

**ROBUST SUMMARY
OF INFORMATION ON**

Substance Group: **PARAFFINIC NAPHTHAS**

Summary prepared by: American Petroleum Institute

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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** : Paraffinic naphtha streams are obtained by alkylation (catalytic reaction), isomerisation (catalytic conversion) and solvent extraction. They contain mostly saturated hydrocarbons, generally in the range C5 to C10 and boil in the range of approximately 90 to 160°C. The paraffinic naphthas typically are composed of the following hydrocarbon classes:

	Content (volume %)
Paraffins	99.4
Olefins	0
Naphthenics	0.6
Aromatics	0

Light Alkylate Naphtha (CAS 64741-66-8) is a typical paraffinic naphtha stream.

The American Petroleum Institute have reported a thorough characterization of a specific sample (API 83-19) of Light Alkylate Naphtha (LAN). The results of this characterization are as follows:

Characterization of API 83-19

Parameter	Method	Units	Value
Gravity	D287	°API	71.3
Density @15°C	D287		0.6970
Molecular weight	D2224	gm/mol	111
Refractive index @ 20°C		RI units	1.3925
Total Sulfur	D3120	ppm/wt	30
Total Nitrogen	Chemil.	ppm/wt	<1
Total Oxygen	NAA	wt%	<0.02
Total Chloride	coulom.	ppm/wt	3
RVP		psia	6.7
Distillation	D86	vol/°F	
IBP/5			98/135
End point			347
Motor Octane No. (MON)			93.5
Research Octane No. (RON)			94.1
Mass Spectrometer Analysis			
Paraffins	D2789	vol%	99.4
Naphthenes	D2789	vol%	0.6
Olefins	D2789	vol%	0
Aromatics	D2789	vol%	0
Indans/Tetralins	D2789	vol%	0
Naphthalenes	D2789	vol%	0
		TOTAL	100

1. General Information

Id P. Naphthas
Date May 20, 2003

Some mammalian toxicology studies have been carried out on a distillate fraction (LAN-D) of a sample of Light Alkylate Naphtha (LAN). The LAN-D and the LAN from which it was derived have been characterized as follows:

Parameter	Unit	LAN	LAN-D
Paraffins	vol. %	99.75	99.97
Olefins	vol. %	0	0.03
Naphthenes	vol. %	0.03	0
Aromatics	vol. %	0	0
Carbon number (vol. %)			
4		0.65	3.25
5		8.09	33.3
6		7.66	18.91
7		8.38	9.81
8		56.76	31.14
9		12.44	3.21
10		5.76	0.39

Ecotoxicity testing and environmental modeling studies have also been carried out on a sample of Light Alkylate Naphtha (LAN). A detailed hydrocarbon analysis (Chevron, 1995) of the sample used for these studies is as follows:

	Total %			Paraffins	
	Olefins	Naphthenes	Aromatics	n-	Total
Total	0	10.76	0.04	1.07	88.63
C4	0	0	0	0.84	0.84
C5	0	0	0	0.23	12.85
C6	0	0	0	0	7.06
C7	0	0	0	0	7.17
C8	0	9.44	0	0	57.58
C9	0	1.29	0	0	2.70
C10	0	0.03	0.04	0.04	0.44

(6) (9)

2.2 BOILING POINT

Value : 39 - 166 °C at
Remark : The paraffinic naphthas boil in the range of approximately 35 to 230 °C.
Sample API 83-19 had an initial boiling point of 135 °F and a final boiling point of 347 °F (equivalent to 57 and 175 °C respectively).

2.3 DENSITY

Type : Relative density
Value : .688 - .701 at 15 °C
Method : ASTM D1298
GLP : No data

(6) (7)

2.4 VAPOUR PRESSURE

Value : 7330 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes

Remark : The sample was identified by CONCAWE as MRD-95-092, gasoline sample CWE4, CAS No. 64741-70-4, isomerase naphtha.

See:
CONCAWE (1995)
Physico-chemical characterization of gasoline samples.
Study No. 104990C. Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

Reliability : (1) valid without restriction

Value : 7860 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes

Remark : The sample was identified by CONCAWE as MRD-95-045, gasoline sample W94/810, CAS No. 64741-70-4, isomerase naphtha.

See:
CONCAWE (1995)
Physico-chemical characterization of gasoline samples.
Study No. 104990C. Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995.

Reliability : (1) valid without restriction

2.5 PARTITION COEFFICIENT

Log pow : 3.11 - 4.54 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65
Year : 2000
GLP : No
Test substance : LAN, see section 1.1.1. for characterization

Remark : Log P values represent the spread of calculated and/or measured values for the C5 to C9 hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.

Reliability : (2) Valid with restrictions

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2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Method : Preparation of Water Soluble Fraction
Year : 1995
GLP : Yes
Test substance : LAN, characterized in section 1.1.1.

Method : Water Accommodated Fractions (WAFs) of LAN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 72 hours in tightly closed systems with minimal headspace.

Result : Gas chromatographic analysis of selected components indicated freshwater and saltwater solubilities of 1.6 and 0.9 ppm respectively. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Concentrations for these components reached equilibrium in freshwater and saltwater by 24 and 12 hours respectively.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LAN components range from <1 to approximately 30 mg/l. 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

(10) (11) (12) (13) (23)

3.1.1 PHOTODEGRADATION

Type : Calculation
Light source : Sun light
Method : Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year : 2000
GLP : No
Test substance : LAN, characterized in section 1.1.1.

Remark : AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O₃. Atmospheric oxidation rates were calculated for the C₅ to C₈ hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Based on a 12-hour day, the range for atmospheric half-lives for LAN constituents is: 1.074 days (2,3,5 trimethyl hexane) to 15.985 days (isopentane).

Result : Indirect Photolysis Yes
Sensitizer OH radical
Conc. of sensitizer 1.50E⁺⁰⁶ radicals/cm³
Rate Constant 0.6691E⁻¹² (isopentane) cm³/mol-sec
to 9.956E⁻¹² (2,3,5 trimethyl hexane)
Half-life 1.074 days to 15.985 days

Reliability : (2) Valid with restrictions

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3.1.2 STABILITY IN WATER

Conclusion : Hydrolysis unlikely
Reliability : (1) Valid without restriction

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3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment and sediment

Remark : Model based on chemical fugacity. Multimedia distribution was calculated for the C₅ to C₉ hydrocarbon components found in LAN, CAS No. 64741-66-8. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LAN sample. Mobility in the aquatic and terrestrial environment is low due to low water solubility and high vapor pressure. The naphtha components will partition rapidly to air, where for the majority of these hydrocarbons will be rapidly oxidized by OH radicals.

Result : Medium % distribution
 Air 99.4 to 100
 Soil 0.01 to 0.27
 Water 0.001 to 0.02
 Sediment <0.001
 Suspended sediment

Test substance : LAN sample is characterized in section 1.1.1.
Conclusion : This complex petroleum mixture is expected to partition primarily to air
Reliability : (2) Valid with restrictions

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3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Mixed, adapted inoculum of domestic activated sludge and soil
Contact time : 56 day(s)
Method : CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year : 1999
GLP : Yes
Test substance : LAN, characterized in section 1.1.1.

Method : Test type:
 Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO₂ headspace test)

Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO₂ production. By day 21 approximately 40% of the test material was degraded, a slight increase to 48% was observed by day 42, but by day 56 degradation had leveled back down to 40%. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63% actual) was observed by day 14, and total blank CO₂ production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21 °C, which deviated from the protocol value of 22 ±2°C. This deviation was not expected to have affected the outcome of this study.

Test Day	% Degradation (sd)	
	Hexadecane	Test Material
3	13.93 (1.85)	0.12 (0.07)
7	34.40 (4.54)	7.84 (7.80)
14	63.17 (0.94)	26.59 (0.85)
21	77.26 (6.52)	40.24 (5.00)
28	90.35 (7.14)	42.41 (2.54)
35	85.13 (n=1)	41.53 (9.90)
42	85.21 (n=1)	48.12 (1.77)
49	96.93 (8.94)	46.55 (1.04)
56	94.69 (4.10)	40.44 (0.76)

Test condition : Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two week adaptation period. Triplicate

test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of soil was obtained at a depth between 5-10 cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substance were added directly to test systems using a 10 microliter Hamilton gas-tight syringe. The volume required to achieve the specified mg carbon/l concentrations were calculated based on % carbon and specific gravity of the respective substance. The test substance % carbon (0.8505) and specific gravity (0.6690 mg/ μ l) information was supplied by the Sponsor. Hexadecane % carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/ μ l) was obtained from Verschueren (1983). Addition of respective substance was performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 (\pm 2°C) in the dark.

Biodegradation by CO₂ determination-test initiation and procedure
On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume (100 ml inoculum/l). Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/l concentration.

Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at

weekly sampling intervals for subsequent CO₂ analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22°C (±2°).

On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc H₃PO₄ was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, then analyzed for CO₂ using gas chromatography-thermal conductivity detection. Quantitation of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability

: (1) Valid without restriction

(19)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static with daily renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	Yes
Year	:	1994
GLP	:	Yes
Test substance	:	LAN, characterized in section 1.1.1.
Method	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002. LL ₅₀ and LC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84. All NOEC values calculated using Fisher's exact test.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 15, 20 and 20, respectively in 0, 1.1, 5.2, 9.7, 19 and 74 mg/l treatments. All surviving organisms exhibited normal behavior. 96-hr LL ₅₀ = 8.2 mg/l, (5.2-9.7 mg/l w/ 95% C.I.) as nominal loading rate 96-hr LC ₅₀ = 305 ppb, (164-384 ppb w/ 95% C.I.) measured concentrations 96-hr NOEL = 5.2 mg/l (as nominal loading rate) 96-hr NOEC = 166 ppb (measured concentrations)
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was Mobil Technical Center well water. Nominal loading rates of 0, 1.1, 5.2, 9.7, 19 and 74 mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl

hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes.

Fish were hatched and raised in-house, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8 liter glass containers with teflon lined caps. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving adequate volume to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Water temperature was 21.2 °C (0.2 °C sd).

Test photoperiod was 16 hrs. light and 8 hr dark.

Dissolved oxygen measurements were between 7.7 and 8.6,

pH values between 7.844 and 8.23.

Reliability

- : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

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4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

- Type** : 48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1994
GLP : Yes
Test substance : LAN, characterized in section 1.1.1.
Method : Statistical Method: (FT - ME) EL₅₀ and EC calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result : Immobility (no. of organisms) at 48 hrs: 0, 0, 0, 12, 13 and 20 for 0, 9, 18, 35, 70 and 140 mg/l treatments. At the 35 and 70 mg/l nominal treatments, 8 and 7 organisms were observed to show lethargic movement, respectively.

48-hr EL₅₀ = 32 mg/l (95% C.I. 18 to 140 mg/l) based upon nominal loading rate.

48 hr EC₅₀ was 556 µg/l (95% C.I. 339 to 1140 µg/l) based on total measured alkyl concentrations.

- 48-hr NOEL = 18 mg/l based upon nominal loading rate.
- 48 hr NOEC was 339 ppb based on total measured alkyl concentrations.
- Test condition** : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was aged well water.
- WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 1.2 liters of water for 24 hr in aluminum foil covered 1-liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately 45 minutes, then drained from the port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes.
- Range finding toxicity studies were conducted at 1.2, 9.9 and 99 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 9, 18, 35, 70, & 140 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.
- Test vessels were teflon cap-sealed 237 ml glass jars with 10 daphnids per jar and were completely filled with test solution.
- During the study test system solutions:
 dissolved oxygen concentration range: 8.0 to 8.5
 pH ranged from 8.00 to 8.2
 temperature was 19.1 to 21.0 °C
 hardness (mg/l) ranged from 180 – 204
 alkalinity (mg/l) was 140-156
 conductivity (µmhos) values were 385 - 390.
- Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1994. The primary culture was obtained from Aquatic Research organisms, Hampton, NH, which was derived from EPA laboratory culture, in Cincinnati, Ohio.
- Reliability** : (2) Valid with restrictions
 Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

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4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
 Exposure period : 96 hour(s)
 Unit : mg/l
 Limit test : No
 Analytical monitoring : Yes
 Method : EPA. 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-8, ES-5.
 Year : 1995
 GLP : Yes
 Test substance : LAN, characterized in section 1.1.1.

Method : Statistical Method: EL₅₀ and EC₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.

Result : All NOEL/NOEC values calculated using Fisher's exact test.
 : Percent inhibition on growth determined by cell density (cells/ml):

96 hour EL₅₀=45mg/l (18-70 mg/l CI @95%)
 96 hour EC₅₀=741µg/l (353-1060 µg/l CI @95%)
 96 hour NOEL=18 mg/l
 96 hour NOEC=741µg/l

Subcultures placed in fresh media (no test substance) after acute testing for nine days indicated that growth inhibition was algistatic in all treatments. No excursions from the protocol were noted. However, range finding and two previous definitive tests were performed and considered inconclusive due to inconsistencies in control and treatment cell densities, which presumably were resolved by modification of the AAP media. Additionally, control growth showed a lag during the first 48 hours of the study.

Concentration (µmg/l)		96hr cell density (cells/ml)	(% Inhibition)
Nominal	Measured.		
Control		5.7x10 ⁴	na
18	0.112	5.53x10 ⁴	3.1
70	0.305	1.27x10 ⁴	77.7
146	0.498	3.46x10 ³	93.9
292	0.610	1.36x10 ³	97.6
1157	0.612	1.60x10 ³	97.2

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.3 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, pH adjusted to 7.5 ± 0.1 with 0.1 NHCl and sterilized by 0.22 micron filtration) in 2.0 liter aspirator bottles, covered with aluminum foil. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass

Erlenmeyer flasks that were completely filled (135 ml) with treatment solution and inoculated with algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media, and transferred every 5-9 days to fresh media. Original algal cultures obtained from American Type Culture Collection (ATCC Strain 22662), Rockville, MD, June 1994. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 18, 70, 146, 292 and 1157 mg/l. The initial algal concentration was 1.0×10^3 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 400 ± 50 -ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0, 24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3 dimethyl butane; 2,4 dimethyl pentane; 2,2,4 trimethyl pentane; 2,5 dimethyl hexane; 2,3,4 trimethyl pentane, 2,3,3 trimethyl pentane and 1-methyl-1-ethyl cyclopentane, which represent 68% composition of the test substance. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of all analytes.

Test temperature was $24 \pm 2^\circ\text{C}$. The pH was 7.5 at test initiation, pH value at test termination not included in report.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

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4.5.1 CHRONIC TOXICITY TO FISH

Species : Pimephales promelas (Fish, fresh water)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD 204
Year : 1999
GLP : Yes
Test substance : LAN, characterized in section 1.1.1.

Method : LL_{50}/LC_{50} and EL_{50}/EC_{50} calculated using linear interpolation. NOEL/NOEC for survival determined by Steel's Many-One Rank Test. NOEL/NOEC for growth determined by Williams Test. TOXSTAT program was used to determine endpoints.

Result : The mean measured concentrations for nominal loading rates of 0.44, 1.0, 2.6, 6.4, 16, and 40 mg/l were 0.011, 0.021, 0.041, 0.10, 0.38, and 0.62 mg/l representing the average of total analytes measured in the new and old WAFs. The

average total analyte concentration in the controls was 0.005 mg/l.
 14-d LL_{50} for survival = 8.0 mg/l (95% C.I. 5.4 - 9.8),
 14-d LC_{50} for survival = 0.15 mg/l (95% C.I. 0.073 - 0.20)
 14-d NOEL for survival = 2.6 mg/l,
 14-d NOEC for survival = 0.041 mg/l.
 Mortality (no. of deaths/treatment) at 14 days: 0, 0, 3, 2, 16, 40, and 40 in the 0, 0.44, 1.0, 2.6, 6.4, 16, and 40 mg/l treatments. All surviving fish in the 6.4 mg/l treatment were lethargic.
 14-d NOEL for growth = 2.6 mg/l,
 14-d NOEC for growth = 0.041 mg/l.
 14-d EL_{50} and EC_{50} for growth could not be calculated because none of the treatment group means were <50% of control.

Since there were significant mortality at the three highest treatments, these treatments were excluded in the analysis of growth data. The mean (standard deviation) for dry weights were 4.08 (0.26), 4.28 (0.20), 4.69 (0.43), and 4.85 (0.38) in the 0, 0.44, 1.0, and 2.6 mg/l treatments.

Dissolved oxygen concentrations in the aged exposure solutions at all loading rates were occasionally below 60% of saturation between day 10 and day 14 due to oxygen consumption by fish and bacteria in the closed test systems and could not be avoided. Light intensity was not measured during the study due to an oversight and had no impact on the results of the study.

Test condition

: Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 170-180 mg/l as $CaCO_3$, total alkalinity of 120mg/l as $CaCO_3$, pH range of 8.0 to 8.2, and a specific conductivity of 490-500 mmhos/cm. Nominal loading rates of 0, 0.44, 1.0, 2.6, 6.4, 16, and 40mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.69 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 1 to 1.25 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with fresh WAFs in which 80% of the old solutions were siphoned and excess debris removed from the exposure vessel prior to refilling with fresh WAF. Renewed solutions were then siphoned again and refilled a second time to achieve an exposure solution of ~96% fresh WAF. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following:

2,3-dimethylbutane, 2,4-dimethylpentane, 2,2,4-trimethylpentane, 2,5-dimethylhexane, 2,3,3-trimethylpentane, and 2,3,4-trimethylpentane. Measured test concentrations of the light alkylate naphtha were based on the concentrations of all analytes. Fish were hatched and raised from laboratory in-house culture. Fish were 8 days old at the start of the test. Test vessels were 1 liter screw-capped glass jars containing 980 ml of WAF with minimal headspace. Four replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Fish were fed 0.15 ml of live brine shrimp nauplii (<48 hr old) twice daily during the test. Water temperature was 24 to 26° C. Test photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen concentrations were 8.7 to 8.9 in the new solutions and 5.7 to 7.8 in the old solutions. pH values were 7.3 to 8.2.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations. Dissolved oxygen in the test solutions were occasionally below 60% of saturation.

(20)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 211
Year : 1999
GLP : Yes
Test substance : LAN, characterized in section 1.1.1.

Method : For NOEL/NOEC, Fisher's Exact Test was used for survival of adult daphnids and Kruskal-Wallis Test with Dunn's Multiple Comparison was used for reproduction. For EL₅₀/EC₅₀, reproduction data were analyzed by linear interpolation. Survival data were not analyzed because survival was >50% at all loading rates. TOXSTAT program was used to determine the endpoints.

Result : The mean measured concentrations for nominal loading rates of 0.44, 1.0, 2.6, 6.4, 16, and 40 mg/l were 0.010, 0.016, 0.032, 0.084, 0.23, and 0.46 mg/l representing the average of total analytes measured in the new and old WAFs. The average total analyte concentration in the controls was 0.005 mg/l.
 21-d EL₅₀ for survival = >40 mg/l
 21-d EC₅₀ for survival = >0.46 mg/l
 21-d NOEL for survival = 16 mg/l
 21-d NOEC for survival = 0.23 mg/l.
 Daphnid immobilization at 21 days: 0, 2, 0, 0, 0, 1, and 4 in the 0, 0.44, 1.0, 2.6, 6.4, 16, and 40 mg/l treatments.
 21-d EL₅₀ for reproduction = 10 mg/l (95% C.I. 8.7-11)
 21-d EC₅₀ for reproduction = 0.14 mg/l (95% C.I. 0.12-0.16)

21-d NOEL for reproduction = 2.6 mg/l

21-d NOEC for reproduction = 0.032 mg/l.

Since there was significant immobilization in the highest treatment, it was excluded in the analysis of reproduction data. The mean numbers (standard deviation) of offspring released per female daphnid were 137 (11), 125 (7), 125 (6), 117 (20), 96 (21), and 28 (10) in the 0, 0.44, 1.0, 2.6, 6.4, and 16 mg/l treatments. The numbers of offspring released in the 6.4 and 16 mg/l treatments were significantly less than the controls. First brood release for organisms exposed to =16 mg/l and the controls occurred by day 8.

Test condition

: Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 170-180 mg/l as CaCO₃, total alkalinity of 120-130 mg/l as CaCO₃, pH range of 8.0 to 8.2, and a specific conductivity range of 500-550 mmhos/cm. Nominal loading rates of 0, 0.44, 1.0, 2.6, 6.4, 16, and 40 mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.69 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 1 to 1.5 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with 70 ml of fresh WAFs added to a second set of beakers. Food was added to the fresh WAFs and daphnids were then transferred from the old test solutions to the fresh WAFs. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following: 2,3-dimethylbutane, 2,4-dimethylpentane, 2,2,4-trimethylpentane, 2,5-dimethylhexane, 2,3,3-trimethylpentane, and 2,3,4-trimethylpentane. Measured test concentrations of the light alkylate naphtha were based on the concentrations of all analytes. Daphnids used in the test were from laboratory in-house culture. Daphnids were =24 hrs old at the start of the test. Test vessels were 70 ml screw-capped glass jars containing 70 ml of WAF with minimal headspace. Ten replicates per treatment and 1 daphnid per replicate were tested for each treatment and the control. Daphnids were fed 0.2 ml of algal suspension (*Ankistrodesmus falcatus*, 4 x 10⁷ cells/ml) and 0.05 ml of a yeast, cereal leaves and digested flaked fish food (YCT) suspension daily during the test. Water temperature was 19 to 21° C. Test photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen concentrations were 9.1 to 9.2 in the new solutions and 8.7 to 9.4 in the old

Reliability : solutions. pH values were 7.5 to 8.5.
(2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

(21)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD ₅₀
Value	:	> 7000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	Undiluted
Year	:	1986
GLP	:	Yes
Test substance	:	Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1
Method	:	Groups of five male and five female fasted rats were given API 83-19 at doses of 5 and 7 g/kg as a single oral dose. The animals were then allowed food and water ad libitum and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded.
Result	:	Clinical signs seen during the study included: hypoactivity, diarrhea, yellow-stained anal area, red discharge from nose, blood-like discharge on or around penile area, pale appearance and one female in the 5 g/kg group died within one hour of dosing. All except two animals had returned to normal by day 3 of the study. The oral LD ₅₀ was found to be greater than 7 g/kg
Reliability	:	(1) Valid without restriction

(4)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC ₅₀
Value	:	> 5 mg/l
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	Air
Exposure time	:	4 hour(s)
Year	:	1987
GLP	:	Yes
Test substance	:	Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-19 at a nominal concentration of 5mg/l for 4 hours. This was achieved by total volatilization of the test material and appropriate dilution with air.

	<p>After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.</p>
Result	<p>: The mean analytical and nominal exposure concentrations were 5.04 ± 0.74 and 6.31 mg/l respectively. All animals survived the study but exhibited languid behavior and a hunched appearance during the exposure. Female body weights were decreased at day 15 but this was attributed to pre-necropsy fasting. At necropsy there were no remarkable findings and histopathology of the lungs was normal.</p>
Reliability	<p>: (1) Valid without restriction</p>

(5)

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	: 4
Year	: 1986
GLP	: Yes
Test substance	: Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1
Method	<p>: The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to a dose of 2 g/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. 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 and the animals were observed for a total of 14 days post-dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.</p>
Result	<p>: A pain response (vocalization) was elicited from all the animals following application of the test material. Apart from skin irritation there were no other clinical signs of toxicity. Skin irritation ranged from slight to severe for erythema and edema, slight to moderate for atonia and coriaceousness and from slight to moderate for desquamation and fissuring. Subcutaneous hemorrhage, blanching and eschar was also observed.</p>
Reliability	<p>: (1) Valid without restriction</p>

(4)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Remark : Not applicable
25.10.2002

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 3.9
Result : Moderately irritating
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1

Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. 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 and again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : The scores for erythema and edema were marginally greater for intact skin than abraded skin, but the difference was not biologically significant.
 Scores for intact skin at each of the observation intervals were:

Time	Erythema	Edema	Irritation score*
24 hours	2.0	1.7	3.5
72 hours	2.5	2.2	4.2
96 hours	2.7	2.8	4.9
7 days	2.5	2.3	4.5
14 days	0.8	1.0	1.2

* Irritation score calculated as the sum of irritation scores for each test site divided by the number of animals at each observation period.

PII is the sum of the 24- and 72- hour total irritation scores divided by 2

Reliability : (1) Valid without restriction

(4)

5.2.2 EYE IRRITATION

Species	:	Rabbit
Concentration	:	Undiluted
Dose	:	0.1 ml
Exposure time	:	
Comment	:	Eyes rinsed 20-30 seconds after exposure (3 rabbits), Eyes not rinsed in remaining animals
Number of animals	:	9
Vehicle	:	None
Result	:	Not irritating
Year	:	1986
GLP	:	Yes
Test substance	:	Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1
Method	:	0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 20 to 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	:	No pain response was elicited on instillation of test material. No corneal or iridial irritation was seen during the study
Reliability	:	(1) Valid without restriction

(4)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1 st : Induction 50 % occlusive epicutaneous 2 nd : Challenge 25 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	Paraffin oil
Result	:	Not sensitizing
Year	:	1986
GLP	:	Yes
Test substance	:	Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1
Method	:	0.4 ml of a 50% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.4 ml of a 25% mixture in paraffin oil) 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.

- Result** : Positive control (2,4-dinitrochlorobenzene), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups. At challenge, a very slight erythema was exhibited by one animal. The other 9 animals had no response. In contrast all 20 of the positive controls responded with reactions ranging from slight to severe irritation. Only one naive control exhibited a very slight erythema upon challenge.
- Reliability** : (1) Valid without restriction (4)

5.4 REPEATED DOSE TOXICITY

- Species** : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 13 weeks
Frequency of treatm. : 6 hours/day, 5 days/week
Doses : 668, 2220, 6646 ppm
Control group : Yes
NOAEL : = 2220 ppm
Method : OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year : 1998
GLP : Yes
Test substance : Distillate of Light alkylate naphtha

- Method** : Groups of 12 male and 12 female rats underwent whole body exposures to 668, 2220 and 6646 ppm LAN-D. Exposures were for 6 hours each day, 5 days per week for 13 weeks. Extra groups of 12 rats of each sex were exposed to the high dose level and also for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 13 weeks exposure.

Neurobehavioural evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14 and 18 recovery groups. Animals were not exposed to LAN-D during these tests.

Following 13 weeks of exposure, 12 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically.

At the end of the 4 week recovery period, 12 animals of each sex from the high and control groups were necropsied and

selected tissues were examined microscopically.

During the study clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and 18 weeks (recovery groups). Body weights and food consumption was measured throughout the study. Blood samples were taken from 12 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements.

At termination (after 13 weeks exposure for the main study and after 18 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) were removed from the control and high dose animals, were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination. Nervous system tissues were selected randomly from 6 rats/sex/group in the high dose and controls at the end of 13 weeks for microscopic examination. Specific brain regions examined were forebrain, cerebral cortex, hippocampus, basal ganglia, midbrain cerebellum and pons and medulla.

Neurobehavioural studies were undertaken as follows:

Motor activity

Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 14 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery

An assessment of the following was made:

- Home cage evaluations for Posture, vocalization, palpebral closure.
- Handling evaluations for reactivity to general stimuli, signs of autonomic function.
- open field behavior: arousal level, gait, urination and defecation frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.
- Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result : There were no mortalities during the study and there were no treatment related signs of toxicity. A possible treatment related sign was an increased incidence of red facial staining in rats of both sexes in the high dose group. Mean body weights, body weight gains and food consumption was unaffected by treatment.

Hematological and clinical chemical measurements were unaffected except for a 5% decrease in hemoglobin, a 5% decrease in hematocrit and a 7% decrease in erythrocytes. The hemoglobin was still decreased (4%) after the 4 week recovery period. However, it was considered that these differences were toxicologically unimportant because they were small and within the historical range for the test laboratory.

Although there were some changes in AST and ALT in high dose females they were not considered to be toxicologically significant because several control animals also had elevated levels for these enzymes in the control groups and also relative to historical controls.

The organ weight changes were few. Absolute and relative kidney weights were increased in the males at all dose levels and they were also elevated in the high dose recovery animals. These increases correlated with the finding of hyaline droplets in the proximal convoluted tubules at microscopy.

Absolute and relative liver weights were observed in the high dose males and females at 13 weeks but the differences had disappeared after the recovery period. There were no pathological findings associated with this increase. The magnitude of the organ weight increases is shown below.

	Dose level (ppm)			
	668	2220	6646	Recovery
Males				
Abs. Kidney wt.	13.2	19.8	27	23
Rel. Kidney wt.		18	30	11
Abs. Liver wt.			21	
Rel. Liver wt.			25	
Females				
Abs. Liver wt.			17	12
Rel. Liver wt.			12	

In the neurobehavioral studies no treatment-related effects were observed in the functional operational battery. In the study of motor activity there were some statistically significant differences, but overall they did not occur in a dose related manner and furthermore were smaller than some of the differences seen during the pre dosing period.

Test condition : The test atmospheres were generated by wholly vaporizing the test material (LAN-D) and diluting with air to achieve the required concentrations. The highest concentration was approximately 75% of the lowest explosive limit.

Nominal concentrations were calculated from the loss of weight from the generation apparatus divided by the total airflow through the chamber during exposure.

Actual exposure concentrations were determined three times daily by gas chromatography.

Particle size determinations were also carried out once during each exposure using an aerodynamic particle sizer. Mean mass aerodynamic diameter (MMAD), geometric standard deviation (GSD) and total mass concentration (TMC) were calculated.

The nominal and actual concentrations for each of the target dose levels were:

Dose group (ppm)	Nominal (ppm)	Actual TMC* (ppm) ($\mu\text{g}/\text{m}^3$)	
0 (Control)	0	0	3.8
675	719	668	3.7
2250	2073	2220	3.9
6750	7127	6669	4.2
6750 (recovery)	6768	6623	3.2

* TMC = Total Mass Aerosol Concentration

Test substance : The test material (LAN-D) was a distillate of a Light Alkylate Naphtha (LAN).

