ROBUST SUMMARY OF INFORMATION ON

Substance Group **Heavy Fuel Oil Category**

Summary prepared by American Petroleum Institute Petroleum HPV Testing Group
Consortium Registration #

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

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 : Residual fuel oils 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 approximately in the range from 83 to greater than 737 °C.

They typically consist of aromatic, aliphatic and naphthenic hydrocarbons, generally having carbon numbers in the range of C7 to greater than C50, together with asphaltenes and smaller amounts of heterocyclic compounds containing sulfur, nitrogen and oxygen.

As an example, these four samples of residual 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%

Because of their complexity and variability, detailed analytical data on heavy fuel oil streams are scarce. Most are characterized only by the parameters used to specify various fuel grades by ASTM or ISO Standards.

Data available for some of the samples for which toxicological information is available are shown below.

Parameter	64741-45-3	64741-62-4	64741-81-7
Sample No.	F-132	API 91-15	API 97-01
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 the boiling range cited in CAS number definitions. The following information is provided as additional data. They represent distillation values (°C) for 54 samples of heavy fuel oil streams and residual fuels analyzed by ASTM D7169 on behalf of the American Petroleum Institute. Distillation ranges may be higher or lower depending on factors such as the source of the crude oil and in the refining process used.

	BP Start	T10	T50	T90	BP END
CAS# 64741-45-3	262	382	521	707	end
CAS# 64741-45-3	257	375	540	713	end
CAS# 64741-45-3	186	359	544	720	end
CAS# 64741-45-3	203	336	495	649	end
CAS# 64741-57-7	296	385	468	543	607
CAS# 64741-57-7	325	399	482	596	end
CAS# 64741-57-7	296	372	443	519	601
CAS# 64741-61-3	182	297	365	422	506
CAS# 64741-61-3	167	297	381	451	617
CAS# 64741-61-3	204	315	377	424	524
CAS# 64741-61-3	160	325	404	495	647
CAS# 64741-62-4	172	327	401	505	737
CAS# 64741-62-4	196	341	417	500	652
CAS# 64741-62-4	145	329	409	513	692
CAS# 64741-67-9	180	204	229	287	404
CAS# 64741-75-9	404	468	518	561	602
CAS# 64741-80-6	217	329	390	448	542
CAS# 64741-81-7	185	274	354	398	446
CAS# 64742-59-2	168	319	432	526	603
CAS# 64742-59-2	208	319	421	500	565
CAS# 64742-78-5	214	299	520	662	716
CAS# 68187-58-6	340	406	563	716	end
CAS# 68333-22-2	292	377	495	672	710
CAS# 68410-00-4	148	283	389	452	536
CAS# 68410-00-4	100	272	381	438	552
CAS# 68410-00-4	172	238	278	330	384
CAS# 68476-33-5	83	229	601	end	717
CAS# 68478-17-1	227	350	429	536	644
CAS# 68478-17-1	257	358	428	514	673
CAS# 68512-62-9	387	531	637	end	720
CAS# 68512-62-9	130	214	590	end	718
CAS# 68553-00-4	182	341	405	482	656
CAS# 68553-00-4	191	290	393	488	650
CAS# 68553-00-4	158	294	433	697	712
CAS# 68607-30-7	347	525	622	end	720
CAS# 68783-08-4	241	315	400	480	591
CAS# 68783-08-4	209	286	387	493	579
CAS# 68783-08-4	256	339	397	492	671
CAS# 68783-08-4	159	230	294	357	571
CAS# 68955-27-1	321	414	481	534	654
CAS# 68955-27-1	401	426	453	484	631
CAS# 70592-76-6	300	366	426	495	603
CAS# 70592-76-6	219	268	316	361	389
CAS# 70592-76-6	212	294	389	483	604
CAS# 70592-76-6	205	316	418	498	594
CAS# 70592-77-7	208	277	348	405	627
CAS# 70592-77-7	247	344	429	528	613
CAS# 70592-77-7	216	300	385	493	623

CAS# 70592-77-7	209	290	371	456	602
CAS# 70592-78-8	296	393	458	544	635
CAS# 70592-78-8	307	403	474	541	619
CAS# 70592-78-8	327	396	477	565	657
CAS# 70913-85-8	394	504	602	707	end
CAS# 70913-85-8	436	517	640	709	end
Min	83	204	229	287	384
Max	436	531	640	720	737
Mean:	237	342	443	511	611
Std Dev:	81	75	90	129	86

Result : For the following petroleum streams in the Heavy Fuels HPV category, boiling ranges were obtained from the CAS 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 measured data supplied by the American Petroleum Institute. 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		
n-heptane	7	6130*
n-undecane	11	55*
n-eicosane	20	$6x10^{-4}$*
n-pentacontane	50	$2x10^{-7}$
iso-alkanes		
iso-heptane	7	8800*
iso-undecane	11	80*
iso-eicosane	20	0.09

iso-pentacontane	50	$3x10^{-13}$
cyclo-alkanes		
1-ring		
methylcyclohexane	7	6130*
pentylcyclohexane	11	50*
tetradecylcyclohexne	20	0.02
tetratetracontylcyclohexane	50	$2x10^{-13}$
2-ring		
2-methyl[4.4.0]bicyclodecane	11	90
2-decyl[4.4.0]bicyclodecane	20	0.03*
2-tetracontyl[4.4.0]bicyclo-decane	50	$2x10^{-13}$
3-ring		
bicyclodecane,1-8-dimethyl	12	33*
3-hexyltricyclotetradecane	20	0.02
3-hexatriacontyltricyclo-tetradecane	50	$2x10^{-13}$
olefins		
1-heptene	7	7900*
1-undecene	11	97*
1-eicosene	20	0.001*
1-pentacontene	50	$3x10^{-13}$
aromatics		
1-ring		
toluene	7	3790*
n-penylbenzene	11	59*
n-tetradecylbenzene	20	0.003
n-pentacontylbenzene	50	$2x10^{-14}$
2-ring		
1-methylnaphthalene	11	7.3*
1-tetradecylnaphthalene	20	$7x10^{-4}$
1-pentacontylnaphthalene	50	$3x10^{-15}$
3-ring		
phenanthrene	14	0.016*
2-hexylphenanthrene	20	$1x10^{-4}$
2-hexatriacontylphenanthrene	50	$5x10^{-16}$
polar/heterocyclic compounds		
quinolines		
quinoline	9	8.0*
4-pentylquinoline	14	0.02
3-butyl-4-propyl-5-butyl-quinoline	20	$1x10^{-4}$
4-hentetracontyl-quinoline	50	$9x10^{-16}$
pyridines		
2-ethyl-pyridine	7	650*
2-nonyl-pyridine	14	0.21
2-pentadecyl-pyridine	20	$8x10^{-4}$
2-pentatetracontyl-pyridine	50	$2x10^{-16}$
carboxylic acids		
cyclopentane-3-methyl-1-carboxylic acid	7	7.6
[4.3.0]bicyclononane-5-		

methyl-1-carboxylic acid	11	0.08
[4.2.4]tricyclotetradecane-11-methyl-1-pentanoic acid	20	$4x10^{-5}$
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	50	$3x10^{-16}$
thiophenes/benzothiophenes		
2-propyl thiophene	7	370
dibenzothiophene	12	0.03*
dibenzothiophene 4,6-dibutyl	20	$1x10^{-5}$
dibenzothiophene 4,6-didecanyl	50	$5x10^{-17}$

Note: values signified with * were cited in the EPI-Suite™ experimental database.

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		
n-heptane	7	4.7*
n-undecane	11	5.7
n-eicosane	20	10.2
n-pentacontane	50	25
iso-alkanes		
iso-heptane	7	3.7
iso-undecane	11	5.7
iso-eicosane	20	10.1

n-pentacontane	50	25
cyclo-alkanes		
methylcyclohexane	7	3.6*
pentylcyclohexane	11	5.6
tetradecylcyclohexane	20	10
tetratetracontylcyclohexane	50	25
2-methyl[4.4.0]bicyclodecane	11	4.6
2-decyl[4.4.0]bicyclodecane	20	9
2-tetracontyl[4.4.0]bicyclodecane	50	24
bicyclodecane,1,8-dimethyl	12	4.9
3-hexyltricyclotetradecane	20	8.1
3-hexatriacontyltricyclotetradecane	50	23
olefins		
1-heptene	7	4.0*
1-undecene	11	5.6
1-eicosene	20	10
1-pentacontene	50	25
aromatics		
1-ring		
toluene	7	2.7*
n-pentylbenzene	11	4.9*
n-tetradecylbenzene	20	8.9
n-pentacontylbenzene	50	24
2-ring		
1-methylnaphthalene	11	3.9*
1-tetradecylnaphthalene	20	8.1
1-pentacontylnaphthalene	50	23
3-ring		
phenanthrene	14	4.5*
2-hexylphenanthrene	20	7.4
2-hexatriacontylphenanthrene	50	22
polar/heterocyclic compounds		
quinolines		
quinoline	9	2.0*
4-pentylquinoline	14	4.7
3-butyl-4-propyl-5-butyl quinoline	20	7.7
4-hentetracontyl quinoline	50	22
pyridines		
2-ethyl pyridine	7	1.7*
2-nonly pyridine	14	5.3
2-pentadecyl pyridine	20	8.2
2-pentatetracontyl pyridine	50	25
carboxylic acids		
cyclopentane-3-methyl-1-carboxylic acid	7	2.0*
[4.3.0]bicyclononane-5-methyl-1-carboxylic acid	11	3.4
[4.2.4]tricyclotetradecane-11-methyl-1-pentanoic acid	20	6.8
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	50	22
thiophenes/benzothiophenes		
2αbutyl thiophene	7	3.3

dibenzothiophene	12	4.4
dibenzothiophene 4,6-dibutyl	20	8.2
dibenzothiophene 4,6-didecanyl	50	23

Note: values signified with * were cited in the EPI-Suite™ experimental database.

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:	Hydroxyl 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		
n-heptane	7	1.6
n-undecane	11	0.9
n-eicosane	20	0.4
n-pentacontane	50	0.2
iso-alkanes		
iso-heptane	7	1.6

iso-undecane	11	0.9
iso-eicosane	20	0.4
n-pentacontane	50	0.2
cyclo-alkanes		
1-ring		
methylcyclohexane	7	1.1
pentylcyclohexane	11	0.7
tetradecylcyclohexane	20	0.4
tetratetracontylcyclohexane	50	0.2
2-ring		
2-methyl[4.4.0]bicyclodecane	11	0.5
2-decyl[4.4.0]bicyclodecane	20	0.3
2-tetraconty[4.4.0]bicyclodecane	50	0.1
3-ring		
bicyclodecane,1,8-dimethyl	12	0.6
3-hexyltricyclotetradecane	20	0.3
3-hexatriacontyltricyclotetradecane	50	0.1
olefins		
1-heptene	7	0.3
1-undecene	11	0.3
1-eicosene	20	0.2
1-pentacontene	50	0.1
aromatics		
1-ring		
toluene	7	2.0
n-pentylbenzene	11	1.1
n-tetradecylbenzene	20	0.5
n-pentacontylbenzene	50	0.2
2-ring		
1-methylnaphthalene	11	0.2
1-tetradecylnaphthalene	20	0.2
1-pentacontylnaphthalene	50	0.1
3-ring		
phenanthrene	14	0.3
2-hexylphenanthrene	20	0.3
2-hexatriacontylphenanthrene	50	<0.1
polar/heterocyclics		
quinolines		
quinoline	9	20.9
4-pentylquinoline	14	0.4
3-butyl-4-propyl-5-butyl quinoline	20	0.3
4-hentetracontyl quinoline	50	<0.1
pyridines		
2-ethyl pyridine	7	5.2
2-nonly pyridine	14	0.9
2-pentadecyl pyridine	20	0.5
2-pentatetracontyl pyridine	50	0.2
carboxylic acids		
cyclopentane-3-methyl-1-carboxylic acid	7	1.1
[4.3.0]bicyclononane-5-methyl-1-carboxylic acid	11	0.5
[4.2.4]tricyclotetradecane-11-methyl-1-pentanoic acid		

	20	0.2
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid		
	50	0.3
thiophenes/benzothiophenes		
2-propyl-thiophene	7	0.4
dibenzothiophene	12	0.4
dibenzothiophene 4,6-dibutyl	20	0.1
dibenzothiophene 4,6-didecanyl	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

Category Chemical :	Heavy fuel oil, various CAS RN
Test Substance :	Heavy fuel, various CAS RN
Test Substance Purity/Composition and Other Test Substance Comments:	
Category Chemical Result Type :	Estimated by calculation
Test Substance Result Type :	Estimated
RESULTS	
Fugacity/Distribution Result Description:	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.

Test Results :

Compound/Compartment	C-Num	Percent Distribution					
		Air	Water	Soil	Sed	Susp. Sed.	Biota
Alkanes							
n-heptane	7	100	<0.1	<0.1	<0.1	<0.1	<0.1
n-undecane	11	93	<0.1	7	<0.1	<0.1	<0.1
n- eicosane	20	<0.1	<0.1	98	2	<0.1	<0.1
n-pentacontane	50	<0.1	<0.1	98	2	<0.1	<0.1
iso-heptane	7	100	<0.1	<0.1	<0.1	<0.1	<0.1
iso-undecane	11	95	<0.1	5	<0.1	<0.1	<0.1
iso- eicosane	20	<0.1	<0.1	98	2	<0.1	<0.1
iso-pentacontane	50	<0.1	<0.1	98	2	<0.1	<0.1
Naphthenes							
methylcyclohexane	7	100	<0.1	<0.1	<0.1	<0.1	<0.1
pentylcyclohexane	11	99	<0.1	0.9	<0.1	<0.1	<0.1
tetradecylcyclohexane	20	<0.1	<0.1	98	2	<0.1	<0.1
tetratetracontylcyclohexane	50	<0.1	<0.1	98	2	<0.1	<0.1
2-methyl[4.4.0]bicylcodecane	11	97	0.1	3	0.1	<0.1	<0.1
2-decyl[4.4.0]bicyclodecane	20	2	<0.1	96	2	<0.1	<0.1
2-tetracontryl[4.4.0]bicyclodecane	50	<0.1	<0.1	98	2	<0.1	<0.1
1,8-dimethyl[4.4.2]tricyclodecane	12	94	0.4	5	0.1	<0.1	<0.1
3-hexyltricyclotetradecane	20	2	<0.1	96	2	<0.1	<0.1
3-hexatriacontyltricyclotetradecane	50	<0.1	<0.1	98	2	<0.1	<0.1
Olefins							
1-heptene	7	100	<0.1	0.1	<0.1	<0.1	<0.1
1-undecene	11	96	<0.1	4	<0.1	<0.1	<0.1
1- eicosene	20	<0.1	<0.1	98	2	<0.1	<0.1
1-pentacontene	50	<0.1	<0.1	98	2	<0.1	<0.1
Aromatics							
toluene	7	99	0.8	0.4	<0.1	<0.1	<0.1
n-pentylbenzene	11	88	0.4	11	0.2	<0.1	<0.1
n-tetradecylbenzene	20	<0.1	<0.1	98	2	<0.1	<0.1
n-pentacontylbenzene	50	<0.1	<0.1	98	2	<0.1	<0.1
1-methylnaphthalene	11	51	6	42	0.9	<0.1	<0.1
1-tetradecylnaphthalene	20	<0.1	<0.1	98	2	<0.1	<0.1
1-pentacontylnaphthalene	50	<0.1	<0.1	98	2	<0.1	<0.1
phenanthrene	14	1	4	93	2	<0.1	<0.1
2-hexylphenanthrene	20	<0.1	<0.1	98	2	<0.1	<0.1
2-hexatriacontylphenanthrene	50	<0.1	<0.1	98	2	<0.1	<0.1
Heterocyclics							
quinoline	9	3	89	8	0.2	<0.1	<0.1
4-pentaquinoline	14	0.5	2	95	2	<0.1	<0.1
3-butyl-4-propyl-5-butyl quinoline	20	<0.1	<0.1	98	2	<0.1	<0.1
4-hentetracontyl quinoline	50	<0.1	<0.1	98	2	<0.1	<0.1
2-ethyl pyridine	7	4	92	4	<0.1	<0.1	<0.1
2-nonyl pyridine	14	0.2	0.5	97	2	<0.1	<0.1
2-pentadecyl pyridine	20	<0.1	<0.1	98	2	<0.1	<0.1
2-pentatetracontyl pyridine	50	<0.1	<0.1	98	2	<0.1	<0.1
Cyclopentane-3-methyl-1-carboxylic acid	7	4	88	8	0.2	<0.1	<0.1
[4.3.0]bicyclononane-5-methyl-1-carboxylinc acid	11	0.5	30	68	1.5	<0.1	<0.1
[4.2.4]tricyclotetradecane-11-methyl-1-penanoic acid	20	<0.1	<0.1	94	2	<0.1	<0.1
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	50	<0.1	<0.1	98	2	<0.1	<0.1
2-propyl thiophene	7	96	1	2	<0.1	<0.1	<0.1
dibenzothiophene	12	3	4	91	2	<0.1	<0.1
dibenzothiophene 4,6-dibutyl	20	<0.1	<0.1	98	2	<0.1	<0.1
dibenzothiophene 4,6-didecanyl	50	<0.1	<0.1	98	2	<0.1	<0.1

Temperature :	20°C
Level of Multi-media Model :	1

Model Input (Water Solubility:)

	Water Solubility mg/L	Reference
Alkanes		
n-heptane	3.4*	1
n-undecane	0.0044*	1
n- eicosane	9×10^{-6}	1
n-pentacontane	5×10^{-21}	1
iso-heptane	2.54*	1
iso-undecane	0.30	1
iso- eicosane	1×10^{-5}	1
iso-pentacontane	5×10^{-21}	1
Naphthenes		
methylcyclohexane	14*	1
pentylcyclohexane	0.4	1
tetradecylcyclohexane	1×10^{-5}	1
tetratetracontylcyclohexane	8×10^{-21}	1
2-methyl[4.4.0]bicylcodecane	2.5	1
2-decyl[4.4.0]bicyclodecane	1×10^{-4}	1
2-tetracontryl[4.4.0]bicyclodecane	5×10^{-20}	1
1,8-dimethyl[4.4.2]tricyclodecane	1.2	1
3-hexyltricyclotetradecane	6×10^{-4}	1
3-hexatriacontyltricyclotetradecane	3×10^{-19}	1
Olefins		
1-heptene	18.2*	1
1-undecene	0.34	1
1- eicosene	1×10^{-5}	1
1-pentacontene	7×10^{-21}	1
Aromatics		
toluene	526*	1
n-pentylbenzene	3.4*	1
n-tetradecylbenzene	4×10^{-4}	1
n-pentacontylbenzene	2×10^{-19}	1
1-methylnaphthalene	25*	1
1-tetradecylnaphthalene	0.002	1
1-pentacontylnaphthalene	1×10^{-18}	1
phenanthrene	1.2	1
2-hexylphenanthrene	8×10^{-4}	1
2-hexatriacontylphenanthrene	5×10^{-19}	1
Heterocyclics		
quinoline	6100*	1
4-pentaquinoline	3.7	1
3-butyl-4-propyl-5-butyl quinoline	0.004	1
4-hentetracontyl quinoline	2×10^{-18}	1
2-ethyl pyridine	3×10^{5}*	1
2-nonyl pyridine	25	1
2-pentadecyl pyridine	0.3	1
2-pentatetracontyl pyridine	8×10^{-20}	1
Cyclopentane-3-methyl-1-carboxylic acid	4900	1
[4.3.0]bicyclononane-5-methyl-1-carboxylinc acid	170	1
[4.2.4]tricyclotetradecane-11-methyl-1-penanoic acid	0.045	1
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	2×10^{-17}	1
2-propyl thiophene	130	1
dibenzothiophene	1.5*	1

dibenzothiophene 4,6-dibutyl	1 X 10^{-4}	1
dibenzothiophene 4,6-didecanyl	6 X 10^{-20}	1

(1) US EPA, 2000 (WSKOWWIN, EPI-Suite V3.10)
Note: Model input data signified by "*" indicates the value was cited by EPI-Suite™ as being from the experimental database (EPA 2000).

Model Input (Vapor Pressure:)

	Vapor Pressure Pa	Reference
Alkanes		
n-heptane	6130*	1
n-undecane	55*	1
n- eicosane	6 x 10^{-4}*	1
n-pentacontane	2 x 10^{-7}	1
iso-heptane	8800*	1
iso-undecane	80*	1
iso- eicosane	0.09	1
iso-pentacontane	3 x 10^{-13}	1
Naphthenes		
methylcyclohexane	6130*	1
pentylcyclohexane	50*	1
tetradecylcyclohexane	0.02	1
tetratetracontylcyclohexane	2 x 10^{-13}	1
2-methyl[4.4.0]bicylcodecane	90	1
2-decyl[4.4.0]bicyclodecane	0.03	1
2-tetracontryl[4.4.0]bicyclodecane	2 x 10^{-13}	1
1,8-dimethyl[4.4.2]tricyclodecane	33	1
3-hexyltricyclotetradecane	0.02	1
3-hexatriacontyltricyclotetradecane	2 x 10^{-13}	1
Olefins		
1-heptene	7900*	1
1-undecene	97*	1
1- eicosene	0.001	1
1-pentacontene	3 x 10^{-13}	1
Aromatics		
toluene	3790*	1
n-pentylbenzene	59*	1
n-tetradecylbenzene	0.003*	1
n-pentacontylbenzene	2 x 10^{-14}	1
1-methylnaphthalene	7.3*	1
1-tetradecylnaphthalene	7 x 10^{-4}	1
1-pentacontylnaphthalene	3 x 10^{-15}	1
phenanthrene	0.016*	1
2-hexylphenanthrene	1 x 10^{-4}	1
2-hexatriacontylphenanthrene	5 x 10^{-16}	1
Heterocyclics		
quinoline	8.0*	1
4-pentaquinoline	0.02	1
3-butyl-4-propyl-5-butyl quinoline	1 x 10^{-4}	1
4-hentetracontyl quinoline	9 x 10^{-16}	1
2-ethyl pyridine	650*	1
2-nonyl pyridine	0.21	1
2-pentadecyl pyridine	8 x 10^{-4}	1
2-pentatetracontyl pyridine	2 x 10^{-16}	1
cyclopentane-3-methyl-1-carboxylic acid	7.6	1
[4.3.0]bicyclononane-5-methyl-1-		

carboxylinc acid	0.08	1
[4.2.4]tricyclotetradecane-11-methyl-1-penanoic acid	4×10^{-5}	1
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	3×10^{-16}	1
2-propyl thiophene	370	1
dibenzothiophene	0.03*	1
dibenzothiophene 4,6-dibutyl	1×10^{-5}	1
dibenzothiophene 4,6-didecanyl	5×10^{-17}	1

(1) US EPA, 2000 (MPBPWIN, EPI Suite V3.10)
Note: Model input data signified by "*" indicates the value was cited by EPI-Suite™ as being from the experimental database (EPA 2000).

Model Input (log K_{ow}:)

	Log Kow	Reference
Alkanes		
n-heptane	4.7*	1
n-undecane	5.7	1
n- eicosane	10.2	1
n-pentacontane	25	1
iso-heptane	3.7	1
iso-undecane	5.7	1
iso- eicosane	10.1	1
iso-pentacontane	25	
Naphthenes		
methylcyclohexane	3.6*	1
pentylcyclohexane	5.6	1
tetradecylcyclohexane	10	1
tetratetracontylcyclohexane	25	1
2-methyl[4.4.0]bicylcodecane	4.6	1
2-decyl[4.4.0]bicyclodecane	9.0	1
2-tetracontryl[4.4.0]bicyclodecane	24	1
1,8-dimethyl[4.4.2]tricyclodecane	4.9	1
3-hexyltricyclotetradecane	8.1	1
3-hexatriacontyltricyclotetradecane	23	1
Olefins		
1-heptene	4.0*	1
1-undecene	5.6	1
1- eicosene	10	1
1-pentacontene	25	1
Aromatics		
toluene	2.7*	1
n-pentylbenzene	4.9*	1
n-tetradecylbenzene	8.9	1
n-pentacontylbenzene	24	1
1-methylnaphthalene	3.9*	1
1-tetradecylnaphthalene	8.1	1
1-pentacontylnaphthalene	23	1
phenanthrene	4.5*	1
2-hexylphenanthrene	7.4	1
2-hexatriacontylphenanthrene	22	1
Heterocyclics		
quinoline	2.0*	1
4-pentaquinoline	4.7	1
3-butyl-4-propyl-5-butyl quinoline	7.7	1
4-hentetracontyl quinoline	22	1
2-ethyl pyridine	1.7*	1
2-nonyl pyridine	5.3	1
2-pentadecyl pyridine	8.2	1
2-pentatetracontyl pyridine	25	1
cyclopentane-3-methyl-1-carboxylic		

acid	2.0*	1
[4.3.0]bicyclononane-5-methyl-1-carboxylinc acid	3.4	1
[4.2.4]tricyclotetradecane-11-methyl-1-penanoic acid	6.8	1
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	22	1
2-propyl thiophene	3.3	1
dibenzothiophene	4.4	1
dibenzothiophene 4,6-dibutyl	8.2	1
dibenzothiophene 4,6-didecanyl	23	1

(1) US EPA, 2000 (KOWWIN, EPI Suite V3.10)
Note: Model input data signified by "*" indicates the value was cited by EPI-Suite™ as being from the experimental database (EPA 2000).

Model Input (Melting Point:)

	Melting Pt °C	Reference
Alkanes		
n-heptane	-90.6*	1
n-undecane	-25.6*	1
n- eicosane	36.8*	1
n-pentacontane	87*	1
iso-heptane	-118.2*	1
iso-undecane	-48.9*	1
iso- eicosane	39.5	1
iso-pentacontane	298	1
Naphthenes		
methylcyclohexane	-126.6*	1
pentylcyclohexane	-58*	1
tetradecylcyclohexane	24*	1
tetratetracontylcyclohexane	300	1
2-methyl[4.4.0]bicylcodecane	-21	1
2-decyl[4.4.0]bicyclodecane	68.8	1
2-tetracontryl[4.4.0]bicyclodecane	300	1
1,8-dimethyl[4.4.2]tricyclodecane	1.47	1
3-hexyltricyclotetradecane	77	1
3-hexatriacontyltricyclotetradecane	300	1
Olefins		
1-heptene	-119.7*	1
1-undecene	-49*	1
1- eicosene	28.5	1
1-pentacontene	297	1
Aromatics		
toluene	-94.9*	1
n-pentylbenzene	-75*	1
n-tetradecylbenzene	16*	1
n-pentacontylbenzene	305	1
1-methylnaphthalene	34.4*	1
1-tetradecylnaphthalene	109	1
1-pentacontylnaphthalene	316	1
phenanthrene	99.2*	1
2-hexylphenanthrene	132	1
2-hexatriacontylphenanthrene	328	1
Heterocyclics		
quinoline	-15*	1
4-pentaquinoline	96	1
3-butyl-4-propyl-5-butyl quinoline	140	1

4-hentetracontyl quinoline	320	1
2-ethyl pyridine	-63*	1
2-nonyl pyridine	64	1
2-pentadecyl pyridine	120	1
2-pentatetracontyl pyridine	330	1
cyclopentane-3-methyl-1-carboxylic acid	32*	1
[4.3.0]bicyclononane-5-methyl-1-carboxylinc acid	81	1
[4.2.4]tricyclotetradecane-11-methyl-1-penanoic acid	150	1
[4.2.4]tricyclodecane-7-eicosyl-1-decacarboxylic acid	330	1
2-propyl thiophene	-3.1	1
dibenzothiophene	97*	1
dibenzothiophene 4,6-dibutyl	160	1
dibenzothiophene 4,6-didecanyl	340	1

(1) US EPA, 2000 (MPBPWIN, EPI Suite V3.10)
Note: Model input data signified by "*" indicates the value was cited by EPI-Suite™ as being from the experimental database (EPA 2000).

Henry's Law Constant :	Calculated by EQC for each constituent
Model Concentration -- Air :	
Model Concentration -- Water :	
Model Concentration -- Soil :	
Model Concentration -- Sediment :	
Results Remarks :	

STUDY/METHOD

Key Study Sponsor Indicator :	Key
Year Study Performed :	
Method/Guideline Followed :	EQC-Equilibrium Criterion Model, Fugacity-Based Level 1
Deviations from Method/Guideline :	
Method/Guideline Description :	The EQC model calculates the distribution of a fixed quantity of conserved (i.e., non-reacting) chemical, in a closed environment at equilibrium, with no degrading reactions, no advective processes, and no intermedia transport processes (e.g., no wet deposition or sedimentation). The medium receiving the emission is unimportant because the chemical is assumed to become instantaneously distributed.
Method/Guideline and Test Condition Remarks :	
GLP :	No
Study Reference :	Trent University. 2003. EQC fugacity-based EQC-equilibrium criterion model, Version 2.02. Canadian Environmental modeling Centre, Trent University, Ontario. URL: http://www.trentu.ca/cemc/ U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V3.10. U.S.

	Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. Potter, T.L. and K.E. Simmons. 1998. Total petroleum hydrocarbon criteria working group series, Volume 2. Composition of petroleum mixtures. Amherst Scientific Publishers, Amherst, Massachusetts. 114 pp. Quann, R.J. and S.B. Jaffe. 1992. Structure-oriented lumping: Describing the chemistry of comples hydrocarbon mixtures. Ind. Eng. Chem. Res. 31(11):2483-2497. Saeger, R.B. and S.B. Jaffe. 2002. Petroleum stream compositional modeling for the petroleum HPV testing group program. ExxonMobil Process Research Laboratories, Paulsboro, NJ.
RELIABILITY/DATA QUALITY	
Reliability :	(2) Reliable with restrictions
Reliability Remarks :	Environmental distribution was estimated using an accepted validated model.

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 1.9×10^5 to 2.7×10^5 at 10 mg/l loading rate, 6.8×10^5 to 27×10^5 at 100 mg/l, and 31×10^5 to 53×10^5 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. (x10^6cells/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 reported as water soluble fraction (mg/L). Data cited in an Environment Canada database (Jokuty et al., 2002;).

Species	Endpoint	Value, mg/l
Neanthes arenaceodentata	96H LC_{50}	4
Capitaella capitata	96H LC_{50}	1
Mysidopsis almyra	48H LC_{50}	1
Palaemonetes pugio	96H LC_{50}	3
Penaeus aztecus	96H LC_{50}	2
Menidia beryllina	96H LC_{50}	2
Fundulus similis	96H LC_{50}	2
Cyprinodon variegates	96H LC_{50}	3

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 Heavy Fuel Oils

Remark :

Heavy Fuel Oil Sample No.	EL50 Alga (*Pseudokirchneriella subcapitata*)	EL50 Invertebrate (*Daphnia magna*)	LL50 Fish (*Oncorhynchus mykiss*)
3	3.3	2	>96
4	3	3.2	>94
5	8	10	79
7	approx. 1	>99	>95
9	>107	>99	>98

Five different heavy fuel oil samples were tested against an alga, invertebrate, and fish using water accommodated fractions (WAF) of each sample. Samples 3, 4, and 5 were tested as full definitive tests, while Samples 7 and 9 were tested as a limit test at a nominal WAF at a loading

rate of 100 mg/L. Data illustrate that fish are less sensitive to HFO WAFs than the algae or invertebrate, while algae appear more sensitive than invertebrates.

These data support the general conclusion for the HFO category that a read-across range of acute toxicity values for these substances is 1 – 100 mg/L when based on loading rates used to prepare WAF exposure solutions.

Reliability : (4) not assignable
Endpoint values cited in company poster presentation; methods referenced appropriate OECD guidelines, but no details were given on methods of WAF preparation or environmental conditions during the tests.
Febbo, et al. [no date]. A multi-tiered aquatic toxicity assessment of heavy fuel oils. ExxonMobil Biomedical Sciences, Annandale, NJ.

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)

Memo : Acute aquatic toxicity of heteroatom-containing constituents in heavy fuel oils and streams.

Remark : Heteroatom compounds exist in crude oil and thus become components of heavy fuel oil streams and products. Sulfur is the most abundant atom in crude oil after carbon and hydrogen, and congeners of thiophene, benzothiophene, dibenzothiophene, and benzonaphthothiophenes have been measured in both crude and residual fuels . Nitrogen content is much lower than sulfur compounds and oxygen compounds lesser yet. All three heteroatoms can exist together in high molecular weight asphaltene and resin molecules. Due to their extremely high molecular weights, asphaltenes and resins are not expected to be bioavailable nor show aquatic toxicity.

It is not known whether specific heteroatom constituents in heavy fuel oils become bioavailable upon entering the aquatic environment, but the test data on heavy fuel oils cited in the robust summaries indicate that in general, heavy fuel oils are not very toxic to aquatic organisms (typical LL/EL50 values for fish and invertebrates are >100 mg/L loading; algal EL50 may fall between 3 and 10 mg/L). To shed some light on the potential toxicity to aquatic biota of heteroatom constituents in heavy fuel oils, the following data were assembled for selected individual heteroatom compounds. Examples of S- and N-heteroatom compounds were chosen at the lower end of the molecular weight spectrum of heavy fuel oils because those would be expected to show the greatest water solubility, and hence bioavailability. However, it should be understood that as individual constituents in heavy fuel oils composed of thousands of compounds, they may not contribute to toxicity in any aqueous preparation.

The table below indicates that the predicted toxicity values of two C12 compounds were flagged by ECOSAR as potentially not being sufficiently soluble to measure an effect. Hydrocarbons within the typical C20-C50 range would be expected to have lower water solubility than those cited in the table (example: solubility of eicosane is $9x10^{-6}$ mg/L; benzo(a)pyrene is 0.0016 mg/L). Residual fuels are principally made of C20 – C50 hydrocarbons, and heteroatom constituents would not be expected to be present in significant concentrations in aqueous preparations. Measured data are provided together with as estimated acute LC/EC50 values derived by the Q-SAR model, ECOSAR.

Compound	C-Num	Water Sol. (mg/L)	Taxon Group	Empirical Data LC/EC50 (mg/L)	Ref.	ECOSAR Estimate
benzo(b)thiophene	8	130	Fish	13.6 (n)	A	11.6
			Invert.	2.9 (n) 63.7 (n) 59 (n)	A B C	13.4
			Algae			8.9
5-methyl benzo(b)thiophene	9	74	Fish			3.9
			Invert.	14 (n)	C	4.7
			Algae			3.2
dibenzothiophene	12	1.5	Fish	0.7 (n)	A	1.8*
			Invert.	>1 (n) 0.42 (n) 0.47 (n)	C A B	1.4
			Algae			1.7*
indole	8	3560	Fish			78
			Invert.			84
			Algae			53

3-methyl indole	9	468	Fish Invert. Algae	8.8 (m)	D	27 30 19
carbazole	12	1.8	Fish	0.93 (m) >1.1 (m) <1.5 (n)	E E E	10.6*
			Invert.	3.35 (m)	E	7.2*
			Algae			5.8*
quinoline	9	6110	Fish	77.8 (m) 0.44 (m) 29.9 (m)	D G H	71
			Invert.	34.5 (m) 42 (n)	G A	77
			Algae	74 (n) 90 (n) >100 (n)	I J J	48

(m) = measured concentrations; (n) = nominal concentrations
Empirical Data References: A = Maas (1990);B = Eastmond et al. (1984); C = Seymour et al. (1997); D = Geiger et al. (1990); E = Brooke (1991); F = Van Vlaardingen et al. (1996); G = Millemann et al. (1984); H = Ramos et al. (1999); I = Ramos et al. (1999); J = Kuhn and Pattard (1990). All empirical data and references may be found in U.S. EPA ECOTOX Database (URL: http://cfpub.epa.gov/ecotox/quick_query.htm).
Notes:
1) Toxicity values are based on a 96-h exposure for fish and algae, and a 48-h exposure for invertebrates.
2) For values indicated by "*", ECOSAR states that the chemical may not be soluble enough to measure the predicted effect.

The above data show that for studies using nominal concentrations, the toxicity values ranged from 0.42 to >100 mg/L. For studies using measured exposure concentrations, the toxicity values range from 0.44 to 77.8 mg/L. These ranges are not substantially different from other classes of hydrocarbon compounds in heavy fuel oils. Because HFOs are composed of many individual constituents, these specific compounds would not be expected to make-up a significant proportion of the sample or partition to the aqueous phase of a WAF preparation to levels that would elicit acute toxicity.

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	:	CAS RN 64741-45-3 sample F-132.
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}
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	:	CAS RN 64741-81-7
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
F-97-01	>5000 mg/kg	ARCO ATX-88-0086

Test substance : Data are available on four samples of vacuum distillate.

The samples are:
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 : CAS RN 64741-62-4 Catalytically cracked clarified oil (API 81-15)

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 : CAS RN 64741-81-7 Coker heavy gas oil, sample F-97-01

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}
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	:	CAS RN 68553-00-4, sample API 78-6
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	:	CAS RN 64741-45-3 sample F-132
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}
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	:	CAS RN 64741-81-7, Visbreaker Gas Oils [3 samples] CAS RN 64741-57-7 Heavy Vacuum Gas Oil
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
Hvy Vac Gas Oil	>2000 mg/kg	Mobil 62443-45

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	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	2
Vehicle	:	None - undiluted
Doses	:	2 g/kg
Year	:	1982
GLP	:	Yes
Test substance	:	CAS RN 4741-62-4 (API 81-15)
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 : CAS RN 64741-81-7 sample F-97-01, Coker heavy gas oil

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}
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 : CAS RN 68553-00-4 Heavy fuel oil API sample 78-6

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 : CAS RN 64741-45-3

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)

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 : CAS RN 64741-81-7 Vacuum tower Bottoms

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 : CAS RN 64741-81-7 Visbreaker Gas Oils [3 samples]
CAS RN 64741-57-7 Heavy Vacuum Gas Oil

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 : CAS RN 64741-62-4, Sample API 81-15

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		Edema	
	Intact	Abraded	Intact	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 : CAS RN 64741-81-7 [F97-01]

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		**Edema**	
	Intact	Abraded	Intact	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)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
Test substance : CAS RN 68553-00-4 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 : CAS RN 64741-45-3

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)

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 : CAS RN 68512-62-9

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 : CAS RN 64741-81-7 VIsbreaker gas oils (3 samples)
CAS RN 64741-57-7 Heavy Vacuum Gas Oil

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 : CAS RN 64741-62-4, Sample API 81-15

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 : CAS RN 64741-81-7 [F97-01]

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)

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 : CAS RN 68553-00-4 Heavy fuel oil, 4 samples

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 : CAS RN 64741-45-3 Sample F-132

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)

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 : CAS RN 68512-62-9 Vacuum Tower Bottoms

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 : CAS RN. 64741-57-7 Heavy Vacuum Gas Oil (HVGO),

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.

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 : CAS RN 64741-62-4, sample API 81-15

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 : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge 50 % occlusive epicutaneous
Number of animals : 10
Vehicle : Mineral oil
Result : Not sensitizing
Year : 1989
GLP : Yes
Test substance : CAS RN 64741-81-7

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)

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 : CAS RN 68553-00-4 Heavy fuels, 4 samples

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 : CAS RN 68553-00-4 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

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-57-7

Test Substance (CAS #): 64741-57-7; Heavy Vacuum Gas Oil Stock (F-113-01)

Test Substance Purity/Composition and Other Test Substance Comments : No information available

Category Chemical Result Type : Measured

Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days; 4 weeks
Frequency of treatm.	Daily, 5 days/week
Doses	0.01, 0.10, 1.00 ml/kg/day (9.3, 93, 930 mg/kg/day)
Control group	Yes, untreated
Method/Guideline followed	Other
GLP	Yes
Year	1993
Post exposure period	None
Test substance	Heavy Vacuum Gas Oil Stock (F-113-01) CAS 64741-57-7
Method/Guideline and Test Condition Remarks	NOTE: After completion of this study, quality control deficiencies were noted with the hematology analyses. It was possible that subtle changes in hematology parameters were not detected. Therefore, a follow-up study was performed, Study No. ATX-930173. The follow-up study included only one dose group, 930 mg/kg/day, the same dose given the high dose group in this study. Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-113-01 dermally once daily, five days per week for four weeks, at doses of 0.01, 0.10, 1.00 ml/kg/day (9.3, 93, 930 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of ten male and ten female rats served as controls (untreated). The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry

parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 93 mg/kg/day (0.1 ml/kg/day)
LOAEL = 930 mg/kg/day (1.0 ml/kg/day)

Females: NOAEL = 93 mg/kg/day (0.0.1 ml/kg/day)
LOAEL = 930 mg/kg/day (1.0 ml/kg/day)

*

Result Remarks

Clinical:
Skin irritation: Slight in 930 mg/kg females

Mortality	***Males***	***Females***
	None	None

Body wt., terminal	**Males**	**Females**
	No difference	↓ 93 (8%), 930 (9%) mg/kg
Body wt., gain	**Males**	**Females**
	No difference	↓ 930 mg/kg
Organ weights	**Males**	**Females**
Liver, rel bw	↑ 930 (10%) mg/kg*	↑ 930 (16%) mg/kg*
Liver, rel brain	No difference	↑ 930 (8%) mg/kg*
Brain, Abs.	No difference	↓ 93 (5%) mg/kg*
Kidney, Abs.	No difference	↓ 93 (10%), 930 (14%) mg/kg*
Kidney, rel brain		↓ 930 (10%) mg/kg*
Hematology	**Males**	**Females**
Hb	↓ 930 (5%) mg/kg	↓ 930 (6%) mg/kg
Hematocrit	↓ 930 (8%) mg/kg	↓ 930 (10%) mg/kg
Serum chemistry	**Males**	**Females**
Cholesterol	No difference	↑ 930 (47%) mg/kg

Histopath (control & high dose)
No test article-related systemic findings
Testes – normal; Ovaries – normal

Note: * = not considered compound-related and/or biologically relevant by study directors

Conclusion

Effects defining LOAEL:
Male (930 mg/kg/day)
Hematocrit, Hb

Female (930 mg/kg/day)
Hematocrit, Hb

Reliability 1 - Reliable without restrictions

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference ARCO. 1993. Twenty-eight day dermal toxicity study in rats administered test article F-113-01. Report no. ATX-890011
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Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-61-3

Test Substance (CAS #): 64741-61-3; Heavy Cycle Oil (F-134)

Test Substance Purity/Composition and Other Test Substance Comments : No information available

Category Chemical Result Measured

Type :

Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days/4weeks
Frequency of treat.	Daily, 5 days/week
Doses	0.01, 0.1, 1.0 ml/kg/day (9.9, 99, 990 mg/kg/day)
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Heavy Cycle Oil (F-134) CAS 64741-61-3
Post exposure period	None
Method/Guiedline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-134 dermally once daily, five days per week for four weeks, at doses of 0.01, 0.1, 1.0 ml/kg/day (9.9, 99, 990 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of ten male and ten female rats served as controls (untreated). The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated). All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist. Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

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NOAEL/LOAEL

Males: NOAEL = 99 mg/kg/day (0.1 ml/kg/day)*
LOAEL = 990 mg/kg/day (1.0 ml/kg/day)

Females: NOAEL = 9.9 mg/kg/day (0.0.01 ml/kg/day)*
LOAEL = 99 mg/kg/day (0.1 ml/kg/day)
*Authors indicate "NOEL" .

Result remarks

Clinical:
Skin irritation: Very slight – Moderate, dose-related

Mortality	*Males*	*Females*
	None	None

Body wt., terminal
Males
↓ 990 (11%) mg/kg/day
Females
No difference

Organ weights

Brain, rel BW
Males
↑ 990 (11%) mg/kg/day

Females
No difference

Liver, Abs
Males
No difference
Females
↑ 990 (28%) mg/kg/day

Liver, rel BW
Males
↑ 990 (19%) mg/kg/day
Females
↑ 990 (33%) mg/kg/day

Liver, rel brain
Males
No difference
Females
↑ 99 (11%), 990 (30%) mg/kg/day

Kidney, rel brain
Males
↓ 990 (10%) mg/kg/day**
Females

No difference

Hematology

RBC	**Males** ↓ 990 (6%) mg/kg/day **Females** ↓ 99 (6%), 990 (9%) mg/kg/day
Hematocrit	**Males** ↓ 990 (10%) mg/kg/day **Females** ↓ 99 (6%), 990 (13%) mg/kg/day
Hb	**Males** ↓ 990 (10%) mg/kg/day **Females** ↓ 99 (4%), 990 (10%) mg/kg/day
Platelets	**Males** No difference **Females** ↓ 990 (33%) mg/kg/day

Serum chemistry

SGOT	**Males** No difference **Females** ↑ 990 (24%) mg/kg/day**
Cholesterol	**Males** No difference

Females

↑ 990 (60%) mg/kg/day

Histopath (sham controls & high dose)

Males

No test article-related systemic findings

Females

No test article-related systemic findings

Testes – normal; Ovaries – normal

Note: ** not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Male (990 mg/kg/day)
Terminal BW; Liver wts; RBC, Hematocrit, Hb

Female (99 mg/kg/day)
Liver wt; RBC, Hematocrit, Hb

Reliability

1 - Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

ARCO. 1992. Twenty-eight day dermal toxicity study in rats administered test article F-134. Report no. ATX-90-0082.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	64741-75-9
Test Substance (CAS #):	64741-75-9; Hydrocracker Recycle Oil (F-127)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	4 weeks; 28 days
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	0.01, 0.05, 0.25 ml/kg/day (8.4, 42, 210 mg/kg/day)
No. of animals/dose	10 animals/sex/group
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Recycle Oil, Hydrocracker (F-127) CAS 64641-75-9
Post exposure period	None

Method/Guideline and Test Condition Remarks

Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-127 dermally once daily, five days per week for four weeks, at a dose of 0.01, 0.05, 0.25 ml/kg/day (8.4, 42, 210 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. A fourth group of ten male and ten female rats served as a control. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks.

The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy.

At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were

analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL /LOAEL

Males: NOAEL = 210 mg/kg/day (0.25 ml/kg/day)*
LOAEL = >210 mg/kg/day (0.25 ml/kg/day)

Females: NOAEL = 210 mg/kg/day (0.25 ml/kg/day)
LOAEL = >210 mg/kg/day (0.25 ml/kg/day)

* Note: Authors indicate "NOEL"

Result remarks

Clinical:
Skin irritation: Very slight - moderate

Mortality	*Males*	*Females*
	None	None

Body wt., terminal	**Males** No difference	**Females** No difference
Organ weights	**Males** No difference	**Females** No difference
Hematology	**Males** No difference	**Females** No difference
Serum chemistry	***Males*** No difference	***Females*** No difference

Histopath (sham controls & high dose)

Males

No test article-related systemic findings

Females

No test article-related systemic findings

Testes – normal; Ovaries – normal

Conclusion Effects defining LOAEL:

Male >210 mg/kg/day (0.25 ml/kg/day)
None – highest dose tested was NOAEL for systemic effects

Female >210 mg/kg/day (0.25 ml/kg/day)
None – highest dose tested was NOAEL for systemic effects

Reliability 1 – Reliable without restrictions

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference ARCO. 1992. Twenty-eight day dermal toxicity study in rats administered test article F-127. Report no. Study No. ATX-900026.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-81-7

Test Substance (CAS #): 64741-81-7; Coker Heavy Gas Oil (F-97-01)

Test Substance Purity/Composition and Other Test Substance Comments : No information available

Category Chemical Result Type : Measured

Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days/4 weeks
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	0.001 (10% test article in acetone), 0.1, 1.0 ml/kg/day (0.93, 93, 930 mg/kg/day)
Control group	Yes, untreated and acetone (1.0 ml/kg)
Method/Guideline followed:	Other
Year	1990
GLP	Yes
Test substance	Coker, Heavy Gas Oil (F-97-01) CAS 684741-81-7
Post exposure period	None
Method/Guideline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F97-01 dermally once daily, five days per week for four weeks, at doses of 0.001 (10% test article in acetone), 0.1, 1.0 ml/kg/day (0.93, 93, 930 mg/kg/day. The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. Two additional groups of ten male and ten female rats served as controls (untreated and acetone). The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated). All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist. Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 0.93 mg/kg/day
LOAEL = 93 mg/kg/day (0.1 ml/kg/day)

Females: NOAEL = 0.93 mg/kg/day
LOAEL = 93 mg/kg/day (0.1 ml/kg/day)

Result remarks

Clinical:
Skin irritation: Slight – moderate, dose related

Mortality	*Males*	*Females*
	None	10% 0.93 mg/kg/day**

Body wt., terminal

Males
No difference
Females
↓ 930 (9%) mg/kg/day

Organ weights

Spleen, Abs
Males
↑ 930 (24%) mg/kg/day
Females
No difference

Spleen, rel bw
Males
↑ 930 (21%) mg/kg/day

Females
↑ 930 (17%) mg/kg/day

Spleen, rel brain
Males
↑ 930 (35%) mg/kg/day

Females
No difference

Liver, Abs
Males

↑ 930 (24%) mg/kg/day
Females
No difference

Liver, rel bw **Males**
↑ 93 (8%), 930 (23%) mg/kg/day

Females
↑ 930 (12%) mg/kg/day

Liver, rel brain **Males**
↑ 930 (35%) mg/kg/day
Females
No difference

Hematology

RBC **Males**
↓ 930 (9%) mg/kg/day
Females
↓ 93 (6%), 930 (9%) mg/kg/day

Hematocrit **Males**
↓ 93 (5%), 930 (11%) mg/kg/day

Females
↓ 930 (10%) mg/kg/day

Hb **Males**
↓ 93 (7%), 930 (13%) mg/kg/day
Females
↓ 930 (11%) mg/kg/day

Neutrophils **Males**
↓ 0.93 (46%) mg/kg/day**

Females
No difference

Lymphocytes **Males**
↑ 0.93 (10%) mg/kg/day**

Females
No difference

Serum chemistry

Males
No difference
Females
No difference

Histopath (acetone & sham controls; 0.93 & 930 mg/kg
No test article-related systemic findings

Testes – normal; Ovaries – normal

Note: ** not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Male (93 mg/kg/day)
↑ Liver wt, rel. bw; ↓ Hematocrit, ↓Hb

Female (93 mg/kg/day)
↓ RBC

Reliability	1 – Reliable without restrictions
Reliability remarks	Similar to guideline study; sufficient detail provided in appendices and tables.
Key study sponsor	Yes
Reference	: ARCO. 1990. Twenty-eight day dermal toxicity study in rats administered test article F97-01. Report no. Study No. ATX-88-0092.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	64741-45-3
Test Substance (CAS #):	64741-45-3; Atmospheric Tower Bottoms (F-132)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days
Frequency of treatm.	Daily, 5 days/week for 4 weeks
Doses	0.01, 0.25, 1.0 ml/kg/day (9.4, 235, 940 mg/kg/day)
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Atmospheric Tower Bottoms (F-132) CAS 64741-45-3
Post exposure period	None
Method/Guideline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-132 dermally once daily, five days per week for four weeks, at doses of 0.01, 0.25, 1.0 ml/kg/day (9.4, 235, 940 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of ten male and ten female rats served as an untreated control. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy.

At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 2350 mg/kg/day (1.0 ml/kg/day)*
LOAEL = > 940 mg/kg/day (1.0 ml/kg/day)
Females: NOAEL = 940 mg/kg/day (1.0 ml/kg/day)*
LOAEL = > 940 mg/kg/day (1.0 ml/kg/day)
* Note: Authors indicate "NOEL"

Result remarks

Clinical:
Skin irritation: None – very minimal in all dosed groups

Mortality	**Males**	**Females**

		None	None
	Body wt., terminal	**Males**	**Females**
		No difference	No difference
	Organ weights	**Males**	**Females**
	Liver, rel BW	No difference	↑ 235 mg/kg (9%)*
	Brain, Abs	↓ 9.4 mg/kg (6%)*	No difference
	Hematology	**Males**	**Females**
	Platelets	No difference	↓ 940 mg/kg (14%)*
	Hb	No difference	↓ 940 mg/kg (6%)*
	HCT	No difference	↓ 940 mg/kg (7%)*
	Serum chemistry	**Males**	**Females**
	Glucose	No difference	↑ 940 mg/kg (22%)*
	Triglycerides	↓ 9.4 mg/kg (35%)*	No difference
	Albumin	↑ 940 mg/kg (8%)*	No difference
	Globulin	↓ 940 mg/kg (11%)*	No difference
	A/G Ratio	↑ 940 mg/kg (17%)*	No difference
	Alk. Phos.	No difference	↓ 940 mg/kg (26%)*
	Potassium	↑ 940 mg/kg (13%)*	No difference
	Chloride	↓ 940 mg/kg (4%)*	↓ 940 mg/kg (6%)*
	Phosphorous	↑ 940 mg/kg (8%)*	No difference

Histopath (control & high dose)
No test article-related systemic findings
Testes – normal; Ovaries – normal

Note: * = not considered compound-related and/or biologically relevant by study directors

Conclusion

Effects defining LOAEL:

Male >940 mg/kg/day (1.0 ml/kg/day)
None, highest dose tested had no systemic effects

Female >940 mg/kg/day (1.0 ml/kg/day)
None, highest dose tested had no systemic effects

Reliability 1 – Reliable without restrictions

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference ARCO. 1992. Twenty-eight day dermal toxicity study in rats administered test article F-132. Report no. Study No. ATX-90-0066.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-57-7

Test Substance (CAS #): 64741-57-7; Heavy Paraffinic Vacuum Distillate (F-128)

Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days; 4 weeks
Frequency of treatm.	Daily, 5 days/week for 4 weeks
Doses	0.1, 1.0, 2.5 ml/kg/day (94, 940, 2350 mg/kg/day)
No. of animals/dose	10/sex/dose
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Vacuum Distillate, Heavy Paraffin (F-128) CAS 64741-57-7
Post exposure period	None
Method/Guideline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-128 dermally once daily, five days per week for four weeks, at doses of 0.1, 1.0, 2.5 ml/kg/day (94, 940, 2350 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of ten male and ten female rats served as an untreated control. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated). All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 2.5 ml/kg/day (2350 mg/kg/day)
LOAEL = > 2.5 ml/kg/day (2350 mg/kg/day)
Females: NOAEL = 0.1 ml/kg/day (94 mg/kg/day)
LOAEL = 1.0 ml/kg/day (940 mg/kg/day)

Result Remarks

Clinical:
Skin irritation: Very slight – slight

Mortality	**Males**	**Females**
	None	None

Body wt., terminal	**Males**	**Females**
	No difference	No difference

Body wt., gain	**Males**	**Females**
	No difference	No difference

Organ weights

Liver, Abs
Males
No difference
Females
↑ 940 (14%), 2350 (25%) mg/kg

Liver, rel BW
Males
↑ 2350 mg/kg (10%)*
Females
↑ 940 (16%), 2350 (28%) mg/kg

Liver, rel Br
Males
No difference
Females
↑ 940 (18%), 2350 (29%) mg/kg

Hematology	**Males**	**Females**
Hb	No difference	↓ 2350 mg/kg (5%)
HCT	No difference	↓ 2350 mg/kg (8%)
Platelets	No difference	↓ 2350 mg/kg (19%)*

Serum chemistry	**Males**	**Females**
Cholesterol	No difference	↑ 2350 mg/kg (51%)
Total Protein	No difference	↑ 2350 mg/kg (7%)*

Histopath (control & high dose)
No test article-related systemic findings
Testes – normal; Ovaries – normal

Note: * = not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Male (>2350 mg/kg/day)
None – highest dose tested was NOAEL for systemic effects

Female (940 mg/kg/day)
↑ Abs & Rel liver wts

Reliability

1 – Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

ARCO. 1992. Twenty-eight day dermal toxicity study in rats administered test article F-128. Report no. Study No. ATX-90-0034

Repeated Dose Toxicity

TEST SUBSTANCE

Category Chemical: 64741-57-7
Test Substance: 64741-57-7; Heavy Vacuum Gas Oil (HVGO)

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil (CRU No. 85244)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348ZV (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-A (%)
85244	6.20	0.00	0.06	2.48	1.86	1.24	0.50	0.0(

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured
Type Repeated dose; 90 day (13 week) dermal exposure
Species Rat
Sex Male/Female
Strain Sprague-Dawley
Route of admin. Dermal
Exposure period 13 weeks
Frequency of treatm. Daily, 5 times/week
Doses 30, 125, 500 & 2000 mg/kg/day
No. of animals/dose 10/sex/dose
Control group Yes, untreated
Method/Guideline followed Other
Year 1988
GLP No data
Test substance Heavy Vacuum Gas Oil (HVGO) Sample 85244 CAS 64741-57-7
Post exposure period None
Method/Guideline and Test Condition Remarks Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off.

Endpoints during the biophase included daily observation of clinical signs and body weights measured weekly. Blood samples were obtained from animals at weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number of red blood cells, and the number of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium.

All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, spleen, thymus. Histological slides on 20 tissues, including

bone marrow, were prepared and examined microscopically by a pathologist. Sperm head morphology was also examined in the control and high dose animals.

Statistical analysis: Not described.

