum distillate. The samples are: Heavy vacuum gas oil Visbreaker HGO Vis gas oil VIBRA VB Mittelol
Reliability	:	(2) valid with restrictions The report was a summary report consolidating the results of several acute studies. Complete experimental details and results were not included. However, the results are consistent and considered to be valid.

(70) (71) (75)

Type	:	LD_{50}
Value	:	= 4320 - 5270 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	10
Vehicle	:	None - undiluted
Doses	:	3.2, 4.0, 4.0, 6.25 & 7.81 g/kg
Year	:	1982
GLP	:	Yes
Test substance	:	Catalytically cracked clarified oil (API 81-15) See section 1.1.1.
Method	:	Undiluted test material was administered orally by gavage to groups of 5 male and 5 female, fasted young adult, Sprague-Dawley rats. Following administration of test material, each animal was observed for pharmacotoxic signs and mortality at hourly intervals for the first six hours and twice daily thereafter for 14 days. Body weights were recorded the day before dosing, before test material administration and again seven and 14 days after dosing. At study termination surviving animals were euthanized and subjected to a gross necropsy examination. Any

abnormalities were recorded.

Result : Pharmacotoxic signs observed included: hypoactivity, ataxia, decreased limb tone, prostration, piloerection, opacity in the left or right eye, red staining around mouth and nose, urogenital and anal areas, brown stain around nose, soft stool, diarrhea, urine stained abdomen, brown stained abdominal and anal region, hair loss from abdominal and anal region, bloating and death.

Weight loss occurred in all dose groups between dosing and day 7 and growth resumed thereafter. The two high dose female groups were exceptions since most animals died before day 7. At necropsy no abnormalities were observed in any animal surviving 14 days. In animals that died during the study the intestinal mucosa was severely reddened and blood was seen on the ventral surface of the animals in the lower dose groups. In the highest dose group, the stomach contained a dark brown, tenacious material and in the mid dose groups intestines also contained a red or brown material.
Mortalities were as follows

Dose (g/kg)	Male	Female
3.2	1/5	1/5
4.0	1/5	3/5
5.0	2/5	2/5
6.25	3/5	5/5
7.81	5/5	5/5

The LD_{50} was estimated to be:
Males: 5.27 g/kg 95% confidence limits 4.03-6.95
Females: 4.32 g/kg 95% confidence limits 2.65-5.47

Reliability : (1) valid without restriction

(7)

Type : LD_{50}
Value : > 5000 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : Undiluted
Doses : Single dose of 5 g/kg
Year : 1988
GLP : Yes
Test substance : Coker heavy gas oil, sample F-97 (See section 1.1.1.)

Method : Undiluted test material was administered orally by gavage to groups of 5 male and 5 female, fasted young adult, Sprague-Dawley rats.
Following administration of test material, each animal was observed hourly for the first four hours and twice daily thereafter for 14 days.
Body weights were recorded the day before dosing, before test material administration and again seven and 14 days after dosing.
At study termination surviving animals were euthanized and subjected to a gross necropsy examination. Any abnormalities were recorded.

Result : No animals died during the study.
Clinical signs included: oral discharge (2/10), nasal discharge (6/10), ocular discharge (1/10), abnormal stools (4/10) and/or lethargy (1/10). All animals were normal by day 4.
All animals gained weight by the end of the study.
At necropsy, kidneys appeared pale in 5/5 males and 2/5 females and mottling was also observed in 2 males and 3 females. In one of the affected females the corpus uteri was slightly enlarged and in the same

		animal the right apical and caudate lobes of the liver were mottled throughout. The LD_{50} was greater than 5 g/kg.
Reliability	:	(1) valid without restriction

(108)

Type	:	LD_{50}
Test substance	:	Residues from reforming processes
Remark	:	No data available

Type	:	LD_{50}
Value	:	> 25 ml/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Undiluted
Doses	:	Single dose of 25 ml/kg
Year	:	1980
GLP	:	Yes
Test substance	:	Heavy fuels, samle API 78-6 (See section 1.1.1.)
Method	:	Undiluted test material was given orally by gavage at a dose of 25 ml/kg to groups of 5 male and 5 female fasted Sprague Dawley rats. Animals were observed daily for signs of toxic or pharmacological signs. Body weights were recorded prior to dosing and again 7 and 14 days after dosing. All animals were sacrificed and subjected to gross autopsy 15 days after dosing.
Remark	:	Acute oral toxicity studies were conducted on three additional fuel oil blends (described in section 1.1.1.) with the following results.

Stream	LD_{50}	Reference
No. 6 Heavy Fuel Oil [CAS 68553-00-4]		
API 78-7	>25 ml/kg	API 27-32774
API 78-8	>25 ml/kg	API 27-32816
API 79-2	5.13 ml/kg	API 27-32813

Result	:	No animals died during the study. After dosing all animals seemed slightly lethargic but recovery was complete the day after dosing. All animals were normal except for grease on the fur, especially around the anal area. This persisted until sacrifice on day 15. The LD_{50} was greater than 25 ml/kg.
Reliability	:	(1) valid without restriction

(3) (4) (5) (6)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Value	:	> 2000 mg/kg bw
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Undiluted
Doses	:	Single dose level of 2 g/kg
Year	:	1992
GLP	:	Yes
Test substance	:	Atmospheric tower bottoms, sample F-132 (See section 1.1.1.)

Method	:	Undiluted test material was applied as a single dose of 2 g/kg to the shorn skin of 5 male and 5 female New Zealand White rabbits. The application site was immediately covered with an occlusive dressing which was left in place for 24 hours. Observations were made hourly for the first 4 hours after dosing and then twice daily for the next 13 days. Body weights were recorded immediately prior to dosing and again 7 and 14 days after dosing. All animals terminated at the end of the study underwent a post mortem examination.
Result	:	No animals died during the study and growth was normal throughout. Four of the ten animals exhibited abnormal stools on day 1 and all animals appeared normal on day 2 throughout the remainder of the study. At necropsy nine of the animals were found to be normal and one male rabbit had dark red foci (6-8mm diam) on the left diaphragmatic lobe. The LD_{50} was greater than 2 g/kg.
Reliability	:	(1) valid without restriction

(121)

Type	:	LD_{50}
Test substance	:	other TS: Atmospheric distillates
Remark	:	Information on gas oils may be used as worst case estimates of the skin irritancy potential of heavy atmospheric distillates.

(23)

Type	:	LD_{50}
Test substance	:	other TS: Vacuum residues
Remark	:	No data

Type	:	LD_{50}
Value	:	> 2000 mg/kg bw
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	3
Vehicle	:	Undiluted
Doses	:	Single dose level of 2 g/kg
Year	:	1988
GLP	:	No data
Test substance	:	Vacuum distillates, HVGO
Method	:	Undiluted test material was applied as a single dose of 2 g/kg to the shorn skin of 3 male and 3 female New Zealand White rabbits. The test site was covered with an occlusive dressing which remained in place for 24 hours. After 24 hours the dressing was removed and any residual test material was wiped from the skin. Animals were observed for signs of toxicity 2 and 4 hours after dosing and daily thereafter (except weekends). Body weights were recorded immediately prior to dosing and again on days 7 and 14 of the study. All animals were necropsied after day 14 of the study.
Remark	:	The LD50s for 3 other samples of heavy vacuum distiilates tested according to the same protocol in the same laboratory are shown below.

Sample	LD_{50}	Report
Visbreaker HGO	>2000 mg/kg	Mobil 62496-99
Vis gas oil VIBRA	>2000 mg/kg	Mobil 62500-03
VB Mittelol	>2000 mg/kg	Mobil 64635-38

Result	:	There were no deaths and all animals gained weight during the study. Soft stool was noted in 5 animals and decreased food consumption was seen in 3 animals on day 1 post dosing. Decreased food consumption and decreased fecal output was also noted in one animal on day 2. No gross

		pathology was noted at necropsy.
Reliability	:	(2) valid with restrictions The report was a summary report consolidating the results of several acute studies. Complete experimental details and results were not included. However, the results are consistent and considered to be valid.

(69) (70) (71) (75)

Type	:	LD_{50}
Value	:	> 2000 mg/kg bw
Species	:	Rrabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	2
Vehicle	:	None - undiluted
Doses	:	2 g/kg
Year	:	1982
GLP	:	Yes
Test substance	:	Cracked residue (API 81-15) See section 1.1.1.
Method	:	Undiluted test material was applied to the dorsal skin of each of 4 male and 4 female rabbits at a dose of 2 g/kg. The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later. The rabbits were observed hourly for the first six hours after dosing for pharmacotoxic signs and mortality, and twice daily for a period of 14 days. Irritation was recorded once daily throughout the observation period. Body weights were recorded just before dosing and again at 7 and 14 days. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Result	:	All animals survived the 14 day observation period and there were no signs of systemic toxicity. There was a slight loss in body weight during the first seven days after dosing, but growth resumed thereafter and at 14 days body weights were greater than they were at the beginning of the study. There were no treatment-related findings at gross necropsy.
Reliability	:	(1) valid without restriction

(7)

Type	:	LD_{50}
Value	:	> 2000 mg/kg bw
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Undiluted
Doses	:	Single dose level of 2 g/kg
Year	:	1989
GLP	:	Yes
Test substance	:	Cracked distillate, sample F-97-01, Coker heavy gas oil (See section 1.1.1.)
Method	:	Undiluted test material was applied as a single dose of 2 g/kg to the shorn skin of 5 male and 5 female New Zealand White rabbits. The application site was immediately covered with an occlusive dressing which was left in place for 24 hours. Observations were made hourly for the first 4 hours after dosing and then twice daily for the next 13 days. Body weights were recorded immediately prior to dosing and again 7 and 14 days after dosing. All animals terminated at the end of the study underwent a post mortem

examination.

Remark : In a study carried out in the same laboratory to the same protocol (ATX-90-0092), the LD_{50} of a sample of Heavy thermocracked distillate was also found to be greater than 2 g/kg.

Result : No animals died during the study. Although the animals gained weight during the first week, there was a minimal weight loss during the second week of the study. Overall there was a weight gain between the first and final day of the study.
The only clinical observations were effects on the skin. These consisted of erythema and edema which was apparent on day 1 and persisted through day 13.
At necropsy, dry skin at the test site was seen in all animals. In two females abnormalities were noted in the kidneys, these were light red to tan color and mottled appearance in one animal and dark patches in the other.
The LD_{50} was greater than 2 g/kg.

Reliability : (1) valid without restriction

(109) (120)

Type : LD_{50}
Test substance : Residue from reforming

Remark : No data

Type : LD_{50}
Value : > 5 ml/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 4
Vehicle : Undiluted
Doses : Single dose of 5 ml/kg
Year : 1979
GLP : No data
Test substance : Heavy fuel oil API sample 78-6, See section 1.1.1.)

Method : Undiluted test material was applied as a single dose of 5ml/kg to the shorn skin of 4 male and 4 female New Zealand White rabbits. The testing site for two males and two females had been abraded prior to application of the test material. The application site was immediately covered with an occlusive dressing which was left in place for 24 hours. Observations were made for 14 days. Body weights were recorded immediately prior to dosing and again 7 and 14 days after dosing. All animals terminated at the end of the study underwent a gross necropsy.

Result : No animals died during the study and there were no clinical signs of systemic toxicity. Two rabbits lost weight during the study but all other animals gained weight normally. Slight erythema was noted in a few animals. Gross post mortem examination revealed two rabbits with slightly congested livers and two that had pitted kidneys, the latter being associated with a common parasite in rabbits.
In addition, three other samples were examined to the same protocol in the same laboratory with the following results.

Sample	LD50	Reference
API 78-7	>5 ml/kg	API 27-32774
API 78-8	>5 ml/kg	API 27-32816
API 79-2	>5 ml/kg	API 27-32813

(3) (4) (5) (6)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : Undiluted
PDII : 3.5
Result : Moderately irritating
Year : 1992
GLP : Yes
Test substance : Atmospheric residue

Method : Undiluted test material (0.5 ml) was applied to four different intact skin sites on each of six New Zealand White rabbits. The treated skin sites were covered with occlusive patches fro 24 hours. After the 24 hour exposure period, the patches were removed and any residual test material was removed by wiping. Observations for skin irritation were made at prescreen, within sixty minutes of patch removal and at 72 hours, 4, 5, 6 and 7 days.

Result : At the 24 hour scoring period, edema was observed in all animals but erythema could not be assessed due to the staining nature of the test material. As the study progressed more sites could be assessed for erythema.
One of the rabbits died on day 5. The average values scored at each of the observation times is summarized below.

	Erythema	Edema
24 hr	NA	2.4
72 hour	1.2	1.6
Day 4	0.8	0.6
Day 5	0.9	0.6
Day 6	0.3	0.4
Day 7	0	0.1

The primary dermal irritation index was 3.5
The authors concluded that the test material was a moderate irritant.

Reliability : (1) valid without restriction

(123)

Test substance : Atmospheric distillate

Remark : Information on gas oils may be used as worst case estimates of the skin irritancy potential of heavy atmospheric distillates.

(23)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 0.18
Result : Not irritating
Year : 1989
GLP : Yes
Test substance : Vacuum residues

Method : Undiluted test material (0.5 ml) was applied to four different skin sites (two intact and two abraded) on each of six New Zealand White rabbits. The treated skin sites wee covered with occlusive patches fro 24 hours. After the 24 hour exposure period, the patches were removed and any residual test material was removed by wiping. Observations for skin irritation were made at prescreen, within sixty minutes of patch removal and at 72 hours, 4, 5, 6 and 7 days.

Result : Due to the staining of the skin at the application sites, it was difficult to assess scores for erythema. Therefore an assessment of erythema was made adjacent to the patch test site. The average scores for erythema and edema at the various observation times are summarized below.

	Erythema		**Edema**	
	Intact	**Abraded**	**Intact**	**Abraded**
24 hours	0.2	0.2	0	0
72 hour	0.1	0.2	0	0
Day 4	0	0	0	0
Day 5	0	0	0	0
Day 6	0	0	0	0
Day 7	0	0	0	0

The authors considered that the test material was not a skin irritant.

Reliability : (1) valid without restriction

(113)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 4 hour(s)
Number of animals : 6
Vehicle : None
Year : 1988
GLP : No data
Test substance : Vacuum distillate

Method : Three 1 sq inch test sites were selected on each flank of each of 3 male and 3 female rabbits (total six sites on each rabbit). The three sites on the right flank were abraded and the three sites on the left flank remained intact.
0.5 ml undiluted test material was applied to each of the six sites on each animal. The anterior and middle test sites were covered with an occlusive patch. The posterior sites were left unoccluded. Following a 4 hour exposure period, the patches were removed from the anterior sites on each flank of each animal and the sites were evaluated for corrosion.
These sites were re evaluated at 48 hours. After the initial evaluation for corrosion, residual test material was wiped from the skin and the site re evaluated using the standard Draize scoring system at 4.5, 28, 52 and 76 hours and again at 7 days.
Following a 24 hour exposure period, the two mid dorsal patches were removed and the rsidual test substance wiped from the skin. These two sites and the posterior sites were then evaluated for irritation at 26 and 72 hours and at 7 days post dosing.
This protocol was followed for four different samples of vacuum distillate.

Result : The results for the sample of heavy vacuum gas oil were as follows:
Mean irritation scores
4 hour occlusion

	Intact skin		**Abraded skin**	
	Erythema	**Edema**	**Erythema**	**Edema**
4.5 hrs	1.2	1.2	1.2	1.0
28 hrs	0.7	0.7	0.8	0.7
52 hrs	0.7	0.7	0.8	0.7
76 hrs	0.5	0.5	0.3	0.3
7 days	0	0	0	0
24 hour occlusion				
26 hrs	1.7	1.3	1.5	1.3

72 hrs	1.0	0.5	1.0	0.7
7 days	0.5	0.5	0.5	0.5
24 hour non-occlusion				
26 hrs	1.8	1.2	1.8	1.3
72 hrs	1.3	1.0	1.3	1.0
7 days	0.3	0.3	0.3	0.3

All four occluded test sites were negative for corrosion at 4 and 48 hours.

The individual scores for the other test materials are not included here. Instead, the following indices were calculated for each of the test materials:

Heavy vacuum gas oil Mobil 62443-45
- 4 h occl. PII 1.2
- 24h occl. PII 2.2
- 24h non occl. PII 2.7

Visbreaker HGO Mobil 62496-99
- 4 h occl. average erythema 1.9
- average edema 1.1
- PII 3.1
- 24h occl. PII 3.1

Vis gas oil VIBRA Mobil 62500-03
- 4 h occl. average erythema 1.3
- average edema 1.0
- PII 2.2
- 24h occl. PII 2.4

VB Mittelol Mobil 64635-38
- 4 h occl. average erythema 1.8
- average edema 1.2
- PII 2.9
- 24h occl. PII 3.6

Reliability : (2) valid with restrictions
The report was a summary report consolidating the results of several acute studies. Complete experimental details and results were not included. However, the results are consistent and considered to be valid.
(69) (70) (71) (75)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 0.2
Method : Draize Test
Year : 1982
GLP : Yes
Test substance : Cracked residue, Sample API 81-15 (See section 1.1.1.)

Method : 0.5 ml of undiluted test material was applied to two areas on the dorsal skin of each of six rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing. After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

At study termination the rabbits were killed with an overdose of carbon dioxide and were subjected to a gross necropsy examination. Any abnormalities were recorded.

Result : The results are given in the following table.

Observation time	Erythema Intact	Erythema Abraded	Edema Intact	Edema Abraded
24 hrs	0	0	0.2	0.2
72 hrs	0	0	0.2	0.3
96 hrs	0	0	0.2	0.3
7 days	2.7	2.7	2.5	2.8
14 days	1.7	1.8	1.2	1.2

Primary dermal irritation Index= 0.2
The primary dermal irritation index is the sum of the irritation scores for 24 and 72 hours (8 values) divided by 4 and rounded to the nearest tenth.

Due to the tar-like nature of the test material all of it could not be removed from the test sites following the 24 exposure period. The remaining test material was probably responsible for the increased dermal irritation observed at the 7 day observation.

There were no gross lesions at necropsy.

Reliability : (1) valid without restriction

(7)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 5.6
Result : Moderately irritating
Year : 1989
GLP : Yes
Test substance : Cracked distillates

Method : Undiluted test material (0.5 ml) was applied to four different skin sites (two intact and two abraded) on each of six New Zealand White rabbits. The treated skin sites were covered with occlusive patches fro 24 hours. After the 24 hour exposure period, the patches were removed and any residual test material was removed by wiping. Observations for skin irritation were made at prescreen, within sixty minutes of patch removal and at 72 hours, 4, 5, 6 and 7 days.