The compositions of the LAN and the LAN-D derived from it are shown in section 1.1.1. above.

The composition and uniformity chamber gas chromatographic results (% weight) were:

Component	Liquid	Vapor	
		At Start	Termination
n-butane	2.442	3.217	3.210
iso-pentane	29.854	33.517	34.343
2,3-dimethylbutane	12.437	11.963	12.977
2-methylpentane	4.064	4.775	4.096
2,4-dimethylpentane	5.923	5.663	5.663
2,3-dimethylpentane	2.904	2.794	2.680
2,2,4-trimethylpentane	18.35	16.897	16.885
2,3,4-trimethylpentane	4.343	3.772	3.578
2,3,3-trimethylpentane	5.258	4.614	4.505
2,2,5-trimethylhexane			

3.096 2.641 2.499

Conclusion : Chamber concentrations were monitored throughout the study. Actual chamber concentrations were close to target concentrations. Particle mass distribution measurements confirmed that no measurable test material was present as aerosol. LAN-D was not a neurotoxicant in the neurobehavioral studies that were conducted. LAN-D did induce a light hydrocarbon nephropathy in the male rats at all exposure levels, but this is regarded as species and sex specific and not relevant for human health risk assessment. Excluding the nephropathy, the NOEL for subchronic toxicity was 2220 ppm and for neurotoxicity was 6646 ppm.

Reliability : (1) Valid without restriction (18)

Type :
Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 28 Days
Frequency of treatm. : Once per day, three times per week for 4 weeks
Doses : 200, 1000 & 2000 mg/kg
Control group : Yes
Year : 1986
GLP : Yes
Test substance : Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1

Method : Prior to the study 5-day range finding study was conducted. The method and results of the range-finding study are not included in this summary.

Undiluted API 83-19 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours which was then removed and the skin was wiped with a dry gauze to remove any residual material. A group of five rabbits of each sex served as sham controls. The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly. At termination blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination. A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination.

Result : No deaths occurred during the study. During the latter half of the study all but one high dose

female looked thin. This was considered to be a treatment-related effect. Apart from skin irritation there were no other treatment-related clinical signs. Weight gains of treated animals over the duration of the study was similar to controls except for the females at 2000 mg/kg. which were significantly reduced. The mean weight for these rabbits was the same at the end of the study as it was on day 1.

A mean irritation score was calculated for each day and overall means were also calculated. (The mean irritation score, MIS, was the sum of irritation scores for both erythema and edema for all animals of a given dose group and sex.)

The overall MIS for each dose group was:

<u>Group/sex</u>	<u>MIS</u>	<u>Classification</u>
2000 mg/kg M	3.5	Moderate irritant
2000 mg/kg F	3.6	Moderate irritant
1000 mg/kg M	2.8	Moderate irritant
1000 mg/kg F	2.7	Moderate irritant
200 mg/kg M	0.5	Minimal irritant
200 mg/kg F	0.5	Minimal irritant
Control M	0	Non irritant
Control F	0	Non irritant

There were no remarkable findings in the hematological data from any of the male or female groups compared to controls.

The only significant clinical chemical finding was an approximately 40% reduction in ALP of the 2000 mg/kg females. All other clinical chemical measurements were unremarkable.

There were few differences in organ weight between the control and treated animals, these were:

- 18% increase in R adrenal weight in 1000 mg/kg males
- 28% increase in L adrenal weight in 1000 mg/kg males
- 37% decrease in R ovary weight of 2000 mg/kg females.

In none of the above was there an associated change in the relative organ weights and the differences were not considered to be treatment-related.

At gross necropsy, treatment - related skin findings consisted of: dry, scaly, rough, fissured, reddened, crusted, and/or thickened skin. There were no other treatment-related findings at necropsy.

Although there were some findings at histopathology, they were not treatment-related except those in the skin.

The skin changes consisted of a slight to moderate proliferative and minimal to moderately severe inflammatory changes in the skin of all animals in the 2000 mg/kg groups.

These skin changes were accompanied by an increased granulopoiesis of the bone marrow. This was considered to be possibly related to stress or other factors resulting from

skin irritation.

Reliability

: (1) Valid without restriction

(3)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Metabolic activation : With and without
Result : Negative
Year : 1985
GLP : Yes
Test substance : Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1

Method : The test material was dissolved in acetone for this assay. Two positive control substances were used viz Ethyl methane sulphonate (EMS) at concentrations of 1.0 & 0.5 µl/ml and 7, 12-DMBA at concentrations of 7.5 & 5.0 µg/ml.

A cytotoxicity study was carried out prior to the mutagenicity assay. The results were difficult to interpret and as a consequence a second study was carried out and the results from this were used to determine the concentrations to be used in the subsequent lymphoma assay. It was established that complete toxicity occurred at 0.05 µl/ml for the non-activated cultures and at 0.5 µl/ml for S-9 activated cultures.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with Aroclor-induced rat liver S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; trifluorothymidine (TFT) was used as the restrictive agent.

Eight non-activated and nine activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 or 0.04 µl/ml and resulted in a range of growth of 6 to 97%. The activated cultures that were cloned were treated with 0.0002, 0.0009, 0.0028, 0.008, 0.02, 0.045, 0.09, 0.7 or 0.75 µl/ml and produced a range of growth from 24 to 109%. Plates were prepared from TFT-restricted and from the Viable cultures (VC) and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was then determined.

The following criteria were used in judging the significance of the activity of the test article.

Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

- Remark** : Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.
- Remark** : Six mouse lymphoma assays were conducted but for technical reasons four of the assays were invalid. In the fifth assay none of the cultures that were cloned, whether in the presence or absence of S-9 activation exhibited mutant frequencies that were greater than those for the solvent control. However, the toxic response in the S-9 activation portion of the assay was erratic and this portion of the assay was repeated. This summary includes information from the fifth and sixth assays only, since they are the only ones considered to be valid.
- Result** : The results of the fifth assay are as follows: After the 2 day recovery period, eight non-activated cultures and nine S-9 activated cultures were cloned based on their degree of toxicity. The mutant frequencies and the percentage total growth at each of the test concentrations is summarized in the following table.

Concentration (μl/ml)	Mutant frequency	% Total growth
<u>Non-Activated</u>		
0.04	0	34
0.035	0.5	3
0.03	0.2	30
0.025	0	46
0.02	0	93
0.015	-0.2	102
0.01	0	79
0.005	0	93
Solvent 1	0.5	
Solvent 2	0.6	
DMBA 7.5 μ l/ml	3.6	27
DMBA 5 μ l/ml	1.9	57
<u>S-9 Activated</u>		
0.75	0.2	101
0.7	0.2	16
0.09	0	88
0.045	-0.1	107
0.02	0	107
0.008	0.1	104
0.0028	0	100
0.0009	0	113
0.0002	-0.1	111
Solvent 1	0.6	
Solvent 2	0.6	
EMS 1 μ l/ml	8.7	3
EMS 0.5 μ l/ml	6.8	29

The sixth assay was with S-9 activation only and the results were as follows:

<u>S-9 Activated</u>		
0.8	0.2	50
0.75	0	84
0.7	-0.1	90
0.65	-0.4	143
0.6	-0.1	99
0.5	-0.1	18
0.45	0.1	89
0.4	-0.1	72
0.35	0.1	76
0.25	-0.3	31
Solvent 1	0.8	
Solvent 2	0.8	
DMBA 7.5 µl/ml	1.4	62
DMBA 5 µl/ml	1.1	86

The authors concluded that according to the criteria used to judge the activity of the test material, the sample produced a negative response in the presence and absence of S-9 activation.

Reliability : (2) Valid with restrictions

(2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : Up to 48 hours after single dose
Doses : 0.3, 1.0 & 3.0 g/kg
Result : Negative
Year : 1985
GLP : Yes
Test substance : Sample API 83-19 is a Light Alkylate Naphtha (LAN) and is characterized in section 1.1.1

Method : The study design was as follows:

Treatment	Animals/sex/sacrifice time		
	6 hrs.	24 hrs.	48 hrs
Corn oil (vehicle)	5	5	5
API 83-19, 3 g/kg	5	5	5
API 83-19, 1 g/kg	5	5	5
API 83-19, 0.3 g/kg	5	5	5
Triethylenemelamine (Positive control)		5	

Test material in vehicle was given intraperitoneally at a

dose of 5 ml/kg to groups of rats as shown above. Corn oil was used as vehicle control and TEM (0.5 mg/kg) as the positive control

Two to four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1 mg/kg).

2 Males and one female in the high dose group died, these were replaced by substitute animals that were killed approximately 50 hours after administration of the test material.

Immediately after sacrifice bone marrow was obtained from the femurs of the animals. The marrow was washed and the cells were fixed before being spread on slides (at least 3 from each animal) for examination.

Slides were scored for chromosomal aberrations.

Where possible, a minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy (1-3).

A mitotic index (= No. of cells in mitosis/500 counted X 100) was calculated and recorded.

The data were evaluated according to the following criteria:

For the test to be considered to be valid, the % of cells in the negative control group demonstrating aberrations of any type, other than gaps, must not exceed 4%.

The % of cells with aberrations in the positive control group must be statistically increased (p=0.05) relative to the vehicle control using Chi-square statistics.

The test material is considered positive when the % of cells with aberrations in any treatment group is significantly increased (p = 0.05) relative to the vehicle control using Chi-square analysis and the number of aberrations per cell is also significantly increased (p =0.05) relative to the vehicle control using t-test statistics.

Result : The dose levels used in the assay were selected on the basis of a preliminary screen in which only one male rat died within 24 hours following the administration of API 83-19 as a single i.p. dose to 4 rats of each sex. In the cytogenetics assay, 5 of 18 males and 4 of 18 females receiving 3 g/kg API 83-19 died within 3 days. At this dose level, there was a weight loss of 10% and 9% in males and females respectively within 48 hours of administration. Other signs of toxicity included piloerection, crusty eyes and noses and excess lacrimation. No sex-related differences were noted in the study and therefore the data for males and females were combined for the cytogenetics evaluation. The results are summarized in the following table.

	0.3 g/kg	1 g/kg	3 g/kg	Positive	Vehicle
<u>Cells with aberrations</u>					
6 hrs	0	2	0		0
24 hrs	1	0	1	171	0
48 hrs	1	0	1		0

<u>Incidence of aberrations (%)</u>					
6 hrs	0	0.4	0		0
24 hrs	0.2	0	0.2	34.2	0
48 hrs	0.2	0	0.3		0
<u>No. Gaps</u>					
6 hrs	0	2	0		0
24 hrs	0	0	0	15	1
48 hrs	0	0	4		1
<u>No. Breaks</u>					
6 hrs	0	2	0		0
24 hrs	1	0	1	197	0
48 hrs	2	0	1		0
<u>Aberrations per cell</u>					
6 hrs	0	0.004	0		0
24 hrs	0.002	0	0.002	2.336	0
48 hrs	0.004	0	0.003		0

NB.1. 500 cells were evaluated for each time point at each dose level.

NB.2. In the API 83-19 and vehicle control groups no rearrangements were observed and no aberrations from severely damaged cells were seen. In contrast 51 rearrangements and 920 aberrations from severely damaged cells were seen in the positive control group.

Reliability : (1) Valid without restriction

(1)

5.8.1 TOXICITY TO FERTILITY

Type : One generation study
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours per day
Frequency of treatm. : Daily
Premating exposure period
 Male : 14 days
 Female : 14 days
Duration of test : Females 7 weeks, males 8 weeks
Doses : 5, 12.5 and 25 g/m³
Control group : Yes
NOAEL parental : > 24700 mg/m³
NOAEL F1 offspring : > 24700 mg/m³
Method : Adaptation of OECD No. 421
Year : 1995
GLP : Yes
Test substance : Distillate of light alkylate naphtha (LAN-D)

Method : The test material was totally vaporized and diluted with air to achieve the desired concentrations for the study. Exposures were conducted in one cubic meter whole-body chambers. Chamber concentrations were monitored three times daily by GC/FID. All animals were housed individually in suspended mesh cages.

10 animals of each sex were exposed 6 hours each day to test material at target concentrations of 5, 12.5 and 25 g/m³. The animals were exposed for 6 hours each day. Parental females were exposed for 14 days prior to mating, throughout mating and gestation days 0-19 (7 consecutive weeks). Dams and their litters were sacrificed on postpartum day 4. Parental males were also exposed for 14 days prior to mating, during mating, throughout the female gestation and post partum period and throughout the female necropsy period (8 consecutive weeks). Rats were mated in a 1:1 ratio and females were monitored for evidence of mating by the examination of a vaginal lavage sample for sperm or vaginal plug. If sperm or a vaginal plug were observed the female was considered to be at day 0 of gestation and the male was removed from the female at this stage. If there was no evidence that mating had occurred the pairs were allowed to remain together up to a period of 2 weeks after which time the female was assumed to be pregnant. All animals were observed for clinical signs at least twice daily throughout the study. Body weights and food consumption were recorded throughout the study. Each litter was examined as soon as possible after delivery to establish number and sex of pups, stillbirths, live births and presence of gross abnormalities. Neonatal survival was monitored and all pups were killed postpartum days 4 or 5. Parental females were killed on gestation day 25 if they had not delivered, otherwise they were killed on postpartum days 4 or 5. At necropsy each parental animal was examined macroscopically for structural abnormalities and pathological changes with emphasis on reproductive organs. Additionally the number of implantation sites and corpora lutea of each female were recorded. Lungs, trachea and larynx were removed in their entirety. The right middle lobe of the lung was weighed, the remaining lobes were fixed for subsequent histopathological examination. The testes and epididymes of the males were weighed and then fixed for histological examination as were the ovaries of the females.

Remark : This study has also been reported in the open literature (Bui et al, 1998) but the open literature publication does not contain as much information as the original laboratory report summarized here.

Result : The chamber concentrations of test material were found to be between 96 and 104% of nominal, the mean highest dose concentration being 24.7 mg/m³. The vapor compositions were also found to be similar to that of the parent test material. No parent animals died or were killed during the study and there were no clinical signs. Body weights and food consumption were unaffected by exposure to test material. Results on reproductive capacity and fertility are summarized in the following table.

Parameter	Treatment group (g/m ³)			
	0	5	12.5	25
Pregnancy (%)	80	80	100	80
Litters with live pups	8	8	9	8
Implantation sites	14.9	16.8	13.9	17.3

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Pups delivered	14.4	15.6	14.3	15.6
Live pups/litter	14.4	14.8	13.8	15.5
No. liveborn	115	118	124	124
Live birth index (%)	100	94	96	99
Pups surviving 4 days	113	114	122	123
Viability index (%)	98	97	98	99
Pup wt./Litter day 1	7.2	7.3	7.1	7.1
Pup wt./Litter day 4	10.8	11.1	11.2	10.5

Test substance : There were no treatment-related findings observed at necropsy. Organ weights were unaffected by treatment and there were no treatment-related histological findings.

The test material (LAN-D) was prepared to be representative of the fraction of light alkylate naphtha to which man would normally be exposed during normal handling and use. It was obtained by the distillation of light alkylate naphtha (LAN) and collecting that fraction that boiled over the temperature range 78 to 145°F. The sample was analyzed and its composition compared to the light alkylate naphtha from which it was derived (See section 1.1.1. above).

The compositions of the distillate and starting material were as follows:

Compound	Weight %	
	LAN-D	LAN
n-butane	3.42	0.84
isopentane	63.59	12.61
n-pentane	1.33	0.23
2,3-dimethylbutane	22.51	4.74
2-methylpentane	6.44	1.57
3-methylpentane	2.26	0.74
2,4-dimethylpentane	0.29	4.09
2,2,4-trimethylpentane	0.06	23.92
2,3,3-trimethylpentane	0	8.99
2,3,4-trimethylpentane	0	11.56

Reliability : (1) Valid without restriction

(8) (22)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

- Species** : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours per day
Frequency of treatm. : Daily
Duration of test : Females 7 weeks, males 8 weeks
Doses : 5, 12.5 and 25 g/m³
Control group : Yes
Method : Adaptation of OECD No. 421
Year : 1995
GLP : Yes
Test substance : Distillate of light alkylate naphtha (LAN-D)
- Method** : This study forms part of the fertility study described in section 5.8.1. where the method is also described.
 For the examination for developmental effects, the pups were sacrificed on day 4 or 5 post partum and were necropsied and examined grossly for any abnormalities.
- Remark** : This study has also been reported in the open literature (Bui et al, 1998) but the open literature publication does not contain as much information as the original laboratory report summarized here.
- Result** : At necropsy, the following incidence of observations (which were not dose related) was recorded:

	Dose group			
	0 N (%)	5 N (%)	12.5 N (%)	25 N (%)
Litters examined	8	8	9	8
Pups examined	113	114	122	123

Observations (Litter incidence)

LIVER

Pale left lateral lobe

0 (0) 1 (0.9) 0 (0) 0 (0)

Patchy tan area both surfaces, all liver lobes

1 (0.9) 0 (0) 0 (0) 0 (0)

LIMBS

Broken rt hind limb

0 (0) 0 (0) 1 (0.8) 0 (0)

THORACIC CAVITY

Adhesion between apex of heart and diaphragm

0 (0) 0 (0) 0 (0) 1 (13)

HEAD

Red focus on rt. side of brain

0 (0) 0 (0) 0 (0) 1 (13)

Red focus on meninges

0 (0) 1 (13) 0 (0) 1 (13)

Depression on right ventricle

0 (0) 1 (0.9) 0 (0) 0 (0)

Red focus (1mmx1mm) on top of brain

	1 (13)	1 (13)	0 (0)	0 (0)
TAIL				
Fleshy tab at tip of tail	1 (13)	0 (0)	0 (0)	0 (0)
V ring (constriction)	0 (0)	0 (0)	0 (0)	1 (0.8)
Necrotic tail tip	1 (13)	1 (13)	0 (0)	0 (0)
TOTAL PUP NECROPSY OBSERVATIONS				
Pup	4 (3.5)	6 (5.3)	1 (0.8)	3 (2.4)
Litter	4 (50)	3 (38)	1 (11)	2 (25)

Reliability

: (2) Valid with restrictions
 This developmental study did not include skeletal staining for an examination of structural abnormalities. Nevertheless the study did not demonstrate skeletal abnormalities by gross observation at necropsy.
 (8) (22)

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**ROBUST SUMMARY
OF INFORMATION ON**

Substance Group: **OLEFINIC NAPHTHAS**

Summary prepared by: American Petroleum Institute

Creation date: DECEMBER 20, 2000

Printing date: MAY 20, 2003

Date of last Update: MAY 20, 2003

Number of Pages: 50

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)

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1. General Information

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Date May 20, 2003.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : The naphtha streams obtained from the catalytic cracking of heavy distillates into lighter fractions contain saturated, olefinic and aromatic hydrocarbons. However, their olefins content is higher than any of the naphtha streams derived by other processes.

The catalytically cracked naphthas boil in the range of approximately -20 to 230°C and contain hydrocarbons in the range C₄ to C₁₀.

The catalytically cracked naphthas typically are composed of the following hydrocarbon classes:

	Approx. Content (volume %)
Paraffins	30
Olefins	46
Naphthenics	10
Aromatics	14

Light catalytically cracked naphtha (CAS 64741-55-5) is a typical olefinic naphtha stream.

The American Petroleum Institute have reported (API, 1987) a thorough characterization of a specific sample of a light catalytically cracked naphtha (API 83-20), which has a high olefinic content and which was used in many of the mammalian toxicity studies. The characterization of this sample is given below:

Sample API 83-20
CAS 64741-55-5

Parameter	Method	Units	Value
API Gravity @60	D287		69.5
Density @ 15°C	D287		0.7033
Molecular weight	D2224	gm/mol	91
Refractive index		RI units	1.4030
Total Sulfur	D3120	wt %	0.035
Total Nitrogen	Chemil.	ppm/wt	6
Total Oxygen	NAA	wt. %	0.099
Total Chloride	coulom.	ppm/wt.	4
RVP		psia	10.3
Distillation	D86	vol/°F	
IBP			99
FBP			334
High olefin mass spectrometer analysis			
Paraffins	D2003/MS	Vol. %	30.6
Olefins	D2003/MS	Vol. %	45.6
Naphthenes	D2003/MS	Vol. %	10.4
Aromatics	D2003/MS	Vol. %	13.1

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Indans/Tetralins	D2003/MS Vol. %	0.3
Naphthalenes	D2003/MS Vol. %	0.0

In addition, some mammalian toxicity studies have been carried out on a lighter, distillate fraction of a sample of Light Catalytically Cracked Naphtha.

The distillate (LCCN-D) and the LCCN from which it was derived were analyzed and the data are shown in the following table.

	LCCN (liquid)	LCCN-D (vapor)
Total Olefins (% vol by MS)	42.44	60.09
Total Naphthenes (% vol by MS)	9.14	2.66
Total Aromatics (% vol by MS)	15.78	1.85
Total Paraffins (% vol by MS)	29.77	35.14
n-Paraffins	5.32	7.11
Benzene (vol %)	1.21	0.79
Carbon No. (vol%)		
4	0.44	1.55
5	24.65	62.35
6	28.22	28.50
7	22.52	6.22
8	16.07	1.27
9	6.85	0.10

For ecotoxicity studies and environmental fate modeling studies another sample of Light catalytically cracked naphtha was used (LCCN). This sample has the following characteristics (Chevron, 1995):

	Olefins	Naphthenes	Aromatics	Paraffins	
				n-	total
Total %	40.52	9.73	19.11	4.86	27.68
C4	0.29	0	0	0.08	0.08
C5	15.65	0.50	0	1.69	6.39
C6	15.27	2.16	1.48	1.71	8.42
C7	7.67	3.39	6.73	0.66	5.76
C8	1.64	2.62	7.69	0.56	4.13
C9	0	1.21	2.57	0.15	2.41
C10	0	0.03	0.61	0	0.40

(6) (11)

2.2 BOILING POINT

Remark : The olefinic naphtha streams boil in the range of approximately -20 to 230 °C

2.4 VAPOUR PRESSURE

Value : 5550 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes

Remark : The sample was identified by CONCAWE as MRD-95-090, gasoline sample CWE2, CAS No. 64741-55-5, a catalytically-cracked light naphtha.

See:
CONCAWE (1995)
Physico-chemical characterization of gasoline samples, study No. 104990C.

Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995

Reliability : (1) valid without restriction

Value : 5930 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes

Remark : The sample was identified by CONCAWE as MRD-95-046, gasoline sample W94/811, CAS No. 64741-54-4, a catalytically-cracked heavy naphtha.

See:
CONCAWE (1995)
Physico-chemical characterization of gasoline samples, study No. 104990C.

Study conducted by Exxon Biomedical Sciences Inc.
CONCAWE, Brussels, 1995

Reliability : (1) valid without restriction

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 2.13 - 4 at 25 °C
pH value : -
Method : Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : No
Test substance : LCCN. See section 1.1.1 for characterization

Remark : Log P values represent the spread of calculated and/or

measured values for the C5 to C9 hydrocarbon components found in LCCN, CAS No. 64741-55-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCCN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.

Reliability : (2) Valid with restrictions (21)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Deg. product :
Method : Preparation of Water Soluble Fraction
Year : 1995
GLP : Yes
Test substance : LCCN. See section 1.1.1 for characterization

Method : Water Accommodated Fractions (WAFs) of LCCN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 72 hours in tightly closed systems with minimal headspace.

Result : Gas chromatographic analysis of selected components indicated freshwater and saltwater solubilities of 4.6 and 4.3 ppm respectively. Measured test concentrations of the light alkylate naphtha were based on the total combined concentrations of benzene, toluene, ethylbenzene, o-xylene and p-xylene, which represent 13% composition of the test substance. Concentrations for these components reached equilibrium in freshwater and saltwater by 24 and 12 hours respectively.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LCCN components range from approximately 3 to 2000 mg/l. 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 (12) (13) (15) (16) (30)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	Calculated												
Media	:	soil, air, water, suspended sediment and sediment												
Method	:	Calculated according to Mackay Level 1												
Year	:	2000												
Remark	:	Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C9 hydrocarbon components found in LCCN, CAS No 64741-55-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCCN sample.												
		The majority of LCCN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals and ozone.												
Result	:	<table border="0"> <thead> <tr> <th><u>Medium</u></th> <th><u>% distribution</u></th> </tr> </thead> <tbody> <tr> <td>Air</td> <td>97 to 100</td> </tr> <tr> <td>Soil</td> <td>0.00 to 1.2</td> </tr> <tr> <td>Water</td> <td>0.01 to 2.7</td> </tr> <tr> <td>Sediment</td> <td><0.001 to 0.02</td> </tr> <tr> <td>Suspended sediment</td> <td><0.001 to 0.02</td> </tr> </tbody> </table>	<u>Medium</u>	<u>% distribution</u>	Air	97 to 100	Soil	0.00 to 1.2	Water	0.01 to 2.7	Sediment	<0.001 to 0.02	Suspended sediment	<0.001 to 0.02
<u>Medium</u>	<u>% distribution</u>													
Air	97 to 100													
Soil	0.00 to 1.2													
Water	0.01 to 2.7													
Sediment	<0.001 to 0.02													
Suspended sediment	<0.001 to 0.02													
Test substance	:	LCCN sample used is characterized in section 1.1.1.												
Conclusion	:	This complex petroleum mixture is expected to partition primarily to air.												
Reliability	:	(2) Valid with restrictions												

(20)

3.5 BIODEGRADATION

Type	:	Aerobic
Inoculum	:	Mixed, adapted inoculum of domestic activated sludge and soil
Contact time	:	56 day(s)
Result	:	Inherently biodegradable
Method	:	CONCAWE test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year	:	1999
GLP	:	Yes
Test substance	:	LCCN. See section 1.1.1 for characterization
Method	:	Type (test type): Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO ₂ headspace test)
Result	:	Test material was inherently biodegradable since it achieved >20% biodegradability based on CO ₂ production. By day 28 approximately 74% of the test material was degraded, then essentially reached a plateau in degradation rate until day 56. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63%

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actual) was observed by day 14, and total blank CO₂ production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21 °C, which deviated from the protocol value of 22 ±2°C. This deviation was not expected to have affected the outcome of this study.

Test Day	% Degradation (sd)	
	Hexadecane	Test Material
3	13.93 (1.85)	16.83 (9.56)
7	34.40 (4.54)	30.99 (0.56)
14	63.17 (0.94)	51.66 (3.33)
21	77.26 (6.52)	54.82 (6.24)
28	90.35 (7.14)	74.30 (1.24)
35	85.13 (n=1)	65.02 (1.37)
42	85.21 (n=1)	74.82 (0.54)
49	96.93 (8.94)	70.78 (6.48)
56	94.69 (4.10)	79.22 (12.28)

Test condition

: Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two week adaptation period. Triplicate test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass-distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of soil was obtained at a depth between 5-10cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substance were added directly to test systems using a 10 microliter Hamilton gas tight syringe. The volume required to achieve the specified mg carbon/l concentrations were calculated based on %carbon and specific gravity of the respective substance. The test substance %carbon (0.8724) and specific gravity (0.7220 mg/μl) information was supplied by the Sponsor. Hexadecane %carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/μl) was obtained from Verschueren (1983). Addition of respective substance was

performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 °C ($\pm 2^\circ\text{C}$) in the dark.

Biodegradation by CO₂ determination-test initiation and procedure. On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume(100 ml inoculum/l). Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/l concentration.

Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO₂ analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22 °C ($\pm 2^\circ\text{C}$).

On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc H₃PO₄ was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, then analyzed for CO₂ using gas chromatography-thermal conductivity detection. Quantitation of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability : (1) Valid without restriction

(24)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	96 Hr Static Acute Toxicity Test w/ Daily Renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring Method	:	Yes
	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year	:	1995
GLP	:	Yes
Test substance	:	LCCN. See section 1.1.1 for characterization
Method	:	Statistical Method: (FT - ME) LL ₅₀ and LC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 0, 1, 0, 0, 4 and 20, respectively 0, 3.0, 7.4, 15, 37 and 74 mg/l treatments. All surviving organisms exhibited normal behavior.
		96-hr LL ₅₀ = 46 mg/l, 37-74 mg/l w/ 95% C.I. (as nominal loading rate)
		96-hr LC ₅₀ = 4.1 mg/l, 3.2-7.0 mg/l mg/l w/ 95% C.I. (measured concentrations)
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was Mobil Technical Center well water. Nominal loading rates of 0, 3.0, 7.4, 15, 37 and 74 mg/l were used to prepare test solutions.
		WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4l of water for 24 hr in 9l glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-PID for concentrations of the following: benzene, toluene, ethylbenzene, and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes.

Fish were hatched and raised in-house, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8l glass containers with teflon lined caps. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving adequate volume to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Reliability : (2) Valid with restrictions
 Water temperature was 21.4-21.8 °C. Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 5.2 and 8.6, pH values between 7.61 and 8.2.
 NOEC values not reported as sublethal effects and moderate mortality (20%) were observed at the 37 ppm (nominal loading) treatment which is reported to be the NOEC.

(27)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : 48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1995
GLP : Yes
Test substance : LCCN. See section 1.1.1 for characterization

Method : Statistical Method: (FT - ME) EL_{50} and EC_{50} calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.

Result : Mortality (no. of deaths/treatment) at 48 hrs: 0, 0, 0, 20, 20 and 20 for 0, 6.4, 13, 25, 51 and 102 mg/l treatments.

48-hr EL_{50} = 18 mg/l (95% C.I. 13 to 25 mg/l) based upon nominal loading rate.

48 hr EC_{50} was 1.4 ppm (95% C.I. 0.99 to 1.95 mg/l); based on total measured concentrations.