NOAEL/LOAEL

Authors determined:
Males: NOAEL = 125 mg/kg/day
LOAEL = 500 mg/kg/day
Females: NOAEL = 125 mg/kg/day
LOAEL = 500 mg/kg/day
Reviewer determined:
LOAEL = 125 mg/kg/day (male and female)

Result

Clinical signs: 2/10 males died in 2000 mg/kg
Skin irritation None
Body wt gain ↓ in both sexes at 2000 mg/kg (Taken from fig in report)

Hematol		**Male**	**Female**
RBC	(125 mg/kg)	↓ 30%	↓ 13%
	(30 mg/kg)		↓6%
Hb	125 mg/kg)	↓ 29%	↓ 18%
	(30 mg/kg)		↓ 8%
HCT	125 mg/kg)	↓ 26%	↓ 13%
	(30 mg/kg)		↓ 8%
Platelets	(125 mg/kg)	↓ 48%	↓ 46%
	(30 mg/kg)		↓ 23%

Serum chemistry		**Male**	**Female**
Total bilirubin	all doses	↓ 21-38% *	↓ 28-39%*
Cholesterol	500 mg/kg	↑ 53%	↑ 47%
	2000 mg/kg	↑ 75%	↑ 98%
Uric acid	2000 mg/kg	↓ 43%	↓ 55%
Glucose	2000 mg/kg		↓ 14%
SDH	2000 mg/kg	↑ 100%	↑ 75%
	500 mg/kg		↑ 63%

* not dose-related

Organ weights (Rel)		**Male**	**Female**
Kidney		↑ 22%	↑ 19% at 2000
Liver	2000 mg/kg	↑ 52%	↑ 87%
	500 mg/kg	↑ 22%	↑ 32%
Spleen	2000 mg/kg	↑ 38%	↑ 21%
	500 mg/kg	↑ 31%	↑ 16%
Thymus	2000 mg/kg	↓ 44%	↓ 54%
	500 mg/kg	↓ 29%	↓ 21%

NB No Absolute weights given in report

Histopath: **Bone marrow** at 2000 mg: decreased erythropoiesis in 9/10 males and 1/10 females.
fibrosis (3/10) and increased vacuoles (4/10) in males
Thymus : lymphocyte depletion at 500 and 2000 mg/kg in both sexes
Spleen: Reduced lymphocytes and megakaryocytes
Sperm evaluation No effects

NOTE: No body wt data given – shown only as diag
No absolute organ weight given

Conclusion

Authors: LOAEL = 500 mg/kg (Authors)
NB Bilirubin was affected at all dose levels.
Hematological parameters affected at 125 mg/kg
Reviewers: LOAEL = 125 mg/kg/day

Reliability	2 – Reliable with restrictions.
Reliability remarks	Poorly reported study and not reported completely.
Key study sponsor	Yes
Reference	Mobil, 1988. Thirteen-week toxicity study by dermal application of Heavy Vacuum Gas Oil (HVGO) to rats. Final Report on study 61590 from Mobil Environmental and Health Science Laboratory, Princeton, NJ. Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Vacuum Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZV API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	64741-62-4
Test Substance (CAS #):	64741-62-4; FCCU Clarified Oil (F-115-01)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days; 4 weeks
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	In acetone 0.01 (1% in acetone), 0.1 (10% in acetone), 1.0 (10% in acetone), 10.0 (10% in acetone), 50.0 (10% in acetone) mg/kg/day Neat 1.0, 10.0, 50.0 mg/kg/day
No. of animals/dose Control group	10/sex/dose Yes, untreated and acetone (.45 ml/kg)
Method/Guideline followed	Other
Year	1993
GLP	Yes
Test substance	FCCU Clarified Oil (F-115-01) CAS 64741-62-4
Post exposure period	None

Method/Guideline and Test Condition Remarks

Ten groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-115-01 dermally once daily, five days per week for four weeks, at doses 0.01 (1% in acetone), 0.1 (10% in acetone), 1.0 (10% in acetone), 10.0 (10% in acetone), 50.0 (10% in acetone) mg/kg/day and 1.0, 10.0, 50.0 mg/kg/day, including a sham and acetone control. The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks.

The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy.

At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

For the nonparametric procedures, the test of equality of means were performed using the Kruskal-Wallis test. If significant differences among the means were indicated, Dunn's Summed Rank test were used to determine which treatment groups differ significantly from control. In addition to the Kruskal-Wallis test,

Jonckheere's test for monotonic trend in the dose response was performed.

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Acetone Group
Males: NOAEL = 1 mg/kg/day*
LOAEL = 10 mg/kg/day
Females: NOAEL = 1 mg/kg/day*
LOAEL = 10 mg/kg/day

Neat Group
Males: NOAEL = 10 mg/kg/day*
LOAEL = 50 mg/kg/day
Females: NOAEL = 1 mg/kg/day*
LOAEL = 10 mg/kg/day

Females: NOAEL = 1 mg/kg/day *
LOAEL = 10 mg/kg/day (acetone group)
* Note: Authors indicate "NOEL"

Result remarks

Clinical:
Skin irritation: Slight

Mortality	*Males*	*Females*
	None	None

Body wt., terminal
Males
No difference
Females
↓ 50.0 neat (13%) mg/kg/day

Organ weights

Brain, rel bw
Males
No difference
Females
↑ 50.0 (neat) (11%) mg/kg/day**

Liver, Abs
Males
↑ 50.0 (acetone) (22%) mg/kg/day
Females
↑ 50.0 (acetone) (24%) mg/kg/day

Liver, rel bw
Males
↑ 10.0 (acetone) (8%), 50.0 (acetone) (29%), 50.0 (neat) (15%) mg/kg/day

Females
↑ 10.0 (acetone) (14%), 50.0 (acetone) (35%), 10.0 (neat) (17%), 50.0 (neat) (25%) mg/kg/day

Liver, rel brain
Males
↑ 10.0 (acetone) (13%), 50.0 (acetone) (24%) mg/kg/day

Females
↑ 50.0 (acetone) (23%), 50.0 (neat) (13%) mg/kg/day

Kidney, Abs **Males**
↓ 50.0 (acetone) (12%) mg/kg/day**
Females
↓ 50.0 (neat) (13%) mg/kg/day**

Kidney, rel brain **Males**
↓ 50.0 (acetone) (9%), 50.0 (neat) (11%) mg/kg/day**
Females
No difference

Thymus, Abs **Males**
↓ 50.0 (acetone) (45%), 50.0 (neat) (23%) mg/kg/day
Females
↓ 50.0 (acetone) (45%), 50.0 (neat) (33%) mg/kg/day

Thymus, rel bw **Males**
↓ 50.0 (acetone) (45%) mg/kg/day
Females
↓ 50.0 (acetone) (39%), 50.0 (neat) (19%) mg/kg/day

Thymus,rel brain **Males**
↓ 50.0 (acetone) (43%), 50.0 (neat) (27%) mg/kg/day
Females
↓ 50.0 (acetone) (45%), 50.0 (neat) (30%) mg/kg/day

Ovaries, Abs **Females**
↓ 50.0 (acetone) (19%) mg/kg/day**

Ovaries, rel bw **Females**
↓ 1.0 (acetone) (17%) mg/kg/day**

Ovaries, rel brain **Females**
↓ 1.0 (acetone) (15%), 50.0 (acetone) (19%) mg/kg/day**

Adrenals, rel brain
Males
No difference
Females
↑ 10.0 (acetone) (20%) mg/kg/day**

Hematology

RBC **Males**
↓ 10.0 (acetone) (12%), 50.0 (acetone) (12%), 50.0 (neat) (14%) mg/kg/day
Females
No difference

Hematocrit **Males**
↓ 10.0 (acetone) (13%), 50.0 (acetone) (13%), 50.0 (neat) (15%) mg/kg/day

Females

↓ 50.0 (acetone) (16%) mg/kg/day

Hb **Males**
↓ 10.0 (acetone) (9%), 50.0 (acetone) (12%), 50.0 (neat) (15%) mg/kg/day
Females
↓ 50.0 (acetone) (16%) mg/kg/day

Neutrophils **Males**
↑ 1.0 (acetone) (138%) mg/kg/day**

	Females No difference
Platelets	**Males** No difference
	Females ↓ 50.0 (neat) (16%) mg/kg/day**

Serum chemistry

BUN	**Males** ↑ 50.0 (acetone) (25%), 50.0 (neat) (28%) mg/kg/day **Females** ↑ 50.0 (acetone) (34%), 50.0 (neat) (17%) mg/kg/day
SGPT	**Males** ↓ 0.1 (acetone) (17%), 10.0 (acetone) (24%), 50.0 (acetone) (28%) mg/kg/day** **Females** No difference
Cholesterol	**Males** No difference **Females** ↑ 10.0 (acetone) (32%), 50.0 (acetone) (96%), 50.0 (neat) (76%) mg/kg/day
Potassium	**Males** No difference **Females** ↓ 10 (neat) (12%), 50.0 (neat) (11%) mg/kg/day**
Glucose	**Males** No difference **Females** ↑ 50.0 (acetone) (27%) mg/kg/day**

Histopath (acetone controls & 50 mg/kg (10% in acetone)
No test article-related systemic findings

Testes – normal; Ovaries – normal

Note: ** not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Acetone Grps
Male 10.0 (acetone) mg/kg/day
↑ Liver wt, rel. bw & brain; ↓RBC, ↓ Hematocrit, ↓Hb
Female 10.0 (acetone) mg/kg/day
↑ Liver wt, rel. bw; ↑ cholesterol

Neat Grps
Male 50.0 mg/kg/day
↑ Liver wt, rel. bw; ↓ thymus wt, abs & rel brain; ↓RBC, ↓ Hematocrit, ↓Hb
Female 10.0 mg/kg/day
↑ Liver wt, rel. bw

Reliability 1 - Reliable without restrictions

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference ARCO. 1993. Twenty-eight day dermal toxicity study in rats administered test

article F-115-01. Report no. ATX-89-0077.

Repeated Dose Toxicity

Test Substance

Category Chemical:

64741-62-4

Test Substance:

64741-62-4; Clarified Slurry Oil (CSO)

Test Substance Purity/Composition and Other Test Substance Comments:

Clarified Slurry Oil (CRU No 86001)

PAC (Polycyclic Aromatic Compound) Content – Report No. 64348 ZA (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-AI (%)
86001	64.20	0.00	2.57	25.68	19.26	6.42	3.21	0.64

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is “aromatic ring class”. “ARC 1 (%)” is the weight percent of PACs that have 1 aromatic ring within the total sample. “ARC 2 (%)” is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure Period	13 weeks
Frequency of treatm.	Daily, 5 days/week
Doses	8, 30, 125, 500 & 2000 mg/kg/day
No. of animals /dose	10/sex/dose
Control group	Yes, untreated
Method/Guideline Followed:	Other
Year	1985
GLP	Yes
Test substance	Clarified Slurry Oil (CSO) Sample 10298102 (CRU 86001)
Post exposure period	None

Method

Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off, but collars remained throughout the weekend, since oil could not be completely removed.

Endpoints during the biophase included daily observation of clinical signs and body

weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, on study days 28, 29 or 30 and 91, 92, or 93. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. The following organs were weighed: lungs, kidneys, adrenals, liver, heart, spleen, thymus, testis, ovary. Histological slides were prepared from the following organs and examined microscopically by a pathologist: colon, kidney, lung, liver, lymph node, ovary, skin, small intestine, spleen, stomach, testis, urinary bladder and any gross lesions.

Statistical analysis: Quantitative data were analyzed for homogeneity of variance. If variances were homogeneous, data were analyzed by analysis of variance followed by multiple t tests or Duncan's multiple range tests. Categorical data were analyzed by a test based on the chi-squared distribution.

NOAEL/LOAEL

Males: NOAEL = < 8 mg/kg/day
LOAEL = 8 mg/kg/day

Females: NOAEL = < 8 mg/kg/day
LOAEL = 8 mg/kg/day

Result

Clinical signs: 2000 mg all animals died/killed
500 mg 85% died or killed
125 mg 5/10 males, 1/10 females died/killed

Body wt gains ↓ at 30 mg (6%) and 125 mg (25%).
8 mg no differences

Skin irritation Not seen at 8, 30 or 125 mg

Hematology	**Males**	**Females**
RBC	↓ (46%)at 125 mg	↓ (24%) at 125 mg
Hb	↓ (49%) at 125 mg	↓ (30%) at 125 mg
HCt	↓ at 30 (53%) and 125	↓ 34%) at 30 and 125

Serum chemistry	**Males**	**Females**
Glucose	↑ (26%) 125 mg	No differences
A/G ratio	↑ (14%) 125 mg	↑ (18%) 125 mg
Uric acid	↓ (33%) 30 mg +	No differences
Total bilirubin	↑ (146%) 125 mg	No differences
Cholesterol	No differences	↑ (43%) 8 mg +
AAT	↑ (200%) 125 mg	No differences
Alk phos.	↑ (72%) 125 mg	↑ (58%) 30 mg +
LDH	↓ (52%) 30 mg +	↓ (79%) 125 mg
Ca	↑ (7%) 125 mg	No differences

Organ wts	**Males**	**Females**
Liver (abs)	No differences	↑ (21%) 8 mg +
Liver (rel)	↑ (13%) 8 mg +	↑ (23%) 8 mg +
Thymus (abs)	↑ (43%) 30 mg +	↓ (67%) 125 mg
Thymus (rel)	↑ (39%) 30 mg +	↓ (38%) 125 mg
Spleen (rel)	↑ (25%) 30 mg	No differences

Histopath

Liver: microcysts, cholangiolitis/cell degeneration, altered focus of hepatocytes at ≥ 8 mg
Necrosis at higher doses
Thymus: hypoplasia/atrophy at ≥ 8 mg
Bone marrow: erythroid hypoplasia at ≥ 30 mg
Ovaries and testes normal

Conclusion

Effects defining LOAEL:
Males: 8 mg/kg.
Effects at LOAEL: Rel liver wt., histopath in thymus and liver
Females: 8 mg/kg
Effects at LOAEL: Cholesterol, Abs and rel liver wt, histopath in thymus and liver

Reliability

- 1 - Reliable without restrictions
-

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

Mobil, 1985. Thirteen-week toxicity study by dermal application of Clarified Slurry Oil (CSO) to rats. Final Report on study 20525 from Mobil Environmental and Health Science Laboratory, Princeton, NJ.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical:

64741-62-4

Test Substance:

64741-62-4; Clarified Slurry Oil (CSO)

Test Substance Purity/Composition and Other Test Substance Comments:

Clarified Slurry Oil (CRU No 86001)

PAC (Polycyclic Aromatic Compound) Content – Report No. 64348 ZA (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86001	64.20	0.00	2.57	25.68	19.26	6.42	3.21	0.64

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type :	Measured
Type	Repeated dose; 2, 4, 8, 10 week dermal and oral exposure
Species	Mouse
Sex	Male
Strain	
Route of admin.	Dermal & Oral
Exposure Period	2, 4, 8 weeks (Oral), 10 weeks (oral and dermal)
Frequency of treatm.	Daily, 5 days/week (total number of weeks were 2, 4. 8 and 10 per protocol listed below)
Doses	1000 mg/kg/day
No. of animals /dose	10/dose
Control group	Yes, untreated
Method/Guideline Followed:	Other
Year	1991
GLP	Yes
Test substance	Clarified Slurry Oil (CSO) Sample 10298102 (CRU 86001)
Post exposure period	None

Method

Dermal

Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle, and left uncovered. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. The animals were 8-9 weeks of age at the initiation of dosing.

Oral

Each treated animal received an amount of CSO calculated from its most recent body weight, the density of the test material, and the dose for that treatment group. The test material was measured by volume in a syringe and administered to the mice by oral gavage using a syringe fitted with an 18 gauge intubation needle. Animals in the control groups were handled in the same manner as the treated animals except that no test material was administered. Animals were dosed each weekday until their scheduled sacrifice.

The treatment groups and time exposure periods were as follows:

1. Control (0 mg/kg/day) – 10 males, oral, 2 weeks
2. CSO (1000 mg/kg/day) – 10 males, oral, 2 weeks
3. Control (0 mg/kg/day) – 10 males, oral, 4weeks
4. CSO (1000 mg/kg/day) – 10 males, oral, 4weeks
5. Control (0 mg/kg/day) – 10 males, oral, 8 weeks
6. CSO (1000 mg/kg/day) – 10 males, oral, 8weeks
7. Control (0 mg/kg/day) – 10 males, dermal, 10 weeks
8. CSO (1000 mg/kg/day) – 10 males, oral, 10 weeks
9. CSO (1000 mg/kg/day) – 10 males, dermal, 10 weeks

Endpoints during the biophase included daily observation of clinical signs and body weights measured weekly.

Animals scheduled for sacrifice (weeks 2, 4, 8 and 10) were fasted overnight, weighed, euthanized with carbon dioxide gas, exsanguinated and necropsied. Gross findings were recorded at necropsy. From all animals sacrificed as scheduled, the liver and thymus were excised and weighed to the nearest milligram. The following tissues, when available, were saved in 10% neutral buffered formalin: bone with marrow (sternum, rib, and femur), gross lesions, liver, thymus, treated skin. Stained sections were prepared and examined microscopically by a pathologist.

Statistical analysis: Body and organ weight data were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnett's test or Tukey's multiple range test, provided that there was statistical significance in ANOVA. Differences between control and treated animals were considered statistically significant only if the probability of the differences being due to chance was less than 5% ($p<0.05$).

NOAEL/LOAEL

Not determined in this study design.

Result

Clinical signs:
Five out of ten “dermals” died during Weeks 9 &10 (Group 9). Because of this dosing for all remaining mice was terminated after 10 weeks. Incidental mortality included one control (missing from cage) and three “orals" which were sacrificed moribund. Two of the three “orals” were apparently misintubated (one had an esophageal perforation; the other had black material in the lung suggestive of the test material) and the cause of illness in the third animal could not be determined. Clinical signs of systemic toxicity were minimal to non-existent in the animals which survived until their scheduled sacrifice. In the animals which died or were sacrificed, signs of systemic toxicity were generally not apparent until a few days prior to their death/sacrifice. The “dermals” showed intense skin irritation (open sores after only one week of dosing). During week 8, four of the “dermals” developed papillomas which, for two of the animals, appeared malignant prior to their death/sacrifice.

Body wt gains
The “orals” gained weight at the same rate as the controls. The "dermals” gained approximately half as much weight as the other animals. After 10 weeks, the mean final body weight of the “dermals" was approximately 10% lower than either the orals or the 'controls'.

Organ wts
In general, the “orals" had statistically significantly ($p<0.05$) heavier absolute and relative liver weights and lighter absolute and relative thymus weights than their respective controls. The only exception to this was the “orals" exposed for 10 weeks. The absolute and relative liver weights of these animals were comparable to their controls and were lighter than those of the "orals” sacrificed after 2, 4 or 8 weeks of CSO exposure. For all "orals", the absolute and relative thymus weights were statistically significantly lighter than the controls. "Dermals” (evaluated only after 10 weeks) had significantly heavier relative liver weights and significantly lighter thymus weights than their respective controls (7.840g vs. 6.072g, liver; .0.042g vs. 0.091g, thymus)

Histopath
Microscopically, after 2 weeks of oral exposure to CSO, most mice showed hepatocyte hypertrophy and foci of mixed inflammatory cells. A few mice also were observed to have yellow-green pigment-bearing macrophages, a trace increase in the number of neutrophils (PMNs) along bile ducts and ductules, microfocal necrosis and occasional apparent loss of small numbers of centrilobular hepatocytes. These same findings were observed after 4 weeks and for some findings the incidence and/or severity of the effect increased. At this time period the incidence of centrilobular hepatocyte loss was tripled and was at its peak for the study. Individual cell necrosis was frequent after 4 weeks but did not peak until the following sacrifice period (after 8 weeks of exposure). After 4 weeks, one mouse had minimal focal fibrosis which was probably the beginnings of centrilobular fibrosis. After 8 weeks, three mice had centrilobular fibrosis and in two of these animals the fibrosis was beginning to bridge central veins. However, probable precursors of centrilobular fibrosis (especially, centrilobular hepatocyte loss and individual cell necrosis) were already diminishing in incidence and/or severity. Thus, after 10 weeks of exposure, the liver of most of the “orals” showed the same

or less discernable morphologic pathology than that which was observed after 2 weeks of oral exposure.
10 weeks of dermal exposure to CSO was associated with hepatocyte hypertrophy; greatly decreased apparent glycogen in hepatocytes, an increase in the prominence of sinusoidal Kupffer cells, a minimal-slight population of large yellow-green pigment-bearing macrophages, quite severe (in several mice) widespread multifocal coagulative necrosis (even liquifactive in some areas or animals) and significant focal or multifocal fibrosis. A majority of these animals had a minimal to slight increase in PMNs along bile ducts/ductules. Mitotic figures were exceptionally numerous in three animals. In the five mice which were found dead, it was judged that the widespread liver injury was severe enough to be a major contributor to, if not the main cause of, illness or death. At the site of administration, there was extensive chronic dermal inflammation and epidermal hyperkeratosis/hyperplasia (regenerative) and three of these mice had histologically malignant squamous cell carcinoma. Dermal administration of CSO also had a minimal effect on sternal marrow which was essentially limited to reduced megakaryocytes.

Conclusion

Based on mortality, body weights, liver weights, and liver and bone marrow pathology, CSO is more toxic to mice when it is administered subchronically by the dermal route than by the oral route. Liver weights and microscopic examination of the liver, following 2, 4 and 8 weeks of oral exposure, indicated definite liver toxicity. However, after 10 weeks of oral administration, mice exhibited only slight morphologic changes in the liver. The observed liver changes were suggestive of the healing of an earlier toxicological insult rather than of on-going toxicity. It would therefore appear that mice exposed orally to CSO developed or manifested, in fewer than 10 weeks, a form of acclimation or adaptation that was profoundly effective in repairing or protecting the liver from the hepatotoxic effects of CSO. Mice exposed dermally to CSO at a dose of 1000 mg/kg/day for 10 weeks had a mortality of 50%, severe skin irritation, slightly decreased body weights, significantly increased liver and reduced thymus weights, reduced megakaryocytes in sterna bone marrow, and severe liver necrosis and fibrosis. Based on a previous study in rats in this laboratory, it also appears that mice are less sensitive to the skin effects of CSO.

Reliability

- 2- Reliable with restrictions
-

Reliability remarks

Non-guideline protocol, report details not complete.

Key study sponsor

No

Reference	Mobil, 1991. Oral and Dermal Administration of Clarified Slurry Oil (CSO) to Male C3H. Final Report on study 63563 from Mobil Environmental and Health Science Laboratory, Princeton, NJ. Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR. API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	64741-62-4
Test Substance (CAS #):	64741-62-4; Carbon Black Oil (F-73-01)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	4 weeks; 28 days
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	0.5, 1.0, 2.5 ml/kg/day (542, 1084, 2710 mg/kg/day)
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1987
GLP	Yes
Test substance	Carbon Black Oil (F-73-01) CAS 64741-62-4
Post exposure period	None
Method/Guideline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-73-01 dermally once daily, five days per week for four weeks, at doses of 0.5, 1.0, 2.5 ml/kg/day (542, 1084, 2710 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of ten male and ten female rats served as controls (untreated). The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy.

At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = <542 mg/kg/day (0.5 ml/kg/day)
LOAEL = 542 mg/kg/day (0.5 ml/kg/day)

Females: NOAEL = <542 mg/kg/day (0.5 ml/kg/day)
LOAEL = 542 mg/kg/day (0.5 ml/kg/day)

Result Remarks

Clinical:
Skin irritation: None
Mortality **Males** **Females**

	None	None
Body wt., terminal		
	Males	
	↓ 542 (11%), 1084 (11%), 2710 (19%) mg/kg/day	
	Females	
	↓ 2710 (11%) mg/kg/day	
Body wt., gain		
	Males	
	↓ 542, 1084, 2710 mg/kg/day	
	Females	
	↓ 542, 1084, 2710 mg/kg/day	
Organ weights		
Brain, Abs	**Males**	
	No difference	
	Females	
	↓ 1084 (6%), 2710 (5%) mg/kg/day*	
Brain, rel BW	**Males**	
	↑ 542 (10%), 1084 (10%), 2710 (22%) mg/kg/day*	

Females

	No difference	
Liver, Abs	**Males**	
	↑ 542(28%), 1084 (33%), 2710 (29%) mg/kg/day	
	Females	
	↑ 542 (27%), 1084 (42%), 2710 (32%) mg/kg/day	
Liver, rel BW	**Males**	
	↑ 542 (44%), 1084 (47%), 2710 (59%) mg/kg/day	
	Females	
	↑ 542 (29%), 1084 (47%), 2710 (47%) mg/kg/day	
Liver, rel brain	**Males**	
	↑ 542 (31%), 1084 (38%), 2710 (33%) mg/kg/day	
	Females	
	↑ 542 (32%), 1084 (51%), 2710 (39%) mg/kg/day	
Spleen, Abs	**Males**	
	No difference	
	Females	
	↓ 2710 (20%) mg/kg/day*	
Spleen, rel BW	**Males**	
	No difference	
	Females	
	↓ 2710 (12%) mg/kg/day*	
Spleen, rel brain	**Males**	
	No difference	
	Females	
	↓ 2710 (16%) mg/kg/day*	
Kidney, Abs	**Males**	
	↓ 1084 (8%), 2710 (15%) mg/kg/day	
	Females	
	↓ 1084 (10%), 2710 (15%) mg/kg/day	
Kidney, rel BW	**Males**	
	↑ 2710 (7%) mg/kg/day	
	Females	
	No difference	
Kidney, rel brain	**Males**	
	No difference	

	Females ↓ 2710 (10%) mg/kg/day
Testes, rel BW	**Males** ↑ 542 (20%), 1084 (20%), 2710 (30%) mg/kg/day*
Ovaries, Abs	**Females** ↓ 1084 (22%), 2710 (28%) mg/kg/day
Ovaries rel Brain	**Females** ↓ 2710 (23%) mg/kg/day

Hematology

Eosinophils	**Males** ↓ 542 (70%), 1084 (100%), 2710 (100%) mg/kg/day*
	Females ↓ 542 (79%), 1084 (100%), 2710 (100%) mg/kg/day*
Hb	**Males** ↓ 542 (8%), 1084 (11%), 2710 (7%) mg/kg/day*
	Females 542 (6%) mg/kg/day*
HCT	**Males** ↓ 542 (7%), 1084 (9%), 2710 (5%) mg/kg/day*
	Females ↓ 542 (5%) mg/kg/day*

Serum chemistry

SGPT	***Males*** ↓ 542 (28%), 1084 (21%), 2710 (23%) mg/kg/day*

Females

	↓ 542 (24%) mg/kg/day*
Alk. Phos.	**Males** No difference
	Females ↑ 1084 (80%), 2710 (78%) mg/kg/day*
BUN	**Males** ↑ 2710 (21%) mg/kg/day*
	Females No difference
Glucose	**Males** ↑ 542 (35%) mg/kg/day*
	Females ↑ 542 (45%), 1084 (31%) mg/kg/day*
Total Protein	**Males** No difference
	Females ↓ 1084 (8%), 2710 (7%) mg/kg/day*

Histopath (sham/control oil controls & high dose)
No test article-related systemic findings
Testes – normal; Ovaries – normal

Note: * = not considered compound-related and/or biologically relevant by study directors

Conclusion

Effects defining LOAEL:

Male (542 mg/kg/day)
Hb, HCT, Liver wts (abs, rel bw, rel brain), BW, BW gain

Female (542 mg/kg/day)
Liver wts (abs, rel bw, rel brain), BW gain

Reliability	- 1 - Reliable without restrictions -
Reliability remarks	Similar to guideline study; sufficient detail provided in appendices and tables.
Key study sponsor	Yes
Reference	ARCO. 1987. Twenty-eight day dermal toxicity study in rats administered test article F-73-01. Report no. ATX-86-0007.

Repeated Dose Toxicity

Test Substance

Category Chemical:

64741-81-7

Test Substance:

64741-81-7; Heavy coker gas oil (HCGO)

Test Substance Purity/Composition and Other Test Substance Comments:

Heavy coker gas oil (CRU No. 86181)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348 ZO (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86181	24.80	0.25	2.48	12.40	7.44	2.48	0.50	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is “aromatic ring class”. “ARC 1 (%)” is the weight percent of PACs that have 1 aromatic ring within the total sample. “ARC 2 (%)” is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type :

Measured

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 days/week
Doses	8, 30, 125 mg/kg/day
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1994
GLP	Yes
Test substance	Joliet Heavy coker gas oil (CRU 86181) CAS 64741-81-7
Post exposure period	None

Method

Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same

procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off as thoroughly as possible.

Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, thyroid, ovaries, uterus, and brain.

The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin:

Adrenals*, esophagus, head (entire), kidneys*, liver *(part of median and right, lateral lobes), pituitary, skeletal muscle*, spleen*, thymus*, tongue and larynx, bone with marrow *(rib sternum, femur), heart* and aorta lachrymal glands, lungs* and bronchi, lymph nodes, cervical mammary gland (with skin), prostate and seminal vesicles*, stomach* (glandular and squamous), uterus* (cervix, corpus, and horns), brain*, eyes* and optic nerve intestine, large* (cecum, colon and rectum), lymph nodes, mesenteric lymph nodes, draining ovaries* and oviducts, salivary glands* (major), spinal cord (cervical, thoracic), thyroid* and parathyroids, trachea, epididymides*, Harderian glands, intestine, small *(duodenum, ileum, jejunum) gross lesions*, pancreas*, sciatic nerve, skin (treated)*, testes*, urinary bladder*, vagina.

NOTE: From all animals, a sample of the right kidney and of the median lobe of the liver were fixed in a formaldehyde-glutaraldehyde mixture (4% and 1%, respectively, in an aqueous buffer).

Tissues marked with an (*) were processed for microscopic examination from all animals in the control group and highest dose group (125 mg/kg). In addition, the skin and thymus from the 30 mg/kg and skin from the 8 mg/kg group were processed. Sections for examination were stained with hematoxylin and eosin, or any special stain deemed necessary. Microscopic examinations were performed by a pathologist.

The left epididymis and testis from the control and 125mg/kg/day male rats were examined. Prior to sample preparation of the testis for examination, the tunica albuginea and corresponding blood vessel were removed and discarded The resulting testicular parenchyma and the cauda epididymis were individually weighed (nearest 0.001 gram) and the weight recorded. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and morphology.

Statistical analysis: Quantitative data (body weight), serum chemistry, hematology, and organ weight data) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnett's test (body weights) and Tukey's Multiple Comparison Test (serum chemistry, hematology and organ weight data), provided that there was statistical significance in ANOVA. 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% ($p<0.05$).

NOAEL/ LOAEL

Males: NOAEL = 8 mg/kg/day
LOAEL = 30 mg/kg/day

Females: NOAEL = 8 mg/kg/day
LOAEL = 30 mg/kg/day

Result

Clinical:
Skin irritation: Moderate in all treated groups

Body wt. gains,		**Males**	**Females**
125 mg/kg		↓ 17%	No differences

Organ weights			**Males**	**Females**
Epididymes		30 mg/kg	↓ 13%	
Liver	(A)	125 mg/kg	↑ 24%	↑ 32%
	(R)	125 mg/kg	↑ 36%	↑ 35%
		30 mg/kg	↑ 16%	↑ 9%
Thymus	(A))	125 mg/kg	↓ 56%	↓ 52%
	(R)	125 mg/kg	↓ 53%	↓ 51%
Heart	(R)	125 mg/kg	↑ 14%	

Hematology	**Males**	**Females**
RBC	↓12% at 125 mg/kg	↓12% at 125 mg/kg
Hb	↓16% at 125 mg/kg	↓15% at 125 mg/kg
Ht	↓5% at 30 mg/kg	↓13% at 125 mg/kg
Platelets	↓31% at 125 mg/kg	30% at 125 mg/kg
MCV	↓4% at 125 mg/kg	-
MCH	↓4% at 30 mg/kg	↓4% at 125 mg/kg
MCHC	-	↓2% at 125 mg/kg

Serum chemistry	**Males**	**Females**
BUN	↑ at 30 mg/kg	
Calcium	↓ at 30 mg/kg	
SDH	↑ at 125 mg/kg	
Glucose		↑ at 125 mg/kg
Creatinine		↑ at 125 mg/kg
Cholesterol		↑ at 125 mg/kg
Triglycerides		↑ at 125 mg/kg
Potassium		↓ at 125 mg/kg

Histopath
Decrease lymphoid tissue in thymus male and female at 125 mg/kg

Conclusion

Drivers of LOAEL: (30 mg/kg/day)
Male
Epididymis wt ↓, Ht ↓, MCH ↓, BUN ↑, Ca ↓

Female
Rel liver wt ↑

Reliability

1 - Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

Mobil, 1994. Thirteen-Week Dermal Administration of Joliet heavy coker gas oil. Final Report on Study 64165 from Mobil Environmental and Health Science Laboratory, Princeton, NJ.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZO.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical:

64741-81-7

Test Substance:

64741-81-7; Heavy Coker Gas Oil (HCGO); Heavy Thermal Cracked Distillate

Test Substance Purity/Composition and Other Test Substance Comments:

Heavy Coker Gas Oil (CRU No. 83366)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348 ZQ (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
83366	12.7	0.1	2.5	5.1	2.5	1.3	0.9	0.1

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type :

Measured

Type	Repeated dose; 13 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 times/week for 13 weeks
Doses	30, 125, 500 and 2000 mg/kg/day
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1994
GLP	Yes
Test substance	Heavy Coker Gas Oil (Paulsboro), Sample 83366, CAS 64741-81-7
Post exposure period	None
Method/Guideline and Test Condition Remarks	Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off as thoroughly as possible.

Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, thyroid, ovaries, uterus, and brain.

The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin:

Adrenals*, esophagus, head (entire), kidneys*, liver *(part of median and right, lateral lobes), pituitary, skeletal muscle*, spleen*, thymus*, tongue and larynx, bone with marrow *(rib sternum, femur), heart* and aorta lachrymal glands, lungs* and bronchi, lymph nodes, cervical mammary gland (with skin), prostate and seminal vesicles*, stomach* (glandular and squamous), uterus* (cervix, corpus, and horns), brain*, eyes* and optic nerve intestine, large* (cecum, colon and rectum), lymph nodes, mesenteric lymph nodes, draining ovaries* and oviducts, salivary glands* (major), spinal cord (cervical, thoracic), thyroid* and parathyroids, trachea, epididymides*, Harderian glands, intestine, small *(duodenum, ileum, jejunum) gross lesions*, pancreas*, sciatic nerve, skin (treated)*, testes*, urinary bladder*, vagina.

NOTE: From all animals, a sample of the right kidney and of the median lobe of the liver were fixed in a formaldehyde-glutaraldehyde mixture (4% and 1%, respectively, in an aqueous buffer).

Tissues marked with an (*) were processed for microscopic examination from all animals in the control group and highest dose group (125 mg/kg). In addition, the skin and thymus from the 30 mg/kg and skin from the 8 mg/kg group were processed. Sections for examination were stained with hematoxylin and eosin, or any special stain deemed necessary. Microscopic examinations were performed by a pathologist.

The left epididymis and testis from the control and 125mg/kg/day male rats were examined. Prior to sample preparation of the testis for examination, the tunica albuginea and corresponding blood vessel were removed and discarded The resulting testicular parenchyma and the cauda epididymis were individually weighed (nearest 0.001 gram) and the weight recorded. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and morphology.

Statistical analysis: Quantitative data (body weight), serum chemistry, hematology, and organ weight data) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnett's test (body weights) and Tukey's Multiple Comparison Test (serum chemistry, hematology and organ weight data), provided that there was statistical significance in ANOVA. 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% ($p<0.05$). Sperm evaluations.

NOAEL/LOAEL

30 mg/kg/day (authors)
Males: NOAEL = <30 mg/kg/day
LOAEL = 30 mg/kg/day

Females: NOAEL = <30 mg/kg/day
LOAEL = 30 mg/kg/day

Result

Clinical
2000 mg all animals terminated early
500 mg all animals terminated early
125 mg 1/10 male, 1/10 females died
30 mg no mortalities

Skin irritation moderate

Terminal Body wt	**Males**	**Females**
	↓ 9% at 30 mg/kg	
	↓ 12% at 30 mg/kg	↓ 13% at 30 mg/kg

Hematol		**Males**	**Females**
RBC	(125 mg/kg)	↓ (9%)	↓ (13%)
Hb	(125 mg/kg)	↓ (10%)	↓ (11%)
HCT	(125 mg/kg)	↓ (10%)	↓ (12%)
Platelets	(125 mg./kg)		↓ (25%)

Chemistry		**Males**	**Females**
SDH	(125 mg/kg)	↓ (38%)	
BUN	(125 mg/kg)		↑ (44%)

Organ wts		**Male**	**Female**
Thymus	(abs)	↓ 48% at 125 mg/kg	↓ 47% at 125 mg/kg
	(rel)	↓ 42% at 125 mg/kg	↓ 43% at 125 mg/kg
Liver	(abs)		↑ 24% at 125 mg/kg
	(rel)	↑ 25% at 125 mg/kg	↑ 34% at 125 mg/kg Adrenals (rel)
			↑ 50% at 125 mg/kg
Spleen	(rel)	↑ 36% at 125 mg/kg	
Testis	(rel)	↑ 16% at 125 mg/kg (not significant)	
		↑ 6% at 30 mg/kg	

Histopath: Thymus, both sexes at 125 mg: lymphoid reduction 14/20)
Spleen males at 125 mg: fibrous foci (6/10)
Bone marrow at 125 mg/kg: focal fibrosis (1/10M, 2/10F)
Sperm morphology unaffected

Conclusion

LOAEL 30 mg/kg/day
Drivers of LOAEL: (30 mg/kg/day)
Male
Terminal BW ↓, Rel testes wt ↑

Female
Terminal BW ↓,

Reliability

1 - Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

Mobil, 1994. Thirteen-Week Dermal Administration of Paulsboro Heavy Coker Gas Oil. Final Report on Study 50391 from Mobil Environmental and Health Science Laboratory, Princeton, NJ.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZQ.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Heavy coker gas oil (HCGO)

Test Substance Purity/Composition and Other Test Substance Comments: Heavy coker gas oil (CRU No. 86272)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348 ZR (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86272	16.20	0.32	4.86	8.10	1.62	0.32	0.16	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 days/ week
Doses	8, 30 and 125 mg/kg/day
No. of animals/dose	10/sex/dose
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1995
GLP	Yes
Test substance	Heavy Coker Gas Oil, Sample 86272, CAS 64741-81-7
Post exposure period	None

Method/Guideline and Test Condition Remarks: Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off as thoroughly as possible.

Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, thyroid, ovaries, uterus, and brain.

The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin:

Adrenals*, esophagus, head (entire), kidneys*, liver *(part of median and right, lateral lobes), pituitary, skeletal muscle*, spleen*, thymus*, tongue and larynx, bone with marrow *(rib sternum, femur), heart* and aorta lachrymal glands, lungs* and bronchi, lymph nodes, cervical mammary gland (with skin), prostate and seminal vesicles*, stomach* (glandular and squamous), uterus* (cervix, corpus, and horns), brain*, eyes* and optic nerve intestine, large* (cecum, colon and rectum), lymph nodes, mesenteric lymph nodes, draining ovaries* and oviducts, salivary glands* (major), spinal cord (cervical, thoracic), thyroid* and parathyroids, trachea, epididymides*, Harderian glands, intestine, small *(duodenum, ileum, jejunum) gross lesions*, pancreas*, sciatic nerve, skin (treated)*, testes*, urinary bladder*, vagina.

NOTE: From all animals, a sample of the right kidney and of the median lobe of the liver were fixed in a formaldehyde-glutaraldehyde mixture (4% and 1%, respectively, in an aqueous buffer).

Tissues marked with an (*) were processed for microscopic examination from all animals in the control group and highest dose group (125 mg/kg). In addition, the skin and thymus from the 30 mg/kg and skin from the 8 mg/kg group were processed. Sections for examination were stained with hematoxylin and eosin, or any special stain deemed necessary. Microscopic examinations were performed by a pathologist.

The left epididymis and testis from the control and 125mg/kg/day male rats were examined. Prior to sample preparation of the testis for examination, the tunica albuginea and corresponding blood vessel were removed and discarded The resulting testicular parenchyma and the cauda epididymis were individually weighed (nearest 0.001 gram) and the weight recorded. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and morphology.
For sperm motion analyses, the left vas deferens was immediately excised from each male and the sperm contents were removed and placed into a buffered solution and incubated. Following incubation, an aliquot of the prepared sample was placed on a siliconized slide and allowed to equilibrate. A minimum of eight fields per sample were videotaped and subsequently analyzed. Characteristics of sperm motion analyzed included percent motile sperm, curvilinear velocity, and linearity.

Statistical analysis: Quantitative data (body weight), serum chemistry, hematology, and organ weight data) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnett's test (body weights) and Tukey's Multiple Comparison Test (serum chemistry, hematology and organ weight data), provided that there was statistical significance in ANOVA. 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% ($p<0.05$).

NOAEL/LOAEL

Males: NOAEL = 30 mg/kg/day
LOAEL = 125 mg/kg/day

Females: NOAEL = 30 mg/kg/day
LOAEL = 125 mg/kg/day

Result

Clinical Limited signs of intoxication in a couple of animals
Skin irritation Moderate to severe
Body wt gain Reduced 20% in males only at 125 mg/kg

Hematology	**Males**	**Females**
Effects only at 125 mg/kg as follows:		
RBC	↓ 13%	
Hb	↓ 11%	↓ 8%
HCT	↓ 10%	
Platelets	↓ 32%	↓ 18%
WBC		↑ 31%
Seg. Neutrophils		↑ 94%
Lymphocytes		↓ 13%

Serum chemistry	**Males**	**Females**
Effects only at 125 mg/kg as follows:		
BUN	↑ 43%	↑ 57%
K		↓ 13%
SDH		↑ 60%

Organ wts		Males	Females
Effects only at 125 mg/kg as follows:			
Liver	(Abs)		↑ 18%
	(Rel)	↑ 24%	↑ 26%
Thymus	(Abs)	↓ 35%	↓ 36%
	(Rel)		↓ 32%

Histopath Thymus at 125 reduction in thymocytes
Bone marrow at 125 increased granulocytes
Sperm morphology OK
Urinalysis No effects
Mobil report of study 64148

Conclusion LOAEL 125 mg/kg/day

Reliability - 1 - Reliable without restrictions
-

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference

Mobil, 1995. Thirteen-Week Dermal Administration of Torrance Heavy Coker Gas Oil to Rats. Final Report on Study 64184 from Mobil Environmental and Health Science Laboratory, Princeton, NJ.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZR.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-81-7

Test Substance (CAS #): 64741-81-7; Heavy Coker Gas Oil (F-136)

Test Substance Purity/Composition and Other Test Substance Comments : No information available

Category Chemical Result Type : Measured

Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	0.01, 0.1, 1.0 ml/kg/day (9.3, 93, 930 mg/kg/day)
Control group	Yes, untreated
Method/Guideline followed	
Year	1992
GLP	Yes
Test substance	Coker, heavy gas oil (F-136) CAS 64741-81-7
Post exposure period	None
Method/Guideline and Test Condition Remarks	Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-136 dermally once daily, five days per week for four weeks, at a dose of .01, 0.1, 1.0 ml/kg/day (9.3, 93, 930 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. A fourth group of ten male and ten female rats served as a control. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated). All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs*

(perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 93 mg/kg/day (0.1 ml/kg/day)*
LOAEL = 930 mg/kg/day (1.0 ml/kg/day)

Females: NOAEL = 93 mg/kg/day (0.1 ml/kg/day)*
LOAEL = 930 mg/kg/day (1.0 ml/kg/day)

*Authors indicate "NOEL"

Result Remarks

Clinical:
Skin irritation: Very slight – Slight, dose-related

Mortality

Males
10% 9.3 & 930 mg/kg/d**ay****
Females
None

Body wt., terminal

Males
No difference

Females
No difference

Organ weights

Liver, Abs
Males
↑ 930 (27%) mg/kg/day
Females
↑ 930 (27%) mg/kg/day

Liver, rel brain
Males

	↑ 930 (28%) mg/kg/day **Females** ↑ 930 (32%) mg/kg/day	
Liver, rel BW	**Males** ↑ 930 (30%) mg/kg/day **Females** ↑ 93 (11%)**, 930 (32%) mg/kg/day	
Hematology	**Males**	**Females**
Hematocrit	↓ 930 (4%) mg/kg/day**	↓ 930 (9%) mg/kg/day**
Hb	No difference	↓ 930 (6%) mg/kg/day**

Serum chemistry

BUN
Males
↑ 9.3 (18%), 93 (21%), 930 (19%) mg/kg/day**
Females
No difference

Cholesterol
Males
No difference

Females

↑ 930 (59%) mg/kg/day

Histopath (sham controls & high dose)

Males

No test article-related systemic findings

Females

No test article-related systemic findings

Testes – normal; Ovaries – normal

Note: ** not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Male (930 mg/kg/day)
Liver wts; Hematocrit

Female (930 mg/kg/day)
Liver wts; Hematocrit, Hb; Cholesterol

Reliability

1 – Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

ARCO. 1992. Twenty-eight day dermal toxicity study in rats administered test article F-136. Report no. Study No. ATX-900098.

Repeated Dose Toxicity

Test Substance

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Visbreaker Gas Oil (VGO); V.B. Mittelol

Test Substance Purity/Composition and Other Test Substance Comments: Visbreaker Gas Oil (CRU No. 86193)

PAC(Polycyclic Aromatic Compound) Content – report no. 64348 ZT (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%) [2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86193	4.20	0.84	2.94	0.38	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 days/week
Doses	0, 8, 30, 125 mg/kg/day
No. of animals/dose	10/sex/dose
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Visbreaker Gas Oil , Sample 86193, CAS 64741-81-7
Post exposure period	None

Method/Guideline and Test Condition Remarks

Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off as thoroughly as possible.

Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. For the control and high dose groups, the following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, thyroid, ovaries, uterus, brain.

The following tissues (when present) from the 0, and 125 mg/kg/day group were processed for microscopic examination:prostate seminal vesicles, epididymis), adrenals, lymph nodes, femur with brain (cerebellum, cerebrum, medulla pons), eye (left) and optic nerve (left), heart, duodenum, colon, kidneys, liver, lung (left lobe) , pancreas, skeletal muscle, salivary glands, pituitary, peripheral nerve, skin* (treated), spinal cord, spleen, sternum with bone marrow, testis (right), ovaries, stomach, thymus, thyroid, uterus, urinary bladder, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. Those tissues marked with (*) were also processed and examined by a qualified pathologist for animals in the 8 and 30 mg/kg/day group.

The left epididymis and testis from the control and 125 mg/kg/day male rats were examined. Prior to sample preparation of the testis for examination, the tunica albuginea and corresponding blood vessel were removed and discarded The resulting testicular parenchyma and the cauda epididymis were individually weighed (nearest 0.001 gram) and the weight recorded. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and morphology.

Statistical analysis: Quantitative data were analyzed for homogeneity of variance (ANOVA), and associated f-test followed by Dunnett's Test (body weights) or Tukey's multiple comparison test (organ weights and hematolology). Differences between control and treated lroups were considered statistically significant only if the probability of the differences being due to chance is less than 5% ($p<0.05$).

NOAEL/LOAEL

NOAEL = 125 mg/kg
LOAEL = >125 mg/kg

Result

Clinical	No findings
Skin irritation	Slight in all treated groups
Body wt gain	No effects
Hematology	No effects
Chemistry	No dose-related findings
Organ wts	No dose-related findings
Histopath	Skin only findings and non-specific reactive hyperplasia in lymph nodes in most instances No effects on sperm morphology

Conclusion

LOAEL > 125 mg/kg/day

Reliability

- 1 - Reliable without restrictions

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

Yes

Reference

Mobil, 1992. Thirteen-Week Dermal Administration of Visbreaker Gas Oil to Rats. Final Report on Study 63237 from Mobil Environmental and Health Science Laboratory, Princeton, NJ

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Visbreaker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348 ZT.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances."

http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

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Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	68476-33-5
Test Substance (CAS #):	68476-33-5; Heavy Fuel Oil (F-92-01)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 4 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	28 days/4 weeks
Frequency of treat.	Daily, 5 days/week for 4 weeks
Doses	0.5, 1.0, 2.0 ml/kg/day (480, 960, 1920 mg/kg/day)
No. of animals/dose	10/sex/dose
Control group	Yes, untreated
Method/Guideline followed	Other
Year	1988
GLP	Yes
Test substance	Heavy Fuel Oil (F-92-01) CAS 68476-33-5
Post exposure period	None

Method/Guideline and Test Condition Remarks

Three groups of ten male and ten female young adult albino Sprague-Dawley rats were administered F-92-01 dermally once daily, five days per week for four weeks, at a dose of 0.5, 1.0, 2.0 ml/kg/day (480, 960, 1920 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. A fourth group of ten male and ten female rats served as a control. The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks.

The animals were observed twice daily for signs of toxicity and viability. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined three times per week during the study (Mondays, Wednesdays and Fridays) and just prior to necropsy.

At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white

blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), NG ratio (calculated).

All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, kidneys, liver, testes, and ovaries. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist.

Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were 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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Mean Draize irritation scores were plotted by group and time. The nonparametric procedures described above were used on this irritation data when appropriate. Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL <480 mg/kg/day (0.5 ml/kg/day)
LOAEL = 480 mg/kg/day (0.5 ml/kg/day)
Females: NOAEL <480 mg/kg/day (0.5 ml/kg/day)
LOAEL = 480 mg/kg/day (0.5 ml/kg/day)

Result remarks

Clinical:
Skin irritation: Mild 1920 mg/kg/day

Mortality	*Males*	*Females*
	None	None

Body wt., terminal	**Males**	**Females**
	↓ 1920 (6%) mg/kg/day	No difference

Organ weights

Liver, Abs	**Males** ↑ 480 (12%), 1920 (16%) mg/kg/day **Females** 480 (21%), 960 (38%), 1920 (21%) mg/kg/day
Liver, rel bw	**Males** ↑ 480 (12%), 960 (15%), 1920 (24%) mg/kg/day **Females** ↑ 480 (19%), 960 (28%), 1920 (25%) mg/kg/day
Liver, rel brain	**Males** ↑ 480 (15%), 960 (14%), 1920 (17%) mg/kg/day **Females** ↑ 480 (20%), 960 (29%), 1920 (22%) mg/kg/day
Kidney, Abs	**Males** ↓ 1920 (8%) mg/kg/day** **Females** No difference
Spleen, Abs	**Males** ↑ 480 (22%), 960 (22%), 1920 (21%) mg/kg/day **Females** ↑ 960 (21%) mg/kg/day
Spleen, rel bw	**Males** ↑ 480 (22%), 960 (28%), 1920 (28%) mg/kg/day **Females** ↑ 960 (24%), 1920 (19%) mg/kg/day
Spleen, rel brain	**Males** ↑ 480 (25%), 960 (25%), 1920 (22%) mg/kg/day **Females** ↑ 480 (21%), 960 (27%) mg/kg/day

Hematology

RBC	**Males** ↓ 960 (12%), 1920 (8%) mg/kg/day **Females** ↓ 480 (8%), 960 (8%), 1920 (7%) mg/kg/day
Hematocrit	**Males** ↓ 480 (6%), 960 (11%), 1920 (8%) mg/kg/day

Females

	↓ 480 (8%), 960 (8%), 1920 (7%) mg/kg/day
Hb	**Males** ↓ 480 (6%), 960 (11%), 1920 (8%) mg/kg/day **Females** ↓ 480 (11%), 960 (10%), 1920 (9%) mg/kg/day
Eosinophils	**Males** No difference **Females** ↓ 960 (91%) mg/kg/day**

Serum chemistry

BUN	**Males** ↑ 960 (27%), 1920 (18%) mg/kg/day** **Females** ↑ 1920 (19%) mg/kg/day**
Glucose	**Males** No difference **Females** ↑ 1920 (12%) mg/kg/day**
SGPT	**Males** ↓ 1920 (18%) mg/kg/day**

Females
No difference

Histopath (sham controls & high dose)

Males
No test article-related systemic findings

Females
No test article-related systemic findings

Testes – normal; Ovaries – normal

Note: ** not considered by study directors to be compound-related and/or biologically relevant

Conclusion

Effects defining LOAEL:

Male 480 mg/kg/day (0.5 ml/kg/day)
Liver wts; Spleen wts; Hematocrit; Hb

Female 480 mg/kg/day (0.5 ml/kg/day)
Liver wts; Spleen wt (rel brain); RBC; Hematocrit; Hb

Reliability - 1 - Reliable without restrictions
-

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference ARCO. 1986. Twenty-eight day dermal toxicity study in rats administered test article F-92-01. Report no. ATX-86-0090.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #): 64741-62-4

Test Substance (CAS #): 64741-62-4; Clarified Slurry Oil (CSO): Petrobase

Test Substance Purity/Composition and Other Test Substance Comments :

Clarified Slurry Oil; (F-179)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091645 (F-179)		0.00	0.70	10.0	30.00	20.00	6.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is “aromatic ring class”. “ARC 1 (%)” is the weight percent of PACs that have 1 aromatic ring within the total sample. “ARC 2 (%)” is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	90 days/13 weeks
Frequency of treat.	Daily, 5 days/week
Doses	0.001, 0.01, 0.05, 0.1, 0.5 ml/kg/day (1.06, 10.6, 53, 106, 530 mg/kg/day)
No. of animals/dose	20 animals/sex/group
Control group	Yes, untreated
Method/Guideline Followed	Other
Year	1993
GLP	Yes
Test substance	Cat. Cracked Slurry Oil (F-179) CAS 684741-62-4
Post exposure period	None
Method/Guideline and Test Condition Remarks	Five groups of twenty male and twenty female young adult albino Sprague-Dawley rats were administered F-179 dermally once daily, five days per week for 13 weeks, at doses of 0.001, 0.01, 0.05, 0.1, 0.5 ml/kg/day (1.06, 10.6, 53, 106, 530 mg/kg/day). The test article was applied to previously clipped sites on the backs of the animals. The site of application was occluded for a period of approximately six hours following application of the test article. The skin was then wiped to remove residual material. One additional group of twenty male and twenty female rats served as controls (untreated). The backs of the control group animals were clipped and the occlusive wrap was applied daily, five days per week, for four weeks. Animals were observed twice daily for viability and daily for signs of toxicity. Dermal irritation at the site of application was evaluated daily just prior to the application of the test article, twenty-four hours after the fifth weekly application and just prior to necropsy. Body weights were determined weekly during the study and just prior to necropsy. Feed consumption was determined weekly beginning at week 6. At the time of necropsy, blood was collected for hematology and clinical chemistry evaluations. Measured hematological parameters were hematocrit, hemoglobin, number of red blood cells, platelets and the number and differential count of white blood cells, and mean corpuscular volume (MCV). The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatinine, glucose, total protein, triglycerides, urea nitrogen, calcium, chloride, iron, phosphorus, potassium, and sodium, globulin (calculated), A/G ratio (calculated). All animals were then killed and necropsied. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, spleen, thymus, testes, and ovaries. Paired tissues were weighed together to obtain a total weight. The following organs were preserved in 10% neutral buffered formalin for possible histological evaluation: accessory genital organs (prostate seminal vesicles, epididymis), adrenals*, aorta, cecum, cervical lymph nodes*, esophagus, femur with articular surface, ileum*, bone and marrow, brain (cerebellum, cerebrum, medulla pons)*, eyes and optic nerve, gonads, heart*, duodenum*, jejunum*, mammary glands, colon*, kidneys*, liver*, lungs* (perfused) with trachea , pancreas*, skeletal muscle, salivary glands*, rectum*, pituitary, peripheral nerve, skin* (untreated and treated), spinal cord, spleen*, sternum with bone marrow*, testes*, ovaries*, stomach*, thymus*, thyroid*, parathyroid glands, uterus, vagina, urinary bladder*, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. For the control and high dose groups, those tissues marked with (*) were stained and sectioned for examination by a qualified pathologist Clinical pathology data, terminal organ weights, and organ to body weight ratios were statistically analyzed. Statistical evaluations of equality of means were

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 have equal variance at the 1 percent level of significance. If the variances are equal, the testing were 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 are indicated, Dunnett's test were used to determine which treatment groups differ significantly *from* control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression was also test for linear lack of fit in the model.

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

Sexes were analyzed separately. All ratios were transformed by the arc sine transformation and Cochran's transformation to stabilize variances. 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.

NOAEL/LOAEL

Males: NOAEL = 1.06 mg/kg/day (0.001 ml/kg/day)*
LOAEL = 10.6 mg/kg/day (0.01 ml/kg/day)

Females: NOAEL = 1.06 mg/kg/day (0.001 ml/kg/day)*
LOAEL = 10.6 mg/kg/day (0.01 ml/kg/day)

*Authors indicate "NOEL".