Result : Due to the staining of the skin at the application sites, it was difficult to assess scores for erythema. Therefore an assessment of erythema was made adjacent to the patch test site. The average scores for erythema and edema at the various observation times are summarized below.

	Erythema Intact	Erythema Abraded	Edema Intact	Edema Abraded
24 hours	2.5	2.7	2.6	2.7
72 hour	2.8	2.8	2.4	3.0
Day 4	2.0	2.0	1.8	2.2
Day 5	2.2	2.0	1.8	2.1
Day 6	2.3	1.9	1.8	1.7
Day 7	2.2	1.8	1.0	0.9

The primary irritation index for intact skin was 5.1 and for abraded skin was 5.6

The authors considered that the test material was moderately irritating.

Reliability : (1) valid without restriction

(112)

Test substance : Reformer residue

Remark : No data

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
Test substance : Heavy fuel oil

Method : Two test sites were prepared either side of the dorsal mid line on each of 3 male and 3 female New Zealand White rabbits. The anterior site of the right side and posterior site of the left side were abraded, the other sites remained intact.
0.5 ml of undiluted test material was applied to each test site and these were then covered with an occlusive dressing. After 24 hours, the patches were removed and any excess test material was removed by wiping. Observations for skin irritation were made at 24 and 72 hours and scoring of reactions were made using the Draize scale.

Four samples of blended No. 6 heavy fuel oil (API 78-6, 78-7, 78-8 and 79-2) were tested according to the above method. The observation times were extended for sample 79-2 to include 7 and 14 days.

Result : Erythema and edema was minimal at either 24 or 72 hours for three of the samples. Sample 79-2 caused severe erythema (scores of 3) in one female rabbit at 24 hours which resolved by 72 hours. In another female treated with sample 79-2, erythema was minimal after 24 hours but increased (score of 2) by 72 hours. For this sample observations were also made at 7 and 14 days and erythema scores for this single animal were 2 and 1 respectively.
A summary of the dermal irritation scores (based on 72 hour readings) is tabulated below for all four samples.

Patch and Exposure (hrs)	Sample			
Erythema	78-6	78-7	78-8	79-2
intact (24 hrs)	0.08	0.08	0.17	1.25
(72 hrs)	0.17	0.08	0	0.67
abraded (24 hrs)	0	0.75	0.42	1.33
(72 hrs)	0.25	0.33	0	0.67
Edema				
intact (24 hrs)	0.17	0.17	0.08	1.0
(72 hrs)	0.08	0	0	0
abraded (24 hrs)	0.58	1.08	0.42	1.25
(72 hrs)	0.08	0.42	0	0
Primary irritation score	0.35	0.73	0.27	1.54

(3) (4) (5) (6)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 3
Vehicle : None
Result : Not irritating
Year : 1991
GLP : Yes
Test substance : Atmospheric residue

Method : 0.1 ml undiluted test material was placed into the conjunctival sac of the right eye of each of three male New Zealand White rabbits. The eyelids were then held closed for approximately one second to prevent loss of test material. The left eye of each animal was untreated and served as control. Eyes were examined 1, 24, 48 and 72 hours after treatment. Fluoroscein was used to assist in the assessment of corneal effects.

Result : There was no evidence of damage to the iris throughout the study period. Fluorescein staining scores were zero for all three animals at all scoring times.
The only responses observed were one hour after treatment and these are shown below. No responses were observed at any other examination time.

Responses one hour after treatment

	Animal		
Cornea	1	2	3
A opacity	1	1	2
B area involved	1	1	3
Cornea score (AxBx5)	5	5	30
Iris			
Conjunctivae			
A redness	2	1	2
B Chemosis	2	2	2
C Discharge	3	3	3
Conjunctivae score (A+B+C) x2	14	12	14

Based on the average score of 0 calculated for all three animals using the 24 and 72 hour readings, the test material was considered to be non-irritant.

Reliability : (1) valid without restriction

(119)

Test substance : Atmospheric distillates

Remark : Information on gas oils may be used as worst case estimates of the eye irritancy potential of heavy atmospheric distillates.

(23)

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Exposure time : 0.5 minute(s)
Comment : Rinsed after (see exposure time)
Number of animals : 12
Vehicle : None
Year : 1989

GLP : Yes
Test substance : Vacuum residues

Method : 0.1 ml undiluted test material was dropped onto the corneal surface of the right eye of each of 12 New Zealand White rabbits. The upper and lower eyelids were held closed for approximately one second to prevent loss of test material. The treated eyes of six rabbits received no further treatment. In the remaining six rabbits 20 to 30 seconds after application of test material, the treated eyes were flushed for one minute with lukewarm water. The untreated control eyes of these six animals were also flushed in a similar manner. Observations of ocular lesions were made 1, 24, 48 and 72 hours after treatment and again 4, 7, 10 and 14 days after treatment. Fluoroscein was used as an aid to assessing ocular effects at all observation times except for the one hour reading.

Result : The test material was extremely viscous and this caused large globules to form and adhere to the eyelids when the eyes were flushed with water. Rinsing of the eye did not caused any observable changes in the consistency of the test material. The incidence of conjunctival redness (Red.) and chemosis (Chem.) are summarized in the following table, together with the average scores at each observation time.

	Unrinsed eyes			**Rinsed eyes**		
	Red.	**Chem.**	**Score**	**Red.**	**Chem.**	**Score**
1 hr	6/6	6/6 (2)	6.7	6/6	6/6 (2)	5.7
24 hr	6/6	6/6 (1)	5.0	6/6	6/6	5.7
48 hr	6/6	6/6	5.0	6/6	6/6	5.0
72 hr	6/6	6/6	4.7	6/6	6/6	4.7
4 day	6/6	6/6	4.0	6/6	6/6	4.3
7 day	4/6	6/6	3.3	6/6	6/6	4.0
10 day	0/6	2/6 (1)	1.0	3/6	1/6 (1)	1.3
14 day	0/6	0/6	0	0/6	0/6	0

Values shown ( ) are the incidence of animals in which a discharge was observed. On the basis of the above results it was concluded that the test material was non-irritant in unrinsed eyes and minimally irritant in rinsed eyes.

Reliability : (1) valid without restriction

(115)

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 6
Method : Draize Test
Year : 1988
GLP : No data
Test substance : Vacuum disitillates (4 samples)

Method : 0.1 ml of test material was instilled into the conjunctival sac of the left eye of 3 male and 3 female rabbits. The untreated eye served as control. Eyes were grossly examined and scored according to the Draize method at 1, 24, 48 and 72 hours.

Result : The total Draize scores for the four test materials are shown in the following table. All responses observed were entirely due to conjunctival redness and swelling. No corneal opacity or iritis was observed in any animal.

Values given are the total Draize scores.

	Time after instillation (hours)			
Test material	**1**	**24**	**48**	**72**
Heavy vacuum gas oil	10	10.3	3.3	0.3
Visbreaker heavy gas oil		1.7	2.3	2.3
Vis gas oil VIBRA		4.0	2.0	1.7
VB MITTELOL		5.3	4.0	2.7

(69) (70) (71) (75)

Species	:	Rabbit
Concentration	:	Undiluted
Dose	:	0.1 ml
Number of animals	:	9
Method	:	Draize Test
Year	:	1982
GLP	:	Yes
Test substance	:	Cracked residue, Sample API 81-15 (See section 1.1.1.)

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result : The presence of brown or light brown test material was noticeable at the observation and scoring. Irritation only lasted for 24 hours after which all eyes were normal.
Primary eye irritation scores recorded in this study are as follows:

	1 Hr.	24 Hrs	48 Hrs	72 Hrs	7 days
Unwashed eyes (6 rabbit mean)	2.3	2.0	0	0	0
Washed eyes (3 rabbit mean)	2.0	2.0	0.0	0.0	0.0

These data demonstrate that the test material was minimally irritating.

Reliability : (1) valid without restriction

(7)

Species	:	Rabbit
Concentration	:	Undiluted
Dose	:	0.1 ml
Exposure time	:	0.5 minute(s)
Comment	:	Rinsed after (see exposure time)
Number of animals	:	12
Vehicle	:	None
Result	:	Not irritating
Year	:	1989
GLP	:	Yes
Test substance	:	Cracked distillates

Method : 0.1 ml undiluted test material was dropped onto the corneal surface of the right eye of each of 12 New Zealand White rabbits. The upper and lower eyelids were held closed for approximately one second to prevent loss of test material. The treated eyes of six rabbits received no further treatment. In the remaining six rabbits 20 to 30 seconds after application of test material, the treated eyes were flushed for one minute with lukewarm water. The untreated control eyes of these six animals were also flushed in a similar manner.
Observations of ocular lesions were made 1, 24, 48 and 72 hours after treatment and again 4 days after treatment. Fluoroscein was used as an aid to assessing ocular effects at all observation times except for the one hour reading.

Result : The incidence of conjunctival redness (Red.) and chemosis (Chem.) are summarized in the following table, together with the average scores at each observation time.

	Unrinsed eyes			Rinsed eyes		
	Red	**Chem**	**Score**	**Red**	**Chem**	**Score**
1 hr	6/6	6/6 (4)	8.3	6/6	6/6 (4)	8.7
24 hr	6/6	5/6	5.7	6/6	4/6 (1)	5.3
48 hr	4/6	3/6	2.3	5/6	3/6	3.3
72 hr	0	0	0	0	0	0
4 day	0	0	0	0	0	0

Values shown ( ) are the incidence of animals in which a discharge was observed.
On the basis of the above results it was concluded that the test material was non-irritant in unrinsed eyes and rinsed eyes.

Reliability : (1) valid without restriction

(114)

Test substance : Reformer residues

Remark : No data

Species : Rabbit
Concentration : Undiluted
Dose : 0.1
Exposure time : 0.5 minute(s)
Comment : Rinsed after (see exposure time)
Number of animals : 9
Vehicle : None
Year : 1980
Test substance : Heavy fuel oil, 4 samples (See section 1.1.1.)

Method : 0.1 ml undiluted test material was placed on the everted lower eyelid of the right eye of each of nine New Zealand White rabbits. The upper and lower eyelids were held together for approximately one second to prevent loss of material. The test eyes of three rabbits (two females, one male) were rinsed for one minute with warm distilled water starting 30 seconds after application of the test material. The test eyes of the other six rabbits were not rinsed. The untreated eyes of all rabbits served as controls.
Scoring of ocular lesions was carried out 24, 48 and 72 hours after application of test material. For two samples the observation period was extended until no irritation was seen. Grading of ocular lesions was according to the Draize scale.

Result : Sample 78-6 (API report No. 27-32814)
No corneal opacities or iridial inflammation was seen in any of the test animals.
Conjunctival irritation was seen in eight rabbits at 24 hours but all were negative at 48 hours.

Sample 78-7 (API report No. 27-32774)
No iridial inflammation was seen in any animal and one rabbit showed corneal opacity at the 24 hour examination.
Conjunctival irritation was apparent in eight animals at 24 hours but this had resolved by 72 hours.

Sample 78-8 (API report No. 32-32816)
Corneal opacities of grade 1 and area 1 were seen in three animals at the 24 and 48 hour observation time. No iridial inflammation was observed in any animal at any time.
Conjunctival irritation was seen in all animals at 24 and 48 hours but by 72 hours this had resolved.

Sample 79-2 (API report No. 27-32813)
Two animals had corneal opacities at the 48 observation. Other rabbits showed opacities at 72 hours and 14 days but these were not considered to be treatment-related.
Conjunctival irritation was present in all rabbits at the 24 hour observation. No irritation was seen by 14 days

The average eye irritation scores for each of the samples were as follows:

	Sample 78-6	78-7	78-8	79-2
Washed eyes				
24 hour	4.67	2.67	7.67	6.67
48 hour	0	1.33	5.0	5.0
72 hour	0	0	0	1.33
7 day	ND	ND	0	0.67
14 day	ND	ND	ND	0
Unwashed eyes				
24 hour	4.0	4.83	7.33	7.33
48 hour	1.0	0.67	4.67	3.83
72 hour	0	0	1.0	1.33
7 day	ND	ND	0	1.0
14 day	ND	ND	ND	0

Reliability : (1) valid without restriction

(3) (4) (5) (6)

5.3 SENSITIZATION

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Result : Not sensitizing
Year : 1992
GLP : Yes
Test substance : Atmospheric residues, Sample F-132, (See section 1.1.1.)

Method : 0.5 ml undiluted test material was applied under occlusion to the shorn skin of 10 guinea pigs. The patch was left in place for six hours after which all covering was removed from the test site. This induction procedure was carried out once each week for three weeks.

Fourteen days after the third induction dose the animals were challenged at a different skin site. The challenge dose of 0.5 ml was applied in the same manner as the induction doses.

24 and 48 hours after each induction and challenge dose an assessment of the treated site was made and scored for response.

The following control groups were included in the study

Challenge control group
received a challenge dose of test material only

Positive control group
received 0.5 ml of a 0.3% solution of DNCB in 80% ethanol once each week during the induction phase.
Challenge dose for the positive controls was 0.5 ml of 0.2%

DNCB in 80% ethanol.

Challenge control group
received the challenge dose of DNCB only.

Result : The following responses were recorded.

Group	Incidence	Severity
F-132 test group	0/10	
F-132 challenge control	0/4	
Positive control	10/10	5.1 & 3.6
DNCB challenge control	2/4	0 & 1.3

These data demonstrate that the test material is not a skin sensitizer.

Reliability : (1) valid without restriction

(122)

Test substance : Atmospheric distillates

Remark : Information on gas oils may be used as worst case estimates of the eye irritancy potential of heavy atmospheric distillates.

(23)

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 9
Result : Not sensitizing
Year : 1989
GLP : Yes
Test substance : Vacuum residue

Method : 0.5 ml undiluted test material was applied under occlusion to the shorn skin of 10 guinea pigs. The patch was left in place for six hours after which all covering was removed from the test site. This induction procedure was carried out once each week for three weeks. Fourteen days after the third induction dose the animals were challenged at a different skin site. The challenge dose of 0.5 ml was applied in the same manner as the induction doses.
24 and 48 hours after each induction and challenge dose an assessment of the treated site was made and scored for response.

The following control groups were included in the study

Challenge control group
received a challenge dose of test material only

Positive control group
received 0.5 ml of a 0.3% solution of DNCB in 80% ethanol once each week during the induction phase.
Challenge dose for the positive controls was 0.5 ml of 0.2% DNCB in 80% ethanol.

Challenge control group
received the challenge dose of DNCB only.

Result : The following responses were recorded.

Group	Incidence	Severity
F-98-01 test group	0/10	
F-98-01 challenge control	0/4	
Positive control	9/9	4.1 & 3.1
DNCB challenge control	4/4	0.8 & 0.8

These data demonstrate that the test material is not a skin sensitizer.

Reliability : (1) valid without restriction

(111)

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction 33 % occlusive epicutaneous
2nd: Challenge 11 % occlusive epicutaneous
Number of animals : 10
Result : Not sensitizing
Year : 1989
GLP : Yes
Test substance : Vacuum distillates

Method : 0.5 ml diluted (1:2 in mineral oil) test material was applied under occlusion to the shorn skin of 10 guinea pigs. The patch was left in place for six hours after which all covering was removed from the test site. This induction procedure was carried out once each week for three weeks. Fourteen days after the third induction dose the animals were challenged at a different skin site. The challenge dose of 0.5 ml was applied as a 1:8 dilution in mineral oil in the same manner as the induction doses. 24 and 48 hours after each induction and challenge dose an assessment of the treated site was made and scored for response.

The following control groups were included in the study
Challenge control group
received a challenge dose of test material only
Positive control group
received 0.5 ml of a 0.3% solution of DNCB in 80% ethanol once each week during the induction phase. Challenge dose for the positive controls was 0.5 ml of 0.2% DNCB in 80% ethanol.
Challenge control group
received the challenge dose of DNCB only.
Vehicle Control
received 0.5 ml mineral oil once each week during the induction phase. Challenge dose of 0.5 ml.

Result : The following responses were recorded.

Group	Incidence	Severity
HVGO test group	1/10	0.1 & 0.0
HVGO challenge control	0/4	0.3 & 0.0
Positive control	10/10	3.6 & 3.3
DNCB challenge control	0/4	1.0 & 0.0

These data demonstrate that the test material is not a skin sensitizer.

Test substance : Heavy Vacuum Gas Oil (HVGO, CAS No. 64741-57-7)
Reliability : (1) valid without restriction

(118)

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Result : Not sensitizing
Method : Beuhler
Year : 1984
GLP : Yes
Test substance : Cracked residues, sample API 81-15 (See section 1.1.1.)

Method : 0.4 ml undiluted test material was applied under an occlusive dressing to

the shaved skin of 10 male Guinea pigs. Six hours after application the dressing was removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. Due to severe irritation at the test site of the positive control animals, the third application was made slightly posterior to the previous site.
Two weeks following the third application a challenge dose was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application.
The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream.

Positive control, vehicle control and naive control groups were included in this study.

Concentrations of positive control were as follows:

Sensitizing doses: 0.4 ml of 0.3% w/v in 80% aqueous ethanol
Challenge dose: 0.4 ml of 0.1% w/v suspension in acetone

Result : During the sensitization phase of the study, dermal irritation included very slight edema and very slight to well define erythema. No dermal irritation was exhibited by either the test group or naive controls following challenge application with undiluted test material.
All 20 Guinea pigs treated with DNCB were sensitized at the end of the study.

Reliability : (1) valid without restriction

(9)

Type : Buehler Test
Species : Guinea pig
Concentration : 1^{st}: Induction undiluted occlusive epicutaneous
2^{nd}: Challenge 50 % occlusive epicutaneous
Number of animals : 10
Vehicle : Mineral oil
Result : Not sensitizing
Year : 1989
GLP : Yes
Test substance : Cracked distillates

Method : 0.5 ml undiluted test material was applied under occlusion to the shorn skin of 10 guinea pigs. The patch was left in place for six hours after which all covering was removed from the test site. This induction procedure was carried out once each week for three weeks. Fourteen days after the third induction dose the animals were challenged at a different skin site. The challenge dose of 0.5 ml was applied as a 50% dilution in mineral oil in the same manner as the induction doses. 24 and 48 hours after each induction and challenge dose an assessment of the treated site was made and scored for response.

The following control groups were included in the study

Challenge control group
received a challenge dose of test material only

Positive control group
received 0.5 ml of a 0.3% solution of DNCB in 80% ethanol once each week during the induction phase.
Challenge dose for the positive controls was 0.5 ml of 0.2% DNCB in 80% ethanol.

Challenge control group
received the challenge dose of DNCB only.

Result : The following responses were recorded.

Group	Incidence	Severity
F-97-01 test group	0/10	
F-97-01 challenge control	0/4	
Positive control	10/10	1.5 & 1.3
DNCB challenge control	0/4	

These data demonstrate that the test material is not a skin sensitizer.