48-hr NOEC = 13 mg/l based upon nominal loading rate.
 48 hr EC_{50} was 0.99 ppm based on total measured concentrations.

Test condition : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was aged well water.

WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 1.2l of water for 24 hr in

aluminum foil covered 1l aspirator bottles fitted with a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately 45 minutes, then drained from the port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes.

Range finding toxicity studies were conducted at 1.3, 10 and 102 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 6.4, 13, 25, 51 and 102 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.

Test vessels were teflon cap-sealed 265 ml glass jars with 10 daphnids per jar and were completely filled with test solution.

During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.6; pH ranged from 7.94 to 8.40; temperature was 19.1 to 20.2 °C; hardness (mg/l) ranged from 172 - 180; alkalinity (mg/l) was 124-132 and conductivity (µmhos) values were 360 - 405.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1994. The primary culture was obtained from Aquatic Research organisms, Hampton, NH, which was derived from EPA laboratory culture, in Cincinnati, Ohio.

Reliability : (2) Valid with restrictions
 Measured concentrations represent only 13-20% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

(28)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : EPA. 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-8, ES-5.
Year : 1995
GLP : Yes
Test substance : LCCN. See section 1.1.1 for characterization

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Method : Statistical Method: LL₅₀ and LC₅₀ calculated using probit analysis. ASTM Special Technical Publication 634. 1977, pp 65-84. All NOEL/NOEC values calculated using Fisher's exact test.

Result : Percent inhibition on growth determined by cell density (cells/ml):
96 hour EL₅₀=64 mg/l (44-111 mg/l CI @95%)
96 hour EC₅₀= 4.6mg/l (2.9-8.8 mg/l CI @95%)
96 hour NOEL=51 mg/l
96 hour NOEC=3.5 mg/l
Subcultures placed in fresh media (no test substance) after acute testing for six days indicated that growth inhibition was algistatic in all treatments, with the exception of the 102 ppm, which was determined to be algicidal. No excursions from the protocol were noted. However, range finding and two previous definitive tests were performed and considered inconclusive due to inconsistencies in control and treatment cell densities, which presumably were resolved by modification of the AAP media. Additionally, control growth showed a lag during the first 72 hours of the study.

Concentration (mg/l)		96hr cell density (cells/ml)	(% Inhibition)
Nominal	Measured		
Control		8.4 x10 ³	na
6.4	0.093	3.2 x10 ⁴	- 281.1
13	0.130	9.73x10 ³	- 16.0
25	0.429	1.99x10 ⁴	-136.9
51	1.87	1.36x10 ³	53.0
102	4.85	2.59x10 ³	69.2

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 4.4 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, pH adjusted to 7.5 ± 0.1 with 0.1NHCl and sterilized by 0.22 micron filtration) in 4.0 liter aspirator bottles. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature in a hood darkened with aluminum foil. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (140 ml) with treatment solution and inoculated with algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media, and transferred every 4-8 days to fresh media. Original algal cultures obtained from American Type Culture Collection (ATCC Strain 22662), Rockville, MD, September 1995. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 6.4, 13, 25, 51 and 102 mg/l The initial algal concentration was 1.0 x 10³ cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 400

+ 50-ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0, 24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, o-xylene and p-xylene, which represent 13% composition of the test substance. Measured test concentrations of the light catalytically cracked naphtha were based on the total combined concentrations of all analytes.

Test temperature was 24 ± 2 °C. The pH was 7.5 at test initiation, pH value at test termination not included in report.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

(29)

4.5.1 CHRONIC TOXICITY TO FISH

Species : Pimephales promelas (Fish, fresh water)
Exposure period : 14 day(s)
Unit : Mg/l
Analytical monitoring : Yes
Method : OECD 204
Year : 1999
GLP : Yes
Test substance : LCCN. See section 1.1.1 for characterization

Method : LL_{50}/LC_{50} and EL_{50}/EC_{50} calculated using linear interpolation. NOEL/NOEC for survival and growth determined by Steel's Many-One Rank Test. TOXSTAT program was used to determine endpoints.

Result : The mean measured concentrations for nominal loading rates of 0.38, 0.99, 2.6, 6.4, 16, and 40 mg/l were 0.009, 0.024, 0.12, 0.28, 0.64, and 3.4 mg/l representing the average of total analytes measured in the new and old WAFs. The average total analyte concentration in the controls was 0.004 mg/l.
 14-d LL_{50} for survival = 23 mg/l (95% C.I. 19 - 26),
 14-d LC_{50} for survival = 1.5 mg/l (95% C.I. 1.1 - 1.8)
 14-d NOEL for survival = 6.4 mg/l,
 14-d NOEC for survival = 0.28 mg/l.
 Mortality (no. of deaths/treatment) at 14 days: 0, 1, 0, 0, 3, 11, and 40 in the 0, 0.38, 0.99, 2.6, 6.4, 16, and 40 mg/l treatments.
 14-d NOEL for growth = 6.4 mg/l,
 14-d NOEC for growth = 0.28 mg/l.
 14-d EL_{50} and EC_{50} for growth could not be calculated because none of the treatment group means were <50% of control.
 Since there were significant mortality at the two highest treatments, these treatments were excluded in the analysis

- of growth data. The mean (standard deviation) for dry weights were 2.49 (0.08), 2.58 (0.21), 2.76 (0.07), 2.67 (0.19), and 2.79 (0.42) in the 0, 0.38, 0.99, 2.6, and 6.4 mg/l treatments. Light intensity was not measured during the study due to an oversight and had no impact on the results of the study.
- Test condition** : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 170-180 mg/l as CaCO₃, total alkalinity of 120-130 mg/l as CaCO₃, pH range of 8.0 to 8.2, and a specific conductivity of 500 mmhos/cm. Nominal loading rates of 0, 0.38, 0.99, 2.6, 6.4, 16, and 40mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.718 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 0.75 to 1.25 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with fresh WAFs in which 80% of the old solutions were siphoned and excess debris removed from the exposure vessel prior to refilling with fresh WAF. Renewed solutions were then siphoned again and refilled a second time to achieve an exposure solution of ~96% fresh WAF. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light catalytically cracked naphtha were based on the concentrations of all analytes. Fish were hatched and raised from laboratory in-house culture. Fish were 10 days old at the start of the test. Test vessels were 1l screw-capped glass jars containing 980 ml of WAF with minimal headspace. Four replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Fish were fed 0.15 ml of live brine shrimp nauplii (<48 hr old) twice daily during the test. Water temperature was 24 to 26° C. Test photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen concentrations were 8.1 to 8.5 in the new solutions and 3.7 to 5.9 in the old solutions. pH values were 7.2 to 8.3.
- Reliability** : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	: Daphnia magna (Crustacea)
Exposure period	: 21 day(s)
Unit	: mg/l
Analytical monitoring	: Yes
Method	: OECD Guide-line 211
Year	: 1999
GLP	: Yes
Test substance	: LCCN. See section 1.1.1 for characterization
Method	: For NOEL/NOEC, Fisher's Exact Test was used for survival of adult daphnids and Kruskal-Wallis Test with Dunn's Multiple Comparison was used for reproduction. For EL ₅₀ /EC ₅₀ , survival data were analyzed using the Spearman-Kärber method and reproduction data were analyzed by linear interpolation. TOXSTAT program was used to determine the endpoints.
Result	: The mean measured concentrations for nominal loading rates of 0.38, 0.99, 2.6, 6.4, 16, and 40 mg/l were 0.007, 0.022, 0.11, 0.27, 0.68, and 3.1 mg/l representing the average of total analytes measured in the new and old WAFs. The average total analyte concentration in the controls was 0.004 mg/l. 21-d EL ₅₀ for survival = 27 mg/l (95% C.I. 26 - 29), 21-d EC ₅₀ for survival = 1.9 mg/l (95% C.I. 1.8 - 2.0) 21-d NOEL for survival = 16 mg/l, 21-d NOEC for survival = 0.68 mg/l. Daphnid immobilization at 21 days: 0, 1, 0, 0, 0, 0, and 10 in the 0, 0.38, 0.99, 2.6, 6.4, 16, and 40 mg/l treatments. 21-d EL ₅₀ for reproduction = 13 mg/l (95% C.I. 12-15), 21-d EC ₅₀ for reproduction = 0.55 mg/l (95% C.I. 0.49-0.64) 21-d NOEL for reproduction = 2.6 mg/l, 21-d NOEC for reproduction = 0.11 mg/l. Since there was significant immobilization in the highest treatment, it was excluded in the analysis of reproduction data. The mean numbers (standard deviation) of offspring released per female daphnid were 150 (9), 139 (12), 141 (7), 139 (10), 123 (10), and 55 (28) in the 0, 0.38, 0.99, 2.6, 6.4, and 16 mg/l treatments. The numbers of offspring released in the 6.4 and 16 mg/l treatments were significantly less than the controls. First brood release for organisms exposed to =6.4 mg/l and the controls occurred by day 8. First brood release for organisms exposed to 16 mg/l occurred on day 10. From day 17 to day 21, immobilized offspring were released in the 16 mg/l treatment.
Test condition	: Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 170-180 mg/l as CaCO ₃ , total alkalinity of 120-130 mg/l as CaCO ₃ , pH range of 8.0 to 8.2, and a specific conductivity of 500 mmhos/cm. Nominal loading rates of 0, 0.38, 0.99,

2.6, 6.4, 16, and 40mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.718 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 45 min to 1.25 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with 70 ml of fresh WAFs added to a second set of beakers. Food was added to the fresh WAFs and daphnids were then transferred from the old test solutions to the fresh WAFs. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light catalytically cracked naphtha were based on the concentrations of all analytes. Daphnids used in the test were from laboratory in-house culture. Daphnids were ≤ 24 hrs old at the start of the test. Test vessels were 70 ml screw-capped glass jars containing 70 ml of WAF with minimal headspace. Ten replicates per treatment and 1 daphnid per replicate were tested for each treatment and the control. Daphnids were fed 0.2 ml of algal suspension (*Ankistrodesmus falcatus*, 4×10^7 cells/ml) and 0.05 ml of a yeast, cereal leaves and digested flaked fish food (YCT) suspension daily during the test. Water temperature was 19 to 21° C. Test photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen concentrations were 8.7 to 8.8 in the new solutions and 8.4 to 9.1 in the old solutions. pH values were 7.2 to 8.2.

Reliability

: (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

(25)

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
Year	:	1986
GLP	:	Yes
Test substance	:	API 83-20 see section 1.1.1.
Method	:	Groups of five male and five female fasted rats were given API 83-20 as a single oral dose of 5 g/kg. The animals were then allowed food and water ad libitum and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded.
Result	:	There were no mortalities during the study. Body weights had increased by day 7 after dosing and further increases were recorded 14 days after dosing. Clinical signs of toxicity were observed during the 24 hours immediately after dosing and appeared normal thereafter. Clinical signs included: hypoactivity, ataxia, diarrhea, lacrimation, yellow-stained anal area, excessive salivation and respiratory congestion. There were no treatment-related lesions observed at necropsy.
Conclusion	:	Oral LD ₅₀ was greater than 5 g/kg for males and females.
Reliability	:	(1) Valid without restriction

(4)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC ₅₀
Value	:	> 5.3 mg/l
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Air
Exposure time	:	4 hour(s)
Year	:	1987
GLP	:	Yes
Test substance	:	API 83-20 see section 1.1.1.
Method	:	A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-20 at a nominal concentration of 5mg/l for 4 hours.

- After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed and subjected to a gross post-mortem examination. For all animals, including those found dead during the study, the lungs were removed, fixed and examined histologically.
- Result** : The mean analytical exposure concentration was measured and found to be 5.28 ±0.55 mg/L. Gravimetric samples, collected on glass fiber filters suggested little or no aerosol in the chamber. Most animals exhibited languid behavior and squinted eyes during the second hour of the exposure. Polypnea was observed in all animals when removed from the chamber at the one hour post exposure observation period. Rhinorrhea was exhibited by two animals on day two of the test. All animals appeared normal subsequently and there were no mortalities during the study. With the exception of one animal (female) all animals had body weights that were considered unremarkable. There were no remarkable gross or microscopic findings.
- Reliability** : (1) Valid without restriction

(5)

5.1.3 ACUTE DERMAL TOXICITY

- Type** : LD₅₀
Value : > 3000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 4
Vehicle : Undiluted
Year : 1986
GLP : Yes
Test substance : API 83-20 see section 1.1.1.

- Method** : The skin of the patched area of four rabbits of each sex had been abraded whilst the other four had intact skin. A weighed quantity of undiluted test material was applied to the dorsal skin of each of 4 male and 4 female rabbits at a dose level of 2.0 g/kg. 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 and the animals were observed for a total of 14 days post-dosing.

- At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded. Since there were 2 mortalities recorded in this study the experiment was repeated using a dose level of 3 g/kg.
- Result** : A pain response was elicited from all animals during application of the test material to the skin. Dermal irritation ranging from slight to severe was observed for

erythema and slight to marked for atonia, desquamation, fissuring and coriaceousness.
At the 2 g/kg dose level, clinical observations included: diarrhea, anorexia and hypoactivity and one female in this dose group died on day 7 following dosing.
There were no clinical signs of toxicity in the 3 g/kg dose group and no animals in this group died.
At necropsy skin lesions were observed more frequently in the 2 g/kg group than the 3 g/kg group.

Conclusion : LD₅₀ was greater than 3 g/kg for both male and female rabbits.

Reliability : (1) Valid without restriction

(4)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Remark : Not relevant

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 3.7
Result : Moderately irritating
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : API 83-20 see section 1.1.1.

Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. 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 and again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : The scores for erythema and edema were marginally greater for abraded skin than intact skin, but the difference was not biologically significant.
Scores for abraded skin at each of the observation intervals were:

<u>Time</u>	<u>Erythema</u>	<u>Edema</u>	<u>Irritation score*</u>
24 hours	1.8	2.0	3.5
72 hours	2.3	1.7	3.8
96 hours	1.5	1.5	2.6
7 days	1.2	0.2	1.2
14 days	0.0	0.0	0.0

* Irritation score calculated as the sum of irritation scores for each test site divided by the number of animals at each observation period.

PII is the sum of the 24- and 72- hour total irritation scores divided by 2

Reliability : (1) Valid without restriction (4)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : See method
Number of animals : 9
Vehicle : None
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : API 83-20 see section 1.1.1.

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control.
 After 20 to 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 : No pain response was elicited from any of the animals when the test material was applied to the corneal surface.
 The primary eye irritation score (=total eye irritation score for all animals divided by the number of animals) was 1.0 after 1 hour for those animals with unwashed eyes compared to 3.3 for those whose eyes had been washed. An irritation score of zero was recorded at all other times.
 No iridial nor corneal irritation resulted from application of the test material.

Reliability : (1) Valid without restriction (4)

5.3 SENSITIZATION

Type : Buehler Test
Species : Guinea pig

5. Toxicity

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- Concentration** : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge 25 % occlusive epicutaneous
- Number of animals** : 10
- Vehicle** : Paraffin oil
- Result** : Not sensitizing
- Classification** : Not sensitizing
- Year** : 1986
- GLP** : Yes
- Test substance** : API 83-20 see section 1.1.1.
- Method** : 0.4 ml of undiluted test material was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application the dressings were removed and the skin wiped to remove residue of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.4 ml of a 25% mixture in paraffin oil) 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 (2,4-dinitrochlorobenzene, as a 0.3% w/v solution in 80% aqueous ethanol), vehicle control (paraffin oil) and naive control groups were included in this study and the procedure for these was the same as for the test groups.
- Result** : No skin reactions were observed following the application of the challenge dose in either the naive controls or the group that had been exposed to test material. Scores of 0.2, 0.3 and 0.5 for erythema were recorded for the paraffin oil controls.

In contrast all positive control animals developed a skin response following the challenge procedure.
- Reliability** : (1) Valid without restriction

(4)

5.4 REPEATED DOSE TOXICITY

- Species** : Rat
- Sex** : Male/female
- Strain** : Sprague-Dawley
- Route of admin.** : Inhalation
- Exposure period** : 15 weeks
- Frequency of treatm.** : 6 hours/day, 5 days/week
- Post exposure period** : 4 weeks
- Doses** : Target: 750, 2500 & 7500 ppm. Actual: 756, 2507 & 7533 ppm
- Control group** : Yes
- Method** : EPA OTS 798.2450
- Year** : 2001

5. Toxicity

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GLP : Yes
Test substance : LCCN-D (Distillate of LCCN) - See section 1.1.1. for characterization of sample

Method : Groups of 16 male and 16 female rats underwent whole body exposures to 750, 2500 and 7500 ppm LCCN-D. Exposures were for 6 hours each day, 5 days per week, for at least 65 exposures, over a period of 15 weeks. Extra groups of 16 rats of each sex were exposed to the high dose level and also for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 15 weeks exposure.

Neurobehavioral evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14/15 and after the 4 week recovery period for the recovery animals. Animals were not exposed to LCCN-D during these tests.

Following 15 weeks of exposure, 16 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically.

At the end of the 4 week recovery period, 16 animals of each sex from the high and control groups were necropsied and selected tissues were examined microscopically.

During the study clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 15 weeks and 20 weeks (recovery groups). Body weights and food consumption were measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements.

At termination (after 15 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. 10 animals of each sex were designated for non-neuropathological examination and 6 of each sex for neuropathological examination.

For the non neuropathology animals, the following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) were removed from the control and high dose animals and were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination. Animals designated for neuropathological examination were

subjected to a detailed examination of central and peripheral nervous tissues.

Neurobehavioral studies were undertaken as follows:

Motor activity

Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 15 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery

An assessment of the following was made:

Home cage evaluations for Posture, vocalization, palpebral closure.

Handling evaluations for reactivity to general stimuli, signs of autonomic function.

Open field behavior: arousal level, gait, urination and defecation frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.

Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result

: No exposure-related clinical observations were noted either during exposure or during non-exposure periods and no ocular abnormalities were observed.

Although the males in the high dose group were slightly lighter than the controls (total weight gain 344g compared to 323g), the difference was not significant. In the females however, the difference (total weight gain 165g compared to 154g) was statistically significant. At the end of the 4 week recovery period body weights of the high dose males and females were comparable to the corresponding controls. During the 4 week recovery period, the high dose males and females had food consumption that were greater (statistically significant) than controls. [Note: actual data not included in the draft publication].

At 15 weeks the following hematological changes were recorded.

7500 ppm males

Decreased hemoglobin concentration (8%)

Decreased hematocrit (7%)

2500 ppm males

Decreased MCHC (3%)

7500 ppm females

Decreased MCHC (4%)

After the 4 week recovery period, all hematological values were considered to be normal.

At 15 weeks there were no abnormal clinical chemistry values. After the 4 week recovery period however, glucose and albumin was raised in the 7500 ppm females by 21 and 15% respectively. Since the values were within the normal range they were not considered to be toxicologically significant.

Neurobehavioral studies

There was no evidence of any effect on motor activity either after 15 weeks exposure or after the 4 week recovery period. There was no evidence of a treatment-related effect in the functional operational battery that was carried out.

Pathology

With the exception of those listed below, absolute and relative organ weights were not affected by treatment.

<u>Parameter</u>	<u>2500 ppm</u>	<u>Group 7500 ppm</u>	<u>Recovery</u>
<u>MALES</u>			
Abs Kidney		21% up	
Rel Kidney	15% up	32% up	
Rel Liver		23% up	
<u>FEMALES</u>			
Rel Kidney		18% up	
Abs Liver			24% up
Rel Liver		12% up	
Rel Brain			9% down

There were no microscopic findings in either the liver or brain of the groups in which organ weight changes had been recorded.

The only treatment-related microscopic changes were found in the nasal turbinates and kidneys as follows.

Nasal turbinates

The following table summarizes the incidence of selected microscopic findings in the nasoturbinal tissues. Numbers in table are male incidence/femlae incidence

	<u>Dose group (ppm)</u>			
	<u>0</u>	<u>750</u>	<u>2500</u>	<u>7500</u>
<u>Incidence at 15 weeks</u>				
No. evaluated	10/10	10/10	10/10	10/10
Goblet cell hypertrophy/hyperplasia				
Score 1	3/2	1/4	1/4	1/1
2	7/6	5/5	7/3	5/5
3	0/1	2/1	2/3	3/3

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Nasal mucosa hyperplasia					
Score	1	0/1	0/4	1/2	1/0
	2	2/3	3/3	6/5	5/5
	3	0/0	0/0	1/1	1/2

Incidence in post-exposure animals

No. evaluated	10/10	0/0	0/0	10/10
Goblet cell hypertrophy/hyperplasia				
Score	1	2/4		2/2
	2	5/2		5/3
	3	3/1		3/0

Nasal mucosa hyperplasia				
Score	1	2/4		2/3
	2	6/4		5/5
	3	0/0		1/0

These findings are considered indicative of exposure to a mild irritant.

Kidney

At the end of 15 weeks exposure several changes were observed and at the end of the 4 week recovery period there was an indication of some reversibility of the kidney effects.

The findings are summarized in the following table.

Finding	Terminal			Post-exposure		
	0	750	2500	7500	0	7500
No of animals evaluated	10	10	10	10	10	10
Bilateral cortex: eosinophilic hyaline droplets in proximal convoluted tubular epithelium with severity greater than or equal to 2	0	3	8	10	0	2
Postive Mallory/Heidenhain staining hyaline droplets in proximal convoluted tubular epithelium with severity greater than or equal to 2	1	4	9	10	1	2
Bilateral interstitium subacute/chronic inflammation	0	0	3	5	1	3
Bilateral cortex/cortico-medullary junction tubules dilated with granular casts	0	0	1	4	0	0
Bilateral cortex convoluted tubular basophilic epithelium	0	0	0	3	0	0

Similar effects were not observed in the females.

In the post exposure animals, brain length and width measurements showed no test-material-related effects.

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Test condition : The test atmospheres were generated by wholly vaporizing the test material (LCCN-D) and diluting with air to achieve the required concentrations.
The highest concentration was approximately 75% of the lower explosive limit.

Actual exposure concentrations were determined six times per exposure session for treated groups and once for controls.

Particle size determinations were carried out once during each exposure using an aerodynamic particle sizer. Mean mass aerodynamic diameter (MMAD), geometric standard deviation (GSD) and total mass concentration (TMC) were calculated.

The actual concentrations for each of the target dose levels were:

Dose group (ppm)	Actual TMC* (ppm) (mg/m³)	
0 (Control)	0	0.005820
750	756	0.005506
2500	2507	0.005085
7500	7533	0.004348

* TMC = Total Mass Aerosol Concentration

Reliability : (1) Valid without restriction

(19)

Species : Rat and Mouse
Sex : Male/female
Strain : Rat - Sprague Dawley, Mouse - CD-1
Route of admin. : Inhalation
Exposure period : 13 weeks
Frequency of treatm. : 6 hours/ day, 5 days/week for 13 weeks
Doses : Target: 500, 2000 & 8000. Actual: 530, 2060 & 7690 mg/m³
Control group : Yes
Year : 1996
GLP : No data
Test substance : LCCN CAS # 64741-55-5

Method : Groups of 10 rats of each sex and 10 mice of each sex were individually housed in inhalation chambers. The rats and mice underwent whole body exposures to LCCN vapors. Exposures were for 6 hours/day, 5 days/week for approximately 13 weeks at nominal concentrations of 500, 2000 or 8000 mg/m³. Extra groups of 10 rats and mice of each sex served as sham and untreated controls. Food and water was available ad lib, except during the exposure periods. Clinical observations were made regularly and body weights were recorded weekly.

At the end of the 13 weeks exposure, the rats were fasted for 16 hours before blood samples were taken for hematological and clinical chemical measurements. All animals were then sacrificed and necropsied. Organs were

weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet and dry weights of the right apical and right middle lung lobes were also recorded. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to determine the number of testicular spermatids.

The following tissues from the high dose and sham treated animals were examined histologically: adrenals, kidney, bone and marrow (sternum), pancreas, brain, submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach, colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver, tracheobronchial lymph nodes, lung (left lobe), nasal turbinates, muscle, urinary bladder, sciatic nerve, and any gross lesions. Additional sections included lung from untreated controls and kidney from 0, 2060 and the 7690 mg/m³ exposure groups.

Result : No treatment-related changes were observed in either species in clinical signs, body weight, clinical chemistry or hematology except four male rats in the high dose group that had lesions on the skin in the scrotal area. This was attributed possibly to an interaction between abrasions of the skin against the floors of the cages and the exposure to the high concentrations of LCCN.

Organ weights were unaffected in either species, except for uterus weights. Uterine weights in the rats were less than untreated controls for all exposed groups, but not less than the sham controls. The actual weights (g) shown below, were not considered to be related to LCCN because they were not dose-related, and there was no difference between the sham and untreated controls. Additionally, no similar effect was observed in the mice.

Untreated controls	0.69 ± 0.17
Sham controls	0.62 ± 0.07
530 mg/m ³	0.55 ± 0.12
2060 mg/m ³	0.52 ± 0.05
7690 mg/m ³	0.54 ± 0.09

No treatment-related abnormalities were observed in any of the organs examined microscopically. The incidence of the occurrence of hyaline droplets in dilated tubules was similar in the controls and the high dose males and was not considered to be relevant.

The number of sperm per gram of cauda epididymis was significantly lower in the 7690 mg/m³ group than in the sham controls but not the untreated controls. The number of epididymal sperm was not significantly affected by exposure. Also, the number of testicular spermatids and the percentage of abnormal sperm in the cauda epididymis were not affected by exposure to 7690 mg/m³ compared to either control group.

Test substance : Vapors of LCCN were generated in a glass countercurrent generator (one for each concentration).

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As liquid LCCN flowed down the coil, nitrogen passed upwards and carried off vapors of the more volatile components. Main stream air was used to dilute the vapor to the required concentration.

Vapor concentration was monitored at approximately hourly intervals during each exposure period.

Concentrations (Target and actual) are shown below.

Target (mg/m ³)	Actual (mg/m ³)
500	530 ± 90
2000	2060 ± 250
8000	7690 ± 730

In addition the composition of neat LCCN (liquid), its static headspace and the inhalation chambers was assessed. The results shown below confirm that the animals had been exposed to the lighter components of LCCN .

Component	% in		Exposure Chamber
	Liquid LCCN	Static Headspace	
Total C4/C5 nonaromatics	18.6	76.5	38.2
n-Butane	0.2	5.5	
Isobutane	-	1.0	0.2
Butenes	1.7	11.4	
n-Pentane	1.4	3.3	7.0
Pentenes	3.8	13.1	
Methyl butene	5.2	11.9	
Total C6 nonaromatics	16.2	14	
n-Hexane	1.0	0.9	
2,3-Dimethylbutane	1.3	1.0	
2-Methylpentane	4.1	6.0	12.2
3-Methylpentane	2.5	2.7	7.5
Methylpentene	1.7	0.9	
Hexenes	2.1	1.1	
Methylcyclopentane	2.3	1.8	
4-Methylcyclopentane	0.6	0.5	
Total C7 nonaromatics	10.2	1.9	
Total C8 nonaromatics	8.0		
n-Octane	0.3		
2,2,4-Trimethylpentane	0.8	0.4	2.7
Total Aromatics	24.0		
Benzene	0.1	0.6	2.3
Toluene	4.6	0.8	4.7
Ethylbenzene	1.5	0.1	
Xylenes	7.6	0.5	2.3

Reliability

: (2) Valid with restrictions

The data presented in the publication are more or less limited to those showing effects. Where no effects are reported , actual data are not shown. Nevertheless, the

study is sound and helpful in assessing the effects of LCCN light ends on this biological endpoint.

(14)

Species : rat

Remark : A further 13 week inhalation study in rats has been reported but the dose concentrations (1500, 2600 & 4500 ppm) were somewhat lower than those in the study reported by Lapin et al. Effects observed were similar to those described by Lapin et al and in addition a trace centrilobular hepatocellular hypertrophy was observed in 50% of the males and 25% of the females in the 4500 ppm group. (API 1987)

A 21-day inhalation study in rats has been reported (Halder et al 1984). Rats were exposed to Light Catalytically Cracked Naphtha at concentrations of 55, 567 and 3628 ppm. The results confirmed the findings in male rat kidneys that have been observed in other studies with rats.

(8) (17)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mouse lymphoma assay
Metabolic activation : With and without
Result : Negative
Year : 1987
GLP : Yes
Test substance : API 83-20 see section 1.1.1.

Method : The test material was dissolved in Ethanol for this assay. Two positive control substances were used viz Ethyl methane sulfonate (EMS) at concentrations of 0.25 & 0.5 µl/ml for non activation assays and 3-methylcholanthrene (MCA) at concentrations of 2.5 & 4.0 µg/ml for activation assays.

A cytotoxicity study carried out prior to the mutagenicity assay established that the sample was highly toxic at 500 nl/ml without activation and lethal at the same concentration in the presence of metabolic activation.

Therefore, for the mutation assay the lymphoma cells were exposed for 4 hours to test material at treatments from 50 to 800 nl/ml without activation and with treatments from 25 to 500 nl/ml with Araclor-induced rat liver S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection. Plates containing colonies of selected cells were incubated for 10 to 14 days after which they were scored for total number of colonies per plate. A mutation frequency was then determined.

Assay evaluation criteria were:

The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency

that is at least 150% of the concurrent background frequency plus 10×10^{-6} . The background frequency is defined as the average mutant frequency of the solvent negative controls. The minimum increase is based on extensive experience which indicates that assay variability increases with higher backgrounds and the calculated minimum increase as defined above is often a repeatable result; statistical analysis for the confidence limits is not yet available.

The observation of a mutant frequency that meets the minimum criterion for a single treated culture within a range of assayed concentrations is not sufficient evidence to evaluate a test material as a mutagen. The following test results must be obtained to reach this conclusion for either activation or non-activation conditions.