Result Remarks

Clinical:
Skin irritation: None

Mortality	*Males*	*Females*
	↑ 530 (35%) mg/kg/day	↑ 530 (10%) mg/kg/day

Body wt., terminal	**Males**	**Females**
	↓ 530 (12%) mg/kg/day	No difference

Organ weights

Brain, Abs
Males
↓ 530 (7%) mg/kg/day**
Females
No difference

Liver, Abs
Males
↑ 53 (19%), 106 (31%), 530 (21%) mg/kg/day
Females
↑ 53 (23%), 106 (26%), 530 (45%) mg/kg/day

Liver, rel bw
Males
↑ 10.6 (11%), 53 (24%), 106 (35%), 530 (37%) mg/kg/day

Females
↑ 10.6 (13%), 53 (23%), 106 (29%), 530 (53%) mg/kg/day

Liver, rel brain
Males
↑ 53 (25%), 106 (36%), 530 (29%) mg/kg/day

	Females
	↑ 53 (24%), 106 (25%), 530 (49%) mg/kg/day
Kidney, Abs	**Males**
	↓ 530 (14%) mg/kg/day**
	Females
	No difference
Kidney, rel brain	**Males**
	↑ 106 (13%) mg/kg/day**
	Females
	No difference
Spleen, Abs	**Males**
	↑ 53 (21%), 106 (39%) mg/kg/day**
	Females
	No difference
Spleen, rel bw	**Males**
	↑ 53 (22%), 106 (39%) mg/kg/day**
	Females
	No difference
Spleen, rel brain	**Males**
	↑ 53 (26%), 106 (45%) mg/kg/day**
	Females
	↑ 53 (16%) mg/kg/day**
Thymus, Abs	**Males**
	↓ 530 (43%) mg/kg/day
	Females
	↓ 530 (56%) mg/kg/day
Thymus, rel bw	**Males**
	↓ 530 (43%) mg/kg/day
	Females
	↓ 530 (50%) mg/kg/day
Thymus,rel brain	**Males**
	↓ 530 (40%), mg/kg/day
	Females
	↓ 530 (55%) mg/kg/day
Lungs, Abs	**Males**
	↑ 53 (15%), 106 (14%) mg/kg/day
	Females
	↑ 53 (12%), 106 (14%), 530 (16%) mg/kg/day
Lungs, rel bw	**Males**
	↑ 53 (21%), 106 (19%), 530 (19%) mg/kg/day
	Females
↑ 10.6 (9%), 53 (13%), 106 (18%) 530 (23%) mg/kg/day	
Lungs, rel brain	**Males**
	↑ 53 (19%), 106 (18%), 530 (14%) mg/kg/day
	Females
	↑ 53 (14%), 106 (14%), 530 (19%) mg/kg/day
Heart, Abs	**Males**
	↑ 106 (16%) mg/kg/day**
	Females
	No difference
Heart, rel bw	**Males**
	↑ 53 (17%), 106 (20%), 530 (17%) mg/kg/day**
	Females
	↑ 530 (11%) mg/kg/day**
Heart, rel brain	**Males**
	↑ 53 (15%), 106 (20%), mg/kg/day**
	Females
	No difference

Hematology

RBC	**Males** ↓ 53 (9%), 106 (18%), 530 (30%) mg/kg/day **Females** ↓ 106 (10%), 530 (22%) mg/kg/day
Hematocrit	**Males** ↓ 53 (9%), 106 (18%), 530 (36%) mg/kg/day

Females

	↓ 53 (7%), 106 (13%), 530 (25%) mg/kg/day
Hb	**Males** ↓ 53 (9%), 106 (16%), 530 (28%) mg/kg/day **Females** ↓ 53 (6%), 106 (11%), 530 (22%) mg/kg/day
Platelets	**Males** ↓ 10.6 (20%), 53 (26%), 106 (34%), 530 (43%) mg/kg/day
	Females ↓ 106 (29%), 530 (55%) mg/kg/day
Total WBC	**Males** No difference
	Females ↑ 10.6 (29%), 53 (45%) mg/kg/day**

Serum chemistry

BUN	**Males** ↑ 53 (61%), 106 (85%), 530 (80%) mg/kg/day **Females** ↑ 106 (31%), 530 (31%) mg/kg/day
Creatinine	**Males** ↑ 530 (14%) mg/kg/day **Females** ↑ 53 (8%), 106 (12%), 530 (9%) mg/kg/day
Albumin	**Males** ↓ 106 (11%) mg/kg/day** **Females** No difference
SGOT	**Males** ↑ 530 (111%) mg/kg/day **Females** ↑ 1.06 (9%)**, 530 (29%) mg/kg/day
Cholesterol	**Males** ↑ 53 (71%), 106 (84%), 530 (61%) mg/kg/day **Females** ↑ 53 (61%), 106 (87%), 530 (103%) mg/kg/day
Triglycerides	**Males** ↑ 53 (66%), 106 (130%) mg/kg/day** **Females** ↑ 10.6 (26%) mg/kg/day**
Sodium	**Males** ↑ 106 (8%), mg/kg/day** **Females** ↑ 10.6 (6%), 53 (7%), 106 (7%), 530 (7%) mg/kg/day**
Phosphorous	**Males** ↑ 106 (14%) mg/kg/day** **Females** No difference
Total Protein	**Males** No difference **Females**

↑ 106 (7%) mg/kg/day**

Alk. Phos. **Males**
No difference
Females
↑ 530 (93%) mg/kg/day

Glucose **Males**
No difference
Females
↑ 106 (14%), 530 (14%) mg/kg/day**

Histopath (sham controls & high dose)
Adrenal glands, bone, bone marrow, thyroid glands, treated skin, liver, lungs, thymus gland and gross lesions were examined from rats in the 1.06, 10.6, 53, 106 mg/kg/day groups

Systemic effects

Bone marrow, cellular depletion
Males
↑ 106 (10%), 530 (40%) mg/kg/day
Females
↑ 106 (5%), 530 (25%) mg/kg/day

Liver, congestion/necrosis/vacuolar change

Males
↑ 53 (10%),106 (20%), 530 (45%) mg/kg/day
Females
↑ 53 (5%), 106 (5%), 530 (15%) mg/kg/day

Thymus; atrophy

Males
↑ 10.6 (5%), 53 (26%), 106 (25%) 530 (63%) mg/kg/day

Females
↑ 106 (30%), 530 (94%) mg/kg/day

Thyroid, chronic inflammation (lymphocytic thyroiditis)

Males
↑ 53 (22%), 106 (11%), 530 (38%) mg/kg/day
Females
↑ 53 (18%), 106 (11%), 530 (15%) mg/kg/day

Testes – normal; Ovaries – normal

Note: **** not considered by study directors to be compound-related and/or biologically relevant

Conclusion **Effects defining LOAEL:**

Male 10.6 mg/kg/day (0.01 ml/kg/day)
↑ Liver wt, rel. bw; ↓ platelets, ↑ thymic atrophy

Female 10.6 mg/kg/day (0.01 ml/kg/day)
↑ Lung wt, rel. bw; Liver wt rel bw

Reliability - 1 - Reliable without restrictions
-

Reliability remarks Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor Yes

Reference

ARCO. 1993. Ninety day (90) dermal toxicity study in rats administered test article F-179. Report no. ATX-910012.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

Repeated Dose Toxicity

Test Substance

Category Chemical:

64741-62-4

Test Substance:

64741-62-4

Test Substance Purity/Composition and Other Test Substance Comments:

Clarified oils (petroleum), catalytic cracked (CRU No. 86484). The test material synonym used in the study report is Syntower Bottoms

PAC (Polycyclic Aromatic Compound) Content – Report No. 64348 ZM (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%) [2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86484	48.80	0.00	0.98	9.76	19.52	9.76	4.88	0.98

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type: Measured

Type	Repeated dose; 90 day (13 week) dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 days/week
Doses	8, 30, 125, 500
No. of animals/dose	10/sex/dose
Control group	Yes two untreated control groups of 10 males and 10 females each
Method/Guideline followed	Other
Year	1988
GLP	Yes
Test substance	CAS 64741-62-4 Clarified oils (petroleum), catalytic cracked (CRU No. 86484). The test material synonym used in the study report is Syntower Bottoms

Post exposure period	None
Method/Guideline and Test Condition Remarks	Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test substance was wiped off as thoroughly as possible. Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin. All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, thyroid, ovaries, uterus, brain. The following tissues (when present) from the 0, 8 and 30 mg/kg/day group were processed for microscopic examination:prostate seminal vesicles, epididymis), adrenals*, lymph nodes*, femur with brain (cerebellum, cerebrum, medulla pons), eye (left) and optic nerve (left), , heart*, duodenum, colon, kidneys*, liver*, lung (left lobe) , pancreas, skeletal muscle, salivary glands, pituitary, peripheral nerve, skin* (treated), spinal cord, spleen, sternum with bone marrow*, testis (right), ovaries, stomach, thymus, thyroid, uterus, urinary bladder, and any gross lesions. Bone marrow smears (femur) were prepared, preserved and maintained. Those tissues marked with (*) were also processed and examined by a qualified pathologist for animals in the 125 and 500 mg/kg/day group. Statistical analysis: Quantitative data were analyzed for homogeneity of variance (ANOVA), and associated f-test followed by Dunnett's Test (body weights) or Tukey's multiple comparison test (organ weights and hematololology). Differences between control and treated lroups were considered statistically significant only if the probability of the differences being due to chance is less than 5% ($p<0.05$).
NOAEL/LOAEL	<u>Males</u> NOAEL = <8 mg/kg/day LOAEL = 8 mg/kg/day <u>Females</u> NOAEL = 8 mg/kg/day LOAEL = 30 mg/kg/day
Result	All of the 500 and 125 mg/kg/day males and the 500 mg/kg/day females died or were sacrificed prior to the scheduled necropsy. In addition, eight of the 125 mg/kg/day females and two of the 30 mg/kg/day males died or were sacrificed prior to the scheduled necropsy. All of the remaining STB-exposed animals survived until the terminal sacrifice. The Group 2 (untreated control) animal were sacrificed. prior to the scheduled necropsy. along with the treated animals for comparison.

Although serum chemistry and organ weight data are given for the 125 mg/kg animals they are based on small numbers because of early termination/death, it follows that these data are to be treated with caution. Organ weight data for these animals are not included in this summary

Clinical signs ↓ food consumption, ↓ motor activity, dyspnea, pallor, petichiae, perineal staining.
Mortality 10/10 males and females died/killed at 500 mg
10/10 males, 8/10 females died/killed at 125 mg
2/10 males, 0/10 females died /killed at 30 mg

Body wt gains > 30 mg significantly less wt gain.
30 mg/kg: 8.8% less but not statistically significant

Hematol at 30 mg/kg	**Males**	**Females**	
	30 mg/kg	**30 mg/kg**	**125 mg/kg**
RBC	↓ 24%	-	↓ 56%
Hb	↓ 26%	-	↓ 58%
HCT	↓ 25%	-	↓ 57%
MCH	-	-	↓ 4%
MCHC	-	-	↓ 2%
Platelets	-	↓ 40%	↓ 90%
		NOTE: Also ↓ 20% at 8 mg/kg	

Serum chem.	**Males**	**Females**	
	30 mg/kg	**30 mg/kg**	**125 mg/kg**
BUN	↑ 134%		↑ 296%
Creatinine	↑ 26%		↑ 19%
Albumin	↓ 10%		
Cholesterol	↑ 45%		
AST	-		↑ 132%
ALT			↑ 68%
Alk phos.			↑ 72%
Triglycerides			↑ 236%
Bilirubin			↑ 75%
Cholesterol		↑ 61%	↑ 88%
Glucose		↑ 41%	↑ 30%
		NOTE:Also ↑ 24% at 8 mg/kg	
Potassium			↓ 20%
Cl	↑ 3%		
SDH			↑ 1075%

Organ wts		**Males**	**Females**	
		30 mg/kg	**30 mg/kg**	**125 mg/kg**
Thymus	(abs)	↓ 42%	↓ 43%	
	(rel)	↓ 39%	↓ 40%	
Liver	(abs)		↑ 26%	
	(rel)	↑ 27%	↑ 32%	
Heart	(rel)	↑ 26%		

Histopath Acute hemorrhage (petichae) in various tissues
Primary changes in bone marrow, liver, lungs at ≥30 mg

Conclusion

LOAEL 8 mg/kg based on decreased platelet count and increased glucose values in females - determined to be test material related. Therefore, NOAEL is less than 8 mg/kg.
Although serum chemistry and organ wt data for the 125 mg/kg animals are available. they are based on small numbers because of early termination/death, it follows that these data are to be treated with caution. Organ wt data for these animals are **NOT** included in this summary

Reliability	1 - Reliable without restrictions
Reliability remarks	Similar to guideline study; sufficient detail provided in appendices and tables.
Key study sponsor	Yes
Reference	Mobil, 1988. Thirteen-Week Dermal Administration of Syntower Bottoms to Rats. Final Report on Study 62710 from Mobil Environmental and Health Science Laboratory, Princeton, NJ. Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Syntower Bottoms. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZM. API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

Repeated Dose Toxicity

Test Substance

Category Chemical (CAS #):	64741-80-6
Test Substance (CAS #):	64741-80-6; Visbreaker Residue (86192)
Test Substance Purity/Composition and Other Test Substance Comments :	No information available
Category Chemical Result Type :	Measured
Type	Repeated dose; 13 week dermal exposure
Species	Rat
Sex	Male/Female
Strain	Sprague-Dawley
Route of admin.	Dermal
Exposure period	13 weeks
Frequency of treatm.	Daily, 5 days/week for 13 weeks
Doses	67% Mix of Visbreaker residue in Stock 141 at 60, 250 and 1000 mg/kg
Control group	Two: Untreated (Sham) and vehicle (Stock 141)
Method/Guideline followed	Other
Year	1992
GLP	Yes
Test substance	Visbreaker residue Sample 86192 CAS 64741-80-6 Administered as 67% concentration in Stock 141 (Highest concentration that could be delivered from a syringe.
Post exposure period	None
Method/Guideline and Test Conditions Remarks	Hair was clipped from the entire trunk of each animal within 24 hours prior to initial treatment; the clipping was repeated weekly throughout the study. The test substance was applied to the back with a syringe and dosing needle; the test substance was spread evenly over the site with the side of the dosing needle. The site was left uncovered and the rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test substance. Sham-exposed controls on the same procedure and schedule as the treated animals. Animals were dosed on 5 consecutive days per week. At 24 hours after the fifth dose, residual test

substance was wiped off as thoroughly as possible.

Endpoints during the biophase included twice daily observation of clinical signs (once over the weekends) and body weights measured weekly. Blood samples were obtained from animals (non-anesthetized) via the orbital venous sinus through a non-heparinized capillary tube, during weeks 5 and 13. Hematological parameters included hematocrit, hemoglobin, number and morphology of red blood cells, and the number and differential count of white blood cells. The following clinical chemistry parameters were analyzed: albumin, alkaline phosphatase, alanine aminotransferase (glutamic puruvic transaminases), aspartate aminotransferase (glutamic oxaloacetic transaminases), cholesterol, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides, urea nitrogen, uric acid, calcium, chloride, phosphorus, potassium, and sodium. Urine samples were also collected for analysis of specific gravity, pH, glucose, occult blood, ketone bodies, albumin, urobilogen, and bilirubin.

All animals were then killed and necropsied. The following organs were weighed: kidneys, adrenals, liver, heart, spleen, thymus, testes, prostate, epididymides, ovaries, uterus, and brain.

The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin:

Adrenals*, esophagus, head (entire), kidneys*, liver *(part of median and right, lateral lobes), pituitary, skeletal muscle*, spleen*, thymus*, tongue and larynx, bone with marrow *(rib sternum, femur), heart* and aorta lachrymal glands, lungs* and bronchi, lymph nodes, cervical mammary gland (with skin), prostate and seminal vesicles*, stomach* (glandular and squamous), uterus* (cervix, corpus, and horns), brain*, eyes* and optic nerve intestine, large* (cecum, colon and rectum), lymph nodes, mesenteric lymph nodes, draining ovaries* and oviducts, salivary glands* (major), spinal cord (cervical, thoracic), thyroid* and parathyroids, trachea, epididymides*, Harderian glands, intestine, small *(duodenum, ileum, jejunum) gross lesions*, pancreas*, sciatic nerve, skin (treated)*, testes*, urinary bladder*, vagina.

NOTE: From all animals, a sample of the right kidney and of the median lobe of the liver were fixed in a formaldehyde-glutaraldehyde mixture (4% and 1%, respectively, in an aqueous buffer).

Tissues marked with an (*) were processed for microscopic examination from all animals in the control group and highest dose group (125 mg/kg). In addition, the skin and thymus from the 30 mg/kg and skin from the 8 mg/kg group were processed. Sections for examination were stained with hematoxylin and eosin, or any special stain deemed necessary. Microscopic examinations were performed by a pathologist.

The left epididymis and testis from the control and 125mg/kg/day male rats were examined. Prior to sample preparation of the testis for examination, the tunica albuginea and corresponding blood vessel were removed and discarded The resulting testicular parenchyma and the cauda epididymis were individually weighed (nearest 0.001 gram) and the weight recorded. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and morphology.

Statistical analysis: Quantitative data (body weight), serum chemistry, hematology, and organ weight data) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnett's test (body weights) and Tukey's Multiple Comparison Test (serum chemistry, hematology and organ weight data), provided that there was statistical significance in ANOVA. 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% ($p<0.05$).

Sperm morphology and count also performed

NOAEL/LOAEL

NOTE: Authors indicate a NOAEL of 250 mg/kg
Determined by reviewer:
Males: NOEL = not determined (<60 mg/kg)
LOEL = 60 mg/kg/day

Females: NOEL = not determined (<60 mg/kg)
LOEL = 60 mg/kg/day

Result remarks

Clinical signs None
Skin irritation None related to compound
Body weight gain ↓ 17% in males at 1000 mg/kg
Hematology RBC (↓ 8%), HB (↓ 11%) and HCT (↓ 9%)in males only at 1000 mg/kg

Serum chemistry		**Males**	**Females**
BUN	1000 mg/kg	↓ 29%	↑ 27%
SDH	1000 mg/kg	↑ 102%	↑ 158%
	250 mg/kg		↑ 143%
ALT	1000 mg/kg	-	
	250 mg/kg	↓ 10%	
	60 mg/kg	↓ 12%	
Total protein	1000 mg/kg	↓ 7%	
Albumin	1000 mg/kg	↓ 10%	
Cl	1000 mg/kg	↓ 3%	
	250 mg/kg	↓ 3%	
Cholesterol	1000 mg/kg		↑ 54%

Organ weights		**Males**	**Females**
Liver (abs)	1000 mg/kg	↑ 23%	↑ 27%
	250 mg/kg	↑ 22%	
	60 mg/kg	↑ 16%	
Liver (rel)	1000 mg/kg	↑ 35%	↑ 30%
	250 mg/kg	↑ 22%	↑ 5%
	60 mg/kg	↑ 12%	↓ 2%%
Spleen (rel)	1000 mg/kg	↑ 23%	
Adrenals (abs)	1000 mg/kg	↑ 24%	
	250 mg/kg	↑ 19%	
	60 mg/kg	↑ 26%	
Adrenals (rel)	1000 mg/kg	↑ 33%	
Kidneys (rel)	1000 MG/KG	↑ 13%	

Histopath No findings in either sex
Sperm evaluation No effects

Conclusion

LOAELof 250 mg/kg was determined by author based on a variety of changes seen at 1000 mg/kg/day, including a decrease in male body weight. However, effects on the following were seen at doses as low as 60 mg/kg, but the results were judged not to be significant due to lack of histopathology:
ALT ↓, abs and relative liver weight ↑, absolute adrenal weight ↑,

Reliability

- 1 - Reliable without restrictions
-

Reliability remarks

Similar to guideline study; sufficient detail provided in appendices and tables.

Key study sponsor

es

Reference

Mobil, 1992. Thirteen-Week Dermal Administration of Visbreaker Residue to Rats. Final Report on study 64002 from Mobil

Environmental and Health Science Laboratory, Princeton, NJ.

-

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 CAS RN 64741-45-3
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) Gas Oil Category CAS RN 68915-97-9 Compositionally similar to Heavy Fuel CAS RN 68783-08-4

Vacuum Residues
No data

Vacuum Distillates CAS RN 64741-57-7
13 week study on Heavy Vacuum Gas Oil * (Ref. Mobil 61590)

Cracked residues CAS RN 64741-62-4
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 CAS RN 64741-81-7
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 CAS RN 68476-33-5
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	:	CAS RN 64741-45-3 sample F-132

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	:	CAS RN 68915-97-9 Gas Oil Category Heavy Atmospheric Gas Oil Compositionally similar to Heavy Fuel CAS RN 68783-08-4 ,

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.

<u>Hematology</u>

Red cell count	Hemoglobin
Hematocrit	White cell count
Platelet count	

<u>Clinical chemistry</u>

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 : CAS RN 64741-57-7 Heavy Vacuum gas oil

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:

Hematology

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 : CAS RN 64741-62-4 Clarified slurry oil

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 : CAS RN 68471-81-7 Visbreaker gas oil

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:

Hematology

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

Clinical chemistry

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 : CAS RN 68476-33-5 Residual fuel oil

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)

Repeated Dose Toxicity

TEST SUBSTANCE

Category Chemical:	64741-62-4
Test Substance:	64741-62-4; Catalytic Cracked Clarified Oil or Clarified Slurry Oil [CSO]
Test Substance Purity/Composition and Other Test Substance Comments:	Catalytic Cracked Clarified Oil (CRU No. 010929) PAC (Polycyclic Aromatic Compound) Content

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
010929	52.0	0.0	1.0	15.6	15.6	10.4	5.2	2.6

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).

	2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured

Type : Subchronic
Species : Rat
Sex : Male/Female
Strain : Sprague-Dawley Charles River Laboratories, Portage MI
Route of admin. : Dermal
Exposure period : 13 Weeks [90 days]
Frequency of treatm. : Daily, 5 days/week
Doses : 0, 5, 25, 50 mg/kg/day
No. of animals/dose : 10 animals/sex/dose
Control group : Yes, untreated [10M, 10F]; Vehicle - Acetone, min 99.0% pure [10M, 10F]
Method/Guideline followed : 1.5mL/kg
OPPTS Guideline 870.3250, 40 CFR 798.2250
OECD Guideline 411
Year : 2012
GLP : Yes
Test substance : Catalytic Cracked Clarified Oil, CRU No. 010929
Post exposure period : None
Method/Guideline and Test Condition Remarks: :

Prior to the initiation of dose administration, and throughout the study as necessary, the hair was clipped from the back (down each side to the ventral surface) and flanks of each animal using an electric clipper; a different set of clippers was used for the sham control group, the vehicle control group, and the test substance-treated groups to avoid potential cross-contamination. Animals were assigned to study groups using a computerized randomization procedure based on body weight stratification in a block design. Doses were based on a 14 day preliminary range finding study at dosage levels of 5, 25, and 100 mg/kg/day which were well tolerated. Dosage levels were selected to cover a range extending from a minimal dosage level to a dosage level which was likely to show signs of toxicity.

Vehicle or test substance was applied evenly to the clipped, unabraded area of skin and spread evenly using a glass rod (to ensure contact with an area of approximately 10% of the body surface area) once daily at doses of 0 [Groups 1, 2], 5, 25 50 mg/kg/day [Groups 3-5] 5 days/week for a minimum 90-day treatment period. No vehicle was applied to the sham control group. All animals wore Elizabethan collars during each 5-day dosing period. At the end of each dosing day, after an approximate 6-hour exposure period, all animals were gently wiped with a paper towel to remove unabsorbed test substance. At the end of each 5 day dosing period, residual test substance was gently removed (as much as possible without inducing irritation of the skin) from all animals using a warm water and mild soap solution (1% Ivory liquid soap in tap water) followed by a deionized water rinse and drying of the animals with a clean paper towel. Following each wash procedure, all animals were transferred to clean cages and the collars removed for a 2-day nondosing period. The mean area of coverage was 10% for males and females in the test substance-treated groups.

All animals were checked twice daily for general condition. Detailed physical examinations, body weight and food consumption measurements were done on a weekly basis. The sites of dose application were examined for dermal effects which were scored following the method of Draize (Draize, 1965) using the 4-step grading system

Samples for clinical pathology (hematology, coagulation and serum chemistry) were taken from all surviving animals. The animals were fasted overnight prior to blood collection. The animals were euthanized by inhalation of isoflurane, and the blood samples were taken from the vena cava as part of the gross necropsy. Parameters evaluated for hematology and coagulation included: total leukocyte count (WBC), erythrocyte count

(RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial thromboplastin time, reticulocyte count (percent, absolute, differential leukocyte count (percent and absolute: neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell), red cell distribution width, hemoglobin distribution width, platelet estimate and red blood cell morphology. The serum chemistry measurements included: albumin, total protein, globulin [by calculation], albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, triglycerides and sorbitol dehydrogenase.

A complete necropsy was conducted on all animals from scheduled necropsy or sacrificed during the study. Animals were anesthetized by isoflurane inhalation and euthanized by exsanguination. Nine animals [50mg/kg 5M, 3F and 25mg/kg 1F] were found dead or sacrificed *in extremis* during the study After sacrifice, organs were taken for weight and/or histological measurements included: adrenals, aorta, bone with marrow, femur with joint, bone marrow smear (sternum), brain (3 sections), cervix, epididymides, eyes with optic nerve, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), heart, kidneys, lacrimal gland, liver (sections of 2 lobes), lungs (including bronchi), lymph nodes (axillary, mandibular, mesenteric), ovaries with oviducts, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary glands, seminal vesicles, skeletal muscle, skin (with mammary gland), skin (treated and untreated skin from areas of dose application), spinal cord (cervical, thoracic, lumbar), spleen, testes, thymus, thyroid (with parathyroid), trachea, urinary bladder, uterus, vagina, and gross lesions. Weights were taken for the following organs: adrenals, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroid, and uterus. Slides were prepared from protocol specified tissue and stained with hematoxylin-eosin for microscopic examination.

NOAEL/LOAEL : NOAEL [No observed effect level] = 5mg/kg/day both sexes
LOAEL [Lowest observed effect level] = 25mg/kg/day both sexes.

Result remarks : Dosing formulation: The analyzed dosing formulations were found to contain 96.4% to 107% of the test substance which was within the WIL Research SOP range of target concentrations for suspensions (85% to 115%) and were homogeneous.

Mortality: Nine rats died or were sacrificed in a moribund condition prior to scheduled sacrifice and deaths were considered treatment related. Of these 8 (5 males and 3 females) were from the 50 mg/kg/day group and one 25mg/kg/day female All 9 had bone marrow depression and centrilobular hepatocellular atrophy and 5 had thrombosis in the heart and renal tubular necrosis.

Body weights: There was also evidence of reduced body weight gain. Terminal body weights of males in the 50 mg/kg/day group were approximately 18% below control values ($p < 0.01$). The body weights of males in the 25 mg/kg/day group were approximately 7% below control values but the difference was not statistically significant. Terminal body weights of females from the 25 and 50 mg/kg/day groups were about 5% below control values, but in both groups the differences were statistically significant. There was little evidence of dermal effects.

Clinical Observations: Test substance-related clinical observations for the surviving animals in the 25 and/or50 mg/kg/day group included pale extremities, reddened and/or swollen ears, and decreased defecation.

Hematology: Test substance-related alterations included lower absolute red blood cell counts, hemoglobin, hematocrit, absolute and relative eosinophil counts, and platelet counts, and higher absolute and relative reticulocyte counts, hemoglobin distribution width (HDW), and red blood cell distribution width (RDW) in all test substance-treated groups. Lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were noted at 25 and/or 50 mg/kg/day group females. Lower mean red blood cell counts, mean hematocrit, mean hemoglobin, mean platelet counts, and higher mean absolute and relative reticulocyte counts, higher RDW and higher HDW were noted in all test substance-treated

male and female groups. The changes were considered to be test substance-related because the changes showed a dose-response in the 5, 25, and 50 mg/kg/day group males and females, and were statistically significant for the 25 and 50 mg/kg/day group males and females when compared with the vehicle control group with the exception of higher mean absolute and relative reticulocyte counts in the 25 mg/kg/day group females. A lower MCH value was noted in the 50 mg/kg/day group females and lower MCV was noted in the 25 and 50 mg/kg/day group females; these changes were statistically significant compared to the vehicle control group. The relationship of these changes to administration of the test substance was uncertain because all individual animal values were within the historical control data reference range. However, the changes were possibly test substance-related given the presence of other test substance-related changes in erythrocyte parameters. Lower mean absolute eosinophil counts were noted in all test substance-treated groups. The change had a dose-response and was statistically significant in all test substance-treated groups when compared with the vehicle control group. Lower mean absolute basophil counts in the 25 and 50 mg/kg/day group males were statistically significantly different compared to the vehicle controls. However the relationship of the change to administration of the test substance was uncertain because all individual animal values were within the historical control data reference range. In the authors opinion the change was considered to be possibly test substance-related given the presence of other test substance-related hematology and bone marrow changes.
However, the reviewer considered that as the white blood cell changes at 5mg/kg were small, generally within historical control ranges, and showed inconsistencies in response between genders, were less likely to have been due to treatment than the effects on red blood cells and not definitive for establishing a NOAEL..

Serum Chemistry: There were higher urea nitrogen levels in all test substance treated groups with statistical significance achieved in the 50 mg/kg/day males. However, as the serum creatinine levels were within normal limits, the higher urea nitrogen levels were considered to have been an indication of dehydration. Other statistically significant findings included higher GGT levels in the 50 mg/kg/day males; higher mean cholesterol levels in the 50 mg/kg/day males and 25 and 50 mg/kg/day females, and lower triglyceride levels in the 50 mg/kg/day females.

Organ weights: Thymus weights, absolute and relative to body weight or brain weight were significantly reduced in both sexes in 25 and 50 mg/kg/day groups. Liver weights, absolute and relative were increased and were significantly different in the 25 and 50 mg/kg/day males and females. Spleen weights were significantly higher in 25 and 50 mg/g group females but the relationship to test article administration was uncertain because there was no correlating histologic changes and no consistent change in males in either group. Significant reductions in absolute brain weights in the 50 mg/kg/day males and absolute kidney weights in the 50 mg/kg/day females was reported, but the differences in kidney weights may have been due to the significant reductions in body weight gains in these groups as the differences were not significant when compared on a "relative to body weight" basis. Other organ weight differences were statistically significant when compared to the vehicle control group but were considered to be a result of a test substance-related effect on final body weight. These included: mean brain, testis, and thyroids and parathyroids weights relative to final body weight in test substance-treated males.

Histopathology: Test substance-related microscopic findings were noted in the bone marrow, liver, kidney, heart, spleen, lymph nodes, thymus, and Peyer's patch as lymphoid depletion, pituitary gland, adrenal cortex, exorbital lacrimal gland, testis, epididymis, ovary, and uterus of the 25 and/or 50 mg/kg/day groups.
In the bone marrow, there was multifocal to coalescing, minimal to severe depletion of hematopoietic cells characterized by decreased cellularity in all erythrocyte, leukocyte, and megakaryocyte cell lines, and increased prominence of bone marrow stromal cells. The bone marrow depletion was often associated with histologic changes in the liver including mild to moderate centrilobular hepatocellular atrophy characterized by loss of centrilobular hepatocytes, lobular collapse, occasional necrosis of scattered individual hepatocytes, and minimal inflammation. These liver changes were consistent with ischemic injury secondary to bone marrow depletion and anemia. One 50mg/kg/day male had atrophy and evidence of active injury, with centrilobular hepatocellular necrosis

characterized by small clusters of necrotic hepatocytes with hemorrhage. Vacuolation of hepatocytes in random areas was also noted in test substance-treated animals. Acute tubular necrosis, most predominantly in proximal renal tubules, was noted in the 50 mg/kg/day group males and females. This change was also consistent with ischemic damage secondary to bone marrow depletion and anemia. Increased amount of intracytoplasmic brown pigment was also observed in proximal renal tubules in test substance-treated males and females. Two males and 3 females in the 25 or 50 mg/kg/day groups had thrombi in the right atrium or ventricle of the heart. Lymphoid depletion was noted in the thymus, spleen, and/or mesenteric, axillary, and/or mandibular lymph nodes in the 25 and 50 mg/kg/day group animals, a change characterized by smaller lymphoid follicles of decreased prominence and scattered necrotic/apoptotic lymphocytes. Lymphoid depletion was also noted in the Peyer's patches in 2 of the 50 mg/kg/day group females. Increased severity of vacuolation of pars distalis in pituitary gland was noted in the 25 and 50 mg/kg/day group males. The vacuolated cells were arranged in individual to small clusters in these animals, compared to the more individualized and scattered population of vacuolated cells in the pituitary gland of the vehicle control group animals. Vacuolation of adrenal cortical cells was noted in the 25 and 50 mg/kg/day group females and males. Necrosis of adrenal cortical cells often associated with hemorrhage was noted in 1 male and 2 females in the 50 mg/kg/day group. Minimal to mild, diffuse bilateral atrophy of exorbital lacrimal glands was noted in 4 males and 2 females in the 50 mg/kg/day group. Higher incidence of sinus erythrocytosis was noted in the mesenteric lymph nodes in the50 mg/kg/day group males
There were also lesions in the male and female reproductive system including increased incidence of seminiferous tubular degeneration in the testis and hypospermia and luminal cellular debris in the epididymis of 3/10 males in the 50 mg/kg/day group. Scattered individual to small numbers of seminiferious tubules contained degenerated spermatogonia, spermatocytes, and/or spermatids with mild disorganization of maturation. One male in the vehicle control group also had seminiferous tubular degeneration and hypospermia; however, the changes were diffuse and unilateral, unlike those in the treated animals. Decreased corpora lutea, increased atretic follicles and atrophy of the uterus with increased prominence of stromal cells were noted in 6/10 of the 50 mg/kg/day females.

Conclusion

NOAEL [No observed effect level] = 5mg/kg/day both sexes
LOAEL [Lowest observed effect level] = 25mg/kg/day both sexes.
Based on increased liver weights, reduced thymus weights and hematologic changes at 25 and 50 mg/kg/day

Reliability : 1 – Reliable without restrictions

Reliability remarks : Conforms to standard US and OECD guidelines and GLPs. Sufficient detail provided in appendices and tables.

Key study sponsor : Yes

Reference : WIL Laboratories 2012. A 90-Day Repeat-Dose Dermal Toxicity Study Utilizing Clarified Oils, Catalytic Cracked in Sprague Dawley Rats. WIL Study # 402023. WIL Research Laboratories, LLC. 1407 George Road, Ashland, OH 44805-8946

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

5.5 GENETIC TOXICITY 'IN VITRO'

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE

Category Chemical:	**Heavy Fuel Oil Category**
Test Substance:	Various Heavy Fuel Oils
Test Substance Purity/Composition and Other Test Substance Comments:	The Heavy Fuel oils tested had total % DMSO extractable PAC contents ranging from approximately 2% in Atmospheric Residuals to 65% in Catalytic cracked stocks.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	

METHOD

Type of Study:	Optimized Ames Assay
System of testing	Salmonella typhimurium TA 98 with metabolic activation
Concentrations:	Various
Year Study Performed:	Various
Method/Guideline Followed:	Optimized Ames assay a modification of the Ames Assay
GLP:	Yes
Positive, Negative and Solvent Control Substance(s):	Various
Method/Guideline and Test Condition Remarks:	The method differed from the standard pre- incubation Ames assay in the following respects. A DMSO extract of the test materials was tested in the assay. The S9 fraction was obtained from Aroclor-induced hamsters. An eightfold concentration of S-9 was used in the assays. Twofold concentration of cofactor NADP was used. The DMSO extracts were tested over a range of concentrations that permitted the construction of a dose-response curve.

The mutagenicity index (MI) is calculated from the slope of the initial portion of the dose response curve expressed in units of revertants per microliter. The mutagenicity index has been demonstrated to be highly correlated with dermal carcinogenic potential, suggesting that oils with MI values < 1 were unlikely to be mutagenic or dermally carcinogenic, oils with MI values $\geq$ 1 but < 2 are mutagenic but indeterminate for dermal carcinogenesis, and oils with MI values $\geq$ 2 are mutagenic and would likely produce skin tumors if tested in mice. The test method was refined to provide the greatest predictive value of gene mutagenicity and potential carcinogenicity for the widest range of high boiling PAC-containing streams [final boiling point approximately ≥650^{0}F, (≥343^{0}C]and thus provides a more sensitive general *Salmonella* protocol for this class of petroleum substances. In 1995, the optimized Ames test was standardized as an ASTM method [ASTM E1687-95].

An assay was judged to be positive if the Mutagenicity Index was equal to or greater than 1.0

TEST RESULTS

Optimized Ames Test results and 1-7 ring PAC Distribution

CAS RN	CRU Number	1-Ring Weight %	2-Ring Weight %	3-Ring Weight %	4-Ring Weight %	5-Ring Weight %	6-Ring Weight %	7-Ring Weight %	Optimized Ames MI
64741-57-7	86281	0.0	0.6	3.6	2.7	1.8	0.7	0.1	11.2
64741-57-7	86010	0.0	0.1	1.3	1.9	1.9	1.3	0.0	7.8
64741-57-7	86179	0.0	0.5	1.0	3.1	2.1	1.0	2.1	7.0
64741-57-7	85244	0.0	0.1	2.5	1.9	1.2	0.5	0.0	5.6
64741-57-7	86176	0.0	0.6	0.9	2.6	1.7	0.9	1.7	5.3
64741-57-7	86189	0.0	0.1	0.2	0.6	1.2	2.5	1.2	3.2
64741-62-4	86196	0.0	1.5	22.5	30.0	15.0	7.5	1.5	860.9
64741-62-4	86185	0.0	1.9	25.5	19.1	12.7	5.1	0.6	774.8
64741-62-4	86001	0.0	2.6	25.7	19.3	6.4	3.2	0.6	739.0
64741-62-4	86002	0.0	1.9	12.3	24.7	12.3	6.2	1.2	726.2
64741-62-4	86180	0.0	1.3	12.7	25.4	12.7	6.4	1.3	688.1
64741-62-4	86066	0.0	0.5	10.5	21.0	10.5	5.3	1.6	555.4
64741-62-4	86015	0.0	0.3	6.2	12.5	9.4	6.2	1.2	466.4
64741-62-4	86484	0.0	1.0	9.8	19.5	9.8	4.9	1.0	437.8
64741-62-4	87279	0.0	0.8	6.1	6.1	4.0	2.0	0.6	168.7
64741-62-4	87278	0.0	0.9	9.1	9.1	6.1	3.0	0.9	167.7
64741-62-4	87277	0.0	0.4	3.8	5.7	5.7	3.8	0.8	141.8
64741-62-4	86123	0.1	4.0	4.0	2.7	2.7	1.2	0.3	33.7
64741-81-7	86181	0.2	2.5	12.4	7.4	2.5	0.5	0.0	142.7
64741-81-7	86161	0.0	0.7	6.0	4.5	3.0	1.5	0.3	122.6
64741-81-7	86272	0.3	4.9	8.1	1.6	0.3	0.2	0.0	111.7
64741-81-7	83366	0.1	2.5	5.1	2.5	1.3	0.9	0.1	89.1
64741-81-7	86194	0.0	0.5	3.2	4.8	4.8	1.6	0.5	76.2
64741-81-7	87213	0.1	4.2	6.3	0.3	0.0	0.0	0.0	13.3
64741-81-7	86230	0.3	2.0	2.7	1.4	0.4	0.1	0.0	3.5

68476-33-5	086104	0.0	1.5	7.3	2.9	1.3	0.6	0.1	84.8
68476-33-5	086108	0.3	2.7	2.7	0.9	0.9	0.7	0.3	21.9
68476-33-5	086119	0.0	2.6	2.6	1.8	0.9	0.6	0.2	8.0
68553-00-4	091674	0.1	2.6	5.2	1.3	1.3	1.3	0.9	23.1
68553-00-4	091675	0.3	6.1	4.6	1.5	0.8	1.5	0.9	17.5

Results Remarks: These data indicate that streams in the Heavy Fuel Oil category are generally mutagenic with the level of activity related to the PAC content and ring distribution profile. Test samples having the same CAS RN may have different mutagenic activity resulting from difference in the composition of starting crude oil and the type and severity of processing. Of this data set only 2 samples of CAS RN 64741-81-7 are not mutagenic [#86193 MI 0.7; #86198 MI 0.0]. Streams derived from Catalytically cracked stock which are higher in biologically PAC [e.g 64741-62-4, 64741-81-7] tend to show greater mutagenic activity.

Conclusion Remarks: Heavy Fuel Oils are considered gene mutations in the Optimized Ames assay

RELIABILITY/DATA QUALITY

Reliability: 1. Reliable without restrictions

Reliability Remarks: This assay is a modification of the Ames Salmonella Assay which has been verified by ASTM E1687-95

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Individual studies can be identified using the CRU number and requested from American Petroleum Institute

Original method publications are:

Blackburn, G.R. Deitch, R.A., Schreiner, C.A. and Mackerer, C.R. 1986. Predicting tumorigenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. Cell Biol. Toxicol 2: 63-84

Blackburn, G.R. Deitch, R.A., Schreiner, C.A. Mehlman, M., and Mackerer, C.R. 1984. Estimation of the dermal carcinogenic activity of petroleum fractions using a modified Ames assay. Cell Biol. Toxicol 1: 67-80

Roy, T.A., Johnson, S.W., Blackburn, G.R., and Mackerer, C.R. 1988. Correlation of mutagenic and dermal carcinogenic activities of mineral oils with polycyclic aromatic compound content. Fund. Appl. Toxicol. 10: 466-376.

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**

Heavy vacuum gas oil CAS RN 64741-57-7

Modified Ames assay	Positive with activation
Cytogenetics assay with Chinese Hamster Ovary cells	Negative with or without activation

Clarified slurry oil CAS RN 64741-62-4

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 Inappropriate test method Data considered unreliable

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

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 : CAS RN 64741-57-7 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 : CAS RN 64741-57-7 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 : CAS RN 64741-62-4 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 : CAS RN 64741-62-4 Catalytically cracked clarified oil (API 81-15)

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 10×10^{-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 40.8×10^{-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 : CAS RN 64741-62-4 Clarified slurry 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)
Cytotoxic concentr. :
Metabolic activation : With and without
Year : 1986
GLP : Yes
Test substance : CAS RN 64741-62-4 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 x 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 : CAS RN 64741-62-4 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 : CAS RN 64741-62-4 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 Aroclor 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)

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'

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE

Category Chemical: **Type in if not listed: 64741-62-4**

Test Substance: **Type in if not listed: 64741-62-4**

Test Substance Purity/Composition and Other Test Substance Comments: Catalytically cracker clarified oil [CCCO], derived from a naphthenic crude oil. Contains high levels of polycyclic aromatic constituents and is highly mutagenic or genotoxic in Salmonella test, mouse lymphoma TK+ test, Syrian Hamster embryo cell transformation test and unscheduled DNA synthesis test, potent inducer of skin tumors in the mouse epidermal carcinogenesis assay. [comments from publication authors.]

Category Chemical Result Type: Measured

METHOD

Type of Study: Cytogenetic

Type of Test: Micronucleus

Route of Administration:	Oral or intraperitoneal
Species:	Mice
Strain:	CD-1
Gender:	Male and female animals from Charles River Canada (Quebec, Canada)
Dose:	Intraperitoneal: 0 (corn Oil), 0.188, 0.375, 0.75, 1.5, and 3.0g/kg; Oral: range finder 0 (corn oil), 1.0, 2.0, 3.0 and 4.0g/kg. Initial comparative studies of oral and intraperitoneal routes: 0 (corn oil), 0.04 (oral only), 0.188, 0.375, 0.75, 1.50 (oral only) g/kg
Year Study Performed:	1999
Method/Guideline Followed:	Other, method of Schmid, 1975
GLP:	Not specified
Duration of Treatment/Exposure Period and Units:	2 days, 48 hour total exposure; one study also included a 48 hour post-treatment sacrifice.
Frequency of Treatment:	2 daily doses, sacrifice 24 hrs after last dose
Positive, Negative and Solvent Control Substance(s):	Positive control: Cyclophosphamide (CAS RN 60555-19-2; Aldrich Chemical) 0.04g/kg in water by gavage Comparative control: Dimethylbenz(a)anthracene (DMBA, CAS RN 57-97-6; Aldrich Chemical) 0.075, 0.15, 0.30g/kg in corn oil or mineral oil by gavage. Corn oil or mineral oil
Post-Exposure Period:	Not applicable
Number of Animals per Sex per Dose:	10 (5 males, 5 females)/group in initial study; subsequent studies 4 (2 males, 2 females)/group
Method/Guideline and Test Condition Remarks:	Male and female mice were employed in all studies and acclimated at least 17 days prior to initiation of study. Mice were approximately 6-9 weeks old and 17-35g at time of dosing, singly housed in wire mesh cages and received food and water *ad libitum*. The micronucleus study was conducted according to the method of Schmid, 1975. Both femurs were removed from each treated mouse, proximal ends were cut and bone marrow was aspirated with fetal bovine serum into a centrifuge tube. Cells were collected by centrifugation and slides were prepared. After fixation in methanol, slides were stained with acridine orange (Hayashi et al., 1983) for approx. 1-2 minutes and evaluated at 400X by fluorescence microscopy. A total of 1000 erythrocytes was counted for each mouse and the total number of polychromatic (PCE) and normochromatic (NCE) erythrocytes were tabulated. An equal distribution of PCE/NCE indicated the absence of test material-induced toxicity. One thousand PCEs were evaluated for the presence of micronuclei (MN). A range-finding study was performed to select doses for the initial oral gavage study. Five separate micronucleus studies were conducted. Initial studies used 10 (5 males, 5 females) mice/groupsubsequent studies used 4 (2 males, 2 females)/group. Test material was administered in 2 consecutive daily doses by gavage or intraperitoneally. Mice were sacrificed approximately 24 hours after the second administration except for one study that utilized a 48 hour cell collection to assure that delayed effects were not missed. Statistics: Standard one-way analysis of variance (ANOVA) at each time period, if significant comparisons of vehicle control to dosed group means were made by Duncan's Multiple range test. Regression analysis performed for dose response.

Residuals from ANOVA anlayzed for normality by Wilk's Criterion. Resdiulas were normally distributed (values > 0.01) in more than 75% of analyses, thus non-parametric analysis was not performed. Sexes were analyzed separately in the initial studies (10 (5 male, 5 female mice/group). In subsequent studies sexes were not separated due to small group size.

TEST RESULTS

Systemic Toxicity:

Mortality was observed at the highest doses in the range-finding study for CCCO and high doses in the DMBA study. See details below.

In the initial study CCCO was administered either orally or intraperitoneally. Orally CCCO was neither clastogenic or toxic to bone marrow cells at doses up to 1.5g/kg. The mean percentage of PCE/1000 total erythrocytes was 49-54, comparable to corn oil controls. To test whether the lack of effect in the oral study was related to the route of administration, CCCO was administered intraperitoneally. CCCO was neither clastogenic nor toxic at levels up to 0.75g/kg. There was no difference in response between collection of cells at 24 or 48 hours (data not shown). These data are summarized in Table 1.

Table 1. Effect of Route on Activity of CCCO in Micronucleus assay

Dose [g/kg, daily for 2 days]	No. of mice/group	MN-PCE/1000	
		Oral	Intraperitoneal
0 [corn oil]	10 (5M, 5F)	1.3	1.8
Positive control[a]	10 (5M, 5F)	9.6	-
0.188 CCCO	10 (5M, 5F)	1.8	0.3
0.375 CCCO	10 (5M, 5F)	1.8	1.3
0.75 CCCO	10 (5M, 5F)	3.0	2.0
1.50 CCCO	10 (5M, 5F)	2.3	-

a – Cyclophosphamide, 0.04g/kg in water
24 hr harvest. No statistically significant increases by either method.

Genotoxic Effect:

A subsequent study with intraperitoneal injection employed higher doses of CCCO at 0.75, 1.5 and 3.0g/kg with 4 (2 males, 2 females /group. Dose related decreases were seen in the frequency of PCE but no significant increase was seen in the frequency of micronucleated PCE at levels up to 3.0g/kg (Table 2) Bone marrow was affected by exposure to CCCO but there was no clastogenic response.

Table 2. CCCO IP injection study

Dose [g/kg, daily for 2 days] 24 hr harvest	No. of mice/group	% PCE (SD)/R[a]	MN-PCE /1000PCE (SD)
0 [corn oil]	4 (2M, 2F)	48.8 (2.7)	1.25 (1.0)
0.75 CCCO	4 (2M, 2F)	41.3 (10.1)	1.75 (1.3)
1.50 CCCO	4 (2M, 2F)	36.8 (7.7)	2.5 (1.7)
3.00 CCCO	4 (2M, 2F)	32.4 (10.1)	1.0 (0.8)

a- SD; R, statistically significant regression coefficient ($p<0.01$)

DMBA, a recognized clastogen and carcinogen, was evaluated to collect dose-reponse information and determine whether vehicles influence clastogenic response. Dose related increases in miconucleated-PCE were observed at doses of 0.075 to 0.3g/kg. Dilution in mineral oil reduced the level of clastogenic activity by 50% compared to the corn oil, suggesting a vehicle effect. To determine if the lack of activity with CCCO could be influenced by vehicle or matrix effects of other components of this complex mixture, a DMSO extract of polycyclic aromatic compounds from CCCO was tested at levels of 1.25, 2.5, and 5.0g/kg in 4 mice by oral gavage. Three of 4 mice died in the 5.0g/kg group and 1 of 4 in the 2.5g/kg group. The PAC fraction of CCCO did not induce cytogenetic effects at any dose level. These results indicate that a matrix effect of CCCO components is not wholly responsible for negative results but does not eliminate the possibility that interactive effects of PAC may retard or eliminate clastogenic activity.

Results Remarks:	DMBA and cyclophosphamide as comparative and positive control respectively induced the expected statistically significant increase in micronucleated polychromatic erythrocytes.
Conclusion:	Catalytically cracked clarified oil did not induce cytogenetic damage in the micronucleus assay in bone marrow of mice. These negative results were not due to reduced gastro-intestinal absorption, route of administration, collection time or matrix effect. CCCO is not a clastogen in this assay system.

RELIABILITY/DATA QUALITY

Reliability:	**1**. Reliable without restriction.
Reliability Remarks:	Adherence to GLPs was not specified in the publication but was not considered to invalidate a Reliability score of 1.
Key Study Sponsor Indicator:	

REFERENCE

Reference:	Przygoda, R.T., Mckee, R.H., Amoruso, M.A., and Freeman, J.J. 1999. Assessment of the utility of the micronucleus test for petroleum-derived materials. Mutation research 438: 145 – 153 Method references: Schmid, W. 1975. The micronucleus test. Mutation Research 31: 9-15. Hayashi, H., Sofuni, T., and Ishidate, M., Jr. 1983. An application of acridine orange fluorescent staining to the micronucleus test. Mutation Research 120: 241-247.

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	**:**	CAS RN 64741-57-7 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 methanol 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.

Heavy Vacuum Gas Oil 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 : CAS RN 64741-62-4 Catalytically cracked clarified oil (API 81-15)

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 1+	2+	Mitotic index
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 : CAS RN 64741-62-4 Clarified slurry oil, API 81-15.

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 : CAS RN 64741-62-4 Clarified slurry oil, API 81-15.

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)
CAS RN 64741-62-4 Clarified slurry oil undiluted 25µl 3 x week (40)	36/40 tumors	17		McKee et al (1990)
CAS RN 64741-62-4 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

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-61-3

Test Substance: 64741-61-3; FCCU Heavy Cycle Oil (HCO)

Test Substance Purity/Composition and Other Test Substance Comments: FCCU Heavy Cycle Oil (F-222)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091686 (F-222)		0.00	4.00	40.00	4.00	0.60	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is “aromatic ring class”. “ARC 1 (%)” is the weight percent of PACs that have 1 aromatic ring within the total sample. “ARC 2 (%)” is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Wilmington, MA)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	12 per dose at 50, 150, or 500 mg/kg dose level of test material 15 per dose for sham control
Concentration:	
Dose:	0, 50, 150, 500 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HCO (F-222) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. HCO 50 mg/kg/day – 12 animals (GD 0-20) 3. HCO 150 mg/kg/day – 12 animals (GD 0-20) 4. HCO 500.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The animals used in the study were between 12 and 13 weeks of age at exposure initiation. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	50		mg/kg/day
NOAEL- Dermal	Maternal	=	Not identified <50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	Not identified <50		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

One female in the control group was sacrificed on GD 15 because of an accidental injury. One female from the 150 mg/kg dose group was found dead on GD 16. Two other females in this dose group were sacrificed in a moribund condition on GD 15 or 16.

Treatment related dermal irritation was noted in animals in the 50, 150 and 500 mg/kg dose groups beginning GD 1 and continued throughout the duration of the study.

Slight to moderate erythema, edema, eschar and dry skin were observed at the test site for animals in the 50 mg/kg dose group. Slight to extreme (primarily slight to moderate) erythema, slight to moderate edema, slight eschar, and slight to extreme (primarily slight to moderate) dry skin were observed at the test site for animals in the 150 mg/kg dose group. Slight to extreme erythema, edema, eschar (primarily slight to moderate), and dry skin were observed at the test site for animals in the 500 mg/kg dose group. Slight fissuring was also noted.

All of the dose groups treated with F-222 exhibited higher incidences of vaginal discharge when compared to the control group. Slight to moderate (primarily slight) vaginal discharge, with a duration of one or two days, was noted for six of the females in the 0 mg/kg dose group. Slight to moderate (primarily slight) vaginal discharge, with a duration of one to five days, was noted for eight of the females in the 50 mg/kg dose group. Slight to extreme vaginal discharge, which occurred over a period of three to ten days, was noted for all of the females in the 150 mg/kg dose group. Slight to moderate vaginal discharge, which occurred over a period of two to eleven days, was noted for 11 of the 12 females in the 500 mg/kg dose group.

In addition to vaginal discharge, paleness, lethargy, no stools, and decreased body temperature were noted for the two females in the 150 mg/kg dose group that were sacrificed in a moribund condition; one of these females also had labored respiration. Paleness, no stools, and decreased body temperature were also noted for one female in the 150 mg/kg dose group before it was found dead. Three of the other females in this dose group were pale in color; one of these females was also lethargic. Three females in this dose group had red, red/black, or yellow stained coats in the perineal region. One female in the 500 mg/kg dose group was lethargic and cold to the touch. Four of the females in this dose group had yellow-stained coats in the perineal/inguinal region. There were no other clinical observations that were considered to be related to treatment with the test article.

Body weights of pregnant females in the 50 mg/kg dose group were significantly lower than those of the control females on GD 12 ($p<0.05$), 16 ($p<O.05$), and 20 ($p<0.01$). Body weights of pregnant females in the 150 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females on GD 4, 8, 12, 16, and 20. Body weights of pregnant females in the 500 mg/kg dose group were significantly lower ($p<0.01$) than

those of the control females on GD 4, 8, 12, 16, and 20. Body weight changes for pregnant females in the 50 mg/kg dose group
were also significantly lower ($p<0.05$) than those of the control females between GD 0 to 4. Body weight changes for pregnant females in the 150 mg/kg dose group were also significantly lower ($p<0.01$) than those of the control females between GD 0 to 4, 12 to 16 and 16 to 20. Body weight changes for females dosed at 500 mg/kg were significantly lower ($p<0.01$) than those of controls throughout gestation.

Absolute food consumption for pregnant females in the 50 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 4 to 8; there was no effect on relative food consumption. Absolute food consumption for pregnant females in the 150 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls throughout gestation. Relative food consumption for pregnant females in this dose group was significantly lower than that of the controls during GD 0 to 4 ($p<0.01$), 4 to 8 ($p<0.01$), and 12 to 16 ($p<0.05$). Absolute food consumption for pregnant females in the 500 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls throughout gestation. Relative food consumption for pregnant females inthis dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4 and 4 to 8.

Dermal irritation (e.g., erythema, edema, eschar, and dry skin) related to administration of the test article was noted at the test site for all dose groups that were treated with the test article.

The death of one female and the moribund condition of two females in the 150 mg/kg dose group may have been due to complications arising from test article treatment. Red/black-stained fur around the vaginal opening, pale tissues and organs, small thymus, and numerous early resorptions in the uterus were noted for the two females in the 150 mg/kg dose group that were sacrificed in a moribund condition. Red fluid on the tail and in the perineal region and resorptions in the uterus were observed for the 150 mg/kg dose group female that was found dead. Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

At a dose of 50 mg/kg, gestation length was significantly longer ($p<0.01$), the number of total and live pups on lactation day 0 was significantly lower ($p<0.01$) and the adjusted mean pup weight and lactation day 0 was significantly decreased ($p<0.05$) compared to the control group. At this dose level, seven of 10 pregnant females delivered a litter. At a dose of 150 mg/kg, the number of total and live pups on lactation day 0 was significantly lower ($p<0.01$) than that of the controls. Although not statistically significant, the adjusted mean pup weight (2 pups) on lactation day 0 was decreased (9%) compared to the control group. At this dose level, one of the 12 pregnant females delivered a litter.