Reliability : (1) valid without restriction

(110)

Test substance : Reformer residues

Remark : No data

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Year : 1980
GLP : No data
Test substance : Heavy fuels, 4 samples (See section 1.1.1.)

Method : Undiluted test material (0.5 ml) was applied under an occlusive patch to the shorn dorsal skin of 10 guinea pigs. Six hours after application the patches were removed.
This procedure was followed three times a week for 3 weeks.
Following a two week rest period a challenge dose was given in exactly the same manner as the induction doses, except that the skin site was a fresh site on each animal.
Skin reactions were graded for erythema and edema 24 hours after each dose.

The following control group was used.
Positive control
Induction with a 0.05% (w/w) dilution of DNCB in ethanol. The test sites were only occluded 5 times during the study.

Result : Three of the samples were not skin sensitizers since the degree of response to the challenge dose was less than that for the positive controls.
Sample 78-7 was considered to be mildly sensitizing.
This was because the challenge scores were in some cases greater than the those for the induction doses.

Material	Result	Reference
API 78-6	Not sensitizing	27-32814
API 78-7	Mildly sensitizing	27-32774
API 78-8	Not sensitizing	27-32816
API 79-2	Not sensitizing	27-32813

Reliability : (2) valid with restrictions
The selection of dose concentrations in this study was on the basis of irritancy studies in rabbits. It is possible that the dose concentrations used were excessive.
The study is not sufficiently robust.

(3) (4) (5) (6)

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction undiluted occlusive epicutaneous 2nd: Challenge undiluted occlusive epicutaneous
Number of animals	:	6
Result	:	Not sensitizing
Year	:	1986
GLP	:	Yes
Test substance	:	Heavy fuel oil sample F-74-01

Method : 0.5 ml undiluted test material was applied under occlusion to the shorn skin of 10 guinea pigs. The patch was left in place for six hours after which all covering was removed from the test site. This induction procedure was carried out once each week for three weeks. Fourteen days after the third induction dose the animals were challenged at a different skin site. The challenge dose of 0.5 ml was applied as a 50% dilution in mineral oil in the same manner as the induction doses.
24 and 48 hours after each induction and challenge dose an assessment of the treated site was made and scored for response. The following control groups were included in the study:

Challenge control group
received a challenge dose of test material only

Positive control group
received 0.5 ml of a 0.3% solution of DNCB in 80% ethanol once each week during the induction phase.
Challenge dose for the positive controls was 0.5 ml of 0.2% DNCB in 80% ethanol.

Challenge control group
received the challenge dose of DNCB only.

Result : The following responses were recorded.

Group	Incidence	Severity
F-74-01 test group	4/10	0.4-0
F-97-01 challenge control	0/4	
Positive control	10/10	3.1 - 2.3
DNCB challenge control	1/4	0.2

These data demonstrate that the test material is not a skin sensitizer.

Reliability : (1) valid without restriction

(106)

5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic

Remark : Dermal studies of up to 13 weeks duration have been reported for streams in this category and all are listed below.
Only one study for each subcategory has been summarized in full and where several studies are available only those of longest duration have been summarized. Studies that have been summarized are indicated * in the following listing.

Atmospheric residues
28 day study on F-132, Atmospheric tower bottoms * (Ref. ATX-90-0066)

Atmospheric distillates
13 week study on Heavy Atmospheric Gas Oil * (Ref. Mobil 63456)

Vacuum Residues
No data

Vacuum Distillates
13 week study on Heavy Vacuum Gas Oil * (Ref. Mobil 61590)

Cracked residues
13 week study on Clarified Slurry oil * (Ref. Mobil 20525)
13 week study on API sample 81-15 (Ref. API 32-32753)
13 week study on Syntower bottoms (Ref. Mobil 62710)
28 day study on API sample 81-15 in rats (Ref. API 33-30442)
28 day dermal study on API sample 81-15 in rabbits (Ref. API 30-32854)

Cracked distillates
13 week study on visbreaker gas oil * (Ref. Mobil 63237)
13 week study on Joliet Heavy coker gas oil (Ref. Mobil 64165)
13 week study on Torrance Heavy coker gas oil (Ref. Mobil 64184)
13 week study on Paulsboro Heavy coker gas oil (Ref. Mobil 50391)

Reformer residues
No data

Residual heavy fuel oil
10 day study on API sample 78-6* (Ref. API 27-32814)
10 day study on API sample 78-7 (Ref. API 27-32774)
10 day study on API sample 78-8 (Ref. API 27-32816)
10 day study on API sample 79-2 (Ref. API 27-32813)
28-day study on F-74-01 (Ref. UBTL, 1987)
(3) (4) (5) (6) (8) (16) (17) (46) (61) (62) (72) (73) (76) (78) (79) (107)

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 28 days
Frequency of treatm. : Once daily, 5 days each week for 4 weeks
Doses : 0.01 (9 mg/kg), 0.25 (231 mg/kg) & 1.0 (927.9 mg/kg) ml/kg
Year : 1990
GLP : Yes
Test substance : Atmospheric residue, sample F-132 (See section 1.1.1.)

Method	:	Three groups of ten male and ten female young adult Sprague Dawley rats were administered F-132 dermally once daily, five days each week for four weeks, at doses of 0.01, 0.25 or 1.0 ml/kg/day. A repeat of the high dose was later conducted due to a possible under-dosing. The test material was applied to the shorn dorsal skin of the animals. The site of application was occluded for a period of at least six hours following dosing. Two groups of ten male and ten female rats served as controls, one group each for the initial and repeat high dose groups. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the application site was evaluated daily just prior to the application of test material. Body weights were recorded three times each week during the study. At necropsy, blood was collected for the following hematological and clinical determinations. Hematology: erythrocyte count, total and differential leucocyte count, hemoglobin, hematocrit and platelet count. Clinical chemistry: sodium, potassium, chloride, calcium, phosphorus, blood urea nitrogen, glucose, creatinine, cholesterol, triglyceride, total protein, albumin, globulin (calculated), A/G ratio (calculated), alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. The following organs were weighed: Adrenal glands, brain, kidneys, liver and testes/ovaries. A wide range of tissues were saved and the following were processed for subsequent histopathological examination. adrenal glands, brain (cerebrum, cerebellum, medulla pons), cervical lymph nodes, gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon, rectum) gross lesions, heart, kidneys (2), liver, lungs, pancreas, salivary glands, skin (treated and untreated), spleen, sternum and bone marrow, testes/ovaries (2), thyroid, thymus, urinary bladder.
Result	:	No animals died or were sacrificed during the study. There wee no clinical observations considered to be treatment-related. No dermal irritation was noted in any of the treatment groups. The only treatment-related finding at gross necropsy was a dark staining of the treated skin site. There were no hematological changes that were considered to be treatment-related. Although some differences were recorded for some of the clinical chemistry parameters, none were considered to be treatment-related. There were no treatment-related differences in body weights or organ weights or organ/body weight ratios. The only treatment-related histopathological findings occurred in the skin and these consisted of trace to mild acanthosis and trace to moderate hyperkeratosis in the high dose animals. The authors concluded that there were no systemic effects at the highest dose level tested.
Reliability	:	(1) valid without restriction

(116)

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	13 weeks
Frequency of treatm.	:	Daily
Doses	:	30, 125 & 500 mg/kg/day

Control group : Yes
NOAEL : = 30 mg/kg bw
Year : 1992
GLP : No data
Test substance : Atmospheric distillate , Sample HAGO

Method : Test material was applied to the shorn skin of groups of 10 male and 10 female rats (approximately 40 days old) at dose levels of 30, 125 and 500 mg/kg. In addition, the test material was applied at a dose level of 500 mg/kg to satellite groups of 10 males for the assessment of male reproductive health. There was a control group of 10 rats of each sex and an additional 10 males that served as controls for the assessment of male reproductive health.
The test material was applied each day, 5 days each week for 13 weeks. All rats were fitted with Elizabethan collars to prevent ingestion of test material. The collars were removed at the end of each week and any residual test material removed from the skin by wiping. Collars were replaced on Mondays before commencement of dosing for the next week. Body weights were recorded before application of the first dose of test material and weekly thereafter.
There were daily observations for clinical signs of toxicity and an assessment and scoring of the treated skin site was made once each week according to the standard Draize scale.
Urine samples were collected during weeks 5 and 13 for urinalysis (pH, specific gravity, bilirubin, urobilinogen, blood, protein, glucose and ketone). Blood samples were taken at the end of the study for the determination of the following clinical chemical and hematological parameters.

Hematology

Red cell count	Hemoglobin
Hematocrit	White cell count
Platelet count	

Clinical chemistry

Sorbitol dehydrogenase	Cholesterol
Alanine aminotransferase	Urea nitrogen
Aspartate aminotransferase	Total protein
Alkaline phosphatase	albumin (A)
Bilirubin	Triglycerides
Inorganic phosphorus	Creatinine
Glucose	Uric acid
Sodium	Potassium
Chloride	Calcium

Globulin(G) and A/G ratios were calculated

All animals surviving to the end of the study were sacrificed and necropsied. The following organs were weighed:

Adrenals	Heart	Spleen
Brain	Kidneys	Thymus
Liver	Ovaries	Uterus
Prostate	Epididymides	Testes

The following tissues/organs were removed from control group and high dose group animals and were fixed for subsequent histopathological examination.

Adrenals (both)	Ovaries (both)
Bone and marrow (sternum)	Pancreas (head)
Brain (3 sections)	Salivary gland (submaxillary)
Eye (left & optic nerve)	Skin (treated 2 sections)
Heart	Spleen
Colon	Stomach (squamous & glandular)

Duodenum	Thymus (both lobes)
Kidneys (both)	Thyroid (both lobes)
Liver (2 lobes)	Urinary bladder
Lung (left lobe)	Uterus (body & horns)
Skeletal muscle (thigh)	Gross lesions
Peripheral nerve (sciatic)	

In addition the following tissues/organs were removed, fixed and examined microscopically from the mid and low dose animals:

Adrenals	Sternum (bone and marrow)
Kidneys (both)	Liver (2 lobes)
Lung	Skin (2 sections plus any gross lesions)
Thymus	Gross lesions.

At the end of the study the epididymides and testes from the male rats in the control and 125 mg/kg groups were removed.
Prior to sample preparation for testis examination, the tunica albuginea and corresponding blood vessels were removed and discarded before the remaining testicular parenchyma and cauda epididymis were weighed.
Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and a morphological assessment was made of testes and epididymides.

Statistical analysis
Body weight, serum chemistry, hematology and organ weight data were analyzed by parametric methods: analysis of variance and associated F-test, followed by Tukey's multiple comparison test (body weight, hematology and organ weight data) or Student-Newman-Keuls multiple comparison test (serum chemistry), provided that there was statistical significance in the analysis of variance.
Differences between control and treated groups were considered statistically significant only if the probability of the differences being due to chance was less than 5% (P<0.05).

Result : Two animals became moribund and were sacrificed in extremis.
One of the animals was a high dose male and the findings were considered to be treatment-related. The other was a low dose male and the findings were considered to be incidental.
There were few clinical findings during the study and these were mostly related to the effects of the Elizabethan collars. In general, skin irritation was slight in the treated groups.
Body weight gains were similar to that of the controls for all groups except the high dose males whose weight gains were significantly less (10%) than controls.

Serum chemistry values in the 30 mg/kg were unaffected by exposure to the test material but some parameters were adversely affected in the rats in the mid and high dose groups. The affected parameters at 13 weeks are shown in the following table together with the % increase (+) or decrease (-) compared to control values. Where no figures are included no significant differences were found.

Parameter	**Male**		**Female**	
	125	**500**	**125**	**500**
Glucose	-	-	-	-
BUN	-	+31%	+27%	+35%
AST	-	-	-	-
ALT	-	-23%	-	-
Alk. Phos.	-	-	-	-
Creatinine	-	-	-	-
Cholesterol	-	-	+39%	+117%
Triglycerides	-	-	-	-
Total protein	-	-	-	+11%
Bilirubin	-	-	-	-

Albumin	-	-	-	-
A/G ratio	-	-	-	-20%
Globulin	-	-	-	+27%
Uric acid	-	-	-	-
Sodium	-	-	-	-
Potassium	+9%	-	-	-
Phosphorus	-	-	-	-
Calcium	-5%	-	-	-
SDH	-	+124%	+68%	+106%
Chloride	-	-	-	-

Hematological parameters were unaffected in the 30 mg/kg group compared to controls. There were however, some differences between the controls and those of the 125 and 500 mg/kg groups. The differences at 13 weeks are shown in the following table with and indication of the magnitude of the difference (%), higher (+) or lower (-). Where no figures are included no significant differences were found.

Parameter	**Male**		**Female**	
	125	**500**	**125**	**500**
RBC Count	-8%	-30%	-	-11%
Hemoglobin	-9%	-31%	-	-13%
Hematocrit	-8%	-30%	-	-12%
MCV	-	-	+3%	-
MCH	-	-	-	-
MCHC	-	-	-	-
Platelets	-	-48%	-	-23%
WBC Count	-	-	-	-

Differential white cell counts were unaffected by exposure to the test material.

At necropsy, the macroscopic findings in both sexes that seemed to be treatment-related were: increased liver size, decreased thymus size, thickening of the limiting ridge between the non-glandular and glandular sections of the stomach and enlarged and reddened lymph nodes.
There were some absolute and some relative organ weight (organ/body weight) differences in the 125 and 500 mg/kg groups but none in the 30 mg/kg group. The differences are shown in the following table as % of control values. (A = absolute weight, R = relative wt). The table lists all the organs that were weighed at necropsy.

Organ	**Male**		**Female**	
	125	**500**	**125**	**500**
Adrenals (A)	-	-	-	-
(R)	-	125%	-	-
Brain (A)	-	-	-	-
(R)	-	-	-	-
Epididymis (A)	-	-		
(R)	-	-		
Heart (A)	-	-	-	112%
(R)	-	117%	-	115%
Kidneys (A)	-	-	-	-
(R)	-	-	-	110%
Liver (A)	-	132%	-	150%
(R)	-	149%	116%	156%
Prostate (A)	-	77.5%		
(R)	-	-		
Spleen (A)	-	-	-	118%
(R)	-	126%	117%	121%
Testes (A)	-	-		

(R)	-	-		
Thymus (A)	-	39%	-	59%
(R)	-	45%	-	61%
Uterus (A)			-	-
(R)			-	-

The only treatment-related changes observed at histopathological examination were confined to animals in the 500 mg/kg groups. These included a severe reduction in hematopoiesis in the bone marrow; 10/10 males were affected compared to 2/10 females. The increases in liver weight that had been observed were attributable to liver hypertrophy and connective tissue formation. Also there were increased areas of hematopoiesis, focal necrosis and individual cell death in this dose group. Although the numbers of circulating lymphocytes were not affected, there was a reduction in the numbers of lymphocytes in the thymus glands of the high dose group animals.
There were no other treatment-related histopathological changes.
There were no treatment-related effects on any of the epididymal sperm parameters or the testicular spermatid parameters that were measured.
Measured parameters included:
Weight of cauda epididymis, No. of sperm/g cauda, No. of sperm/cauda, Testis weight, No. spermatids/g testis and No. sperm/testis.

Reliability : (2) valid with restrictions
Although it is not stated in the report that the study was conducted to GLP, it nevertheless is described fully and is considered to be reliable.
(77)

Test substance : Vacuum residues

Remark : Data summarized in the test plan and robust summaries for asphalt may be used to predict the toxicity of this subgroup of heavy petroleum streams.

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 13 weeks
Frequency of treatm. : Daily
Doses : 30, 125, 500 & 2000 mg/kg/day
Control group : Yes
NOAEL : = 125 mg/kg bw
Year : 1988
GLP : No data
Test substance : Vacuum distillates

Method : Undiluted heavy vacuum gas oil was applied at doses of 0, 30, 125, 500 and 2000 mg/kg/day to the shorn skin of groups of ten male and ten female Sprague Dawley rats. The males weighed between 220 and 230 g and the females weighed between 160 and 170 g at the start of the study.
The material was applied 5 days each week for 13 weeks. Collars were fitted to the animals to prevent oral ingestion.
Body weights were recorded weekly throughout the study and clinical observations were made daily. Skin irritation was assessed weekly. At 5 and 13 weeks, blood samples were taken for measurement of the following hematological and clinical chemical parameters:

<u>Hematology</u>

Red blood cell count	Hemoglobin
Hematocrit	White blood cell count
Differential WBC count	MCV, MCH & MCHC caclulated

Clinical chemistry

Glucose	Urea nitrogen
Uric acid	Total protein
Albumin	Globulin (calculated)
Albumin/Globulin ratio	Calcium
Alkaline phosphatase	Alanine aminotransferase
Aspartate aminotransferase	Lactate dehydrogenase
Sorbitol dehydrogenase	Creatinine
Cholesterol	Triglycerides
Total Bilirubin	Calcium
Phosphorus	Sodium
Potassium	Chloride

At the end of the study (13 weeks) all surviving animals were sacrificed and a gross necropsy examination was performed. The following organs were weighed:

Adrenals	Kidneys	Spleen
Brain	Liver	Testes
Epididymes	Ovaries	Thymus
Heart	Prostate	Uterus

The following tissues in the high dose group animals were examined microscopically:

Adrenals (both)	Ovaries (both)
Bone & marrow (sternum)	Pancreas (head)
Brain (3 sections)	Salivary gland (submaxillary)
Eye & optic nerve	Skin (treated, 2 sections)
Heart Colon	Duodenum
Stomach	Kidneys (both)
Testes (both)	Liver (2 lobes)
Thymus (both lobes)	Lung (left lobe)
Thyroid (both lobes)	Muscle (skeletal, thigh)
Urinary bladder	Peripheral nerve (sciatic)
Gross lesions	

Histopathological examination was only undertaken on thymus, spleen and sternum for the 500 mg/kg/day animals and thymus only for the 125 mg/kg/day animals.

Result : Two males and one female in the high dose group died during the study. The male deaths were considered to be compound related but the female death was considered incidental.
Growth rates of males and females in the highest dose group were reduced compared to controls. At 13 weeks the males weighed 20% less and the females 15% less than controls.
At 2000 mg/kg/day males and females had reduced erythrocytes and reduced platelets at 5 and 13 weeks. Similar effects were also found in the 500 mg/kg/day females.

Clinical chemical changes in males and females at 2000 mg/kg/day consisted of:

- twofold increase in sorbitol dehydrogenase
- twofold increase in cholesterol
- 50% reduction in uric acid

In addition in females at 500 mg/kg/day, glucose was reduced and in the 500 mg/kg males cholesterol was increased.