A dose-related or toxicity-related increase in mutant frequency should be observed. It is desirable to obtain this relation for at least three doses, but this depends on the concentration steps chosen for the assay and the toxicity at which mutagenic activity appears.

If an increase of about two times the minimum criterion or greater is observed for a single dose near the highest testable toxicity, as defined in the Assay acceptance criteria, the test material will be considered mutagenic. Smaller increases at a single dose near the highest testable toxicity will require confirmation by a repeat assay.

Treatments that induce less than 10% relative growth are included in the assay, but are not used as primary evidence for mutagenicity as it relates to risk assessment.

In the assay reported in this particular study, under non-activation conditions, the test material was excessively toxic at 300 nl/ml. Five treatments from 50 to 250 nl/ml were therefore chosen for the analysis of mutant induction and non-detectable to moderate toxicities were induced (relative growths 205.3% to 25.5%). None of the assayed treatments induced a mutant frequency that exceeded the minimum criterion of 89.7×10^{-6} . However, since it is desirable to include highly toxic treatments (10 to 20% relative growth) in an analysis, another non activation assay was performed in an attempt to obtain a wider range of toxicities.

In the second assay the test material was analyzed for mutant induction from 50 to 150 nl/ml.

In the presence of metabolic activation six treatments from 75 to 400 nl/ml were analyzed for mutant induction and a wide range of toxicities was induced (86.3 to 6.9% relative growths). The minimum criterion for mutagenesis in this assay was a mutant frequency exceeding 81.4×10^{-6} . None of the acceptable treatments induced a mutant frequency that exceeded the minimum criterion. One treatment with less than 10% relative growth (400 nl/ml) induced a mutant frequency

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Remark : that exceeded the minimum criterion, but the treatment was not acceptable for analysis because it did not fulfill the requirements of the assay evaluation criteria. A second assay was therefore performed at treatments ranging from 200 to 300 nl/ml.

: Two other olefinic naphtha streams have been tested in a mouse lymphoma assay. The results are summarized below.

<u>Sample</u>	<u>Result</u>	<u>API Report</u>
API 81-03	Negative With or without S-9	32-31300
API 81-04	Negative without S-9 Equivocal with S-9	32-31710

Result : S-9 source: Araclor-induced rat liver

: Only the results of the second assays are summarized since the first assay was not considered acceptable (for the reasons given in the method section above).

<u>Test condition</u>	<u>Cloning efficiency</u>	<u>Relative growth (%)</u>	<u>Mutant frequency (10E⁻⁶ units)</u>
<u>Non activation</u>			
Solvent control	100.5	100	49.1
Solvent control	109.7	100	42.5
Solvent control	107.5	100	45.6
EMS 0.25 µl/ml	86.8	82.5	286.7
EMS 0.4 µl/ml	72.7	56.2	469.7
Sample 83-20			
50 nl/ml	128.4*	144.7	38.5
100 nl/ml	100.1*	88.8	58.2
150 nl/ml	78.5*	81.6	60.5
<u>S9 activation</u>			
Solvent control	114.7	100	46.5
Solvent control	121.0	100	44.3
Solvent control	100.2	100	57.2
MCA 2.5 µg/ml	87.3	55.5	235.1
MCA 4 µg/ml	73.2	53.5	210.0
Sample 83-20			
200 nl/ml	88.7*	62.4	48.3
250 nl/ml	88.1*	68.8	65.5
250 nl/ml	64.3*	8.6	66.2
300 nl/ml	63.7*	11.1	74.3
300 nl/ml	59.7*	7.3	96.7

* Cloning efficiency relative to solvent control

In the non activation assay, at most, low toxicities were induced without inducing significant increases above the background mutant frequency (average of solvent controls). Higher toxicities could not be assayed because of a very sharp toxicity curve; a small increase in concentration from 150 to 175 nl/ml was excessively toxic. The test material was, therefore, considered non mutagenic without activation

at concentrations that approached excessive toxicity.

In the activation assay, the 250 and 300 nl/ml treatments were duplicated to determine reproducibility.

Low and high toxicities were induced by the assayed treatments (68.8 to 7.3% relative growths).

For a treatment to be considered mutagenic in this trial, a mutant frequency exceeding 84.0×10^{-6} was required. One treatment at 300 nl/ml induced a mutant frequency that exceeded this criterion but the increase was observed at less than 10% relative growth and a duplicate treatment at the same concentration was inactive.

The test material was, therefore, considered non-mutagenic with activation in this assay.

In the assays used in this evaluation, the average cloning efficiencies for the solvent controls varied from 70.5% and 105.9% without activation to 96.2% and 112.0% with activation, which demonstrated acceptable cloning conditions for the assays.

The negative control mutant frequencies were all within the expected range and the positive control compounds yielded mutant frequencies that were greatly in excess of the background.

Sample 83-20 is considered inactive in the mouse lymphoma assay, with and without metabolic activation.

Reliability : (1) Valid without restriction (2) (3) (7)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary (CHO) cells
Metabolic activation : With and without
Year : 1988
GLP : Yes
Test substance : API 81-03

Method : A cytotoxicity study was performed in order to select dose levels for the SCE assay.
 For the SCE assay CHO cells were seeded in duplicate for each treatment condition and were incubated at 37°C in a humidified atmosphere for 16 to 24 hours.
 Treatment was carried out by re-feeding two complete sets of flasks with complete medium for the non activation study or with Aroclor-induced rat liver S-9 reaction mixture for the activated study to which was added 50 µl of dosing solution of test control or article in solvent or solvent alone. An untreated control of cells in complete medium was also included.

In the non-activation study the cells were exposed for 28 hours. Two hours after exposure 0.01 mM BrdUrd was added to the treatment medium. At the end of the treatment period, the treatment medium was removed, the cells were rinsed and were then exposed to colcemid (0.1 µg/ml) for a further 2 hours.

In the activation study exposure was for 2 hours. After the exposure period, the treatment medium was removed, the cells were washed with PBS, re-fed with medium containing BrdUrd and then incubated for a further 28 hours. Colcemid was added at a final concentration of 0.1 µg/ml for the last 2 hours of incubation.

For activated and non-activated assays, metaphase cells were harvested 2 hours after addition of colcemid. Cells were collected and fixed and stored until slides were prepared.

Slides were coded and scored without regard to treatment group. Only cells with 20 = 2 centromeres were selected for evaluation of SCEs. A total of 4 doses were scored including the highest test article dose where sufficient second-division metaphase cells were available. SCEs were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. The percentage of cells in first (M1), second (M2) or third division (M3) metaphase was also recorded for a total of 100 metaphase cells scored.

TEM was used as positive control at a concentration of 0.025 µg/ml. in the non-activated assay.

In the activated assay CP was used at a concentration of 2.5 µg/ml.

The solvent vehicle for the test article was used as the solvent control.

A test was deemed valid if the mean SCE/cell in the untreated control did not exceed 13 and the mean SCE/cell for the positive control must be at least double that of the negative control.

A test material is considered positive if it induces a doubling in SCE frequency over the solvent control at a minimum of three consecutive dose levels or if a dose responsive and statistically significant increase is observed over a minimum of 3 dose levels affected.

A statistically significant increase at one or more dose levels with no evidence of a dose response is assessed as equivocal or as negative according to the magnitude of the response and the number of dose levels affected.

Result

- : Dose levels for the SCE assay were selected following a preliminary toxicity test based upon cell proliferation after treatment relative to the solvent control. CHO cells were exposed to solvent alone and to nine concentrations of test material ranging from 1 to 0.0001 µl/ml in the absence and presence of an S-9 reaction mixture. The test material was partially insoluble at 1 µl/l. Further dilutions were completely soluble. Based on the growth inhibition and cell cycle delay, dose levels of 0.3, 0.2, 0.1 and 0.05 µl/ml were selected for use in the assay without metabolic activation. For the assay with metabolic activation concentrations of 0.2, 0.1, 0.05 and 0.03 µl/ml were selected.

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A harvest time of 30 hours after treatment initiation was selected to assure collection of enough analyzable second division metaphases at the high dose.

Summarized results for the assays are as follows:

Treatment/ replicate	Cell cycle kinetics			SCEs/ chromosome	Group mean SCEs/ cell (\pm SD)
	M1	M2	M3		
<u>Without metabolic activation</u>					
Untreated A	2	98	0	0.5	9.92 (3.16)
B	5	95	0	0.48	
Acetone A	4	96	0	0.52	9.94 (3.03)
B	5	95	0	0.50	
API 81-03 (μ /ml)					
0.05 A	3	97	0	0.51	9.88 (3.12)
B	2	98	0	0.51	
0.1 A	12	88	0	0.54	10.32 (3.01)
B	9	91	0	0.52	
0.2 A	6	94	0	0.51	9.94 (3.11)
B	5	95	0	0.52	
0.3 A	1	99	0	0.53	10.34 (2.58)
B	4	96	0	0.53	
TEM (μ g/ml)					
0.025 A	0	65	35	2.79	59.02 (12.31)
B	0	81	19	3.21	
<u>With metabolic activation</u>					
Untreated A	2	97	1	0.52	10.10 (3.12)
B	2	95	3	0.51	
Acetone A	4	96	0	0.45	9.38 (3.36)
B	2	98	0	0.51	
API 81-03 (μ /ml)					
0.03 A	3	97	0	0.48	8.82 (2.46)
B	5	95	0	0.44	
0.05 A	8	92	0	0.54	10.54 (2.7)
B	5	95	0	0.56	
0.1 A	6	94	0	0.59	11.20 (3.45)
B	3	97	0	0.55	
0.2 A	2	96	2	0.48	10.10 (2.59)
B	1	94	5	0.57	

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CP (ug/ml)						
2.5	A	2	98	0	1.63	
	B	4	96	0	1.72	32.72 (6.51)

In the assay with metabolic activation, the group mean SCEs/cell were significantly increased compared to controls, P= 0.05 for 0.05 µl/ml concentration and P= 0.01 for the 0.02 µl/ml concentration.
Positive controls in both assays were significant P=0.01

The ositive and negative controls fulfilled the requirements for a valid test.

API 81-03 did not induce an increase in sister chromatid exchanges in CHO cells when tested in the absence of metabolic activation. However the test material did induce a small but statistically significant increase in SCEs at two intermediate dose levels in the presence of metabolic activation which was concluded to be equivocal.

Test substance : Sample API 81-03 is a light catalytically cracked naphtha stream.
The PONA analysis for this sample is

Type	Vol. %
Paraffins	42.8
Olefins	36.5
Naphthenes	10.2
Aromatics	10.2
Indans/tetralins	0.3
Naphthalenes	0

Reliability : (1) Valid without restriction

(10)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Doses : 0.3, 1.0 and 3.0 g/kg
Result : Negative
Year : 1985
GLP : Yes
Test substance : API 81-04

Method : Test material was given intraperitoneally as a solution in corn oil at a rate of 5 ml/kg to groups of 15 rats of each sex at three different dose levels (0.3, 1.0 & 3.0 g/kg). A group of 15 rats of each sex were given corn oil and these animals served as vehicle controls. A group of 5 animals of each sex to be used as positive controls was dosed with triethylenemelamine (TEM) at a level of 0.5 mg/kg and these animals were killed 24 hours afterwards. Two to four hours prior to being killed the rats were given a single ip dose of colchicine (1 mg/kg).
For each dose level of test material and the negative

controls 5 rats of each sex were killed 6, 24 and 48 hours after dosing.

Immediately following sacrifice bone marrow was aspirated from the femur. The marrow was washed and the cells were fixed before being spread on slides (at least 3 slides were prepared from each animal).

Slides were stained and scored without regard to treatment group. Where possible, a minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy.

The ratio of the number of cells in mitosis per 500 cells counted x 100 is defined as the mitotic index.

Remark

The data on chromosomal aberrations for the treated animals was compared to that for the negative controls.
: In a separate study in which exposure was by inhalation at 63, 297 and 2046 ppm, 6hr/day for 5 days, there was no evidence of a light catalytic cracked naphtha causing chromosomal aberrations in rats. (API Report 32-31300)

Result

: There was a 9% weight loss in males 48 hours after receiving 3 g/kg API 81-04 and a 2% weight loss in females at the same time and dose level.

Clinical signs of toxicity in the 3 g/kg group included lethargy in both sexes and increased tearing as indicated by a crusty appearance of fur around the eyes of the male animals. Animals in the vehicle control group appeared normal.

The report includes for each animal the following information:

number of cells scored, mitotic index, modal chromosome number, number of gaps, breaks (chromatid and chromosome) and fragment, number of rearrangements (exchange figures, dicentric and ring) and number of severely damaged cells. Treatment with API 81-04 did not affect any of these parameters.

There were no apparent sex differences and consequently the data for both sexes are combined in the following summary table.

500 cells were examined for every treatment at each time period shown below.

No rearrangements were recorded for either the vehicle control or any of the groups treated with API 81-04, whereas 13 rearrangements were recorded for the positive control group.

No aberrations from severely damaged cells were recorded for either the vehicle control or API 81-04 groups but 80 were recorded for the positive control group.

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Time (hrs)	Incidence of aberrations (%)	Total No. of Gaps	Breaks	Aberrations per cell
Corn oil control (5 ml/kg)				
6	0.8	5	4	0.008
24	0.4	3	2	0.004
48	0.4	2	2	0.004
API 81-04 (3 g/kg)				
6	1	3	5	0.010
24	0	5	0	0
48	0.4	1	2	0.004
API 81-04 (1 g/kg)				
6	0.6	3	3	0.006
24	0.4	2	2	0.004
48	0.4	0	2	0.004
API 81-04 (0.3 g/kg)				
6	1.0	1	5	0.010
24	0	2	0	0
48	0.2	3	1	0.002
TEM 0.5 mg/kg)				
24	16.6	22	126	0.438

The data above demonstrate that API 81-04 did not cause chromosomal aberrations in either male or female rats at the dose levels tested.

Test substance : API 81-04 is an olefinic naphtha stream with the following characterization

Type	Vol. %
Paraffins	34.6
Olefins	29.2
Naphthenes	14.5
Aromatics	21.1
Indans/tetralins	0.5
Naphthalenes	0.1

Reliability : (1) Valid without restriction

(1) (3)

Type : Sister chromatid exchange assay
Species : Mouse
Sex : Male/female
Strain : B6C3F1
Route of admin. : i.p.
Doses : 200, 1200 and 2400 mg/kg
Result : Positive
Year : 1988
GLP : Yes
Test substance : API 81-03

Method : Dose levels were selected on the basis of a dose range finding study that had been conducted previously.

Six experimental groups of five male and five female mice were used for the SCE assay. Four hours prior to administration of test material, the mice were anesthetized with Metofane and an agar-coated 50 mg BrdUrd pellet was implanted subcutaneously in the lower abdominal region.

The test material in corn oil or the corn oil alone were administered by ip injection at a rate of 10 ml/kg body weight. The positive control (CP) was injected ip at a dose level of 10 mg/kg. The positive control (API 81-15) was administered at a dose of 4 g/kg, which was administered by ip injection at a rate of 10 ml/kg.

All mice were weighed immediately prior to administration of test dose. Colchicine, used to arrest dividing cells in metaphase, was administered ip at 1 mg/kg to all mice two to four hours prior to sacrifice.

24 to 26 hours after BrdUrd pellet implantation the mice were sacrificed. Marrow was collected from both femurs. After washing and fixing bone marrow cells slides were prepared for subsequent staining and examination. Two to five slides were prepared from each animal.

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 base upon 500 cells counted.

The percentage of first, second and third-division metaphase cells was also recorded as the number per 100 cells counted.

A test article is considered to induce a positive response if a dose-related increase in SCEs/metaphase is observed relative to the vehicle control.

The test is considered valid if the mean number of SCE per second division metaphase cell must not exceed 8 SCEs/cell/animal in the negative (vehicle) control. The mean SCE/cell/animal for the positive control animals must be statistically increased relative to the vehicle control using the Mann Whitney test ($P= 0.05$)

Result : There was little or no apparent weight loss between the pretreatment body weights and those at the time of colchicine dosing. No clinical signs of toxicity were observed.

No mitotic delay or adverse effect on mitotic indices were observed at any test article dose level.

The results are summarized in the following table.

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Sex	Range of mean SCEs/cell for individual animals		Average SCEs/cell per mouse(1,2)
-----	--	--	----------------------------------

Corn oil

M	4.68	5.84	5.44 ±0.47 (5.64)
F	5.28	7.36	6.25 ±0.86 (6.06)

API 81-03 (2.4 mg/kg)

M	7.06	10.46	8.88 ±1.24 (9.14)**
F	8.16	11.58	9.61 ±1.4 (9.12)**

API 81-03 (1.2 mg/kg)

M	8.58	10.2	9.15 ±0.65 (8.96)**
F	8.92	12.28	10.5 ±1.49 (10.2)**

API 81-03 (0.2 mg/kg)

M	7.54	9.28	8.52 ±0.71 (8.86)**
F	9.2	11.44	10.0 ±0.92 (10.14)**

API 81-15 positive control (4 g/kg)

M	6.68	9.28	7.94 ±0.93 (7.94)**
F	7.28	8.54	7.86 ±0.58 (7.56)*

CP (10 mg/kg)

M	36.6	44.18	40.3 ±3.53 (38.5)**
F	18.34	31.44	25.5 ±5.38 (25.06)**

1 Mean± standard deviation (median SCEs/cell)

2 * P=0.05 ** P=0.01

There was a significant increase in SCEs/cell when analyzed by sex.

Pairwise comparisons by sex of each treatment group with its vehicle control were significantly different.

CP and 81-15 also caused an increase in SCEs/cell/mouse in both males and females.

The negative and positive controls fulfilled the requirements for determination of a valid test. Therefore, API 81-03 was shown to be positive in the SCE assay.

Test substance

: Sample API 81-03 is a light catalytically cracked naphtha stream.

The PONA analysis for this sample is

Type	Vol. %
Paraffins	42.8
Olefins	36.5
Naphthenes	10.2
Aromatics	10.2
Indans/tetralins	0.3
Naphthalenes	0

Reliability

: (1) Valid without restriction

(9)

5.7 CARCINOGENICITY

Remark : No data

5.8.1 TOXICITY TO FERTILITY

Type : Reproductive/developmental toxicity screening study, (OECD 421)
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Frequency of treatm. : 6 hours/day, 7 days/week
Premating exposure period
Male : 2 weeks
Female : 2 weeks
Doses : Target: 750, 2500 & 7500 ppm. Actual: 752, 2512 & 7518 ppm
Control group : Yes
NOAEL parental : = 2500 ppm
other: NOAEL for : = 7500 ppm
reproductive performance and developmental toxicity
Method : OECD combined repeated dose and reproductive/developmental toxicity screening test
Year : 1999
GLP : Yes
Test substance : LCCN-D See section 1.1.1.

Method : Groups of 10 rats of each sex were exposed to 750, 2500 or 7500 ppm. LCRN-D for 6 hours /day, seven days/week. A group of 10 rats of each sex served as sham treated controls. Parental females were exposed for 14 consecutive days prior to mating, throughout mating and days 0-19 of gestation. Dams and their litters were sacrificed on post partum day 4. Unmated females and parental males were exposed to the test material for 14 days prior to mating, throughout mating and for 23 additional days following completion of the mating period. These animals were sacrificed shortly after the last litters were delivered reached post partum day 4.

Mating

Within each group one male was co-housed with the same female until evidence of mating was observed (presence of sperm in vaginal smear or copulatory plug). The day of mating was designated day 0 of gestation. Following mating, the females were housed individually and continued their exposures to test material until day 19 of gestation. Females not showing evidence of mating following a 14 day mating period continued their exposures. If such a female showed signs of being pregnant it was removed from the exposure regimen and observed for parturition.

Observations

All parental animals were regularly observed for mortality and gross pharmacologic signs. A physical examination, including palpation for tissue masses was carried out daily 30 mins. after removal from the exposure chambers. Body weights and food consumption were measured throughout the study.

From day 20 of gestation, females (pregnant and non-pregnant) were observed for signs of parturition. As soon as possible after delivery, litters were observed for the number of live and dead pups and for any abnormalities. Litters were also observed twice daily for unusual findings and dead pups. On days 0 and 4 of lactation, the pups were counted, weighed and their sex was determined by external observation.

Pathology

Males were killed as a group shortly after the last litters delivered had reached day 4 of lactation.

Females with litters that reached day 4 of lactation were killed the next day or shortly thereafter.

Unmated females and those that did not deliver were killed 23 days after completion of the mating period.

At post mortem, a complete macroscopic examination was carried out on all adult animals. The following organs were weighed and organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, lung, spleen, epididymes, testes and thymus. Post mortem examination of females included a count of uterine implantation scars when present.

Pups were sacrificed on day 4 of lactation and underwent a complete macroscopic examination and a determination of sex by internal examination. All pups were preserved with viscera intact. Pups found dead at birth and that died prior to day 4 of lactation also underwent a gross external and internal examination. Dead pups were not eviscerated, but were preserved intact.

27 tissues were preserved from all adult animals in all dose groups. Ovaries, testes, epididymes, nose with nasal turbinates, and any grossly observed abnormalities were processed and sections examined histologically for all males and female parental animals in the control and highest dose group. Four sections were prepared and examined microscopically of the skull containing the nasal turbinates. These were

- area between upper incisor and incisive papilla
- area between incisive papilla and first palatal ridge
- area between second palatal ridge and first upper molar
- area between first upper molar and nasopharynx.

Result

- : All animals survived to scheduled sacrifice. Red staining on the snout was seen with increasing frequency in the mid and high dose animals of both sexes throughout the study. Microscopic examination of the nasal turbinates of the sham-exposed and high dose animals did not reveal any significant changes.

Although all treated groups gained slightly less weight than the sham treated controls the differences were not statistically significant. Food consumption was comparable in all groups. Apart from those listed below, absolute and relative organ weights were unaffected by treatment.

High dose Males

Absolute kidney weight increased (18%)
Relative kidney weights increased (24%)
Relative liver weights increased (15%)

High dose females.

Absolute spleen weights increased by (19%)
Relative spleen weights increased by (19%)

At necropsy, no organs appeared abnormal.

Microscopic examination of kidneys from one high dose male with a dilated renal pelvis at necropsy revealed hyaline droplet formation and tubular dilatation of tubules in the cortico-medullary junction. This finding is consistent with male-rat-specific light hydrocarbon nephropathy. No test-related microscopic changes were observed in the testes or epididymes of adult male rats or ovaries of adult female rats in the high dose group.

Reproductive/fertility effects

All groups had a fertility index of >90% and all groups had a live birth index greater than or equal to 98%. Data are summarized below.

Parameter	Dose group (ppm)			
	0	750	2500	7500
Females on study	10	10	10	10
Litters with liveborn	9	8	9	10
Implantation sites	155	126	139	160
Mean	17.2	15.8	15.4	16
Pups delivered (total)	149	110	132	152
Liveborn	149	108	131	151
Live birth index (%)	100	98	99	99
Pups dying				
Day 0	0	2	1	1
Days 1-4	4	2	2	1
Pups surviving 4 days	145	106	129	150
Viability index (%)	97	98	99	99
pup sex distribution				
Day 0 M/F (ratio)	72/77	50/58	65/66	87/64
Day 4 M/F (ratio)	72/73	49/57	65/64	87/63
Pup weight/litter (g)				
Day 0	6.3	6.6	6.4	6.4
Day 4	9.9	10.8	10.1	10.3

External and internal examination of pups sacrificed on day 4 of lactation were unremarkable.

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Test condition : The sample that was used has been described in section 1.1.1. The LCCN-D was wholly vaporized using a countercurrent volatilization chamber. The volatilized LCCN-D was diluted with air to achieve the desired atmospheric concentrations.

The target and actual chamber concentrations are as follows:

Target concentration (ppm)	Actual concentration (ppm)	Total mass aerosol concentration ($\mu\text{g}/\text{m}^3$)
0	0	4.8 ± 4.5
750	752 ± 35	4.1 ± 3.5
2500	2512 ± 66	3.7 ± 3.3
7500	7518 ± 146	4.1 ± 3.9

The LCCN-D was characterized pre- and post study. The results (given in weight %) of the characterization are as follows:

Component	LCCN-D liquid	Study start	Study end
n-Butane	0.43	0.40	0.43
n-Pentane	3.28	3.23	3.24
iso-Pentane	15.22	15.87	15.74
1-Pentane	2.82	2.64	2.70
2-Pentene (trans)	7.30	6.96	7.02
2-Pentene (cis)	4.12	3.96	4.02
2-Methyl-2-butene	10.81	10.37	10.43
2-Methyl-1-butene	5.60	5.23	5.33
Cyclopentane	1.34	1.28	1.31
n-Hexane	1.56	1.58	1.56
Methylcyclopentane	1.95	2.15	2.12
2,3-Dimethylbutane	1.36	2.30	2.27
2-Methylpentane	5.57	6.28	6.15
3-Methylpentane	3.08	3.18	3.12
1-Methylcyclopentane	1.23	1.24	1.25
Benzene	1.15	1.30	1.20
2-Methylhexane	1.09	1.20	1.17

Reliability : (1) Valid without restriction

(23)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : During days 0 to 19 of gestation inclusive.
Frequency of treatm. : 6 hours each day
Doses : Target: 2000 & 8000 mg/m^3 . Actual: 2150 & 7660 mg/m^3
Control group : Yes

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Year : 1996
GLP : No data
Test substance : LCCN CAS # 64741-55-5

Method : Four groups of 15 presumed-pregnant female rats were assigned to the following groups: Untreated controls, sham-treated controls, 2000 and 8000 mg/m³ test material. Exposures were for 6 hours each day on days 0 to 19 of gestation. All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation. On day 20 each female was sacrificed and all organs were examined grossly. Serum samples were analyzed for a variety of parameters, including serum iron and lactic dehydrogenase. The number of corporea lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early resorptions and live and dead fetuses recorded. Each fetus was identified for its sex, was weighed and the crown-rump distance was measured. Each fetus was examined for external anomalies. Half the fetuses were fixed in Bouin's solution and examined for visceral anomalies and the remaining fetuses were prepared for examination for skeletal anomalies.

Result : There were no treatment-related clinical abnormalities or differences in body weight. Results of the reproductive parameters are listed below.

Parameter	No treat	Control Sham treat	LCCN	LCCN
			2150 mg/m ³	7660 mg/m ³
Females mated	15	15	15	15
Females pregnant	14	13	14	15
Corpora lutea	18	18	16	18
Implantation sites	16	16	14	16
Primplantation loss (&)	10	12	14	8
Viable fetuses/litter	15	14	14	15
Resorptions	0.7	0.6	0.8	1.7 *a
Resorptions (%)	4.6	3.9	4.7	10.4 *a
Dams with resorptions	9	5	8	13 *b

*a Significant difference from untreated and sham treated controls

*b Significant difference from sham treated controls

It is clear that with the exception of resorptions, no other parameter was affected by exposure.

During the external examination of fetuses, a sham treated animal had gastroschisis and one fetus from the 2150 mg/m³ group had a tail that was short and filamentous.

Fetal body weights and crown-rump lengths were unaffected by treatment.

No visceral abnormalities were observed.

There was an increased number of skeletal variations in animals housed in the exposure chambers (exposed and sham treated controls) when compared to the untreated controls. The authors concluded that these alterations were not related to LCCN since they occurred at the same incidence in the sham treated controls as well. The findings are tabulated below.

The numbers of fetuses with the specific anomaly are shown. The numbers in parenthesis are the % of fetuses.

Parameter	No treat	Control Sham treat	LCCN	LCCN
			2150 mg/m ³	7660 mg/m ³
Caudal vertebrae: transverse process incompletely ossified	18(16)	42(40)	41(40)	45(39)
Sacral vertebrae transverse process incompletely ossified	7(6)	23(22)	17(17)	28(24)
Incompletely ossified sternebrae	83(75)	80(76)	91(89)	101(88)

Test substance : A description of atmosphere generation is given in a publication by the same authors in section 5.4.

Reliability : Actual concentrations in this study were:
 2150 ±260 and 7660 ±570 mg/m³.
 (1) Valid without restriction

(14)

- (1) American Petroleum Institute (1985)
Activity of API 81-04 in the acute in vivo cytogenetics assay in male and female rats
API Med. Res. Pub. 32-32288
- (2) American Petroleum Institute (1985)
L5178Y TK +/- Mouse lymphoma mutagenesis assay of API 81-04
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**ROBUST SUMMARY
OF INFORMATION ON**

Substance Group: **NAPHTHENIC NAPHTHAS**

Summary prepared by: American Petroleum Institute

Creation date: 22 February 2001

Printing date: MAY 20, 2003

Date of last Update: MAY 20, 2003

Number of Pages: 39

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)

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Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : The naphtha streams that are rich in naphthenes are obtained from the atmospheric distillation of crude oil. The streams contain saturated and aromatic hydrocarbons, mainly in the range C₄ to C₁₀ and boil in the range of approximately minus 39 to 200 °C

The naphthenic naphthas typically are composed of the following hydrocarbon classes:

	Approx. Content (volume %)
Paraffins	72
Olefins	<0.1
Naphthenics	21
Aromatics	7

Sweetened Naphtha (CAS No. 64741-87-3) is typical of the streams in this category.