The number of implantation sites was significantly lower ($p<0.01$) for females in the 500 mg/kg dose group, suggesting increased pre-implantation loss at this dose level; none of the 10 pregnant females delivered a litter. For the 50 and 150 mg/kg dose groups, there were no significant differences in number of implantation sites, proportion of dead pups on lactation day 0, proportion of pups surviving to lactation day 4, proportion of males on lactation days 0 and 4 or external pup alterations. External pup alterations noted included: cold to touch, small,
cannibalized, pale, dark in color, anal region inflamed, no milk in stomach and moribund.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**50**	**150**
Body wt –final (g)	422.8	363.0b	300.1b
Body wt – lactation day 0	315.9	305.0	292.0
Body wt – lactation day 4	323.3	314.5	NA
GD 0-4 wt gain (g)	22.9	15.2a	10.2b
GD 4-8 wt gain (g)	19.2	15.3	15.0
GD 8-12 wt gain (g)	23.8	20.2	17.8
GD 12-16 wt gain (g)	33.0	18.9	-15.3b

GD 16-20 wt gain (g)	57.7	27.5	-0.7b	-
Lactation day0-4 wt gain (g)	11.3	6.5	NA	N

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
NA=not applicable

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**50**	**150**
Number of dams pregnant	14	10	12
Dams with resorptions	0	1	4
Implantation sites - Mean	15.9	15.4	15.1
Number of litters with live pups	13	7	1
Total pups/litter (day 0)	14.8	9.0b	5.0b
Live pups/litter (day 0)	14.4	8.1b	2.0b
Proportion surviving to day 4 (%)	95	81	0
Pup weights (g) – mean, day 0	6.47	5.91a	5.87
Pup weights (g) – mean, day 4	9.41	8.74	NA

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
NA=not applicable

Conclusion:

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.
The systemic maternal NOAEL for dermal exposure to HCO during GD 0-20 was not be identified (<50 mg/kg/day); the LOAEL= 50 mg/kg/day based on increased vaginal discharge, decreased body weight, body weight changes, and food consumption.

The developmental NOAEL for dermal exposure to HCO during GD 0-20 was not identified (<50 mg/kg/day); the LOAEL = 50 mg/kg/day based on a decreased number of total and live pups on lactation day 0 and decreased pup body weights on lactation day 0.

Note the dermal NOAEL was determined to be < 50 mg/kg since dermal irritation occurred at all dose levels.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-222 Dermally During GD 0 to 20. Report ATX-91-0270.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR.

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec

2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	64741-57-7
Test Substance:	64741-57-7; Heavy Vacuum Gas Oil (HVGO)
Test Substance Purity/Composition and Other Test Substance Comments:	HVGO (F-225) PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%) [2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091689 (F-225)		0.00	0.40	4.00	1.00	0.40	0.10	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Wilmington, MA)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	12 per dose at 50, 150, or 500 mg/kg dose level of test material 15 per dose for sham control
Concentration:	
Dose:	0, 50, 150, 500 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation Day (GD) 0 to 20
Frequency of Treatment:	Once per day

Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HVGO (F-225) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. HVGO 50 mg/kg/day – 12 animals (GD 0-20) 3. HVGO 150 mg/kg/day – 12 animals (GD 0-20) 4. HVGO 500.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The animals used in the study were between 12 and 13 weeks of age at exposure initiation. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period). Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded. Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day0, pup alterations at lactation day0, male pups at days 0 and 4, survival of pups at lactation day4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	150		mg/kg/day
NOAEL- Dermal	Maternal	=	50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	150		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:	No mortalities occurred during the study. Slight erythema, eschar, and dry skin were observed at the test site for animals in the 50 mg/kg dose group. Slight to moderate (primarily slight) erythema and slight dry skin were observed at the test site for animals in the 150mg/kg dose group. Slight to moderate erythema, slight edema and slight dry skin were observed at the test site for animals in the 500 mg/kg dose group. All of the dermal irritation findings at the treated site were observed to be reversible by the end of the study period. Since eschar was only observed in one animal of the 50 mg/kg dose group, it is not considered to be treatment related. Rather, it is more probable that it is a result of shaving irritation. The occurrence of vaginal discharge was higher than that of the control group for females in the 500 mg/kg dose group. Seven females in the 500 mg/kg dose group had vaginal discharge for one to four days between GD 13 and 21. Vaginal discharge was also observed in two females in the 50 mg/kg dose group on GD 15 and 16. At a dose of 500 mg/kg, gestation length was significantly increased ($p<0.01$) when compared to that of the control group. There were no other clinical observations that were considered to be related to treatment with the test article. At a dose of 50 mg/kg, there were no significant differences in body weights or body weight changes when compared with the control group. At a dose of 150 mg/kg, there were no significant differences in body weights when compared with the control group. Body weights of pregnant females in the 500 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females on GD 4, 8, 12, 16, and 20 and on Lactation Day 4. Body weight changes for pregnant females in the 150 mg/kg dose group were also significantly lower ($p<0.05$) than those of the control females between GD 0 to 4. Body weight changes for females dosed at 500 mg/kg were significantly lower ($p<0.01$) than those of controls between GD 0 to 4, 12 to 16, and 16 to 20, and Lactation Days 0 to 4. There were statistically significant ($p<0.01$) dose response relationships between treatment groups for the intervals with decreased body weights and body weight changes. At a dose of 50 mg/kg, there were no statistically significant differences in absolute or relative food consumption when compared with that of the control group. At a dose of 150 mg/kg, there were no significant differences in absolute food consumption. Relative food consumption for pregnant females in the 150 mg/kg dose group was significantly lower than that of the control group during GD 0 to 4. Absolute food consumption for pregnant females in the 500 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4, 4 to 8, 12 to 16, and Lactation Days 0 to 4. Relative food consumption for pregnant females in the 500 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4 and 4 to 8 and Lactation Days 0 to 4. Relative food consumption for pregnant females in the 500 mg/kg dose group was significantly higher ($p<0.01$) than that of the controls during GD 16 to 20. The higher relative food consumption was considered to be related to the lower body weights at this interval. There were statistically significant ($p<0.01$) dose response relationships between treatment groups for the intervals with decreased absolute food consumption and decreased or increased relative food consumption.

The papillary process lobe of the liver appeared mottled, extending all of the way through the cut surface in one female in the sham control group. The lobe was white-yellow and light red in color. No visible lesions were noted at necropsy for females in the 50 and 150 mg/kg dose groups. One female in the 500 mg/kg dose group had red vaginal discharge and a dead fetus in the uterus at the time of necropsy. Another female in this dose group had thickened uterine walls and one early resorption in the uterus.

There were no significant effects on delivery and litter data at a dose of 50 mg/kg. At a dose of 150 mg/kg, pup body weights on Lactation Days 0 and 4 were significantly lower ($p<0.01$) than those of the controls. The number of total and live pups on Lactation Day 0 were significantly lower ($p<0.01$) than those of the control group. The proportion of pups dead on Lactation Day 0 was significantly higher ($p<0.05$) than that of the control group. Pup body weights on Lactation Days 0 and 4 were significantly lower ($p<0.01$) than those of the control group. Three of the pregnant females in the 500 mg/kg dose group did not deliver a litter and one of the pregnant females delivered only dead pups. These effects on delivery and litter data were considered to be related to dermal administration of the test article.

The proportion of pups surviving to Lactation Day 4 and the proportion of males on Lactation Day 4 were significantly lower ($p<0.05$) for the 500 mg/kg dose group when compared to that of the control group.

These differences were not considered to be toxicologically significant because a number of the deaths between Lactation Days 0 and 4 may have been caused by wet bedding in the nesting boxes.

For all dose groups, there were no significant differences in the number of implantation sites or external pup alterations.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	50	150	500
Body wt –final (g)	423.9	411.0	418.5	345.7b
Body wt – lactation day 0	320.8	313.1	318.4	300.6
Body wt – lactation day 4	336.7	329.8	331.4	295.9b
GD 0-4 wt gain (g)	23.7	18.6	16.8a	10.7b
GD 4-8 wt gain (g)	16.0	16.2	12.9	11.3
GD 8-12 wt gain (g)	23.3	21.5	25.2	21.3
GD 12-16 wt gain (g)	28.7	28.3	30.5	12.6 b
GD 16-20 wt gain (g)	59.7	55.6	57.1	26.8b
Lactation day0-4 wt gain (g)	15.9	16.9	13.0	-4.7b

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	150	500
Number of dams pregnant	15	10	12	11
Number of dams with resorptions	0	0	0	1
Number of dams that delivered	15	10	12	8
Implantation sites -	16.4	16.2	17.1	16.0

	Mean | | | | | **Number of litters with live pups** | 15 | 10 | 12 | 8 **Total pups/litter (day 0)** | 15.1 | 14.6 | 14.8 | 4.6b **Live pups/litter (day 0)** | 14.9 | 14.4 | 14.7 | 4.1b **Proportion surviving to day 4 (%)** | 97 | 97 | 92 | 82 **Pup weights (g) – mean, day 0** | 6.723 | 6.795 | 6.221b | 5.445b **Pup weights (g) – mean, day 4** | 10.188 | 9.404 | 8.832b | 7.084b a)Statistically different from control (p<0.05) b)Statistically different from control (p<0.01) Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.
Conclusion:	The systemic maternal NOAEL for dermal exposure to HVGO during GD 0-20 was determined to be 50 mg/kg/day; the LOAEL= 150 mg/kg/day based decreased body weight changes and food consumption. The developmental NOAEL for dermal exposure to HVGO during GD 0-20 was determined to be 50 mg/kg/day; the LOAEL = 150 mg/kg/day based on decreased pup body weights on lactation days 0 and 4. Note the dermal NOAEL was determined to be < 50 mg/kg since dermal irritation occurred at all dose levels.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-225 Dermally During GD 0 to 20. Report ATX-91-0270. Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

TEST SUBSTANCE

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Heavy Coker Gas Oil (HCGO); Heavy Thermal Cracked Distillate

Test Substance Purity/Composition and Other Test Substance Comments: HCGO (F-274)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
094625 (F-274)		7.00	9.00	7.00	5.00	2.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Wilmington, MA)

Other Strain: Not applicable

Gender: Females (non treated males used for mating)

Number of Animals per Dose: 12 per dose at 1, 50 or 250 mg/kg dose level of test material
15 per dose for sham control

Concentration:

Dose: 0, 1, 50, 250 mg/kg/day

Year Study Performed : 1994

Method/Guideline Followed: Other

GLP: No information

Exposure Period: Gestation Day (GD) 0 to 20

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The study was designed to determine the developmental toxicity of HCGO (F-274) following dermal administration to female rats daily for days 0 through day 20 of gestation.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug:

1. Sham control 0 mg/kg/day – 15 animals (GD 0-20)
2. HCGO 1 mg/kg/day – 12 animals (GD 0-20)

3. HCGO 50 mg/kg/day – 12 animals (GD 0-20)
4. HCGO 250.mg/kg/day – 12 animals (GD 0-20)

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	1		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:

No mortalities occurred during the study.

Dermal irritation related to administration of the test article was noted for females dosed at 1 mg/kg beginning GD 3 and continuing through GD 15. Slight to moderate (primarily slight) erythema was observed at the test site. Slight dry skin was also observed at the test site. Dermal irritation related to administration of the test article was noted for animals dosed at 50 mg/kg beginning as early as GD 2 and continuing throughout the duration of the study. Slight to moderate (primarily slight) erythema, edema, eschar and dry skin were observed at the test site. Dermal irritation related to administration of the test article was noted for females dosed at 250 mg/kg beginning GD 2 and continuing throughout the duration of the study. Slight to severe erythema (primarily slight and moderate) and eschar (primarily slight) were observed at the test she. Slight to moderate edema and dry skin were also observed at the test site. Vaginal discharge was observed in one female on GDs 19 and 20.

Scratches on the back were observed in several rats within each of the treated and control groups. The scratches were observed within the first eight days of gestation and are considered to be a result of aggressive behavior exhibited during mating. Shaving irritation was noted in one female of each of the dose groups and sham control group. Collar irritation was also noted in one female of the sham control group. Slight skin irritation remote from the treatment site was also noted in one female in each of the 1, 50 and 250 mg/kg groups. These observations are considered to be unrelated to treatment with F-274.

Body weights of pregnant females in the 1 mg/kg dose group were not significantly different than those of the control females throughout the duration of the study. Body weight changes for pregnant females in the 1 mg/kg dose group were significantly lower than those of the control females on GDs 0 to 4 ($p<0.05$). Body weights of pregnant females in the 50 mg/kg dose group were significantly lower than those of the control females on GDs 16 ($p<0.05$) and 20 ($p<0.01$). Body weight changes for pregnant females in the 50 mg/kg dose group were significantly lower than those of control females on GDs 0 to 4 ($p<0.05$), 4 to 8 ($p<0.01$), 12 to 16 ($p<0.05$) and 16 to 20 ($p<0.05$). Body weights of pregnant females in the 250 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females on GDs 4, 8, 12, 16 and 20. Body weight changes for pregnant females in the 250 mg/kg dose group were significantly lower than those of control females between GDs 0 to 4 ($p<0.01$), 4 to 8 ($p<0.01$), 8 to 12 ($p<0.05$), 12 to 16 ($p<0.01$) and 16 to 20 ($p<0.01$). The effects on body weight and body weight change observed at the 50 and 250 mg/kg dose levels are considered to be treatment related. A dose dependent correlation between dose and decreased body weight as well as body weight change was observed at these dose levels. The decrease in body weight change in the 1 mg/kg dose group between GDs 0 to 4 is not considered to be treatment related since this was not observed throughout the rest of the study and a dose related response was not observed.

Absolute and relative food consumption of pregnant females in the 1 mg/kg dose group were not significantly different than those of the control females throughout the duration of the study.Absolute food consumption of pregnant females in the 50 mg/kg dose group was significantly lower than those of the control females during GDs 4 to 8 ($p<0.01$), 8 to 12 ($p<0.01$), 12 to 16 ($p<0.05$), 16 to 20 ($p<0.05$) and Lactation Days 0 to 4 ($p<0.01$). Relative food consumption of pregnant females in the 50 mg/kg dose group was significantly lower than those of control females during GDs 4 to 8 ($p<0.01$), and Lactation Days 0 to 4 ($p<0.01$). Absolute and relative food consumption of pregnant females in the 250 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females during GDs 0 to 4, 4 to 8, 8 to 12, 12 to 16 and 16 to 20. The effects on absolute and relative food consumption observed in the 50 and 250 mg/kg dose groups are considered to be treatment related since they are consistently observed throughout the treatment period. In addition, there appears to be a correlation between dose and decreases in absolute and relative food consumption at these doses.

At necropsy, slight dermal irritation related to administration of test article was noted in one female of the 50 mg/kg dose group and in 10 females of the 250 mg/kg dose group. Multiple red foci were noted in the thymus of one female in the sham control and one female in the 50 mg/kg dose group. These findings are considered to be incidental in nature and not treatment related. Early resorption sites were noted in the uteri of two females in the 250 mg/kg dose group; with a red fluid filling the uterus of one of these females. The uterus of a third female in the 250 mg/kg dose group was filled with a clear fluid. An atrophied thymus, pale lungs, masses on each uterine horn, enlarged heart, spleen and liver were noted in another female in the 250 mg/kg dose group. Early resorption sites in the uterus are considered to be treatment related since they were observed only in the high dose group. The multiple lesions observed in one animal of the 250 mg/kg dose group are not considered to be treatment related since no evidence of similar findings was observed in other females of this or the lower dose groups. The gestation length in the 50 mg/kg dose group was statistically longer ($p<0.05$) than that of the sham treated controls.

Total pups per litter and live pups per litter in the 50 mg/kg dose group were significantly less ($p<0.05$) than in the sham control group. No females in the 250 mg/kg dose group delivered litters. The number of implantation sites in females of the 250 mg/kg dose group were significantly less ($p<0.01$) than in the sham control group. There were no statistically significant differences observed in any of the other parameters evaluated when the F-274 treated groups were compared to the sham control group.

Average pup body weights for the 1 and 50 mg/kg dose groups were not significantly different than that of controls. The following pup observations of hematoma, tip of tail black, eschar, missing tail, red anal region, pale in color and lethargy occurred sporadically and are considered to be incidental in nature.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**1**	**50**	**250**
Body wt –final (g)	409.9	402.0	352.8b	266.0b
Body wt – lactation day 0	300.0	296.2	289.6	NA
Body wt – lactation day 4	319.2	314.6	302.7	NA
GD 0-4 wt gain (g)	27.7	21.7a	22.6a	15.3b
GD 4-8 wt gain (g)	26.4	24.3	20.8b	18.7b
GD 8-12 wt gain (g)	26.4	25.9	24.8	21.4a
GD 12-16 wt gain (g)	34.9	34.1	20.1a	-9.5b
GD 16-20 wt gain (g)	66.7	70.7	38.0b	0.7b
Lactation day0-4 wt gain (g)	19.1	18.4	13.1	NA

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
NA= Not applicable

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**1**	**50**	**250**
Number of dams pregnant	15	10	12	10
Number of dams with resorptions	0	0	0	2
Number of dams that delivered	15	10	10	0
Implantation sites - Mean	17.5	16.8	16.1	12.8
Number of litters with live pups	15	10	10	NA
Total pups/litter (day 0)	16.1	16.0	10.1a	NA
Live pups/litter (day 0)	15.4	15.9	9.9a	NA
Proportion surviving to day 4 (%)	91	97	87	NA
Pup weights (g) – mean, day 0	6.64	6.58	6.26	NA
Pup weights (g) – mean, day 4	10.27	10.06	9.96	NA

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
NA= Not applicable

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability

to produce and carry a conceptus, or a direct effect on the embryo/fetus.

Conclusion: The systemic maternal NOAEL for dermal exposure to HCGO during GD 0-20 was determined to be 1 mg/kg/day; the LOAEL= 50 mg/kg/day based on decreased body weight, body weight changes, food consumption and relative food consumption.

The developmental NOAEL for dermal exposure to HCGO during GD 0-20 was determined to be 1 mg/kg/day; the LOAEL = 50 mg/kg/day based on decreased number of total and live pups delivered per litter.

Note the dermal NOAEL was determined to be < 1 mg/kg since dermal irritation occurred at all dose levels.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-274 Dermally During GD 0 to 20. Report ATX-93-0069.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 68410-00-4

Test Substance: 68410-00-4; Distillates, Crude Oil (DCO); VDF Diesel

Test Substance Purity/Composition and Other Test Substance Comments: Distillates, Crude Oil (F-194)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091647 (F-194)		0.10	4.00	4.00	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	15, 15, 14 for 125, 250 and 1000 mg/kg dose level of DCO, respectively 19 per dose for sham control
Concentration:	
Dose:	0, 125, 250, 1000 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of DCO (F-194) following dermal administration to female rats daily for days 0 through day 20 of gestation, or days 5 through 9 of gestation (1000 mg/kg/day group). Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. *Sham control 0 mg/kg/day – 19 animals (GD 0-20) 2. DCO 125 mg/kg/day – 15 animals (GD 0-20) 3. DCO 250 mg/kg/day – 15 animals (GD 0-20) 4. DCO 1000 mg/kg/day – 14 animals (GD 5-9)** *Shared with study number ATX-91-0129 **Dosing adjustment based on initial study indicating severe irritation and poor mating performance At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-3 on GD 0 through GD 20. The group 4 (1000 mg/kg/day) animals received a shortened dosing regimen of GD 5 through GD 9. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight (0.0, 125, 250 mg/kg/day groups) or GD 4 (1000 mg/kg/day group) body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in

appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that

	highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)*

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	125		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	125		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	Not determined (<125)		mg/kg/day

***Determined by reviewer – skin irritation excluded as endpoint**

Results Remarks:

The animals used in the study were between 13 and 14 weeks of age at exposure initiation.

There were no mortalities observed during the study.

Slight to extreme (primarily slight to moderate) erythema, edema, eschar, and dry skin were observed at the test site for the 125 mg/kg/day group beginning on GD 1 and throughout the study. For the 250 mg/kg/day group slight to extreme (primarily moderate to extreme) erythema, edema, eschar, and dry skin were observed at the test site beginning GD 2 and throughout the study. Slight fissuring was also noted. The 1000 mg/kg/day dose group, beginning GD 6 and continuing throughout the duration of the study, showed slight to extreme (primarily moderate to extreme) erythema, edema, eschar, and dry skin were observed at the test site, as well as slight fissuring. [Note: administration of test article for the high dose group began on GD 5.]

Higher incidences of yellow-stained coat in the perineal region and alopecia were noted for females in the 1000 mg/kg/day dose group. The alopecia was generally noted in areas surrounding the test site and was considered to be due to irritation caused by test article that had spread to these areas. There were no other clinical observations that were considered to be related to treatment with the test article.

There were no effects on body weights or body weight changes at a dose of 125 mg/kg/day. Body weights and body weight changes of pregnant females in the 2500 and 1000 mg/kg/day dose group were significantly lower than those of the control females at various points during gestation and postnatal time period, per the table below.

There was no effect on absolute food consumption for females in the 125 mg/kg/day dose group. Relative food consumption for females in this group was significantly lower ($p<0.05$) than that of the controls during GD 4 to 8. Because the effect was isolated and did not occur at the 250 mg/kg/day dose level, it was considered incidental and unrelated to treatment with test article. Absolute food consumption for the 250 mg/kg/day dose group was significantly lower ($p<0.05$) than that of the controls during GDs 4 to 8. There were no effects on relative food consumption. For the 1000 mg/kg/day dose group, absolute food consumption was significantly lower ($p<0.01$) during GD 4 to 8 and 8 to 12. It was significantly higher ($p<0.05$) during GD 0 to 4; however, this was prior to treatment with the test article. Relative food consumption the 1000 mg/kg/day dose group was significantly lower ($p<0.01$) than that of the controls during GD 4 to 8 and 8 to 12 and significantly higher ($p<0.01$) on GD 12 to 16 and 16 to 20. An increase (not statistically significant), in

absolute food consumption occurred after treatment with the test article was discontinued, and with a slower recovery in body weight, resulted in a higher relative food consumption.

At necropsy, dermal irritation related to administration of the test article was noted for females in all of the dose groups that received the test article. Alopecia was also noted only in females in the 1000 mg/kg/day/day dose group.

Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

Pup body weights on lactation day 0 were decreased at doses of 125, 250, and 1000 mg/kg/day. Pup body weights were also decreased on lactation day 4 at doses of 250 and 1000 mg/kg/day.

For all dose groups, there were no significant differences in gestation length, number of implantation sites, external pup alterations, the 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. External pup alterations observed during lactation Days 0-4 included: cold/cool to touch, small, cannibalized, lethargic, pale, laceration/scab/eschar, discolored area, hematoma, swollen navel, and erythema and edema in perineal/genital region.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	125	250	1000
Body wt day 0	273.5	278.1	270.4	277.5
Body wt –final (g)	424.7	424.4	389.9b	393.4a
Body wt – lactation day 0	331.3	319.4	296.5b	300.4b
Body wt – lactation day 4	341.0	338.6	314.0b	315.9b
GD 0-4 wt gain (g)	25.4	22.1	21.3	27.8
GD 4-8 wt gain (g)	16.8	16.6	11.9	-15.0b
GD 8-12 wt gain (g)	20.6	17.8	19.5	4.8b
GD 12-16 wt gain (g)	34.6	34.5	23.9a	36.6
GD 16-20 wt gain (g)	58.1	56.9	48.5	61.6
Lactation day 0-4 wt gain (g)	9.7	19.3	18.9	16.7

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	125	250	1000
Dams with resorptions	0	0	0	0
Implantation sites	15.6	18.0	15.6	16.6
Number of litters with live pups	15	11	14	12
Total pups/litter (day 0)	14.0	16.5	14.6	15.5
Live pups/litter (day 0)	13.9	16.4	14.4	15.2
Pup weights (g) – mean, day 0	6.618	6.217a	6.104b	6.309a
Pup weights (g) – mean, day 4	10.104	9.573	9.000a	8.669b

a)Statistically different from control ($p<0.05$)

	b)Statistically different from control (p<0.01)
Conclusion:	Determined by reviewer: The maternal NOAEL for dermal exposure to DCO during gestation days 0-20 was determined to be 125 mg/kg/day (LOAEL= 250 mg/kg/day based on decreased body weights, body weight changes and food consumption). Note: the authors determined that the NOAEL is <125 mg/kg/day based on skin irritation; irritation was observed at every dose level tested. The developmental NOAEL for dermal exposure to DCO during gestation days 0-20 was determined to be less than 125 mg/kg/day (LOAEL = 125 mg/kg/day based on a decreased pup body weights on lactation day 0). Administration of the test article at a higher dose level (1000 mg/kg/day), but for a shorter dosing period (GD 5-9) produced signs of both maternal and developmental toxicity.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Non guideline study, but with adequate detail to make NOAEL determination.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-194 Dermally During Gestation Days 0 to 20. Report ATX-91-0128 Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	68410-00-4
Test Substance:	68410-00-4; Distillates, Crude Oil (DCO); VDF Diesel
Test Substance Purity/Composition and Other Test Substance Comments:	Distillates, Crude Oil (F-215) PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091681 (F-215)		0.20	4.00	4.00	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that

	have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Wilmington, MA)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	12 at 50, 150, or 500 mg/kg dose level of DC 15 per dose for sham control
Concentration:	
Dose:	0, 50, 150, 500 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of DCO (F-215) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. DCO 50 mg/kg/day – 12 animals (GD 0-20) 3. DCO 150 mg/kg/day – 12 animals (GD 0-20) 4. DCO 500.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was

based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	500		mg/kg/day
NOAEL- Dermal	Maternal	=	150		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	150		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

There were no mortalities observed during the study.

Slight eschar was noted at the control site of one sham control animal on GD 12 to 17. Slight to extreme (primarily slight to moderate) erythema, slight to moderate edema, slight to moderate eschar, and slight to extreme (primarily slight to moderate) dry skin were observed at the test site for animals in the 50 mg/kg dose group. Slight to extreme (primarily slight to moderate) erythema, edema, eschar, and dry skin were observed at the test site for animals in the 150 mg/kg dose group. Slight to extreme (primarily moderate to extreme) erythema, edema, eschar, and dry skin were observed at the test site for animals in the 500 mg/kg dose group. Slight fissuring was noted at the test site for two animals on one or two days. The occurrence of vaginal discharge was slightly higher than that of the control group for females treated with the test article. This difference was not considered to be related to the test article because the vaginal discharge was slight, the duration was limited (one or two days), it occurred soon after mating or at the time that the vascular membrane becomes visually apparent, and did not occur in a dose-dependent manner.

Yellow, yellow/brown, yellow/orange, or red/yellow stained coats were noted for eight females in the 500 mg/kg dose group. The staining was slight to severe in nature, and occurred in the perineal and abdominal regions. Alopecia, erythema, edema, and eschar were noted for a few of the females in the 500 mg/kg dose group in regions generally located adjacent to the test site. These findings were considered to have been caused by the spread of the test article/irritation beyond the test site.

There were no other clinical observations that were considered to be related to treatment with the test article.

There were no statistically significant differences in body weights or body weight changes at doses of 50 and 150 mg/kg. Body weight changes for females dosed at 500 mg/kg were significantly lower than those of controls between GD 0 to 4, 4 to 8, 12 to 16, and 16 to 20, with a statistically significant dose response relationship between treatment groups. Body weight changes for females dosed at 500 mg/kg were significantly higher than those of controls between lactation days

0 and 4, with a statistically significant dose response relationship between treatment groups; this increase in body weight change was considered to be related to a recovery from treatment with the test article.

There were no statistically significant differences in absolute or relative food consumption for pregnant females in the 50 mg/kg dose group when compared to the control group. There were no significant differences in absolute food consumption for pregnant females in the 150 mg/kg dose group. Relative food consumption for pregnant females in the 150 mg/kg dose group was significantly higher than that of the controls during GD 16 to 20. Absolute food consumption for pregnant females in the 500 mg/kg dose group was significantly higher than that of the control group during lactation days 0 to 4. Relative food consumption for pregnant females in the 500 mg/kg dose group was significantly higher than that of the controls during GD 12 to 16 and 16 to 20 and lactation days 0 to 4. There were statistically significant dose response relationships between treatment groups for the increased absolute and relative food consumption values that occurred during the latter part of gestation and during lactation. The higher absolute and relative food consumption values at a dose of 500 mg/kg were considered to be related to a
recovery from treatment with the test article. The higher relative food consumption value at a dose of 150 mg/kg was a single occurrence during GD 16 to 20 and is not considered to be treatment related.

Dermal irritation (e.g., erythema, edema, eschar, and dry skin) related to administration of the test article was noted at the test site for all dose groups that were treated with the test article.

Alopecia was noted for the 500 mg/kg dose group in areas generally located adjacent to the test site. These findings were considered to have been the result of the spread of test article/irritation beyond the test site.

Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

There were no significant effects on delivery and litter data at a dose of 50 mg/kg. At a dose of 150 mg/kg, pup body weights on lactation days 0 and 4 were significantly lower than that of the controls. At a dose of 500 mg/kg, the proportion of pups surviving to lactation day 4 was significantly lower than that of the controls. Pup body weights on lactation days 0 and 4 were significantly lower than those of controls on lactation days 0 and 4. For all dose groups, there were no significant differences in gestation length, number of implantation sites, the number of total and live pups on lactation day 0, external pup alterations, proportion of dead pups on
lactation day 0, or the proportion of males on lactation days 0 and 4.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**50**	**150**	**500**
Body wt –final (g)	426.1	418.2	414.1	355.8b
Body wt – lactation day 0	324.2	321.9	317.1	267.0b
Body wt – lactation day 4	335.8	337.2	330.8	291.4b
GD 0-4 wt gain (g)	21.3	21.9	17.4	8.7b
GD 4-8 wt gain (g)	17.1	16.5	16.1	1.5b
GD 8-12 wt gain (g)	24.1	24.3	24.0	20.7
GD 12-16 wt gain (g)	32.0	32.5	27.6	18.2b
GD 16-20 wt gain (g)	60.8	55.8	63.7	41.9b
Lactation day 0-4 wt gain (g)	11.6	15.3	13.7	27.4a

a)Statistically different from control ($p<0.05$)

b)Statistically different from control (p<0.01)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	150	500
Implantation sites - Mean	16.0	15.6	17.0*	16.6
Number of litters with live pups	15	11	9	12
Total pups/litter (day 0)	15.2	13.5	15.3**	15.8
Live pups/litter (day 0)	14.9	13.5	14.9	15.1
Proportion surviving to day 4 (%)	97	99	99	70b
Pup weights (g) – mean, day 0	6.55	6.65	6.10	5.56
Pup weights (g) – mean, day 4	9.89	10.52	8.41	6.94

* One female was omitted from the statistical analysis because the number of implantation sites was miscounted.
** One female was omitted from the statistical analysis because the number of pups was miscounted.
a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.

Conclusion:

The systemic maternal NOAEL for dermal exposure to DCO during GD 0-20 was determined to be 150 mg/kg/day (LOAEL= 500 mg/kg/day based on decreased body weight and body weight changes during gestation).

Note the dermal NOAEL was determined to be < 50 mg/kg since dermal irritation occurred at all dose levels.

The developmental NOAEL for dermal exposure to DCO during GD 0-20 was determined to be 50 mg/kg/day (LOAEL = 150 mg/kg/day based on a decreased pup body weights on lactation days 0 and 4).

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-215 Dermally During GD 0 to 20. Report ATX-91-0263.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	68783-08-4
Test Substance:	68783-08-4; Full Range Gas Oil (FRGO)
Test Substance Purity/Composition and Other Test Substance Comments:	Full Range Gas Oil (F-275) PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%) [2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
094626 (F-275)		0.70	4.00	1.00	0.70	0.50	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Wilmington, MA)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	12 at 50, 250, or 500 mg/kg dose level of FRGO 15 per dose for sham control
Concentration:	
Dose:	0, 50, 250, 500 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation Day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of FRGO (F-275) following dermal administration to female rats daily for days 0 through day 20 of

gestation.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug:

1. Sham control 0 mg/kg/day – 15 animals (GD 0-20)
2. FRGO 50 mg/kg/day – 12 animals (GD 0-20)
3. FRGO 250 mg/kg/day – 12 animals (GD 0-20)
4. FRGO 500.mg/kg/day – 12 animals (GD 0-20)

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:

The animals used in the study were between 9 and 10 weeks of age at exposure initiation.

There were no mortalities observed during the study.

Dermal irritation related to administration of the test article was noted for females dosed at 50 mg/kg on GD 5 - 8 and 18, and lactation day 4. Slight erythema was observed at the test site of one female on GD 5 - 8. Slight· dry skin on GD 18 and slight eschar on lactation day 4 were observed at the test site of another female. Dermal irritation related to administration of the test article was noted in one female dosed at 250 mg/kg on GD 9 and another female on lactation day 4.

Slight erythema was observed at the test site of one female on GD 9 and slight eschar was observed at the test site of the other female on lactation day 4. Dermal irritation related to administration of the test article was noted in females dosed at 500 mg/kg beginning GD 1 and continuing throughout the duration of the study. Slight erythema and dry skin were also observed at the test site. The gestation length in "the 500 mg/kg dose group was statistically longer than that of

the sham treated controls. The nipple size of one female in the 500 mg/kg dose group was noted to be very small.

Scratches on the back were observed in several rats within each of the treated and control groups. The scratches were observed within the first eight days of gestation and are considered to be a result of aggressive behavior exhibited during mating. Shaving irritation was noted in one female of the sham control group. Collar irritation was noted in another female of the sham control group.

Body weights of pregnant females in the 50 mg/kg dose group were significantly lower than those of the control females on GD 0, 4, 8, 12, 16 and 20. Body weights of pregnant females in the 250 mg/kg dose group were significantly lower than those of the control females on GD 12, 16 and 20. Body weight changes for pregnant females in the 250 mg/kg dose group were significantly lower than those of control females on GD 0 to 4, 4 to 8, 8 to 12 and 16 to 20. Body weights of pregnant females in the 500 mg/kg dose group were significantly lower than those of the control females on GD 4, 8, 12, 16, 20 and lactation days 0 and 4. Body weight changes for pregnant females in the 500 mg/kg dose group were significantly lower than those of control females between GD 0 to 4, 8 to 12, 12 to 16, 16 to 20 and lactation days 0 to 4. The differences between mean body weights of the sham control and the 50 mg/kg dose group are not considered to be treatment related since body weight changes were not affected at this dose and mean body weight was significantly lower on GD O. As a result of the design of this study, strict randomization according to body weight is not performed. Animals are assigned randomly to dose groups as they demonstrate positive evidence of mating. As a result of this procedure, the mean body weight for the animals in the 50 mg/kg dose group was significantly lower than that of the sham control group at initiation of dosing. The significant decreases in body weight and the lack of a corresponding difference in body weight change in the 50 mg/kg dose group appear to be an inadvertent result of the experimental design rather than a treatment related effect.

The effects on body weight and body weight change observed at the 250 and 500 mg/kg doses are considered to be treatment related since the effect appears to become more marked over the treatment period and there appears to be a dose dependent correlation between dose and decrease in body weight as well as body weight change at these doses.

Absolute food consumption of pregnant females in the 50 mg/kg dose group was significantly lower than those of the control females during GD 4 to 8, while relative food consumption was not significantly different than controls throughout the duration of the study. Absolute food consumption of pregnant females in the 250 mg/kg dose group was significantly lower than those of the control females during GD 0 to 4 and 4 to 8 . Relative food consumption of pregnant females in the 250 mg/kg dose group was significantly lower than those of control females during GD 4 to 8. Absolute food consumption of pregnant females in the 500 mg/kg dose group were significantly lower than those of the control females during GD 0 to 4, 4 to 8, 8 to 12, 16 to 20 and lactation days 0 to 4 . Relative food consumption was significantly lower than those of the control females during GD 0 to 4, 4 to 8 and lactation days 0 to 4. Since the effects observed early in the treatment period (GD 0 to 12) on absolute and relative food consumption appear to be dose dependent, these statistically significant effects observed in the 250 and 500 mg/kg dose groups are considered to be treatment related. Decreased absolute and relative food consumption observed in the 500 mg/kg dose group on lactation days 0 to 4 is considered to be a secondary effect of treatment with F-275 since litter size was decreased in this group and one female appeared to have stopped lactating during this period.

Slight dermal irritation related to administration of test article was noted in one female in each of the 50 and the 250 mg/kg dose groups and two females in the 500 mg/kg dose group. Multiple red foci were noted in the thymus of one female in the sham control group. The left ovaries of one female in the 250 mg/kg dose

group and one female in the 500 mg/kg dose group were surrounded by a clear fluid filled sac. The kidneys of one female in the 500 mg/kg dose group were noted to have a mottled appearance. These findings are considered to be incidental in nature and not treatment related. Early resorption sites were noted in the uteri of two females in the 500 mg/kg dose group. Early resorption sites in the uterus are considered to be treatment related since they were observed only in the high dose group and they correspond to decreased litter size observed at this dose level.

Total pups per litter and live pups per litter in the 250 mg/kg and 500 mg/kg dose groups were significantly decreased when compared to the sham control group. Four females in the 500 mg/kg dose group did not deliver litters. The proportion of dead pups in the 500 mg/kg dose group was significantly greater than in the sham control group on lactation day 0. Two of the females in the 500 mg/kg dose group that did deliver litters delivered only one pup each that were both found dead on lactation day 0. A third female in the 500 mg/kg dose group that delivered two pups was noted to have very small nipples on lactation day 3. On lactation day 4 these two pups were found dead, with no milk in their stomachs, apparently because the dam had stopped lactating. The proportion of male pups in 500 mg/kg dose group was significantly greater than in the sham controls on lactation days 0 and 4 . There were no statistically significant differences observed in any of the other parameters evaluated when the F-275 treated groups were compared to the sham control group. Average pup body weights were significantly lower than that of the sham controls on lactation days 0 and 4 for the 250 mg/kg and 500 mg/kg dose groups. The following pup observations of lethargic and purple, isolated from litter, hematoma, tip of tail black, left eye slightly swollen and dark red, eschar and missing tail occurred sporadically and are considered to be incidental in nature.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**50**	**250**	**500**
Body wt –final (g)	409.9	382.8a	378.4b	314.0b
Body wt – lactation day 0	300.0	284.6	293.6	279.7a
Body wt – lactation day 4	319.2	306.3	308.8	279.7b
GD 0-4 wt gain (g)	27.7	26.9	20.4b	17.8b
GD 4-8 wt gain (g)	26.4	24.4	22.2a	23.6
GD 8-12 wt gain (g)	26.4	27.8	21.7a	20.9a
GD 12-16 wt gain (g)	34.9	33.3	28.9	11.3b
GD 16-20 wt gain (g)	66.7	63.5	56.8a	18.8b
Lactation day 0-4 wt gain (g)	19.1	21.6	15.3	0.5b

a)Statistically different from control
b)Statistically different from control

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**50**	**250**	**500**
Implantation sites - Mean	17.5	16.9	16.6	16.8
Number of litters with live pups	15	11	12	8
Total pups/litter (day 0)	16.1	15.4	13.3b	4.4b
Live pups/litter (day 0)	15.4	15.2	12.8b	4.0b
Proportion surviving to day 4 (%)	97	96	98	92

	Pup weights (g) – mean, day 0 | 6.56 | 6.50 | 6.56 | 6.01 **Pup weights (g) – mean, day 4** | 10.27 | 10.57 | 10.27 | 8.71 a)Statistically different from control b)Statistically different from control Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.
Conclusion:	The systemic maternal NOAEL for dermal exposure to FRGO during GD 0-20 was determined to be 50 mg/kg/day (LOAEL= 250 mg/kg/day based on decreased body weight, body weight changes and food consumption during gestation. The developmental NOAEL for dermal exposure to FRGO during GD 0-20 was determined to be 50 mg/kg/day (LOAEL = 250 mg/kg/day based on a decreased number of total and live pups delivered and decreased pup body weights on lactation days 0 and 4).
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-275 Dermally During GD 0 to 20. 1994. Report ATX-93-0071. Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-45-3

Test Substance: 64741-45-3; Atmospheric Tower Bottom (ATB)

Test Substance Purity/Composition and Other Test Substance Comments: ATB (F-228)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091691 (F-228)		0.10	0.30	2.00	2.00	2.00	0.60	0.10

1) Percent of DMSO-extractable materials (mostly PACs), determined by the

	PAC 2 method as described in API (2008). 2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Wilmington, MA)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	12 per dose at 50, 333, or 1000 mg/kg dose level of test material 15 per dose for sham control
Concentration:	
Dose:	0, 50, 333, 1000 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation Day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of ATB (F-228) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. ATB 50 mg/kg/day – 12 animals (GD 0-20) 3. ATB 333 mg/kg/day – 12 animals (GD 0-20) 4. ATB 1000.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The animals used in the study were between 12 and 13 weeks of age at exposure initiation. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day0, pup alterations at lactation day0, male pups at days 0 and 4, survival of pups at lactation day4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	333		mg/kg/day
NOAEL- Dermal	Maternal	=	50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	333		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:

One female in the 333 mg/kg dose group was unsuccessful in delivering her litter and was sacrificed moribund. No other mortality occurred in this phase of the study.

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. No dermal irritation was observed at the test site for animals in any of the test article dose groups.

One animal in the 333 mg/kg dose group was unsuccessful in delivering her litter and was noted as being cold to touch, pale in color, lethargic, and as having red colored urine on GD 23. She was sacrificed moribund. This is not considered to be related to test article exposure. At a dose of 1000 mg/kg, the gestation length (days) was significantly longer ($p<0.01$) than that of the control group. There were no other clinical observations that were considered to be related to treatment with the test article.

Body weight changes for pregnant females in the 1000 mg/kg dose group were significantly lower ($p<0.05$) than those of the control females between GD16 to 20. The changes in female body weight· appear to be influenced by two females which had reduced litter sizes. This finding is considered 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.

Relative food consumption for pregnant females in the 50 mg/kg dose group was significantly lower ($p<0.05$) than that of the controls during GD16 to 20 and significantly higher ($p<0.05$) than that of the controls during Lactation Days 0 to 4. These differences are not considered to be related to treatment with the test article since the relative food consumption was not significantly different at the higher dose levels of 333 and 1000 mg/kg. There were no other effects on absolute or relative food consumption at any of the dose levels.

The 333 mg/kg female sacrificed moribund on GD 23 was observed to have dark red fluid in the bladder and uterus and the uterus contained dead and live fetuses. These findings were not considered to be related to treatment with the test article but rather are attributed to the dystocia. No lesions related to administration of the test article

were noted for females in any of the dose groups.

At a dose of 333 mg/kg, the number of implantation sites was significantly decreased ($p<0.05$) compared to that of the control group. This difference is not considered to be related to treatment with the test article since the number of implantation sites was not significantly lower at the higher dose of 1000 mg/kg. At a dose of 50 mg/kg, the live pup weights on Lactation Day 4 were significantly lower ($p<0.05$) than those of the control group; however, this difference is not considered to be related to treatment with the test article since a clear dose response was not observed. In addition, excellent pup survival was observed at this dose level, which would not be expected if the decreased body weight was, in fact, biologically relevant. At a dose of 1000 mg/kg, the live pup weights on Lactation Days 0 and 4 were significantly lower ($p<0.05$) than those of the control group.

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.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	50	333	1000
Body wt –final (g)	424.5	423.3	416.5	404.7
Body wt – lactation day 0	325.1	319.3	327.4	317.4
Body wt – lactation day 4	334.8	326.5	335.9	326.0
GD 0-4 wt gain (g)	23.5	24.5	18.4	19.4
GD 4-8 wt gain (g)	16.5	18.1	16.3	14.7
GD 8-12 wt gain (g)	22.7	21.1	24.0	19.8
GD 12-16 wt gain (g)	32.8	33.8	33.7	30.8
GD 16-20 wt gain (g)	59.1	57.8	51.7	43.1a
Lactation day0-4 wt gain (g)	9.7	7.7	8.4	9.4

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	333	1000
Number of dams pregnant	15	12	10	11
Number of dams with resorptions	0	0	0	0
Number of dams that delivered	15	12	9	11
Implantation sites - Mean	16.4	17.2	14.0a	17.0
Number of litters with live pups	15	12	9	11
Total pups/litter (day 0)	14.0	16.0	13.1	11.4
Live pups/litter (day 0)	13.9	15.9	12.9	10.9
Proportion surviving to day 4 (%)	87	95**	94	84*
Pup weights (g) – mean, day 0	6.681	6.283	6.647	6.132a
Pup weights (g) – mean, day 4	8.969	7.745a**	9.066	7.621 a*

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

*one litter excluded from statistical analysis because of the number of accidental deaths in the litter.
**One litter was inadvertently sacrificed early.

Conclusion:

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.
The systemic maternal NOAEL for dermal exposure to ATB during GD 0-20 was determined to be 333 mg/kg/day; the LOAEL= 1000 mg/kg/day based on decreased body weight changes and an increase in gestation length.

The developmental NOAEL for dermal exposure to ATB during GD 0-20 was determined to be 333 mg/kg/day; the LOAEL = 1000 mg/kg/day based on a decrease in pup body weights on Lactation Days 0 and 4.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-228 Dermally During GD 0 to 20. Report ATX-91-0267.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil; Hydrocracker Feed Oil

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil (F-276)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
094627 (F-276)		9.00	9.00	0.20	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type: Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	11, 12, 11 at 1.0, 250 and 500 mg/kg dose level of HVGO, respectively 15 per dose for sham control
Concentration:	
Dose:	0, 1.0, 250, 500 mg/kg/day
	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation Day (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HVGO (F-276) following dermal administration to female rats daily for days 0 through day 20 of gestation.

Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows:

1. Sham control (0 mg/kg/day) – 15 animals; 13 animals at GD 0
2. HVGO 1.0 mg/kg/day –11 animals; 11 animals at GD 0
3. HVGO 250 mg/kg/day – 12 animals; 12 animals at GD 0
4. HVGO 500 mg/kg/day – 11 animals; 11 animals at GD 0

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based upon the day -7 body weight for the pre-mating period and the GD 0 body weight for the gestation period.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for

changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:

The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	1		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	1		mg/kg/day

Results Remarks:

The animals used in the study were between 9 and 10 weeks of age at exposure initiation.

There was no mortality observed during the study period.

Dermal irritation related to administration of the test article was noted for females dosed at 1.0 mg/kg beginning premating day -5 and continuing throughout the duration of the study. Slight to moderate (primarily slight) erythema and edema, and slight dry skin and eschar were observed at the test sites. Dermal irritation related to administration of the test article was noted for females dosed at 250 and 500 mg/kg beginning premating day -5 and continuing throughout the duration of the study. Slight to severe (primarily moderate for the 250 mg/kg dose group and primarily severe for the 500 mg/kg dose group) erythema, edema, dry skin and eschar were observed at the test sites. One animal in the 500 mg/kg dose group exhibited fissuring at the test site on premating day -2.

One animal in the 1.0 mg/kg dose group and one animal in the 500 mg/kg dose group exhibited slight vaginal discharge on GD ay 0. Two animals in the 500 mg/kg dose group exhibited moderate staining of the coat (perineal region) on premating day -1. These findings are considered to be incidental in nature and not treatment related. Scratches on the back were observed in several rats within the sham control, 1.0 and 250 mg/kg dose groups. The scratches were observed within the first four days of gestation and are considered to be a result of aggressive behavior exhibited during mating.

Body weights of pregnant females dosed at 1.0 mg/kg were not significantly different from those of the control females throughout the duration of the study. Body weight changes were significantly higher than those of the control females between GD 4 and 8. Body weights of pregnant females dosed at 250 mg/kg were significantly lower than those of the control females on GD 4, 8, 12, 16 and 20 and on lactation days 0 and 4. Body weight changes were significantly lower than those of the control females between GD 0 and 4 and 12 and 16. Body weights of pregnant females dosed at 500 mg/kg were significantly lower than those of the control females on premating day -1; on GD 0, 4, 8, 12, 16, 20; and on lactation days 0 and 4. Body weight changes were significantly lower than those of the control females between premating days -7 and -1; between GD 0 and 4, 12 and 16 and 16 and 20. The effects on body weight and body weight change observed at the 250 and 500 mg/kg dose levels are considered to be treatment related. A dose dependent correlation between dose and decreased body weight as well as body weight change was

observed at these dose levels. The increase in mean body weight change in the 1.0 mg/kg dose group between GD4 and 8 is not considered to be treatment related since the effect did not persist throughout the remainder of the study and was not observed at higher dose levels; also the control females mean body weight change for the same time period appears to be abnormally low.

Absolute and relative food consumption of pregnant females dosed at 1.0 mg/kg were not significantly different than those of the control females throughout the duration of the study. Absolute food consumption of pregnant females dosed at 250 mg/kg was significantly lower than that of the control females between premating days -7 and -1. Relative food consumption was significantly lower than that of the control females between premating days -7 and -1. Relative food consumption was significantly higher than that of the control females between GD12 and 16, 16 and 20 and between Lactation Days 0 and 4. Absolute food consumption of pregnant females dosed at 500 mg/kg was significantly lower than that of the control females between premating days -7 and -1. Relative food consumption was significantly lower than that of the control females between premating days -7 and -1. Relative food consumption was significantly higher than that of the control females between GD4 and 8, 8 and 12, 12 and 16 and 16 and 20. The decreases in absolute and relative food consumption observed in the 250 and 500 mg/kg dose groups between premating Days -7 and -1 are considered to be treatment related. A dose dependent correlation between dose and decreased absolute food consumption as well as relative food consumption was observed at these dose levels. The increases in relative food consumption observed in the 250 and 500 mg/kg dose groups are considered to be treatment related in that they occurred in a dose dependent manner over more than one consecutive (4 day) measurement interval.

Dermal irritation related to administration of the test article was noted in all of the females in the 250 and 500 mg/kg dose groups and in 5 of the 11 females in the 1.0 mg/kg dose group. The axillary lymph nodes of two animals in the 250 mg/kg dose group and one animal in the 500 mg/kg dose group were noted as being enlarged. The cervical lymph nodes of three animals in the 500 mg/kg dose group were also noted as being enlarged. These findings are considered to be secondary to the dermal irritation and therefore are indirectly considered to be treatment related. The right ovary of one of the sham control animals was surrounded by a clear fluid filled sac. A red foci was noted on the right side of the thymus of one animal in the 500 mg/kg dose group. These findings are considered to be incidental in nature and not treatment related.

The number of implantation sites for the 250 and 500 mg/kg dose groups was significantly lower than that of the control group. Total pups per litter and live pups per litter were significantly less in the 250 and 500 dose groups than in the control group. The number of pups surviving to day 4 of lactation was significantly less in the 500 mg/kg dose group than in the control group. There were no statistically significant differences observed in any of the other parameters evaluated when the F-276 treated groups were compared to the sham control group.

Average pup body weights for the 250 mg/kg dose group were significantly lower than that of the controls on lactation day 0. Average pup body weight for the 500 mg/kg dose group were significantly lower than that of the controls on lactation days 0 and 4. The following pup observations: cold to the touch, pale in color, hematoma, tip of tail black in color or missing, dry skin, right forelimb cannibalized and eschar occurred sporadically and are considered to be incidental in nature and not treatment related.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	1.0	250	500

Body wt day -7	215.4	213.8	212.8	207.9
Body wt day -1	221.6	224.2	214.7	198.8b
Body wt –final (g)	401.0	416.7	358.7b	331.4 b
Body wt – lactation day 0	304.5	310.3	273.4b	259.9b
Body wt – lactation day 4	320.9	319.2	298.3a	284.2b
Premating day -7 to -1 wt gain (g)	19.4	19.5	17.3b	16.5b
GD 0-4 wt gain (g)	25.6	24.2	23.5	24.8
GD 4-8 wt gain (g)	24.8	25.9	23.6	24.8
GD 8-12 wt gain (g)	26.1	26.9	25.5	26.9
GD 12-16 wt gain (g)	27.9	29.3	27.8	28.7
GD 16-20 wt gain (g)	30.2	32.1	29.8	31.1
Lactation day 0-4 wt gain (g)	38.2	38.1	42.8	37.1

a)Statistically different from control
b)Statistically different from control

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**1.0**	**250**	**500**
Implantation sites (mean)	16.4	17.5	15.5a	12.4a
Number of litters with live pups	12	11	12	12
Total pups/litter (day 0)	16.1	16.1	13.9a	10.8b
Live pups/litter (day 0)	15.7	16.1	13.9a	10.3b
Proportion pups surviving to day 4 (%)	97	99	99	84a
Pup weights (g) – mean, day 0	6.70	6.57	6.03b	5.58b
Pup weights (g) – mean, day 4	10.72	10.20	9.83	8.44b

a)Statistically different from control
b)Statistically different from control

Conclusion:

Given the design of the study and the results observed, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The systemic maternal NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL= 250 mg/kg/day based on vaginal discharged, decreased body weights, body weight changes, and decreased absolute and relative food consumption).

The NOAEL for dermal irritation could not be determined, i.e., < 1 mg/kg, since dermal irritation occurred at all doses tested.

The developmental NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL = 250 mg/kg/day based on decreased number of implantation sites, total and live pups on lactation day 0, and decreased pup body weights on lactation days 0).

RELIABILITY/DATA QUALITY

Reliability: Valid without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-276 Dermally During GD -7 to 20. Report ATX-93-0073.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil (F-196)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091649 (F-196)		0.10	0.30	3.00	2.00	2.00	0.70	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females (non treated males used for mating)

Number of Animals per Dose: 15 per dose level of HVGO
20 per dose for sham control

Concentration:

Dose:	0, 1.0, 250, 1000 mg/kg/day
	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	GD (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HVGO (F-196) following dermal administration to female rats daily for one week prior to mating through day 20 of gestation. Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows: 1. Sham control (0 mg/kg/day) – 20 animals; 16 animals at GD 0 2. HVGO 1.0 mg/kg/day –15 animals; 10 animals at GD 0 3. HVGO 250 mg/kg/day – 15 animals; 9 animals at GD 0 4. HVGO 1000 mg/kg/day – 15 animals; 8 animals at GD 0 At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based upon the day -7 body weight for the pre-mating period and the GD 0 body weight for the gestation period. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period). Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded. Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25. The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females,

including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	1.0		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	1.0		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

One female in the 1000 mg/kg dose group was found dead on GD 22. There were no other mortalities during the study.

Slight eschar was noted on one day for one female in the sham control group. Slight erythema was noted on one day for one female in the 250 mg/kg dose group. Because the erythema was slight, of limited duration, and was noted for only one animal in the dose group, it was not considered to be related to the test article. Sporadic dermal irritation related to administration of the test article was noted for females dosed at 1000 mg/kg on premating days -6, -5, -4; mating days 0, 1; GD 0, 1, and 9-18. Slight erythema and eschar were observed at the test site. After 12 days of dosing, animals in the 1000 mg/kg dose group had accumulated test article over the body surfaces, therefore, there is the possibility of oral exposure via preening.

The incidence of vaginal discharge was slightly higher in the 250 mg/kg dose group; vaginal discharge was noted as early as GD 13 and as late as GD 20. A higher incidence of vaginal discharge was noted for females in the 1000 mg/kg dose group; vaginal discharge was observed as early as day 12 and as late as day 22 of gestation. A positive correlation was noted between vaginal discharge and resorptions. One animal that died on test exhibited tremors and lethargy on gestation days 20-21.

There were no other clinical observations that were considered to be related to treatment with the test article.

There were no effects on body weights or body weight changes at a dose of 1.0 mg/kg. Body weights of pregnant females in the 250 mg/kg dose group were significantly lower than those of the control group on GD 16 and 20. Body weights of females dosed at 1000 mg/kg were significantly lower than those of the controls on Day -1 of the premating period. Body weights of pregnant females in the 1000 mg/kg dose group were also significantly lower than those of the control females during GD 4 , 8 , 12 , 16 , and 20. Body weight changes for females dosed at 250 mg/kg were significantly lower than those of controls between Days -7 and -1 of the premating period. Body weight changes for pregnant females dosed at 250 mg/kg were significantly lower than those of controls for GD 0 to 4. Body weight changes for females dosed at 1000 mg/kg were significantly lower than those of controls between days -7 and -1 of the premating period. Body weight changes for pregnant females in the 1000 mg/kg dose group were also significantly lower than those of the control females for GD 0 to 4, 12 to 16, and 16 to 20.

There were no effects on absolute or relative food consumption at a dose of 1.0 mg/kg. Absolute and relative food consumption for females in the 250 mg/kg dose group were significantly lower than that of the controls during days -7 to -1 of the premating period. Absolute and relative food consumption for pregnant females in the 250 mg/kg dose group were significantly lower than that of the controls during GD 0 to 4. Absolute and relative food consumption for females in the 1000 mg/kg dose group were significantly lower than that of the controls during days -7 to -1 of the premating period. Absolute food consumption for pregnant females in the 1000 mg/kg dose group was significantly lower than that of the controls during GD 0 to 4 and 16 to 20.