At gross necropsy, relative thymus weights were reduced in the 500 (by 25%) and 2000 mg/kg/day (by 50%) animals of both sexes. Relative liver weights were also increased at 500 and 2000 mg/kg/day for both sexes.

Histological examination revealed decreased erythropoeisis and fibrosis of

the bone marrow in the 2000 mg/kg/day males.
There was a reduction in thymic lymphocytes in the 2000 mg/kg/day groups (marked for males and moderate for females) and a slight reduction in the 500 mg/kg/day groups for both sexes.

No effects were found on either sperm morphology or in the results of the urinalysis.

The NOEL for both males and females was found to be 125 mg/kg/day.

Test substance : The sample of Heavy vacuum gas oil was produced by the vacuum distillation of crude oil.
It was a dark amber liquid with a boiling range of approximately 657 to 1038 °F.
The sample originated from the Beaumont crude unit B (CRU #85244) and contained:
54% paraffins
35% polycyclic aromatic hydrocarbons
2% nitrogen-containing polycyclic aromatic hydrocarbons
9% residuals.

Reliability : (1) valid without restriction

(72)

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 13 weeks
Frequency of treatm. : Daily, 5 days each week for 13 weeks
Doses : 8, 30, 125 & 500 mg/kg/day
Control group : yes, concurrent no treatment
NOAEL : < 8 mg/kg bw
Year : 1986
GLP : No data
Test substance : Cracked residues, sample CSO

Method : Groups of ten male and ten female, 5-6 week old Sprague-Dawley rats were used in this study.
Undiluted test material was applied to the shorn skin of the animals at dose levels of 8, 30, 125, 500 and 2000 mg/kg/day. Applications were made once each day, five days each week for 13 weeks. Ten males and ten females were used as controls and these animals did not receive any test material. The test sites remained uncovered and to prevent ingestion all animals were fitted with collars.
Animals were weighed weekly and were monitored once daily for reaction and twice daily for moribundity and mortality.
Blood samples were collected during weeks 5 and 13 and hematological determinations were made of: red blood cell count, hematocrit, hemoglobin content, white blood cell count and differential white cell count. The serum was analyzed for glucose, urea nitrogen, uric acid, total protein, albumin, albumin/globulin ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, cholesterol, triglycerides, total and direct bilirubin, calcium, phosphorus, sodium, potassium and chloride.
During weeks 5 and 13, freshly voided urine was examined for color and clarity and pH, presence of occult blood, glucose, protein, ketones, bilirubin and bilirubinogen were determined using reagent strips. Specific gravity of the urine was measured using a protometer.
Following 13 weeks of treatment, the animals were starved overnight and then euthaized with carbon dioxide. All animals underwent a complete necropsy. Heart, liver, spleen, thymus, adrenals, gonads and kidneys were weighed. The following tissues were taken, processed for histology and

examined microscopically: gonads, small intestine, kidneys, liver, treated skin, spleen, stomach, thymus, urinary bladder, prostate and seminal vesicles, uterus, bone marrow and all gross lesions.
Although statistical analyses were carried out, the techniques used are not described in the published paper.

Remark : This study report is available both as a laboratory report and as a publication in the open literature (Cruzan et al, 1986). The laboratory report was used to prepare the robust summary. The publication reference is given for completeness.

Result : All rats in the highest dose group (2000 mg/kg/day) died or were killed in a moribund condition during the second week of the experiment. Survival was as follows:

	Male	**Female**
Control	10	100
8 mg/kg/day	10	100
30 mg/kg/day	9	10
125 mg/kg/day	3**	6***
500 mg/kg/day	2	1*
2000 mg/kg/day	0	0

No of * indicate number of rats dying shortly after blood samples were taken.
Some treated rats in dose groups 125 mg/kg/day and greater were lethargic and/or having thin appearance. This was usually a prelude to dying.
Body weights were affected by treatment. The body weights at the end of the study, expressed as a percentage of the corresponding controls are listed below.

Dose group	**Male**	**Female**
8 mg/kg/day	96%	96%
30 mg/kg/day	94%	93%
125 mg/kg/day	74%	78%
500 mg/kg/day	47%	67%

Skin irritation was not seen in rats in the 8, 30 or 125 mg/kg/day dose groups. Barely perceptible erythema was observed in 1 rat and thickened, slightly leathery skin in 4 rats on day 8 of the 500 mg/kg/day group.

Recorded differences in hematological parameters after 13 weeks exposure to test material are tabulated below. Values given are percentage increases (+) or decreases (-) compared to control.

	Dose group (mg/kg/day)					
	Males			**Females**		
Parameter	**30**	**125**	**500**	**30**	**125**	**500**
Hematocrit	-15%	-53%	-21%	-14%	-34%	-25%
Hemoglobin			-49%		-30%	
lymphocyte			-35%		-24%	
Mature neutrophils			+88%			

The serum chemistry data revealed that the liver was the primary target organ. Percentage of control values shown as Increases (+) or decreases (-) are shown in the following table.

	Dose group (mg/kg/day)					
	Males			**Females**		
	30	**125**	**500**	**30**	**125**	**500**
glucose			-25			
Total protein			-12			
A/G ratio		+14	+12		+18	+13
Urea N				+31	+46	
Uric acid	-33	-40	-47	-29	-53	-12

Bilirubin						
(total)					+80	+400
(direct)					+400	+400
Triglycerides			+560			+300
Aspartate amino transferase		+200	+53			+302
Alanine aminotransferase			+265			+230
Alk. phos.		+72	+241	+58	+127	+250
Lactate dehydrogenase	-52	-70	-79		+79	+70
Ca		+7	+6			+11

At 13 weeks there was an increased frequency of elevated glucose levels (100 mg/l) in the urine of rats dosed at 30 mg/kg/day or greater.

	Male	**Female**
Control	0/10	0/10
8 mg/kg	0/10	0/10
30 mg/kg	1/9	2/10
125 mg/kg	4/6	2/10
500 mg/kg	1/2	2/2

Liver weights of males and females were increased at all dose levels compared to controls. The liver to body weight ratios expressed as a percentage of controls were as follows

	Male	**Female**
8 mg/kg	13%	23%
30 mg/kg	23%	34%
125 mg/kg	54%	41%

There were insufficient number of rats at 500 mg/kg to allow meaningful comparison.
There was also a dose related decrease in thymus weights. Male thymus weights were decreased in the males by 43 and 89% in the 30 and 125 mg/kg/day groups respectively. In the females at 125 mg/kg/day thymus weights were 50% less than the controls.

Pathology

Treated skin site
Effects were slight and consisted of slight epidermal hyperplasia and trace to slight chronic inflammation in the superficial dermis.

Liver
Several animals had livers that were yellow-green color, friable texture and cobblestone appearance, indicating possible pathological effects.
Microscopic examination of the liver indicated that panlobular hepatocellular degeneration was probably the major cause of death in the 200 mg/kg/day animals.
In rats dosed at 125 and 500 mg/kg/day, there were prominent centrilobular and midzonal changes (hepatocyte degeneration, necrosis and fibrosis). In some of the 500 mg/kg/day animals these changes extended to post necrotic cirrhosis with separation of liver lobules into nodules.
The hepatic architecture was further distorted by the presence of extensive hepatocyte hypertrophy, areas of multinucleated large hepatocytes, numerous microcysts, acute and/or chronic active cholangitis/cholangiolitis and bile duct hyperplasia.
Overlying these diverse changes, most animals dosed at 125 and 500 mg/kg/day had considerable widespread lobular disarray, scattered areas of apparent bile duct and portal tract loss and areas characterized by loss of central veins and probable marked reduction of blood supply to the liver cells. Most animals at 8 and 125 mg/kg/day had minimal but discernible

levels of cholangiolitis/cell degenaration/disarray and microcysts.
The following table summarizes the major findings and the dose levels at which they were observed.

Major lesion observed	Lowest dose level affected (mg/kg/day)
Hepatocellular degeneration	125
Hypertrophy of hepatocytes	125
Multinucleated large hepatocytes	125
Vacuolation, fine	125
Necrosis, submassive/bridging	30
Fibrosis, zonal/bridging	30
Microcysts (extra vascular spaces)	8
Cholangiolitis/cell degeneration/ disarray	8
Altered focus of hepatocytes	8

Thymus
At 30 mg/kg/day and greater the thymus was grossly small and microscopically showed hypoplasia/atrophy. The severity of size reduction was dose-related. Some females at 8 mg/kg/day were also affected.

Bone marrow
Erythroid hypoplasia was found in the bone marrow of animals dosed at 125 mg/kg/day and greater. Slight changes were found in3/20 rats at 30 mg/kg/day. In some cases, there was also hypoplasia of the myeloid and megakaryocytic elements.

A No Adverse Effect Level was not established in this study.

Test substance : An analysis of the test material provided the following information. The percentage shown is the average of six determinations.

Chemical class	Weight (%)	Major identified components
Paraffins	13.8	C10-C30 alkanes, normal, branched and cyclic
Diaromatics	10.5	C1-C8 alkylnaphthalenes and C1-C5 alkylbiphenyls
3-ring PAH	26.5	C1-C7 alkylated derivatives of fluorene, phenanthrene and anthracene
4-ring PAH	20.7	C1-C4 alkylated derivatives of pyrene, benzofluorenes, chrysene, benz(a)anthracene, naphthacene, and triphneylene
5-ring PAH	10.6	C1-C4 alkylated derivatives of benzofluoranthenes, perylene, benzopyrenes and benzoanthrylenes
Residue	22.2	Carbazole and C1-C6 alkylcarbazoles, benzocarbazoles and C1-C4 alkylbenzcarbazoles

Reliability : (1) valid without restriction

(39) (62)

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	13 Weeks
Frequency of treatm.	:	Daily, five times each week for 13 weeks
Doses	:	8, 30 & 125 mg/kg/day
Control group	:	Yes
NOAEL	:	> 125 mg/kg bw
Year	:	1992
GLP	:	Yes
Test substance	:	Cracked distillates, Visbreaker gas oil CAS 68471-81-7

Method : Undiluted visbreaker gas oil was applied at doses of 0, 8, 30 and 125 mg/kg/day to the shorn skin of groups of ten male and ten female Sprague Dawley rats. The animals were approximately 48 days old at the start of the study.
The material was applied 5 days each week for 13 weeks. Collars were fitted to the animals to prevent oral ingestion.
Body weights were recorded weekly throughout the study and clinical observations were made daily. Skin irritation was assessed weekly. At 5 and 13 weeks, blood samples were taken for measurement of the following hematological and clinical chemical parameters:

<u>Hematology</u>

Red blood cell count	Hemoglobin
Hematocrit	White blood cell count
Platelet count	MCV, MCH & MCHC caclulated

<u>Clinical chemistry</u>

Urea nitrogen	Total protein
Albumin	Globulin (calculated)
Albumin/Globulin ratio	Alkaline phosphatase
Alanine aminotransferase	Aspartate aminotransferase
Sorbitol dehydrogenase	Creatinine
Cholesterol	Triglycerides
Total Bilirubin	Potassium
Chloride	Sodium

Also at weeks 5 and 13, urine samples were collected for the following determinations: bilirubin, glucose, protein, specific gravity, blood, ketone, pH and urobilinogen.

At the end of the study (13 weeks) all surviving animals were sacrificed and a gross necropsy examination was performed. The following organs were weighed:

Adrenals	Kidneys	Spleen
Brain	Liver	Testes
Epididymes	Ovaries	Thymus
Heart	Prostate	Uterus

The following tissues in the high dose group animals were examined microscopically:

Adrenals (both)	Brain (3 sections)
Bone & marrow (sternum)	Eye (left)
Heart	Intestine, large (colon)
Kidneys (both)	Intestine, small (duodenum)
Liver (2 lobes)	Lung (left lobe)
Ovaries (both)	Muscle, skeletal (thigh)
Optic nerve (left)	Pancreas (head)

Nerve, peprpipheral (sciatic)	Prostate
Seminal vesicles	Salivary gland (submaxillary)
Skin, treated	Spleen
Stomach (squamous & glandular)	Testis (right)
Thymus	Uterus (body & horns)
Thyroid gland	Urinary bladder
Epididymis (right)	Gross lesions

The skin was examined at all dose levels.

The left epididymis and testis from nine control males and ten 125 mg/kg/day males were used for spermatozoa/spermatid evaluations. The tunica albuginea and corresponding blood vessels were removed from the testes and the resulting testicular parenchyma and cauda epididymis were individually weighed. Testes were prepared for spermatid counts and epididymes were prepared for spermatozoa counts and morphological examination.

Result : There were no deaths during the study and, with the exception of the occurrence of skin irritation, no clinical signs of toxicity were observed. There were no compound-related effects on: body weight, urinalysis, hematology or clinical chemistry.
At necropsy there were no treatment-related findings, with the exception of effects on the skin.
The only organ weight effect was a reduction in uterus weight in the 30 mg/kg/day animals, but this was not recorded in any other dose group.
Treatment with visbreaker gas oil did not cause any changes in testicular spermatid or epididymal spermatozoa count nor in sperm morphology.

The only treatment-related finding was skin irritation.
Irritation occurred in a dose-related manner, but there was also wide variation in each group. The group mean irritation scores (and ranges) at week 14 are shown in the following table.

Dose group (mg/kg/day)		Erythema	Edema	CDS*	Sum of means
Males					
8		0.4	0.1	1.8	2.3
	range	0-1	0-1	1-5	1-7
30		0.7	0.3	2.4	3.4
	range	0-1	0-1	1-5	1-7
125		0.8	0.4	4.1	5.3
	range	0-2	0-2	2-5	2-9
Females					
8		0.3	0.1	1.5	1.9
	range	0-1	0-1	1-5	1-6
30		0.9	0.6	2.5	4.0
	range	0-2	0-2	1-5	1-9
125		1.5	1.3	4.1	6.9
	range	0-2	0-2	2-5	2-9

* CDS = Chronic deterioration of the skin

Microscopic examination of the skin revealed thickened epidermis with parakeratosis, chronic inflammation in the subcutis, ulcers and increased mitosis in the epidermal basal cells. The skin changes were more severe in females than the males. Lymph nodes were enlarged predominantly in the high dose animals and microscopic examination revealed non-specific reactive hyperplasia in most instances.

Test substance : The test material was described as V. B. Mittelol (Visbreaker gas oil). Identification: CRU No. 86193
A sample of Visbreaker gas oil (believed to be the same as this sample) was reported to contain 0.38% 3-7 ring PACs (Feuston et al, 1994)
Reliability : (1) valid without restriction

(46) (76)

Test substance : Reformer residues

Remark : No data

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 28 days
Frequency of treatm. : Daily, 5 days/week
Doses : 0.5 (496 mg/kg), 1.0 (992 mg/kg), 2.5 (2480 mg/kg) ml/kg
Control group : Yes
Year : 1987
GLP : Yes
Test substance : Heavy fuels

Method : Three groups of ten male and ten female young adult Sprague Dawley rats were administered heavy fuel oil (CAS no. 68476-33-5) dermally once daily, five days each week for four weeks, at doses of 0.5, 1.0 or 2.5 ml/kgbw/day. The test material was applied to the shorn dorsal skin of the animals. The site of application was occluded for a period of at least six hours following dosing. A group of ten male and ten female rats served as a sham-treated control group.

The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the application site was evaluated daily just prior to the application of test material. Body weights were recorded three times each week during the study.

At necropsy, blood was collected for the following hematological and clinical determinations.
Hematology: erythrocyte count, total and differential leucocyte count, hemoglobin, and hematocrit.
Clinical chemistry: glucose, blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein

The following organs were weighed: liver, kidneys, testes/ovaries, brain, and spleen.

A wide range of tissues were preserved in formalin and the following were processed for subsequent histopathological examination.
spleen, liver, kidneys (2), testes/ovaries (2), brain (cerebrum, cerebellum, pons), skin (treated and untreated), bone marrow, and gross lesions.
Microscopic examination was performed of tissues from the control and high dose animals.

Body weights, clinical pathology, terminal body weights, and absolute and relative organ body weight and organ to brain weight data of the control groups were statistically compared to the treated group data of the same sex, using the Dunnett's t Test at the 5% probability level.
Result : The test material produced minimal reversible dermal irritation at all dose levels. Daily observations of the animals found no compound-related

effects.

There were no other compound-related findings at necropsy other than staining of the skin at the exposure site by the test article.

Eosinophil counts were significantly lower for the mid-dose and high-dose males. SGPT levels were significantly lower for the low- and high-dose females and the high-dose males. Glucose levels were significantly higher for the mid- and high-dose females and high-dose males. Total protein levels were significantly lower for the low-dose males. Hemoglobin levels were significantly lower for the high-dose males. Upon comparison and review of historic data, the study directors concluded the significant values obtained from the hematology or clinical chemistry assays were within normal limits and did not exhibit any clear dose-related trends.

Relative liver weights were significantly higher for the females in all dose groups and in the high-dose males. With the exception of the liver/brain weight ratios in the low-dose males, liver/body weight and liver/brain weight ratios were significantly higher for both sexes in all dose groups. Spleen/body weight ratios were significantly higher for the low and mid-dose females and the high-dose males. The spleen/brain weight ratios were significantly higher for the low-dose females and the high-dose males. The changes in relative spleen weights were not thought to be dose-related by the study directors.

Histopathology findings observed in the non-dermal tissues included eosinophilic casts in the kidneys of both control and high-dose rats. This finding was considered to be a spontaneous lesion expected in Sprague Dawley rats. Pulmonary inflammation was observed in two control males and hepatic inflammation was observed in a high-dose male. Hyperkeratosis (minimal severity) at the test compound application site was seen in the high-dose rats. The dermal lesion at the skin application site occurred only in treated rats and was considered to be related to the dermal application of the test material.

Test substance : Residual fuel oil
Reliability : (1) valid without restriction

(107)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Various

Remark : Several in-vitro genetic toxicity studies have been reported for heavy fuel oil streams. They are listed below together with an indication of the results of the studies.
Summaries of each of the studies are included in the following section.