An API sample of Sweetened naphtha that has been used for some of the toxicology studies for this group has been characterized as follows (API, 1997):

Sample API 81-08
CAS 64741-87-3

Parameter	Method	Units	Value
API Gravity @60	D287		76.9
Density @ 15°C	D287		0.6782
Molecular weight	D2224	gm/mol	81
Refractive index		RI units	1.3892
Total Sulfur	D3120	ppm/wt	1170
Total Nitrogen	Chemil.	ppm/wt	<1
Total Oxygen	NAA	wt. %	0.42
Total Chloride	coulom.	ppm/wt.	<1
RVP		psia	10.2
Distillation	D86	vol/°F	
IBP			102
FBP			238
Mass spectrometer analysis			
Paraffins	D2789/MS	Vol. %	72.1
Olefins	D2789/MS	Vol. %	<0.1
Naphthenes	D2789/MS	Vol. %	20.9
Aromatics	D2789/MS	Vol. %	6.9
Indans/Tetralins	D2789/MS	Vol. %	0.1
Naphthalenes	D2789/MS	Vol. %	0.0

Three different naphtha stream (Light straight run naphthas) have been used for the ecotox studies and their compositional details were also used for fate and effects modelling.

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The three samples High, Moderate and Low naphthenic contents, are as follows:

Moderate naphthenic content
Chevron sample (Chevron, 1995)
CAS No. 64741-46-4
Detailed hydrocarbon analysis

	Olefins Naphthenes		Aromatics	Paraffins	
				n-	total
Total%	0.72	22.41	3.06	73.31	31.13
C4	0.03	0.00	0.00	5.85	5.58
C5	0.085	1.73	0.00	38.80	16.27
C6	0.36	6.24	0.70	18.18	6.26
C7	0.05	7.11	1.12	5.58	2.00
C8	0.00	5.31	0.96	3.22	0.79
C9	0.00	1.95	0.25	1.20	0.13
C10	0.00	0.07	0.03	0.46	0.08

High naphthenic content
CONCAWE sample W94/809
CAS No. 64741-46-4
Density (g/ml @ 16°C) 0.7587
Sulfur (ppm) <10

Detailed hydrocarbon analysis (Method ASTM D 5134-92)

	Olefins Naphthenes		Aromatics	Paraffins	
				n-	i-
Total%	2.18	33.92	17.26	18.88	26.83
C4	0.019	0.00	0.00	0.141	0.059
C5	0.090	0.138	0.00	0.592	0.468
C6	0.066	2.578	0.756	1.565	1.341
C7	0.663	10.265	5.218	3.887	3.811
C8	0.074	11.036	9.044	8.407	9.409
C9	1.161	9.117	2.080	3.762	8.834
C10	0.103	0.778	0.153	0.778	0.103
C11	0.00	0.009	0.007	0.009	0.145

Low naphthenic content
CONCAWE sample CWE3
CAS No. 64741-46-4
Density (g/ml @ 16°C) 0.6662
Sulfur (ppm) 83

Detailed hydrocarbon analysis (Method ASTM D 5134-92)

	Olefins Naphthenes		Aromatics	Paraffins	
				n-	i-
Total%	1.04	12.23	3.27	48.19	34.02
C4	0.00	0.00	0.00	0.006	0.000
C5	0.085	4.047	0.00	31.91	8.228
C6	0.830	6.696	2.252	16.139	23.917
C7	0.119	1.056	0.382	0.647	1.241
C8	0.00	0.303	0.334	0.263	0.324
C9	0.00	0.165	0.243	0.162	0.178

(9) (12) (15)

2.1 MELTING POINT

Remark : Not relevant

2.2 BOILING POINT

Value : ca. 49 - 177 °C at 1013 hPa
Decomposition : No

Remark : The samples which were used by the API in its toxicity assessments for this Group were prepared by the fractionation of two types of crude oil, using a pilot plant still and separating cuts in a distillation range of 120 to 350°F (49 to 177°C).

These figures represent a typical boiling range for light straight-run naphtha, CAS No. 64741-46-4.

The standard oil industry method for determination of boiling range is ASTM D86.

(11) (20)

2.4 VAPOUR PRESSURE

Value : 1290 hPa at 37.8 °C
Decomposition :
Method : ASTM D5191
Year : 1995
GLP : Yes
Test substance : LSRN-Hi Naphthenic, CONCAWE sample W94/809, See section 1.1.1. for characterization

Remark : The sample was identified by CONCAWE as MRD-95-044, gasoline sample W94/809, CAS No. 64741-46-4, a light straight-run naphtha.

Reliability : (1) valid without restriction

(15)

Value : 9150 hPa at 37.8 °C
Decomposition :
Method : ASTM D5191
Year : 1995
GLP : Yes
Test substance : LSRN (Low Naphthenic), CONCAWE sample CWE3, See section 1.1.1. for characterization

Remark : The sample was identified by CONCAWE as MRD-95-091, gasoline sample CWE3, CAS No. 64741-46-4, a light straight-run naphtha.

Reliability : (1) valid without restriction

(15)

2.5 PARTITION COEFFICIENT

Log pow : = 2.13 - 4 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65
Year : 2000
GLP : No
Test substance : LSRN (Low Naphthenic], CONCAWE sample CWE3, See section 1.1.1. for characterization

Remark : Log P values represent the spread of calculated and/or measured values for C5 to C7 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).

Reliability : (2) Valid with restrictions (15) (22)

Log pow : = 2.13 - 4.76 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : No
Test substance : LSRN (Moderate Naphthenic] Chevron analysis-See section 1.1.1. for characterization

Remark : Log P values represent the spread of calculated and/or measured values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific moderate naphthenic (19.7%) LSRN sample. Calculated SAR result for surrogate structures contained in program database (smilecas.dat). Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard.

Reliability : (2) Valid with restrictions (22)

Log pow : = 2.73 - 4.85 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65
Year : 2000
GLP : No
Test substance : LSRN [Hi Naphthenic] CONCAWE sample W94/809, See section 1.1.1. for characterization

Remark : Log P values represent the spread of calculated and/or measured values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).

Reliability : (2) Valid with restrictions (15) (22)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Method : Preparation of Water Soluble Fractions
Year : 1995
GLP : Yes
Test substance : LSRN-Hi Naphthenic, CONCAWE sample W94/809, See section 1.1.1. for characterization

Method : Water Accommodated Fractions (WAFs) of LSRN were prepared at 100 mg/L loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.

Result : Gas chromatographic analysis of TEX (toluene, ethyl benzene, and xylenes) components indicated freshwater solubility of 5.7-7.9 ppm (as TEX). Measured test concentrations of the LSRN were based on the total combined concentrations of TEXN which represent approximately 13% composition of the test substance. Concentrations for these components reached equilibrium by 19 hours.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. 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 (13) (14) (16) (17) (18)

Method : Preparation of Water Soluble Fraction
Year : 1995
GLP : Yes
Test substance : LSRN-Low Naphthenic, CONCAWE sample CWE39, See section 1.1.1. for characterization

Method : Water Accommodated Fractions (WAFs) of LSRN were prepared at 1000 mg/l loading in freshwater and equilibrated for 24 hours in tightly closed systems with minimal headspace.

Result : Gas chromatographic analysis of BTEX components indicated freshwater solubility at 24 hours of 4.9 ppm as benzene.

Conclusion : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for gasoline components range from approximately 3 to 2000 mg/l. 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

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	<p>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.</p>	
Reliability	: (2) Valid with restrictions	(13) (16) (17) (18) (24)
Method	: Preparation of water soluble fraction	
Year	: 1995	
GLP	: Yes	
Test substance	: LSRN-Moderate (19.7%) Naphthenic, See section 1.1.1. for Chevron characterization	
Method	: Water Accommodated Fractions (WAFs) of LSRN were prepared at 50 mg/L loading in freshwater and saltwater and equilibrated for 48 hours in tightly closed systems with minimal headspace	
Result	: Gas chromatographic analysis of LSRN components 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene in WAFs indicated freshwater solubility of 1.62-1.81 ppm and 1.50 mg/l in saltwater. Concentrations for these components reached equilibrium by 24 hours.	
Conclusion	: Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. 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	(4) (13) (16) (17) (18)

3.1.1 PHOTODEGRADATION

Type : Calculated
Light source : Sun light
INDIRECT PHOTOLYSIS
Sensitizer : OH
Method : Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year : 2000
GLP : No
Test substance : LSRN-Low Naphthenic, CONCAWE sample CWE39, See section 1.1.1. for characterization

Remark : AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O₃. Atmospheric oxidation rates were calculated for the C₅ to C₉ hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 1.262 days (cyclohexane) to 15.985 days (isopentane)

Result : Indirect Photolysis
 Sensitizer: OH radical
 Conc. of sensitizer: $1.50E^{+06}$ OH radicals/cm³
 Rate Constant: $0.6991 E^{-12}$ (isopentane) to $8.4783 E^{-12}$ (cyclohexane) cm³/molecule-sec
 Half-life: 1.262 to 15.985 days

Reliability : (2) Valid with restrictions

(15) (23)

Type : Calculation
Light source : Sun light
INDIRECT PHOTOLYSIS
Sensitizer : OH
Method : Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year : 2000
GLP : No
Test substance : LSRN-Hi Naphthenic, CONCAWE sample W94/809, See section 1.1.1. for characterization

Remark : AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O₃. Atmospheric oxidation rates were calculated for the C₅ to C₉ hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 0.789 days (m-xylene) to 5.486 days (benzene).

Result : Indirect Photolysis
 Sensitizer: OH radical
 Conc. of sensitizer: $1.50E^{+06}$ OH radicals/cm³
 Rate Constant: $1.9498E^{-12}$ (benzene) to $13.5606 E^{-12}$ (m-xylene) cm³/molecule-sec
 Half-life: 0.789 to 5.486 days

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Reliability	: (2) Valid with restrictions	(15) (23)
Type	: Calculation	
Light source	: Sun light	
Relative intensity	: = 1 based on intensity of sunlight	
INDIRECT PHOTOLYSIS		
Sensitizer	: OH	
Method	: Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson	
Year	: 2000	
GLP	: No	
Test substance	: LSRN-Moderate (19.7%) Naphthenic, See section 1.1.1 for characterization by Chevron	
Remark	: AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O ₃ . Atmospheric oxidation rates were calculated for the C5 to C9 hydrocarbon components found in LSRN, CAS No. 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific moderate naphthenic LSRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LSRN constituents is: 0.789 days (m-xylene) to 15.985 days (isopentane).	
Result	: Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E ⁺⁰⁶ OH radicals/cm ³ Rate Constant: 0.6691E ⁻¹² (isopentane) to 13.5606E ⁻¹² (m-xylene) cm ³ /molecule-sec Half-life: 0.789 to 15.985 days	
Reliability	: (2) Valid with restrictions	(23)

3.1.2 STABILITY IN WATER

Test substance	: LSRN-Hi Naphthenic, CONCAWE sample W94/809, Se section 1.1.1. for characterization	
Conclusion	: Hydrolysis unlikely	
Reliability	: (1) Valid without restriction	(19)
Test substance	: LSRN-Low Naphthenic, CONCAWE sample CWE39, See section 1.1.1. for characterization	
Conclusion	: Hydrolysis unlikely	
Reliability	: (1) Valid without restriction	(19)
Test substance	: LSRN-Moderate (19.7%) Naphthenic, See section 1.1.1. for characterization	
Conclusion	: Hydrolysis unlikely	
Reliability	: (1) Valid without restriction	(19)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment, sediment
Year : 2000

Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample.

The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

<u>Medium</u>	<u>% distribution</u>
Air:	97 to 99.97
Soil:	0.03 to 1.2
Water:	0.008 to 2.7
Sediment	0.00 to 0.02
Suspended sediment	0.00

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.

Reliability : (2) Valid with restrictions

(15) (21)

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment, sediment
Method : Calculated
Year : 2000

Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific low naphthenic LSRN sample.

The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

<u>Medium</u>	<u>% distribution</u>
Air:	98.89 to 99.98
Soil:	0.01 to 0.11
Water:	0.01 to 1.00
Sediment	<0.001
Suspended sediment	<0.001

Reliability : (2) Valid with restrictions

(15) (21)

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment, sediment

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Method : other: Calculated
Year : 2000

Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific moderate naphthenic (19.7%) LSRN sample. The majority of LSRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

<u>Medium</u>	<u>% distribution</u>
Air:	97 to 99.97
Soil:	0.00to 1.2
Water:	0.013 to 2.7
Sediment	0.00 to 0.03
Suspended Sediment	0.00

Test substance : Light Straight Run Naphtha (LSRN)-Moderate (19.7%) Naphthenic, No. 64741-46-4

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.

Reliability : (2) Valid with restrictions

(21)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	: Static with daily renewal
Species	: Pimephales promelas (Fish, fresh water)
Exposure period	: 96 hour(s)
Unit	: mg/l
Limit test	: No
Analytical monitoring	: yes
Year	: 1996
GLP	: Yes
Test substance	: LSRN-Moderate naphthenic content see section 1.1.1.
Method	: No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002. LL ₅₀ and LC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	: Mortality (no. of deaths/treatment) at 96 hrs: 0, 0, 0, 6, 20 and 21 in 0, 3.1, 6.3, 13, 25 and 50 mg/l treatments. Abnormal behavior (surfacing, erratic swimming, quiescence) was observed at 96 hrs for 6 organisms in the 13 mg/l treatment. 96-hr LL ₅₀ = 15 mg/l, 6.3-25 mg/L w/ 95% C.I. (as nominal loading rate) 96-hr LC ₅₀ = 0.689 mg/l, 0.289-0.962 mg/l w/ 95% C.I. (measured concentrations) 96-hr NOEL = 6.3 mg/l (nominal); 96-hr NOEC = 0.287 mg/l (measured) based on lack of mortality and abnormal effects for these treatments. A low boiling point naphtha sample w/ CAS no. 8030-30-6 (different from the sample used in toxicity testing, but similar in composition) was used to validate the analytical method being developed to identify water soluble hydrocarbons in aqueous 24 hour equilibrated samples. This does not appear to have affected the results of the study.
Test condition	: Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by blending naturally hard well water with water that had been demineralized by reverse osmosis. Nominal loading rates of 0, 3.1, 6.3, 13, 25 and 50 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface,

and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes.

Fish were hatched and raised from ABC Laboratories' in-house culture, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8 liter glass containers with teflon lined caps. Fish were acclimated to the test water and temperature approximately 72 hr before the test, and were not fed during this 72 hr period. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control, with the exception of the 50 mg/l treatment, where 11 organisms instead of 10 were placed in one replicate. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving approximately one liter of solution to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Water temperature was 21-22 °C. Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 7.3 and 8.8, pH values between 8.1 and 8.3. Hardness values ranged from 134 to 144 mg/l; alkalinity values ranged from 144 to 154 mg/l and conductivity values ranged from 300 to 340 microsiemens.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

(3)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : 48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : Procedure patterned after:1991 ASTM method E729-88a and 1985 USEPA TSCA Test Guidelines: Daphnid Acute Toxicity Test. Fed. Reg., vol. 50 (No. 188) Sept 27, 1985, 797.1300.
Year : 1996

GLP	:	Yes
Test substance	:	LSRN-Moderate naphthenic content see section 1.1.1.
Method	:	Statistical Method: (FT - ME) EL ₅₀ and EC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	<p>Immobility (no. of organisms) at 48 hrs: 1, 3, 0, 0, 19 and 20 for 0, 3.0, 6.0, 12, 24 and 48 mg/l treatments.</p> <p>At the 3 and 12mg/l nominal treatments, 1 and 20 organisms were observed at the bottom of the test chambers, respectively.</p> <p>48-hr EL₅₀ = 18 mg/l based upon nominal loading rate (95% C.I. 12 to 24 mg/l)</p> <p>48 hr EC₅₀ was 0.65 mg/l (95% C.I. 0.47 to 0.83 mg/l); based on total measured concentrations.</p> <p>48-hr NOEL = 6.0 mg/l based upon nominal loading rate.; 48 hr NOEC was 0.24 ppm based on total measured concentrations.</p>
Test condition	:	<p>Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were a blend of aged well water and reverse osmosis well water.</p> <p>WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 2.4l of water for 24 hr in aluminum foil covered 2.5 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately one hour, then drained from the port and used for testing. Samples were also analyzed by purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes.</p> <p>Range finding toxicity studies were conducted at 0.5, 1.0, 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 3.0, 6.0, 12, 24 and 48 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.</p> <p>Test vessels were teflon cap-sealed 8 oz. glass jars with 10 daphnids per jar and were completely filled to overflowing with approximately 273 ml test solution.</p> <p>During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.5; pH ranged from 8.3 to 8.4; temperature was 20 to 21 °C; hardness (mg/l) ranged from 132 - 140; alkalinity (mg/l) was 142-150 and conductivity (µmhos) values were 280 - 300.</p>

Reliability : Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from 11 day culture maintained in-house since October 1996.
 : (2) Valid with restrictions
 Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAF may be equally toxic and should have been quantitated to determine total measured concentrations.

(1)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1997
GLP : Yes
Test substance : LSRN-Moderate naphthenic content see section 1.1.1.

Method : Statistical Method: EL₅₀ and EC₅₀ calculated using nonlinear logistics sigmoid model (SAS). All NOEL/NOEC values based on visual review and Dunnett's test for significance.

Result : Percent inhibition on growth determined by cell density (cells/ml):
 96 hour EL₁₀=2.7 mg/l (1.9-3.5 mg/l CI @95%)
 96 hour EL₅₀=6.4mg/l (5.7-7.1 mg/l CI @95%)
 96 hour EL₉₀=15 mg/l (12-18 mg/l CI @95%)
 96 hour NOEL=1.9 mg/l

 96 hour EC₁₀=0.1 mg/l (0.061-0.15 mg/l CI @95%)
 96 hour EC₅₀= 0.26 mg/l (0.22-0.30 mg/l CI @95%)
 96 hour EC₉₀=0.66 mg/l (0.50-0.83 mg/l CI @95%)
 96 hour NOEC=0.0326 mg/l

Subcultures of the 31 mg/l treatment cultures were placed in fresh media (no test substance) after acute testing for ten days and indicated that growth inhibition was algistic in this treatment. Conduct of the range-finder and definitive tests were acceptable (no repeats). No excursions from the protocol were noted which would have affected the integrity of the study.

Concentration (mg/l)		
Nominal	Measured	96hr cell density (cells/ml)
Control	(<LOQ)	43.58 x10 ⁴
1.9	(0.0322)	42.332 x10 ⁴
4.0	(0.130)	29.25 x10 ⁴
7.8	(0.329)	18.42 x10 ⁴

	16	(0.704)	1.74×10^4
	31	(1.29)	0.04×10^4
Test condition	:	<p>Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 9.4-9.6l of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 µg/l EDTA chelator, pH adjusted to 7.5 ± 0.1 with 0.1 NHCl and sterilized by 0.45 micron filtration) in 9.5 liter aspirator bottles. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. The contents were stirred with teflon coated stir bars in the mixing vessels which were placed on magnetic stir plates at room temperature. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45 minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (148 ml) with treatment solution and inoculated with 3 day old algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media. Original algal cultures (stock UTEX-1648) obtained from Dept of Botany, Culture Collection of Algae, University of Texas at Austin, 1996. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 1.9, 4.0, 7.8, 16 and 31 mg/l The initial algal concentration was 1.0×10^3 cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination 400 +50 ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell densities were determined by direct microscopic examination. Samples at 0,24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: 2-methyl-pentane, cyclohexane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light straight run naphtha were based on the total combined concentrations of all analytes.</p>	
Reliability	:	<p>Test temperature was 24-26 °C. Test solution pH ranged from 8.0 to 8.5.</p> <p>(2) Valid with restrictions Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.</p> <p>(2)</p>	

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	:	10
Vehicle	:	Undiluted
Year	:	1982
GLP	:	Yes
Test substance	:	API 81-08 See section 1.1.1. for characterization
Method	:	<p>The test material was administered undiluted, as a single oral dose to groups of 5 male 5 female rats at a single dose level of 5 g/Kg. The dose volume was 7.35 ml/Kg based on an average bulk density of 0.68 g/ml. Food had been withheld from the rats overnight prior to dosing, but they had free access to water. Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days. The rats were weighed the day before dosing and then at 7 and 14 days after dosing.</p> <p>At study termination all animals were killed with carbon dioxide and subjected to a gross necropsy and abnormalities were recorded.</p>
Result	:	<p>No animals died during the study.</p> <p>Clinical signs of intoxication included diarrhea and mucoid diarrhea.</p> <p>Although there was a reduction in body weight following fasting, body weight were increasing by seven and 14 days post dosing.</p> <p>At necropsy no visible lesions were observed in 4 of 5 males and 2 of 5 females. In the right kidney of one male the renal pelvis was mildly dilated and a cervical lymph node was enlarged.</p> <p>In the females dilation of the pelvis of the kidney was observed in one animal, a cervical lymph node was enlarged in another animal and in a third animal mild hydrometra of the uterus was observed.</p> <p>The Oral LD₅₀ was greater than 5 g/Kg.</p>
Reliability	:	(1) Valid without restriction

(5)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC₅₀
Value : > 5.2 mg/l
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 10
Vehicle : Air
Exposure time : 4 hour(s)
Method :
Year : 1986
GLP : Yes
Test substance : API 81-08 See section 1.1.1. for characterization

Method : A group of 5 male and 5 female rats were exposed by whole body inhalation to API 81-08 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.

Result : The actual chamber concentrations were found to be 5.2 mg/l. No deaths occurred during the study. There were no unusual pharmacotoxic signs or behavior observed in the control animals. There was however, a slight incidence of nasal discharge (2/5 males and 1/5 females) during the exposure period but none during the following 14 day observation period. The body weight gains for the males exposed to API 81-08 was considered normal but the female body weight gains were marginally less than that of the controls on day 14 post exposure (8.2% compared to 13.8% increase over pre-exposure body weight). No significant macro or microscopic changes were observed that were considered to be treatment related.

Reliability : (1) Valid without restriction

(7)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD₅₀
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 8
Vehicle : Undiluted
Year : 1982

GLP	:	Yes
Test substance	:	API 81-08 See section 1.1.1. for characterization
Method	:	<p>The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to a dose of 2 g/Kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. 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. Collars were used to restrain the animals during the application period. The animals were observed for a total of 14 days post-dosing. Body weights were recorded just prior to dosing and again seven and 14 days after dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.</p>
Result	:	<p>No animals died during the study and no clinical signs of intoxication were observed. Normal growth was observed throughout the study. At necropsy, the only visible lesions seen were on the skin of two animals in which the test site was reddened in one together with crusted appearance and mild crusting was observed in the other rabbit.</p>
Reliability	:	(1) Valid without restriction

(5)

5.2.1 SKIN IRRITATION

Species	:	Rabbit
Concentration	:	Undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	None
PDII	:	1.2
Result	:	Slightly irritating
Method	:	Draize Test
Year	:	1982
GLP	:	Yes
Test substance	:	API 81-08 See section 1.1.1. for characterization
Method	:	<p>0.5 ml of undiluted test material was applied to two areas on each rabbit. 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.</p>

Body weights were recorded just prior to application of the test material and weekly thereafter throughout the study. At study termination, all surviving animals were euthanized with an overexposure of carbon dioxide, subjected to a gross necropsy and abnormalities were recorded.

Result : The scores for erythema and edema at each of the observation times were as follows:

	Erythema		Edema	
	Intact	Abraded	Intact	Abraded
24 h	0.7	0.7	1.0	0.7
72 h	1.0	0.7	0	0
96 h	0.5	0.2	0	0
7 days	0.3	0.3	0	0
14 days	0	0	0	0

The Primary dermal Irritation index was 1.2

Growth rates were normal throughout the study and there were no visible lesions at necropsy.

Reliability : (1) Valid without restriction

(5)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : Rinsed after 30 sec exposure in 3 rabbits; 6 rabbits unwashed
Number of animals : 9
Vehicle : None
Result : Not irritating
Method : Draize Test
Year : 1982
GLP : Yes
Test substance : API 81-08 See section 1.1.1. for characterization

Method : 0.1 ml of undiluted test material was placed in the everted lower eyelid 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. At the 72 hour and seven day readings, sodium fluorescein was used to aid in revealing possible corneal injury. Body weights were recorded just prior to treatment and one week afterwards. At termination of the study, the rabbits were euthanized by an overexposure of carbon dioxide and were subjected to a gross necropsy. Any abnormalities found were recorded.

Result : One hour after application of the test material the average score for irritation was 2.0 and 0.7 for unwashed and washed eyes respectively and the 24 hour readings were 0.3 and 0 respectively. All other scores throughout the study were 0. Growth was normal throughout the study and there were no visible lesions at necropsy.

Reliability : (1) Valid without restriction

(5)

5.3 SENSITIZATION

Remark : No data

5.4 REPEATED DOSE TOXICITY

Remark : No data

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Test concentration : 12.5 - 300 µl/ml
Cycotoxic concentr. : 0.05µl/ml without activation; 0.5µl/ml with activation
Metabolic activation : With and without
Result : Negative
Year : 1985
GLP : Yes
Test substance : API 81-08 See section 1.1.1. for characterization

Method : Based on a preliminary test, ethanol was selected as solvent for this assay. Concentrations of 0.061 to 1000 µl/ml appeared soluble in the assay medium and no change in color was noted.
 Two positive control substances were used viz Ethyl methane sulphonate (EMS) at a concentration of 0.5 µl/ml in the assay without activation and Dimethylnitrosamine (DMN) at a concentrations of 0.3 µl/ml.

A cytotoxicity study was carried out prior to the mutagenicity assay. The results were difficult to interpret and as a consequence a second study was carried out and the results from this were used to determine the concentrations to be used in the subsequent lymphoma assay. It was established that complete toxicity occurred at 0.05 µl/ml for the non-activated cultures and at 0.5 µl/ml for Araclor-induced rat liver S-9 activated cultures.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; 5-trifluorothymidine (TFT) was used as the restrictive agent.

Eight non-activated and nine activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 or 0.04 µl/ml and resulted in a range of growth of 6 to 97%. The

activated cultures that were cloned were treated with 0.0002, 0.0009, 0.0028, 0.008, 0.02, 0.045, 0.09, 0.7 or 0.75 µl/ml and produced a range of growth from 24 to 109%. Plates were prepared from TFT and from the viable culture (VC) and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was then determined.

The following criteria were used in judging the significance of the activity of the test article.

Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Result

: The data from each of the 3 trials that were considered valid are tabulated below.

Conc. (µl.ml)	Relative growth (%)	Mutant frequency (10⁶ units)
<u>TRIAL 1 No activation</u>		
15.6	118.6	18.1
31.3	64.4	27
62.5	97.8	15.7
125	78.2	24.2
250	20.1	48.8
Solvent control 1	100	13.9
Solvent control 2	100	20.3
Untreated control	191.7	21.5
EMS 0.5 µl/ml	17.4	258.2
<u>TRIAL 1 with S-9 activation</u>		
15.6	78.1	59.1
31.3	53.8	49.3
62.5	63.3	49
125	46.5	79.7
250	46.3	41.6
Solvent control 1	100	34
Solvent control 2	100	24
Untreated control	100.7	30.5
DMN 0.3 µl/ml	5	327.5
<u>TRIAL 4 No activation</u>		
12.5	47.8	19.5
25	49.7	19.2
50	37.7	13.5
100	113.3	8.5

200	86.2	9.3
300	19.8	36.4
Solvent control 1	100	18.3
Solvent control 2	100	18.5
Untreated control	163.9	16.2
EMS 0.5 µl/ml	13.5	700
<u>TRIAL 4 with S-9 activation</u>		
12.5	81.6	52.3
25	60.2	85.7
50	57.3	59.1
100	44.7	63.8
200	71.8	21
300	3.1	19.3
Solvent control 1	100	23.2
Solvent control 2	100	22.9
Untreated control	78.2	22.2
DMN 0.3 µl/ml	8.8	469.4
<u>TRIAL 5 with S-9 activation</u>		
150	76.9	13.6
150	28.4	25.2
200	42.5	24
200	41.9	15.3
250	59.6	24.2
250	15.6	31.1
300	4.9	30.2
300	7.3	32
Solvent control 1	100	27.1
Solvent control 2	100	19.2
Solvent control 3	100	22.4
Solvent control 4	100	24.5
Untreated control 1	63.8	31
Untreated control 2	49.9	29.2
DMN 0.3 µl/ml	16.6	352.9
DMN 0.3 µl/ml	2.2	333.3

The authors judged the data using the following criteria.

TRIAL 1

Non activation conditions.

The percent relative growths of the assayed treatments ranged from 118.6% to 20.1% which demonstrated non-detectable to moderate toxicities.

The minimum criterion for mutagenesis in this assay was a mutant frequency that exceeding 37.8×10^{-6} . The highest, most toxic treatment (250 µl/ml) induced a mutant frequency that exceeded the minimum criterion, but the increase in the mutant frequency was not accompanied by an increase in the total mutant clones. In order to determine if the increase was repeatable, another nonactivation assay was performed.

Activated assay

Test material was assayed at concentrations ranging from 15.6 to 250 µl.ml. The minimum criterion for mutagenesis in his assay was a mutant frequency exceeding 54.2×10^{-6} . Two treatments induced mutant frequencies that exceeded the

minimum criterion, but the increases were sporadic and unrelated to dose or toxicity. Another assay was therefore performed.

TRIAL 4Non activated assay.

The test material was assayed at concentrations ranging from 12.5 to 300 µl/ml. In order for a treatment to be considered mutagenic in this assay, a mutant frequency of 36.5×10^{-6} was required. None of the assayed treatments induced mutant frequencies that exceeded the minimum criterion. The observed toxicities ranged from non toxic to moderate toxicity. Although it is preferable to consider results from treatments that induce high toxicity, it was not possible in this assay because of a sharp toxicity curve. The test material was therefore considered non mutagenic without activation in this assay at treatments that approached lethality.

Activated assay.

Concentrations ranging from 12.5 to 300 µl/ml were used in this assay and low to very high toxicity was induced. Sporadic increases in the mutant frequency were induced. The minimum criterion for mutagenesis in this assay was a mutant frequency exceeding 44.2×10^{-6} and three treatments did exceed the minimum criterion (25, 50 & 100 nl/ml). However, the highest concentrations assayed were non-mutagenic. A further assay was therefore performed.