Decreased thymus size was noted at necropsy for one, two, three, and six females in the 0.0, 1.0, 250 and 1000 mg/kg dose groups, respectively. One of three in the 250 mg/kg dose group and five of six in the 1000 mg/kg dose group were noted as very small. The decreased thymus size was considered toxicologically significant at the 250 and 1000 mg/kg dose levels. The 1000 mg/kg female that was found dead also had red vaginal discharge, a pale liver, and a uterus with early and late resorption sites.

Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

There were no effects on delivery or litter data at a dose of 1.0 mg/kg. For females dosed at 250 mg/kg, the number of total and live pups on lactation day 0 were significantly lower than that of the controls. Body weights of pups in the 250 mg/kg dose group were also significantly lower than those of the control group on lactation days 0 and 4. None of the pregnant females dosed at 1000 mg/kg delivered a litter. For the 1.0 and 250 mg/kg dose groups, there were no significant differences (when compared to the control group) in gestation length, number of implantation sites, external pup alterations, 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. There was no significant difference between the 1000 mg/kg dose group and the controls for the number of implantation sites.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**1.0**	**250**	**1000**
Body wt day -7	257.7	258.5	258.0	257.7
Body wt day -1	265.4	264.5	257.8	250.5a
Body wt –final (g)	410.9	416.8	374.2a	281.6b
Body wt – lactation day 0	308.8	312.5	293.3	NAc
Body wt – lactation day 4	316.9	316.9	301.2	NAc
Premating day -7 to -1 wt gain (g)	7.7	6.0	-0.2	-7.3b
GD 0-4 wt gain (g)	24.8	22..3	11.0b	14.3b
GD 4-8 wt gain (g)	12.8	13.8	14.1	11.5
GD 8-12 wt gain (g)	20.5	23.3	19.1	15.6
GD 12-16 wt gain (g)	27.3	29.6	20.1	-2.6b
GD 16-20 wt gain (g)	61.6	64.7	51.0	-8.6b
Lactation day 0-4 wt gain (g)	8.1	4.4	7.9	NAc

a)Statistically different from control
b)Statistically different from control
c) Not applicable – no females delivered

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**1.0**	**250**	**1000**
Implantation sites (mean)	15.8	16.9	14.7	15.3
Number of litters with live pups	16	10	9	(c)
Total pups/litter (day 0)	14.4	15.3	10.9b	(c)
Live pups/litter (day 0)	13.8	14.9	10.3b	(c)
Proportion pups surviving to day 4 (%)	97	82	98	(c)
Pup weights (g) – mean, day 0	6.43	6.38	6.02a	(c)
Pup weights (g) – mean, day 4	9.58	9.51	8.23b	(c)

a)Statistically different from control
b)Statistically different from control

Conclusion:

c)No females delivered

Given the design of the study and the results observed, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The maternal NOAEL for dermal exposure to HVGO during gestation days GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL= 250 mg/kg/day based on vaginal discharge, decreased body weights, body weight changes, decreased food consumption and decreased thymus size).

The developmental NOAEL for dermal exposure to HVGO during gestation days GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL = 250 mg/kg/day based on decreased number of total and live pups on lactation day 0, and decreased pup body weights were lower on lactation days 0 and 4).

RELIABILITY/DATA QUALITY

Reliability: Valid without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-196 Dermally During GD -7 to 20. 1994. Report ATX-91-0130.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil (HVGO); VDF Gas Oil

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil; (F-197)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091650 (F-197)		0.00	0.40	4.00	2.00	0.60	0.20	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	15 per dose level of HVGO 20 per dose for sham control
Concentration:	
Dose:	0, 1, 250.0 (241 = corrected dose), 1000.0 (965 = corrected dose) mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation day (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None

Method/Guideline and Test Condition Remarks:

The study was designed to determine the developmental toxicity of HVGO (F-197) following dermal administration to female rats daily for one week prior to mating through day 20 of gestation.

Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows:

1. *Sham control (0 mg/kg/day) – 20 animals
2. HVGO 1 mg/kg/day – 15 animals
3. HVGO 241 mg/kg/day – 15 animals
4. HVGO 965 mg/kg/day – 15 animals

*Shared with study number ATX-91-0127

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based upon the day -7 body weight for the pre-mating period and the GD 0 body weight for the gestation period.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for

changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:

The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	241		mg/kg/day
NOAEL- Dermal	Maternal	=	1		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	241		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	1		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

There were no mortalities during the study.

No dermal irritation was noted for the sham control or the 1 mg/kg/day dose. Slight to moderate erythema, edema and eschar, and dry skin were observed at site of administration at the two highest dose levels (241 and 965 mg/kg/day). A higher incidence of vaginal discharge was noted for females in the 241 mg/kg/day dose group; vaginal discharge was observed as early as GD 14 and as late as GD 21. For the 965 mg/kg/day group vaginal discharge was noted as early as GD 12 and as late as GD 23. There were no other clinical observations that were considered to be related to treatment with the test article. Paleness, decreased body temperature, and/or lethargy were noted for three females in the 965 mg/kg/day dose group; these findings were associated with an increased incidence of vaginal discharge.

There were no effects on body weights or body weight changes at a dose of 1 mg/kg/day. Per the table below, mean body weights were significantly decreased in the 241 mg/kg/day groups at various points during gestation and post-natal period. Significant differences were noted in body weight for the 965 mg/kg/day females during the pre-mating and gestational periods. Body weight changes for the 241 and the 965 mg/kg/day dose groups were significantly lower during both the pre-mating and gestational periods.

There were no effects on absolute or relative food consumption at a dose of 1 mg/kg/day. Absolute and relative food consumption for females in the 241 mg/kg/day dose group was significantly lower ($p<0.01$) than that of the controls during days -7 to -1 of the pre-mating period. Absolute food consumption in this dose group was significantly lower during GD 0 to 4 ($p<0.01$), 4 to 8 ($p<0.01$), 8 to 12 ($p<0.01$), and 12 to 16 ($p<0.05$). Relative food consumption the 241 mg/kg/day dose group was significantly lower than that of the controls during GD 0 to 4 ($p<0.01$). Absolute and relative food consumption in the 965 mg/kg/day dose group was significantly lower ($p<0.01$) than that of the controls during days -7 to -1 of the pre-mating period. Absolute food consumption in this group was significantly lower than that of the controls during GD 0 to 4 ($p<0.05$), 8 to 12 ($p<0.05$), 12 to 16 ($p<0.01$), and 16 to 20 ($p<0.01$). Relative food consumption was significantly

lower ($p<0.05$) during GD 12 to 16.

A uterus with a late resorption was noted at necropsy for one female in the 965 mg/kg/day dose group. Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

There were no effects on delivery and litter data at a dose of 1 mg/kg. At a dose of 241 mg/kg/day, the number of total and live pups delivered were significantly lower ($p<0.05$); the proportion of pups surviving to lactation day 4 was significantly lower ($p<0.01$); and pup body weights were significantly lower ($p<0.05$) on both lactation days 0 and 4. At a dose of 965 mg/kg/day, none of the females delivered a litter (pregnancy was confirmed through examination of the uterine horns at necropsy).

For the 1 and 241 mg/kg dose groups, there were no significant differences in gestation length, the number of implantation sites, external pup alterations, or the proportion of males on lactation days 0 and 4.

There was no significant difference in the number of implantation sites between the controls and the 965 mg/kg dose group.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**1**
Body wt day -7	264.0	263.3
Body wt day -1	272.3	274.1
Body wt –final (g)	428.1	440.3
Body wt – lactation day 0	323.9	323.0
Body wt – lactation day 4	337.0	341.9
Premating day -7 to -1 wt gain (g)	8.3	10.8
GD 0-4 wt gain (g)	20.2	22.2
GD 4-8 wt gain (g)	19.1	21.3
GD 8-12 wt gain (g)	20.0	19.7
GD 12-16 wt gain (g)	31.5	31.0
GD 16-20 wt gain (g)	59.6	60.3
Lactation day 0-4 wt gain (g)	13.1	18.9

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c)No females delivered

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**1**
Dams with resorptions	0	0
Implantation sites	15.9	17.4
Number of litters with live pups	18	9
Total pups/litter (day 0)	14.9	16.1
Live pups/litter (day 0)	14.7	14.8
Proportion pups surviving to day 4	0.985	0.970
Pup weights (g) – mean, day 0	6.625	6.557
Pup weights (g) – mean, day 4	9.873	9.624

a)Statistically different from control ($p<0.05$)

Conclusion:

b)Statistically different from control (p<0.01)
c)No females delivered
The maternal NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1 mg/kg/day (LOAEL= 241 mg/kg/day based on increased vaginal discharge, decreased body weights, body weight changes and decreased food consumption)

The developmental NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1 mg/kg/day (LOAEL = 241 mg/kg/day based on a decreased total and live pup numbers, proportion of pups surviving to lactation day 4, and decreased pup body weights on lactation day 0 and 4)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-197 Dermally During Gestation Days -7 to 20. Report ATX-91-0131.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil; Hydrocracker Fresh Feed

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil (F-201)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091654 (F-201)		0.10	0.40	4.00	3.00	0.90	0.40	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	15 per dose level of HVGO 20 per dose for sham control
Concentration:	
Dose:	0, 1.0, 250, 1000 mg/kg/day
	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation day (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HVGO (F-201) following dermal administration to female rats daily for one week prior to mating through day 20 of gestation. Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows: 1. Sham control (0 mg/kg/day) – 20 animal; 16 animals at GD 0 2. HVGO 1.0 mg/kg/day –15 animals; 10 animals at GD 0 3. HVGO 250 mg/kg/day – 15 animals; 9 animals at GD 0 4. HVGO 1000 mg/kg/day –15 animals; 12 animals at GD 0 At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based upon the day -7 body weight for the pre-mating period and the GD 0 body weight for the gestation period. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-

health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and

determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal		1.0		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)		1.0		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

One female in the 1000 mg/kg dose group was sacrificed in a moribund condition.

Slight eschar was noted on one day for one female in the sham control group. Slight erythema was noted on one day for one female in the 1.0 mg/kg dose group. Because the erythema was slight, of limited duration, and was noted for only one animal in the dose group, it was not considered to be related to the test article. Treatment related dermal irritation was observed at the test site in the 250 and 1000 mg/kg dose groups consisting of slight erythema and eschar.

The dermal irritation was noted beginning premating day -2 extending to GD 16 for the 250 mg/kg dose group and premating day -6 to GD 17 for the high dose group. After 12 days of dosing, animals in the 1,000 mg/kg dose group had accumulated test article over the body surfaces.

The incidence of vaginal discharge (primarily slight) was slightly higher in the 250 mg/kg dose group; vaginal discharge was noted as early as GD 12 and as late as GD 22. A higher incidence of vaginal discharge was noted for females in the 1000 mg/kg dose group; vaginal discharge was observed as early as day 12 and as late as day 24 of gestation.

Ocular and nasal discharge were observed more frequently for the 1000 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 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 significantly lower than those of the control group on GD 4, 8, 12, 16, and 20 and on lactation day 4.

Body weights of females dosed at 1,000 mg/kg were significantly lower than those of the controls on day -1 of the premating period. Body weights of pregnant females in the 1,000 mg/kg dose group were also significantly lower than those of the control females during GD 4, 8, 12, 16, and 20. Body weight changes for females dosed at 1.0 mg/kg were significantly higher than those of controls for lactation days 0 to 4; this difference was not considered to be toxicologically significant.

Body weight changes for females dosed at 250 mg/kg were significantly lower

than those of controls between Days -7 and -1 of the premating period. Body weight changes for pregnant females dosed at 250 mg/kg were significantly lower than those of controls for GD 0 to 4, 12 to 16, and 16 to 20 and lactation days 0 to 4. Body weight changes for females dosed at 1000 mg/kg were significantly lower than those of controls between Days -7 and -1 of the premating period. Body weight changes for pregnant females in the 1,000 mg/kg dose group were also significantly lower than those of the control females for GD 0 to 4, 8 to 12,12 to 16, and 16 to 20.

Relative food consumption for females in the 1.0 mg/kg dose group was significantly lower than that of the controls during Days -7 to -1 of the premating period. Absolute and relative food consumption for pregnant females in the 1.0 mg/kg dose group was significantly higher than that of the controls during lactation days 0 to 4. These differences were not considered to be toxicologically significant. Absolute and relative food consumption for females in the 250 mg/kg dose group were significantly lower than that of the controls during days -7 to -1 of the premating period. Absolute food consumption for pregnant females in the 250 mg/kg dose group was significantly lower than that of the controls during GD 0 to 4 and lactation Days 0 to 4. Relative food consumption for pregnant females in the 250 mg/kg dose group was significantly lower than that of the controls during GD 0 to 4. Relative food consumption for pregnant females in the 250 mg/kg dose group was significantly higher than that of the controls during GD 12 to 16; this difference was not considered to be toxicologically significant. Absolute and relative food consumption for females in the 1,000 mg/kg dose group were significantly lower than that of the controls during days -7 to -1 of the premating period. Absolute food consumption for pregnant females in' the 1,000 mg/kg dose group was significantly lower than that of the controls during GD 16 to 20. Relative food consumption for pregnant females in the 1000 mg/kg dose group was significantly higher than that of the controls during
GD 8 to 12; this difference was not considered to be toxicologically significant.

Decreased thymus size was noted at necropsy for one of the control females, three females in the 250 and six females in the 1,000 mg/kg dose groups. The decreased thymus size in the 250 and 1000 mg/kg dose groups was considered to be treatment related. The 1,000 mg/kg female sacrificed in a moribund condition also had a pale liver, pale and enlarged kidneys, enlarged cervical lymph nodes, and a hemorrhagic uterus with early and late resorption sites.

Although other findings were observed at the time of necropsy, they were considered incidental and unrelated to test article treatment.

For females dosed at 250 mg/kg, the number of implantation sites was significantly lower than that of the control group, suggesting increased pre-implantation loss for the females dosed at 250 mg/kg. The number of total and live pups on lactation day 0 were also significantly lower than that of the controls. Body weights of pups in the 250 mg/kg dose group were also significantly lower than those of the control group on lactation days 0 and 4. The number of implantation sites for females in the 1,000 mg/kg dose group was lower than that of the control group. While this difference was not statistically significant, it was considered to be toxicologically significant based upon the decreased number of implantation sites noted at 250 mg/kg. The number of females that delivered was also decreased in the 250 and 1000 mg/kg dose groups. Six out of nine females in the 250 mg/kg dose group and zero out of twelve of the pregnant females dosed at 1,000 mg/kg delivered a litter.

For the 1.0 and 250 mg/kg dose groups, there were no significant differences in gestation length, external pup alterations, 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.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	1.0	250	1000
Body wt day -7	257.7	259.3	258.2	255.5
Body wt day -1	265.4	263.9	252.0a	248.9b
Body wt –final (g)	410.9	412.0	342.0b	270.7b
Body wt – lactation day 0	308.8	300.9	300.3	NAc
Body wt – lactation day 4	316.9	320.6	292.4a	NAc
Premating day -7 to -1 wt gain (g)	7.7	4.6	-6.2b	-6.6b
GD 0-4 wt gain (g)	24.8	18.3	10.7b	-5.6b
GD 4-8 wt gain (g)	12.8	13.7	15.0	10.8
GD 8-12 wt gain (g)	20.5	23.8	19.2	13.0b
GD 12-16 wt gain (g)	27.3	23.4	12.9 a	-10.9b
GD 16-20 wt gain (g)	61.6	69.1	28.2b	-8.9b
Lactation day 0-4 wt gain (g)	8.1	19.7b	-3.4a	NAc

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) Not applicable – no females delivered

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	1.0	250	1000
Implantation sites (mean)	15.8	16.1	13.6b	14.3
Number of litters with live pups	16	9	6	(c)
Total pups/litter (day 0)	14.4	14.8	8.5b	(c)
Live pups/litter (day 0)	13.8	14.8	7.5b	(c)
Proportion pups surviving to day 4 (%)	97	94	89	(c)
Pup weights (g) – mean, day 0	6.54	6.61	5.74	(c)
Pup weights (g) – mean, day 4	9.56	9.10	6.07	(c)

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c)No females delivered

Conclusion:

Given the design of the study and the results observed, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The maternal NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL= 250 mg/kg/day based on decreased body weights, body weight changes, skin irritation and decreased thymus size).

The developmental NOAEL for dermal exposure to HVGO during GD -7 to 20 was determined to be 1.0 mg/kg/day (LOAEL = 250 mg/kg/day based on decreased implantation sites, decreased number of total and live pups on lactation day 0, and decreased pup body weights were lower on lactation days 0 and 4).

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-201 Dermally During GD -7 to 20. Report ATX-913-0135.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil (HVGO); VDF Gas Oil

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil; (F-197)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091650 (F-197)		0.00	0.40	4.00	2.00	0.60	0.20	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Portage, MI)

Other Strain: Not applicable

Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	25 per dose for level
Concentration:	
Dose:	0, 50, 100, 250 mg/kg/day
Year Study Performed :	1993
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study)
GLP:	Yes
Exposure Period:	Gestation day (GD) 0-19
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of HVGO (F-197) administered percutaneously to presumed pregnant rats. Prior to the initiation of dosing with the test substance, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of sperm in a vaginal smear or a copulatory plug, the individual, presumed pregnant females were randomly assigned to four treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of evidence of mating: 1. Vehicle control (acetone) 0 mg/kg/day – 25 animals (GD 0-19) 2. HVGO 50 mg/kg/day – 25 animals (GD 0-19) 3. HVGO 100 mg/kg/day – 25 animals (GD 0-19) 4. HVGO 250 mg/kg/day – 25 animals (GD 0-19) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. Suspensions of F-197 were prepared daily at concentrations of 0 (vehicle, acetone), 50, 100 and 250 mg/mL such that doses of 0, 50, 100, and 250 mg/kg/day, respectively, were administered at a volume of 1 mL/kg. Animals in all groups were treated on GD 0 through GD 19. Each treatment day, animals were dosed by even application of the test substance to their shaved backs, using a blunt-tipped glass syringe. The test substance dose was calculated from each rat's most recent body weight. Rats were fitted with Elizabethan collars to minimize ingestion of test substance. Controls were handled in the same manner but with application of the vehicle only. Elizabethan collars were applied just prior to dosing and were removed after a 6 hour exposure period. At the time of collar removal, any excess test article was wiped off with a cloth dipped in acetone and dried with a clean cloth. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Skin reactions were graded using the Draize and National Research Council standards. Individual body weights and food consumption were recorded daily during presumed gestation. All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation, and a gross necropsy of the thoracic and abdominal viscera was performed. The abdomen of each rat was opened, and the intact uterus was excised and examined for pregnancy. To confirm the pregnancy status, uteri from rats that appeared non-pregnant were examined while transilluminated

and pressed between two glass plates. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other maternal tissues were discarded.

Corpora lutea in each ovary were recorded. The number and distribution of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Each fetus was removed from the uterus, placed in an individual container, weighed, and examined for weighed and examined for sex and gross external alterations. Live fetuses were sacrificed.

Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations by using an adaptation of Wilson's sectioning technique. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red, and examined for skeletal alterations.

STATISTICAL ANALYSES: Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Maternal body weights, body weight changes, feed consumption values, and litter averages for fetal body weights, percent male fetuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test and ANOVA, when appropriate [i.e., Bartlett's Test was not significant (P>0.05)]. If the analysis of variance was significant (P<0.05), Dunnett's Test was used to identify the statistical significance of the 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, when less than or equal to 75% ties were present. When more than 75% ties were present, Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant (P<0.05), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures described for the Kruskal-Wallis Test.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	50		mg/kg/day
NOAEL- Dermal	Maternal	=	Not determined (<50)		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	100		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

No deaths occurred during the conduct of this study. Skin reactions related to administration of the test substance
occurred in the 50, 100 and 250 mg/kg/day dose groups. Significantly increased (P<0.05 to P<0.01) numbers of rats in the 50, 100 and 250 mg/kg/day dose group had erythema (grade 1). One rat in the 250 mg/kg/day dose group had grade 2 erythema. Significant (P<0.01) desquamation (grade 1) occurred in one rat at the 100 mg/kg/day dose and six rats in the 250 mg/kg/day dose. One 50 mg/kg/day dose group rat also had grade 1 edema. This observation was considered unrelated to the test substance because the incidence was not dose-dependent.

All other clinical observations were unrelated to administration of the test substance because: 1) the incidences were not dose-dependent; 2) the values were not significantly increased, as compared with the control group values; or 3) the observations commonly occur in this strain of rat. These observations included localized alopecia,
lacrimation, chromorrhinorrhea and lesions located on the head, neck or forelimb. The only necropsy observation (moderate dilation of the pelvis of the left kidney) was considered unrelated to test substance administration because it was not a dose~dependent event and occurred in only one rat in the 100 mg/kg/day dose group.

Maternal body weight and body weight gains were significantly reduced in the 100 and 250 mg/kg/day dose group at various points for for the entire dosing period per the table below. Body weight gains were significantly reduced (P<0.05) in the 50 mg/kg/day dose group on GD 19 to 20. This reduction was not biologically important and considered unrelated to the test substance administration.

Absolute and relative feed consumption values were reduced or significantly reduced (P<0.05to P<0.01) in the 50, 100 and 250 mg/kg/day dose groups on days 9 to 12 of gestation. Absolute and/or relative feed consumption values were significantly reduced (P<0.05to P<0.01) in the 100 and 250 mg/kg/day dose groups on days 3 to 6, 6 to 9 and 0 to 20 of gestation. These values were also reduced or significantly reduced (P<0.05to P<0.01) in the 250 mg/kg/day dose group on days 0 to 3 of gestation.

There were 20, 19, 19 and 21 rats pregnant and Caesarean-sectioned on day 20 of gestation in the 0, 50, 100 and 250 mg/kg/day dose groups, respectively. Litter sizes and the number of live fetuses were significantly reduced (P<0.05) in the 250 mg/kg/day dose group. Litter averages for total resorptions, early resorptions and percent resorbed conceptuses and the number of dams with resorptions were increased in the 250 mg/kg/day dose group. Live fetal body weights and female fetal body weights were significantly reduced (P<0.01) in the 250 mg/kg/day dose group. There were no statistically significant or biologically important differences in the litter averages for corpora lutea, implantations and sex ratios. There were no late resorptions. No dam resorbed all conceptuses, and the numbers of dams with viable fetuses were comparable among the four dose groups.

Fetal alterations were classified as: 1) malformations (irreversible changes which occur at low incidences in this species and strain); or 2) variations (relatively common developmental changes in this species and strain, including minor reversible delays or accelerations in development).

The average number of caudal vertebral ossification sites per fetus was significantly reduced (P<0.01) in the 250 mg/kg/day dose group, as compared with the control group value. The fetal and litter incidences of bifid thoracic vertebral centra and, incompletely ossified sternebrae tended to be increased in the 250 mg/kg/day dose group.
These delays in ossification were considered effects of the test substance and

associated with reduced fetal body weights in this dose group. No gross external or soft tissue alterations in the fetuses were observed at doses as high as 250 mg/kg/day. The incidences that occurred were neither dose-dependent nor statistically significant or the alterations occurred in only one 250 mg/kg/day dose group fetus.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	50	100	250
Body wt –final (gr)	412.6	408.0	408.2	388.9
GD 0-3 wt gain (gr)	18.4	16.8	15.2	11.0 a
GD 3-6 wt gain (gr)	15.6	15.7	16.0	12.8
GD 6-9 wt gain (gr)	14.2	15.3	13.9	10.9
GD 9-12 wt gain (gr)	23.3	20.8	20.4	19.8
GD 12-15 wt gain (gr)	21.8	22.8	21.9	21.1
GD 15-19 wt gain (gr)	61.2	57.5	60.0	56.0
GD 19-20 wt gain (gr)	19.4	16.7	17.7	13.0 b
GD 0-20 wt gain (gr)	174.0	165.7	165.b	144.7 1b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	100	250
Corpora lutea	20.2	18.7	19.8	18.7
Implantation sites – mean	16.4	16.0	17.0	15.8
Live fetuses – total	312	282	300	288
Live fetuses - mean	15.6	14.8	15.8	13.7b
Litter size	15.6	14.8	15.8	13.7b
Viable male fetuses (%)	51.3	52.5	48.5	47.5
Total resorptions (mean)	0.8	1.2	1.2	2.0
Dams with resorptions	10	12	13	16

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Fetal Endpoints

Dose (mg/kg/day)	0	50	100	250
Fetal weights (gr)	3.60	3.62	3.68	3.41b
Litters evaluated	20	19	19	21
Live fetuses - total	312	282	300	288
Dead fetuses – dead	0	0	0	0
% Resorbed conceptuses per litter	5.9	7.6	7.2	12.7
Litters with any alteration (N;%)c	5(25.0)	8(42.1)	5(26.3)	10(47.6)cd
Fetuses with any alteration (N;%)c	6(1.9)	9(3.2)	8(2.7)	14(4.9)cd
Fetuses with any alteration per litter (mean %)c	1.84	3.40	2.52	4.84

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) See text for discussion of results.
d) Some of the specific fetal alterations in this group were judged to be test substance related based on criteria described above.

Conclusion:
The maternal NOAEL for dermal exposure to HVGO during GD 0-19 was determined to be <50 mg/kg/day (LOAEL = 50 mg/kg/day based on skin irritation and feed consumption).

Reviewer's note: reduced feed consumption did not result in significantly lower body weights or body weight gains at this dose level (50 mg/kg).

The developmental NOAEL for dermal exposure to HVGO during GD 0-19 was determined to be 100 mg/kg/day. (LOAEL = 250 mg/kg/day based on decreased fetal body weights, and increased variations in fetal skeletal ossification.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1993. Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of F-197 Administered Percutaneously to Crl:CD®BRK VAF/Plus® Presumed Pregnant Rats. Report ATX-92-0154.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; Clarified Slurry Oil (CSO): Petrobase

Test Substance Purity/Composition and Other Test Substance Comments: Clarified Slurry Oil; (F-179)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091645 (F-179)		0.00	0.70	10.0	30.00	20.00	6.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	15 per dose level of CSO per details below 20 per dose for sham control
Concentration:	
Dose:	0, 0.05, 10.0, 250 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation day (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of CSO (F-179) following dermal administration to female rats daily for one week prior to mating through day 20 of gestation. Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows: 1. Sham control (0 mg/kg/day) – 20 (18 animals at GD 0) 2. CSO 1.0 mg/kg/day – 15 (11 animals at GD 0) 3. CSO 241.0 mg/kg/day – 15 (12 animals at GD 0) 4. CSO 965.0 mg/kg/day – 15 (14 animals at GD 0) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based upon the day -7 body weight for the pre-mating period and the GD 0 body weight for the gestation period. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	10		mg/kg/day
NOAEL- Dermal	Maternal	=	0.05		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	10		mg/kg/day

Results Remarks:

The animals used in the study were between 13 and 14 weeks of age at exposure initiation.

There were no mortalities 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 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 than those of the control females throughout most of gestation.

Body weight changes for females dosed at 10.0 or 250.0 mg/kg were significantly lower 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 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 10.0 and 250 mg/kg dose groups were significantly lower than that of the controls during Days -7 to -1 of the premating period. At a dose of 10.0 mg/kg, absolute and relative food consumption for pregnant females was significantly lower than that of the controls during GD 0 to 4; relative food consumption was also significantly lower ($p<0.05$) than that of controls during GD 4 to 8. Absolute food consumption for pregnant females in the 250 mg/kg dose group was significantly lower than that of the control females throughout gestation; relative food consumption was significantly lower than that of controls during GD 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.

Fertility rates of 39 to 50% were noted for all groups during the study. The reason for these low fertility rates was not determined. Because decreased fertility was also noted in the control group, it was not considered to be related to the test article

Signs of developmental toxicity considered to be related to administration of F-179 was limited to the 250 mg/kg dose group; none of the females in this dose level 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.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	0.05	10	250
Body wt day -7	284.	282.20	286.33	284.73
Body wt day -1	293.05	285.07	286.53	261.73b
Body wt –final (g)	424.29	455.60	419.17	271.17 b
Body wt – lactation day 0	349.00	355.33	348.50	NAc
Body wt – lactation day 4	354.60	350.40	357.40	NAc
Premating day -7 to -1 wt gain (g)	8.25	2.87	0.20b	-23.00b
GD 0-4 wt gain (g)	27.00	29.60	16.50	-4.67b
GD 4-8 wt gain (g)	16.29	16.80	13.33	9.17
GD 8-12 wt gain (g)	15.29	19.60	21.80	9.17
GD 12-16 wt gain (g)	23.57	30.60	19.20	-26.50b
GD 16-20 wt gain (g)	41.0	65.80	46.33	3.50b
Lactation day 0-4 wt gain (g)	11.50	-13.33	5.50	NAc

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c)Not applicable - no females delivered

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	0.05	10	250
Dams with resorptions	1	0	1	6
Implantation sites (mean)	11.7	14.4	10.5	9.0
Number of litters with live pups	6	5	5	0
Total pups/litter (day 0)	13.0	13.2	10.8	(c)
Live pups/litter (day 0)	12.7	13.0	10.8	(c)
Proportion pups surviving to day 4 (%)	97.4	81.5	92.6	(c)
Pup weights (g) – mean, day 0	6.747	6.557	6.280	(c)
Pup weights (g) – mean, day 4	9.815	9.215	9.004	(c)

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c)No females delivered

Conclusion:

Given the design of the study and the results observed, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The maternal NOAEL for dermal exposure to CSO during gestation days -7 to 20 was determined to be 0. 05 mg/kg/day (LOAEL= 10.0 mg/kg/day based on decreased body weight changes and food consumption).

The developmental NOAEL for dermal exposure to CSO during gestation days -7 to 20 was determined to be 10.0 mg/kg/day (LOAEL = 250.0 mg/kg/day based on a 100% resorption rate – none of the females delivered).

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-179 Dermally During GD -7 to 20. 1994. Report ATX-91-0155.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; FCCU Clarified Oil, Carbon Black Oil (CBO)

Test Substance Purity/Composition and Other Test Substance Comments: CBO (F-229)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.%[1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091692 (F-229)		0.00	3.00	20.00	30.00	10.00	4.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Wilmington, MA)

Other Strain: Not applicable

Gender: Females (non treated males used for mating)

Number of Animals per Dose: 12 per dose at 0.05, 10, or 50 mg/kg dose level of test material
15 per dose for sham control

Concentration:	
Dose:	0, 0.05, 10, 50 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation Day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of CBO (F-229) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. CBO 0.05 mg/kg/day – 12 animals (GD 0-20) 3. CBO 10 mg/kg/day – 12 animals (GD 0-20) 4. CBO 50.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The animals used in the study were between 12 and 13 weeks of age at exposure initiation. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period). Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded. Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day0, pup alterations at lactation day0, male pups at days 0 and 4, survival of pups at lactation day4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	50		mg/kg/day
NOAEL- Dermal	Maternal	=	10		mg/kg/day

LOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	10		mg/kg/day

Results Remarks:

No mortality occurred during the study.

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. Slight erythema, eschar and/or dry skin were observed at the test site for one or more animals in the 0.05 mg/kg test article dose group. Slight erythema was observed at the test site for one or more animals in the 10 mg/kg test article dose group. Since the dermal irritation observed in the treated groups was noted with a similar degree and frequency in the control group, these findings are not considered to be treatment related. In addition, one animal in the 50 mg/kg dose group was recorded as having pale eyes on GD 17 - 21. This finding is also not considered to be treatment related.

In the 50 mg/kg test article dose group, there was an increased incidence of vaginal discharge and the gestation length (days) was significantly longer ($p<0.01$) than that of the control group. There were no other clinical observations that were considered to be related to treatment with the test article.

Body weights for pregnant females in the 50 mg/kg dose group were significantly lower ($p<0.05$) than those of the control females on GD 16 and significantly lower ($p<0.01$) than those of the
control females on GD 20. Body weight changes for pregnant females in the 50 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females between GD 0 to 4 and 16 to 20. They were also significantly lower ($p<0.05$) than those of the control females between GD 4 to 8 and Lactation Days 0 to 4. There were no other effects on body weights or body weight changes at any of the dose levels.

Mean absolute food consumption for pregnant females in the 50 mg/kg dose group was significantly lower ($p<0.01$) than that of the control females between GD 16 to 20. Relative food consumption for pregnant females in the 10 mg/kg dose group was significantly higher ($p<0.05$) than that of the control females during GD 12 to 16. This difference is not considered to be
related to treatment with the test article since the relative food consumption was not significantly different at the higher dose level of 50 mg/kg. There were no other effects on absolute or relative food consumption at any of the dose levels.

No lesions related to administration of the test article were noted for females in any of the dose groups. At a dose of 50 mg/kg, the total number of pups delivered and the total number of live pups delivered were significantly lower ($p<0.01$) than the control females. The proportion dead on Lactation Day 0 was significantly higher ($p<0.01$) than the control females. The proportion males was significantly lower ($p<0.05$) than the control group on Lactation Days 0 and 4. At a dose of 50 mg/kg, the live pup weights on Lactation Day 0 were significantly lower ($p<0.01$) than those of the control group.

For all dose groups, there were no significant differences for the number of implantation sites, proportion surviving to Lactation Day 4 or external pup alterations.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**0.05**	**10**	**50**
Body wt –final (g)	424.5	420.3	420.3	357.9b
Body wt – lactation day 0	325.1	329.9	316.3	327.8

Body wt – lactation day 4	334.8	337.9	328.1	318.0
GD 0-4 wt gain (g)	23.5	25.9	22.7	14.4b
GD 4-8 wt gain (g)	16.5	21.3	16.5	11.5a
GD 8-12 wt gain (g)	22.7	21.8	22.6	19.7
GD 12-16 wt gain (g)	32.8	32.3	33.4	20.3
GD 16-20 wt gain (g)	59.1	53.6	59.6	20.2b
Lactation day0-4 wt gain (g)	9.7	8.0	11.8	-9.8a

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**0.05**	**10**	**50**
Number of dams pregnant	15	8	11	10
Number of dams with resorptions	0	0	0	0
Number of dams that delivered	15	8	11	6
Implantation sites - Mean	16.4	14.0	16.7	15.5
Number of litters with live pups	15	8	11	6
Total pups/litter (day 0)	14.0	13.0	15.4	6.5b
Live pups/litter (day 0)	13.9	12.9	15.1	4.5b
Proportion surviving to day 4 (%)	87	83	95	74
Proportion males – day 0	49	49	48	25a
Proportion males – day 4	55	55	48	30a
Pup weights (g) – mean, day 0	6.681	6.338	6.453	5.598b
Pup weights (g) – mean, day 4	8.969	8.744	8.567	7.418

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Conclusion:

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The systemic maternal NOAEL for dermal exposure to CBO during GD 0-20 was determined to be 10 mg/kg/day; the LOAEL= 50 mg/kg/day based on decreased body weight on GD 16 and 20, decreased body weight changes between GD 0-4, 4-8, 16-20 and between Lactation Days 0-4, an increased incidence of vaginal discharge and an increase in gestation length.

The developmental NOAEL for dermal exposure to CBO during GD 0-20 was determined to be 10 mg/kg/day; the LOAEL = 50 mg/kg/day based on decreased total and live pups per litter, increased proportion dead on Lactation Day 0, decreased proportion of males on Lactation Days 0 and 4, and a decrease in pup body weights on Lactation Day 0.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-229 Dermally During GD 0 to 20. Report ATX-91-0267.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64742-86-5

Test Substance: 64742-86-5; Hydrodesulfurized Heavy Vacuum Gas Oil (HHVGO)

Test Substance Purity/Composition and Other Test Substance Comments: HHVGO (F-227)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.%[1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091690 (F-227)		0.10	0.70	3.00	2.00	1.00	0.30	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Wilmington, MA)

Other Strain: Not applicable

Gender: Females (non treated males used for mating)

Number of Animals per Dose: 12 per dose at 50, 333, or 1000 mg/kg dose level of test material
15 per dose for sham control

Concentration:	
Dose:	0, 50, 333, 1000 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	No information
Exposure Period:	Gestation Day (GD) 0 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HHVGO (F-227) following dermal administration to female rats daily for days 0 through day 20 of gestation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Females that exhibited positive signs of mating were randomly assigned to four treatment groups. Males were not treated. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of sperm in a vaginal smear or a copulatory plug: 1. Sham control 0 mg/kg/day – 15 animals (GD 0-20) 2. HHVGO 50 mg/kg/day – 12 animals (GD 0-20) 3. HHVGO 333 mg/kg/day – 12 animals (GD 0-20) 4. HHVGO 1000.mg/kg/day – 12 animals (GD 0-20) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The animals used in the study were between 12 and 13 weeks of age at exposure initiation. The test material was administered to groups 2-4 on GD 0 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. Test article was applied to alternating sites (intrascapular and lumbar regions). 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 was wiped from the application site. The dose administered was based upon the GD 0 body weight. With the exception of test article application, control animals underwent the same procedures as treated animals. Dosing was based on the results of an irritation pre-screening test conducted prior to initiation of the developmental study. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Individual body weights were recorded at receipt, near the end of the quarantine period, on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period). Each litter was observed daily during lactation day0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded. Each female that mated was sacrificed by overexposure to carbon dioxide

and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 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 was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup was recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0, male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value	Value or Lower	Upper	Units:

		Description:	Concentration:	Concentration:	
LOAEL – Dermal	Maternal	=	333		mg/kg/day
NOAEL- Dermal	Maternal	=	50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	333		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day

Results Remarks:

One female in the 1000 mg/kg dose group was found dead on GD 16. No other mortalities occurred in this phase of the study.

No dermal irritation was observed at the test site for animals in the 50 mg/kg dose group. Slight dermal irritation (erythema, eschar, and/or dry skin) was observed at the test site for four animals in the 333 mg/kg dose group. The duration of the irritation was less than one week for all of the animals. Slight to extreme (primarily slight) erythema, slight to moderate edema, and slight eschar were observed at the test site for nine of the animals in the 1000 mg/kg dose group.

The incidence of vaginal discharge was higher than that of the control group for females in the 333 and 1000 mg/kg dose groups. One female in the 333 mg/kg dose group and two females in the 1000 mg/kg dose group were pale in color for four to 11 days. One female in the 333 mg/kg dose group was cold to touch for one day. Red/black stained coat in the perineal region was noted for two females in the 1000 mg/kg dose group. One female in the 1000 mg/kg dose group was found dead on GD 16. There were no other clinical observations that were considered to be related to treatment with the test article.

At a dose of 50 mg/kg, there were no significant differences in body weights or body weight changes when compared to the control group. Body weights of pregnant females in the 333 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females on GD 4, 8, 12, 16, and 20. Body weights of pregnant females in the 1000 mg/kg dose group were significantly lower ($p<0.01$) than those of the control females on GD 4, 8, 12, 16, and 20.

Body weight changes for pregnant females in the 333 mg/kg dose group were also significantly lower than those of the control females between GD 0 to 4 ($p<0.01$), and 16 to 20 ($p<0.05$). Body weight changes for females dosed at 1000 mg/kg were significantly lower ($p<0.01$) than those of controls between GD 0 to 4, 12 to 16, and 16 to 20.

At a dose of 50 mg/kg, there were no significant differences in absolute or relative food consumption when compared with the control group. Absolute food consumption for pregnant females in the 333 mg/kg dose group was significantly lower than that of the controls during GD 0 to 4 ($p<0.01$), 4 to 8 ($p<0.01$), and 8 to 12 ($p<0.05$). Relative food consumption for pregnant females in the 333 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4 ($p<0.01$) and 4 to 8 ($p<0.01$) and during Lactation Days 0 to 4 ($p<0.05$). Absolute food consumption for pregnant females in the 1000 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4, 4 to 8, 12 to 16, and 16 to 20 ($p<0.01$). Relative food consumption for pregnant females in the 1000 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during GD 0 to 4 and 4 to 8.

In one female in the sham control group, the papillary process lobe of the liver appeared mottled, extending all of the way through the cut surface. The lobe was white-yellow and light red in color. No lesions related to administration of the test article were noted for females in the 50 and 333 mg/kg dose groups. The 1000 mg/kg female found dead on GD 16 had red stained coat in the perineal, inguinal, and abdominal regions; a red stained tail; red nasal

discharge; a gastrointestinal tract filled with black fluid; a pale liver and kidneys; and a uterus that contained both early and late resorptions. An additional female had two early resorptions in the uterus. Another female in this dose group exhibited slight eschar on the left flank and eschar in the cervical region.

At a dose of 50 mg/kg, the proportion of males on Lactation Day 0 was significantly lower ($p<0.05$) than that of the control group. This difference was not considered to be related t6 treatment with the test article because the proportion of males on Lactation Day 0 was not significantly lower at a higher dose of 333 mg/kg. At a dose of 333 mg/kg, the number of total and live pups on Lactation Day 0 was significantly lower ($P<0.01$) than that of the control group.

Pup body weights for the 333 dose group were significantly lower than those of the controls on Lactation Days 0 ($p<0.05$) and 4 ($p<0.01$). One of the pregnant females in this dose group delivered only dead pups. At a dose of 1000 mg/kg, none of the 12 pregnant females in the group delivered a litter. For the 50 and 333 mg/kg dose groups, there were no significant differences in the number of implantation sites, gestation length, proportion dead on Lactation Day 0, proportion surviving to Lactation Day 4, proportion of males on Lactation Day 4, or external pup alterations. There were no significant differences in the number of implantation sites between the 1000 mg/kg dose group and the control group.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	50	333	1000
Body wt –final (g)	423.9	420.5	366.2b	299.1b
Body wt – lactation day 0	320.8	317.9	306.4	NA
Body wt – lactation day 4	336.7	331.5	313.8	NA
GD 0-4 wt gain (g)	23.7	19.8	2.9b	-5.7b
GD 4-8 wt gain (g)	16.0	16.9	16.4	16.7
GD 8-12 wt gain (g)	23.3	22.5	19.0	19.2
GD 12-16 wt gain (g)	28.7	28.3	21.8	-7.0b
GD 16-20 wt gain (g)	59.7	58.5	36.4a	2.0b
Lactation day0-4 wt gain (g)	15.9	13.6	7.8	NA

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
NA=not applicable

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	333	1000
Number of dams pregnant	15	11	12	12
Number of dams with resorptions	0	0	0	3
Number of dams that delivered	15	11	11*	0
Implantation sites - Mean	16.4	17.0	25.8	15.4
Number of litters with live pups	15	11	11	NA
Total pups/litter (day 0)	15.1	15.3	9.5b	NA
Live pups/litter (day 0)	14.9	14.7	8.6b	NA
Proportion surviving to day 4 (%)	97	94	89	NA
Pup weights (g) –	6.723	6.538	6.080a	NA

mean, day 0				
Pup weights (g) – mean, day 4	10.188	10.016	7.080b	NA

a) Statistically different from control ($p<0.05$)
b) Statistically different from control ($p<0.01$)
NA=not applicable
*One dam in this dose group delivered only dead pups

Conclusion:

Given the design of the study and the results observed, it was not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus. The systemic maternal NOAEL for dermal exposure to HHVGO during GD 0-20 was determined to be 50 mg/kg/day; the LOAEL= 333 mg/kg/day based decreased body weight, body weight changes and food consumption.

The developmental NOAEL for dermal exposure to HHVGO during GD 0-20 was determined to be 50 mg/kg/day; the LOAEL = 333 mg/kg/day based on decreased number of total and live pups on lactation days 0, and a decrease in pup body weight on lactation days 0 and 4. In addition, one female in the 333 mg/kg group failed to deliver.

Note the dermal NOAEL was determined to be 50 mg/kg since dermal irritation occurred at dose levels greater than 333 mg/kg/day

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination for the endpoints measured.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-227 Dermally During GD 0 to 20. Report ATX-91-0266.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil (HVGO); VDF Gas Oil

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil; (F-196)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.%	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)

	[1]							
091649 (F-196)		0.10	0.30	3.00	2.00	2.00	0.70	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Portage, MI)

Other Strain: Not applicable

Gender: Females (non treated males used for mating)

Number of Animals per Dose: 25 per dose for level

Concentration:

Dose: 0, 75, 150, 300 mg/kg/day

Year Study Performed : 1993

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study)

GLP: Yes

Exposure Period: Gestation day (GD) 0-19

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The study was designed to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of HVGO (F-196) administered percutaneously to presumed pregnant rats.

Prior to the initiation of dosing with the test substance, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of sperm in a vaginal smear or a copulatory plug, the individual, presumed pregnant females were randomly assigned to four treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of evidence of mating:

1. Vehicle control (acetone) 0 mg/kg/day – 25 animals (GD 0-19)
2. HVGO 75 mg/kg/day – 25 animals (GD 0-19)
3. HVGO 150 mg/kg/day – 25 animals (GD 0-19)
4. HVGO 300 mg/kg/day – 25 animals (GD 0-19)

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

Suspensions of F-196 were prepared daily at concentrations of 0 (vehicle, acetone), 75, 150 and 300 mg/mL such that doses of 0, 75, 150, and 300 mg/kg/day, respectively, were administered at a volume of 1 mL/kg. Animals in all

groups were treated on GD 0 through GD 19. Each treatment day, animals were dosed by even application of the test substance to their shaved backs, using a blunt-tipped glass syringe. The test substance dose was calculated from each rat's most recent body weight. Rats were fitted with Elizabethan collars to minimize ingestion of test substance. Controls were handled in the same manner but with application of the vehicle only. Elizabethan collars were applied just prior to dosing and were removed after a 6 hour exposure period. At the time of collar removal, any excess test article was wiped off with a cloth dipped in acetone and dried with a clean cloth.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Skin reactions were graded using the Draize and National Research Council standards.

Individual body weights and food consumption were recorded daily during presumed gestation.

All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation, and a gross necropsy of the thoracic and abdominal viscera was performed. The abdomen of each rat was opened, and the intact uterus was excised and examined for pregnancy. To confirm the pregnancy status, uteri from rats that appeared non-pregnant were examined while transilluminated and pressed between two glass plates. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other maternal tissues were discarded.

Corpora lutea in each ovary were recorded. The number and distribution of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Each fetus was removed from the uterus, placed in an individual container, weighed, and examined for weighed and examined for sex and gross external alterations. Live fetuses were sacrificed.

Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations by using an adaptation of Wilson's sectioning technique. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red, and examined for skeletal alterations.

STATISTICAL ANALYSES: Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Maternal body weights, body weight changes, feed consumption values, and litter averages for fetal body weights, percent male fetuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test and ANOVA, when appropriate [i.e., Bartlett's Test was not significant ($P>0.05$)]. If the analysis of variance was significant ($P<0.05$), Dunnett's Test was used to identify the statistical significance of the 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, when less than or equal to 75% ties were present. When more than 75% ties were present, Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant ($P<0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures described for the Kruskal-Wallis Test.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	Not determined < 75		mg/kg/day
NOAEL- Dermal	Maternal	=	75		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	Not determined <75		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	75		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

No deaths occurred during the conduct of this study. No skin reactions were related to percutaneous administration of the test substance at doses as high as 300 mg/kg/day. Erythema (grades 1 or 2) occurred in two 75 mg/kg/day dose group rats and one 300 mg/kg/day dose group rat. This observation was unrelated to the test substance because the incidences were not dose dependent.

All other clinical observations were unrelated to administration of the test substance because: 1) the incidences were not dose-dependent; 2) the values were not significantly increased, as compared with the control group values; or 3) the observations commonly occur in this strain of rat. These observations included lacrimation, localized alopecia, chromorrhinorrhea, chromodacryorrhea, dental problems, swollen limbs, swollen and purple ears, umbilical hernia and adhesions of the stomach, spleen, left kidney and left ovary.

Maternal body weights and body weight gains were reduced or significantly reduced ($P<0.05$ to $P<0.01$) in the 75, 150 and 300 mg/kg/day dose groups at various points throughout the dosing period, per the table below.

Absolute and relative feed consumption values were significantly reduced ($P<0.05$ to $P<0.01$) in the 75, 150 and 300 mg/kg/day dose groups for the entire dosing period (calculated as days 0 to 20 of gestation). Within the dosing period, absolute and relative feed consumption values were reduced or significantly reduced
($P<0.05$ to $P<0.01$) in these dose groups.

Percutaneous administration at doses of 150 and 300 mg/kg/day caused biologically important and/or significant reductions ($P<0.01$) in litter sizes and live fetuses and, increases or significant increases ($P<0.01$) in resorptions (total and early resorptions) in these groups. The number of dams with any resorptions was also
significantly increased ($P<0.01$) in the 300 mg/kg/day dose group. Reflecting these effects, the percentage of

resorbed conceptuses per litter tended to be increased in the 150 mg/kg/day dose group and was significantly increased (P<0.01) in the 300 mg/kg/day dose group.

Fetal body weights (total and male and female) were reduced or significantly reduced (P<0.05 to P<0.01) in the 75, 150 and 300 mg/kg/day dose groups. All other Caesarean-sectioning and litter parameters were unaffected by doses of the test substance as high as 300 mg/kg/day.

Litter averages for corpora lutea, implantations, late resorptions and sex ratios did not demonstrate any significant or biologically important differences. No dam resorbed all conceptuses, and the numbers of dams with viable fetuses were comparable among the four dose groups.

Fetal alterations were classified as: 1) malformations (irreversible changes which occur at low incidences in this species and strain); or 2) variations (relatively common developmental changes in this species and strain, including minor reversible delays or accelerations in development).

The 75, 150 and 300 mg/kg/day doses of the test substance were the possible cause of increased incidences of eye malformations in the fetuses [decrease in size, evident as small eye bulge(s) at gross external examination, microphthalmia at soft tissue examination, or small eye sockets at skeletal examination]. The fetal incidence of
microphthalmia was significant (P<0.01) in the 75 mg/kg/day dose group. The absence of a clear dose-dependent incidence for this malformation may be interrelated with the dose-dependent increases in resorption (embryo-fetal deaths) in the 150 and 300 mg/kg/day dose groups.

The litter and fetal incidences of bifid thoracic vertebral centra were significantly increased (P<0.01) in the 75, 150 and 300 mg/kg/day dose groups. This variation in vertebral ossification was related to the test substance because: 1) the litter and fetal incidences were significantly increased; and 2) the incidences exceeded the ranges observed historically.

Reductions in the average number of ossified caudal vertebrae per fetus occurred in the 300 mg/kg/day dose group. This event was related to the test substance because: 1) it was associated with delays in ossification commonly observed with reduced fetal body weights; and 2) the incidence exceeded the range observed
Historically.

No other gross external or soft tissue alterations in the fetuses were caused by percutaneous administration of F-196 to the dams at doses as high as 300 mg/kg/day. All other fetal alterations occurred at incidences within the control ranges observed historically, and there were no other incidences that significantly differed from those of
the concurrent control group. Alterations that occurred only in the high dose group occurred in no more than one fetus.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	75	150	300
Body wt –final (gr)	367.1	3.54.5	346.3b	331.3b
GD 0-3 wt gain (gr)	14.0	10.5	6.6b	3.0b
GD 3-6 wt gain (gr)	9.0	8.3	8.4	9.6
GD 6-9 wt gain (gr)	11.1	10.3	10.6	9.4
GD 9-12 wt gain (gr)	14.4	11.9	12.4	10.2b
GD 12-15 wt gain (gr)	17.4	16.6	15.5	13.1b
GD 15-18 wt gain (gr)	37.2	36.1	32.8	29.9b
GD 19-20 wt gain (gr)	16.9	13.6	11.8b	10.0b

GD 0-20 wt gain (gr)	132.3	120.5a	109.6b	95. 8b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**75**	**150**	**300**
Corpora lutea	16.5	16.3	16.2	16.1
Implantation sites – mean	14.7	14.3	13.1	13.9
Live fetuses – total	331	294	297	231
Live fetuses - mean	13.8	13.4	11.9	10.0b
Litter size	13.8	13.4	11.9	10.0b
Viable male fetuses (%)	52.0	52.0	52.1	55.3
Total resorptions (mean)	0.9	1.0	1.2	3.9b
Dams with resorptions	12	9	14	21b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Fetal Endpoints

Dose (mg/kg/day)	**0**	**75**	**150**
Fetal weights (gr)	3.41	3.27	3.22a
Litters evaluated	24	22	25
Live fetuses - total	331	294	297
Dead fetuses – dead	0	0	0
% Resorbed conceptuses per litter	5.8	6.4	9.2
Litters with any alteration (N;%)c	7 (29.2)	10 (45.4)c	11 (44.0)c
Fetuses with any alteration (N;%)c	11 (3.3)	15 (5.1)c	15 (5.0)cc
Fetuses with any alteration per litter (mean %)c	3.35	6.27cd	4.89cd

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) See text for discussion of results.
d) Some of the specific fetal alterations in this group were judged to be test substance related based on criteria described above.

Conclusion:

The maternal NOAEL for dermal exposure to HVGO during GD 0-19 was determined to be <75 mg/kg/day (LOAEL = 75 mg/kg/day based on reduced maternal weight gains and feed consumption).

The developmental NOAEL for dermal exposure to HVGO during GD 0-19 was determined to be <75 mg/kg/day. (LOAEL = 75 mg/kg/day based on decreased fetal body weights, microphthalmia and delayed ossification)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1993. Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of F-196 Administered Percutaneously to Crl:CD®BRK VAF/Plus® Presumed Pregnant Rats. Report ATX-92-0012.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR.

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic

ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances."
http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-57-7

Test Substance: 64741-57-7; Heavy Vacuum Gas Oil (HVGO)

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Vacuum Gas Oil (CRU No. 85244)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348ZV (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%) [2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
85244	6.20	0.00	0.06	2.48	1.86	1.24	0.50	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity screen

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 10 per dose, except for an additional group of 8 animals exposed at 500 mg/kg on GD 10-19 used to obtain bioavailability data

Concentration:

Dose: Developmental study, GD 0-19:
0 (remote), 0 (proximate), 30, 125, 500 and 1000 mg/kg/day.
Bioavailability study, GD 10-12:
500 mg/kg/day

Year Study Performed : 1988

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (10/group versus 20).

GLP: No information

Exposure Period: GD 0-19

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks:

The study was designed to obtain data on the influence of HVGO on parameters of reproductive performance during gestation (implantation, litter size) and viability and development of the embryo/fetus. The study was also designed to include clinical chemistry analyses of maternal sera, and bioavailability analyses of HVGO in maternal blood, placentae and fetuses.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

5. *Remotely-housed dermal control (0 mg/kg/day) – GD 0-19 – 10 animals
6. Proximately-housed dermal control (0 mg/kg/day) – GD 0-19 – 10 animals
7. HVGO 30 mg/kg/day – GD 0-19 – 10 animals
8. HVGO 125 mg/kg/day – GD 0-19 – 10 animals
9. HVGO 500 mg/kg/day – GD 0-19 – 10 animals
10. HVGO 1000 mg/kg/day – GD 0-19 – 10 animals
11. Radio-labeled HVGO 500 mg/kg/day – GD 10-12 – 8 animals (bioavailability study group).

*Because inhalation of the test material could not be ruled out, a separate control group was not housed in the same animal room (remote-housed control).

The exposure levels were based on results of a 13 week study previously conducted on the same material.

Developmental study (Groups 1-6):
The test material was administered to groups 3-6 on GD 0-19. Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality or abortion. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. The ovaries and uterus of each rat were excised and examined grossly. The thoracic and abdominal cavities were exposed and all organs were examined grossly for evidence of pathosis. The thymus and liver of each animal in Groups 1-6 were removed, trimmed of excess tissue,

and weighed to the nearest 0.001 gram. The liver and thymus were preserved in 10 % formalin. No histopathology was performed for these tissues.

The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. An "early resorption" was defined as a reabsorbed dead conceptus in which it was not grossly evident that organogenesis had occurred; a "late resorption" was defined similarly but as one in which it was evident that organogenesis had occurred. A "live fetus" was defined as a fetus which responded to a stimulus, such as touch; a "dead fetus" did not respond to stimuli, nor did it demonstrate the autolysis characteristic of late resorptions. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each fetus was gendered, weighed and grossly examined for anomalies, malformations and variations.
The following definitions and terminology were used in describing fetal findings:

1) Anomaly: Any deviation (malformation or variation) from "normal."
2) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
3) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.

After gross evaluation, fetuses in each litter were equally distributed into two groups, and preparation begun for either soft tissue or skeletal evaluations. Approximately half of the fetuses were randomly assigned for examination of soft tissues (visceral) and were fixed in Bouin's solution, using a modification of the Wilson's technique with sectioning by razor blade. The other half were fixed in 95% ethanol, macerated in potassium hydroxide, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal anomalies.

<u>Bioavailability Study (Group 6)</u>
Eight presumed-pregnant female rats were used in the bioavailability experiments. From gestation day 0 through the morning of gestation day 10, the rats were housed in stainless steel cages with wire bottoms and fronts. On gestation days 10, 11, and 12, the rats were housed in metabolism cages. Two HVGO mixtures were used in the experiments. One mixture contained two radio labeled surrogates, 14-C-carbazole and 3-H-benzo(a)pyrene (BaP) while the other mixture contained only14-C-phenanthrene. Five rats were treated in this manner with HVGO containing the dual radiolabels; three rats were treated in another experiment with HVGO containing only 14-C-phenanthrene. On GD 10, the hair was clipped from the dorsal trunk of each animal and the radiolabeled test material was

applied to the skin within a protective device designed to contain the administered dose. A mesh screen was attached to the protective device, and each rat was fitted with an Elizabethan collar. The same procedure was repeated on GD 11 and 12, except the needle tip with the test material was inserted through the mesh screen in order to apply the test material.