Test	**Result**
Atmospheric residues	No data
Atmospheric distillates	No data
Vacuum residues	No data
Vacuum distillates	
Heavy vacuum gas oil	
Modified Ames assay	Positive with activation
Cytogenetics assay with Chinese Hamster Ovary cells	Negative with or without activation
Cracked residues	

Clarified slurry oil	
Modified Ames assay	Positive with or without activation
Mouse lymphoma assay	Positive with or without activation
Sister chromatid exchange assay	Positive with or without activation
Cell transformation assay	Negative without activation Positive with activation
Unscheduled DNA synthesis	Positive
Bacterial forward mutation assay	Negative with or without activation

Residual fuel oil

Ames assay	Negative with or without activation
Bacterial forward mutation assay	Negative

Test substance : Atmospheric residues

Remark : No data

Test substance : Atmospheric distillates

Remark : No data, but information on gas oils may be used for an estimate of genotoxicity

Test substance : Vacuum residues

Remark : No data

Type : Ames assay (modified)
System of testing : Salmonella Typhimurium TA 98
Test concentration : 5, 7, 10, 15, 20, 30, 40 & 50 µl/plate
Metabolic activation : With
Result : Positive
Year : 1985
GLP : No data
Test substance : Heavy vacuum gas oil

Method : DMSO extraction was performed on
a solution of heavy vacuum gas oil dissolved in cyclohexane
Petroleum crude oil (positive control)
Stock 642-100 (positive control)
Refrigerator oil (negative control)
The extracts were prepared by mixing 2 ml of test material with 3 ml cyclohexane to homogeneity. 10 ml DMSO was added and mixed for 30 minutes. After 30 minutes, the mixture was centrifuged at 1000 rpm and 22°C for 5 minutes. The DMSO layer was removed and stored in amber bottles at 4 °C until required for the mutagenicity assay.

For the mutagenicity asay, the extracts were tested in strain TA98 according to the following regimens.
The DMSO extracts of heavy vacuum gas oil and NBS1582 were delivered at doses of 50 µl, 40 µl, 30 µl, 20 µl, 15 µl, 10 µl, 7 µl and 5 µl/50 µl. The DMSO extracts of refrigerator oil and stock 642-100° CNN were

delivered at a volume of 50 µl. The metabolic activation mixture contained eightfold higher concentration of hamster liver homogenate (S-9) and a twofold higher level of NADP than used in the standard assay.

Positive control chemicals were 2.0 µg 2-aminoanthracene, 5.0 µg benzo(a)pyrene and 25.0 µg 2-nitrofluorene, in 50 µl DMSO per bacterial plate.

The S-9 fraction was prepared from livers of 6-8 week old Syrian-Golden male hamsters induced with Aroclor 1254.

The appropriate dilution of the test material was incubated for 20 minutes at 37 °C with phosphate buffer for tubes not requiring activation or S-9 mix for tubes requiring activation and 0.1 ml Salmonella broth culture. Agar was added after preincubation and this mix was overlayed on medium in Petri dishes. The plates were incubated for 48 hours at 37 °C. After incubation the number of revertant colonies was counted.

Analysis of data
The mean number of revertants/plate for each dose was calculated. If a dose-related doubling of revertants relative to the mean solvent control was not reached, the mutagenicity index was considered to be zero.
If a doubling was reached, the triplicate revertant values at all doses (including solvent control) was plotted versus dose on an arithmetic scale. The slope of the dose response curve was taken as the mutagenicity index.

Result : The mutagenicity index for heavy vacuum gas oil was reported to be 5.6
No data are provided for the other oils tested.

Reliability : (4) not assignable
Few data are provided in the report.

(60)

Type : Cytogenetic assay
System of testing : Chinese hamster ovary cells
Test concentration : 5, 8, 10, 12 & 15 µl/ml
Metabolic activation : With and without
Result : Negative
Year : 1987
GLP : No data
Test substance : Heavy vacuum gas oil

Result : Metaphase analysis was performed at the highest concentration of test material as well as the controls. This concentration did not demonstrate a significant elevation of aberrant cells compared to the solvent control with or without metabolic activation whereas the positive control has a significant proportion of aberrant cells (33%).

Reliability : (4) not assignable
This information is taken from a compilation of available data. No details of the study are provided.

(67)

Type : Modified Ames assay
System of testing : Salmonella typhimurium TA98
Metabolic activation : With and without
Result : Positive
Year : 1986
GLP : Yes
Test substance : Clarified slurry oil

Method : Four trials were conducted. Two trials employed the use of rat liver homogenate at the standard concentration (10%)
whilst the other two used the rat liver homogenate at an eightfold concentration (80%) in the assay. In the assays using a higher

concentration of S-9 mix, the concentration of NADP was also increased threefold.
In all other respects the method used was the standard Ames assay.
The test material (API 81-15) was tested as a solution in DMSO.
Concentrations of material tested were 1000, 5000, 10,000, 25,000 and 50,000µg/plate.

A positive response was recorded if there was a two-fold or greater increase in revertants per plate.

Remark : This study was carried out as part of a method development program. It was designed to optimize the conditions for testing petroleum streams. The study included several petroleum streams, including clarified slurry oil (API 81-15), as test materials.

Result : The detailed results are provided in the report but only the summarized result for API 81-15 is shown below.

	Maximum-fold increases in TA98 revertants/plate			
	10% S-9 mix		80% S-9 mix	
	Trial 1	Trial 2	Trial 1	Trial 2
API 81-15	13.1	27.8*	44.0	46.3*

* In trial 2, the sample was tested over a lower dose range (33-3333 µg/plate) in order to demonstrate a dose response.

Although the study was conducted to determine the effect of altering the S-9 concentration on the assay outcome, it also clearly demonstrated that API 81-15 was mutagenic in both the standard and modified Ames assays.

Reliability : (1) valid without restriction

(19)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma L5178Y cell line
Metabolic activation : With and without
Result : Positive
Year : 1985
GLP : Yes
Test substance : Catalytically cracked clarified oil (API 81-15) See section 1.1.1.

Method : Non-Activation assay
Cultures of mouse lymphoma cells were exposed to the test material for four hours at doses that were selected during a cytotoxicity study that had been carried out previously.
Following exposure, the cells were washed and placed in growth medium for two or three days to allow recovery, growth and expression of the induced TK-/- phenotype. Cell counts were made daily and appropriate dilutions were made to allow optimal growth rates.
At the end of the expression period, 3×10^6 cells for each dose were seeded in soft agar plates with selection medium and resistant (mutant) colonies were counted after 10 days incubation. To determine the actual number of cells capable of forming colonies, a portion of the cell suspension was also cloned in normal (non-selective) medium. The ratio of resistant colonies to total viable cell number is the mutant frequency.

Activation Assay
The activation assay was run concurrently with the non-activation assay. The only difference was the addition of the S9 fraction of rat liver homogenate and necessary co factors during the four hour treatment period. The final concentrations of the activation system components in the cell suspension were:
2.4 mg NADP/ml; 4.5 mg isocitric acid/ml; 50 µl S9/ml.

S9 homogenate was obtained from Araclor-induced rat liver.

Evaluation criteria
The minimum condition considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that exceeds 150% of the concurrent background frequency by at least 10 x 10^{-6}

Result : The test material was immiscible with water, DMSO and ethanol at 100 µl/ml but formed an opaque brown liquid with acetone at the same concentration.
Stocks were prepared by performing serial dilutions in acetone just prior to each assay. The mutation assays were then initiated by performing final dilutions of the stocks into the assay medium containing the lymphoma cells. The test material appeared miscible in the assay medium without activation from 0.061 nl/ml to 31.3 nl/ml but a brown precipitate was noted at the top of the treatments from 62.5 to 1000 nl/ml.

The results of the assay are summarized below.

	Rel Susp. growth (% of control)	Total mutant colonies	Total viable	Rel cloning eff.	Rel growth (%)	Mutant frequency $10E^{-6}$units
Non activation assay						
Solvent control (acetone)						
	100	73	289	100	100	25.3
	100	53	262	100	100	20.2
Untreated control						
	242.2	51	208	75.5	182.9	24.5
EMS (µl/ml)						
0.5	64.2	710	90	32.7	21	788.9
API 81-15 (nl/ml)						
7,8100	206.6	33	153	55.6	114.9	21.6
15,6000	144.7	43	161	58.5	84.6	26.7
31,3000	114.9	41	174	63.2	72.6	23.6
62,5000	92.7	57	175	63.5	58.9	32.6
125,000	101.8	73	154	55.9	56.9	47.4
Activation assay						
Solvent control (acetone)						
	100	89	299	100	100	29.8
	100	85	195	100	100	43.6
Untreated control						
	69.5	96	266	107.7	74.9	36.1
DMN (µl/ml)						
0.3	57.5	243	63	25.5	14.7	385.7
API 81-15 (nl/ml)						
9770	49.9	132	260	105.2	52.5	50.8
1,9500	38.9	162	204	82.5	32.1	79.4
3,9100	35.5	194	181	73.2	26	107.2
7,8100	14.2	188	106	42.9	6.1	177.4
15,6000	3.4	115	58	35.2	1.2	198.3
31,3000	6.5	196	123	39.3	2.6	159.3

Interpretation of results
Under non-activation conditions, the minimum criterion for mutagenesis is 40.8 x 10^{-6}. The highest concentration assayed induced a mutant frequency that just exceeded the minimum criterion, suggesting weak

mutagenic activity.

In the presence of metabolic activation, the minimum criterion mutant frequency is 64.8×10^{-6}. A dose-dependent increase in the mutant frequency was induced at concentrations above 0.977 nl/ml. Increases in the total mutant clones were also induced, even at treatments that were excessively toxic. The test material was, therefore, positive in this assay.

The negative control mutant frequencies were all within normal background and the positive control materials yielded mutant frequencies greatly in excess of background.

Reliability : (1) valid without restriction

(14)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary cells (CHO)
Test concentration : 5 to 100 µg/ml without activation; 100 to 5000 µg/ml with activation
Metabolic activation : With and without
Year : 1985
GLP : Yes
Test substance : Clarified oil

Result : SCEs were not increased in the absence of S-9 but were increased in the presence of S-9.

(15)

Type : Cell transformation assay
System of testing : BALB/3T3 Mouse embryo cells
Test concentration : 1, 3,, 6 & 9 µg/ml (without activation). 10, 30, 100 & 300 µg/ml (with activation)
Cycotoxic concentr. :
Metabolic activation : With and without
Year : 1986
GLP : Yes
Test substance : Clarified slurry oil

Method : The test material was tested as a solution in acetone. The positive control substance used in the non activation study was N-Methyl N'-nitro-N-nitrosoguanidine (MNNG). For the study with metabolic activation, benzo(a)pyrene was used as the positive control substance.
The S-9 was prepared from Araclor-induced male rat liver.

Exponentially growing 3T3 clone A31-1 cells were seeded for each treatment condition at 25 cells/dish in triplicate for determination of cytotoxicity and at 1×10^{4} cells/dish in 15 replicates for determination of phenotypic transformation.
Time of initiation was designated day 0.
Dilutions of test material and control substances to suitable concentrations for testing were prepared immediately prior to use.
Treatment was accomplished by adding two concentrations of test substance, solvent or positive control to an equal volume of Eagle's minimum essential medium in a dish. Cells were exposed to four concentrations of test material as well as solvent and positive controls for 3 days in the non-activated assay and 4 hours in the activated assay.
Following the exposure period, all treatment materials were withdrawn, the cells were washed once with Hank's balanced salt solution and re-fed with 5ml complete growth medium.
After 70-10 days incubation, the concurrent toxicity dishes were fixed with methanol, stained with 10% Giemsa and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation dishes were fixed, stained and scored for morphologically transformed Type II and Type III foci according to Reznikoff's criteria.

Dose levels for the transformation assay were selected following a preliminary toxicity screen.
It was found that the test material was insoluble in treatment medium at final concentrations of 300 and 1000 µg/ml and was partially soluble at 100 µg/ml. Concentrations below 100 µg/ml were soluble. Survival ranged from 0 to 99%.
Solubility was similar in the presence of activation.
Survival ranged from 31 to 100% in the presence of 100 µl S-9/ml and from 5 to 98% in the presence of 20 µl S-9/ml.
Based on these findings dose levels of 1, 3, 6 and 9 µg/ml in the absence of S-9 and 10, 20, 30, 100 and 300 µg/ml in the presence of 100 µl S-9/ml were selected for the assay.

Evaluation of results
The cytotoxic effects of each treatment condition were expressed relative to the solvent control (relative cloning efficiency).
The transformation frequency for each treatment condition was expressed as the number of transformed foci per surviving cell. For test conditions in which no Type III foci were observed, transformation frequencies were expressed as less than the frequency obtained with one Type III focus.
The number of Type II and Type iii foci per total dishes scored are also recorded.
The transforming potential of each treatment condition was compared to that of the solvent control using a special application of the Poisson distribution.

Result : The results are tabulated below.

	RCE(a)	Dishes with foci per total dishes		Total Foci per total dishes		
		Type II	Type III	Type II	Type III	TF(b)
Treatment						
Without metabolic activation						
Acetone (2µl/ml)						
	100	1/15	1/15	2/15	1/15	0.14
API 81-15 (µg/ml						
1	96	0/14	2/14	0/14	2/14	0.32
3	91	1/15	0/15	1/15	0/15	<0.16
6	85	0/15	2/15	0/15	2/15	0.33
9	66	0/14	0/14	0/14	0/14	<0.23
MNNG (0.5 µg/ml)						
	6	9/15	9/15	18/15	15/15	33.33**
With metabolic activation						
Acetone (2µl/ml)						
	100	1/14	0/14	1/14	0/14	<0.18
API 81-15 (µg/ml						
10	69	4/15	1/15	6/15	1/15	0.25
30	38	1/14	1/14	1/14	1/14	0.48
100	21	2/14	3/14	2/14	3/14	2.68*3
300	18	3/12	0/12	3/12	0/12	<0.19
BaP (12.5 µg/ml)						
	10	6/14	7/14	6/14	8/14	14.29**

(a) Relative cloning efficiency
(b) Transformation frequency (x 10 -4)
* P<0.05
** P<0.01

On the basis of the data shown it is concluded that the test material was negative without metabolic activation, but positive with metabolic activation.

Reliability : (1) valid without restriction

(18)

Type : Unscheduled DNA synthesis
System of testing : Primary rat hepatocyte cultures
Result : Positive
Year : 1985
GLP : Yes
Test substance : Clarified slurry oil

Method : Preparation of hepatocyte cultures
Primary rat liver cell cultures were derived from the livers of two adult male F-344 rats. Each rat was anesthetized and the hepatocytes were isolated by liver perfusion with a collagenase solution and inoculated into culture dishes containing coverslips in supplemented Williams' medium.
After 1.5 to 2 hours incubation, the non-viable cells (those not attached to the coverslips) were washed out of the cultures and the viable cells were used immediately for the UDS assay.

The test material and controls were diluted in DMSO. The final concentration of DMSO was maintained at 1% when diluted in the culture medium.
Three controls were used in the study: a negative solvent control, an untreated medium control and a positive control (2-acetylaminofluorene)

For the preliminary UDS assay, three cultures were used for each of 10 dilutions of 81-15, for the positive control and both negative controls. The maximum concentration of 81-15 tested was 1000 µg/ml.
Cultures were exposed simultaneously to the test material and to 10 µCi/ml 3H-thymidine for 20 hours. After exposure all cultures were washed with medium, swelled in hypotonic solution, fixed and washed with water. The coverslips were mounted on slides, dipped in Kodak NTB-2 emulsion and exposed at -20°C for 7 days prior to development.
Cells were stained in methyl green Pyronin Y. After determining the appropriate concentrations based on cytotoxicity and positive responses, a replicate experiment was performed to ensure reproducibility. The UDSassay was repeated at six non-cytotoxic concentrations of 81-15.

Measurement of UDS
Quantitative autoradiographic grain counting was accomplished using colony counters.
50 morphologically unaltered cells on a randomly selected area of the slide were counted. The highest count from two nuclear size areas areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grans/nucleus (NG).
The percentage of cells in repair was calculated as the percentage of cells with at least +5NG. 150 cells were scored for each concentration reported for each experiment.

Criteria for interpretation
Positive
A test material is considered positive if UDS is markedly elevated above that in the solvent control.
Negative
A material is considered negative if testing has been performed to the limits of solubility or cytotoxicity, or at 5000 µg/ml and if UDS is not significantly elevated above that of the solvent control.

Remark : This study included three test materials, one of which was API 81-15. Only the information relating to the 81-15 is included in this summary.

Result : Cytotoxicity was observed at 1000 µg/ml in the preliminary experiment and at 1000 and 500 µg/ml in the replicate study.

The preliminary experiment was performed at concentrations between 1 x 10^{-6} and 1000 µg/ml. A precipitate was observed adhering to the sides of the tubes at 100 and 1000 µg/ml. UDS was measured at 81-15 concentrations between 1 x 10^{-4} and 100 µg/ml in the preliminary experiment and between 0.5 and 100 µg/ml in the replicate experiment. The results are tabulated below.

Treatment	Preliminary assay		Replicate assay	
	N.G	**%IR**	**N.G.**	**%IR**
Control medium	-4.1	3	-3.7	11
DMSO control -	7.2	5	-9.3	0
2-AA	28.6	94	60.3	99
81-15				
1 x10 -4 µg/ml	-5.4	3	NT	
0.001 µg/ml	-7.4	1	NT	
0.01 µg/ml	-7.2	1	NT	
0.1 µg/ml	-6.8	1	NT	
0.5 µg/ml	NT		-3.3	3
1 µg/ml	7.8	56	-6.6	3
5 µg/ml	NT		12.7	67
10 µg/ml	51.1	98	19.5	87
50 µg/ml	NT		59.7	97
100 µg/ml	49.8	99	33.2	93
500 µg/ml	NT		*	
1000 µg/ml	*		*	

% IR Percentage of cells in repair
NT Not tested at the concentration shown
* Cytotoxicity observed, slides unscorable.

The presence of a dose response, positive net grain count and an increased number of cells in repair indicate that sample 81-15 is genotoxic in this assay.

Reliability : (1) valid without restriction

(11)

Type : Bacterial forward mutation assay
System of testing : Chinese hanster ovary cells (CHO)
Test concentration : 0.1, 1, 3, 10 & 30 µg/ml without activation. 0.1, 1, 10, 100 & 200 µg/ml with activation
Metabolic activation : Wth and without
Result : Negative
Year : 1985
GLP : Yes
Test substance : Clarified slurry oil

Method : A cytotoxicity pre-screen was carried out before conducting the assay. Based on the results of this pre-screen the following dose levels, using DMSO as a solvent, were selected for evaluation in duplicate cultures:

Without S-9 activation 0.1, 1, 3, 10 and 30 µg/ml
With S-9 activation 0.1, 1, 10, 100 and 200 µg/ml.

S-9 was prepared from Araclor induced rat liver.
Two positive control substances were used. For the assay without activation, ethylmethane sulfonate (EMS) was used at a concentration of 200 µg/ml whilst for the assay without activation dimethylnitrosamine (DMN) was used at a concentration of 100 µg/ml.