TRIAL 5Activated assay

The test material was assayed in duplicate at concentrations ranging from 150 to 300 nl/ml. A wide range of toxicities were induced. The sporadic increases in mutant frequency observed in Trials 1 and 4 were not repeatable. None of the treatments induced mutant frequencies that exceeded the minimum criterion of 48.4×10^{-6} . The test material was therefore considered non-mutagenic with activation in this assay.

Reliability : (2) Valid with restrictions (multiple assays needed to get usable studies)

(6)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours/day, for 5 days
Doses : 0, 65, 300 & 2050 ppm, nominal concentrations
Result : Negative
Year : 1986
GLP : Yes

- Test substance** : API 81-08 See section 1.1.1. for characterization
- Method** : Groups of 10 male and 10 female Sprague Dawley rats were exposed (whole body) to nominal concentrations of 65, 300 and 2050 ppm of test material. Animals were exposed to vapor of the test material 6 hours each day for 5 consecutive days. A positive control group of 10 rats of each sex were given a single dose (0.8 mg/kg) of TEM intraperitoneally 24 hours before sacrifice. A negative control group of 10 rats of each sex were exposed to air only. For the treated and negative control groups bone marrow was harvested 6 hours after the final exposure. For the positive control group the bone marrow was harvested 24 hours after administration of the TEM.
- Three hours prior to sacrifice by carbon monoxide the rats were given a single intraperitoneal dose of colchicine (4 mg/kg). Immediately after sacrifice bone marrow was obtained from the tibiae of the animals. The marrow was washed and the cells were fixed before being spread on slides for examination. Routinely 50 spreads were prepared for each animal. The location of cells bearing aberrations were identified. A mitotic index based on at least 500 cells counted was also recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read. Slides were scored for chromosomal aberrations.
- The authors give the following as the criteria for a positive response and data interpretation. Gaps were not counted as significant aberrations. Indicators of genetic damage were considered to be: Open breaks, configurations resulting from the repair of breaks. The latter included translocations, multiradials, rings, multicentrics etc. Reunion figures such as these were weighted slightly higher than breaks since they usually resulted from more than one break. The number of cells with aberrations per animal was also considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of mutagenic potential. Often it is not possible to locate 50 suitable metaphase spreads for each animal, even after preparing additional spreads. Possible causes for this appear to be related to cytotoxic effects which alter the duration of the cell cycle, kill the cell or cause clumping of the chromosomes. Additional information can be gained from the mitotic index which also appears to reflect cytotoxic effects. The type of aberration, its frequency and its correlation to dose in a given time period was considered in evaluating a test article as being mutagenically positive or negative. Statistical analysis employed a Kruskal-Wallis test of aberrations per cell on a per animal basis.
- Result** : The mean exposure chamber concentrations were found to be: 0, 69±18, 293±42 and 2012±16 ppm.

No signs of toxicity were observed in the rats during the exposure phase of the study. The results of the cytogenetic evaluation are summarized in the following table. NB. Mean values without standard errors are given in the table, although these data are available in the report.

	Exposure concentration (ppm)			Control	
	69	293	2012	Positive	Negative
Total No. of cells					
Male	470	500	410	400	500
Female	500	500	500	474	500
M+F	970	1000	910	874	1000
Frequency of structural aberrations					
Male	.009	.006	.029	>.708	.016
Female	0	>.014	.030	>.970	.008
M+F	.005	>0.01	.029	>.853	.012
Frequency of numerical aberrations					
Male	.012	0	.013	.023	.01
Female	.012	.016	.006	.015	.008
M+F	.01	.008	.01	.019	.009
% Cells with structural aberrations/animal					
1 or more					
Male	.9	.4	2.2	20	.6
Female	0	1.4	2.6	19	.8
M+F	.5	.9	2.4	19.5	.7
2 or more					
Male	0	.2	.4	11.3	.4
Female	0	.2	.4	14.4	0
M+F	0	.2	.4	12.9	.2
%MI					
Male	6.5	6.6	3.8	1	5.7
Female	4.1	4.8	4.5	1	4.2
M+F	5.3	5.7	4.2	12.9	.2

On the basis of the above data, the authors concluded that there was no evidence of a clastogenic effect of the test material and that there was no significant increase in chromosomal aberration in the dosed animals when compared to the negative controls.

Test condition

: Vapor of the test material was generated by bubbling nitrogen through heated distillation columns packed with glass beads. The test material was delivered to the top of the glass beads using syringe pumps, a different delivery rate being used for each target dose level. Chamber concentrations were monitored hourly each exposure period. Results of chamber monitoring are:

	<u>Target concentration (ppm)</u>	<u>Actual concentration (ppm)</u>
	0	0
	65	69 ± 18
	300	293 ± 42
	2050	2012 ± 16

Reliability : (1) Valid without restriction

(8)

5.7 CARCINOGENICITY

Species : Mouse
 Sex : Male
 Strain : C3H
 Route of admin. : Dermal
 Exposure period : Lifetime
 Frequency of treatm. : Twice weekly.
 Post exposure period : None
 Doses : 50 µl/application
 Control group : Untreated, solvent and positive controls
 Year : 1989
 GLP : Yes
 Test substance : API 81-08 See section 1.1.1. for characterization

Method

: The study summarized here was designed to evaluate the carcinogenicity of 12 different petroleum refinery streams. Only the information relating to the control groups and the group exposed to API 81-08 is included in this summary.

50 µl of undiluted test material was applied twice weekly to the shorn dorsal skin to a group of 47 male mice for 139 weeks. An untreated group of 50 male mice served as untreated controls. A further 50 male mice used as solvent controls received 50 µl of toluene twice weekly for 2 years and BaP at concentrations of 0.01% and 0.05% in toluene was applied twice weekly to a further two groups of 50 male mice.

Body weights of the mice were recorded prior to study initiation, weekly for the first 13 weeks of the study and every 4 weeks until termination at 139 weeks.

Observations were made daily for morbidity and mortality and any clinical signs of toxicity. All tumors that developed were recorded and their progression noted.

A gross necropsy was performed on all animals dying during the study or killed at termination. Special attention was paid to any dermal and subcutaneous masses.

Liver, kidneys, lungs and gonads were weighed for each animal at necropsy and group mean organ weight and organ/body weight ratios were calculated.

The test skin site (including dermal and subcutaneous tumors) and control skin site were examined histopathologically as were any suspected dermal and systemic neoplasms.

Result

: The body weights of the mice treated with 81-08 did not differ from those of controls throughout the study. No clinical signs of systemic toxicity were observed in animals treated with 81-08. Observations of preputial gland swelling and penile prolapse increased in all groups with age. penile prolapse occurred in virtually all mice by 2 years.

Virtually no dermal lesions were observed in the untreated control group. However mice treated with toluene had an average of 100% incidence of mild or moderate desquamation and an average of 10 to 20% incidence of mild irritation and scabbing. The incidence of scabbing increased to 40% in older mice.

Dermal lesions in mice treated with 0.01% BaP were similar to the toluene controls. Although they initially had less irritation than the animals treated with toluene, the incidence of mild irritation increased to approximately 50% after 2 years.

To begin with, the mice treated with 0.05% BaP had similar lesions to the toluene controls. However, the incidence of irritation increased to 50 to 100% from weeks 60 to 78.

Dermal lesions in mice treated with 81-08 were very similar to the toluene controls, but with slightly less irritation and scabbing.

Survival of the mice treated with 81-08 was better than that

for any of the control groups as shown in the following table

Group	Survival % at month				
	6	12	18	24	30
Untreated	90	90	86	62	18
Toluene	96	94	76	52	10
BaP 0.01%	100	98	84	38	0
BaP 0.05%	100	86	2	0	0
81-08	98	98	89	56	19

A variety of non-neoplastic lesions other than those at the treated skin site were observed at histopathological examination but these occurred in all groups and were not considered to be treatment related.

Lesions at the treated skin site are summarized as follows:

Lesion	Solvent control	Group			
		Toluene control	BaP 0.01%	BaP 0.05%	81-08
Dermal inflammation					
% affected	0	36	20	24	6
Severity		1.7	1.6	1.6	1.3
Hyperkeratosis					
% affected	0	96	90	92	94
Severity		2	2	2	2
Acanthosis					
% affected	0	76	80	78	87
Severity		1.8	1.7	1.7	1.9
Epidermal crusting					
% affected	2	84	82	86	75
Severity	1	1.8	1.7	2.1	1.9
Dermal pigmentation					
% affected	0	78	12	2	87
Severity		1.6	1	1	1.9
Dermal fibrosis					
% affected	0	68	54	36	96
Severity		1.6	1.4	1.4	2
Ulceration					
% affected	0	18	12	6	0
Severity		1.6	1.7	1.7	
Dermal neoplasms					
% affected ***	0	8	64	98	6

* % mice affected

** Severity on a scale
1 = minimal
2 = mild
3 = moderate

4 = severe
*** % mice with neoplasms

The percent of mice with systemic neoplasms in the control and 81-08 animals was as follows:

Neoplasm	Group				
	Solvent control	Toluene control	BaP 0.01%	BaP 0.05%	81-08
Primary liver neoplasm					
Benign	4	2	6	0	4
Malignant	32	20	30	4	23
Primary lung neoplasm					
Benign	2	4	2	2	0
Malignant	0	0	0	0	0
Other neoplasms*					
Benign	0	2	0	0	2
Malignant	2	0	0	0	4
Total neoplasms	40	28	38	6	33
Group mean longevity**	103	100	96	61	108

* Includes malignant lymphomas that were observed in multiple sites, including treated skin

** Longevity shown in weeks

The data on dermal neoplasms that developed during the study are summarized in the following table

	Solvent control	Toluene control	BaP 0.01%	BaP 0.05%	81-08
% mice developing benign dermal neoplasm	0	0	0	4	4
% mice developing malignant dermal neoplasm	0	8	56	98	2
& mice with multiple tumors	0	0	28	60	0
Average tumors/mouse	0	.08	1	1.82	.06
No. mice with metastases	0	0	6	12	0
Mean latency (weeks)		111	86	49	113

5. Toxicity

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Date May 20, 2003

Tumorigenic activity				
FEN *	43	49	47	46
% **	9	65	100	7

* FEN = No. of animals alive at the time of appearance of the median tumor plus any mice that died from tumor before that time OR when median latency is over 60 weeks,
FEN = No. of animals alive at 60 weeks plus any mice that died with tumor before 60 weeks.

** % of mice developing tumors = No. developing tumors divided by FENx100

Test substance : The tumor data of the 81-08 group were compared using a Chi square test with the data from the untreated and solvent controls and no significant differences were found.
API 81-08 was applied undiluted.
The solvent control group was treated with toluene.
Benzo(a)pyrene was applied at concentrations of 0.01 and 0.05% in toluene.

(10)

5.8.1 TOXICITY TO FERTILITY

Remark : No data

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Remark : No data

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- (11) American Society for Testing and Materials (ASTM), 1991
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**ROBUST SUMMARY
OF INFORMATION ON**

Substance Group: **AROMATIC NAPHTHAS**

Summary prepared by: American Petroleum Institute

Creation date: **MARCH 1, 2001**

Printing date: **MAY 20, 2003**

Date of last Update: **MAY 20, 2003**

Number of Pages: **49**

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 : Aromatic naphtha streams are obtained from the catalytic reforming of mainly n-alkane and cycloparaffinic feedstocks into aromatic and branched chain hydrocarbons. The hydrocarbons are mainly in the range C5 to C12 and boil in the range of approximately 30 to 220°C.

A typical aromatic naphtha is composed of the following hydrocarbon classes in the approximate proportions shown:

**Content
(volume %)**

Paraffins	32
Olefins	0.5
Naphthenics	4
Aromatics	63.5

Full range catalytically reformed naphtha (CAS 64741-66-8) is a typical aromatic naphtha stream and the American Petroleum Institute (API, 1987) have characterized a specific sample (API 83-05) of a Full range catalytic reformed naphtha. The results of this characterization are as follows:

Characterization of API 83-05		CAS No. 64741-66-8	
<u>Parameter</u>	<u>Method</u>	<u>Units</u>	<u>Value</u>
Gravity	D287	°API	44.2
Density @15°C	D287		0.8045
Molecular weight	D2224	gm/mol	96
Refractive index @ 20°C		RI units	1.4592
Total Sulfur	D3120	ppm/wt	<1
Total Nitrogen	Chemil.	ppm/wt	<1
Total Oxygen	NAA	wt%	<0.01
Total Chloride	coulom.	ppm/wt	<1
RVP		psia	3.0
Distillation	D86	vol/°F	
IBP/5			136/168
End point			392
Motor Octane No. (MON)			93.5
Research Octane No. (RON)			94.1
Mass Spectrometer Analysis			
Paraffins	D2789	vol%	32.1
Naphthenes	D2789	vol%	3.7
Aromatics	D2789	vol%	63.3
Indans/Tetralins	D2789	vol%	0.7
Naphthalenes	D2789	vol%	0.2
		TOTAL	100

Some mammalian toxicology studies have been carried out on a distillate

1. General Information

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fraction (LCRN-D) of a sample of Light Catalytically Reformed Naphtha (LCRN). The LCRN-D and the LCRN from which it was derived have been characterized as follows:

Parameter	Sample	
	LCRN	LCRN-D
Paraffins (% by MS)	63.76	88.3
Olefins (% by MS)	0.96	1.37
Naphthenes (% by MS)	2.28	1.24
Aromatics (% by MS)	33	9.09
Benzene (vol %)	6.93	4.65
Carbon No. (vol%)		
4	1.02	3.6
5	23.34	59.11
6	25.76	25.18
7	45.27	11.65
8	4.56	0.46
9	0.05	0

Ecotoxicology and fate/effects modeling have been carried out on several catalytically reformed naphtha streams. The information on each of the samples that have been used is shown below:

Concawe sample MRD-95-047 (gasoline sample W94/812)
CAS No. 64741-63-5, a light reformat.

Parameter

Density (g/ml @16°C) 0.8026

Sulfur (ppm) 17

Detailed hydrocarbon analysis ASTM D5134-92

	Olefins		Naphthenes	Aromatics	Paraffins	
	n-	i-			n-	i-
Total %	0.93	2.52		63.38	11.92	20.80
C4	0.055	0.00		0.00	3.617	1.815
C5	0.248	0.200		0.00	4.336	7.093
C6	0.281	0.306		4.305	5.712	2.224
C7	0.316	0.300		20.285	1.011	3.751
C8	0.009	0.171		29.123	0.263	1.146
C9	0.000	0.145		8.926	0.020	0.090
C10	0.000	1.392		0.461	0.000	0.748
C11	0.000	0.007		0.262	0.117	0.441

Concawe sample MRD-95-089 (gasoline sample CWE1)

CAS No. 68919-37-9, a full range reformat.

Parameter

Density (g/ml @16°C) 0.8012

Sulfur (ppm) 6.72

Detailed hydrocarbon analysis (ASTM D5134-92)

	Olefins		Naphthenes	Aromatics	Paraffins	
	n-	i-			n-	i-
total %	1.64	2.65		63.84	9.44	20.84
C4	0.04	0.00		0.00	1.621	0.536
C5	0.726	0.125		0.00	3.328	6.090
C6	0.520	0.354		8.759	2.925	7.179
C7	0.301	0.226		22.726	1.220	4.482
C8	0.054	0.114		22.605	0.690	0.162

1. General Information

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C ₉	0.00	0.235	8.762	0.011	0.071
C ₁₀	0.00	1.591	0.639	0.000	0.950

Sample identified by Chevron Research as a light catalytically reformed naphtha CAS No. 64741-63-5

Detailed hydrocarbon analysis

	Olefins	Naphthenes	Aromatics	Paraffins total	n-
total %	0.90	2.36	39.40	57.34	17.51
C ₄	0.00	0.00	0.00	0.81	0.78
C ₅	0.34	0.26	0.00	19.45	8.05
C ₆	0.27	0.62	8.37	16.23	4.69
C ₇	0.28	1.18	29.77	17.70	3.59
C ₈	0.01	0.27	1.26	3.12	0.40

(16) (17) (20)

2. Physico-Chemical Data

Id A. Naphthas
Date May 20, 2003

2.2 BOILING POINT

Value : ca. 35 - 220 °C
Remark : See General substance information, Section 1.1.1.

2.4 VAPOUR PRESSURE

Value : 4630 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes
Test substance :
Remark : The sample was identified by CONCAWE as MRD-95-089, gasoline sample CWE1, CAS No. 68919-37-9, a reformat full range.
Reliability : (1) valid without restriction (21)

Value : 5500 hPa at 37.8 °C
Method : ASTM D5191
Year : 1995
GLP : Yes
Remark : The sample was identified by CONCAWE as MRD-95-047, gasoline sample W94/812, CAS No. 64741-63-5, a light reformat.
Reliability : (1) valid without restriction (21)

2.5 PARTITION COEFFICIENT

Log pow : = 2.13 - 4.54 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : No
Test substance : Light Catalytically Reformed Naphtha
Remark : Log P values represent the spread of calculated and/or measured values for C5 to C9 hydrocarbon components found in LCRN, CAS No 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).
Reliability : (2) Valid with restrictions (17) (28)
10.12.2001
Log pow : = 2.13 - 4.76 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65.

2. Physico-Chemical Data

Id A. Naphthas
Date May 20, 2003

Year : 2000
GLP : No
Test substance : Full -Range Catalytically Reformed Naphtha (FRCRN)-
CAS No. 68955-35-1; API sample 83-05

Remark : Log P values represent the spread of calculated and/or
measured values for C5 to C9 hydrocarbon components found in
FRCRN, CAS No 68955-35-1. Detailed hydrocarbon analysis was
used to identify the components of this FRCRN (63% aromatics) sample.
Calculated SAR result for surrogate structures contained in program
database (smilecas.dat).
Calculation based on an atom/fragment contribution method of
W. Meylan and P. Howard.

Reliability : (2) Valid with restrictions

(16) (28)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	Yes
Test substance	:	Light Catalytically Reformed Naphtha See section 1.1.1. For characterization reported by Chevron
Method	:	Water Accommodated Fractions (WAFs) of LCRN were prepared at 50 mg/l loading in freshwater and saltwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Result	:	Gas chromatographic analysis of selected components freshwater and saltwater solubilities of 13.7 and 14.0 ppm respectively. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Concentrations for these components reached equilibrium by 24 hours.
Conclusion	:	Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LCRN components range from approximately 3 to 2000 mg/l. 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 (1) (18) (21) (23) (24)
Method	:	Preparation of Water Soluble Fraction
Year	:	1995
GLP	:	Yes
Test substance	:	Full -Range Catalytically Reformed Naphtha (FRCRN)- CAS No. 68955-35-1; API sample 83-05. See section 1.1.1
Method	:	Water Accommodated Fractions (WAFs) of CONCAWE Reformate light naphtha (LCRN), CAS no. 64741-63-5 (CONCAWE sample ID W94/812) were prepared at 100 mg/l loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Remark	:	Detailed hydrocarbon analysis was used to identify the components of this CONCAWE Light Cracked Naphtha (63% aromatics) sample. The analysis indicated that the

- composition of the CONCAWE LCRN sample was essentially identical to the composition of API 83-05 FRCRN sample. Therefore the water solubility information for the CONCAWE LCRN sample is applicable to the FRCRN sample.
- Result** : Gas chromatographic analysis of LCRN components benzene, toluene, ethylbenzene, ortho, meta and para-xylene in WAFs indicated freshwater solubility of 6.3 ppm. Concentrations for these components reached equilibrium by 48 hours.
- Conclusion** : Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for FRCRN components range from approximately 3 to 2000 mg/l. 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
- (18) (19) (21) (23) (24)

3.1.1 PHOTODEGRADATION

Type : Calculated
Light source : Sun light
Relative intensity : = 1 based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer : OH
Method : Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year : 2000
GLP : No
Test substance : Light Catalytically Reformed Naphtha.
 See section 1.1.1. for characterization

Remark : AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O₃. Atmospheric oxidation rates were calculated for the C₅ to C₈ hydrocarbon components found in LCRN, CAS No. 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample. Based on a 12-hour day, the range for atmospheric half-lives for LCRN constituents is: 1.498 days (2,3 dimethyl pentane) to 15.985 days (isopentane).

Result : Indirect photolysis:
 Conc. of sensitizer: 1.50E⁺⁰⁶ OH radicals/cm³
 Rate constant: 0.6691E⁻¹² cm³/mol-sec (isopentane)
 to 7.1392E⁻¹² (2,3 dimethyl pentane)
 Half life: 1.498 to 15.985 days

Reliability : (2) Valid with restrictions

(29)

Type : Calculated
Light source : Sun light
INDIRECT PHOTOLYSIS
Sensitizer : OH
Method : Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year :
GLP : No
Test substance : Full -Range Catalytically Reformed Naphtha (FRCRN) - CAS No. 68955-35-1; API sample 83-05. See section 1.1.1. for characterization

Remark : AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O₃. Atmospheric oxidation rates were calculated for the C₅ to C₉ hydrocarbon components found in FRCRN, CAS No. 68955-35-1. Detailed hydrocarbon analysis was used to identify the components of this specific FRCRN (63% aromatics) sample. Based on a 12-hour day, the range for atmospheric half-lives for FRCRN constituents is: 0.641 days (1, 2, 4 trimethylbenzene) to 15.985 days (isopentane).

3. Environmental Fate and Pathways

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Date May 20, 2003

Result : Indirect photolysis:
Conc. of sensitizer: $1.50E^{+06}$ OH radicals/cm³
Rate constant: $0.6691E^{-12}$ cm³/mol-sec (isopentane)
to $16.698E^{-12}$ (1,2,4 trimethyl benzene)
Half life: 0.641 to 15.985 days
Reliability : (2) Valid with restrictions (29)

3.1.2 STABILITY IN WATER

Test substance : Light Catalytically Reformed Naphtha.
See section 1.1.1. for characterization
Conclusion : Hydrolysis unlikely
Reliability : (1) Valid without restriction (26)

Test substance : Full -Range Catalytically Reformed Naphtha (FRCRN) - CAS No. 68955-35-1; API sample 83-05. See section 1.1.1. for characterization
Conclusion : Hydrolysis unlikely
Reliability : (1) Valid without restriction (26)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level 1
Media : Soil, air, water, suspended sediment and sediment
Method : Calculated according to Mackay Level I
Year : 2000

Remark : Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C8 hydrocarbon components found in LCRN, CAS No 64741-63-5. Detailed hydrocarbon analysis performed by Chevron Research was used to identify the components of this specific LCRN sample (see section 1.1.1.).

The majority of LCRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.

Result :

<u>Medium</u>	<u>% distribution</u>
Air	97 to 99.98
Soil	0.01 to 0.8
Water	0.01 to 2.7
Sediment	0.00
Suspended sediment	0.00

Conclusion : The constituents of this complex petroleum mixture are expected to partition primarily to air.
Reliability : (2) Valid with restrictions (27)

3. Environmental Fate and Pathways

Id A. Naphthas
Date May 20, 2003

Type	:	Calculated												
Media	:	Soil, air, water, suspended sediment, and sediment												
Method	:	Calculated according to Mackay Level I												
Year	:	2000												
Remark	:	Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C9 hydrocarbon components found in FRCRN, CAS No 68955-35-1. Detailed hydrocarbon analysis was used to identify the components of this specific FRCRN (63% aromatics) sample. The majority of FRCRN components will partition rapidly to air, where these hydrocarbons will be rapidly oxidized by OH radicals.												
Result	:	<table><thead><tr><th><u>Medium</u></th><th><u>% distribution</u></th></tr></thead><tbody><tr><td>Air</td><td>96.5 to 99.98</td></tr><tr><td>Soil</td><td>0.01 to 1.83</td></tr><tr><td>Water</td><td>0.01 to 2.7</td></tr><tr><td>Sediment</td><td><0.001 to 0.03</td></tr><tr><td>Suspended Sediment</td><td><0.001</td></tr></tbody></table>	<u>Medium</u>	<u>% distribution</u>	Air	96.5 to 99.98	Soil	0.01 to 1.83	Water	0.01 to 2.7	Sediment	<0.001 to 0.03	Suspended Sediment	<0.001
<u>Medium</u>	<u>% distribution</u>													
Air	96.5 to 99.98													
Soil	0.01 to 1.83													
Water	0.01 to 2.7													
Sediment	<0.001 to 0.03													
Suspended Sediment	<0.001													
Test substance	:	Full -Range Catalytically Reformed Naphtha (FRCRN) - CAS No. 68955-35-1; API sample 83-05. See section 1.1.1. for characterization.												
Conclusion	:	The constituents of this complex petroleum mixture are expected to partition primarily to air.												
Reliability	:	(2) Valid with restrictions												

(27)

3.5 BIODEGRADATION

Type	:	Aerobic
Inoculum	:	Mixed, adapted inoculum of domestic activated sludge and soil
Contact time	:	56 day(s)
Result	:	Inherently biodegradable
Method	:	CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year	:	1999
GLP	:	Yes
Test substance	:	Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization reported by Chevron.
Method	:	Type (test type): Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO ₂ headspace test)
Result	:	Test material was inherently biodegradable since it achieved >20% biodegradability based on CO ₂ production. By day 28 approximately 96% of the test material was degraded, then essentially reached a plateau in degradation rate until day 56. The test was considered valid according to CONCAWE criteria, as >60% biodegradation of positive control (63% actual) was observed by day 14, and total blank CO ₂ production at termination was less than 15% of the organic carbon added as test substance. Temperature ranged from 18 to 21° C, which deviated from the protocol value of 22 ± 2°C. This deviation was not expected to have affected the outcome of this study.

Test Day	% Degradation (sd)	
	Hexadecane	Test Material
3	13.93 (1.85)	30.85 (3.85)
7	34.40 (4.54)	53.71 (3.52)
14	63.17 (0.94)	77.25 (3.65)
21	77.26 (6.52)	87.17 (8.87)
28	90.35 (7.14)	96.17(5.26)
35	85.13 (n=1)	107.9 (n=1)
42	85.21 (n=1)	96.95 (6.37)
49	96.93 (8.94)	92.02 (n=1)
56	94.69 (4.10)	84.92 (0.51)

Test condition : Mixed inoculum prepared from soil and activated sludge was incubated with test substance or hexadecane (positive control) during a two-week adaptation period. Triplicate test systems were incubated for both the test substance and hexadecane fed inoculum. Two additional, similar test substances were concurrently incubated in separate 160 ml test systems using the same inoculum and acclimation procedure. Duplicate blank control test systems were prepared which consisted of the mixed inocula in mineral medium but no test or positive control substance. Test medium consisted of glass-distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) prepared as described in ISO method.

Acclimation procedure-Activated sludge from aeration basin of Wareham Wastewater Treatment Plant (Mass., U.S.A.) was sieved through 2 mm and centrifuged at 1000 rpm for 10 minutes. After removal of supernatant the concentrated solids were diluted to 5 mg/ml suspended solids with reagent grade water. Soil was collected from a site located in a mixed hardwood and pine forest (Mass., U.S.A.). Site of sampling was cleared of debris and approximately 500 g of soil was obtained at a depth between 5-10cm from the soil surface. Soil was air-dried, sieved through a 2 mm sieve, and analyzed for moisture content (38%).

Test vessels (160 ml serum bottles) were filled with 103 ml of mineral medium containing 50 mg/l of yeast extract and 50 mg/l (dry weight) washed activated sludge, then approximately 0.16g of sieved soil (0.1 g dry wt) was added to each bottle. Test or reference substance were added directly to test systems using a 10 microliter Hamilton gas tight syringe. The volume required to achieve the specified mg carbon/l concentrations were calculated based on % carbon and specific gravity of the respective substance. The test substance % carbon (0.8856) and specific gravity (0.7325 mg/μl) information was supplied by the Sponsor. Hexadecane % carbon (0.8496) was calculated from the empirical formula and specific gravity (0.7749 mg/μl) was obtained from Verschueren (1983). Addition of respective substance was performed on an incremental basis to the appropriate vessels as follows: 4, 8 and 8 mg C/l were added on days 0, 7 and 11, respectively. Test vessels were sealed with butyl rubber septa/aluminum crimp caps and incubated at 22 °C (± 2°C) in the dark.

Biodegradation by CO₂ determination
test initiation and procedure

On day 14 of the acclimation phase, all test system inoculum from blanks, positive control, and each of the three test substances was combined and filtered through glass wool, and aerated prior to use. The aerated mixed inoculum was then added to mineral medium to achieve 10% concentration based on total volume (100 ml inoculum/l). Test vessels (160 ml serum bottles) were filled with 103 ml of inoculated mineral medium. Respective test systems were dosed with either test substance or hexadecane as described for the acclimation procedure to achieve 20 mg carbon/l concentration. Duplicate test systems for each test substance, positive control and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO₂ analysis. After test system preparation, all vessels were placed in a walk-in chamber and incubated in the dark at 22 °C (± 2 °C).

On days 3, 7, 14, 21, 28, 35, 42, 49 and 56, 1ml of conc H₃PO₄ was injected through the septum of each sacrificed test vessel. The acidified samples were shaken for 1 hr at 200 ppm, then analyzed for CO₂ using gas chromatography-thermal conductivity detection. Quantitation of inorganic mg C/l evolved was determined by linear regression analysis based on response factors for sodium carbonate standards spanning 1-30 mg carbon/l concentrations.