On GD 13, 24 hrs after the administration of the last HVGO dose, animals were sacrificed and maternal blood was collected. Necropsies were performed and the uterine contents located and examined for the number of normal and resorbed fetuses for each dam. The individual fetal units were removed, and the amniotic fluid was collected from the isolated placenta. The embryo was separated from the yolk sac and rinsed with water to remove residual amniotic fluid. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights or volumes of the pooled samples determined. Maternal tissues collected for radioactivity analysis included the following: thymus, liver, heart, brain, small intestine, large intestine, kidneys, spleen, stomach, ovaries, urinary bladder, lungs, muscle, retroperitoneal fat, femur bone and residual carcass.

Determination of radioactivity in blood, urine and cage wash was accomplished by measuring the amount of carbon-14 labeled carbon dioxide and H-3 labeled water produced from direct combustion of duplicate samples. Samples were oxidized for three minutes and the carbon dioxide and water generated from the combustion were separated and trapped in a cocktail fluid. Carbon-14 and hydrogen-3 radioactivities were measured. Fecal samples were homogenized, combusted and the radioactivity measured.

The placentae, urteri, embryos, and yolk sacs were homogenized in an equivalent volume of water, and aliquots of the homogenate were combusted. Maternal tissues were treated in the same manner, although six tissues including the ovaries, urinary bladder, muscle, fat, bone and residual carcass were combusted directly without homogenization or dilution. In all cases, the trapped carbon dioxide and water were measured for radioactivity by liquid scintillation counting. Samples of the amniotic fluid were also combusted directly without dilution. Duplicate analyses were performed whenever possible. The sensitivity of the radioactivity allowed for the detection of 0.005% of the applied dose.

The systemic dermal absorption of the three radio labeled surrogates was determined by summing the total carbon-14 or hydrogen-3 radioactivities found in the urine, urine/cage washings, feces and collected maternal and embryonic tissues at the end of 72 hours. Tissue concentrations of carbazole, phenanthrene and benzo(a)pyrene (BaP) were calculated based on the radioactivity found per gram or per ml. The total amount of a radiolabeled surrogate in the tissues was calculated as a percent of the total applied radioactive dermal dose over three days.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated 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 the 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. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere . Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Report no. 64348 ZQ- how to reference??)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	500		mg/kg/day
NOAEL- Dermal	Maternal	=	125		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	500		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	125		mg/kg/day

Results Remarks:

The animals used in the study were approximately 6 weeks old at receipt and approximately 9 weeks old at exposure initiation.

The red nasal exudate and chromodacryorrhea that were observed in control and HVGO-exposed groups are common in animals that are collared. Also, neck lesions were observed in control and HVGO-exposed groups, in spite of the protective soft rubber tubing that lines the inner surface of the cardboard collar. Scratches and/or flaking of skin were observed on the backs of a few of the animals from control and HVGO-exposed group

Signs of dermal irritation were limited to one dam exposed to 500 mg/kg/day HVGO. A bloody discharge from the vagina, a sign of some degree of litter resorption, was observed only in two dams exposed to HVGO at a dose level of 1000 mg/kg/day. At the time that the discharge was observed, one of these animals was pale in color. Six 1000 mg/kg/day-exposed animals and one 125 mg/kg/day-exposed animal had decreased stool. This was not unexpected since these animals, in general, consumed less food than the other animals.

A dose-related decrease in mean body weights and body weight changes was observed at various points during gestation in dams exposed to HVGO doses of 500 mg/kg/day and higher, per the table below. At these doses, the decreased body weights reflect the decrease in litter sizes. In general, a decrease in food consumption was observed only at the two highest dose levels (500 and 1000 mg/kg/day). At many of the time points, however, the amount of food consumed was not significantly different ($p > 0.05$) than the amount consumed by control animals.

The thymus of a limited number of dams exposed to 1000 mg/kg/day appeared to be smaller than the thymus of control animals. This observation was confirmed by weighing the thymus from dams in all of the groups. Lungs that were pale in color were observed only in HVGO-exposed groups. The significance of this finding was not known. No significant differences ($p >0.05$) in liver weight were observed in HVGO-exposed rats in comparison to the control animals.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0 Rem.	0 Prox.	30	125	500	1000 GD 10-12
Body wt –final (gr)	390	397	387	381	347bd	311bd
GD 0-3 wt gain (gr)	17	14	II	6	-5bd	-14bd
GD 3-6 wt gain (gr)	14	15	II	14	17	22
GD 6-10 wt gain (gr)	17	21	15	16	16	21
GD 10-13 wt gain (gr)	17	14	17	17	17	11
GD 13-16 wt gain (gr)	27	26	28	30	15ac	10bd
GD 16-20 wt gain (gr)	58	66	60	56	46c	21bd
GD 0-20 wt gain (gr)	150	156	142	139	105bd	70bd
Gravid uterus (gr)	74.6	78.5	74.1	78.4	52.2	31.3
Carcass (gr)	315.7	318.4	313.1	302.3	294.9c	279.9bd
Net wt change from day 0 (e)	75.3	77.2	68.4	60.2	53.0ad	39.1bd
Thymus weight (g)-absolute	0.254	0.281	0.304	0.259	0.221	0.126ac
Thymus weight (g)-relative – not reported	--	--	--	--	--	--
Liver weight - absolute (g)	16.02	16.52	16.00	16.74	17.26	16.69
Liver weight (g)-relative	5.067	5.181	5.113	5.545	5.855ac	5.923ac

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)
e) = Carcass weight minus day 0 body wt.

The number of implantation sites and percent pre-implantation loss were not affected by exposure to HVGO for GD 1-19. The number of dams with resorptions, the number of resorptions and the litter size were significantly different from the controls at a dose of 500 mg/kg/day and higher.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	0 Rem.	0 Prox.	30	125	500	1000 GD 10-12
Implantation sites – total	135	146	149	124	156	141
Implantation sites – mean	15.0	14.6	14.9	15.5	15.6	15.7
Preimplantation loss (%)	8..6	8.6	5.7	2.9	1.2	2.8
Viable fetuses	125	140	138	115	100	52
Litter size (e)	13.9	14.0	13.8	14.4	10.0ac	5.8bd
Viable male fetuses (%)	58	49	47	48	50	54
Resorptions (mean)	1.1	0.6	1.1	1.1	5.6bd	9.9bd
Resorptions (mean %)	7.1	4.5	7.7	7.3	35.1bd	63.8bd
Dams with resorptions (%)	58	50	70	63	100ac	100c

a)Statistically different from remote control (p<0.05)

b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)
e) Number of viable fetuses/number of litters evaluated.

No statistically significant differences in serum chemistry were observed between the two groups of control animals. Therefore statistical analyses were performed between the remote control and HVGO-exposed groups only. Significant differences were observed for six serum components, all of which demonstrated a dose-response
effect. Under the conditions of the study, there was indication of dose-related hepatotoxicity as characterized by marked increases in serum aspartate aminotransferase and sorbitol dehydrogenase activities. There was equivocal evidence of an effect on the kidneys as shown by a significant increase in serum urea nitrogen. The dose related responses that were observed for serum triglycerides and iron are likely a result of a secondary effect of HVGO as a result of resorption. It has been previously observed that the dams that resorb their litters have a serum profile that is similar to nonpregnant animals.

At the time of cesarean section, all fetuses were viable. Fetal body weights were significantly reduced ($p < 0.05$) in fetuses exposed in utero to HVGO at doses of 500 mg/kg/day and higher. Although fetuses exposed to HVGO had reduced crown-rump lengths in comparison to the lengths of the proximate control fetuses, the differences were not significantly different ($p > 0.05$) when compared to the lengths of remote control fetuses.

At the time of external examination, malformations were observed in one fetus exposed in utero to 1000 mg/kg/day HVGO. This fetus was edematous (general accumulation of serum in the cellular tissues of the body) and pale
in color. Also, both hind paws were malformed; the digits were reduced in size (brachydactyly) with a subcutaneous hematoma (a circumscribed dermal effusion of blood) located at the distal most aspect of each of the digits.

In the skeletal examination, there was no significant increase in skeletal malformations among the exposed groups compared to the control groups. A variety of skeletal variations were observed in HVGO-exposed and control fetuses. Some skeletal variations (mostly unossified or incompletely ossified bones) were seen at a higher incidence among the HCGO-exposed groups, particularly at doses of 500 mg/kg/day and higher. __ fetuses with vertebral malformations were observed among the litters of dams given 500 mg/kg/day, but no individual skeletal malformation was significantly increased compared to controls at any dose level.

Visceral malformations were restricted to two fetuses from the 500 mg/kg/day group. One fetus had a reduction in the size of one of its eyes (microphthalmia) and another fetus had a diaphragmatic hernia (protrusion of the liver into the thoracic cavity) which displaced the heart from a left-sided to a right-sided position.

Fetal Endpoints - Weight and Gross Exam

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**30**	**125**	**500**	**1000 GD 10-12**
Fetal weights (gr)	3.5	3.7	3.5	3.6	3.2ad	3.0bd
Crown-rump length (mm)	34.1	34.7	34.3	33.8	32.9c	32.0a

Litters evaluated	9	10	10	9	9	10
Fetuses - live	125	140	138	128	100	52
Fetuses – dead	0	0	0	0	0	6
Total gross exam anomalies (fetal incidence; %)	0; 0.0	0; 0.0	0; 0.0	0; 0.0	0; 0.0	1; 1.9
Total gross exam anomalies (litter incidence; %)	0; 0.0	0; 0.0	0; 0.0	0; 0.0	0; 0.0	1; 17

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Fetal Endpoints - Skeletal

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**30**	**125**	**500**	**1000 GD 10-12**
Litters evaluated	9	10	10	8	10	6
Fetuses - live	65	72	72	59	52	28
Fetuses – dead	0	0	0	0	0	0
Total skeletal changes (fetal incidence; %)	60; 92	59; 82	66; 92	49; 83	52; 100d	28; 100c
Total skeletal changes (litter incidence; %)	9; 100	10; 100	10; 100	8; 100	10; 100	6; 100

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Fetal Endpoints – Soft Tissue

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**30**	**125**	**500**	**1000 GD 10-12**
Litters evaluated	9	3	0	8	10	6
Fetuses - live	60	19	0	56	48	24
Fetuses – dead	0	0	0	0	0	0
Total soft tissue anomalies (fetal incidence; %)	0; 0.0	0; 0.0	*	0; 0.0	3; 63	1; 4.2
Total soft tissue anomalies (litter incidence; %)	0; 0.0	0; 0.0	*	0; 0.0	2; 20	1; 17

*dose group not examined for this endpoint
a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Bioavailability Analyses
The dermal penetration of 14-C-carbazole (38.7% of total applied dose absorbed) occurred more extensively
than either 14-C-phenanthrene (17.3% of applied dose absorbed) or 3-H-BaP (8.8% of applied dose absorbed). In spite of the dermal bioavailability of 14 -C-carbazole, 14-C-phenanthrene and 3-H-BaP in the dam, the amount of radio labeled material found in the embryo was very low. These findings indicate that although 14-C-carbazole, 14-C-phenanthrene and 3-H-BaP are capable of

Conclusion:	reaching the embryo, they do not accumulate to any significant degree in the embryo. The results suggest that the placenta may be an effective barrier against the transplacental transport of these HVGO components to the embryo. The maternal NOAEL for dermal exposure to HVGO during GD 0-19 was determined to be 125 mg/kg/day (LOAEL= 500 mg/kg/day based on decreased body weight, body weight gains and food consumption, increased relative liver weight and aberrant serum chemistry) The developmental LOAEL for dermal exposure to HVGO during GD 0-19 was determined to be 125 mg/kg/day (LOAEL = 500 mg/kg/day based on increased resorptions and decreased fetal body weight)

RELIABILITY/DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	Key

REFERENCE

Reference:	Mobil. 1988. Developmental Toxicity Screen in Rats Exposed Dermally to Heavy Vacuum Gas Oil. 1988. Mobil Environmental and Health Sciences Laboratory Report 61801. Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Vacuum Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZV API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	64741-62-4
Test Substance:	64741-62-4; Clarified Slurry Oil (CSO)
Test Substance Purity/Composition and Other Test Substance Comments:	Clarified Slurry Oil (CRU No 86001) PAC (Polycyclic Aromatic Compound) Content – Report No. 64348 ZA (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86001	64.20	0.00	2.57	25.68	19.26	6.42	3.21	0.64

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity study
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females, presumed pregnant (non treated males used for mating)
Number of Animals per Dose:	20 per dose, except for an additional group of 4 animals exposed at 1000 mg/kg of radiolabeled material on GD 9-12 used to obtain bioavailability data
Concentration:	
Dose:	Developmental study: 0, 10, 100, 1000 mg/kg/day Bioavailability study: 1000 mg/kg/day
Year Study Performed :	1988
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study), except for limited gestation period exposure (GD 9-12); study designed to evaluate specific tissue malformation – esophagus.
GLP:	No information
Exposure Period:	GD 9-12
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to detect the teratogenic potential of CSO. The study was also designed to include clinical chemistry analyses of maternal sera, bioavailability/bioaccumulation of LCGO in maternal tissues, placentae, and fetuses, and postnatal survival of neonates. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid: 12. Sham control (0 mg/kg/day) – GD 9-12 – 20 animals 13. CSO10 mg/kg/day – GD 9-12 – 20 animals 14. CSO 100 mg/kg/day – GD 9-12 – 15 animals 15. CSO 1000 mg/kg/day – GD 9-12– 15 animals 16. CSO 1000 mg/kg/day – GD 9-12 – 4 animals; residue analyses group Developmental study (Groups 1-4): The test material was administered to groups 2-4 on GD 9-12. Hair was clipped from the dorsal trunk of each animal on GD 9. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. To minimize ingestion of test

material rats were fitted with Elizabethan collars on GD 0. Controls were handled in the same manner, minus application of the test material. Control animals were clipped, collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, abortion or premature delivery. All unusual findings were noted.

Individual body weights were recorded on days 0, 6, 9, 13, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-6, 6-9, 9-13, 13-20, 0-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and livers were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea per ovary was recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each live fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

4) Anomaly: Any deviation (malformation or variation) from "normal."
5) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
6) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.
7) Incidental: An incidental finding is generally an accidental event, e.g., accidentally, tip of tail was cut off.

After gross evaluation, all fetuses in each litter were fixed in Bouin's solution for subsequent soft tissue evaluation using a modification of Wilson's technique. The head and thoracic regions were evaluated for palatal and esophageal anomalies, respectively; no other soft tissues were evaluated.

<u>Bioavailability Study (Group 9)</u>

From GD 0-8, pregnant females were housed in stainless steel cages with wire bottoms and fronts. On GD 9, 10, 11, and 12, the rats were housed in metabolism cages. The CSO used in the bioavailability study contained two radioactive surrogates, carbon-14 radiolabeled carbazole and hydrogen-3 radiolabeled benzo(a)pyrene (BaP). On GD 9, the hair was clipped from the dorsal trunk of each animal and the radiolabeled test material (1000 mg/kg) was applied to the skin within a protective device designed to contain the administered dose. A mesh screen was attached to the protective device, and

each rat was fitted with an Elizabethan collar. The same procedure was repeated on GD10, 11 and 12, except the needle tip with the test material was inserted through the mesh screen in order to apply the test material.

On GD 13, 24 hrs after the administration of the last CSO dose, animals were sacrificed ether overexposure and maternal blood was collected. Necropsies were performed and the uterine contents located and examined for the number of normal and resorbed fetuses for each dam. The individual fetal units were removed, and the amniotic fluid was collected from the isolated placenta. The embryo was separated from the yolk sac and rinsed with water to remove residual amniotic fluid. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights or volumes of the pooled samples determined. Maternal tissues collected for radioactivity analysis included the following: blood, thymus, liver, small intestine, large intestine, kidneys, stomach, and ovaries.

Determination of radioactivity in blood, urine and cage wash was accomplished by measuring the amount of carbon-14 labeled carbon dioxide and H-3 labeled water produced from direct combustion of duplicate samples. Samples were oxidized for three minutes and the carbon dioxide and water generated from the combustion were separated and trapped in a cocktail fluid. Carbon-14 and hydrogen-3 radioactivities were measured. Fecal samples were homogenized, combusted and the radioactivity measured.

The placentae, uteri, embryos, and yolk sacs were homogenized in an equivalent volume of water, and aliquots of the homogenate were combusted. Maternal tissues were treated in the same manner, although the ovaries, and amniotic fluid were combusted directly without homogenization or dilution. In all cases, the trapped carbon dioxide and water were measured for radioactivity by liquid scintillation counting. Samples of the amniotic fluid were also combusted directly without dilution. Duplicate analyses were performed whenever possible. The sensitivity of the radioactivity allowed for the detection of 0.005% of the applied dose.

The systemic dermal absorption of the two radiolabeled surrogates was determined by summing the total carbon-14 or hydrogen-3 radioactivities found in the urine, urine/cage washings, feces and collected maternal and embryonic tissues at the end of 72 hours. Tissue concentrations of carbazole and benzo(a)pyrene (BaP) were calculated based on the radioactivity found per gram or per ml. The total amount of a radiolabeled surrogate in the tissues was calculated as a percent of the total applied radioactive dermal dose over three days.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were collected, processed and analyzed (Tukey'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 the 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. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an

	analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	100		mg/kg/day
NOAEL- Dermal	Maternal	=	10		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	100		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	10		mg/kg/day

Results Remarks:

The female animals used in the study were approximately 8 weeks old at receipt and approximately 10 weeks old at exposure initiation.

The red nasal exudate and chromodacryorrhea that were observed in control and CSO-exposed groups are common in animals that are collared. Also, neck lesions were observed in control and LCGO-exposed groups in spite of the protective soft rubber tubing that lines the inner surface of the cardboard collar. Scratches were observed on the backs of a few of the animals at the time of the first clipping and probably occurred during mating activity.

CSO did not produce much dermal irritation. One high-dose dam had flaking of the skin and three high-dose animals developed scabs at the site of application. The fur of animals in the high-dose group, including the face and paws as well as the entire body, was discolored by CSO. In view of this, the possibility of ingestion at the high dose level could not be excluded. Bloody discharge from the vagina, usually a sign of some degree of litter resorption, was observed in all of the groups exposed to CSO. However, at cesarean section, the one 10 mg/kg dam and two of the five 100 mg/kg dams which had bloody discharges did not have any resorptions. One 100 mg/kg and three 1000 mg/kg animals had decreased stool.

A dose-related decrease in mean body weights, body weight changes, and net body weights was observed in CSO-exposed dams. At the 1000 mg/kg level, the decreased body weights reflect the decrease in litter sizes. A decrease in food consumption was observed in the mid- and high-dose groups for gestation day intervals 9-13 (period of dosing) and 13-20.

Liver, thymus, spleen, axillary lymph nodes and brachial lymph nodes appeared to be adversely affected by exposure to CSO. Liver weights were significantly increased and thymus weights were significantly decreased at 1000 mg/kg.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	10	100	1000
Body wt –at delivery (gr)	399.9	484.8	378.9a	313.3b
GD 0-6 wt gain (gr)	29	30	31	29
GD 6-9 wt gain (gr)	16	18	16	14
GD 9-13 wt gain (gr)	25	19	–1b	–8b
GD 13-20 wt gain (gr)	89	94	89	36b
GD 0-20 wt gain (gr)	159	160	135b	71b
Gravid uterus (gr)	86.3	79.8	71.7	18.4b

Carcass (gr)/Final body weight	319.6	325.3	305.2	294.9b
Net wt change from day 0 (c)	79.8	81.4	63.4b	52.4b
Thymus weight (g)-absolute	0.301	0.350	0.252	0.071b
Thymus weight (g)-relative	0.094	0.108	0.082	0.024b
Liver weight - absolute (g)	16.61	17.18	16.41	18.85b
Liver weight (g)-relative	5.20	5.28	5.38	6.39b

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) = Carcass weight minus day 0 body wt

At 1000 mg/kg, the following parameters appeared to be adversely affected by CSO exposure: number of dams with resorptions (increased), number of resorptions (increased) litter size (decreased).

Statistically significant differences between the data from control and treated animals were observed for a total of sixteen parameters: uric acid, urea nitrogen, lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, cholesterol, triglycerides, total protein, total bilirubin, albumin, calcium, inorganic phosphorus, potassium, albumin/globulin ratio, sorbitol dehydrogenase, and iron. A linear relationship between dose and serum level was found for all of these components. When the historical reference values are taken into consideration, the dose-response curves for uric acid, aspartate aminotransferase, alkaline phosphatase, cholesterol, triglycerides, total bilirubin, albumin, inorganic phosphorus, albumin/globulin ratio, sorbitol dehydrogenase, and iron at the 1000 mg/kg dose level fall outside the normal range as defined by the l0th to 90th percentiles of the historical data.

Summary of Mean Selected Reproduction Data (Groups 1-4)

Dose (mg/kg/day)	**0**	**10**	**100**	**1000**
Implantation sites – mean	299	299	310	310
Viable fetuses- total	278	276	274	42
Litter size (c)	14.6	14.5	13.7	2.1b
Viable male fetuses (%)	53	51	49	48
Resorptions (mean)	1.1	1.2	1.8	13.4b
Resorptions (mean %)	7.3	7.7	10.9	86.4b
Dams with resorptions (%)	63	68	65	100b

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) Number of viable fetuses/number of litters evaluated.

At the time of cesarean section all fetuses were viable. However, there was an increase in in utero death at 1000 mg/kg. Fetal body weights were significantly reduced in fetuses exposed in utero to CSO at a dose level of 1000 mg/kg. Anomalous development, primarily edema and paw malformations, was significantly increased at 1000 mg/kg. Although statistical significance was not achieved at 100 mg/kg for some of these same findings, they are considered to be of biological significance. One fetus exposed in utero to 100 mg/kg has situs inversus; this finding is believed to be of a spontaneous nature. Due to the high incidence of resorption observed in the high-dose group, a dose related response was not evident for some of the observed effects. Cleft palate was

the only other visceral finding noted at the time of evaluation. It was observed only at the 1000 mg/kg level at a litter incidence of 40% and a fetal incidence of 14%.

Fetal Endpoints – Weight, Gross and Soft Tissue Examination (Groups 1-4)

Dose (mg/kg/day)	0	10	100	1000
Fetal weights (g)	3.5	3.5	3.3	2.5b
Litters evaluated	19	19	20	10
Fetuses - live	278	276	274	42
Fetuses – dead	0	0	0	0
Gross Exam (fetal incidence; %)	3; 1.1	2; 0.7	5; 1.8	11; 26.0
Gross Exam (litter incidence; %)	2; 11.0	2; 11.0	3; 15.0	5; 50.0b
Total fetal soft tissue (fetal incidence; %)*	0; 0.0	0; 0.0	0; 0.0	6; 14.0
Total fetal soft tissue (litter incidence; %)*	0; 0.0	0; 0.0	1; 5.0	4; 40.0

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Bioavailability/Bioaccumulation Analyses
The dermal penetration of 14-C-carbazole occurred more extensively and rapidly than 3H- BaP absorption over a treatment period. In spite of the dermal bioavailability of 14-C-carbazole and 3H- BaP in the dam, the amount of radiolabel led material found in the embryo was very low. The amount of 14-C-carbazole and 3-H-BaP found in the embryo was less than 0.01% of the radiolabeled dose, compared to that found in the maternal tissues (0.5-2.2%) of the radioactive dose. The placenta appears to be an effective barrier against the transport of carbazole and BaP to the embryo. There is no evidence the 14-C-carbazole or 3-H- BaP accumulates selectively in the embryo.

Conclusion:

The maternal NOAEL for dermal exposure to CSO for GD 9-12 was identified at 10 mg/kg/day. (LOAEL= 100 mg/kg/day based on significant decrease in body weight and food consumption.)

The developmental NOAEL for dermal exposure to CSO for GD 9-12 was identified at 10 mg/kg/day. (LOAEL = 100 mg/kg/day based on external fetal anomalies.)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=2)

Reliability Remarks: Not guideline study but has sufficient detail

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil.1988. Teratology Study Rats Exposed Dermally to Clarified Slurry Oil. Mobil Environmental and Health Sciences Laboratory Report 62492.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Clarified Slurry Oil. 1991. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZA.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; Syntower Bottoms (STB)

Test Substance Purity/Composition and Other Test Substance Comments: Syntower Bottoms (CRU No 86484)

PAC (Polycyclic Aromatic Compound) Content 64348 ZM (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86484	48.80	0.00	0.98	9.76	19.52	9.76	4.88	0.98

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity study

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 15 per dose, except for an additional group of 4 animals exposed at 500 mg/kg on GD 10-12 used to obtain bioavailability data

Concentration:

Dose: Developmental study:
0, 4, 8, 125, 500 mg/kg/day
Bioavailability study:
500 mg/kg/day

Year Study Performed : 1990??

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (15/group versus 20).

GLP: No information

Exposure Period: GD 0-19 (5 groups); GD 10-12 (2 groups)

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks:

The study was designed to detect the effects of exposure to STB on parameters of reproductive performance during gestation (implantation, litter size) and viability and development of the embryo/fetus. An experimental group in which STB was administered only on GD 10-12 was included in the study to complement the bioavailability/bioaccumulation assays. The study was also designed to include clinical chemistry analyses of maternal sera, bioavailability/bioaccumulation of LCGO in maternal tissues, placentae, and fetuses, and postnatal survival of neonates.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

17. Sham control (0 mg/kg/day) – GD 0-19 – 15 animals
18. STB "4 mg/kg/day" (8mg/kg/day) – GD 0, 2, 4, 6, 8, 10, 14, 16, 18 – 15 animals. *
19. STB 8 mg/kg/day – GD 0-19 – 15 animals
20. STB 30 mg/kg/day – GD 0-19 – 15 animals
21. STB 125 mg/kg/day – GD 0-19 – 15 animals
22. STB 500 mg/kg/day – GD 10-12 – 15 animals; included as an additional group because was anticipated that administration throughout the complete gestation period may result in a high incidence of fetal lethality. This is a period during which fetuses are susceptible to abnormal development.
23. Radiolabeled STB 500 mg/kg/day – GD 10-12 – 4 animals; residue analyses group

*Considered to be "4 "mg/kg/day based on dosing of 8 mg/kg/day on alternate days of during gestation period.

Developmental study (Groups 1-6:
The test material was administered to groups 3-5 on GD 0-19. Group 2 animals were administered test material on alternate days during gestation (GD 0, 2, 4, 6, 8, 10, 14, 16, and 18). Group 6 females were similarly treated but administration of test material was restricted to a period of gestation during which fetuses are susceptible to abnormal development (GD 10-12). Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared
and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, abortion or premature delivery. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and livers were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. The ovaries and uterus of each rat were

excised and examined grossly. The number of corpora lutea per ovary was recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each live fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

8) Anomaly: Any deviation (malformation or variation) from "normal."
9) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
10) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.

Approximately half of the fetuses were randomly assigned for examination of soft tissues (viscera) following fixation in Bouin's solution, using a modification of the Wilson's technique. The other half were fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

Bioavailability Study (Group 9)

From GD 0-9, pregnant females were housed in stainless steel cages with wire bottoms and fronts. On GD 10, 11, and 12, the rats were housed in metabolism cages. The STB used in the bioavailability study contained two radioactive surrogates, carbon-14 radiolabeled carbazole and hydrogen-3 radiolabeled benzo(a)pyrene (BaP). On GD 10, the hair was clipped from the dorsal trunk of each animal and the radiolabeled test material (500 mg/kg) was applied to the skin within a protective device designed to contain the administered dose. A mesh screen was attached to the protective device, and each rat was fitted with an Elizabethan collar. The same procedure was repeated on GD 11 and 12, except the needle tip with the test material was inserted through the mesh screen in order to apply the test material.

On GD 13, 24 hrs after the administration of the last STB dose, animals were sacrificed ether overexposure and maternal blood was collected. Necropsies were performed and the uterine contents located and examined for the number of normal and resorbed fetuses for each dam. The individual fetal units were removed, and the amniotic fluid was collected from the isolated placenta. The embryo was separated from the yolk sac and rinsed with water to remove residual amniotic fluid. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights or volumes of the pooled samples determined. Maternal tissues collected for radioactivity analysis included the following: thymus, liver, small intestine, large intestine, kidneys, stomach, and ovaries.

Determination of radioactivity in blood, urine and cage wash was accomplished by measuring the amount of carbon-14 labeled carbon dioxide and H-3 labeled water produced from direct combustion of duplicate samples. Samples were oxidized for three minutes and the carbon dioxide and water

generated from the combustion were separated and trapped in a cocktail fluid. Carbon-14 and hydrogen-3 radioactivities were measured. Fecal samples were homogenized, combusted and the radioactivity measured.

The placentae, uteri, embryos, and yolk sacs were homogenized in an equivalent volume of water, and aliquots of the homogenate were combusted. Maternal tissues were treated in the same manner, although the ovaries, and amniotic fluid were combusted directly without homogenization or dilution. In all cases, the trapped carbon dioxide and water were measured for radioactivity by liquid scintillation counting. Samples of the amniotic fluid were also combusted directly without dilution. Duplicate analyses were performed whenever possible. The sensitivity of the radioactivity allowed for the detection of 0.005% of the applied dose.

The systemic dermal absorption of the two radiolabeled surrogates was determined by summing the total carbon-14 or hydrogen-3 radioactivities found in the urine, urine/cage washings, feces and collected maternal and embryonic tissues at the end of 72 hours. Tissue concentrations of carbazole and benzo(a)pyrene (BaP) were calculated based on the radioactivity found per gram or per ml. The total amount of a radiolabeled surrogate in the tissues was calculated as a percent of the total applied radioactive dermal dose over three days.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated by ANOVA followed by Duncan's multiple range 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 the 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. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	4		mg/kg/day
NOAEL- Dermal	Maternal	=	Not identified <4		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	4		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	Not identified		mg/kg/day

Results Remarks:

The female animals used in the study were approximately 9 weeks old at receipt and approximately 11 weeks old at exposure initiation.

The red nasal exudate and chromodacryorrhea that were observed in control and STB-exposed groups are common in animals that are collared. Also, neck lesions were observed in control and LCGO-exposed groups in spite of the protective soft rubber tubing that lines the inner surface of the cardboard collar. Scratches were observed on the backs of a few of the animals at the time of the first clipping and probably occurred during mating activity.

Findings attributable to STB exposure included vaginal bleeding (generally a sign of some degree of litter resorption) observed in the treated groups at dose levels at or above 8 mg/kg/day. One of the four females in the 8 mg/kg/day group which had red vaginal discharge had no resorptions; another exhibited the discharge on GD 20 which may be indicative of premature delivery. Historically, red vaginal discharge has been observed in the laboratory facility in control animals as well as in animals that had no resorption. The vaginal bleeding may have contributed to the paleness observed in animals exposed at the 30 and 125 mg/kg/day dose levels since this finding Was noted either during or following vaginal bleeding for these animals, Several exposed females had decreased stool. This finding was noted more frequently in the 125 mg/kg/day group. Scabs were observed at the site of
application in two females exposed to “4” mg/kg/day; one of these two females also had slight erythema at the dosing site.

The mean body weights for the 30 and 125 mg/kg/day groups were significantly reduced throughout most of the gestation period. Animals administered test material for a limited period of gestation (GD 10-12; 500 mg/kg/day) weighed significantly less following the period of STB exposure. The body weights for the 8 mg/kg/day group were significantly reduced toward the end of gestation. All STB-exposed groups gained significantly less weight overall than that of the control group; this finding was dose-related. Net maternal body weight changes are significantly reduced in STB exposed groups dosed at 8, 125, and 500 mg/kg/day. Although the body weight change for the 30 mg/kg/day group was low compared to the control group, significance was not achieved due to an increase in variability caused by several outlying animals in this dose group.

The amount of food consumed by females exposed to STB was lower than that consumed by the control group at each of the intervals measured. This reduction was significant throughout gestation for the 125 mg/kg/day group and during early to mid-gestation for females exposed to STB at dose levels of “4”, 8, and 30 mg/kg/day. A significant decrease in food consumption was observed for the 500 mg/kg/day group during the latter part of gestation which reflects the time at which these animals were exposed to
the test material (GD 10-12).

Although the thymus appeared small in females from all groups, the incidence was higher in the 125 and 500
mg/kg/day groups. A significant reduction in absolute and relative thymus weight was noted in animals exposed to STB at dose levels of 125 and 500 mg/kg/day. Absolute liver weights were significantly reduced at the 125 mg/kg/day dose level. This finding was not unexpected due to total fetal resorption for this group. As observed in other studies at this laboratory, the size of the liver increases during pregnancy; however when all fetuses are resorbed, the female returns to the “nonpregnant” state and the liver returns to a “normal” size.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	"4"	8	30	125	500 (GD 10-12)
Body wt –at delivery (gr)	428.7	392.1	382.6b	338.7b	256.2b	342.3b
GD 0-3 wt gain (gr)	13	4	3	-2b	24b	15
GD 3-6 wt gain (gr)	17	12	10	12	5a	13
GD 6-10 wt gain (gr)	21	19	16	20	15	23
GD 10-13 wt gain (gr)	18	16	16	13	12	-14b
GD 13-16 wt gain (gr)	20	25	21	4b	-15b	11b
GD 16-20 wt gain (gr)	58	59	52a	27b	-2b	35b
GD 0-20 wt gain (gr)	165	135a	117b	81b	-8b	82b
Gravid uterus (gr)	87.9	75.6	59.8b	22.9b	2.6b	23.7b
Carcass (gr)/Final body weight	340.8	321.5	322.6	316.7	253.7b	318.6
Net wt change from day 0 (e)	76.3	64.3	55.3a	59.1	-10.3b	58.2a
Thymus weight (g)-absolute	0.243	0.212	0.238	0.197	0.075b	0.098b
Thymus weight (g)-relative	0.0768	0.0686	0.0736	0.0818	0.0294 b	0.0310 b
Liver weight - absolute (g)	17.646	16.635	16.741	16.822	13.866 b	17.796
Liver weight (g)-relative	6.1497	6.1484	6.1883	6.2349	6.4484	6.6784

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) Statistically different from matched control ($p<0.05$)
d) Statistically different from matched control ($p<0.01$)
e) = Carcass weight minus day 0 body wt

The number and percent resorptions were significantly increased at the 30 mg/kg/day level and above. The threefold increase at the 8 mg/kg/day level was considered to be biologically significant. Litter size was significantly decreased at dose levels of 8 mg/kg/day and above.

Adverse effects on serum components were noted at the 125 mg/kg/day dose level. Aberrant serum chemistry values were obtained for urea nitrogen, aspartate aminotransferase, cholesterol, triglycerides, total protein, albumin, albumin/globulin ratio, uric acid, inorganic phosphorus, calcium and iron. A linear relationship (>99% confidence level) was found between dose and serum levels for aspartate aminotransferase, cholesterol, triglyceride, total protein, albumin, albumin/globulin ratio, inorganic phosphorus, calcium and iron. When historical serum reference values were taken into consideration, the dose-response curves for each of these serum components at 125 mg/kg/day dose level and for iron and albumin at the 30 mg/kg/day level fell outside the normal range as defined by the 10^{th} and 90^{th} percentiles of historical data.

Summary of Mean Selected Reproduction Data (Groups 1-6)

Dose (mg/kg/day)	0	"4"	8	30	125	500 (GD 10-12)
Implantation sites – mean	17.8	15.8	16.1	15.5	14.7	16.1
Viable fetuses-total	180	159	161	47	0	56
Litter size (c)	16.4	13.3	11.5b	3.5b	0.0b	3.7b
Viable male fetuses (%)	51	56	54	49	--	54
Resorptions (mean)	1.5	2.5	4.6	11.3b	14.7b	12.3b
Resorptions (mean %)	0.4	16.5	28.9	78.1b	100.0b	74.5b
Dams with resorptions (%)	9	12	12	13	12	15

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) Number of viable fetuses/number of litters evaluated.

A significant decrease in mean fetal body weight was observed in male fetuses from dams exposed to greater than "4" mg/kg/day (8 mg/kg/day and above.

At the time of fetal gross examination, two fetuses exposed in utero to 500 mg/kg/day (GD 10-12) were edematous and one fetus had a kinked tail. The incidence of each observation alone was not significant; however, the total number of affected fetuses observed in this group was significantly greater than that observed in the control group. One fetus in the 30 mg/kg/day group exhibited hyperflexion of both forelimbs.

Fetal Endpoints – Weight and Gross Examination (Groups 1-6)

Dose (mg/kg/day)	0	"4"	8	30	125	500 (GD 10-12)
Fetal weights (g)	3.5	3.5	3.2	2.9b	*	2.6b
Litters evaluated	11	12	14	10	0	11
Fetuses - live	190	159	161	47	0	58
Fetuses – dead	0	0	0	0	0	0
Gross Exam (fetal incidence; %)	0; 0.0	0; 0.0	0; 0.0	1; 2.1	--	3; 5.4a
Gross Exam (litter incidence; %)	0; 0.0	0; 0.0	0; 0.0	1; 10	--	3; 27

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
*No viable fetuses

A significant increase in total rib malformations was observed for the 500 mg/kg/day group (GD 10-12). Other malformations observed in the study appeared randomly and at a low frequency throughout the groups. Incomplete ossification of the nasal bones, vertebrae and sternebrae were the most commonly observed variations noted in the STB-exposed groups. In general, the incidence was dose-related.

Fetal Endpoints – Skeletal Malformations and Skeletal Variations (Groups 1-6)

Dose (mg/kg/day)	0	"4"	8	30	125	500 (GD 10-12)
Litters evaluated	11	12	14	10	0	11
Fetuses - live	94	83	63	25	0	30
Fetuses – dead	0	0	0	0	0	0
Total skeletal observations (fetal incidence; %)	86;91	73; 88	60; 96	25; 100	--	30; 100
Total skeletal observations (litter incidence; %)	11; 100	12; 100	14; 100	10; 100	--	11; 100

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

A significant increase in fetuses having cleft palate was observed for the 500 mg/kg/day (GD 10-12) group. Isolated incidences of other malformations were noted throughout the control and STB –exposed groups. These findings were not statistically significant from the control group. The most commonly noted variation was distention of the ureters. This finding, although present in the control fetuses, was observed significantly more in fetuses exposed in utero to 8 and 500 mg/kg/day.

Fetal Endpoints – Soft Tissue Anomalies (Groups 1-6)

Dose (mg/kg/day)	0	"4"	8	30	125	500 (GD 10-12)
Litters evaluated	11	12	14	9	0	10
Fetuses - live	96	78	73	22	0	26
Fetuses – dead	0	0	0	0	0	0
Total fetal soft tissue (fetal incidence; %)*	7; 8.1	7; 9.2	18;23b	4;18	--	11; 42b
Total fetal soft tissue (litter incidence; %)*	6; 55	5; 42	10; 71	3; 33	--	5; 50

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Bioavailability/Bioaccumulation Analyses

The dermal penetration of 14-C-carbazole occurred more extensively and rapidly than 3H- BaP absorption over a 72 hour period. About 27.5% of the total applied 14-C radioactive dose (three applications) was dermally absorbed. In comparison 3.3 % of the total applied 3-H-benzo(a)pyrene dose was systemically absorbed. At the end of 72 hours, 2.5% of the 14-C-carbazole was found in the maternal tissues and less than 0.01% of the 14-C radioactive dose was detected in the embryo. The majority of the 14-C-radiolabel in the maternal tissues was found in the large intestines (0.81% of the radioactive dose), maternal blood (0.69 %), liver (0.30%), and small intestines (0.26%). The amount of 3-H-BaP found in maternal tissues at the end of 72 hours was 0.8% of the tritiated dose and the amount found in the embryo was less than 0.01% of the radiolabeled dose. Most of the tritium (3-H) was found in ht large and small intestines (0.47% and 0.12% of the radiolabeled dose, respectively), liver (0.12%) and maternal blood (0.09%).

In spite of the dermal bioavailability of 14C-carbazole and 3H- BaP in the dam, the amount of radiolabel led material found in the embryo was very low.

	Less than 0.01% of the 14-C-carbazole or 3-H-benzo(a)pyrene in the radioactive doses was detected in the embryos on gestation day 13. The placenta appears to be an effective barrier against the transport of carbazole and BaP to the embryo. There is no evidence the 14C-carbazole or 3H- BaP accumulates selectively in the embryo.
Conclusion:	The maternal NOAEL for dermal exposure to STB for GD 0-19 could not be identified (<4 mg/kg/day). (LOAEL= 4 mg/kg/day based on decreased body weight gain) The developmental NOAEL for dermal exposure for GD 0-19 could not be identified (<4 mg/kg/day). (LOAEL = 4 mg/kg/day based on a potentially biologically significant increase in resorptions and decreased litter size. Neither was statistically significant, but the authors determined biological significance.

RELIABILITY/DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	Key

REFERENCE

Reference:

Mobil. 1989. Developmental Toxicity Study in Rats Exposed Dermally to Ferndale Syntower Bottoms. Mobil Environmental and Health Sciences Laboratory Report 62934.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Syntower Bottoms. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZM.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	64741-62-4
Test Substance:	64741-62-4; Clarified Slurry Oil (CSO); Cat Cracked Clarified Oil
Test Substance Purity/Composition and Other Test Substance Comments:	Clarified Slurry Oil (F-179) PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091645 (F-179)		0.00	0.70	10.00	30.00	20.00	6.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs

with 2 aromatic rings, and so forth to 7 aromatic rings

Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Portage, MI)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	25 per dose for vehicle (acetone) control 25 per dose level of 0.05 mg/kg/day CSO per administration schedule listed below 70 per dose level of 1, 50 and 250 mg/kg/day per administration schedule listed below (10 per subgroup)
Concentration:	
Dose:	0, 0.05, 1, 50, 250 mg/kg/day
Year Study Performed :	1992
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation day (GD) 0-19 (two dose groups) Three dose groups were divided into the following subgroup schedule: GD 0-2, GD 3-5, G 12-14, GD 15-17, or GD 18-19.
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the critical period effect of dermal administration of CSO (F-179) on major organogenesis in the developing rat conceptus. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of sperm in a vaginal smear or a copulatory plug, the individual, presumed pregnant females were randomly assigned to five treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of evidence of mating: 1. Vehicle control (acetone) 0 mg/kg/day – 25 animals (GD 0-19) 2. CSO 0.05 mg/kg/day – 25 animals (GD 0-19) 3. CSO 1.0 mg/kg/day – 70 animals (7 subgroups according to GD)*

4. CSO 50.0 mg/kg/day – 70 animals total (7 subgroups according to GD)*
5. CSO 250.0 mg/kg/day – 70 animals total (7 subgroups according to GD)*

*Subgroups were as follows: GD 0-2, GD 3-5, GD 6-8, GD 9-11, GD 12-14, GD 15-17, or GD 18-19.

At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed.

Suspensions of F-179 were prepared daily at concentrations of 0 (vehicle, acetone), 0.05, 1.0, 50, and 250 mg/mL such that doses of 0. 0.05, 1.0, 50 and 250 mg/kg/day, respectively, were administered at a volume of 1 mL/kg. The test material was administered to groups 1 and 2 GD 0 through GD 19; Groups 3-5 received test material according to their assigned subgroups of GD 0-2, GD 3-5, GD 6-8, GD 9-11, GD 12-14, GD 15-17, or GD 18-19. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls, dosed at GD 0 through 19, were handled in the same manner but with application of the vehicle only. 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 was wiped off with a cloth dipped in acetone and dried with a clean cloth.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights and food consumption were recorded daily during presumed gestation.

The abdomen of each rat was opened, and the intact uterus was excised and examined for pregnancy. Uteri from rats that appeared nonpregnant were examined while pressed between two glass plates to confirm pregnancy status. The thoracic and abdominal cavities were examined for gross lesions. Gross lesions were preserved in neutral buffered 10% formalin.

Corpora lutea in each ovary were counted. The number and distribution of implantations, early and late resorptions and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Each fetus was removed from the uterus, placed in an individual container, weighed, and examined for weighed and examined for sex and gross external alterations. Live fetuses were sacrificed by immersion in the appropriate fixative.

Approximately one-half of the fetuses in each litter were preserved in Bouin's solution, and the remaining fetuses in each litter were preserved in alcohol. Fetuses in dose groups 1 and 2 that were preserved in Bouin's solution were examined for soft tissue alterations by using a variation of Wilson's sectioning technique. The remaining fetuses in these two dose groups that were preserved in alcohol were cleared, stained with alizarin red S and examined for skeletal alterations.

STATISTICAL ANALYSES: Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Maternal body weights, body weight changes, feed consumption values, and litter averages for fetal body weights, percent male fetuses, fetal ossification sites and percent fetal

alterations were analyzed using Bartlett's Test and ANOVA, when appropriate [i.e., Bartlett's Test was not significant (P>0.05)]. If the analysis of variance was significant (P<0.05), Dunnett's Test was used to identify the statistical significance of the 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, when less than or equal to 75% ties were present. When more than 75% ties were present, Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant (P<0.05), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures described for the Kruskal-Wallis Test.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	Not identified for GD 0-19		mg/kg/day
NOAEL- Dermal	Maternal	=	0.05		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	Not identified for GD 0-19		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	0.05		mg/kg/day

Results Remarks:

The animals used in the study were between 10-11 weeks of age at exposure initiation.

No deaths occurred during the conduct of this study. No skin irritation occurred and no clinical or necropsy observations were related to the test substance at doses as high as 250 mg/kg/day. The clinical observations that occurred in the 0.05 mg/kg/day dose group were single events and not significant. These observations included dental problems and chromodacryorrhea. Clinical and necropsy observations that occurred in the 1, 50 and 250 mg/kg/day dose groups, when dosing occurred at different intervals during gestation, were also considered unrelated to the test substance because: 1) they were single events; or 2) they are commonly observed in this strain of rat. These observations included localized alopecia, dental problems, mouth lesion. ovarian cyst, mass on the back, distended uterine horn and cervix apparently filled with blood.

The 0.05 mg/kg/day dose did not affect maternal body weight gains, body weights or absolute and relative feed
consumption values (GD 0-20). Maternal body weight gains during the dosing periods were reduced by administration of the test article at levels of 1, 50 and 250 mg/kg/day at all intervals between days 3 through 17 of gestation. In fact, the 50 and 250 mg/kg/day doses reduced maternal body weight gains during the dosing period for each interval examined. Maternal body weights were unaffected. These reductions were biologically important and/or statistically significant (P<0.05 to P<0.0l). These three doses of the test substance also caused biologically

important and/or significant reductions (P<0.05 to P<0.0l) in absolute and/or relative feed consumption values during the treatment periods, when administered at intervals between days 6 through 19 of gestation. The 50 and 250 mg/kg/day doses of the test substance also caused biologically important or significant changes in absolute and/or relative feed consumption values during the treatment periods, when administered on days 0 through 2 or 3 through 5 of gestation. During the post-dosing period, relative feed consumption values remained significantly reduced (P<0.05 to P<0.01) at the 1, 50 and 250 mg/kg/day dose levels, when these doses were administered on days 15 through 17 or 18 and 19 of gestation.

The 0.05 mg/kg/day dose of the test substance also did not adversely affect the offspring (there were no effects on embryo-fetal viability, sex, body weight. or external, soft tissue and skeletal morphology).

The authors conclude that the most sensitive indicator of the potential developmental toxicity of F-179 was early resorption; fetal body weights and morphology were unaffected by the doses of the test substance tested in this study. The critical periods for causing embryo deaths (early resorptions) were days 6 through 8 and 9 through 11 of gestation, with the earlier period being the most sensitive. Both the 50 and 250 mg/kg/day doses of F-179 increased early resorptions in these groups, when the test substance was administered on days 6 through 8 of gestation. The 250 mg/kg/day level also resulted in increased early resorptions when administered on days 9 through 11 of gestation. Reflecting test substance effects, the percentage of resorbed conceptuses per litter tended to be increased in the 50 mg/kg/day dose group and was significantly increased (P<0.05) in the 250 mg/kg/day dose group, when the test substance was administered on days 6 through 8 of gestation. This parameter tended to be increased at the 250 mg/kg/day dose level, when the test substance was administered on days 9 through 11 of gestation. There were no other adverse effects on the conceptuses at the 50 or 250 mg/kg/day doses of the test substance.

All fetal alterations that occurred in this study were considered unrelated to the test substance because: 1) the incidences were not significant, as compared to the control group values; and 2) the incidences were either not dose-dependent or were single events and within the ranges observed historically. Fetal sex ratios, body weights and gross external, soft tissue or skeletal morphology were unaffected by these doses of test substance, when administered on days 0 through 19 of gestation and days 0 through 2, 3 through 5, 6 through 8, 9 through 11, 12 through 14, 15 through 17 or 18 and 19 of gestation, respectively.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0 *	0.05*	1d	50d	250d
Body wt –final (gr)	407.5	404.5	c	C	c
GD 0-3 wt gain (gr)	13.2	12.0	c	D	d
GD 3-6 wt gain (gr)	15.9	14.1	d	D	d
GD 6-9 wt gain (gr)	12.8	14.8	d	D	d
GD 9-12 wt gain (gr)	18.3	17.5	d	D	d
GD 12-15 wt gain (gr)	23.6	22.7	c	D	d
GD 15-18 wt gain (gr)	42.9	41.1	e	D	d
GD 18-20 wt gain (gr)	15.0	15.5	c	D	d
GD 19-20 wt gain (gr)	34.1	34.8	c	E	d
GD 0-20 wt gain (gr)	160.8	157.1	c	C	c

*GD 0-19 dosing period

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) No different from control, regardless of GD dosing period.
d) Significant difference from controls occurred: see text for explanation.
e) Exact interval not measured; however differences were observed at some point within this interval.

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	0.05	1	50	250
Implantation sites – mean	16.1	15.8	c	C	c
Viable fetuses	344	362	c	C	c
Litter size	15.0	15.1	c	C	c
Viable male fetuses (%)	51	52	c	C	c
Resorptions (mean)	1.1	0.8	c	E	d
Resorptions (mean %)	7.0	4.6	c	E	d
Dams with resorptions	13	11	c	C	c

*GD 0-19 dosing period
a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) No different from control, regardless of GD dosing period.
d) Significant difference from controls occurred: see text for explanation.
e) Judged to be biologically significant.

Fetal Endpoints

Dose (mg/kg/day)	0	0.05	1	50	250
Fetal weights (g)	3.5	3.5	c	C	c
Litters evaluated	23	24	c	C	c
Fetuses - live	344	362	c	C	c
Fetuses – dead	0	0	c	C	c
Gross exam anomalies (fetal incidence; %)	3.5	4.4	c	C	c
Gross exam anomalies (litter incidence; %)	3.69	4.32	c	C	c
Total skeletal alterations (fetal incidence; %)	3.4 (N=177)	3.2 (N=187)	c	C	c
Total skeletal alterations (litter incidence; %)	13.0	8.3	c	C	c
Total fetal soft tissue (fetal incidence; %)	0 (N=167)	1.2 (N=175)	c	C	c
Total fetal soft tissue (litter incidence; %)	0	8.6	c	C	c

*GD 0-19 dosing period
a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) No different from control, regardless of GD dosing period.
d) Significant difference from controls occurred: see text for explanation.

Conclusion: The maternal NOAEL for dermal exposure to CSO during GD 0-19 was determined to be 0.05 mg/kg/day. (the LOAEL was not identified for GD 0-19

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The developmental NOAEL for dermal exposure to CSO during GD 0-19 was determined to be 0.05 mg/kg/day. (the LOAEL was not identified for GD 0-19)

RELIABILITY/DATA QUALITY

Reliability: Valid with Restrictions (KS=2)

Reliability Remarks: Non-guideline study; research study to determine critical period of developmental toxicity; adequate detail provided

Key Study Sponsor Indicator: Key

REFERENCE

Reference: ARCO, 1992. Critical Period Developmental toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of F-179 Administered Percutaneously to Crl:CD®BRK VAF/Plus® Presumed Pregnant Rats. Report ATX-91-0042.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; Clarified Slurry Oil (CSO); Cat Cracked Clarified Oil

Test Substance Purity/Composition and Other Test Substance Comments: Clarified Slurry Oil (CRU No. 86001)

PAC Content – report no. 64348 ZA (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86001	64.20	0.00	2.57	25.68	19.26	6.42	3.21	0.64

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity screen

Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females, presumed pregnant (non treated males used for mating)
Number of Animals per Dose:	10 per dose, except for an additional group of 5 animals exposed at 125 mg/kg on GD 0-19 used to obtain residue data
Concentration:	
Dose:	Developmental study, GD 0-19: 0 (remote), 0 (proximate), 8, 30, 125, 250 mg/kg/day Developmental study, GD 0, 2, 4, 6, 8, 10, 14, 16, 18: 8 mg/kg/day [Note: since the dose was administered on alternate days throughout gestation, this was considered to be the 4 mg/kg/day group] Residue Group, GD 0-19: 125 mg/kg/day
Year Study Performed :	1987
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (10/group versus 20),
GLP:	No information
Exposure Period:	GD 0-19 (7 groups); GD 0, 2, 4, 6, 8, 10, 14, 16, 18 (1 group)
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to obtain data on the influence of CSO on parameters of reproductive performance during gestation (implantation, litter size) and viability and development of the embryo/fetus. An additional experimental group was initially added in order to include residue analyses of maternal blood, fetuses, and placentae. However, the dams assigned to this group resorbed their entire litters, precluding any analyses. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (<u>in situ</u> or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid: 1. *Remotely-housed dermal control (0 mg/kg/day) – GD 0-19 2. Proximately-housed dermal control (0 mg/kg/day) – GD 0-19 3. CSO 8 mg/kg/day – GD 0-19 – 10 animals 4. CSO 30 mg/kg/day – GD 0-19 – 10 animals 5. CSO 125 mg/kg/day – GD 0-19 – 10 animals 6. CSO 250 mg/kg/day – GD 0-19 – 10 animals 7. CSO 8mg/kg/day – GD 0, 2, 4, 6, 8, 10, 14, 16, 18 – 10 animals. ** 8. CSO 125 mg/kg/day – GD 0-19 – 5 animals; residue analyses group *Because inhalation of the test material could not be ruled out, a separate control group was not housed in the same animal room (remote-housed control). Subsequent analyses of air samples indicated that no single compound was detected above the limit of detection of 0.2 mg/m3. **Considered to be “4 “mg/kg/day based on dosing of 8 mg/kg/day on alternate days of during gestation period. The exposure levels were based on results of a 13 week study previously conducted on the same material. <u>Developmental study (Groups 1-7):</u>

The test material was administered to groups 3-6 and group 8 on GD 0-19. Group 7 animals were administered test material on alternat days during gestation (GD 0, 2, 4, 6, 8, 10, 14, 16, and 18). Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality or abortion. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and liver of each animal exposed to 0, "4", and 250 mg/kg/day were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea per ovary were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each live fetus was gendered, weighed and grossly examined. Approximately half of the fetuses were randomly assigned for examination of soft tissues (viscera) following fixation in Bouin's solution, using a modification of the Wilson's technique. The other half were fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

Residue Study (Group 8)
An additional experimental group was initially added in order to include residue analyses of maternal blood, fetuses, and placentae. These analyses were not performed because the dams assigned to this group resorbed their entire litters.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated by ANOVA followed by Duncan's multiple range 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 the 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. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	8		mg/kg/day
NOAEL- Dermal	Maternal	=	"4"		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	8		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	"4"		mg/kg/day

Results Remarks:

The animals used in the study were approximately 9 weeks old at receipt and approximately 11 weeks old at exposure initiation.

The majority of clinical observations were noted in both the control and treated groups and appear not to be treatement related. Alopecia was observed in some of the animals exposed to CSO. Findings were not considered to be test material related due to the low incidence and lack of a dose response relationship.

Except for two dams exposed to 4 mg/kg/day which exhibited erythema, flaking and scabs, there were no signs of dermal irritation in the CSO exposed animals. One dam exposed to 125 mg/kg/day was found dead in her cage on GD 18. Vaginal bleeding, a sign of some degree of litter resorption was observed in all exposed groups exposed to CSO at doses of 8 mg/kg/day or greater.

Mean body weights, body weight gains and net body weights decreased in a dose-related fashion at doses of 8 mg/kg/day or greater. Except at 8 mg/kg/day, the decreased body weights reflect the decrease in litter sizes observed at these doses. In general, animals exposed to CSO 8 mg/kg/day or greater consumed less food than the controls.

Maternal necropsy result showed a reduced size of the thymus at doses greater than 8 mg/kg/day. Thymus weight measurements confirmed this observation , reflecting a significant decrease ($p<0.05$) in mean weight at the 250 mg/kg/day, but not at the "4" mg/kg/day level. [note: 8 mg/kg/day weight not determined.] In addition, there was a significant reduction ($p<0.05$) in liver weights in the high dose rats. However, when the data are expressed in terms of mean organ-to-body weight ratios, results of the high-dose group were higher than the other groups examined.