The CHO-K1-BH4 cells were seeded into flasks and treated (day 0) with the test material and control substances at the concentrations shown

above. Following 19 hours incubation after treatment, the cells were harvested and a cell number was determined for each culture. An aliquot of each culture was diluted in Saline G to a density of 1000 cells/ml and 0.2 ml were then added to each of 3 plates containing 5 ml of F12FCM5 (200 cells/plate). These plates were used to determine the relative cell survival following treatment and were incubated for 7 days before the colonies were fixed, stained and counted. An additional aliquot yielding 1 x 10^6 cells was subcultured for phenotypic expression into a 100 mm dish containing 10 ml of F12FCM5. Subcultures were performed on days 3 and 5 with selection on day 7.
Selection was accomplished by taking cells from each culture and plating them in medium containing TG (6-thioguanine).

Mutant frequency, expressed as TG r mutants/10^6 clonable cells was calculated by dividing the total number of mutant clones by the number the number of cells plated, corrected for the cloning efficiency of the cells at the time of mutant selection.

Interpretation of results
A test article is considered positive if it exhibits a dose-dependent increase in mutation induction with at least one dose resulting in a mutant frequency of > 50 Tg r mutants/10^6 clonable cells.

Result : There was no dose-dependent increase in the mutant frequencies of the cultures treated with the sample of API 81-15. See table below.

Dose	**Rel. initial Survival (%)**	**Total No mutants**	**Cloning efficiency (%)**	**Mutation Frequency (mean)**
Without activation				
Untreat.	99.2	1	83	
	100.8	2	85.3	1.7
DMSO	108.1	2	81	2.5
	96	7	80.7	5.6
EMS	53.1	107	68.8	
	53.1	109	62.7	164.6
API 81-15 (µg/ml)				
0.1	87.9	2	77.5	
0.1	85.1	3	80	3.2
1.0	80.2	14	85.2	
1.0	67.1	18	91.8	18.0
3.0	45.6	0	88.3	
3.0	52.8	1	85.2	0.6
10	33.1	2	75.5	
10	31.4	1	74	2.0
30	17	13	86	
30	10.6	4	100.7	9.6
With activation				
Untreat.	93.8	4	85.8	
	98.7	2	77.8	3.6
DMSO	99.7	6	95.7	
	98.2	3	77	5.2
DMN	14.3	102	43.5	

	20.5	124	44.2	257.4
API 81-15 (µg/ml)				
0.1	76.5	2	79.5	
0.1	78.5	3	73.7	3.3
1.0	70.5	0	89.3	
1.0	65.8	1	87.8	0.6
10	51.2	4	97	
10	55.5	11	82.7	8.7
100	22	15	82	
100	33.8	7	86.8	13.2
200	16	15	96.7	
200	9.4	16	93.8	16.4

It is concluded that the test material was negative in this assay.
(10)

Test substance : Cracked distillates

Remark : No data

Test substance : Reformer residues

Remark : No data

Type : Ames test
System of testing : Salmonella typhimurium, 4 strains
Metabolic activation : With and without
Result : Negative
Year : 1985
GLP : No data
Test substance : Heavy fuels
Reliability Due to the inappropriate test method, the study is not reliable.
Remark : This study was reported fully in an open literature publication. However a standard Ames assay has been shown to be inappropriate for petroleum products. Consequently, the study is not summarized here.
(126)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : Rat
Sex : Male/female
Route of admin. : Dermal
Exposure period : 90 days
Doses : 30, 125, 500 & 2000 mg/kg/day
Result : Negative
Year : 1987
GLP : No data
Test substance : Heavy vacuum gas oil

Method : Groups of ten male and ten female rats were exposed dermally to Heavy vacuum gas oil (HVGO) at daily dose levels of 0, 30, 125, 500 or 2000 mg/kg/day, five days each week for 13 weeks. At the end of the 13

weeks exposure, the animals were killed and the femurs were taken from five animals per sex per dose group except for 125 mg/kg/day females and 2000 mg/kg/day males. Three bone marrow slides were prepared from each animal.
The slides were air dried, fixed in absolute mathanol and stained with acridine orange. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored to determine the prcentage of micronucleated erythrocytes.

A statistical analysis was conducted and if a significant increase in micronuclei over the control values occurred it was taken as an indicaton that the test material was clastogenic.

Result : The individual raw data are given in the report together with summarized data.
There were no differences between the control values and those for any of the treated groups for:
polychromatic erythrocytes/ normochromatic erythrocytes
% micronucleated PCEs
or % micronucleated NCEs

In view of the negative results, the data are not summarized here.

API 81-15 was negative in the micronucleus assay.

Reliability : (1) valid without restriction

(68)

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : 5 days
Doses : 0.1, 0.3 & 1 g/kg/day
Result : Negative
Year : 1985
GLP : Yes
Test substance : Catalytically cracked clarified oil (API 81-15) See section 1.1.1.

Method : Groups of adult male and female Sprague-Dawley rats were given test material by gavage, once each day for five days at the dose levels shown in the table below. In addition, triethylenemelamine (TEM) at a dose level of 1 mg/kg was administered to a group of male and female rats as a single intraperitoneal dose 24 hours before the end of the study; these groups served as positive controls. Negative controls consisted of groups of rats that were given corn oil orally at the same times as the dosing of the test material.

Treatment	No. animals Male	Female
1 g/kg/day	13	13
0.3 g/kg/day	10	10
0.1 g/kg/day	10	10
TEM 0.1 g/kg ip*	10	10
Corn oil	10	10

Three hours prior to being killed with CO_2, animals were injected i.p. with 4 mg/kg of colchicine. After the animal was killed, the adhering soft tissue and epiphyses of both tibiae were removed and the marrow was flushed from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and was then re suspended in 0.075M KCl. The centrifugation was repeated and the pellet re suspended in fixative (methanol:acetic acid, 3:1). The fixative was

changed once and left overnight. Cells in fixative were dropped onto glass slides which were then air dried and stained with 5% Giemsa.
Slides were coded and scored for chromosomal aberrations.

50 spreads were read for each animal where feasible.
A mitotic index based on at least 500 counted cells was also recorded.
The index was calculated by scoring the number of cells in mitosis per 500 cells on each read slide.

Statistical evaluation was performed by Student's t-tests.

Data interpretation and evaluation
Gaps were not counted as significant aberrations.
Open breaks were considered as indicators of genetic damage as were configurations resulting from the repair of breaks. The latter included translocations, multiradials, rings, multicentrics, etc. Reunion figures such as these were weighed slightly higher than breaks since they usually resulted from more than one break.
Cells with more than one aberration were considered to indicate more genetic damage than those with evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of mutagenic potential.

The type of aberration, its frequency and its correlation to dose in a given time was considered in evaluating the test material as being positive or negative.

Result : The data are given in the report for males, females and as male and female pooled data.
The structural aberration frequencies in negative control males and females, both separately and pooled were similar to those obtained previously in the test laboratory. The data summarized below, are the pooled data for males and females.

Dose	**Total No of cells**	**% cells with aberrations**		**Mitotic index**
		1+	**2+**	
Negative control corn oil	929	0.4	0	5.0
Positive control TEM, 0.8 mg/kg	400	57.5**	48.5**	0.9
API 81-15				
0.1 g/kg	950	0.4	0	4.8
0.3 g/kg	900	0.6	0	4.5
1.0 g/kg	929	0.8	0	4.6

**P < 0.01
At all dose levels of test material, the number of cells with structral aberrations did not differ significantly from those for the negative control whereas those for the positive controls were elevated.

Sample 81-15 was negative in this assay.

Reliability : (1) valid without restriction

(14)

Type : Sister chromatid exchange assay
Species : Mouse
Sex : Male/female
Strain : B6C3F1

Route of admin. : i.p.
Exposure period : Four hours
Doses : 0.4, 2.0 & 4.0 g/kg
Result : Positive
Year : 1985
GLP : Yes
Test substance : Clarified slurry oil, API 81-15. See section 1.1.1.

Method : Prior to treatment with the test material, 30 male and 30 female mice were anesthetized and an agar coated 50 mg BRdU pellet was implanted subcutaneously in the lower abdominal region.
Four hours after implantation of the pellet, groups of five males and five females were given a single intraperitonelal dose of 0.4, 2 or 4 g/kg of test substance in a dose volume of 10 ml/kg. A positive control group of five animals of each sex was given cyclophosphamide at a level of 10 mg/kg. Colchicine (1 mg/kg) was administered intraperitoneally to all mice 2 hours before sacrifice to arrest mitosis.
24 to 26 hours after BRdU pellet implantation, the mice were sacrificed. Both femurs were exposed, cut just above the knee and the marrow was aspirated into cold Hank's solution.
The cells were collected by centrifugation, resuspended in warm hypotonic solution and then incubated for approximately 10 minutes at 37 °C to swell the cells. The cells were collected by centrifugation, resuspended in two consective changes in Carnoy's fixative, capped and stored overnight at approximately 4 °C.
Two to four drops of fixed cells were dropped onto a wet slide and air dried. Two to five slides were prepared for each animal and after staining were examined microscopically.
Metaphase cells were examined. Where possible, a minimum of 50 second-division metaphase spreads from each animal were examined and scored for SCEs and chromosome number. The mitotic index was recorded as the percentage number of cells in mitosis based on 500 cells counted. The percentage of first, second and third division metaphase cells was also recorded as the number per 100 cells counted.

Evaluation of test results
The test material is considered to induce a positive response if a dose-related increase ($p < 0.05$, one way ANOVA, studentized range test) in SCEs/metaphase is observed relative to the vehicle control.

Result : The results are shown in the following table.

Treatment (sex)	No. of mice	Range of SCEs/cell	Average SCEs/cell per mouse
Corn oil (M)	4	4.86-6.18	5.43±0.60
(F)	5	5.91-7.44	6.73±0.68
API 81-15			
4 g/kg (M)	5	6.76-11.18	8.83±1.60*
(F)	5	7.82-10.46	9.26±0.95*
2 g/kg (M)	4	6.84-9.5	8.43±1.15*
(F)	5	7.14-10.42	8.06±1.36
0.4 g/kg (M)	5	6.28-8.62	7.43±1.0
(F)	5	5.84-8.94	7.22±1.17
CP (M)	5	16.54-33.97	24.61±7.39**
(F)	5	25.56-43.38	31.60±7.24**

* P< 0.05
** P< 0.01

Under the conditions of the assay, API 81-15 did induce a statistically significant and dose-responsive increase in SCEs/metaphase in male and female mice.

Reliability : (1) valid without restriction

(13)

Type : Unscheduled DNA synthesis
Species : Rat
Sex : Male
Strain : Fischer 344
Route of admin. : Gavage
Exposure period : 2 and 12 hours
Doses : 50, 200 & 1000 mg/kg
Result : Positive
Year : 1985
GLP : Yes
Test substance : Slurry oil, API 81-15. see setion 1.1.1.

Method : Groups of three male F-344 rats were treated by gavage with test material at doses of 50, 200 and 1000 mg/kg in a dose volume of 3 ml/kg. Animals were treated 2 and 12 hours before sacrifice. A positive control group was given 2-acetylaminofluorene in corn oil 12 hours prior to sacrifice. The negative control was corn oil.

Primary hepatocyte cultures were obtained from the livers of the treated rats. The cells were inoculated into 6-well culture dishes containing cover slips in supplementd William's medium. After 1.5 to 2 hours the cultures were washed to remove non-viable cells (those not attached to the cover slips).

Cultures were incubated in William's medium containing 10 µCi/ml ^{3}H-thymidine for 4 hours, followed by 14 to 16 hours in William's medium containing 0.25mM unlabelled thymidine.
Cultures were then washed, swelled in a hypotonic solution, fixed and washed with water. The cover slips were mounted, dipped in Kodak NTB-2 emulsion and exposed at -20 °C for 12 to 14 days prior to development.
Cells were stained with 1% methyl-green Pyronin Y.

Quantitative autoradiographic grain counting was accomplished using colony counters.
50 morphologically unaltered cells on a randomly selected area of the slide were counted. The highest count from two nuclear size areas over the most heavily labelled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains/nucleus (NG).
The percentage of cells in repair was calculated as the percentage of cells with at least +5NG.

A minimum of 3 slides were scored for each of 3 animals, for a minimum total sample of 3 animals, 9 slides, and 450 cells/dose/time point.

Criteria for interpretation
Positive
A test material is considered positive if UDS is markedly elevated above that in the solvent control.
The presence of a dose-response, changes in the frequency distribution of cellular responses, increases of the percentage of cells in repair and reproducibility of data were all considered in classifying the test material as "positive" or "negative". No other statistical methods were used in analyzing the data.

Negative
A test material was considered negative if UDS was not markedly elevated above that in the solvent control.
A material is considered negative if testing has been performed to the limits of solubility or cytotoxicity, or at 5000 µg/ml and if UDS is not significantly elevated above that of the solvent control.

Result : The results are tabulated below.

Treatment	Dose (mg/kg)	Time	NG (hr)	% in repair
Corn oil		12	-3.6	3
2-AA	50	12	19	87
81-15	50	2	-6.2	1
		12	-5.4	1
	100	2	-5.8	1
	100	12	-2.8	16
	1000	2	-0.9	14
	1000	12	9.5	58

These results indicate that 81-15 is a genotoxic agent in this assay.

Reliability : (1) valid without restriction

(12)

5.7 CARCINOGENICITY

Species : Mouse

Remark : Available dermal carcinogenicity studies have been summarized by CONCAWE (CONCAWE, 1998) and Bingham et al (Bingham et al 1980) and have also been reviewed by IARC (IARC, 1989).
A tabulation of the studies that have been summarized by CONCAWE is shown below.

Dosing regime	Result*	Mean latency (weeks)	Reference
Steam cracked tar 15 mg 3 x week (100)	38/62 tumors	43	Smith et al (1951)
Clarified slurry oil undiluted 25µl 3 x week (40)	36/40 tumors	17	McKee et al (1990)
Sample API 81-15, 10% in toluene 50 µl 2 x day (100)	49/50 tumors 48 malignant 1 benign	22	API 1989
Sample API 81-15, 1% in toluene 50 µl 2 x day (100)	45/50 tumors 44 malignant 1 benign	72	API 1989
Sample API 81-15, 0.1% in toluene 50 µl 2 x day (100)	2/50 tumors 2 benign	113	API 1989

* Numbers given are the number of animals with tumors/number in group

An abbreviated version of a summary table in Bingham et al follows:

Potencies of two blended fuel oils for the skin of C3H mice
(Explanation of headings given below)

Base blend stock	Cracked residue added	Dose (mg)	No of mice	FEN	No. mice with tumor benign	malignant
A	0	20	19	17	1	1
		50	20	17	3	7 (58.8)
B	0	20	40	23	0	1 ....
A	5	20	30	27	15	8 (41.5)
		50	30	27	13	8 (28.3)
B	5	20	40	31	9	11 (49.1)
		50	28	27	9	9 (36.9)
A	10	20	30	26	19	7 (40.4)
		50	30	25	22	3 (32.2)
B	10	20	40	35	22	13 (40.5)
		50	30	30	9	18 (26.7)
A	20	20	25	23	12	9 (25.2)
B	20	20	29	28	11	16 (23.4)

Base blend stocks were
A Cracked bunker fuel
B West Texas uncracked residuum
Cracked residue added was cat cracked clarified oil at the concentrations shown

Dosage was applied twice weekly

FEN is number alive at time appearance of median tumor plus number of tumor-bearing mice which died.

Number in parentheses is the averasge time of appearance of papillomas (weeks)

(21) (28) (29) (51) (59) (101)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0-20 incl. of gestation
Frequency of treatm. : Daily
Doses : 50, 333 & 1000 mg/kg/day
Control group : Yes
NOAEL maternal tox. : = 333 mg/kg bw
NOAEL teratogen. : = 333 mg/kg bw

Year : 1994
GLP : Yes
Test substance : Atmospheric residues

Method : Groups of 12 presumed-pregnant rats (approximately 11-12 weeks old) were distributed into the following groups:

Group	Dose level (mg/kg/day)	Gestation days of administration
1	0	0-20
2	50	0-20
3	333	0-20
4	1000	0-20

The control animals received the carrier, corn oil, at a dose of 2 ml/kg. With the exception of test article application, these animals underwent the same procedures as the other treatment groups.

The test material was applied daily to the shorn dorsal skin at the dose levels shown above and for the duration indicated. The rats were fitted with collars to prevent oral ingestion of the applied material.

Observations of the dams were made daily for clinical signs and body weights and food consumption were recorded regularly throughout the study. Each litter was observed daily during Days 0 (day of parturition) through 4 of lactation for signs of toxicity and mortality. Each pup was examined externally for abnormalities. On lactation Days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed with carbon dioxide and necropsied; one female was sacrificed moribund and necropsied. Females that delivered a litter were necropsied on Day 4 of lactation, and those that did not deliver a litter or if all pups were dead by Lactation Day 4 or delivered all dead pups were necropsied on presumed Gestation Day 25. The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The number of implantation sites within the uterine horns was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide in an attempt to reveal any implantation sites. If no implantation sites were observed, the animal was considered to be non pregnant. Dead pups were removed and examined externally. If there were no external abnormalities, the pups were discarded. On Day 4 of lactation, all surviving pups were sacrificed with an intraperitoneal injection of euthanasia solution and discarded.

Statistical evaluation of female body weight and food consumption data equality of means was done by an appropriate one way analysis of variance and a test for ordered response in the dose groups. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1 percent level of significance. If the variances were equal, the testing was done using parametric methods, otherwise, non-parametric techniques were used.

For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used. If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.

For the non-parametric procedures, the test of equality of means was performed using the Kruskal-Wallis test. If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment groups differed significantly from control. In addition to the Kruskal-Wallis test, Jonckheere's test for monotonic trend in the dose response was performed.

The test for equal variance (Bartlett) was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance.

Result : During the gestation and lactation periods slight to moderate (primarily slight) erythema and eschar and slight edema and dry skin were observed, both on treated and untreated skin in the carrier control group. There were no other clinical observations (including dermal irritation) that were considered to be related to treatment with the test article.

One dam in the 333.0 mg/kg dose group was unsuccessful in delivering her litter and was sacrificed moribund. The study directors did not consider this death to be related to test article exposure. No other mortality occurred in this phase of the study.

Body weight changes for pregnant females in the 1000 mg/kg/day dose group were significantly lower ($p<0.05$) than those of the control females between Gestation Days 16 to 20. The laboratory report notes that the changes in female body weights appear to be influenced by two females which had reduced litter sizes. The study directors considered this finding to be treatment related; however, it may be significantly influenced by a decrease in fetal mass. There were no other effects on body weight or body weight changes at any of the dose levels.

There were no compound-related effects on either absolute (g/animal/day) or relative (g/kg body weight/day) food consumption in the dams.

At necropsy, no lesions related to administration of the test article were noted for dams in any of the dose groups.