Reliability : (1) Valid without restriction

(33)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	96 Hr Static Acute Toxicity Test w/ Daily Renewal
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring Method	:	Yes
	:	No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year	:	1998
GLP	:	Yes
Test substance	:	TS: Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization reported by Chevron.
Method	:	Statistical Method: (FT - ME) LL ₅₀ and LC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	Mortality (no. of deaths/treatment) at 96 hrs: 1, 0, 1, 0, 1 and 20 in 0, 3.1, 6.3, 13, 25 and 50 mg/l treatments. Abnormal behavior (surfacing, erratic swimming) was observed at 96 hrs for 3 organisms in 13 mg/l and 7 fish in 25 mg/l treatments. 96-hr LL ₅₀ = 34 mg/l, 25-50 mg/l w/ 95% C.I. (as nominal loading rate) 96-hr LC ₅₀ = 11 mg/l, 8.2-17.2 mg/l w/ 95% C.I. (measured concentrations) 96-hr NOEL = 3.1 mg/l (nominal); 96-hr NOEC = 1.03 mg/l (measured) based on lack of mortality and abnormal effects for these treatments.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by blending naturally hard well water with water that had been demineralized by reverse osmosis. Nominal loading rates of 0, 3.1, 6.3, 13, 25 and 50 mg/l were used to prepare test solutions. WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 9.4 liters of water for 24 hr in 9 liter glass bottles. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was added into each bottle, and the bottles were capped tightly with a positive pressure siphoning apparatus. The siphoning apparatus consisted of a teflon lined neoprene stopper housing two teflon tubes. One tube extended to the bottom of the bottle for removal of the WAF solution, the other tube ended above the WAF surface, and was used to control air pressure during siphoning. During WAF preparation, parafilm was used to seal the external joint between the neoprene stopper and glass bottle, and the bottles were covered with aluminum foil. After stirring for 24 hrs using 25% or less vortex, the contents of the WAF solution bottles were allowed to settle for approximately 45

minutes to two hours, then siphoned by the positive pressure apparatus port and used for testing. Samples were also analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes.

Fish were hatched and raised from ABC Laboratories' in-house culture, and were acclimated prior to experimentation for a minimum of 14 days on a 16/8hr light/dark cycle. Test vessels were 3.8 liter glass containers with teflon-lined caps.

Fish were acclimated to the test water and temperature approximately 72 hr before the test, and were not fed during this 72 hr period. Two replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removal of most of the test solution (leaving approximately one liter of solution to avoid stressing test organisms) and replacing it with fresh WAF solution prepared at least 24 hrs prior to use.

Water temperature was 21-22 °C. Test photoperiod was 16 hrs. light and 8 hr dark. Dissolved oxygen measurements were between 6.0 and 8.5, pH values between 7.7 and 8.5. Hardness values ranged from 138 to 148 mg/l; alkalinity values ranged from 150 to 158 mg/l and conductivity values ranged from 299 to 313 microsiemens.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations. (2)

Type : Predicted value for fish toxicity
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : = 2.09 calculated
Method : Predicted

Method : Calculated based on hydrocarbon block principle. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalised by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below).

Result : Estimated 96 hour(s) fish acute toxicity LL₅₀: 2.09 mg/l
Reliability : (2) Valid with restrictions (30)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	48 Hr Static Acute Toxicity Test with 24Hr Renewal in Closed Systems
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	Procedure patterned after:1991 ASTM method E729-88a and 1985 USEPA TSCA Test Guidelines: Daphnid Acute Toxicity Test. Fed. Reg., vol. 50 (No. 188) Sept 27, 1985, 797.1300.
Year	:	1998
GLP	:	Yes
Test substance	:	TS: Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization reported by Chevron.
Method	:	Statistical Method: (FT - ME) EL ₅₀ and EC ₅₀ calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84.
Result	:	<p>Immobility (no. of organisms) at 48 hrs: 0, 0, 0, 15, 20 and 20 for 0, 3.0, 6.0, 12, 24 and 48 mg/l treatments. At the 6 and 12mg/l nominal treatments, 20 and 5 organisms were observed at the bottom of the test chambers, respectively.</p> <p>48-hr EL₅₀ = 10 mg/l based upon nominal loading rate (95% C.I. 6 to 12 mg/l);</p> <p>48 hr EC₅₀ was 2.6 mg/l (95% C.I. 1.06 to 3.6 mg/l); based on total measured concentrations.</p> <p>48-hr NOEL = 3 mg/l based upon nominal loading rate.;</p> <p>48 hr NOEC was 0.465 ppm based on total measured concentrations.</p>
Test condition	:	<p>Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were a blend of aged well water and reverse osmosis well water.</p> <p>WAFS were prepared for each test concentration by mixing the appropriate mass of substance in 2.4 liters of water for 24 hr in aluminum foil covered 2.5 liter aspirator bottles fitted w/ a port near the bottle bottom. The bottles were filled to neck height with water to minimize volatility. A measured amount of test substance was pipetted below the water surface. After stirring for 24 hrs using 25% or less vortex, the contents of the aspirator bottles were allowed to settle for approximately one hour, then drained from the port and used for testing. Samples were also analyzed by purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes.</p> <p>Range finding toxicity studies were conducted at 0.5, 1.0, 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies</p>

were conducted at 3.0, 6.0, 12, 24 and 48 mg/l loading, using WAFS which were divided into duplicate aliquots and tested.

Test vessels were teflon cap-sealed 273 ml glass jars with 10 daphnids per jar and were completely filled with test solution.

During the study test system solutions: dissolved oxygen concentration range: 8.0 to 8.5; pH ranged from 8.2 to 8.4; temperature was 20 to 21 °C; hardness (mg/l) ranged from 146 - 152; alkalinity (mg/l) was 158-168 and conductivity (µmhos) values were 312 - 317.

Daphnia magna, were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house since January 1998.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations. (3)

Type : Predicted value for invertebrate 48 hour acute toxicity
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : = .9 calculated

Method : Calculated based on hydrocarbon block principle. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below).

Result : Estimated 48 hour(s) Daphnid acute toxicity EL₅₀: 0.9 mg/l.
Reliability : (2) Valid with restrictions (30)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : No specific guideline was described as being used to conduct the test, however report references 1991 ASTM method E729-88a and 1982 EPA Support Documents for Environmental Testing: EPA 560/6-82-002.
Year : 1998
GLP : Yes
Test substance : TS: Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization reported by Chevron.

Method : Statistical Method: EL₅₀ and EC₅₀ calculated using nonlinear logistics sigmoid model (SAS). All NOEL/NOEC values based on visual review and Dunnett's test for

Result : significance.
: Percent inhibition on growth determined by cell density (cells/mL):
96 hour EL₁₀=6.0 mg/l (3.1-8.8 mg/l CI @95%)
96 hour EL₅₀=8.5mg/l (7.3-9.8 mg/l CI @95%)
96 hour EL₉₀=12 mg/l (9.9-14 mg/l CI @95%)
96 hour NOEL=5.0 mg/l

96 hour EC₁₀=1.1 mg/l (0.41-1.8 mg/l CI @95%)
96 hour EC₅₀= 1.7mg/l (1.4-2.1 mg/l CI @95%)
96 hour EC₉₀=2.7 mg/l (2.1-3.4 mg/l CI @95%)
96 hour NOEC=0.866 mg/l

Subcultures of the 10, 20 and 40 mg/l treatment cultures were placed in fresh media (no test substance) after acute testing for ten days indicated that growth inhibition was algistatic in all treatments. No excursions from the protocol were noted which would have affected the integrity of the study.

Concentration		
nominal (mg/l)	measured (mg/l)	96hr cell density (cells/ml)
Control	0.0147	40.5 x10 ⁴
1.3	0.126	40.92 x10 ⁴
2.5	0.211	42.33 x10 ⁴
5.0	0.866	41.17 x10 ⁴
10	2.12	11.11 x10 ⁴
20	5.26	0.70 x10 ⁴
40	13.3	0.04 x10 ⁴

Test condition : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.4 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 ug/l EDTA chelator, pH adjusted to 7.5 ±0.1 with 0.1NHCl and sterilized by 0.45 micron filtration) in 2.5 liter aspirator bottles. The mixing vessels were sealed with foil covered stoppers, covered with aluminum foil and the contents mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed from the spout at the base of each bottle and used for testing. Test vessels were 125ml glass Erlenmeyer flasks that were completely filled (148 ml) with treatment solution and inoculated with 6 day old algae. Algal cells obtained from testing laboratory cultures grown in sterile, nutrient enriched AAP media. Original algal cultures (stock UTEX-1648) obtained from Dept of Botany, Culture Collection of Algae, University of Texas at Austin, 1997. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and compound microscope. Twelve replicates were prepared for each treatment level. Nominal treatment levels were 0, 1.3, 2.5, 5.0, 10, 20 and 40 mg/l. The initial algal concentration was 1.0 x 10³ cells/ml. All test replicates were placed on a shaker table at 100 oscillations per minute during the study and exposed to continuous fluorescent light, illumination range 371 to 442 ft candles. Triplicate samples were taken daily for cell counts and analytical testing. Cell

densities were determined by direct microscopic examination. Samples at 0, 24, 48, 72 and 96 hrs were also analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, 2-methyl-pentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene, which represent more than 50% composition of the test substance. Measured test concentrations of the light catalytically reformed naphtha were based on the total combined concentrations of all analytes.

Test temperature was 24-25 °C. Test solution pH ranged from 7.5 to 8.9.

Reliability : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

(4)

4.5.1 CHRONIC TOXICITY TO FISH

Species : Pimephales promelas (Fish, fresh water)
Exposure period : 14 day(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD 204
Year : 1999
GLP : Yes
Test substance : CAS No. 64741-55-5; Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization

Method : LL₅₀/LC₅₀ and EL₅₀/EC₅₀ calculated using linear interpolation. NOEL/NOEC for survival determined by Steel's Many-One Rank Test. NOEL/NOEC for growth determined by Williams Test. TOXSTAT program was used to determine endpoints.

Result : The mean measured concentrations for nominal loading rates of 0.39, 1.0, 2.6, 6.3, 16, and 40 mg/l were 0.079, 0.15, 0.38, 0.80, 5.2, and 15 mg/l representing the average of total analytes measured in the new and old WAFs. The average total analyte concentration in the controls was 0.030 mg/l.
 14-d LL₅₀ for survival = 5.2 mg/l (95% C.I. 4.4 - 7.0),
 14-d LC₅₀ for survival = 0.67 mg/l (95% C.I. 0.58 - 0.93)
 14-d NOEL for survival = 2.6 mg/l,
 14-d NOEC for survival = 0.38 mg/l.
 Mortality (no. of deaths/treatment) at 14 days: 1, 0, 0, 2, 28, 40, and 40 in the 0, 0.39, 1.0, 2.6, 6.3, 16, and 40 mg/l treatments.
 14-d NOEL for growth = 2.6 mg/l,
 14-d NOEC for growth = 0.38 mg/l.
 14-d EL₅₀ and EC₅₀ for growth could not be calculated because none of the treatment group means were <50% of control.
 Since there were significant mortality at the three highest treatments, these treatments were excluded in the analysis

- of growth data. The mean (standard deviation) for dry weights were 2.11 (0.09), 1.96 (0.08), 2.05 (0.28), and 2.29 (0.16) in the 0, 0.39, 1.0, and 2.6 mg/l treatments. Dissolved oxygen concentrations in the old exposure solutions in the 2.6mg/l treatments were consistently below 60% saturation and could not be avoided with the closed exposure system. Oxygen concentrations in the 6.3 mg/l aged WAF were =4 mg/l between days 3 and 11 and could have contributed to fish mortality in that treatment.
- Test condition** : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 160-170 mg/l as CaCO₃, total alkalinity of 120 mg/l as CaCO₃, pH range of 7.9 to 8.1, and a specific conductivity range of 480 to 500 mmhos/cm. Nominal loading rates of 0, 0.39, 1.0, 2.6, 6.3, 16, and 40mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.742 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 1 to 1.5 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with fresh WAFs in which 80% of the old solutions were siphoned and excess debris removed from the exposure vessel prior to refilling with fresh WAF. Renewed solutions were then siphoned again and refilled a second time to achieve an exposure solution of ~96% fresh WAF. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, methylpentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light catalytically reformed naphtha were based on the concentrations of all analytes. Fish were hatched and raised from laboratory in-house culture. Fish were 8 days old at the start of the test. Test vessels were 1l screw-capped glass jars containing 980 ml of WAF with minimal headspace. Four replicates per treatment and 10 organisms per replicate were tested for each treatment and the control. Fish were fed 0.15 ml of live brine shrimp nauplii (<48 hr old) twice daily during the test. Water temperature was 24 to 26° C. Test photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen concentrations were 8.2 to 8.9 in the new solutions and 3.6 to 5.8 in the old solutions. pH values were 7.2 to 8.2.
- Reliability** : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be

equally toxic and should have been quantitated to determine total measured concentrations. Low dissolved oxygen could have contributed to fish mortality.

(35)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	: Daphnia magna (Crustacea)
Exposure period	: 21 day(s)
Unit	: mg/l
Analytical monitoring	: Yes
Method	: OECD Guide-line 211
Year	: 1999
GLP	: Yes
Test substance	: CAS No. 64741-55-5, Light Catalytically Reformed Naphtha. See section 1.1.1. for characterization.
Method	: For NOEL/NOEC, Fisher's Exact Test was used for survival of adult daphnids and Williams Test was used for reproduction. For EL ₅₀ /EC ₅₀ , survival data were analyzed using the Spearman-Kärber method and reproduction data were analyzed by linear interpolation. TOXSTAT program was used to determine the endpoints.
Result	: The mean measured concentrations for nominal loading rates of 0.39, 1.0, 2.6, 6.3, 16, and 40 mg/l were 0.069, 0.15, 0.36, 0.80, 3.8, and 13 mg/l representing the average of total analytes measured in the new and old WAFs. The average total analyte concentration in the controls was 0.026 mg/l. 21-d EL ₅₀ for survival = 26 mg/l (95% C.I. 22 - 29), 21-d EC ₅₀ for survival = 7.5 mg/l (95% C.I. 6.3 - 8.7) 21-d NOEL for survival = 16 mg/l, 21-d NOEC for survival = 3.8 mg/l. Daphnid immobilization at 21 days: 0, 1, 0, 0, 1, 1, and 10 in the 0, 0.39, 1.0, 2.6, 6.3, 16, and 40 mg/l treatments. 21-d EL ₅₀ for reproduction = 14 mg/l (95% C.I. 12-16), 21-d EC ₅₀ for reproduction = 3.2 mg/l (95% C.I. 2.3-3.7) 21-d NOEL for reproduction = <0.39 mg/l, 21-d NOEC for reproduction = <0.069 mg/l. Since there was significant immobilization in the highest treatment, it was excluded in the analysis of reproduction data. The mean numbers (standard deviation) of offspring released per female daphnid were 122 (14), 94 (32), 87 (21), 113 (30), 92 (17), and 53 (26) in the 0, 0.39, 1.0, 2.6, 6.3, and 16 mg/l treatments. The numbers of offspring released at all loading rates were significantly less than the controls. First brood release for organisms exposed to =6.3 mg/l and the controls generally occurred by day 8. Some or all offspring from 4 broods (1 brood at 0.39 mg/l, 2 broods at 1.0 mg/l, 1 brood at 2.6 mg/l) were immobile at the observation time.
Test condition	: Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water was prepared by fortifying well water according to the formula for hard water (USEPA, 1975, EPA-660/3-75-009) and filtering through Amberlite

XAD-7 resin to remove potential organic contaminants. The water used in this study had a total hardness range of 160-170 mg/l as CaCO₃, total alkalinity of 120 mg/l as CaCO₃, pH range of 7.9 to 8.1, and a specific conductivity range of 480 to 500 mmhos/cm. Nominal loading rates of 0, 0.39, 1.0, 2.6, 6.3, 16, and 40mg/l were used to prepare test solutions. WAFs were prepared for each test concentration by mixing the appropriate volume of substance in 9.4l of fortified well water for 24 hr in 9.5l screw-capped glass jars. The volume of test substance added was based on the experimentally determined density of 0.742 g/ml. After stirring for 24 hrs with a vortex of no more than 25% of the solution depth, the contents of the WAF solution bottles were allowed to settle for 1 to 1.5 hrs prior to use. The WAF was removed from an outlet port located 2 cm from the bottom of the jar directly into each exposure vessel. A control solution was prepared similarly except without test substance addition. Test solutions were renewed daily with 70 ml of fresh WAFs added to a second set of beakers. Food was added to the fresh WAFs and daphnids were then transferred from the old test solutions to the fresh WAFs. Duplicate samples of freshly prepared WAFs and composited replicate old test solutions were collected each day and analyzed by Purge & trap/GC-FID for concentrations of the following: pentane, methylpentane, benzene, toluene, ethylbenzene, ortho, meta and para-xylene. Measured test concentrations of the light catalytically reformed naphtha were based on the concentrations of all analytes. Daphnids used in the test were from laboratory in-house culture. Daphnids were 24 hrs old at the start of the test. Test vessels were 70 ml screw-capped glass jars containing 70 ml of WAF with minimal headspace. Ten replicates per treatment and 1 daphnid per replicate were tested for each treatment and the control. Daphnids were fed 0.2 ml of algal suspension (*Ankistrodesmus falcatus*, 4×10^7 cells/ml) and 0.05 ml of a yeast, cereal leaves and digested flaked fish food (YCT) suspension daily during the test. Water temperature was 19 to 21° C. Test photoperiod was 16 hrs light and 8 hr dark. Dissolved oxygen concentrations were 8.6 to 9.1 in the new solutions and 8.4 to 10.0 in the old solutions. pH values were 7.1 to 8.4.

Reliability

- : (2) Valid with restrictions
Measured concentrations may not represent 100% of components, remaining hydrocarbon components in WAFs may be equally toxic and should have been quantitated to determine total measured concentrations.

(34)

5.1.1 ACUTE ORAL TOXICITY

Type : LD₅₀
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 10
Vehicle : Undiluted
Doses : Male: 5, 6, 6.5, 7 & 9.8; Females 3.57, 4.29, 5, 7 & 9.8 g/kg
Year : 1985
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : The test material was administered undiluted, as a single oral dose to groups of 5 male 5 female rats at dose levels ranging from 3.57 to 9.8 g/kg. The dose volume varied per dosage level based on an average bulk density of 0.8 g/ml. Food had been withheld from the rats overnight prior to dosing, but they had free access to water. Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days. The rats were weighed the day before dosing and then at 7 and 14 days after dosing. All animals, whether dying during the study or surviving to termination were subjected to a gross necropsy and any abnormalities were recorded.

Result : Clinical signs seen during the study included: hypoactivity, diarrhea, brown-stained anal area, ataxia, prostration, red stained nose and mouth, lacrimation, dyspnea, yellow-stained abdomen, hair loss on abdomen, decreased limb tone, piloerection, hair loss on front legs, excess salivation, yellow- or reddish-brown-stained urogenital region, tremors and death. All mortality occurred within the first three days after dosing. All surviving animal had returned to normal by day 11 except for those with hair loss.

At necropsy, there were few findings in the animals that survived to termination.

Mortalities and body weights were as follows:

Dose level (g/kg)	Body weights (g)		Mortality	No with lesions at necropsy
	Initial	Terminal (fasted)		
<u>Male</u>				
5	285	336	0/5	0/5
6	315	370	1/5	1/5
6.5	324	386	1/5	1/5
7	306	-	5/5	4/5
9.8	310	-	5/5	5/5
<u>Female</u>				
3.57	212	227	0/5	1/5

5. Toxicity

Id A. Naphthas
Date May 20, 2003

4.29	199	233	0/5	0/5
5	202	235	3/5	3/5
7	210	213	3/5	2/5
9.8	208	-	5/5	5/5

Typically at non-lethal dose levels the lesions frequently observed included: presence of dark colored material in the stomach, glandular mucosa of stomach with dark red to black area. Additionally at the highest dose levels the urinary bladder contained a red fluid in 4 of the five males examined.

The estimated LD₅₀ values and 95% confidence limits were:

Males: 6.62 g/kg (6.2 - 7.08)
Females: 5.39 g/kg (3.23 - 6.86)

Reliability : (1) Valid without restriction

(7)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 5.22 mg/l
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : Air
Doses : 5 mg/l
Exposure time : 4 hour(s)
Year : 1984
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-05 at a nominal concentration of 5mg/l for 4 hours.
After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure.
On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically.

Result : The exposure chamber TWA concentration was determined to be 5.22 ± 0.14 mg/l.
No animal died during the study and no clinical signs of systemic toxicity were observed.
There were no significant gross observations at necropsy and no histological changes were observed in the lungs.
The 4 hour LC₅₀ was therefore greater than 5.22 mg/l.

Reliability : (1) Valid without restriction

(5)

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	: 4
Vehicle	: Undiluted
Doses	: 2 g/kg
Year	: 1985
GLP	: Yes
Test substance	: API 83-05. See section 1.1.1. for characterization.
Method	: The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A weighed quantity of undiluted test material (equivalent to a dose of 2 g/Kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. 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. Collars were used to restrain the animals during the application period. The animals were observed for a total of 14 days post-dosing. Body weights were recorded just prior to dosing and again seven and 14 days after dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.
Result	: There were no mortalities during the study and there were no clinical signs of toxicity with the exception of one rabbit. This animal had soft stools, diarrhea, hypoactivity and an inflamed urogenital area during the last three days of the study. All animals had gained weight by the end of the study. Dermal irritation occurred during the study and this ranged from slight to severe for erythema, slight to marked for edema and slight to marked for atonia, desquamation, coriaceousness and fissuring. At necropsy, the only findings in the males were on the treated area of the skin and were consistent with the gross observations of irritation. In the females similar skin lesions were observed and in addition, the vagina was reddened in 3 of the four animals and in one of these the trachea contained a red liquid on the inside walls and the lungs had multiple red pinpoint foci on all lobes.
Reliability	: (1) Valid without restriction

(7)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 3.1
Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each rabbit. 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 and again at 5, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : The scores for erythema and edema at each of the observation times were as follows:

	Erythema		Edema	
	Intact	Abraded	Intact	Abraded
24 h	1.2	1.5	1.5	1.8
72 h	1.5	1.5	1.7	1.8
5 days	1.0	1.3	1.5	1.7
7 days	0.8	1.0	1.0	1.0
14 days	0	0	0	0

The Primary dermal Irritation index was 3.1

Growth rates were normal throughout the study and there were no clinical signs of systemic toxicity.

Reliability : (1) Valid without restriction

(7)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : .1 ml
Comment : Rinsed eye after 30 sec exposure in 3 rabbits only
Number of animals : 9
Vehicle : None
Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : 0.1 ml of undiluted test material was dripped onto 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. At the 72 hour and seven day readings, sodium fluorescein was used to aid in revealing possible corneal injury. Body weights were recorded just prior to treatment and one week afterwards. At termination of the study, the rabbits were euthanized by an overexposure of carbon dioxide and were subjected to a gross necropsy. Any abnormalities found were recorded.

Result : No signs of systemic toxicity were observed during the study.
 The primary eye irritation scores were as follows:

Observation period	Primary eye irritation score	
	Unwashed*	Washed**
1 hour	7.2	7.3
24 hour	5.5	2.7
48 hour	4.3	2.0
72 hour	3.0	2.0
7 day	1.0	1.3
14 day	0	0

* Mean of six rabbits

** Mean of three rabbits

In rabbits whose eyes had not been washed irridial irritation that had occurred had subsided by 24 hours and all corneal involvement had subsided by 48 hours. No corneal or irridial irritation was observed in the group whose eyes had been washed followed by application of test material.

Reliability : (1) Valid without restriction

(7)

5.3 SENSITIZATION

Type	: Buehler Test
Species	: Guinea pig
Concentration	: 1 st : Induction 50 % occlusive epicutaneous 2 nd : Challenge 25 % occlusive epicutaneous
Number of animals	: 20
Vehicle	: Paraffin oil
Result	: Not sensitizing
Method	: Beuhler
Year	: 1986
GLP	: Yes
Test substance	: API 83-05. See section 1.1.1. for characterization.
Method	: 0.4 ml of a 50% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.4 ml of a 25% mixture in paraffin oil) 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 (2,4-dinitrochlorobenzene), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups. The positive control was used at a concentration of 0.3% in 80% aqueous ethanol for the induction doses and at 0.1% w/v in acetone for the challenge dose.
Result	: There was no abnormal appearance in any of the animals exposed to the test material during the study. The skin reactions to the challenge dose are summarized as follows: Test material No dermal reactions by any animal Naive control Very slight erythema in 2/20 animals Vehicle control No dermal reactions by any animal Positive control Very slight to moderate irritation by all 20 animals. The reactions of 16 of the animals exceeded the highest reaction observed in the naive positive control animals. Naive positive control 10/20 animals exhibited very slight erythema.
Reliability	: (1) Valid without restriction

5.4 REPEATED DOSE TOXICITY

Species	: Rat
Sex	: Male/female
Strain	: Sprague-Dawley
Route of admin.	: Inhalation
Exposure period	: 13 weeks
Frequency of treatm.	: 6 hours/day, 5 days/week
Post exposure period	: Recovery group 4 weeks
Doses	: 750, 2500 and 7500 ppm
Control group	: Yes
NOAEL	: = 2500 ppm
Method	: OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	: 2000
GLP	: Yes
Test substance	: LCRN-D See section 1.1.1. for characterization.

Method : The method used was described in OECD guideline 413. Groups of 16 male and 16 female rats underwent whole body exposures to 750, 2500 and 7500 ppm LCRN-D. Exposures were for 6 hours each day, 5 days per week for 13 weeks. Extra groups of 16 rats of each sex were exposed to the high dose level and also for a recovery control group. These animals were maintained untreated for 28 days following cessation of the 13 weeks exposure.

Neurobehavioural evaluations of motor activity and functional activity were performed pretest and during weeks 5, 9, 14 and 19 (recovery groups). Animals were not exposed to LCRN-D during these tests.

Following 13 weeks of exposure, 16 animals/sex/group were necropsied and microscopic examination was performed on selected tissues. Nervous tissue from 6 rats/sex/group was also examined microscopically.

At the end of the 4 week recovery period, 16 animals of each sex from the high and control groups were necropsied and selected tissues were examined microscopically.

During the study, clinical observations were made twice daily. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and 19 weeks (recovery groups). Body weights and food consumption was measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements.

At termination (after 13 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. 10 animals of each sex were designated for non-neuropathological examination and 6 of each sex for neuropathological examination.

For the non-neuropathology animals, the following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymes), thymus and uterus. Brain lengths and widths were measured for each rat.

A wide range of tissues (39) was removed from the control and high dose animals, were fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were also removed from the nervous system (central and peripheral) of all animals for subsequent special staining and histopathological examination. Animals designated for neuropathological examination were subjected to a detailed examination of central and peripheral nervous tissues. N

Neurobehavioural studies were undertaken as follows:

Motor activity

Locomotor activity was monitored as the number of beam breaks in an activity box. Monitoring sessions were for 60 minutes, divided into twelve 5-minute intervals. Evaluation was made pretest and during weeks 5, 9, 14 and at the end of the 4 week recovery period. [A detailed description of the evaluation and analysis is provided in the publication but is not included here.]

Functional Operational Battery

An assessment of the following was made:

Home cage evaluations for Posture, vocalization, palpebral closure.

Handling evaluations for reactivity to general stimuli, signs of autonomic function.

open field behavior: arousal level, gait, urination and defecation frequency, convulsions, tremor, abnormal behavior, piloerection and exophthalmos.

Reflex assessments for: response to visual and auditory stimuli, tail pinch, pupillary function.

Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

Result

: There were no mortalities during the study and there were no treatment-related signs of toxicity. The ophthalmic examinations did not reveal any treatment-related effects. Mean body weights, body weight gains and food consumption were unaffected by treatment.

No treatment-related effects were recorded in the Functional Operational Battery. In the examinations of motor activity, there were no treatment-related effects recorded during the 13 week exposure period but a slight increased activity was found in the highest treatment group after the 4 week recovery period.

After 13 weeks exposure there was a significant decrease in total WBC count (36%) and lymphocyte counts in the high dose males and a slight decrease in neutrophil counts for the mid dose males. A trend towards decreased WBC (2.1%) and lymphocyte counts was also seen in the mid dose males and high dose females. After the 4 week recovery period, leukocyte values were comparable to control values. However, MCV was slightly decreased (2.8%) in the high dose males. It was concluded that these changes were suggestive of a reversible slight effect of the LCRN-D.

Clinical chemistry parameters were unaffected by treatment.

After 13 weeks exposure relative kidney weights in the high dose males were increased (15.9%) and this correlated with the occurrence of hyaline droplets in the proximal convoluted tubules. This finding has been described as a "light hydrocarbon nephropathy" and is sex and species specific and is not relevant for human health risk assessment.

In the high dose males decreased absolute (25.7%) and relative (22%) spleen weights were also recorded. It was concluded that this was associated with the minor hematological changes that had been observed. These differences were not apparent after the recovery period and no abnormal microscopic findings were found in either the spleen or bone marrow.

Brain length and width measurements were unaffected by treatment and there were no abnormal microscopic findings in the brain, spinal cord or peripheral nerves.