Summary of Selected Maternal Weight Parameters

Dose	0	0	8	30	125	250	"4"	125

(mg/kg/day)	Rem.	Prox.						
Body wt –final (g)	396	397	376	316 bd	272bd	243bd	396	260 bd
GD 0-3 wt gain (g)	17	17	12	7	1bd	-2bd	9	-3bd
GD 3-6 wt gain (g)	16	13	10	12	6	2b	16	11
GD 6-10 wt gain (g)	19	23	20	18	19	14c	16	18
GD 10-13 wt gain (g)	20	20	18	13	7bd	9ac	22	8
GD 13-16 wt gain (g)	24	26	22	2ad	-10bd	-13bd	22	-4ac
GD 16-20 wt gain (g)	62	64	60	26bd	9bd	-1bd	68	-10bd
GD 0-20 wt gain (g)	158	162	141	78bd	35bd	9bd	152	22bd
Thymus weight (g)-absolute (relative weight not determined)	0.249	0.295	ND	ND	ND	0.061ac	0.280	ND
Liver weight (g)-absolute	15.808	16.578	ND	ND	ND	13.543ac	16.485	ND
Liver weight (g)-relative	5.03	5.20	ND	ND	ND	5.60ac	5.18	ND

a) Statistically different from remote control ($p<0.05$)
b) Statistically different from remote control ($p<0.01$)
c) Statistically different from proximate control ($p<0.05$)
d) Statistically different from proximate control ($p<0.01$)

For clinical chemistry parameters, statistical analyses were performed only between the remote control and CSO-exposed groups. Differences were seen for seven serum parameters, all of which demonstrated a dose response effect. A linear relationship was found between dose and serum level for all of these components but cholesterol. Of these findings, only the increased levels of alkaline phosphatase and cholesterol, indicative of liver toxicity, appear to be related to CSO exposure.

At Cesarean section, the number of implantation sites and percent preimplantation loss were not observed to be affected by exposure to CSO. The parameters affected by CSO were the number of dams with all resorptions, number of resorptions (increased at levels of 30 mg/kg/day or more- $p<0.01$) and litter size (decreased at levels of 30 mg/kg/day or more – $p<0.01$).

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**8**	**30**	**125**	**250**	**"4"**	**125**
Implantation sites - total	164	157	146	141	107	135	166	35
Implantation sites – mean	16.4	15.7	16.2	15.7	15.3	15.0	16.6	11.7
Preimplantation loss (%)	10.8	12.4	3.6	7.7	12.1	11.5	10.8	22.2
Viable	154	143	128	41	2	0	148	0.0

fetuses								
Litter size (e)	15.4	14.3	14.2	4.8bd	0.4bd	0.0bd	14.8	0.0
Viable male fetuses	80	75	65	19	2	0.0	82	0.0
Viable female fetuses	74	68	63	22	0.0	0.0	66	0.0
Resorptions (mean)	1.0	1.4	2.0	10.9bd	14.9bd	15.0bd	1.0	11.7bd
Resorptions (mean %)	6.0	8.9	11.7	69.9bd	97.1bd	100.0bd	10.8	100.0bd
Dams with resorptions (%)	70	70	78	89	100	100	80	100

a) Statistically different from remote control (p<0.05)
b) Statistically different from remote control (p<0.01)
c) Statistically different from proximate control (p<0.05)
d) Statistically different from proximate control (p<0.01)
e) Number of viable fetuses/number of litters evaluated.

Fetuses from pregnant females exposed to CSO at dose levels of 30 and 125 mg/kg/day were smaller (decreased body weight and crown rump lengths than fetuses from the control and low dose groups. Abnormal external fetal development was observed in fetuses at dosages of 8, 30 and 125 mg/kg/day. Anomalies included micrognathia, kinked tail and edema. Of the six fetuses that were affected, only three of the fetuses exposed to CSO were observed at the time of soft tissue and skeletal evaluations.

Visceral anomalies observed in viable fetuses included enlarged ventricles of the brain, displacement of the esophagus from a left-sided position to a right-sided position, and anomalous development of the heart. A variety of skeletal variations and malformations were observed in CSO-exposed and control fetuses, however the degree of aberrant development in the controls was not as severe as the CSO-exposed fetuses. Although the number of adverse findings was limited, they were judged to be possibly test material related. Abnormal external and visceral development was observed in all of the dead fetuses.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**8**	**30**	**125**	**250**	**"4"**	**125**
Fetal weights (g)	3.5	3.5	3.4	2.7bd	2.3ac	--	3.5	--
Litters evaluated	10	10	9	7	1	0	10	0
Fetuses - live	80	75	66	22	1	0	77	0
Fetuses – dead	0	0	0	0	0	0	0	0
Gross fetal exam anomalies (%)	0	0	0.8	2.4	50	--	--	--

a) Statistically different from remote control (p<0.05)
b) Statistically different from remote control (p<0.01)
c) Statistically different from proximate control (p<0.05)
d) Statistically different from proximate control (p<0.01)

Fetal Endpoints – Skeletal Malformations and Skeletal Variations

Dose (mg/kg/day)	0 Rem.	0 Prox.	8	30	125	250	"4"	125
Litters evaluated	10	10	9	7	1	0	10	0
Fetuses - live	80	75	66	22	1	0	77	0
Fetuses – dead	0	0	0	0	0	0	0	0
Total skeletal malformations (fetal incidence; %)	1; 1.3	0; 0.0	1; 1.5	1; 4.5	0; 0.0	--	2; 2.6	--
Total skeletal malformations (litter incidence; %)	1: 10	0; 0.0	11; 1	1; 14	0; 0.0	--	1; 10	--
Total skeletal variations (fetal incidence; %)	25; 31	51; 68b	34; 52a	20; 91b	1; 100	--	50; 65b	--
Total skeletal variations (litter incidence; %)	7; 70	10; 100	9; 100	7; 100	1; 100	--	10; 100	--

a) Statistically different from remote control ($p<0.05$)
b) Statistically different from remote control ($p<0.01$)
c) Statistically different from proximate control ($p<0.05$)
d) Statistically different from proximate control ($p<0.01$)

Fetal Endpoints – Soft Tissue Anomalies

Dose (mg/kg/day)	0 Rem.	0 Prox.	8	30	125	250	"4"	125
Litters evaluated	10	10	9	7	2	0	10	0
Fetuses - live	74	68	62	19	1	0	71	0
Fetuses – dead	0	0	0	2	1	0	01	0
Total fetal soft tissue malformations (fetal incidence; %)	0; 0.0	0; 0.0	0; 0.0	2; 9.5a	1; 50ac	--	0; 0.0	--
Total fetal soft tissue malformations (litter incidence; %)	0; 0.0	0; 0.0	0; 0.0	2; 29	1; 50	--	0; 0.0	--

a) Statistically different from remote control (p<0.05)
b) Statistically different from remote control (p<0.01)
c) Statistically different from proximate control (p<0.05)
d) Statistically different from proximate control (p<0.01)

Conclusion: The maternal NOAEL for dermal exposure to CSO during GD 0-19 was determined to be "4" mg/kg/day (LOAEL= 8 mg/kg/day based on vaginal discharge observations, decreased body weight; decreased food consumption, and atrophy of the thymus)

The developmental NOAEL for dermal exposure to CSO during GD 0-19 was determined to be "4" mg/kg/day (LOAEL = 8 mg/kg/day based on increased number and percent resorptions; decreased fetal body weight and crown-rump length, and increased fetal anomalies)

The authors also note that developmental toxicity was observed at concentrations that also produced overt maternal toxicity.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Comparable to guideline study

Key Study Sponsor Indicator: Key

REFERENCE

Reference: Mobil. 1987. Clarified Slurry Oil Developmental Toxicity Study in Rats. Mobil Environmental and Health Sciences Laboratory Report 50541.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Clarified Slurry = Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348 ZA

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; Clarified Slurry Oil (CSO)

Test Substance Purity/Composition and Other Test Substance Comments: Clarified Slurry Oil (CRU No. 86001)

PAC (Polycyclic Aromatic Compound) Content – Report No. 64348 ZA (Mobil, 1991)

Sample #	DMSO wt.%[1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86001	64.20	0.00	2.57	25.68	19.26	6.42	3.21	0.64

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs

	that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Oral; gavage
Other Route of Administration:	
Type of Exposure:	Developmental toxicity study
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females, presumed pregnant (non treated males used for mating)
Number of Animals per Dose:	12 per dose
Concentration:	
Dose:	0, 125, 500, 2000 mg/kg/day (per gestation days described in methods below)
Year Study Performed :	1990
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (12 group versus 20 and gestation day exposures were limited to single dose on specific days).
GLP:	No information
Exposure Period:	GD 11-14 (single doses on each day per method described below)
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The primary objectives of this study were to evaluate the effects of CSO on pregnant female rats during gestation (food consumption, body weight gain, and viability and development of the offspring and to compare those data with data previously obtained using the dermal route of exposure. This developmental toxicity study was designed to detect, in a relatively short period of time, both reproductive and developmental effects which might be related to a single oral exposure of CSO. The study also provides a means to evaluate viability and normal development of the fetus during specific periods of organogenesis. Selection of dose levels and the day chosen to examine dose-response (gestation day 12) were based on the results of a pilot study. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid: 24. Control (0 mg/kg/day CSO–tap water at 2000 mg/kg) – GD 11-14 – 12 animals 25. CSO 2000 mg/kg/day – GD 11– 12 animals 26. CSO 125 mg/kg/day – GD 12 – 12 animals 27. CSO 500 mg/kg/day – GD 12 – 12 animals 28. CSO 2000 mg/kg/day – GD 12 – 12 animals 29. CSO 2000 mg/kg/day – GD 13 – 12 animals 30. CSO 2000 mg/kg/day – GD 14 – 12 animals

The test material was administered to groups 2-7 via oral gavage on one of GD 11-14, an interval during which the developing conceptus is believed to be sensitive to teratogenic insult by refinery streams. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Controls were administered water on GD 11-14.

Each presumed-pregnant female was observed at least once a day throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery and/or death. All unusual findings were noted.

Individual body weights were recorded on days 0, 6, 11-15, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-6, 6-11, 11-15 and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and the reproductive organs were examined grossly for evidence of pathosis. Thymus and liver weights were measured to the nearest 0.001 gram, and preserved in neutral buffered formalin. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Each live fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

11) Anomaly: Any deviation (malformation or variation) from "normal."
12) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
13) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.
14) Incidental: An incidental finding is generally an accidental event, e.g., accidentally, tip of tail was cut off.

After gross evaluation, all fetuses in each litter were fixed in Bouin's solution for subsequent soft tissue evaluation using a modification of Wilson's technique. 'The head and thoracic regions were evaluated for palatal and esophageal anomalies, respectively; no other soft tissues were evaluated.

After gross evaluation, fetuses in each litter were equally distributed into two groups, and preparation began for either soft tissue or skeletal evaluations. Approximately one-half of the fetuses in each litter were randomly distributed to soft tissue (viscera) or skeletal evaluation groups. Fetuses assigned to the soft tissue analysis group were fixed in Bouin's solution and examined for anomalies using a modification of Wilson's technique. The other half were pooled, fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated using Tukey's

test. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)*

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	500		mg/kg/day
NOAEL- Dermal	Maternal	=	125		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	125		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	Not identified <125		mg/kg/day

***Determined by reviewer**

Results Remarks:

The animals used in the study were approximately 9 weeks old at receipt and approximately 11 weeks old at exposure initiation.

One female in group 2 (2000 mg/kg/GD 11) died on gestation day 19. Gross necropsy revealed a hole in the left bronchus and fluid in the thoracic cavity. Although it appeared that the damage was a result of the dosing procedure, the source of the hole (mechanical damage during dosing or accidentally cut at the time of necropsy) was uncertain and no definite conclusions were reached. Data for this female have been excluded from the summaries.

Incidental findings included red nasal exudate and chromodacryorrhea. These observations are common signs of stress in rats and are not considered to be test material-related. One female in the 2000 mg/kg (GD 11) group exhibited scabs in the abdominal area. The cause of this finding is uncertain.

Findings attributable to CSO exposure included vaginal bleeding, perineal staining, and decreased stool. Red vaginal discharge is generally a sign of some degree of litter resorption and is probably the case in this study since the percentage of resorptions was high in animals which exhibited the discharge. One female in the 2000 mg/kg (GD 14) group was sacrificed moribund on gestation day 19. She had severe red vaginal discharge and was very pale. No gross findings were noted for this dam at the time of necropsy, however, upon uterine examination, her entire litter was found to be dead. Since this dam was sacrificed moribund prior to gestation day 20, and cesarean section data are excluded from the summary tables.

Mean body weights for the 500 and 2000 mg/kg groups were significantly reduced during the latter part of gestation. A significant reduction in overall maternal body weight gain (GD 0-20) and net body weight change was also observed for these same groups. A dose-response was observed for overall and net body weight gain for groups treated on gestation day 12. Mean maternal body weight changes indicate that all CSO-exposed groups began

to lose a significant amount of weight the day following CSO administration. Females exposed to CSO at dose levels of 500 mg/kg or greater consumed significantly less food than the control group during mid and/or late gestation. This period reflects the times at which these animals were administered CSO.

The thymus appeared small in females from the 500 and 2000 mg/kg groups, however, the incidence was higher in the 2000 mg/kg groups. A significant reduction in absolute and relative thymus weight was noted in animals exposed to CSO at dose levels of 500 mg/kg or greater. This finding appeared to be dose-related for those groups dosed on gestation day 12. Relative liver weights were significantly increased at the 2000 mg//kg (GD 13 and 14) dose levels.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0 (GD 11-14)**	**2000 (GD 11)**	**125 (GD 12)**	**500 (GD12)**	**2000 (GD 12)**	**2000 (GD 13)**	**2000 (GD 14)**
Body wt –at delivery (gr)	411	326	415	374	325	355	363
GD 0-6 wt gain (gr)	34	36	35	33	36	34	36
GD 6-11 wt gain (gr)	29	28	28	23	28	27	28
GD 11-12 wt gain (gr)	6	-16b	6	4	6	6	5
GD 12-13 wt gain (gr)	5	-11b	-8b	9b	15b	5	4
GD 13-14 wt gain (gr)	5	-7b	11	-2	-10b	-11b	7
GD 14-15 wt gain (gr)	8	10	9	16	-9b	-8b	-13b
GD 15-20 wt gain (gr)	76	41b	74	67	45b	51b	44b
GD 0-20 wt gain (gr)	163	80b	155	132b	80b	105b	112b
Gravid uterus (gr)	80.4	21.4 b	77.6	68.4	23.0 b	55.4 b	65.7a
Carcass (gr)	331	304a	337	305	302a	299b	297b
Net wt change from day 0 (g)	82.1	58.9 b	77.8	63.5b	57.3 b	49.8 b	46.4b
Thymus weight (g)-absolute	0.353	0.09 4b	0.285	0.196 b	0.08 4b	0.09 9b	0.091b
Thymus weight (g)-relative	0.106	0.03 1b	0.084	0.064 b	0.02 8b	0.03 8b	0.031b
Liver weight - absolute (g)	15.602	15.1 67	16.69 2	15.08 8	15.6 94	16.4 86	16.585
Liver weight (g)-relative	4.709	4.98 4	4.938	4.939	5.19 7	5.48 8b	5.705b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) = Carcass weight minus day 0 body wt

The following parameters appeared to be adversely affected by CSO exposure: The number and percent resorptions were significantly increased at 2000 mg/kg (GD 11 and 12). The greater than threefold increase at 2000 mg/kg on GD 13 is considered to be of biological significance. At 2000 mg/kg (GD 11 and 12), litter size was also decreased significantly, and the fetal sex

ratio was significantly altered in the 2000 mg/kg (GD 11) group. The biological significance of this finding is questionable since no other CSO-exposed group showed a similar pattern.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	0 (GD 11-14)	2000 (GD 11)	125 (GD 12)	500 (GD12)	2000 (GD 12)	2000 (GD 13)	2000 (GD 14)
Implantation sites – total	173	171	181	150	171	194	167
Implantation sites – mean	16.7	15.5	16.5	15.0	15.6	16.2	16.7
Preimplantation loss (%)	6.9	6.1	6.1	4.2*	8.3	7.9	2.0
Viable fetuses - total	163	41	186	135	41	153	151
Litter size (c)	14.8	3.7b	15.1	13.5	3.8b	12.8	15.2
Viable male fetuses (%)	56	37	49	49	50	50	50
Resorptions (mean)	0.9	11.8b	1.4	1.5	11.7b	3.3	1.5
Resorptions (mean %)	6.2	75.9b	8.2	10.0	75.6b	20.4	8.9
Dams with resorptions (%)	64	100	73	70	100	92	70

*number of females evaluated =9; data from one female not available
a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) Number of viable fetuses/number of litters evaluated.

A significant decrease in mean fetal body weight was observed in male fetuses from dams exposed to CSG at dose levels of 500 mg/kg or greater and in all viable fetuses in the 2000 mg/kg groups.

A significant increase in fetal external anomalies was observed for all 2000 mg/kg groups. The most commonly noted malformations involved the mouth (cleft palate), the hind- and forepaws (brachydactyly), and the tail (kinked, fleshy tab at the tip of the tail). Two fetuses exposed in utero to 500 mg/kg (GD 12) had hindpaw malformations; one fetus had syndactyly and one fetus had brachydactyly. Although these findings were not statistically significant, they are consistent with those of the 2000 mg/kg groups and are considered to be CSO-related.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	0 (GD 11-14)	2000 (GD 11)	125 (GD 12)	500 (GD12)	2000 (GD 12)	2000 (GD 13)	2000 (GD 14)
Fetal weights (gr)-mean	3.6	2.6b	3.4	3.3	2.5b	2.8b	2.9b
Litters evaluated	11	7	11	10	9	12	10
Fetuses - live	163	41	166	135	42	154	152

Fetuses – dead	0	0	0	0	1	1	1
Total gross exam anomalies (fetal incidence; %)	0;0.0	10; 24b	0;0.0	2; 1.5	18; 43b	75; 49b	34; 22b
Total gross exam anomalies (litter incidence; %)	0;0.0	5;71b	0;0.0	2; 20	9; 100b	11; 92b	9; 90b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

A significant increase in skeletal malformations was noted for the 500 and 2000 mg/kg groups. The most commonly observed malformations included misshapen cervical and caudal vertebrae, misshapen clavicle and costal cartilage, and fore- and hindpaw phalanges absent, misshapen, or fused. CSO-exposed fetuses also had a significantly higher incidence of incompletely ossified skeletal structures.

Fetal Endpoints – Skeletal Malformations and Skeletal Variations

Dose (mg/kg/day)	**0 (GD 11-14)**	**2000 (GD 11)**	**125 (GD 12)**	**500 (GD12)**	**2000 (GD 12)**	**2000 (GD 13)**	**2000 (GD 14)**
Litters evaluated	11	7	11	10	9	12	10
Fetuses - live	84	23	86	70	24	79	79
Fetuses – dead	0	0	0	0	0	0	0
Total skeletal malformations (fetal incidence; %)	1; 1.2	15; 65b	3; 3.5	31; 44b	18; 75b	46; 57b	30;38b
Total skeletal malformations litter incidence; %)	1; 9.1	7; 100b	8; 27	10; 100b	9; 100b	11; 92b	10; 100b
Total skeletal variations (fetal incidence; %)	72; 86	23; 100	71; 83	66; 94	24; 100	79; 100b	70; 100b
Total skeletal variations litter incidence; %)	11; 100	7; 100	11; 100	10; 100	9; 100	12; 100	10; 100

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

A significant increase in fetuses having cleft palate was observed for the 2000 mg/kg groups. This malformation was also observed in one fetus from the 125 mg/kg group and one fetus in the 500 mg/kg group. Although not detected at the time of external examination due to the location of the cleft palate (soft palate), this finding is consistent with the 2000 mg/kg groups and is probably a CSO-related effect. Other CSO-related findings included

diaphragmatic hernia at the 2000 mg/kg dose administered on GD 11, 12, and 13 and ectopic (right-sided) esophagus at 2000 mg/kg (GD 13).

Dose (mg/kg/day)	0 (GD 11-14)	2000 (GD 11)	125 (GD 12)	500 (GD12)	2000 (GD 12)	2000 (GD 13)	2000 (GD 14)
Litters evaluated	11	7	11	10	6	12	10
Fetuses - live	79	18	80	85	17	74	73
Fetuses – dead	0	0	0	0	0	0	0
Total fetal soft tissue (fetal incidence; %)	3; 3.8	7; 39	2; 2.5	6; 12	11; 65b	52; 70b	18; 25b
Total fetal soft tissue (litter incidence; %)	2; 18	5; 71a	2; 18	5; 50	6; 100b	12; 100b	7; 70a

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Conclusion:

Determined by reviewer:
The maternal NOAEL for a single oral exposure to CSO on one of GD 11-14 was determined to be 125 mg/kg/day (LOAEL= 500 mg/kg/day based on significantly lower body weight gain, decreased net maternal weight gain, decreased food consumption, and decreased thymus weight)

The developmental NOAEL for a single oral exposure to CSO on one of GD 11- 14 could not be identified (< 125 mg/kg); (LOAEL= 125mg/kg/day based on significantly increased soft tissue malformations and skeletal variations)
A dose response for developmental toxicity was observed for those groups dosed on gestation day 12.

RELIABILITY/DATA QUALITY

Reliability: Valid With Restriction (KS=2)

Reliability Remarks: Non guideline study; research protocol; adequate experimental details.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil. 1990. Developmental Toxicity Study in Rats Exposed Orally to a Single Dose of Clarified Slurry Oil. Mobil Environmental and Health Sciences Laboratory Report 63122.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Clarified Slurry Oil. 1991. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZA.

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-62-4

Test Substance: 64741-62-4; Syntower Bottoms (STB)

Test Substance Purity/Composition and Other Test Substance Comments: Syntower Bottoms (CRU No 86484)

PAC (Polycyclic Aromatic Compound) Content 64348 ZM (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86484	48.80	0.00	0.98	9.76	19.52	9.76	4.88	0.98

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Oral; gavage

Other Route of Administration:

Type of Exposure: Developmental toxicity study

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 11 per dose

Concentration:

Dose: 0, 125, 500, 2000 mg/kg/day (per gestation days described in methods below)

Year Study Performed : 1990

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (11 group versus 20 and gestation day exposures were limited to single dose on specific days).

GLP: No information

Exposure Period: GD 11-15 (single doses on each day per method described below)

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The primary objectives of this study were to evaluate the effects of STB on pregnant female rats during gestation (food consumption, body weight gain, and viability and development of the offspring and to compare those data with data previously obtained using the dermal route of exposure. This developmental toxicity study was designed to detect, in a relatively short period of time, both reproductive and developmental effects which might be related to a single oral exposure of STB. The study also provides a means to evaluate viability and normal

development of the fetus during specific periods of organogenesis (GD 11-15). Dose levels and days of administration were selected based on the results of a previously conducted study on clarified slurry oil.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

31. Control (0 mg/kg/day STB –tap water at 2000 mg/kg) – GD 11-15 – 11 animals
32. STB 2000 mg/kg/day – GD 11– 11 animals
33. STB 125 mg/kg/day – GD 12 – 11 animals
34. STB 500 mg/kg/day – GD 12 – 11 animals
35. STB 2000 mg/kg/day – GD 12 – 11animals
36. STB 2000 mg/kg/day – GD 13 – 11 animals
37. STB 2000 mg/kg/day – GD 14 – 11 animals
38. STB 2000 mg/kg/day – GD 15 – 11 animals

The test material was administered to groups 2-8 via oral gavage on one of GD 11-15, an interval during which the developing conceptus is believed to be sensitive to teratogenic insult by refinery streams. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Controls were administered water on GD 11-15.

Each presumed-pregnant female was observed at least once a day throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery and/or death. All unusual findings were noted.

Individual body weights were recorded on days 0, 6, 11-18, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-6, 6-11, 11-16 and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and the reproductive organs were examined grossly for evidence of pathosis. Thymus and liver weights were measured to the nearest 0.001 gram, and preserved in neutral buffered formalin. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Each live fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

15) Anomaly: Any deviation (malformation or variation) from "normal."
16) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
17) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.
18) Incidental: An incidental finding is generally an accidental event, e.g., accidentally, tip of tail was cut off.

After gross evaluation, all fetuses in each litter were fixed in Bouin's solution for

subsequent soft tissue evaluation using a modification of Wilson's technique. 'The head and thoracic regions were evaluated for palatal and esophageal anomalies, respectively; no other soft tissues were evaluated.

After gross evaluation, fetuses in each litter were equally distributed into two groups, and preparation began for either soft tissue or skeletal evaluations. Approximately one-half of the fetuses in each litter were randomly distributed to soft tissue (viscera) or skeletal evaluation groups. Fetuses assigned to the soft tissue analysis group were fixed in Bouin's solution and examined for anomalies using a modification of Wilson's technique. The other half were pooled, fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated using Tukey's test. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)*

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	500		mg/kg/day
NOAEL- Dermal	Maternal	=	125		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	500		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	125		mg/kg/day

***Determined by reviewer**

Results Remarks:

The animals used in the study were approximately 9 weeks old at receipt and approximately 11 weeks old at exposure initiation.

One female in Group 7 (2000 mg/kg/GD 14) was sacrificed moribund on gestation day 15. Gross necropsy revealed a small tear in the esophagus indicating a probable mis-dose. Data for this female have been excluded from all summary tables.

Incidental findings included red nasal exudate and chromodacryorrhea (red discharge around the eyes). These are common signs of stress in rats and are not attributed to STB-exposure. Two females in the STB-exposed groups had alopecia (hairloss) in the abdominal area.

Treatment-related findings included red vaginal discharge, perineal staining, and decreased stools, all of which occurred in a dose-related manner. Red vaginal discharge is usually indicative of some degree of litter resorption. In this study, this

was probably the case since the females with red vaginal discharge had large numbers of resorptions. Perianal staining was noted for three STB-exposed females.

In general, the mean body weights of the groups exposed the 2000 mg/kg were significantly less than that of the control group during the latter part of gestation; the effect became apparent for each group approximately 2-3 days post-dose. The mean maternal body weight changes indicate that all STB-exposed females actually began to lose weight immediately following STB exposure. This weight loss was statistically significant at all STB dose levels. Overall body weight gain (gestation days 0 to 20) was significantly reduced for all females exposed to STB at a dose level of 2000 mg/kg. Although all 2000 mg/kg groups had a lower mean net body weight gain than the control group, the reduction was only statistically significant for those groups dosed on gestation days 13, 14, or 15.
Overall, the amount of food consumed by females exposed to STB at 2000 mg/kg was significantly lower than that consumed by the control group during the mid to late gestation period. This period reflects the time at which these animals were administered STB. Gravid uterine weight was significantly less than that of the control group at a dose level of 500 mg/kg and above. This may be attributed to resorption of fetuses as well as decreased fetal weights at those dose levels.

Both absolute and relative thymus weights of females exposed to STB at dose levels of 500 mg/kg or higher were significantly reduced. Liver weights did not appear to be adversely affected. No other STB-related findings were noted at the time of necropsy.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0 (GD 11-15)**	**2000 (GD 11)**	**125 (GD 12)**	**500 (GD12)**	**2000 (GD 12)**	**2000 (GD 13)**	**2000 (GD 14)**	**2000 (GD 15)**
Body wt – at delivery (gr)	422	357	404	401	357	381	377	368
GD 0-6 wt gain (gr)	35	33	32	33	34	36	34	37
GD 6-11 wt gain (gr)	27	27	27	32	28	28	27	28
GD 11-12 wt gain (gr)	3	-10b	5	5	6	7	6	7
GD 12-13 wt gain (gr)	8	-12b	-3b	-11b	-12b	6	7	5
GD 13-14 wt gain (gr)	5	-4a	10	-5b	-11b	-11b	5	8
GD 14-15 wt gain (gr)	10	13	7	22b	-1b	-13b	-8b	7
GD 15-160 wt gain (gr)	8	12	10	9	14	0	-9b	-11b
GD 16-20 wt gain (gr)	72	34b	66	60	50a	70	64	28b
GD 0-20	169	91b	153	146	108b	122b	125b	108b
Gravid uterus	89.5	25.8	79.4	6S.7 b	47.9 b	66.6 b	71.8 a	61.0b

(gr)								
Carcass (gr)	332	332	324	333	309	314	306	307
Net wt change from day 0 (g)	79.3	65.7	73.3	76.2	60.3	55.7 a	54.1 b	47.5b
Thymus weight (g)-absolute	0.314	0.129b	0.244	0.216 b	0.117b	0.106b	0.117b	0.154 b
Thymus weight (g)-relative	0.094	0.039b	0.075	0.065 b	0.038b	0.033b	0.038b	0.049 b
Liver weight - absolute (g)	17.419	17.560	16.824	17.231	18.668	17.039	16.449	17.338
Liver weight (g)-relative	5.237	5.305	5.181	5.175	5.387	5.430	5.371	5.651

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) = Carcass weight minus day 0 body wt

Group 2 (2000 mg/kg/GD 11) had an unusually high percentage of preimplantation loss (14.6% ± 25.8). This effect IS attributed to one female in the group who had only two implantation sites and sixteen corpora lutea. The cause of this is unknown, but since implantation occurs on or about gestation day 6, the finding is not STB-related. In general, live litter size was slightly decreased for all STB-exposed groups. Statistical significance was achieved for groups 2 and 5 (2000 mg/kg/GD 11 and 12, respectively). A corresponding (slight) increase in the number and percent resorptions was also seen in all STB-exposed groups with significance again being achieved for groups 2 and 5. The number of dams with resorptions was significantly higher at dose levels of 500 and 2000 mg/kg.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	**0 (GD 11-15)**	**2000 (GD 11)**	**125 (GD 12)**	**500 (GD12)**	**2000 (GD 12)**	**2000 (GD 13)**	**2000 (GD 14)**	**2000 (GD 15)**
Implantation sites – total	185	1181	156	177	166	167	167	153
Implantation sites – mean	16.8	16.5	15.6	16.1	15.1	16.7	16.7	15.3
Preimplantation loss (%)	1.6	14.6	4.1	10.6	11.5	5.5	4.9	12.0
Viable fetuses - total	182	49	147	150	109	149	154	125
Litter size (c)	16.5	4.5b	14.8	13.6	9.9b	14.9	15.4	13.1
Viable male fetuses (%)	47	55	54	46	51	53	48	39

Resorptions (mean)	0.3	11.9 b	0.8	2.5	5.2b	1.8	1.3	2.2
Resorptions (mean %)	1.6	69.7 b	5.0	15.7	34.2 b	11.2	7.7	13.7
Dams with resorptions (%)	27	100b	50	100b	100b	80a	70	80a

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) Number of viable fetuses/number of litters evaluated.

A decrease in fetal weights was observed in fetuses from dams exposed to STB at a dose level of 500 mg/kg and above with significance being achieved at the 2000 mg/kg level. A significant increase in fetal external malformations was observed at 2000mg/kg/GDs 11-14. The malformations generally involved the mouth (cleft palate), the hindlimb (brachydactyly, adactyly) and the tail (fleshy tab at the tip of the tail and shortened tail). Adactyly and brachydactyly were noted in one fetus in the 500 mg/kg group and gastroschisis was noted in one fetus in the 125 mg/kg group. The adactyly/brachydactyly in the 500 mg/kg group was consistent with the findings in the 2000 mg/kg groups and is probably STB-related. Gastroschisis occurs spontaneously and has been seen in control animals at this facility. The incidence of hematomas (8 variation) on the forepaws and hindpaws of fetuses from dams exposed to STB at 2000 mg/kg/GD 15 was statistically significant. Other fetal variations, such as edema and malrotated hindlimbs also occurred in fetuses from dams exposed to STB and were not observed in the fetuses from control dams.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	**0 (GD 11-15)**	**2000 (GD 11)**	**125 (GD 12)**	**500 (GD12)**	**2000 (GD 12)**	**2000 (GD 13)**	**2000 (GD 14)**	**2000 (GD 15)**
Fetal weights (gr)- mean	3.6	2.7b	3.5	3.2	2.9b	3.0b	3.1a	2.9b
Litters evaluated	11	9	10	11	11	10	10	10
Fetuses - live	182	49	147	150	109	149	154	125
Fetuses – dead	0	1	1	0	0	0	0	6
Total gross exam anomalies (fetal incidence; %)	0;0.0	6; 12b	1.0.7	1;0.7	15; 14b	40;60b	19; 29b	9; 6.9b
Total gross exam anomalies (litter incidence; %)	0;0.0	5; 56b	1; 10	1; 9.1	7; 64	8; 80b	8; 80b	4; 40a

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

There was a significant increase in malformations at 500 mg/kg and above when administered on gestation days 11-14. Malformations included misshapen cervical

transverse process, shortened tail, and hind paw phalanges fused, misshapen, or missing. Although there were no malformations noted for the 2000 mg/kg/GD 15 fetuses, there was a significant increase in incomplete ossification of many skeletal structures.

Fetal Endpoints – Skeletal Malformations and Variations

Dose (mg/kg/day)	0 (GD 11-15)	2000 (GD 11)	125 (GD 12)	500 (GD12)	2000 (GD 12)	2000 (GD 13)	2000 (GD 14)	2000 (GD 15)
Litters evaluated	11	9	10	11	11	10	10	10
Fetuses - live	182	49	147	150	109	149	154	125
Fetuses – dead	0	1	1	0	0	0	0	6
Total skeletal malformations (fetal incidence; %)	1; 1.1	6; 22b	2; 2.7b	17; 22b	33; 58b	40; 53b	33; 41b	0.; 0.0
Total skeletal malformations litter incidence; %)	1; 9.1	4; 44	1; 10	7; 64a	11; 100b	10; 100b	9; 90b	0; 0.0

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

There was a significant increase in visceral malformations at 2000 mg/kg regardless of the gestation day of administration. Among the findings were small and/or lobular lungs (gestation days 11, 12, and 13), small spleen (days 11 and 15), ectopic and small kidneys (days 11 and 14), cleft palate (days 12, 13, and 14), right-sided esophagus (days 12 and 13), heart anomalies (days 12 and 13), and diaphragmatic hernia (days 12 and 13). In addition, a few of the above mentioned malformations were noted as isolated occurrences at 500 mg/kg. Variations of the urinary tract (dilatation of renal pelvis and distended ureters) were seen significantly more often in the 125. 500 and 2000 (GD 15) mg/kg groups than in the controls.

Fetal Endpoints – Soft Tissue Malformations and Variations

Dose (mg/kg/day)	0 (GD 11-15)	2000 (GD 11)	125 (GD 12)	500 (GD12)	2000 (GD 12)	2000 (GD 13)	2000 (GD 14)	2000 (GD 15)
Litters evaluated	11	7	10	11	11	10	10	10
Fetuses - live	90	22	73	72	52	73	74	80
Fetuses – dead	0	0	0	0	0	0	0	0
Total fetal soft tissue (fetal incidence	5; 5.6	8; 36b	2; 2.7	7; 9.7	32; 62b	52; 71b	16;22 b	14; 23b

; %)*								
Total fetal soft tissue (litter incidence ; %)*	3; 27	3; 43	2; 20`	5; 45	10; 91b	9; 90b	7; 70	5; 50

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Conclusion:

Determined by Reviewer:
The maternal NOAEL for a single oral exposure to STB on one of GD 11- 15 was determined to be 125 mg/kg/day (LOAEL= 500 mg/kg/day based on red vaginal discharge, significant weight loss at the time of exposure, a decrease in net body weight gain, and a decrease in absolute and relative thymus weight.)

The developmental NOAEL for a single oral exposure to STB on one of GD 11-15 was determined to be 125 mg/kg. (LOAEL= 500 mg/kg/day based on the number and percent of dams with resorptions, and a significant increase in fetal skeletal and visceral anomalies)

RELIABILITY/DATA QUALITY

Reliability: Valid With Restriction (KS=2)

Reliability Remarks: Non guideline study; research protocol; adequate experimental details.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil. 1990. Developmental Toxicity Study in Rats Exposed Orally to a Single Dose of Syntower Bottoms. Mobil Environmental and Health Sciences Laboratory Report 63123.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Syntower Bottoms. Mobil Environmental and Health Sciences Laboratory Report No. 64348 ZM.

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Heavy Coker Gas Oil (HCGO); Heavy Thermal Cracked Distillate

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Coker Gas Oil (F-200)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091653 (F-200)		0.00	0.90	20.00	5.00	0.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).

	2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	15 per dose level of HCGO 20 per dose for sham control
Concentration:	
Dose:	0, 0.1, 50, 250 mg/kg/day
Year Study Performed :	1994
Method/Guideline Followed:	Other
GLP:	Yes
Exposure Period:	Gestation day (GD) -7 to 20
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to determine the developmental toxicity of HCGO (F-200) following dermal administration to female rats daily for one week prior to mating through day 20 of gestation. Females were randomly assigned to four treatment groups and dosing began one week prior to the start of mating (GD -7) and throughout mating. Males were not treated. Mating was confirmed by detection of sperm in a vaginal smear or a copulatory plug. Females that exhibited positive signs of mating (GD 0) also received the test article through presumed GD 20. The treatment groups and time exposure periods were as follows: 1. *Sham control (0 mg/kg/day) – 20 animals 2. HCGO 0.1 mg/kg/day – 15 animals (via solution of 1.0% concentration of test article in acetone) 3. HCGO 50 mg/kg/day – 15 animals (neat material) 4. HCGO 250 mg/kg/day – 15 animals (neat material) *Shared with study number ATX-91-0133 At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. The test material was administered to groups 2-4 on GD -7 through GD 20. The test article was applied to previously clipped, intact dermal sites on the backs of female animals. 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 was wiped from the application site. With the exception of test article application, control animals underwent the same procedure as the other treatment groups. The dose administered was based

upon the day -7 body weight for the premating period and the GD 0 body weight for the gestation period.

Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted.

Individual body weights were recorded at receipt, near the end of the quarantine period, on days -7 and -1 (premating period), on days 0, 4, 8, 12, 16, and 20 of gestation, and on days 0 and 4 of lactation. Individual food consumption was measured for days -7 to -1 (premating); for GD intervals 0-4, 4-8, 8-12, 12-16, and 16-20; and for days 0-4 of lactation (postnatal period).

Each litter was observed daily during lactation day 0 (day of parturition) through 4 for signs of toxicity and mortality. On lactation days 0 and 4, the weight and sex of each live pup was recorded.

Each female that mated was sacrificed by overexposure to carbon dioxide and necropsied. Females that delivered a litter were necropsied on day 4 of lactation and those that did not deliver a litter were necropsied on presumed GD 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The ovaries were examined and the number of corpora lutea was determined for each female that delivered. The number of implantation sites was recorded for all females, including those that appeared non-gravid. Dead pups were removed, examined externally and discarded. On lactation days 0 and 4, the sex and weight of each pup were recorded. On day 4 of lactation, all surviving pups were examined externally, sacrificed with carbon dioxide, and discarded.

STATISTICAL ANALYSES: Data for female body weight and food consumption were evaluated by ANOVA. First, Bartlett's test was performed to determine if the dose groups had equal variance at the 1percent 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 reproductive and litter data, i.e., 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 lactation day 0, pup alterations at lactation day 0,

male pups at days 0 and 4, survival of pups at lactation day 4) were 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.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	50		mg/kg/day
NOAEL- Dermal	Maternal	=	0.1		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	50		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	0.1		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

One female in the 250 mg/kg/day dose group was found dead on GD 18. There were no other mortalities during the study.

Slight erythema was noted on day 2 for one female in the 0.1 mg/kg/day dose group. Because the erythema was slight, of limited duration, and was noted for only one animal in the dose group, it was not considered to be related to the test article. Erythema, edema, eschar, and dry skin were observed at site of administration at the two highest dose levels (50 and 250 mg/kg/day). Irritation ranged from slight to severe, and was believed to be related to test article administration. A higher incidence of vaginal discharge was noted for females in the 250 mg/kg/day dose group; vaginal discharge was observed as early as GD 13 and as late as GD 23 of gestation. There were no other clinical observations that were considered to be related to treatment with the test article.

There were no effects on body weights or body weight changes at a dose of 0.1 mg/kg/day. Mean body weights and body weight gains were significantly decreased in both the 50 and 250 mg/kg/day groups at various points during gestation per the table below. The 250 mg/kg/day group also had decreased body weights during the pre-mating period.

There were no effects on absolute or relative food consumption at doses of 0.1 and 50 mg/kg/day. Effects on absolute or relative food consumption were only apparent in the 250 mg/kg/day group, being significantly lower ($p<0.01$) than that of the controls during days -7 to -1 of the premating period. Absolute food consumption for pregnant females in the 250 mg/kg dose group was significantly lower ($p<0.01$) than that of the controls during most of gestation.

Necropsy evaluations indicate that dermal irritation related to administration of

the test article was noted for females in the 50 and 250 mg/kg/day dose groups. Decreased thymus size (no thymus weight data) was also noted for three females in the 250 mg/kg/day dose group. The uterus of two animals in this dose group also showed resorptions. There were no other necropsy findings that were considered to be related to the test article.

The total number of live pups and pup body weights were significantly lower ($p<0.01$) for those delivered from females dosed at 50 mg/kg/day. The number of implantation sites for females in the 250 mg/kg/day dose group was significantly lower ($p<0.01$) than that of the control group, suggesting increased pre-implantation loss at this dose. Only one of the pregnant females dosed at 250 mg/kg/day delivered a litter, and this litter did not survive to lactation day 4.

At 50 mg/kg/day, the number of total and live pups on lactation day 0 was decreased and pup body weights were lower on both lactation days 0 and 4. At 250 mg/kg/day, none of the pups in the one litter delivered survived to lactation day 4. For all dose groups, there were no significant differences in gestation length, external pup alterations, or the proportion of males on lactation days 0 and 4.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**0.1**	**50**	**250**
Body wt day -7	251.2	251.7	249.9	251.3
Body wt day -1	257.4	260.7	256.7	246.1a
Body wt –final (g)	415.3	414.3	389.2a	276.6b
Body wt – lactation day 0	307.8	307.2	301.9	283.0
Body wt – lactation day 4	325.7	323.1	315.3	--
Premating day -7 to -1 wt gain (g)	6.20	9.00	6.73	-5.20b
GD 0-4 wt gain (g)	26.00	23.87	17.15b	14.08b
GD 4-8 wt gain (g)	14.00	15.40	15.23	8.25a
GD 8-12 wt gain (g)	21.71	18.60	17.08	11.25b
GD 12-16 wt gain (g)	27.82	30.20	25.69	-1.45b
GD 16-20 wt gain (g)	66.78	65.93	54.38b	·3.40b
Lactation day 0-4 wt gain (g)	17.83	15.93	13.38	--

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	**0**	**0.1**	**50**	**250**
Dams with resorptions	0	0	0	2
Implantation sites	16.8	16.7	15.7	12.6b

Number of litters with live pups	18	15	13	1
Total pups/litter (day 0)	15.6	15.9	12.8b	10.0c
Live pups/litter (day 0)	15.2	15.3	12.8b	4.0c
Pup weights (g) – mean, day 0	6.49	6.51	5.98a	6.06c
Pup weights (g) – mean, day 4	9.67	9.77	8.68	c

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c)Only one female delivered a litter; no pups survived to lactation day 4

Conclusion: The maternal NOAEL for dermal exposure to HCGO during GD -7 to 20 was determined to be 0.1 mg/kg/day (LOAEL= 50 mg/kg/day based on decreased body weight and body weight changes)

The developmental NOAEL for dermal exposure to HCGO during GD -7 to 20 was determined to be 0.1 mg/kg/day (LOAEL = 50 mg/kg/day based on a decreased total and live pup numbers as well as decreased pup body weights.)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1994. A Developmental Toxicity Screen in Female Sprague-Dawley Rats Administered F-200 Dermally During Gestation Days -7 to 20. Report ATX-91-0134.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Heavy Coker Gas Oil (HCGO); Heavy Thermal Cracked Distillate

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Coker Gas Oil (CRU No. 83366)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348 ZQ (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)

83366	12.7	0.1	2.5	5.1	2.5	1.3	0.9	0.1

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity screen

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Raleigh, N.C.)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 10 per dose, except for an additional group of 5 animals exposed at 125 mg/kg on GD 10-12 used to obtain bioavailability data

Concentration:

Dose: Developmental study, GD 0-19 and GD 10-12:
0 (remote), 0 (proximate), 8, 30, 125, 250 mg/kg/day
Bioavailability study, GD 10-12:
125 mg/kg/day

Year Study Performed : 1987

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (10/group versus 20),.

GLP: No information

Exposure Period: GD 0-19 (6 groups); GD 10-12 (2 groups)

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The study was designed to obtain data on the influence of HCGO on parameters of reproductive performance during gestation (implantation, litter size) and viability and development of the embryo/fetus. An additional experimental group was added in order to assess the bioavailability/bioaccumulation of HCGO in a select number of maternal tissues, fetuses and placentae.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

1. *Remotely-housed dermal control (0 mg/kg/day) – GD 0-19
2. Proximately-housed dermal control (0 mg/kg/day) – GD 0-19
3. HCGO 8 mg/kg/day – GD 0-19 – 10 animals
4. HCGO 30 mg/kg/day – GD 0-19 – 10 animals
5. HCGO 125 mg/kg/day – GD 0-19 – 10 animals
6. HCGO 250 mg/kg/day – GD 0-19 – 10 animals
7. HCGO 125 mg/kg/day – GD 10-12 – 10 animals; included as an additional group because was anticipated that administration throughout

the complete gestation period may result in a high incidence of fetal lethality. This is a period during which fetuses are susceptible to abnormal development.

8. Radiolabeled HCGO 125 mg/kg/day – GD 10-12 – 5 animals; bioavailability group

*Because inhalation of the test material could not be ruled out, a separate control group was not housed in the same animal room (remote-housed control). Subsequent analyses of air samples indicated that no single compound was detected above the limit of detection of 0.2 mg/m3.

The exposure levels were based on results of a 13 week study previously conducted on the same material and on data obtained in a developmental study on a similar material; 8 mg/kg/day was selected as the lowest dose.

Developmental study (Groups 1-7):
The test material was administered to groups 3-6 on GD 0-19. Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Group 7 females were similarly treated but administration of test material was restricted to a period of gestation during which fetuses are susceptible to abnormal development (GD 10-12).

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality or abortion. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and liver of each animal in groups 1-7 were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each fetus was gendered, weighed and grossly examined. Approximately half of the fetuses were randomly assigned for examination of soft tissues (viscera) following fixation in Bouin's solution, using a modification of the Wilson's

technique. The other half were fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

Bioavailability Study (Group 8)
From GD 0-9, pregnant females were housed in stainless steel cages with wire bottoms and fronts. On GD 10, 11, and 12, the rats were housed in metabolism cages. The HCGO used in the bioavailability study contained two radioactive surrogates, carbon-14 radiolabeled carbazole and hydrogen-3 radiolabeled benzo(a)pyrene (BaP). On GD 10, the hair was clipped from the dorsal trunk of each animal and the radiolabeled test material was applied to the skin within a protective device designed to contain the administered dose. A mesh screen was attached to the protective device, and each rat was fitted with an Elizabethan collar. The same procedure was repeated on GD 11 and 12, except the needle tip with the test material was inserted through the mesh screen in order to apply the test material.

On GD 13, 24 hrs after the administration of the last HCGO dose, animals were sacrificed and maternal blood was collected. Necropsies were performed and the uterine contents located and examined for the number of normal and resorbed fetuses for each dam. The individual fetal units were removed, and the amniotic fluid was collected from the isolated placenta. The embryo was separated from the yolk sac and rinsed with water to remove residual amniotic fluid. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights or volumes of the pooled samples determined. Maternal tissues collected for radioactivity analysis included the following: thymus, liver, heart, brain, small intestine, large intestine, kidneys, spleen, stomach, ovaries, urinary bladder, lungs, muscle, retroperitoneal fat, femur bone and residual carcass.

Determination of radioactivity in blood, urine and cage wash was accomplished by measuring the amount of carbon-14 labeled carbon dioxide and H-3 labeled water produced from direct combustion of duplicate samples. Samples were oxidized for three minutes and the carbon dioxide and water generated from the combustion were separated and trapped in a cocktail fluid. Carbon-14 and hydrogen-3 radioactivities were measured. Fecal samples were homogenized, combusted and the radioactivity measured.
The placentae, urteri, embryos, and yolk sacs were homogenized in an equivalent volume of water, and aliquots of the homogenate were combusted. Maternal tissues were treated in the same manner, although six tissues including the ovaries, urinary bladder, muscle, fat, bone and residual carcass were combusted directly without homogenization or dilution. In all cases, the trapped carbon dioxide and water were measured for radioactivity by liquid scintillation counting. Samples of the amniotic fluid were also combusted directly without dilution. Duplicate analyses were performed whenever possible. The sensitivity of the radioactivity allowed for the detection of 0.005% of the applied dose.

The systemic dermal absorption of the two radiolabeled surrogates was determined by summing the total carbon-14 or hydrogen-3 radioactivities found in the urine, urine/cage washings, feces and collected maternal and embryonic tissues at the end of 72 hours. Tissue concentrations of carbazole and benzo(a)pyrene (BaP) were calculated based on the radioactivity found per gram or per ml. The total amount of a radiolabeled surrogate in the tissues was calculated a s a percent of the total applied radioactive dermal dose over three days.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated using Duncan's multiple range 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 the 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. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere . Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008 and Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	30		mg/kg/day
NOAEL- Dermal	Maternal	=	8		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	125		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	30		mg/kg/day

Results Remarks:

The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The majority of clinical observations were noted in both the control and treated groups and appear to be a
result of animals being collared and/or related to mating activity. Alopecia was observed in some of the animals exposed to HCGO. One female exposed to HCGO at a dose level of 125 mg/kg/day had swollen hind paws. Findings were not considered to be test material related due to the low incidence and lack of a dose response relationship.

Erythema and flaking of skin (with or without scabs) at the site of administration were observed in all of the groups exposed to HCGO. Eschar was observed at the two highest dose levels (125 and 250- mg/kg/day) and fissuring was observed in one animal from each of the 30, 125 and 250 mg/kg/day groups. Irritation ranged from moderate (low doses) to severe (high doses). Vaginal bleeding, a sign of some degree of litter resorption was observed in all exposed groups, GD 0-19, dosed greater than 30 mg/kg/day. Group 7, dosed on GD 10-12 at 125 mg/kg/day did not display vaginal bleeding.

Mean body weights, body weight gains uterine weights and net body weights decreased in a dose-related fashion at doses of 30 mg/kg/day or greater for animals exposed GD 0-19. In general, animals exposed to HCGO at a level of 125 mg/kg/day or greater consumed less food than the controls.

Maternal necropsy result showed a reduced size of the thymus at the 125 mg/kg/day (Groups 5 and 7) and 250 mg/kg/day level, later confirmed by thymus weight measurements ($p<0.05$). Pale lungs were observed in treated animals only; the significance is not known. In addition, there was a significant reduction ($p<0.05$) in liver weights in the high dose rats. Relative liver weights were higher in dams exposed to HCGO throughout gestation, but was significant ($p<0.05$)

only at the 125 mg/kg/day level. It was speculated that the liver weight profiles in animals that have a high incidence of resorptions (i.e., 250 mg/kg/day group) resemble nonpregnant animals which generally have lower liver weights.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0 Rem.	0 Prox.	8	30	125	250	125 (GD 10-12)
Body wt –final (g)	417	432	415	403c	351bd	297bd	419
GD 0-3 wt gain (g)	14	15	20	15	9	9	16
GD 3-6 wt gain (g)	15	18	17	14	12	11	16
GD 6-10 wt gain (g)	22	21	17	14	12	11	16
GD 10-13 wt gain (g)	19	19	21	19	13	6bd	17
GD 13-16 wt gain (g)	26	30	24	23	15ad	0bd	24
GD 16-20 wt gain (g)	73	70	65	65	37bd	1bd	67
Gravid uterus (g)	91.1	84.4	82.3	79.1	39.6bd	9.8bd	73.3a
Thymus weight (g)-absolute (relative weight not determined)	0.333	0.377	0.328	0.359	0.246ac	0.177ac	0.306bd
Liver weight (g)-absolute	17.605	18.783	18.061	18.196	18.481	16.256ac	18.640
Liver weight (g)-relative	5.40	5.40	5.43	5.62	5.91bd	5.66	5.40
Carcass (g)	326.7	347.2	333.0	323.8c	312.6d	286.9bd	345.7
Net wt change from day 0 (e)	77.0	89.3	81.4	74.6	63.8d	33.2bd	87.8

a)Statistically different from remote control ($p<0.05$)
b)Statistically different from remote control ($p<0.01$)
c)Statistically different from proximate control ($p<0.05$)
d)Statistically different from proximate control ($p<0.01$)
e) = Carcass weight minus day 0 body wt

For the GD 0-19 groups, HCGO exposures at 125 mg/kg/day and higher adversely affected the number of dams with all resorptions, the number of resorptions, and litter size in an apparent dose-related manner. All other parameters were not significantly different from the control animals. A significant decrease in litter size was also observed in Group 7 (125 mg/kg/day, GD 10-12) but only when compared to the remote control animals. No maternal toxicity was observed in this group at the dose level administered.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	0 Rem.	0 Prox.	8	30	125	250	125 (GD 10-12)
Implantation sites - total	157	168	148	168	163	169	154
Implantation sites – mean	17.4	16.8	16.4	16.8	16.3	16.9	15.4
Viable fetuses	154	155	140	150	74	7	129
Litter size (e)	17.1	15.6	15.6	16.0	7.4bd	0.8bd	12.9b
Viable male	94	86	72	72a	32a	5	61a

fetuses							
Viable female fetuses	60	69	68	78a	42a	2	68a
Resorptions (mean)	0.3	1.3	0.9	1.8	8.9bd	16.1bd	2.6
Resorptions (mean %)	1..8	7.8	5.3	10.5	54.6bd	95.6bd	15.3
Dams with resorptions (%)	33	60	56	60	100b	100b	80

a)Statistically different from remote control ($p<0.05$)
b)Statistically different from remote control ($p<0.01$)
c)Statistically different from proximate control ($p<0.05$)
d)Statistically different from proximate control ($p<0.01$)
e) Number of viable fetuses/number of litters evaluated.

For clinical chemistry parameters, statistical analyses were performed only between the remote control and HCGO-exposed groups. Differences were seen for eleven serum parameters, all of which demonstrated a dose response effect. There was an indication of dose-related hepatotoxicity as characterized by marked increases of serum aspartate aminotransferase and sorbitol dehydrogenase activities. There was equivocal evidence of an effect of the kidneys as shown by a significant increase in serum urea nitrogen concentration in animals at 250 mg/kg/day. It was concluded that the dose-related responses that were observed for serum triglycerides, iron, albumin and albumin/globulin ratio are likely a secondary effect of HCGO as a result of resorption, since it previously has been noted that dams that resorb their litters have a serum profile that is similar to nonpregnant animals.

Fetuses from animals exposed at doses of 125 and 250 mg/kg/day weighed significantly less than fetuses from the control groups. Crown-rump length was significantly decreased among the female fetuses (but not male fetuses) from dams exposed to HCGO at dose levels of 125 and 250 mg/kg/day in the GD 0-19 groups.

In the external fetal examination, a slight increase in external anomalies was observed at 125 and 250 mg/kg/day; the increase was statistically significant among fetuses, but not among litters. A single fetus with edema was observed at both 125 and 250 mg/kg/day. A single case of “slightly reduced lower jaw” was also noted at 125 mg/kg/day; one dead fetus had micrognathia at 250 mg/kg/day.

The soft tissue examination did not reveal any statistically significant increase in anomalies. One fetus in the 125 mg/kg/day group (GD 10-12, but not GD 0-19) demonstrated displacement of the esophagus from a left-sided to a right-sided position, which was classified as a malformation (not ever observed in control fetuses from any study conducted at the laboratory). Four fetuses from two dams exposed to 125 mg/kg/day (GD 0-19) had distended ureters, which was classified as a variation.