Developmental data

		Dose (mg/kg)		
Parameter	**0**	**50**	**333**	**1000**
Number + evidence mating				
	15	12	12	12
Number pregnant				
	15	12	10	11
Gestation Length (Days)				
	22.1	22.1	22.4	22.8**
Number of Implantation sites				
	16.4	17.2	14.0*	17.0
Number litters w/ live pups				
	15	12	9	11
Mean number live pups				
- Day 0	13.9	15.9	12.9	10.9
- Day 4	(87%)	(95%)	(94%)	(84%)
Proportion males				
- Day 0	0.49	0.49	0.53	0.55
- Day 4	0.54	0.47	0.54	0.54
Mean wt (g) live pups				
- Day 0	6.68	6.28	6.64	6.13*

-	Day 4	8.96	7.74*	9.06	7.62*

* (p<0.05)
** p<0.01

For all dose groups, there were no significant differences for the total pups per litter, proportion dead Lactation Day 0, proportion surviving to Lactation Day 4, proportion males Lactation Days 0 and 4 or external pup alterations.

The study directors considered decreased body weight changes and the increase in gestation length at a dose of 1,000.0 mg/kg to be signs of compound-related maternal toxicity.

Signs of developmental toxicity considered by the study directors to be compound-related included decreased pup body weights on Lactation Days 0 and 4 at a dose of 1,000.0 mg/kg. The study directors did not think the reduced number of implantation sites seen in the 333 mg/kg/day group were treatment-related since the number of implantation sites were not significantly lower at the higher dose of 1000.0 mg/kg/day. Similarly, the reduced live pup weights on Lactation Day 4 in the 50 mg/kg/day group were not considered to be related to treatment with the test article since the two higher doses were normal. In addition, the report notes that excellent pup survival was observed at this dose level, which would not be expected if the decreased body weight was, in fact, biologically relevant.

The authors concluded that for maternal toxicity and signs of developmental toxicity the no-observable-adverse-effect level (NOAEL) was 333.0 mg/kg/day.

Test substance : CASRN 64741-45-3
Residues (petroleum), atm. Tower
A complex residuum from the atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly greater than C20 and boiling above approximately 350 °C (662°F). This stream is likely to contain 5 wt % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

Reliability : (1) valid without restriction

(124)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0 to 19 of gestation
Frequency of treatm. : Daily
Doses : 8, 30, 125 & 500 mg/kg/day
Control group : Yes
NOAEL maternal tox. : = 30 mg/kg bw
NOAEL teratogen. : = 30 mg/kg bw
Year : 1991
GLP : No data
Test substance : Atmospheric distilate, HAGO

Method : Prior to dosing, females approximately 13 weeks old were paired. The subsequent appearance of a vaginal plug or the presence of spermatozoa in vaginal lavage fluid was taken to indicate that mating had occurred. This was taken to be day 0 of the study.
The presumed-pregnant rats were distributed into the following groups each of 12 animals:

	Dose level (mg/kg/day)
Prenatal groups	
Group 1	0 (sham control)
Group 2	8
Group 3	30
Group 4	125
Group 5	500
Postnatal groups	
Group 6	0 (sham control)
Group 7	125

The test material was applied daily from days 0 to 19 of gestation to the shorn dorsal skin at the dose levels shown above. The rats were fitted with collars to prevent oral ingestion of the applied material. Observations were made daily for clinical signs.

Postnatal group
Dams and their litters were observed on post partum days 0 to 4 for signs of pathosis and/or death. On postpartum day 0 pups were also examined for external malformations. Pups were also examined daily for presence of milk in their stomachs and absence of milk was recorded.
Body weights and food intakes were recorded throughout the study except that food intakes were not recorded postpartum. Offspring were weighed according to gender.

Prenatal group
Each female was sacrificed on day 20 of presumed gestation and the reproductive organs examined. The uterus and ovaries were removed, the remaining organs were examined grossly and the liver and thymus were weighed. The liver was fixed for subsequent histopathology.
The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined grossly and all remarkable findings recorded. Uterus weights were also determined.
The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals assigned to prenatal groups and the following hematological and clinical chemical measurements/calculations were made.

Hematology

Hematocrit	Hemoglobin
Mean corpuscular volume (MCV)	Platelet count
Mean corpuscular hemoglobin (MCH)	RBC count
Mean corpuscular hemoglobin concentration (MCHC)	RBC morphology
	WBC count

Clinical chemistry

Alanine aminotransferase	Glucose
Albumin	Lactate dehydrogenase
Albumin/globulin ratio	Inorganic phosphorus
Alkaline phosphatase	Potassium
Aspartate aminotransferase	Sodium
Bilirubin (total)	Sorbitol dehydrogenase
Calcium	Total protein
Chloride	Triglycerides
Cholesterol	Urea nitrogen
Creatinine	Uric acid
Globulin	

Fetuses were examined and half were preserved for examination of soft

tissue abnormalities, the remainder being differentially stained for skeletal examination.
Animals in the Postnatal groups were sacrificed either on day 4 postpartum if they had surviving offspring or day 25 of gestation if they had not given birth. The reproductive organs were examined grossly, the liver and thymus was weighed and the liver preserved for histological examination. Surviving pups were sacrificed on postpartum day 4 and no further examination of these was undertaken.

Statistical analysis
Maternal biophase data, cesarian section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's exact or Dunnet's test.

Thymus and liver weight data were statistically evaluated using Tukey's test.

Hematology and serum chemistry data were analyzed for analysis of variance followed by comparisons using Tukey's test.

For all statistical analyses, differences between control and treated groups were considered to be significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$)

Result : Skin irritation which ranged from slight to moderate occurred in a few animals in each of the groups exposed to gas oil. However, there was no obvious dose response effect.
A red vaginal discharge (normally indicative of litter resorption) was observed in 7/11 animals in the 500 mg/kg group. A red vaginal discharge was also observed in one female of the pre- and postnatal groups at 125 mg/kg. The report comments that such an observation has been noted in control animals and therefore in this study it is unclear as to whether the observation was related to the administration of gas oil.

The dams in the 8 and 30 mg/kg groups were unaffected by exposure. The only differences were observed in the 125 and 500 mg/kg groups and these are listed below.

Parameter	125 mg/kg	500 mg/kg
Body weight	Reduced	Reduced
Overall weight gain	-20% *	-65% **
Food consumption	Reduced ** first 13 days	Reduced ** throughout
Thymus weight (abs.)		-53% **
Thymus weight (rel.)		-46% **
Liver weight (rel.)		+16% **
Platelets		-25% *
Segmented neutrophils	-30% *	
Triglycerides		-68% **
Total protein		+20% **
Albumin		+27% **
Calcium		+8% **
Blood urea nitrogen		+38% *
Alkaline phosphatase		+95% **

* P< 0.05
** P< 0.01

Reproductive evaluations
No effects were recorded in the 8 and 30 mg/kg groups.
Preimplantation losses in both the 125 and 500 mg/kg groups

were more than twice that of controls; the difference, however, was not statistically significant. Two females in each of these two groups had few implantation sites relative to the number of eggs ovulated.
Three of these four animals also had a reduced number of corpora lutea. However, since ovulation had occurred prior to the start of treatment with gas oil this was not regarded as a treatment-related effect.
There was a significant increase in the mean number/percent resorptions in the 500 mg/kg group.

Fetal evaluations
Mean fetal body weights were significantly decreased for all viable fetuses in the 500 mg/kg prenatal group and in the males pups of the 125 mg/kg group. There was one dead fetus in the 125 mg/kg prenatal group and two dead fetuses in the 500 mg/kg group. The fetus in the 125 mg/kg prenatal group was severely malformed while the two fetuses in the 500 mg/kg group were not malformed. However, these findings were considered to be incidental.
There was a significant increase in incomplete ossification of a number of skeletal structures (nasal bones, thoracic centra, caudal centra, sternebrae, metatarsal and pubis) in the 125 and 500 mg/kg groups. There were no treatment-related abnormalities found in the soft tissues.

Postnatal group findings
At necropsy, the absolute and relative liver weights of the 125 mg/kg females were significantly increased.

Litter data
Exposure to gas oil did not adversely affect pup survival or development. Pups from gas oil exposed females were significantly smaller than control pups but the gas oil exposed females had significantly larger litters overall and pups in larger litters tend to be smaller than pups from smaller litters.

Reliability : (1) valid without restriction

(74)

Test substance : Vacuum residues

Remark : No data

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Frequency of treatm. : Daily
Duration of test : Days 0-19 incl. of gestation
Doses : 30, 125, 500 & 1000 mg/kg/day
Control group : Yes
NOAEL maternal tox. : = 125 mg/kg bw
NOAEL teratogen. : = 125 mg/kg bw
GLP : No data
Test substance : Heavy vacuum gas oil

Method : Groups of 10 presumed-pregnant rats (approximately 9-10 weeks old) were distributed into the following groups:

Group	Dose level (mg/kg/day)	Gestation days of administration
1	0 (remote control)	0-19
2	0 (proximate control)	0-19
3	30	0-19
4	125	0-19
5	500	0-19

6	1000	0-19
7*	500 (bioavailability)	10-12

* Group size was 5 at start but increased to 8 after study initiation.

The test material was applied daily to the shorn dorsal skin at the dose levels shown above and for the duration indicated. The rats were fitted with collars to prevent oral ingestion of the applied material.
Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gasoil that had been applied to other animals.

Observations were made daily for clinical signs and body weights and food consumption were recorded regularly throughout the study.

Each female was sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly.
The thymus and liver were removed from each animal and weighed and then preserved in formalin but not examined further. The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined.
The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and a range of clinical chemical measurements were made of the following:

Alanine aminotransferase (ALT)	Glucose
Albumin	Iron
Albumin/globulin ratio	Phosphorus, inorganic
Alkaline phosphatase (ALP)	Potassium
Bilirubin, total	Sodium
Calcium	Sorbitol dehydrogenase (SDH).
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid.

Fetuses were examined and half were preserved in Bouin's solution for examination of soft tissue abnormalities, the remainder were being differentially stained for subsequent skeletal examination.

Statistical analysis

Maternal biophase and cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's Exact or Dunnet's Test.
Fetal skeletal and visceral data were evaluated statistically by ANOVA followed by group comparisons using Fisher's Exact test.
Thymus and liver weights were evaluated statistically using Student-Newman-Keul's test.
Statistical analyses of clinical chemistry data were performed separately on individual serum components using SAS procedures. First the F-test was employed to do an analysis of variance on the serum data obtained from control and exposed groups. Next, the Student-Newman-Keul's multiple comparison test was employed to identify the specific group subsets within the serum data sets identified as having nonrandom variance.
In general, for all statistical tests, differences between control and treated groups were considered statistically significant if the probability of the difference being due to chance was less than 5% ($P<0.05$).

Result : Parental animals.

There were no clinical signs attributable to exposure to HVGO other than in the highest dose group in which 2 rats had a red vaginal discharge, one animal was pale in color and six had decreased stool. The latter observation was probably associated with smaller food consumption in this group. Although food consumption was generally also less associated body weight decrease.
At doses in excess of 125 mg/kg/day there was a decrease in mean body weights of the dams which reflected the decreased litter sizes for these groups.
At gross necropsy it was noted that the lungs appeared pale in a few animals; 4 animals were affected at the highest dose and only one in the 500 mg/kg/day group.
Mean thymus weights of animals in the highest dose group were approximately half those of the control groups. Although absolute liver weights were unaffected by exposure to HVGO, mean relative liver weights were increased (approximately 15%) in groups exposed to doses greater than 125 mg/kg/day.

Observations of Dams at Caesarean section.
Parameters with treatment-related effects are shown below.

	Dose group (mg/kg/day)					
	0(R)	**0(P)**	**30**	**125**	**500**	**1000**
Dams with viable fetuses	9/9	10/10	10/10	8/10	10/10	6/10
Dams with all resorptions	0	0	0	0	0	3
Mean litter size of viable fetuses	13.9	14	13.8	14.4	10	5.8
Resorptions Mean	1.1	0.6	1.1	1.1	5.6	9.9
% Dams with resorptions	56	50	70	63	100	100

Parameters unaffected were:
- No. premature births
- Female mortality
- No. corporea lutea
- No. implantation sites
- Pre-implantation losses
- Viable male fetuses
- Viable female fetuses
- No. dead fetuses

Fetal evaluations

Fetal body weights were significantly reduced in fetuses exposed in utero to HVGO at doses in excess of 125 mg/kg/day.
Although there were differences between control and treated crown-rump lengths they were not statistically significant.
At the time of external examination, malformations were observed in one fetus in the 1000 mg/kg/day group. The fetus was edematous and pale in color. Both hindpaws were malformed; the digits were reduced in size with a subcutaneous hematoma located at the distal most aspect of each of the digits.
Malformations of the vertebral column were restricted to the 500 mg/kg/day group.
Although a variety of skeletal malformations were observed in treated and control groups the degree of aberrant development in control fetuses was

not as severe as in the HVGO-exposed groups.
Visceral malformations were restricted to two fetuses in the 500 mg/kg/day group. One fetus had microphthalmia and the other fetus had a diaphragmatic hernia which displaced the heart from the left to right hand side.

The authors concluded that the maternal NOAEL was 125 mg/kg/day and that the fetal NOAEL was also 125 mg/kg/day

Test substance : The sample of Heavy vacuum gas oil (CAS 64741-57-7) was produced by the vacuum distillation of crude oil.
It was a dark amber liquid with a boiling range of approximately 657 to 1038 °F and density 0.93 g/ml.
The sample (CRU #85244) originated from the Beaumont crude unit B and contained:
54% paraffins
35% polycyclic aromatic hydrocarbons
2% nitrogen-containing polycyclic aromatic hydrocarbons
9% residuals

Reliability : (2) valid with restrictions
The report evaluated was incomplete but nevertheless was sufficient to identify the relevant effects of exposure to the test material.
(80)

Species : Rat
Sex : Female
Strain : Crl:CD(SD)BR VAF/Plus
Route of admin. : Dermal
Exposure period : Days 0-19 gestation
Frequency of treatm. : Daily
Duration of test :
Doses : 0.05, 1, 10, 50 & 250 mg/kg/day
Control group : Yes
NOAEL maternal tox. : = 0.05 mg/kg bw
NOAEL teratogen. : = 0.05 mg/kg bw
Method :
Year : 1995
GLP : Yes
Test substance : Clarified slurry oil

Method : Undiluted test material was applied to the shorn skin of groups of 24 presumed-pregnant rats at doses of 0.05, 1, 10, 50 or 250 mg/kg.
Application was made daily on days 0 through 19 of gestation. The application sites were not covered and to prevent ingestion of the test material, the animals were fitted with collars throughout the study. A group of 24 presumed-pregnant rats were shaved only and served as negative controls.
Daily observations were made for clinical signs and local skin reactions were assessed before each application of test material. Body weights were recorded on days 0, 6, 9, 12, 15, 18 and 20 of gestation and food consumption was recorded daily.
On day 20 of gestation the animals were sacrificed with carbon dioxide and examined for gross lesions. The gravid uterus was weighed and examined for: number and placement of implantation sites, signs of early or late resorptions, live and dead fetuses. The number of corpora lutea were was identified in each ovary. Uteri from non pregnant rats were examined while pressed between two glass slides for confirmation of the status of pregnancy.
All fetuses were individually identified, weighed, sexed and examined for gross external alterations.
Approximately half the fetuses from each litter were examined for soft tissue alterations using Wilson's sectioning technique. The remaining

fetuses were stained with Alizarin red S and examined for skeletal alterations.

Fetal alterations were defined as:
1. Malformations (irreversible changes which occur at low incidences in the species and strain used.
2. Variations (common findings in the species/strain used, and/or reversible delays or accelerations in development.

Statistical analysis
Comparisons were made with the concurrent control group.
Continuous data and litter averages were analyzed for homogeneity and, if homogenous were further analyzed by analysis of variance or covariance. Dunnett's test was used to identify the statistical significance for individual groups. If the data were not homogenous, analyses were made using Kruskal-Wallis test. If this was significant, Dunn's method of multiple comparison was used to identify the statistical significance of individual groups. For count data with greater than 75%ties, Fisher's exact test was used.
Proportion data were analyzed using the variance test for homogeneity of the binomial distribution.

Remark : This study also included groups of animals that were given CSO in a pulsed dosing regime. This was included to ascertain whether there wee any critical gestational phases for developmental effects. The results of this portion of the study demonstrated that the effects on embryo-fetal development were due to early death and not to death of malformed conceptuses.
This aspect of the study has not been summarized here.

Result : There were no signs of skin irritation in the study; no deaths occurred and no dam aborted or prematurely delivered a litter. With the exception of the 0.05 mg/kg/day group there were significant reductions in food consumption. This was accompanied by significant dose-related reductions in maternal body weight in the same groups. Gravid uterine weights and corrected maternal body weight averages (Day 20 body weight - gravid uterine weight) were also significantly reduced in a dose-related manner.
Clinical and necropsy observations are summarized in the following table. Numbers shown are No. affected/No. examined.

	Dose level (mg/kg/day)				
	0.05	**1**	**10**	**50**	**250**
Clinical observations					
Red vaginal exudate	9/24*	5/24		14/24**	19/24**
Emaciation					6/24**
Swollen dark anogenital area					2/24
Slight dehydration					1/24
Necropsy observations					
One placenta					2/24
Two placentas				1/24	
Three placentas					1/24
Uterus contained one placenta					1/24

* P<0.05
** P<0.01

The fetal litter data are summarized in the following table.
The values given are mean values.
The data show that effects occurred in a dose-related manner and that the 0.05 g/kg/day was unaffected by treatment.

	Dose level (mg/kg/day)					
	0	**0.05**	**1**	**10**	**50**	**250**
Dams caesarean sectioned (%)						
	100	96	100	100	95.8	95.8

Live fetuses						
	14.3	15.1	9.3	4.9	0.9*	0*
Total resorptions						
	0.6	0.8	5.0*	9.4*	14*	14.3*
Early resorptions						
	0.6	0.8	4.7*	9.2*	13.9*	14.1*
% dead or resorbed conceptuses/litter						
	4.1	4.6	33.8*	43.6*	67.6*	-
Fetal body weights (g/litter)						
	3.52	3.54	2.94*	3.02*	2.62*	-

* P<0.01

There were no treatment-related incidences of fetal malformations. However, increased incidences of fetal variations that are generally interpreted as reversible delays in development associated with significant decreases in body weight were produced in fetuses from the 1 to 50 mg/kg/day dose groups. These variations included moderate dilation of the renal pelvis, slight dilation of the lateral ventricles of the brain, bifid thoracic vertebral centrum and decreased average numbers of ossified caudal vertebrae, metacarpals and hindpaw phalanges. No fetal alterations (malformations or variations) were observed in the 0.05 mg/kg/day group.

In summary, Clarified slurry oil caused a dose-related increase in maternal toxicity at dose of 1 mg/kg/day or greater. It also caused fetal developmental effects at these maternally toxic doses. At 0.05 mg/kg/day, CSO did not cause either maternal toxicity or developmental effects on the fetus.

Reliability : (1) valid without restriction

(50)

Species : Rat
Sex : Male
Strain : Crl:CD(SD)BR VAF/Plus
Route of admin. : Dermal
Exposure period : 70 days
Frequency of treatm. : Daily
Doses : 0.1, 1, 10, 50 & 250 mg/kg/day
Control group : Yes
other: NOAEL paternal tox : = 1 mg/kg bw
other: Male reproductive : > 250 mg/kg bw
Year : 1992
GLP : Yes
Test substance : Clarified slurry oil

Method : Groups of 10 proven breeders (approximately 11-12 weeks old) were distributed into the following groups:

Group	Dose level (mg/kg/day)
1	0
2	0.1
3	1.0
4	10
5	50
6	250

The male rats were given appropriate percutaneous dosages of the test substance for 70 days before a seven-day cohabitation period with untreated virgin female rats. Two female rats were assigned to cohabitation with each male rat. Day 0 of presumed gestation was identified on the basis of the presence of spermatozoa in a smear of the vaginal contents or a copulatory plug in situ.

The male rats were examined daily for viability, adverse clinical observations and/or effects of the test substance. During the dosage period, the rats were examined once daily for skin reactions, immediately before application of the test substance. During the post-dosage period, skin reactions were evaluated weekly. Body weights and feed consumption values were recorded daily during the dosage period. The male rats were sacrificed by carbon dioxide asphyxiation after completion of the cohabitation period. The testes, epididymides (right and left whole and the left cauda epididymis), seminal vesicles (with and without their fluid contents), prostate gland, pituitary gland and brain were excised and individually weighed. The left testis and epididymis were used for evaluation of the spermatozoa, which included determination of testicular spermatid count and concentration, and cauda epididymal spermatozoa count, concentration and motility, and evaluation of the epididymal fluid for debris and unexpected cell types. The right testis and epididymis (caput, corpus and cauda regions), seminal vesicles, prostate gland, pituitary gland and gross lesions were retained in neutral buffered 10% formalin for possible future histological evaluation.

The female rats were not administered the test substance, but were examined daily for viability and clinical observations, and body weights were recorded on days 0, 6 and 14 of presumed gestation. On day 14 of presumed gestation, the female rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic and abdominal viscera was performed. Gross lesions were preserved in neutral buffered 10% formalin; all other tissues were discarded. The uterus of each rat was examined for pregnancy, number and distribution of implantations, early resorptions and live and dead embryos. Uteri of apparently nonpregnant rats were examined while pressed between two glass plates to determine pregnancy status. The number of corpora lutea in each ovary was recorded. All embryos were discarded.

All proportion data was analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Body weight and feed consumption data, as well as male reproductive organ weights, spermatid count, sperm count, motility and morphology were analyzed using Bartlett's Test of Homogeneity of Variance and the Analysis of Variance. If the Analysis of Variance was significant and appropriate [i.e., Bartlett's Test was not significant ($P>0.05$)], Dunnett's Test was used to identify the statistical significance of individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($P=0.05$)], the Kruskal-Wallis Test was used if less than or equal to 75% ties were present. In cases where statistical significance occurred, Dunn's method of multiple comparison was used to identify statistical significance of individual groups. If there were greater than 75% ties, Fisher's Exact Test was used. Sperm motility data that was expressed as percentages was initially subjected to arcsine transformation and then analyzed, as indicated above, by parametric methods. Data obtained at Caesarean-sectioning was evaluated by the Kruskal-Wallis Test.

Result : No deaths and no skin reactions were caused by the test material.

The 50 and 250 mg/kg/day dosages increased the numbers of pale rats in these dosage groups. No other clinical or necropsy observations were caused by the test substance. One rat in the 250 mg/kg/day dosage group had small, pale seminal vesicles and prostate and a small pituitary.

All organ weights and their body and brain weight ratios were comparable among the six dosage groups. The 10, 50 and 250 mg/kg/day dosages of the test substance reduced the absolute prostate weights and tended to reduce the ratios of prostate weights to brain weights in these dosage groups. These observations were interrelated with the reduced body weights in these dosage groups; the ratios of prostate weights to terminal body weights were unaffected.

Administration of 10, 50 and 250 mg/kg/day dosages caused initial body weight losses that were generally followed by reduced body weight gains and resulted in reduced body weight gains for the entire dosage period. Reflecting these reductions in body weight gains, body weights in the 250 mg/kg/day dosage group tended to be reduced after day 22 of dosage, and body weights in the 10, 50 and 250 mg/kg/day dosage groups tended to be reduced on day 70 of dosing.

Absolute (g/day) feed consumption values tended to be reduced in the 10 mg/kg/day dosage group and were significantly reduced ($P<0.05$ to $P<0.01$) in the 50 and 250 mg/kg/day dosage groups during the first three weeks of dosage. Absolute feed consumption values in the 250 mg/kg/day dosage group were also reduced on days 57 to 70 of dosing. Relative (g/kg/day) feed consumption value tended to be reduced in the 10 mg/kg/day dosage group and were significantly reduced ($P<0.05$ to $P<0.01$) in the 50 and 250 mg/kg/day dosage groups during the first week of dosage. Relative feed consumption values were also reduced during the second week of dosage in the 50 mg/kg/day dosage group and through the third week of dosage in the 250 mg/kg/day.

Mating and fertility parameters were unaffected at any of the dose levels. Mating incidences were comparable among the dosage groups. All male rats sired at least one litter, and seven to nine male rats in each dosage group sired two litters.

The female rats assigned to cohabitation with male rats dosed with test material had no biologically important differences in clinical and necropsy observations or the averages for body weights, body weight changes, or absolute and relative feed consumption values. Litter averages for corpora lutea, implantations, and live embryos and resorptions did not significantly differ among the six dosage groups. There were no dead embryos, and no dam resorbed all conceptuses.

The study directors concluded that the paternal no-observable-adverse-effect-level (NOAEL) was 1 mg/kg/day. The 10, 50 and 250 mg/kg/day doses reduced body weights and feed consumption values; the 50 and 250 mg/kg/day dosages also caused clinical observations.

The reproductive NOAEL for the male rats was higher than 250 mg/kg/day (no mating, fertility or testicular parameters in the male rats were affected by the highest dosage tested).

Test substance : CASRN 64741-62-4
Reliability : (1) valid without restriction

(24)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period :
Frequency of treatm. : Daily
Duration of test : 1 week prior to mating through Day 20 of gestation
Doses : 0.05, 10, 250 mg/kg/day
Control group : Yes
NOAEL maternal tox. : = 0.05 mg/kg bw

other: NOAEL repro/dev. tox. : = 10 mg/kg bw
Method :
Year : 1994
GLP : Yes
Test substance : Carbon black oil (CAS 64741-62-4) (Cracked residue)

Method :

Group Number	Treatment	Dose Level (mg/kg)	Number of Females
1	Sham Control	0.00	20
2	CBO	0.05	15
3	CBO	10.00	15
4	CBO	250.00	15

Female Sprague-Dawley rats (approximately 13-14 weeks old) were administered carbon black oil dermally (clipped) once per day beginning one week prior to the initiation of mating, throughout mating, and through Day 20 of gestation. Elizabethan collars were applied just prior to dosing and were removed no sooner than 6 hours later. At the time of collar removal, any excess test article noted was wiped from the site. .Male rats to which the females were mated were not administered test compound. Each female was cohabited with one male nightly and was examined daily for positive evidence of mating (presence of sperm in a vaginal smear or a copulatory plug). On the day a female showed evidence of mating (considered to be Day 0 of gestation), cohabitation with the male ceased. The mating procedure was continued daily until at least eight females in each group showed evidence of mating.

Each female was observed twice daily for viability and once daily for signs of toxicity. Body weights were recorded for each female at receipt; near the end of the quarantine period; on Days -7 and -1 (premating); on Days 0, 4, 8, 12, 16, and 20 of gestation; and on Days 0 and 4 of lactation. Food consumption was similarly measured beginning on Day -7. On Day 4 of lactation or on Gestation Day 25 for females that did not deliver a litter, each female was sacrificed and subjected to a gross necropsy including an examination of the uterine horns. The ovaries and uterine horns of each female were examined to determine the number of corpora lutea and implantation sites, respectively.

Each litter was observed daily during Days 0 (day of parturition) through 4 of lactation for signs of toxicity and mortality. Pups were examined daily for external abnormalities. On Days 0 and 4 of lactation, each pup was weighed and its sex was determined. Dead pups were removed, examined externally and discarded. On Day 4 of lactation, all surviving pups were examined externally, sacrificed and discarded.

Female body weight and food consumption data were analyzed by an appropriate one way analysis of variance and a test for ordered response in the dose groups. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1 percent level of significance. If the variances were equal, the testing was done using parametric methods, otherwise, nonparametric techniques were used.

For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used. If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.

For the nonparametric procedures, the test of equality of means was performed using the Kruskal-Wallis test. If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment groups differed significantly from control. In addition to the Kruskal-Wallis test, Jonckheere's test for monotonic trend in the dose response was performed.

The test for equal variance (Bartlett) was conducted at the 1% level of

significance. All other tests were conducted at the 5% and 1% level of significance.
For the number of implantation sites, gestation length, total number of pups per litter and number of live pups per litter, normal probability plots of the residuals and plots of residuals by treatment group were used to judge whether or not departure from the assumptions of normality and homogeneous variance were sufficient to invalidate the usual ANOVA analysis. If the usual analysis was invalid, a "weighted" General Linear Model (GLM) analysis was used, where the weights were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.
All proportions (dead pups at Day 0, pup alterations at Day 0, male pups at Days 0 and 4, survival of pups at Day 4) were analyzed by the "weighted" GLM with the litter size as the "weights." Average live pup weight at Days 0 and 4 was analyzed by the "weighted" GLM, with litter size as the "weights" and as a covariate in the model. The assumption was made that these weights were proportional to the reciprocal of the variances.

For all proportions and mean pup weight data, values were first derived within the litter, and group mean values were derived as a mean of the individual litter mean values.

Result : No deaths occurred during the study.

A higher incidence of vaginal discharge was noted during Days 13 through 22 of gestation for females in the 250 mg/kg dose group. There were no other clinical observations that were considered to be related to treatment with the test article.

Body weights of females dosed at 250 mg/kg were significantly lower ($p<0.01$) than those of the controls on Day -1 of the premating period. Body weights of pregnant females in the 250 mg/kg dose group were also significantly lower ($p<0.01$) than those of the control females throughout most of gestation.

Body weight changes for females dosed at 10 or 250 mg/kg were significantly lower ($p<0.01$) than those of controls between Days -7 and -1 of the premating period. Body weight changes for pregnant females in the 250 mg/kg dose group were also lower ($p<0.01$) than those of the control females between Gestation Days 0 to 4, 12 to 16, and 16 to 20.

Absolute and relative food consumption for females in the 10 and 250 mg/kg dose groups were significantly lower ($p<0.01$) than controls during Days -7 to -1 of the premating period. At the 10 mg/kg dose level, absolute and relative food consumption for pregnant females was significantly lower ($p<0.05$) than that of the controls during Gestation Days 0 to 4; relative food consumption was also significantly lower ($p<0.05$) than that of controls during Gestation Days 4 to 8. Absolute food consumption for pregnant females in the 250 mg/kg dose group was significantly lower ($p<0.01$) than that of the control females throughout gestation; relative food consumption was significantly lower ($p<0.05$) than that of controls during Gestation Days 0 to 4, 4 to 8, 8 to 12, and 12 to 16.

Decreased thymus size was noted at necropsy for all females in the 250 mg/kg dose group. There were no other necropsy findings that were considered to be related to the test article.

None of the pregnant females dosed at 250.00 mg/kg delivered a litter (Pregnancy was confirmed through examination of the uterine horns at necropsy).

There were no significant differences between the dose groups that delivered a litter and the control group with respect to gestation length, total and live pups delivered, external pup alterations, pup body weights, proportion of pups dead on Lactation Day 0, proportion of pups surviving to Lactation Day 4, or the proportion of males on Lactation Days 0 and 4. None of the dose groups exhibited a significant difference from the control group for number of implantation sites.

There were no significant differences between the dose groups that delivered a litter and the control group with respect to gestation length, total and live pups delivered, external pup alterations, pup body weights, proportion of pups dead on Lactation Day 0, proportion of pups surviving to Lactation Day 4, or the proportion of males on Lactation Days 0 and 4. None of the dose groups exhibited a significant difference from the control group for number of implantation sites.

The study directors considered the following signs of maternal toxicity to be related to administration of the test material: a higher incidence of vaginal discharge at a dose of 250 mg/kg; decreased body weights, body weight changes, and food consumption at doses of 10 and 250 mg/kg; and decreased thymus size at a dose of 250 mg/kg. Signs of developmental toxicity considered to be compound-related were limited to the 250 mg/kg dose group; none of the females in this dose level delivered a litter.

The study directors concluded the no-observable-adverse-effect levels (NOAEL) were 0.05 mg/kg for maternal toxicity and 10 mg/kg for signs of developmental toxicity.

Reliability : (1) valid without restriction

(125)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Frequency of treatm. : Daily
Duration of test : Days 0-9 of gestation
Doses : 8, 30, 125 and 250 mg/kg/day
Control group : Yes
Year : 1987
GLP : No data
Test substance : Cracked distillates

Method : Presumed-pregnant rats were distributed into the following groups each of 10 animals:

Prenatal groups	**Dose level (mg/kg/day)**	**Days of administration**
Group 1	0 (sham control, remote)	
Group 2	0 (sham control, proximate)	
Group 3	8	0-19
Group 4	30	0-19
Group 5	125	0-19
Group 6	250	0-19
Group 7*	125	10-12
Group 8*	125	10-12

* Groups 7 and 8 were used for a bioavailability study. Results of this portion of the study are not included in this robust summary.

The test material was applied daily to the shorn dorsal skin at the dose levels and days of gestation shown above.
The rats were fitted with collars to prevent oral ingestion of the applied

material.
Observations were made daily for clinical signs.
Body weights were recorded on days 3, 6, 10, 13, 16 and 20 of gestation.
Food consumption was also determined for gestation day intervals 0-3, 3-6, 6-10, 10-13, 13-16 and 16-20.
Each female rat was sacrificed on its 20th day of gestation. The thoracic and abdominal cavities and all organs were examined grossly. The thymus and liver of each animal in groups 1-7 were removed, weighed and preserved in fixative although these organs were not examined microscopically.
The ovaries and uterus of each rat were excised and examined grossly.
The number of corpora lutea per ovary of each pregnant female was counted and recorded. The ovaries of non-pregnant females were examined and then discarded.
The weight of the intact uterus was recorded and the uterine contents were exposed and the number and location of implantations (early or late) and live and dead fetuses was recorded.
At necropsy, blood samples were taken from all animals and the following clinical chemical measurements/calculations were made.

Alanine aminotransferase	Glucose
Albumin	Iron
Albumin/globulin ratio	Lactate dehydrogenase
Alkaline phosphatase	Inorganic phosphorus
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Sorbitol dehydrogenase
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid

Fetal evaluations
Each live fetus was identified as to sex, weighed and examined for external anomalies. Half the fetuses were preserved for examination of soft tissue abnormalities, the remainder being differentially stained for skeletal examination.

Result : Treatment-related clinical observations consisted of erythema, flaking, scabbing, edema, eschar and fissuring and the occurrence of a red vaginal discharge.
Erythema and flaking was observed in all animals in all treatment groups. Scabbing occurred in fewer animals but nevertheless occurred in all treatment groups. Eschar and fissuring occurred in the highest two dose groups only.
Vaginal bleeding was observed in all dose groups exposed to test material at doses of 30 mg/kg/day and higher. The incidences (incidence/group of 10 animals) are shown below

Dose (mg/kg) Group	**0 Prox.**	**0. Rem.**	**8**	**30**	**125**	**250**
Dermal effects						
Erythema	0	0	10	10	10	10
Flaking	0	0	10	10	10	10
Scabs	0	0	3	5	6	10
Edema	0	0	1	4	3	4
Eschar	0	0	0	0	2	7
Fissuring	0	0	0	1	1	1
Non-dermal effects						
Red vaginal discharge						
	0	0	0	3	6	9

There was a dose related decrease in mean body weight gains over the period day 0 to day 20. The authors determined the net body weight change from day 0 to day 20 by subtracting the gravid uterus weight from the body weight at day 20 and subtracting the day 0 body weight from this value. Thus, the net body weight change for each group was calculated as follows:

Dose group	Net body weight gain
Proximate control	77
Remote control	89.3
8 mg/kg	81.4
30 mg/kg	74.6
125 mg/kg	63.8*
250 mg/kg	33.2*

* significantly different from control.

Food consumption was slightly reduced in the groups exposed to test material at doses of 125 and 250 mg/kg/day.

At necropsy, the only treatment-related observation was an apparent reduction in thymus size which was noted at all treatment levels. Organ weight measurements, confirmed that thymus weights were reduced and in addition, liver weights were also increased. These changes, expressed as percentages of the value for the remote controls are summarized below.

Group	Absolute Thymus weight	Absolute Liver weight	Relative Liver weight
8 mg/kg	-1.5%	+3%	-2%
30 mg/kg	+8%	+3%	-4%
125 mg/kg	-26%*	+5%	-9%
250 mg/kg	-47%*	-8%	-5%

Clinical chemical values were affected only at the highest dose of 250 mg/kg as follows:
Triglycerides decreased by 52%
Albumin increased by 36%
A/G ratio increased by 33%
Inorganic phosphorus increased by 43%
Iron 2.5 times higher than control.

The only reproductive parameters adversely affected were:
Number of dams with all resorptions: 50% at 250 mg/kg/day
Number of resorptions: increased ≥125 mg/kg/day
Litter size decreased ≥125 mg/kg/day
Fetal body weights decreased ≥125 mg/kg/day
Crown rump length reduced ≥125 mg/kg/day

Abnormal external development was observed in viable and non-viable fetuses exposed to test material at 125 and 250 mg/kg/day. The anomalies observed included reduced (shortened) lower jaw and edema. Visceral anomalies included displacement of esophagus from a left-sided to a right-sided position and distension of the ureturs. Malformations of the vertebral column were restricted to fetuses of dams exposed to the test material. Although there was a variety of skeletal malformations in the study, the degree of aberrant development observed was not as severe in the control groups as the groups exposed to test material.

The authors concluded that the NOAEL for maternal and fetal toxicity was 30 mg/kg/day.

Reliability : (1) valid without restriction

(66)

Test substance : Reformer residues

Remark : No data

Test substance : Heavy fuels

Remark : No data

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