In the skeletal examination, there was no significant increase in skeletal malformations among the exposed groups compared to the control groups. One or two fetuses with vertebral malformations were observed among the litters of dams given 30 or 125 mg/kg/day, but no individual skeletal malformation was significantly increased compared to controls at any dose level. Some skeletal variations (mostly unossified or incompletely ossified bones) were seen at a higher incidence among the HCGO-exposed groups, particularly at 125 and 250 mg/kg/day.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	**0**	**0**	**8**	**30**	**125**	**250**	**125 (GD**

	Rem.	Prox.					10-12)
Fetal weights (g)	3.5	3.6	3.5	3.5	3.1ad	2.9bd	3.7
Litters evaluated	9	10	9	10	10	5	10
Fetuses - live	154	155	140	150	74	7	129
Fetuses – dead	0	0	0	0	0	1	0
Gross exam anomalies (fetal incidence; %)	0; 0.0	0; 0.0	2; 1.4	1:0.7	4;5.4ac	2;25bd	2; 1.6
Gross exam anomalies (litter incidence; %)	0; 0.0	0; 0.0	1;11	1;10	3;30	2;40	2; 20

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Fetal Endpoints – Skeletal Malformations and Skeletal Variations

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**8**	**30**	**125**	**250**	**125 (GD 10-12)**
Litters evaluated	9	10	9	10	10	3	10
Fetuses - live	77	80	74	77	39	4	66
Fetuses – dead	0	0	0	0	0	0	0
Total skeletal malformations (fetal incidence; %)	0; 0.0	0; 0.0	0; 0.0	4;; 5.2	2; 5.1	0; 0.0	0; 0.0
Total skeletal malformations (litter incidence; %)	0; 0.0	0; 0.0	0; 0.0	1;;10	2;;20	0; 0.0	0; 0.0
Total skeletal variations (fetal incidence; %)	67;; 87	72; 90	66; 89	73; 95	39; 100a	4;100	57; 88
Total skeletal variations (litter incidence; %)	9; 100	10; 100	9; 100	10; 100	10; 100	3; 100	10; 100

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Fetal Endpoints – Soft Tissue Anomalies

Dose (mg/kg/day)	**0 Rem.**	**0 Prox.**	**8**	**30**	**125**	**250**	**125 (GD 10-12)**
Litters evaluated	9	10	9	10	10	4	10
Fetuses - live	76	75	66	73	35	3	63
Fetuses – dead	0	0	0	0	0	1	0
Total fetal soft tissue (fetal incidence; %)	7;9.2	4;5.3	2;3.0	3;4.1	7;20c	1;25	2;3.2
Total fetal soft tissue (litter incidence; %)	3;33	3;30	2;22	2;20	4;40	1;25	2;20

a)Statistically different from remote control (p<0.05)

b)Statistically different from remote control (p<0.01)
c)Statistically different from proximate control (p<0.05)
d)Statistically different from proximate control (p<0.01)

Bioavailability/Bioaccumulation Analyses
The dermal penetration of C-14 carbazole occurred more extensively and rapidly than H-3 BaP absorption. In spite of the the dermal bioavailability of radiolabeled material, the amount found in the embryo was very low. This indicates that although radiolabeled carbazole and BaP are capable of reaching the embryo, they do not accumulate there to a significant degree. The results suggest that the placenta may be an effective barrier against the transplacental transport of thes HCGO components to the embryo.

Conclusion:

The maternal NOAEL for dermal exposure to HCGO during GD 0-19 was determined to be 8 mg/kg/day (LOAEL= 30 mg/kg/day based on vaginal discharge observations, decreased body weight; decreased food consumption)

The developmental LOAEL for dermal exposure to HCGO during GD 0-19 was determined to be 30 mg/kg/day (LOAEL = 125 mg/kg/day based on increased number and percent resorptions; decreased fetal body weight and crown-rump length; increased fetal anomalies).

The authors also note that developmental toxicity can occur even at concentrations that do not produce overt maternal toxicity based on reduced litter size in animals exposed during GD 10-12 only.

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Comparable to guideline study

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil. 1987. Developmental Toxicity Screen in Rats Exposed Dermally to Heavy Coker Gas Oil. 1987. Mobil Environmental and Health Sciences Laboratory Report 50431.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZQ.

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Heavy Coker Gas Oil (HCGO)

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Coker Gas Oil (CRU No. 86181)

PAC (Polycyclic Aromatic Compound) Content – report no. 64348 ZO (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86181	24.80	0.25	2.48	12.40	7.44	2.48	0.50	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity study

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 15 per dose

Concentration:

Dose: 0, 8, 30, 125, 250 mg/kg/day

Year Study Performed : 1994???

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (15/group versus 20)

GLP: No information

Exposure Period: GD 0-19

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The primary objectives of this study were to evaluate the effects of dermal JHCGO exposure on female rats during gestation and to determine if such exposure adversely affects fetal viability and development.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

39. Sham control -0 mg/kg/day – GD 0-19
40. HCGO 8 mg/kg/day – GD 0-19
41. HCGO 30 mg/kg/day – GD 0-19
42. HCGO 125 mg/kg/day – GD 0-19
43. HCGO 250 mg/kg/day – GD 0-19

The exposure levels were based on results of a subchronic toxicity study using this material.

The test material was administered to groups 2-5 on GD 0-19. Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, abortion or premature delivery. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. The abdominal cavity was exposed and blood collected for hematology and serum chemistry analysis. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and liver of each animal in groups 1-7 were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram. Only the livers of pregnant females were preserved in 10% formalin. The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples were collected at the time of sacrifice from the aorta of each rat and analyzed for clinical chemistry and hematology analyses. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. A thin smear of blood was made for determination of red blood cell morphology, nucleated RBCs and white blood cell differentials [seven components including segmented neutrophils (SEG) and lymphocytes (LYM)].

Whole blood from each dam was allowed to clot for approximately thirty minutes and centrifuged to obtain the serum. Samples were analyzed for the following biochemical parameters: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

1. Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal

survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
2. Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.
3. Incidental: An incidental finding is generally an accidental event, e.g., accidentally the tip of the tail was cut off.

After gross evaluation, fetuses were submerged in cold water until no response to stimuli was evident. Fetuses in each litter (except one litter in group 2 for which all fetuses were inadvertently prepared for skeletal exam) were distributed equally into two groups, and prepared for soft tissue (viscera) or skeletal evaluations. Fetuses assigned to the soft tissue analysis group were fixed in Bouin's solution. Fetuses assigned to the skeleton analysis group were fixed in ethanol. Although fetuses were not evaluated for abnormal visceral or skeletal development, they were, however, stored in the tissue archives of the laboratory should it be deemed necessary at a later date to evaluate them.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated using analysis of variance and Tukey's Test. Statistical analyses of serum chemistry and hematology data were analyzed using "CLINPATH" (Grosse System). 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere . Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Report no. 64348 ZQ- how to reference??)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	8		mg/kg/day
NOAEL- Dermal	Maternal	=	Not identified <8		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	30		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	8		mg/kg/day

Results Remarks:

The animals used in the study were approximately 7 weeks old at receipt and approximately 10 weeks old at exposure initiation.

Incidental observations were noted in both the control and treated groups and appear to be a result of animals being collared and/or related to mating activity. Scratches appeared on one female in the 250 mg/kg group during the latter part of gestation. She was probably scratching in response to the

irritation of the treated skin, per the authors of the report. Several females developed neck lesions in spite of the protective soft rubber tubing that lined the inner surface of the cardboard collar.

HCGO-related observations were also reported during gestation. Skin irritation was present in all groups exposed to JHCGO. The irritation ranged from slight at 8 mg/kg (erythema and flaking) to severe at 125 and 250 mg/kg (thickening of the skin, fissuring of the skin, and open sores). Clinical signs of maternal toxicity were evident and, in some cases, severe at 125 and 250 mg/kg. Red vaginal discharge was observed at 30 mg/kg and above; the incidence increased with increasing dose level. In all cases, the discharge could be attributed to resorption of offspring. Several females at the 125 and 250 mg/kg dose levels became pale and their skin became cool to the touch following the onset of the red vaginal discharge. One female in the high dose group (250 mg/kg) was sacrificed moribund on gestation day 16. She had no stool, was emaciated and cool to the touch, and had severe vaginal bleeding (red vaginal discharge). Uterine examination revealed 20 implantation sites, all of which were resorbed. Another female in this group exhibited decreased activity
and labored breathing on gestation day 17.

Mean body weights, body weight gains uterine weights and net body weights decreased in a dose-related fashion at doses of 30 mg/kg/day or greater for animals exposed GD 0-19. In general, animals exposed to HCGO at a level of 125 mg/kg/day or greater consumed less food than the controls.

In general, the mean body weights of all groups treated with HCGO were significantly lower than the mean weights of the control group throughout most of gestation. It should be noted that on gestation day 0 there were no significant differences among the mean body weights for the groups. Overall mean body weight gain (gestation days 0-20) decreased with increasing dose level. Mean body weight gains were significantly reduced at 30, 125, and 250 mg/kg. At 30 mg/kg. the significance was apparent when overall body weight gain was calculated. The decrease in body weight gain was more severe at the 125 and 250 mg/kg dose levels and achieved statistical significance for nearly all intervals measured. Although not statistically significant,
body weight gain was also reduced throughout the gestational period at 8 mg/kg. Net body weight gain was significantly reduced at 125 and 250 mg/kg. Statistical significance was not achieved for the mean net body weight changes at 8 and 30 mg/kg, however both were reduced compared to the control mean value.

Food consumption was significantly decreased in all groups treated with JHCO during many of the intervals evaluated. The number of intervals during which food consumption was significantly reduced, as well as the amount of reduction, increased with increasing dose level.

There were no remarkable maternal necropsy findings. The mean absolute liver weight for the high-dose group (250 mg/kg) was significantly reduced. Under normal conditions, liver weight increases during pregnancy.
It was speculated that the liver weight profiles in animals that have a high incidence of resorptions (i.e., 250 mg/kg/day group) resemble nonpregnant animals which generally have lower liver weights. Calculation of relative weights shows that the mean relative liver weights were significantly increased at 125 and 250 mg/kg. Absolute thymus weights were significantly reduced at 30 mg/kg and above. Relative thymus weights decreased with increasing dose level, but statistical significance was achieved only at 125 and 250 mg/kg.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	8	30	125	250
Body wt –final (g)	415.5	393.7	384.9a	314.6b	261. 9b
GD 0-3 wt gain (g)	15	8	3a	-2b	-15b
GD 3-6 wt gain (g)	14	9	16	13	13
GD 6-10 wt gain (g)	20	20	20	17	20
GD 10-13 wt gain (g)	22	17	18	16a	10b
GD 13-16 wt gain (g)	25	24	20	3b	-22b
GD 16-20 wt gain (g)	66	67	61	22b	7b
GD 0-20 wt gain (g)	163	145	138a	69b	14b
Gravid uterus (g)	80.6	77.7	70.2	21.6b	5.0b
Carcass (g)	334.9	316.0	314.7a	293.0b	256.9b
Net wt change from day 0 (c)	82.3	67.7	67.7	47.0b	8.5b
Thymus weight (g)-absolute	0.292	0.259	0.218a	0.135b	0.069b
Thymus weight (g)-relative	0.087	0.082	0.069	0.046b	0.026b
Liver weight (g)-absolute	18.004	17.65 7	18.016	17.196	15.l35b
Liver weight (g)-relative	5.382	5.586	5.729	5.869b	5.874a

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) = Carcass weight minus day 0 body wt

For the GD 0-19 groups, HCGO exposures at 125 mg/kg/day and higher adversely affected the number of dams with all resorptions, the number of resorptions, and litter size in an apparent dose-related manner. The incidence of resorption was also increased at 30 mg/kg and. although not statistically significant, is considered to be biologically significant. All other parameters were not significantly different from the control animals. A significant decrease in litter size was also observed in Group 7 (125 mg/kg/day, GD 10-12) but only when compared to the remote control animals. No maternal toxicity was observed in this group at the dose level administered.

Viable litter size was significantly reduced at 125 and 250 mg/kg. Both mean number and percent resorptions were significantly increased at these same dose levels as was the number of dams with resorptions. Overall, resorption increased with increasing dose level. The increase at 30 mg/kg is considered to be biologically significant since approximately one-half of the females in this group had between 14 and 39 percent fetal resorption (the mean for the control group was 4.9 percent resorption). The biological significance of the increase in percent resorption for the 8 mg/kg group is uncertain. The statistical significance achieved at 30 mg/kg for the number of male and female fetuses is not considered to be biologically significant and can be attributed to the unusually high number of males and low number of females in the control group.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	0	8	30	125	250
Implantation sites - total	231	205	231	233	187
Implantation sites – mean	15.4	15.8	15.4	15.5	14.4

Viable fetuses – total	220	187	200	52	3
Litter size (c)	14.7	14.4	13.3	3.5b	0.2b
Viable male fetuses (%)	61	55	49	58	33
Viable female fetuses (%)	39	45	51	42	67
Resorptions (mean)	0.7	1.4	2.1	12.1b	14.2b
Resorptions (mean %)	4.9	8.6	13.4	78.0b	98.6b
Dams with resorptions (%)	67	69	93	100	100

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) Number of viable fetuses/number of litters evaluated.

Statistically significant differences ($p<0.05$) were found between the untreated and treated animals for red blood cell count, hemoglobin, mean corpuscular volume, hematocrit, mean corpuscular hemoglogin, platelet count, segmented neutrophils, lymphocytes and monocytes. A linear relationship (>99% confidence level, Pearson's correlation coefficient) was found between the dose and blood level for all of the above except segmented neutrophils. lymphocytes and monocytes. When the historical hematology reference values are taken into consideration. the dose response curves for all the affected parameters fell outside the normal range as defined by the 10th and 90th percentiles of the historical data.

Statistically significant differences were found between the untreated and treated animals for glucose. urea nitrogen, creatinine. triglycerides. total protein, bilirubin. albumin, sodium, inorganic phosphorus. calcium. Sorbitol dehydrogenase and chloride. A linear relationship (>99% confidence level. Pearson's correlation coefficient) was found between the dose and blood level for all the above components except bilirubin. The dose response curves for all the above except creatinine fell above the normal range as defined by the 10th and 90^{th} percentiles of the historical data. The levels of serum glucose, triglycerides, albumin and *A/G* ratio are noticeably different between non-pregnant and pregnant rats on gestation day 20. Serum data indicates that with the exception of *A/G* ratio, the above serum components in rats treated at 125 and 250 mg/kg/day are comparable with the normal range of non-pregnant animals.

Fetal body weights, a parameter of body growth and development, were significantly decreased for all viable fetuses at the 125 and 250 mg/kg dose levels.

Gross external fetal examinations indicated isolated incidences of variations and malformations at 8, 30, and 125 mg/kg. Kinked tail was noted in two fetuses; one in the 8 mg/kg dose group and one in the 125 mg/kg dose group. One fetus (30 mg/kg) had gastroschisis (protrusion of the intestines through a fissure in the abdominal wall). These scattered findings did not appear to be related to test material administration.

Fetal skeletal evaluations showed a statistically significant increase in incompletely ossified or unossified sternebrae at dose level of 8 mg/kg/day. This indicates significant growth retardation at 8 mg/kg. The fetal visceral evaluations showed isolated incidences of variations and malformations. These scattered findings were not dose related and did not appear to be related to test material administration.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	0	8	30	125	250
Fetal weights (g)	3.6	3.5	3.4	2.9b	2.9a
Litters evaluated	15	13	15	13	2
Fetuses - live	220	187	200	52	3
Fetuses – dead	0	0	0	0	0
Gross exam anomalies (fetal incidence; %)	0; 0.0	2; 1.1	1; 0.5	2a; 3.8	0;0.0
Gross exam anomalies (litter incidence; %)	0; 0.0	1; 7.7	1; 6.7	2; 15	0; 0.0

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Fetal Endpoints – Skeletal Malformations and Skeletal Variations

Dose (mg/kg/day)	0	8	30	125	250
Litters evaluated	15	13	15	13	2
Fetuses - live	113	103	103	30	2
Fetuses – dead	0	0	0	0	0
Total skeletal observations (fetal incidence; %)	36; 32	48a; 47	38; 37	11; 37	2; 100
Total skeletal observations (litter incidence; %)	8; 53	8; 62	9; 60	7; 52	2; 100

a)Statistically different from (p<0.05)
b)Statistically different from control (p<0.01)

Fetal Endpoints – Soft Tissue Anomalies

Dose (mg/kg/day)	0 Rem.	8	30	125	250
Litters evaluated	15	0	15	9	1
Fetuses - live	106	0	96	22	1
Fetuses – dead	0	0	0	0	0
Total fetal soft tissue (fetal incidence; %)	8; 7.5	--	8; 8.3	3; 14	0; 0.0
Total fetal soft tissue (litter incidence; %)	7; 47	--	5; 33	3; 33	0; 0.0

a)Statistically different from remote control (p<0.05)
b)Statistically different from remote control (p<0.01)

Conclusion:

The maternal NOAEL for dermal exposure to HCGO during GD 0-19 was not identified (<8 mg/kg/day). (LOAEL= 8 mg/kg/day based on increased thymus weights (absolute and relative) and liver weights (relative).

The developmental NOAEL for dermal exposure to LCO during GD 0-19 was determined to be 8 mg/kg/day (LOAEL = 30 mg/kg/day, based on increased number and percent resorptions.)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Comparable to guideline study

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil. 1994. Developmental Toxicity Study in Rats Exposed Dermally to Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report 64168.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in

Heavy Coker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348ZO.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 64741-81-7

Test Substance: 64741-81-7; Visbreaker Gas Oil (VGO); V.B. Mittelol

Test Substance Purity/Composition and Other Test Substance Comments: Heavy Coker Gas Oil (CRU No. 86193)

PAC(Polycyclic Aromatic Compound) Content – report no. 64348 ZT (Mobil, 1991)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86193	4.20	0.84	2.94	0.38	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity study

Species: Rat

Other Species: Not applicable

Mammalian Strain: Sprague-Dawley (Charles River, Kingston, NY)

Other Strain: Not applicable

Gender: Females, presumed pregnant (non treated males used for mating)

Number of Animals per Dose: 15 per dose

Concentration:

Dose: GD 0-19:
0, 30, 125, 250 mg/kg/day

Year Study Performed : 1994

Method/Guideline Followed: Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (15/group versus 20).

GLP: No information

Exposure Period: GD 0-19

Frequency of Treatment: Once per day

Post-Exposure Period: None

Method/Guideline and Test Condition Remarks: The primary objectives of this study were to assess the potential of VBO to produce maternal and/or developmental toxicity when applied dermally to pregnant rats throughout gestation, and to obtain additional data (primarily on resorptions and fetal body weights). Dose levels were chosen based on the chemical composition of the material and the results of a previous subchronic dermal study conducted with VBO.

Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (in situ or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid:

44. Control (0 mg/kg/day) – GD 0-19 – 14 animals
45. VBO 30 mg/kg/day – GD 0-19 – 15 animals
46. VBO 125 mg/kg/day – GD 0-19 – 15 animals
47. VBO 250 mg/kg/day – GD 0-19 – 15 animals

The test material was administered to groups 2-4 via dermal application on GD 0-19. Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Controls were handled in the same manner, minus application of the test material. Control animals were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, abortion or premature delivery. All unusual findings were noted. Effects of the test material on the skin at the site of application were scored weekly. Erythema and edema were evaluated using the Draize scales. The skin was also examined and scored for chronic deterioration, flaking, thickening, stiffening, cracking, and sloughing.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20.

Each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The number of corpora lutea per ovary and the weight of the gravid uterus were recorded. The ovaries in nonpregnant females were grossly examined and then discarded. In the uterus, the number and location of implantations, early and late resorptions, and live and dead fetuses were

recorded. An "early resorption" was defined as a reabsorbed dead conceptus in which it was not grossly evident that organogenesis had occurred; a "late resorption" was defined similarly but as one in which it was evident that organogenesis had occurred. A "live fetus" was defined as a fetus which responded to a stimulus, such as touch; a "dead fetus" did not respond to stimuli, nor did it demonstrate the autolysis characteristic of late resorptions. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation

Each fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

4. Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
5. Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.
6. Incidental: An incidental finding is generally an accidental event, e,g., accidentally the tip of the tail was cut off.

Following gross examination, fetuses in each litter were distributed equally into two groups, and prepared for soft tissue (viscera) or skeletal evaluations. Fetuses assigned to the soft tissue analysis group were fixed in Bouin's solution. Fetuses assigned to the skeleton analysis group were fixed in ethanol. Although fetuses were not evaluated for abnormal visceral or skeletal development, they were, however, stored in the tissue archives of the laboratory should it be deemed necessary at a later date to evaluate them.

Statistical analysis:
Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. 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% ($p<0.05$).

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC species, so that highly alkylated PACs are excluded from measurement. (API, 2008; Mobil, 1991)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)*

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	125		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	Not identified (>250)		mg/kg/day

NOAEL - Dermal	Offspring (F1)	=	250		mg/kg/day

***Determined by reviewer**

Results Remarks:

The animals used in the study were approximately 8 weeks old at receipt and approximately 10 weeks old at exposure initiation.

No animals associated with this study died or were sacrificed prior to the scheduled necropsy. Two animals from group 2 were excluded from the study. The reason for the exclusion was a miscalculated gestation day 0 date based on higher than average fetal body weights. No clinical signs indicative of systemic toxicity were observed during the study. Most clinical signs were local effects from the collars (e.g., neck irritation, chromodacryorrhea, reddish nasal discharge), and are not considered to be test material related.

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Skin irritation was present in all groups exposed to VBO. The irritation ranged from slight at 30 mg/kg (erythema and flaking) *to* severe at 125 mg/kg and 250 mg/kg (scabbing of the skin, fissuring of the skin and open sores).

Final body weights for the treated animals did not significantly differ from the controls. Mean body weight changes for the high-dose group (250 mg/kg) were significantly lower than the control group for GD 0-20. Mean body weight changes of the 125 and 30 mg/kg were not significantly different from the control group.

There were no significant differences in food consumption values of the exposed animals relative to control animals.

There were no significant maternal necropsy findings.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**30**	**125**	**250**
Body wt –final (gr)	407.1	414.8	409.9	388.7
GD 0-3 wt gain (gr)	8	7	7	0
GD 3-6 wt gain (gr)	18	17	12	16
GD 6-10 wt gain (gr)	17	20	17	15
GD 10-13 wt gain (gr)	21	19	22	18
GD 13-16 wt gain (gr)	29	19	25	23
GD 16-20 wt gain (gr)	56	63	60	50
GD 0-20 wt gain (gr)	149	146	1441	122a
Gravid uterus (gr)	81.9	85.1	86.3	71.7
Carcass (gr)	325.2	329.7	323.6	317.0
Net wt change from day 0 (e)	66.7	61.0	57.2	50.1

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c) = Carcass weight minus day 0 body wt

Even though it was not statistically significant, an increase in resorptions with increasing dose level was observed.

Summary of Mean Selected Reproduction Data

Dose (mg/kg/day)	**0**	**30**	**125**	**250**
Implantation sites	192	188	248	198

– total				
Implantation sites – mean	14.8	15.7	16.3	14.1
Preimplantation loss (%)	1.2	0.4	0.5	13.3
Viable fetuses - total	186	176	231	179
Litter size (c)	14.3	14.7	15.4	12.8
Viable male fetuses (%)	53	50	48	51
Resorptions (mean)	0.5	1.0	1.1	1.4
Resorptions (mean %)	4.2	6.9	6.7	8.8
Dams with resorptions (%)	38	67	80	71

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) Number of viable fetuses/number of litters evaluated.

There were no significant differences in fetal body weights from exposed animals relative to control fetal body weights.

There were isolated incidences of variations and malformations observed at the time of external examination of fetuses. Hematoma on tip of the tail was noted in one fetus and missing eye bulge was found in another one in the control group. Fleshy tab tip of tail was noted in two fetuses, one in the 125 mg/kg group and one in 250 mg/kg group. Protruding tongue was noted in one fetus in the 250 mg/kg group. Due to this low incidence of seemingly unrelated observations and the lack of a dose response, the observed external anomalies are not considered to be treatment related.

Fetal Endpoints – Weight and Gross Examination

Dose (mg/kg/day)	**0**	**25**	**50**	**125**
Fetal weights (gr)- mean	3.7	3.8	3.7	3.8
Litters evaluated	13	12	15	14
Fetuses - live	186	176	230	179
Fetuses – dead	0	0	0	0
Total gross exam anomalies (fetal incidence; %)	2; 1.1	0; 0.0	1; 0.4	2; 1.1
Total gross exam anomalies (litter incidence; %)	2; 15	0; 0.0	1; 6.7	2; 14
Total skeletal changes (fetal incidence; %)	*	*	*	*
Total skeletal changes (litter incidence; %)	*	*	*	*
Total soft tissue anomalies (fetal incidence; %)	*	*	*	*

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
*not examined; tissues saved in archives

Conclusion:

Determined by Reviewer:
The maternal NOAEL for dermal exposure to VBO during GD 0-19 was determined to be 250 mg/kg/day (LOAEL= 250 mg/kg/day based on significantly lower body weight gain)

The developmental NOAEL for dermal exposure to LCO during GD 0-19 was determined to be 250 mg/kg/day (LOAEL = not identified (>

250 mg/kg/day)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Comparable to guideline study

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

Mobil. 1994. Developmental Toxicity Study in Rats Exposed Dermally to V.B. Mittelol. Mobil Environmental and Health Sciences Laboratory Report 64643.

Mobil. 1991. Characterization and Quantitation of Polynuclear Aromatics in Visbreaker Gas Oil. Mobil Environmental and Health Sciences Laboratory Report no. 64348 ZT.

API. 2008. PAC Analysis Task Group, "The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances." http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical: 68410-00-4

Test Substance: 68410-00-4; Distillates, Crude Oil (DCO); VDF Diesel

Test Substance Purity/Composition and Other Test Substance Comments: Distillates, Crude Oil (F-215)

PAC Content – report no. 65726-ZA-ZR (Mobil, 1994)
Distillates, Crude Oil (F-215)

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
091681 (F-215)		0.20	4.00	4.00	0.00	0.00	0.00	0.00

1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008).
2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.

Category Chemical Result Type : Measured

Unable to Measure or Estimate Justification :

METHOD

Route of Administration: Dermal, non-occluded

Other Route of Administration:

Type of Exposure: Developmental toxicity

Species: Rat

Other Species: Not applicable

Mammalian Strain:	Sprague-Dawley (Charles River, Portage, MI)
Other Strain:	Not applicable
Gender:	Females (non treated males used for mating)
Number of Animals per Dose:	25 per dose for level
Concentration:	
Dose:	0, 50, 250, 500 mg/kg/day
Year Study Performed :	1993
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study)
GLP:	yes
Exposure Period:	Gestation day (GD) 0-19
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of DCO (F-215) administered percutaneously to presumed pregnant rats. Prior to the initiation of dosing with the test substance, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of sperm in a vaginal smear or a copulatory plug, the individual, presumed pregnant females were randomly assigned to four treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of evidence of mating: 1. Vehicle control (acetone) 0 mg/kg/day – 25 animals (GD 0-19) 2. DCO 50 mg/kg/day – 25 animals (GD 0-19) 3. DCO 250 mg/kg/day – 25 animals (GD 0-19) 4. DCO 500 mg/kg/day – 25 animals (GD 0-19) At the completion of mating, all males and those females that did not exhibit positive evidence of mating were sacrificed. Suspensions of F-215 were prepared daily at concentrations of 0 (vehicle, acetone), 50, 100 and 250 mg/mL such that doses of 0, 50, 100, and 250 mg/kg/day, respectively, were administered at a volume of 1 mL/kg. Animals in all groups were treated on GD 0 through GD 19. Each treatment day, animals were dosed by even application of the test substance to their shaved backs, using a blunt-tipped glass syringe. The test substance dose was calculated from each rat's most recent body weight. Rats were fitted with Elizabethan collars to minimize ingestion of test substance. Controls were handled in the same manner but with application of the vehicle only. Elizabethan collars were applied just prior to dosing and were removed after a 6 hour exposure period. At the time of collar removal, any excess test article was wiped off with a cloth dipped in acetone and dried with a clean cloth. Upon initiation of treatment, each female was observed twice daily for viability. Each rat was observed at least once a day throughout gestation until sacrifice for changes in appearance, behavior, excretory function, and general signs of ill-health or abortion. All unusual findings were noted. Skin reactions were graded using the Draize and National Research Council standards. On GD 9, the post-dosing observation for one rat in the 250 mg/kg/day dose group was inadvertently performed at 6 hours, rather than at 60 minutes, post-dosing. On GD 0, the post-dosing observations for eight rats in the 500 mg/kg/day dose group were inadvertently performed at 8 hours, rather than 60 minutes post-dosing. These deviations did not affect the outcome of the study because no

adverse clinical observations occurred other than skin reactions, and are documented in the raw data.

Individual body weights and food consumption were recorded daily during presumed gestation.

All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation, and a gross necropsy of the thoracic and abdominal viscera was performed. The abdomen of each rat was opened, and the intact uterus was excised and examined for pregnancy. To confirm the pregnancy status, uteri from rats that appeared non-pregnant were examined while transilluminated and pressed between two glass plates. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other maternal tissues were discarded.

One rat (GD 1) died as the result of an accident and was necropsied on the day the event occurred using the procedures described for rats that survived to GD 20. Pregnancy status was not confirmed because death occurred before implantation.

Corpora lutea in each ovary were recorded. The number and distribution of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Each fetus was removed from the uterus, placed in an individual container, weighed, and examined for weighed and examined for sex and gross external alterations. Live fetuses were sacrificed.

Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations by using an adaptation of Wilson's sectioning technique. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red, and examined for skeletal alterations.

STATISTICAL ANALYSES: Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Maternal body weights, body weight changes, feed consumption values, and litter averages for fetal body weights, percent male fetuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test and ANOVA, when appropriate [i.e., Bartlett's Test was not significant ($P>0.05$)]. If the analysis of variance was significant ($P<0.05$), Dunnett's Test was used to identify the statistical significance of the 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, when less than or equal to 75% ties were present. When more than 75% ties were present, Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant ($P<0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures described for the Kruskal-Wallis Test.

PAC Analysis:
The percent of each ring class was conducted in a separate study and determined by the PAC 2 method as described elsewhere. Briefly the PAC 2 is a single analytical method that involves solvent extraction (DMSO) and an analysis of the DMSO-extracted concentrate of PACs by gas chromatography with an FID or MS detector. The DMSO extraction procedure is selective for the less polar PAC

species, so that highly alkylated PACs are excluded from measurement. (API, 2008; and Mobil, 1994)

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	250		mg/kg/day
NOAEL- Dermal	Maternal	=	50		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	Not determined >500		mg/kg/day
NOAEL - Dermal	Offspring (F1)	=	500		mg/kg/day

Results Remarks:

The animals used in the study were between 12 and 13 weeks of age at exposure initiation.

No deaths were caused by the test substance. One control group rat died as the result of an accident (necropsy revealed extensive hemorrhage present ventral to the cervical vertebrae and surrounding the esophagus and trachea, observations compatible with trauma).

Skin reactions related to administration of the test substance occurred in the 250 and 500 mg/kg/day dose groups.
Increased or significantly increased (P<0.01) erythema (grades 1 and 2) edema (grade 1), atonia (grades 1 and 2), desquamation (grades 1 and 2), fissuring (grade 1), exfoliation and eschar occurred in the 250 and 500 mg/kg/day dose group rats. Increased or significantly increased (P<0.01) numbers of 500 mg/kg/day dose group rats also had erythema (grade 3), atonia (grade 3) and fissuring (grade 2).

Vocalization occurred in eight 500 mg/kg/day dose group rats. This clinical observation was considered an effect of the test substance because it occurred in the high dose group. The only other clinical observation (localized alopecia) was considered unrelated to administration of F-215 because the sign occurred in only one 250 rat in the mg/kg/day dose group. The only necropsy observation occurred in the control group rat that died, as described previously.

Maternal body weights and body weight gains were significantly reduced in the 250 and 500 mg/kg/day dose groups at various points and during gestation, per the table below. No effects were seen at the 50 mg/kg/day dose level.

Feed consumption was not affected at dose levels of 50 mg/kg/day. Absolute feed consumption values were significantly reduced (P<0.05) in the 500 mg/kg/day dose group on GD 3 to 6. Relative feed consumption values in this group were significantly reduced (P<0.05) on GD 0 to 3 and significantly increased (P<0.05) on GD days 12 to 15. Percutaneous administration of doses as high as 250 mg/kg/day on days GD 0 through 19 did not significantly affect absolute or relative feed consumption values.

All other Caesarean-sectioning and litter parameters were unaffected by doses of the test substance as high as 500 mg/kg/day. Litter averages for implantations and sex ratios did not demonstrate any significant or biologically important differences. The average number of corpora lutea was significantly reduced (P<0.0l) in the 250 mg/kg/day dose group. This event was considered unrelated to the test substance because the incidence was not dose-dependent. No dam resorbed all conceptuses, and the numbers of dams with viable fetuses were comparable
among the four dose groups.

Percutaneous administration of F-215 at doses as high as 500 mg/kg/day did not affect Caesarean-sectioning or litter observations. There were 24, 20, 20 and 22 rats pregnant and Caesarean sectioned in the 0 (vehic1e), 50, 250 and 500 mg/kg/day dose groups, respectively, on GD 20. There were no biologically important differences in litter averages for corpora lutea, implantations, litter sizes, live fetuses, resorptions (early and late), fetal body weights,· percent resorbed conceptuses and sex ratios. No dam resorbed all conceptuses, and the numbers of dams with resorptions and viable fetuses were comparable among the four dose groups.

Male fetal body weights were significantly increased (P<0.05) in the 50 and 250 mg/kg/day dose groups. These effects were not dose-dependent observations and were judged to be unrelated to the test substance and interrelated with differences in litter sizes among the four dose groups. When values for litters of less than ten fetuses were excluded from analyses, fetal body weights did not significantly differ.

Fetal alterations were classified as: 1) malformations (irreversible changes which occur at low incidences in this species and strain); or 2) variations (relatively common developmental changes in this species and strain, including minor reversible delays or accelerations in development).

No gross external, soft tissue or skeletal alterations in the fetuses were caused by test article administration at doses as high as 500 mg/kg/day. The significant increases (P<0.01) in fetuses with any alteration observed and in the fetal incidences of incompletely ossified pubes and ischia in the 50 mg/kg/day dose group were unrelated to the test substance because: 1) the values were not dose-dependent; and 2) the litter incidences were not significant.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	0	50	250	500
Body wt –final (gr)	414.7	409.8	387.6b	365.2b
GD 0-3 wt gain (gr)	12.2	11.8	12.5	7.4a
GD 3-6 wt gain (gr)	16.4	16.0	13.6	9.2 b
GD 6-9 wt gain (gr)	18.5	16.4	17.0	15.2
GD 9-12 wt gain (gr)	21.2	21.3	20.8	21.1
GD 12-15 wt gain (gr)	25.3	22.4	16.3a	16.6b
GD 15-18 wt gain (gr)	45.5 1	42.4	38.8a	21.1b
GD 18-20 wt gain (gr)	34.5	32.9	27.0b	25.7b
GD 0-20 wt gain (gr)	173.8	163.2	146.0b	122.4b

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Summary of Mean Selected Reproduction and Litter Data

Dose (mg/kg/day)	0	50	250	500
Corpora lutea	20.4	19.0	19.2	19.3
Implantation sites – mean	16.0	14.4	15.0	15.0
Live fetuses – total	366	277	290	318
Live fetuses - mean	15.2	13.8	14.5	14.4
Litter size	15.2	13.8	14.5	14.4
Viable male fetuses (%)	51.9	49.7	50.4	49.8
Total resorptions (mean)	0.8	0.6	0.4	0.6
Dams with resorptions	13	8	8	9

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Fetal Endpoints

Dose (mg/kg/day)	0	50	250	500
Fetal weights (gr)	3.73	3.83c	3.87c	3.75
Litters evaluated	24d	20	20	22
Live fetuses - total	366	277	290	318
Dead fetuses – dead	0	0	0	0
% Resorbed conceptuses per litter	4.9	3.8	3.6	3.7
Litters with any alteration (N;%)e	4(16.7)	7(35.0)	5(25.0)	4(18.2)
Fetuses with any alteration (N;%)e	4(1.1)	13(4.7)b e	8(2.8)	4(1.1)
Fetuses with any alteration per litter (mean %)e	1.18	4.43	3.02	1.49

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)
c) Male fetal body weight was significantly different, but judged not to be biologically significant (see text)
d) Excludes values for one rat which died as the result of an accident on day 1 of presumed gestation.
e)See text for discussion of results.

Conclusion: The maternal NOAEL for dermal exposure to DCO during GD 0-19 was determined to be 50 mg/kg/day (LOAEL = 250 mg/kg/day based on skin irritation, decreased body weight and body weight gains).

The developmental NOAEL for dermal exposure to DCO during GD 0-19 was determined to be 500 mg/kg/day. (LOAEL = not determined, >500 mg/kg/day; the highest dosage tested did not result in effects on embryo-fetal viability or fetal body weights or morphology.)

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Non guideline study, but with adequate detail to make NOAEL determination.

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

ARCO. 1993. Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of F-215 Administered Percutaneously to Crl:CD®BRK VAF/Plus® Presumed Pregnant Rats. Report ATX-92-0155.

Mobil. 1994. Characterization and Quantitation of Polynuclear Aromatics. Mobil Environmental and Health Sciences Laboratory Report no. 65726-ZA-ZR

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	64741-62-4

Test Substance:	64741-62-4; Catalytic Cracked Clarified Oil or Clarified Slurry Oil [CSO]
Test Substance Purity/Composition and Other Test Substance Comments:	Catalytic Cracked Clarified Oil (CRU No. 010929) PAC (Polycyclic Aromatic Compound) Content Sample # / DMSO wt.% [1] / 1-ARC (%)[2] / 2-ARC (%) / 3-ARC (%) / 4-ARC (%) / 5-ARC (%) / 6-ARC (%) / 7-ARC (%) 010929 / 52.0 / 0.0 / 1.0 / 15.6 / 15.6 / 10.4 / 5.2 / 2.6 1) Percent of DMSO-extractable PACs, determined by the PAC 2 method as described in API (2008). 2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Dermal, non-occluded
Other Route of Administration:	Not applicable
Type of Exposure:	Developmental toxicity study
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley Charles River Laboratories, Raleigh, NC
Other Strain:	Not applicable
Gender:	Females, presumed pregnant (non treated males used for mating)
Number of Animals per Dose:	25/group
Controls	Untreated [Sham} controls, vehicle control – Acetone 1.5mL/kg
Doses:	0, 5, 25, 50 mg/kg/day
Year Study Performed :	2012
Method/Guideline Followed:	OECD 414 Prenatal Developmental Toxicity Test; EPA OCSPP 870.3700 Prenatal Developmental Toxicity Test
GLP:	Yes
Exposure Period:	Gestation day 0-19, 6 hours/day
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	Crl:CD(SD) sexually mature female rats [approx. 79 days old at receipt] were received in good health from Charles River Laboratories, Inc., Raleigh, NC. The day following receipt, all animals were weighed and clinical observations were recorded. Each rat was uniquely identified by a Monel® metal ear tag displaying the animal number and housed for 14 days for acclimation purposes. During the acclimation period, the rats were observed twice daily for mortality and changes in general appearance and behavior. Animals were acclimated to wearing Elizabethan-style collars on an incremental basis, starting with approximately 1 hour and ending with approximately 24 hours of acclimation, starting approximately 1 week prior to the initiation of dose application. Animals were housed individually except during breeding in a room with a 12 hour light/12 hour dark cycle and received food and water *ad libitum*. The room temperature and humidity controls were set to maintain environmental conditions of 71 ± 5^0F (22 ± 3^0C) and 50 ± 20%, respectively. Females were approximately 13 weeks old when paired for breeding with sexually mature untreated

male rats of same strain and source. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage and verified by a second biologist. Each mating pair was examined daily. The day on which evidence of mating was identified was termed gestation day 0 and the animals were separated. The bred females were assigned to groups using a computer program which randomized the animals based on stratification of the gestation day 0 body weights in a block design. Animals not assigned to study were euthanized by carbon dioxide inhalation and discarded. Body weight values ranged from 224g to 284g on gestation day 0.

On the day prior to the initiation of dose administration, and as often as necessary thereafter, the hair was clipped (in a manner that would not abrade the skin) from the dorsal scapular area; repeated clippings were performed prior to or at least 2-4 hours after dose administration. A different set of clippers was used for each group to avoid the potential for cross-contamination. Catalytic cracked clarified oil in acetone was applied once daily from gestation days 0 through 19 for 6 hours each day. Exposure levels were 5, 25, and 50 mg/kg/day administered at a dosage volume of 1.5 mL/kg. The vehicle or test substance was applied evenly to the clipped, unabraded skin and spread evenly using a glass rod (to ensure contact with an area of approximately 10% of the total body surface area). At the end of the 6-hour exposure period, the test sites were gently patted using a disposable paper towel in an effort to remove any remaining test substance or vehicle from the skin. If needed, the test site could be gently patted with gauze moistened with mineral oil and then patted again with dry gauze or a dry disposable paper towel.

All animals were observed twice daily for mortality and moribundity and clinical observations recorded. Body weights and food consumption were recorded at GD 0, 3, 6, 9, 12, 15, 18, and 20. Mean body weight changes were calculated for each interval and GD 0-20. Collars were removed during weighing. Gravid uterine weight was collected and net body weight (the gestation day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the gestation day 0-20 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

Pathology Maternal and developmental: On gestation day 20, females were euthanized by carbon dioxide inhalation and a laparohysterectomy was performed on each surviving female. The cranial, thoracic, abdominal, and pelvic cavities were opened and the contents examined. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn. Tissue retained were treated skin, untreated skin (right hind limb), liver, thymus, brain and all gross lesions. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. Females that died or were euthanized (by carbon dioxide inhalation) *in extremis* during the course of the study were similarly examined and tissue retained in 10% neutral-buffered formalin. The number and location of implantation sites, corpora lutea, and viable fetuses were recorded. Recognizable fetuses were examined externally and preserved in 10% neutral-buffered formalin for possible future analysis. Remaining tissue was discarded. The liver, brain, and thymus were weighed from all animals euthanized *in extremis* or at the scheduled necropsy. Organ to brain weight ratios were calculated.

Intrauterine data were summarized using 2 methods of calculation. An example of each method of calculation follows:

1. Group Mean Litter Basis:

$$\text{Postimplantation Loss/Litter} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Group}}{\text{No. Gravid Females/Group}}$$

2. Proportional Litter Basis:

$$\text{Summation Per Group (\%)} = \frac{\text{Sum of Postimplantation Loss/Litter (\%)}}{\text{No. Litters/Group}}$$

where

$$\text{Postimplantation Loss/Litter (\%)} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Litter}}{\text{No. Implantation Sites/Litter}} \times 1$$

Fetal Evaluation: Each viable fetus was subjected to a visceral examination using a fresh dissection technique to include the heart and major blood vessels [Stuckhardt and Poppe, 1984]. The sex of each fetus was confirmed by internal examination. Fetal kidneys were examined and graded for renal papillae development. Heads from approximately one-half of the fetuses in each litter were

placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining one-half of the fetuses were examined by a midcoronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol.
Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S and Alcian Blue and examined for skeletal malformations and developmental variations.
External, visceral, and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

The fetal developmental findings were summarized by: 1) presenting the incidence of a given finding both as the number of fetuses and the number of litters available for examination in the group; and 2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per Group (\%)} = \frac{\text{Sum of Viable Fetuses Affected/Litter (\%)}}{\text{No. Litters/Group}}$$

Where:

$$\text{Viable Fetuses Affected/Litter (\%)} = \frac{\text{No. Viable Fetuses Affected/Litter}}{\text{No. Viable Fetuses/Litter}} \times$$

Statistical Analysis: Mean maternal body weights (absolute and net), body weight changes (absolute and net), and food consumption, gravid uterine weights, numbers of corpora lutea, implantation sites, and viable fetuses, fetal body weights (separately by sex and combined), and organ weights were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test or a two-sample t-test, as appropriate, was used to compare the test substance-treated groups to the vehicle control group and the vehicle control group to the sham control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and postimplantation loss, and fetal sex distribution), total fetal malformations and developmental variations (external, visceral, skeletal, and combined) and each particular external, visceral, and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test substance-treated groups to the vehicle control group and the vehicle control group to the sham control group.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
EL dermal	Maternal	=	25		mg/kg/d
EL dermal	Maternal	=	5		mg/kg/d
EL dermal	Offspring	=	25		mg/kg/d
EL dermal	Offspring	=	5		mg/kg/d

Results Remarks:

Dosing Formulations: The analyzed dosing formulations were within WIL Research's SOP range for suspensions (85% to 115%) and were homogeneous

Mortality: Two females in the 50 mg/kg/day group died prior to termination, one on GD18 and the other on GD19. These deaths were assumed to have been treatment-related but the causes of death was not determined. Both females were noted with yellow and/or red material around the urogenital area, nose, and eyes and decreased defecation at the daily examinations intermittently throughout the treatment period until the day of death; single occurrences of red material in the urogenital area were noted for each female 1 to 2 hours following dose administration. On the day prior to death, both females were noted with a pale body. All other females in all groups survived to termination on GD 20.

Clinical findings: Red and/or yellow material in the urogenital area were noted in all groups, including the sham and vehicle control groups, generally throughout the treatment period (gestation days 0-20) but occurred at a much greater frequency in the test substance-treated groups. Red vaginal discharge were

noted for 3 surviving females in the 50 mg/kg/day group during gestation days 16-19 at 1 to 2 hours following dose administration. In addition, 2 surviving females in the 50 mg/kg/day group were noted with a pale body during gestation days 17-19 at the daily examinations. There were no remarkable dermal observations

Maternal Body Weight, weight gains and Gravid uterus: Test substance-related mean body weight losses were noted in the 25 and 50 mg/kg/day groups at the start of the treatment period (gestation days 0-3); the difference was significant ($p<0.01$) at 50 mg/kg/day. Mean body weight gains in the 25 and 50 mg/kg/day groups were similar to the vehicle control group during gestation days 3-6, 6-9, and 9-12. Lower mean body weight gains were noted in the 25 and 50 mg/kg/day groups throughout the remainder of the treatment period (gestation days 12-20). As a result, significantly ($p<0.01$) lower mean body weight gains were noted at 25 and 50 mg/kg/day when the entire treatment period (gestation days 0-20) was evaluated. Mean body weights in the 25 and 50 mg/kg/day groups were significantly ($p<0.01$) lower (up to 10.6% and 22.3%, respectively) than the vehicle control group during gestation days 18-20 and 12-20, respectively. A lower mean net body weight in the 50 mg/kg/day group and lower mean net body weight changes in the 25 and 50 mg/kg/day groups were also noted; the differences were significant ($p<0.01$). Mean net body weight in the 25 mg/kg/day group was similar to the vehicle control group.

Gravid uterine weights in the 25 and 50 mg/kg/day groups were significantly ($p<0.01$) lower than the vehicle control group and were attributed to the decreased number of viable fetuses and lower mean fetal weights noted at these exposure The decreased number of viable fetuses and lower fetal weights in the 25 and 50 mg/kg/day groups also contributed to the lower body weights and body weight losses/reduced body weight gains in these groups, especially during the latter part of gestation

	Sham control	Acetone control	5mg/kg	25mg/kg
Gravid Uterine Weight	84.0 ± 9.0	76.8 ± 17.3	76.1 ± 8.6	46.7 ± 22.4**
Net Extra-Uterine Wt Gain	48.2 ± 12.9	49.7 ± 10.3	46.4 ± 14.2	37.3 ± 11.1**

Mean maternal body weights, body weight gains, net body weight, net body weight gain, and gravid uterine weight in the 5mg/kg/day group were similar to the vehicle control group. Differences from the vehicle control group were slight and not statistically significant. Lower mean food consumption in the 25and 50mg/kg/day groups corresponded to the changes in body weight and weight gain. Maternal food consumption in the 5 mg/kg/day group was generally similar to the vehicle control group throughout the treatment period. However, a significantly ($p<0.05$) lower mean food consumption value (g/kg/day only) was noted in the 5 mg/kg/day group when the overall treatment period (gestation days 0-20) was evaluated. However, no corresponding effects on g/animal/day food consumption, mean body weights, or mean body weight gains were noted in this group; therefore, the difference in mean food consumption at 5 mg/kg/day was not considered test substance-related.

Maternal organ weights and macroscopic findings: At the scheduled necropsy on gestation day 20, no test substance-related gross internal findings were observed at any dosage level. Test substance-related, significantly ($p<0.05$ or $p<0.01$) lower mean thymus weights (absolute and relative to brain) were noted in the 25 and 50 mg/kg/day groups compared to the vehicle control group. Mean liver (absolute and relative to brain) weights and absolute brain weights in the 25 and 50 mg/kg/day groups were similar to the vehicle control group. No test substance-related effects on organ weights (absolute and relative to brain weight) were observed at 5 mg/kg/day.

Laparohysterectomy findings and fetal weights [Table 1]: The examination of uteri revealed that the number of gravid females was similar across groups. There were no statistically significant differences in numbers of corpora lutea or implantation sites. However, the number of early resorptions was significantly increased, and the number of viable fetuses was significantly decreased in the 25 and 50 mg/kg/day groups. The mean litter proportions of postimplantation loss in the 25 and 50 mg/kg/day groups (42.8% and 82.8% per litter, respectively) were significantly ($p<0.01$) higher than the vehicle control group (6.5% per litter). This increase in postimplantation loss was the result of an increased mean litter proportion of early resorptions at 25 mg/kg/day and increased mean litter

proportions of early and late resorptions at 50 mg/kg/day. Corresponding significantly (p<0.01) lower mean litter proportions of viable fetuses were noted in the 25 and 50 mg/kg/day groups (57.2% and 17.2% per litter, respectively) when compared to the vehicle control group (93.5% per litter). The mean numbers of viable fetuses at 25 and 50 mg/kg/day (8.5 and 2.7 per litter, respectively) were also significantly (p<0.01) lower than the vehicle control group (13.4 per litter). One and 8 females in the 25 and 50 mg/kg/day groups, respectively, had 100% post-implantation loss (0.0% viable fetuses). Additionally, in the 25 and 50 mg/kg/day groups, significantly (p<0.01) lower mean male (3.4 g and 2.7 g, respectively), female (3.1 g and 2.6 g, respectively), and combined (3.2 g and 2.7 g, respectively) fetal weights were noted compared to the vehicle control group values (3.8 g, 3.6 g, and 3.7 g, respectively). Intrauterine growth and survival were unaffected at 5mg/kg/day

Table 1 Results of laparahysterectomy and fetal examination from dams treated dermally with CSO

Parameter	Sham control	Acetone Control	5 mg/kg/day
Number of Gravid Females	24	24	25
Corpora Lutea: Total Mean ± SD	381 15.9 ± 1.54	370 15.4 ± 2.89	390 15.6 ± 1.83
Implantation Sites Total Mean ± SD	374 15.6 ± 1.69	346 14.4 3.12	372 14.9 ± 1.62
Viable fetuses Mean ± SD			
Male	7.8 ± 2.5	7.0 ± 2.4	6.6 ± 2.6
Female	6.7 ± 2.5	6.4 ± 2.6	7.0 ± 2.4
Total	14.4 ± 1.47	13.4 ± 3.0	13.6 ± 1.4
Dead Fetuses			
Combined Sexes	0.0	0.0	0.0
Resorptions Totals/group: Mean ± SD			
Early	28 1.2 ± 1.6	24 1.0 ± 1.2	29 1.2 ± 1.1
Late	0 ± 0.0	0 ± 0.0	3 0.1 ± 0.3
Postimplantation losses Total/group; Mean ± SD	28 1.2 ± 1.6	24 1.0 ± 1.2	32 1.3 ± 1.2
Fetal weight Mean ± SD			
Male fetuses (g)	3.8 ± 0.32	3.8 ± 0.31	3.5 ± 0.26
Female fetuses (g)	3.7 ± 0.35	3.6 ± 0.32	3.5 ± 0.25
Combined Fetal weight (g)	3.7 ± 0.29	3.7 ± 0.28	3.6 ± 0.29

** p = 0.01

Fetal Examinations: Few malformations were observed in 4(2), 0(0), 3(2), 0(0), and 1(1) fetuses (litters) in sham control, vehicle control and 5, 25, 50mg/kg/day groups, respectively (Table 2).

Table 2: Summary of Malformations of fetuses from dams treated with CSO

Observation	Sham control	Acetone Control	5 mg/kg/day
Number fetuses examined (no. of litters)	346 (24)	322 (24)	340 (25)
Localized fetal edema	1	0	0
Micropthalmia and/or anopthalmia	0	0	1
Visceral examination – situs inversis	1	0	1
Skeletal examination			
Vertebral anomaly with or without associated rib anomaly	0	0	2
Sternebrae misaligned	1	0	0
Sternoschisis	2	0	0
Total Number fetuses with malformations	4	0	3

External	1	0	1
Soft Tissue	1	2	0
Skeletal	3	0	2

A significantly ($p<0.01$) higher incidence of fetal developmental variations was observed in the 50 mg/kg/day group (82.9% per litter) compared to the vehicle control group (33.0% per litter). This was due to the significantly ($p<0.01$) higher percent per litter of skeletal variations (82.9% per litter versus 32.6% per litter in the vehicle control group). Increased mean litter proportions of sternebra(e) nos. 5 and/or 6 unossified, reduced ossification of the vertebral arches, reduced ossification of the skull, and pubis unossified were noted in the 50 mg/kg/day group. In addition, a decreased mean litter proportion of cervical centrum no. 1 ossified was noted at 50 mg/kg/day. An increased mean litter proportion of reduced ossification of the skull was also noted in the 25 mg/kg/day group. These findings were considered secondary to the reduced fetal weights noted in the 25 and 50 mg/kg/day groups (Table 3). No significant test substance-related fetal malformations or developmental variations were observed in the 5 mg/kg/day group fetuses.

Table 3: Skeletal variations in fetuses from dams treated dermally with CSO

	Acetone Control	5 mg/kg/day	25 mg/kg/day
Developmental Variations- Absolute number (% per litter			
Sternebrae #5 and/or 6 unossified	48 (14.6)	40 (11.8)	35 (14.7)
Cervical centrum # 1 ossified	33 (9.8)	63 (18.5)	15 (11.3)
Reduced ossification of vertebral arches	2 (0.5)	1 (0.3)	3 (1.1)
Reduced ossification of the skull	1 (0.2)	2 (0.6)	5 (1.8)
Pubis unossified	1 (0.3)	0 (0.0)	0 (0.0)

* p =0.05 Dunnett's test

Conclusion:

The NOAEL for both maternal and fetal toxicity was 5.0 mg/kg/day. The LOAEL = 25mg/kg based on decreased maternal weight and weight gain, food consumption and organ weight changes and significant reductions in fetal survival and fetal weight and an increased incidence in early resorptions. Developmental delays were also observed secondary to reduced fetal weights, but the frequency of malformations was not increased. CSO acted at a fetal toxicant at levels which also produce maternal toxicity; fetal effects were not a unique result of CSO exposure. No significant soft tissue or skeletal malformations were observed.

RELIABILITY/DATA QUALITY

Reliability: 1 - Reliable without restrictions

Reliability Remarks: Conforms to standard US and OECD guidelines and GLPs. Sufficient detail provided in appendices and tables

Key Study Sponsor Indicator: Yes

REFERENCE

Reference:

WIL Laboratories 2012. A Dermal Prenatal Developmental Toxicity Study of Clarified Oils, Catalytic Cracked in Rats. WIL Study # 402016. WIL Research Laboratories, LLC. 1407 George Road, Ashland, OH 44805-8946

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	:	CAS RN 64741-45-3

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 : CAS RN 68915-97-9 Gas Oil Category Heavy Atmospheric Gas Oil Compositionally similar to Heavy Fuel CAS RN 68783-08-4

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 : CAS RN 64741-57-7 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	:	CAS RN 64741-62-4 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 : CAS RN 64741-62-4 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).

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 RN 64741-62-4)

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 : CAS RN 64741-81-7 Coker heavy Gas Oil,

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

(1) (1) RR spreadsheet data

(2) Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem, and G.M. Hightower (1974)
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Marine Biology. 27:75-88.

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American Petroleum Institute Report 27-32814

(4) API (1980)
Acute toxicity tests
API 78-7 #6 Heavy fuel oil (API gravity 17.1/0.8%S
American Petroleum Institute Report 27-32774

(5) API (1980)
Acute toxicity tests
API 78-8 #6 Heavy fuel oil (API gravity 23.1/0.2%S
American Petroleum Institute Report 27-32816

(6) API (1980)
Acute toxicity tests
API 79-2 #6 Heavy fuel oil (API gravity 5.2/1.2%S
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American Petroleum Institute Med.Res.Publ. 30-31854

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(11) API (1985)
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In vivo sister chromatid exchange assay
API 81-15, catalytically cracked clarified oil
(CAS 64741-62-4)
American Petroleum Institute HESD Publ. 32-32254

(14) API (1985)
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in the rat bone marrow cytogenetic assay
in the mouse lymphoma forward mutation assay
catalytic cracked clarified oil API sample 81-15
American Petroleum Institute Med. Res. Publ. 32-30534

(15) API (1985)
Sister chromatid exchange assay in Chinese Hamster Ovary
(CHO) cells.
Catalytic cracked clarified oil.
API sample 81-15 CAS 64741-62-4
American Petroleum Institute Report No. 32-32750

(16) API (1985)
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hydrocarbon in rats (API 81-15) catalytically cracked
clarified oil (CAS 64741-62-4)
American Petroleum Institute Med. Res. Publ. 32-32753

(17) API (1986)
Four-week dermal range-finding toxicity study in rats
API 81-15, catalytically cracked clarified oil
(CAS 64741-62-4)
American Petroleum Institute HESD Publ. 33-30442

(18) API (1986)
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(CAS 64741-62-4)
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American Petroleum Institute, Washington, DC.

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