Test condition

: The LCRN-D was pumped onto the central glass helix of a volatilization chamber. Nitrogen was passed upwards through the chamber over the heated coil and the volatilized material was suitably diluted with air to achieve the desired concentration. A separate volatilization chamber was used for each dose concentration. During the study the exposure chamber concentrations were monitored hourly. The composition of the vapor (vol. %) is shown in the following table:

Parameter	LCRN-D vapor
Olefins	1.37
Paraffins	88.3
Naphthenes)	1.24
Aromatics	9.09
Benzene	4.65
Carbon No.	
4	3.6
5	59.11
6	25.18
7	11.65
8	0.46
9	0

There was a gas chromatographic analysis of the LCRN-D at the beginning and at the end of the study. The results (expressed in wt %) were as follows:

Component	LCRN-D vapor (wt %)	
	Study Beginning	Study Termination
n-Butane	3.34	3.16
n-Pentane	20.38	20.43
Isopentane	35.31	34.70
1-Pentene	0.05	0.05
2-Methyl-2-butene	0.35	0.36
2-Methyl-1-butene	0.20	0.20
2,2-Dimethylbutane	2.22	2.22
n-Hexane	4.27	4.34
Methylcyclopentane	0.48	0.49
2,3-Dimethylbutane	1.54	1.59
2-Methylpentane	6.62	6.73
3-Methylpentane	4.54	4.62
Benzene	6.42	6.61
2-Methylhexane	1.65	1.67
3-Methylhexane	1.83	1.86
n-Heptane	0.90	0.91
Toluene	5.65	5.76

Reliability : (1) Valid without restriction

(31)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 13 weeks
Frequency of treatm. : 6 hours/day, 5 days/week
Doses : 410, 1970 and 8050 ppm
Control group : Yes
NOAEL : = 1970 ppm
Year : 1996
GLP : No data
Test substance : Partially vaporized full range catalytic reformed naphtha

Method : Groups of 15 rats of each sex were individually housed in 1m³ inhalation chambers. The rats underwent whole body exposures to partially vaporized full range catalytic reformed naphtha (FRCRN). Exposures were [6 hours/day, 5 days/week] for 13 weeks at nominal concentrations of 500, 2000 and 8000 mg/m³. Two extra groups of 15 rats of each sex served as sham and untreated controls. (NB. This is not stated in the publication but from other comments in the paper, it is clear that exposure was not continual during the study). Water was available ad lib, but food was withheld during the exposure periods. Clinical observations were made regularly and body weights were recorded weekly.

At the end of the 13 weeks exposure, blood samples were taken for hematological and clinical chemical measurements. The rats were then sacrificed and necropsied. Organs were weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet weight of the right middle lung lobe was also weighed. The lobes were then dried and their dry weights determined. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to determine the number of testicular spermatids.

The following tissues from the high dose animals were examined histologically: adrenals, bone and marrow (sternum), pancreas (head), brain (three sections), submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach (squamous and glandular), colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver, tracheobronchial lymph nodes, lung (left lobe), nasal turbinates (four sections), thigh muscle, urinary bladder, sciatic nerve, and any gross lesions. In addition, tracheobronchial lymph nodes and any gross lesions from untreated control animals were also evaluated.

Result

- : There were no treatment-related clinical signs during the study, no effects on serum chemistry values or parameters of the male reproductive system a terminal sacrifice. Body weights of males were exposed t the mid and high dose groups were higher than the controls throughout the study and the differences were statistically significant in the high dose group from week 10 onwards.

WBC count was significantly lower in sham treated controls and all three treated groups in both sexes compared to untreated controls. Additionally the WBC count was decreased by approximately 24% in the high dose females when compared to the sham controls. No other parameters were affected.

The only organ weights affected were the liver and kidney. In the male high dose group, mean kidney weight was approximately 13% greater than the sham treated animals (but not the untreated controls), and the liver weight was approximately 14% greater.

No treatment-related gross lesions were observed at necropsy and no treatment-related abnormalities were noted during microscopic examination. Because of the lack of effects in the histology, no tissues were examined from the lower dose groups.

Test substance

- : Test atmospheres were generated by partially vaporizing FRCRN. The concentrations in the chamber were adjusted by dilution with air. Concentrations were monitored throughout the study. The actual concentrations for each of the dose levels are shown below.

Parameter	Exposure group		
	Low	Medium	High
Target conc. (mg/m ³)	500	2000	8000
Actual conc. (mg/m ³)	410	1970	8050
Butane	4.33	3.91	4.05
Methylbutane	20.56	17.26	17.55
Pentane	13.24	11.44	11.86
Hexane	6.53	5.71	6.36
Heptane	2.32	2.35	2.33
Benzene	2.19	4.93	5.79
Toluene	10.02	12.23	10.93
m- and p- Xylenes	3.57	4.05	3.4
2- Ethyltoluene	0.43	0.35	0.17
Trimethylbenzene	0.01	0.01	0.04

Reliability

: (2) Valid with restrictions

The publication is not clear in its description of the frequency and duration of exposures. However, it is assumed that the exposures are 6 hours/day, 5 days/week since this would be consistent with other studies reported from the same laboratory.

(22)

Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 6 hours
Frequency of treatm. : 3 times per week for 28 days (12 applications total)
Doses : 200, 1000 & 2000 mg/kg
Control group : Yes, concurrent no treatment
Year : 1986
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : Prior to the study 5-day range finding study was conducted. The method and results of he range-finding study are not included in this summary.

Undiluted API 83-05 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours, which was then removed and the skin was wiped with a dry gauze to remove any residual material.

A group of five rabbits of each sex served as sham controls. The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly.

At termination, blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination.

A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination. Microscopic examination was undertaken for the control and high dose groups only.

Result : Two males in the 2000 mg/kg group and one male in the 1000 mg/kg died during the study. The deaths occurred on days 12 and 17 for the highest dose group and day 19 at the mid dose group. There were no clinical signs of intoxication in any other animal on the study.

At 200 and 1000 mg/kg there were no treatment related effects on body weight gains over the study period although there were isolated differences between treated and control animals during the study. At the highest dose level, the females showed no weight gain and the males had an overall weight loss.

A mean irritation score was calculated for each day and overall means were also calculated. (The mean irritation score, MIS, was the sum of irritation scores for both erythema and edema for all animals of a given dose group and sex.)

The overall MIS for each dose group was:

Group/sex	MIS	Classification
2000 mg/kg M	5.1	Severe irritant
2000 mg/kg F	4.9	Severe irritant*
1000 mg/kg M	4.3	Moderate irritant
1000 mg/kg F	4.1	Moderate irritant
200 mg/kg M	2.9	Moderate irritant
200 mg/kg F	2.5	Moderate irritant
Control M	0	Non irritant
Control F	0	Non irritant

*Severe irritation was observed in the high dose females during the study and the authors concluded that even though the overall MIS for this group led to a moderate classification, a severe classification would be more appropriate.

Some differences were observed between the control and treated groups for a few hematological and clinical chemical parameters. The differences from control values are shown below. However, the authors concluded that since the values fell within the normal range for the laboratory, they should not be regarded as treatment related.

Dose group	Parameter	Difference
1000 mg (M)	WBC	25% higher
	Alkaline phosphatase	37% lower
2000 mg (M)	Hemoglobin	5% lower
	Blood urea nitrogen	24% lower
	Alkaline phosphatase	35% lower
	(F) SGPT	26% lower

There were also a few differences between control and treated animals for absolute and relative organ weight for a small number of organs. Since there was no associated histopathological findings and since the differences were not dose-related, they were not considered to be significant.

At necropsy, few gross findings were recorded other than effects on the treated skin. The findings in the liver of males and females of treated and control groups consisted of areas of discoloration and were considered to incidental to treatment.

Histological changes were mainly confined to the skin except for two males that died in the highest dose group. The kidneys of these two animals contained slight to moderate tubular degeneration.

The changes in the skin consisted of slight to moderate proliferative and inflammatory changes at the highest dose group. Concurrent with these changes in the skin there was an increased granulopoiesis of the bone marrow. Increased granulopoiesis was recorded for all the high dose group males and females examined and for one control male. The authors considered that this was probably related to the stress or other factors associated with the skin irritation.

Reliability : (1) Valid without restriction

Type	: Sub-acute
Remark	: Two other inhalation studies have been reported (Halder et al, 1984), but since they were of shorter duration (21 days) than those summarized above and also were only with male rats, they are not summarized in full here. The studies were: Light catalytically reformed naphtha (31% aromatics) at dose concentrations of 544, 1591 and 5522 ppm. Heavy catalytically reformed naphtha (93% aromatics) at concentrations of 215, 587 and 2132 ppm. In the study with the LCRN renal changes typical of light hydrocarbon nephropathy were observed. In the study with HCRN there were no kidney changes but lung irritation was apparent.

(25)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	: Mouse lymphoma assay
System of testing	: Forward mutation assay using cell line L5178Y TK+/-
Cytotoxic concentr.	: 250µl/ml, lethal at 500µl/ml
Metabolic activation	: With and without
Year	: 1985
GLP	: Yes
Test substance	: API 83-05. See section 1.1.1. for characterization.
Method	: The test material was dissolved in acetone for this assay. Three positive control substances were used viz Ethyl methane sulphonate (EMS) at concentrations of 0.25 & 0.4 µl/ml for the non activation assay, Dimethylnitrosamine (DMN) at a concentration of 0.3 and Methylcholanthrene (MCA) at a concentration of 2.5 µg/ml for the activation assay. Aroclor-induced rat liver was the source of the S-9 homogenate for the activation assay. A cytotoxicity study was carried out prior to the mutagenicity assay. The test material was lethal at a concentration of 500 µl/ml and highly toxic at 250 µl/ml without S-9. These results were used to select a dose range of 6.25 to 500 µl/ml for the non-activation assay and 3.13 to 400 µl/ml for the activation assay. For the mutation assay, the lymphoma cells were exposed for 4 hours to test material. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; 5-Trifluorothymidine (TFT) was used as the restrictive agent. six non-activated and six activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 6.25, 25, 37.5 50, 75 and 100 µl/ml of test material and

resulted in a range of growth of 30 to 97% compared to the solvent control. The activated cultures that were cloned were treated with 18.8, 37.5, 75, 100, 150 and 200 µl/ml of test material. This resulted in growth ranging from 4.6 to 67.9% compared to solvent control.

Plates were prepared from TFT and from the viable cultures (VC) and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. A mutation frequency was then determined.

The following criteria were used in judging the significance of the activity of the test article.

Positive - if there is a positive dose response and one or more of the 3 highest doses exhibit a mutant frequency which is two-fold greater than background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Remark : Mouse lymphoma forward mutation assays have been carried out on two other aromatic naphtha samples. The results were:

Sample No.	Aromatic content (vol. %)	Response with S-9	without S-9
83-04	42.1	negative	negative
83-06	89.8	negative	positive
Laboratory 1		equivocal	equivocal
Laboratory 2			

Result : The mutant frequencies and the percentage total growth at each of the test concentrations is summarized in the following table.

Concentration (µl/ml)	Mutant frequency	% Relative growth
<u>Non-Activated</u>		
6.25	24.2	97.3
25	22.5	64.3
37.5	18.2	32.6
50	23	47.8
75	39.6	59.4
100	22.3	29.6
Solvent 1	100	22.7
Solvent 2	100	30.6
Untreated control	20.7	110.6

EMS 0.25 µl/ml	364.5	53.8
EMS 0.4 µl/ml	504.5	23.2

S-9 Activated

18.8	54.2	67.9
37.5	57.3	56.1
75	72.1	60.3
100	85.2	32.8
150	73	27.4
200	146.2	4.6
Solvent 1	31.3	100
Solvent 2	30.8	100
Untreated control	42.1	123.9
DMN 0.3 µl/ml	258.8	12.7
MCA 2.5 µl/ml	243.6	78.5

The authors concluded that the test material was not mutagenic in the non-activated assay because there was no dose response relationship and furthermore the mutant frequency was not significantly different from the solvent and untreated controls.

The minimum criteria for indicating mutagenesis would have been 47×10^{-6} . Since the 100 µl/ml treatment represented a close approach to the excessively toxic treatment at 150 nl/ml, this assay was considered sufficient to evaluate the test material as non-mutagenic under non-activation conditions.

In the presence of the S-9 mix, the test material was converted into one or more mutagenic products.

The minimum criterion for a significant response was a mutant frequency exceeding 62.1×10^{-6} . This value was exceeded for 4 of the 6 analyzed cultures. The response was dose related.

The results were judged sufficient to evaluate the test material as mutagenic in the presence the metabolic activation system.

Reliability : (1) Valid without restriction

(8) (10) (11) (14)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : One single dose given
Doses : 0.26, 0.82 & 2.42 g/kg
Result : Negative
Year : 1985
GLP : Yes
Test substance : API 83-05. See section 1.1.1. for characterization.

Method : Two studies were carried out. In the first study, the test material did not induce a significant increase in the percentage of aberrant cells above the controls in either sex. Furthermore, the positive control (TEM at a dose of 0.8

mg/kg)) did not induce a significant elevation in the percentage of cells with structural aberrations. The assay was, therefore, repeated using a higher dose of TEM. In this robust summary, only the results of the repeat study are described.

The study design was as follows:

Treatment	Animals/sex/sacrifice time		
	6 hrs.	24 hrs.	48 hrs
Corn oil (vehicle)	5	5	5
API 83-05, 2.42 g/kg	5	5	5
API 83-05, 0.82 g/kg	5	5	5
API 83-05, 0.26 g/kg	5	5	5
Triethylenemelamine (Positive control)		5	

Test material in vehicle was given intraperitoneally at a dose of 5 ml/kg to groups of rats as shown above. Corn oil was used as vehicle control and TEM (1.5 mg/kg) as the positive control.

Four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (4 mg/kg).

One male in the 2.42 g/kg group and one male in the 0.82 g/kg dose group died immediately after dosing, these were replaced by substitute animals.

Immediately after sacrifice, bone marrow was obtained from the tibiae of the animals. The marrow was washed and the cells were fixed before being spread on slides (at least 3 from each animal) for examination.

Slides were scored for chromosomal aberrations.

Where possible, a minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy (1-3).

A mitotic index (= No. of cells in mitosis/500 counted x 100) was calculated and recorded.

The type of aberration, its frequency, the statistical significance of any increases and its correlation to dose in a given time period will all be considered in evaluating a test article as being mutagenically positive or negative. Criteria for a positive response are generally a statistically significant dose-related increase in the number of structural aberrations at three dose levels. The final decision is based on scientific judgment.

Remark : Similar cytogenetics assays have been reported for two other aromatic naphtha samples (API 83-04 and API 83-06, approx. 42 and 90% aromatics respectively) and both were negative.

Result : The dose levels used in the assay were selected on the basis of a preliminary screen.

In the cytogenetics assay, one male died at each of the dose levels 2.42 and 0,82 g/kg, the mortality occurred immediately after dosing. Toxic signs included lethargy and a moribund appearance at the high dose and slow uncoordinated movement in the mid dose group.

The results of the cytogenetics evaluations are summarized

5. Toxicity

Id A. Naphthas
Date May 20, 2003

in the following table.

MALES

	Dose level (g/kg)			Positive control	Vehicle control
	0.26	0.82	2.42		
% Cells with 1 or more aberrations					
6 hrs	0.5	0.4	1.0	32.4	0.5
24 hrs	0.4	0.8	1.0		0
48 hrs	0	1.6	0.5		0.8
% Cells with 2 or more aberrations					
6 hrs	0	0	0	10.8	0.5
24 hrs	0	0	0		0
48 hrs	0	0	0		0
Frequency of structural aberrations					
6 hrs	.005	.004	.01	.708	.05
24 hrs	.004	.008	.01		0
48 hrs	0	.016	.005		.008
Frequency of numerical aberrations					
6 hrs	.005	0	.016	.008	.015
24 hrs	.008	.008	.01		.005
48 hrs	.01	.008	0		.004
Mitotic Index					
6 hrs	4.1	3.6	2.4	6.3	5.4
24 hrs	4.4	5.7	5.5		5.0
48 hrs	6.5	5.1	5.2		5.7

FEMALES

Cells with 1 or more aberrations					
6 hrs	0	0.5	1.6	33.2	0.8
24 hrs	0	0.4	1.5		0
48 hrs	0	1.2	0.8		1.2
Cells with 2 or more aberrations					
6 hrs	0	0	0	13.20	0
24 hrs	0	0	0.5		0
48 hrs	0	0	0		0.5
Frequency of structural aberrations*					
6 hrs	0	.005	.016	0.804	0.008
24 hrs	0	.004	.02		0
48 hrs	0	.012	.008		0.012
Frequency of numerical aberrations*					
6 hrs	.005	.005	.020	0.020	0
24 hrs	.01	.016	.005		0.020
48 hrs	.008	0	.012		0.005
Mitotic Index					
6 hrs	5.4	6.3	3.1	4.7	6.1
24 hrs	4.9	5.4	4.1		4.8
48 hrs	5.5	4.9	7.0		5.2

* Frequency based upon the aberration frequency per cell per animal

Note that for simplicity only, mean values without standard errors are shown in the above table although they are given in the laboratory report.

On the basis of the criteria defined for assessing the results, the authors concluded that API 83-05 was not mutagenic in this assay.

Reliability : (1) Valid without restriction

(6) (9) (15)

5.8.1 TOXICITY TO FERTILITY

Type : Reproductive/developmental toxicity screening study, (OECD 421)
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Frequency of treatm. : 6 hours/day, 7 days/week
Premating exposure period
 Male : 2 weeks
 Female : 2 weeks
Doses : Target conc.: 750, 2500 & 7500 ppm. Actual conc.: 750, 2490 & 7480 ppm
Control group : Yes
Method : OECD combined repeated dose and reproductive/developmental toxicity screening test
Year : 2000
GLP : Yes
Test substance : LCRN-D. See section 1.1.1. for characterization

Method : Groups of 10 rats of each sex were exposed to 750, 2500 or 7500 ppm. LCRN-D for 6 hours /day, seven days/week. A group of 10 rats of each sex served as sham treated controls. Parental females were exposed for 14 consecutive days prior to mating, throughout mating and days 0-10 of gestation. Dams and their litters were sacrificed on post partum day 4. Unmated females and parental males were exposed to the test material for 14 days prior to mating, throughout mating and 18 additional days following completion of the mating period. These animals were sacrificed shortly after the last litters were delivered reached post partum day 4.

Mating

Within each group one male was co-housed with the same female until evidence of mating was observed (presence of sperm in vaginal smear or copulatory plug). The day of mating was designated day 0 of gestation. Following mating, the females were housed individually and continued their exposures to test material until day 19 of gestation. Females not showing evidence of mating following a 14 day mating period continued their exposures. If such a female showed signs of being pregnant, it was removed from the exposure regimen and observed for parturition.

Observations

All parental animals were regularly observed for mortality and gross pharmacologic signs. A physical examination, including palpation for tissue masses was carried out daily 30 mins. after removal from the exposure chambers.

Body weights and food consumption were measured throughout the study.

From day 20 of gestation, females (pregnant and non-pregnant) were observed for signs of parturition.

As soon as possible after delivery, litters were observed for the number of live and dead pups and for any abnormalities. Litters were also observed twice daily for unusual findings and dead pups.

On days 0 and 4 of lactation, the pups were counted, weighed and sex was determined by external observation.

Pathology

Males were killed as a group shortly after the last litters delivered had reached day 4 of lactation.

Females with litters that reached day 4 of lactation were killed the next day or shortly thereafter.

Unmated females and those that did not deliver were killed 23 days after completion of the mating period.

At post mortem, a complete macroscopic examination was carried out on all adult animals. The following organs were weighed and organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, lung, spleen, epididymes, testes and thymus. Post mortem examination of females included a count of uterine implantation scars when present.

Pups were sacrificed on day 4 of lactation and underwent a complete macroscopic examination and a determination of sex by internal examination. All pups were preserved with viscera intact. Pups found dead at birth and that died prior to day 4 of lactation also underwent a gross external and internal examination. Dead pups were not eviscerated, the intact pups were preserved.

27 tissues were preserved from all adult animals in all dose groups. Ovaries, testes, epididymes, nose with nasal turbinates, and any grossly observed abnormalities were processed and sections examined histologically for all males and female parental animals in the control and highest dose group. Four sections were prepared and examined microscopically of the skull containing the nasal turbinates. These were

- area between upper incisor and incisive papilla
- area between incisive papilla and first palatal ridge
- area between second palatal ridge and first upper molar
- area between first upper molar and nasopharynx.

Result

- : All parental animals survived to scheduled sacrifice and no treatment related clinical signs were observed. Except for a slight reduction in body weights in the high dose males there were no other effects on either body weight or food consumption. When compared to the controls, at week 3 the decrease in weight of the high dose males was 3.8% and

at week 7 was 7.8.

The only treatment related organ weight changes was an increase in relative kidney (15%) and relative liver (5%) weights in the high dose males. No other organ weight changes were recorded.

There were no treatment-related microscopic changes in the testes, epididymes, ovaries or nasal turbinates in the animals in the high dose group.

Reproductive/fertility effects

All groups had a mating index and a fertility index of 100% and all animals in all groups had mated within 4 days of cohabitation.

Delivery and litter data did not demonstrate any effects of treatment see data summarized below.

Parameter	Dose group (ppm)			
	0	750	2500	7500
Females on study	10	10	10	10
Litters with liveborn	10	10	10	10
Implantation sites	147	154	155	154
Mean	14.7	15.1	15.5	15.4
Pups delivered (total)	145	151	146	145
Liveborn	142	151	143	144
Live birth index (%)	98	100	98	99
Pups dying				
Day 0	0	1	1	1
Days 1-4	2	4	0	0
Pups surviving 4 days	140	146	142	143
Viability index (%)	99	97	99	99
pup sex distribution				
Day 0 M/F (ratio)	63/79	67/84	69/74	68/76
Day 4 M/F (ratio)	63/77	64/82	68/74	68/75
Pup weight/litter (g)				
Day 0	6.0	6.6	6.2	6.1
Day 4	9.3	8.9	9.2	9.6

External and internal examination of pups sacrificed on day 4 of lactation resulted in only one pup in a single litter of the control group with abnormalities.

Reliability : (1) Valid without restriction

(32)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
 Sex : Male/female
 Strain : Sprague-Dawley
 Route of admin. : Inhalation
 Exposure period : Gestation days 6-19 inc.
 Frequency of treatm. : 6 hours/ day
 Doses : Target concentrations: 2000 and 8000 ppm. Actual: 2160 and 7800 ppm
 Control group : Yes
 NOAEL maternal tox. : = 7800 ppm
 NOAEL teratogen. : = 7800 ppm

- Year** : 1996
GLP : No data
Test substance : Partially vaporized full range catalytic reformed naphtha
- Method** : Groups of 11 or 12 presumed pregnant female rats were exposed 6 hours each day from days 6-19 of gestation to whole body exposures of 2000 or 8000 ppm partially vaporized FRCRN. Two extra groups served as untreated and sham treated controls.
All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation.
On day 20 each female was sacrificed and blood samples removed for serum chemistry evaluations. Parameters measured were the same as those in the subchronic study by the same authors, and in addition included iron and lactic dehydrogenase.
- All organs were examined grossly and liver and thymus weights were recorded. In addition, the number of corpora lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early and late resorptions and live and dead fetuses were recorded.
- Each fetus was gendered, weighed and grossly examined for external abnormalities. Half the fetuses were fixed in Bouin's fluid and examined subsequently for soft tissue abnormalities. Remaining fetuses were stained with Alizarin red and examined for skeletal anomalies.
- Result** : There were no adverse effects on maternal body weight gain, liver weight or thymus weight.
In the high dose group, maternal serum glucose levels were significantly decreased (1.5%) and potassium levels increased (1%) relative to the untreated controls.
- Reproductive performance during gestation and in-utero survival and development of concepti were unaffected by treatment. Furthermore, there were no treatment-related increases in gross abnormalities or anomalies of soft or skeletal tissues.
- Reliability** : (1) Valid without restriction

(22)

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**ROBUST SUMMARY
OF INFORMATION ON**

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Summary prepared by: American Petroleum Institute

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1.1.1 GENERAL SUBSTANCE INFORMATION

- Substance type** : Petroleum product
Physical status : Liquid
- Remark** : Gasoline is a complex combination of hydrocarbons consisting primarily of paraffins, cycloparaffins, aromatic and olefinic hydrocarbons having carbon numbers predominantly greater than C₃ and boiling in the range of 30°C to 260°C.

To achieve acceptable physical and combustion properties, gasoline is prepared by blending naphtha streams, each of which will consist predominantly of one or other chemical type.

The streams normally used are:

Paraffinic streams derived from alkylation, isomerization and solvent extraction

Olefinic streams derived from catalytic cracking

Naphthenic streams derived from crude oil distillation

Aromatic streams derived from catalytic reforming

The American Petroleum Institute prepared a blend of naphtha streams which was considered to be typical of US gasoline in the middle 1970s. The blend was designated PS-6 gasoline. The benzene content of PS-6 was adjusted upwards to 2.0% by volume, although this would never happen in normal refinery practice. The proportions of the various naphtha streams used to prepare the PS-6 gasoline blend were:

<u>Naphtha stream/Component</u>	<u>% vol.</u>
Paraffinic naphtha streams	22.0
Olefinic naphtha streams	52.1
Aromatic naphtha streams	21.3
Benzene	0.8
n-butane	3.8

A more comprehensive characterization of PS-6 gasoline was reported by MacFarland et al. as follows:

Research Octane No.	92.0
Motor Octane No.	84.1
Reid vapor pressure (Lbs)	9.5
Distillation	
Initial boiling point (°C)	93
Final boiling point (°C)	428
API Gravity	60.6
Gum, ASTM D381 (mg/gal)	1
Sulfur (ppm)	97
Phosphorus (g/gal)	<0.005
Lead (g/gal)	<0.05
Stability (hrs)	>24
Hydrocarbon analysis, ASTM D1319	
Aromatics (vol%)	26.1

1. General Information

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Olefins (vol%)	8.4
Saturate (vol%)	65.5
Benzene (%)	2.0

An unleaded gasoline, tested in an inhalation teratology study had the following physical chemical characteristics:

API Gravity	57.4
Reid vapor pressure (Lbs)	8.8
Initial boiling point (°C)	88
Final boiling point (°C)	378
Sulfur (ppm)	157
Vapor density	3.4
Paraffins (vol%)	47
Olefins (vol%)	4
Naphthenes (vol%)	10
Aromatics (vol%)	39
C6 Aromatics (%)	1.2
C7 Aromatics (%)	10.2
C8 Aromatics (%)	16.3
C9-plus Aromatics (%)	11.3

(23)

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 300 ppm
Short term exposure limit value
Limit value : 500 ppm
Time schedule : 8 hour(s)

(1)

2. Physico-Chemical Data

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2.2 BOILING POINT

Value : ca. 93 - 428 °C at
Year : 1984
GLP : No data
Test substance : API PS-6 gasoline

Reliability : (1) Valid without restriction (25)

2.3 DENSITY

Type : Relative density
Value : ca. 50 at °C
Year : 1984
Test substance : API PS-6 gasoline

Reliability : (1) Valid without restriction (25)

2.5 PARTITION COEFFICIENT

Log pow : = 2.13 - 4.5 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : No
Test substance : Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5

Remark : Log P values represent the spread of calculated and/or measured values for C5 to C8 hydrocarbon components found in gasoline, CAS No 86290-81-5. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).

Reliability : (2) Valid with restrictions (13) (27)

Log pow : = 2.73 - 4.85 at 25 °C
Method : Calculated by LOGKOWWIN ver. 1.65.
Year : 2000
GLP : No
Test substance : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). Test material was added to 2.4 liters of sterilized AAP test media (enriched with 515 mg/l of sodium bicarbonate, 300 µg/l EDTA)

Remark : Log P values represent the spread of calculated and/or measured values for C5 to C9 hydrocarbon components found in LSRN, CAS No 64741-46-4. Detailed hydrocarbon analysis was used to identify the components of this specific high naphthenic LSRN sample. Calculation based on an atom/fragment contribution method of W. Meylan and P. Howard. Calculated SAR result for surrogate structure contained in program database (smilecas.dat).

Reliability : (2) Valid with restrictions (13) (27)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Method	: Preparation of Water Soluble Fraction
Year	: 1995
GLP	: Yes
Test substance	: Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Method	: Water Accommodated Fractions (WAFs) of LSRN were prepared at 100 mg/l loading in freshwater and equilibrated for 48 hours in tightly closed systems with minimal headspace.
Result	: Gas chromatographic analysis of BTEX components indicated freshwater solubility at 24 hours of 3.1, 3.1, $6.9E^{-3}$, and 0.92 ppm (as BTEX, respectively).
Conclusion	: Individual components of complex petroleum substances have specific and differing solubilities. Calculated and measured water solubilities for LSRN components range from approximately 3 to 2000 mg/l. 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 (7) (14) (16) (17) (31)

2.14 ADDITIONAL REMARKS

Memo	: Modeled data on the significant constituents are shown in the attached spreadsheet
Attached document	:  Gasoline_AD2644.xls

3. Environmental Fate and Pathways

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3.1.1 PHOTODEGRADATION

Type	: Calculated
Light source	: Sun light
INDIRECT PHOTOLYSIS	
Sensitizer	: OH
Method	: Calculated by AOPWIN ver. 1.89. Method based on the work of R. Atkinson
Year	: 2000
GLP	: No
Test substance	: Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Remark	: AOPWIN ver. 1.89 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight and in contact with O ₃ . Atmospheric oxidation rates were calculated for the C5 to C8 hydrocarbon components found in gasoline. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. Based on a 12-hour day, the range for atmospheric half-lives for gasoline constituents is: 0.789 days (m-xylene) to 15.985 days (isopentane).
Result	: Indirect Photolysis Sensitizer: OH radical Conc. of sensitizer: 1.50E ⁺⁰⁶ OH radicals/cm ³ Rate Constant: 0.6991 E ⁻¹² (isopentane) to 13.5606 E ⁻¹² (m-xylene) cm ³ /molecule-sec Half-life: 0.789 to 15.985 days
Reliability	: (2) Valid with restrictions

(13) (28)

3.1.2 STABILITY IN WATER

GLP	:
Test substance	: Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6), CAS No. 86290-81-5
Conclusion	: Hydrolysis unlikely
Reliability	: (1) Valid without restriction

(18)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	: Calculated
Media	: Soil, air, water, suspended sediment, sediment
Method	: Calculated according to Mackay Level I
Year	: 2000
Remark	: Model based on chemical fugacity. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Values represent the spread of calculated values for C5 to C8 hydrocarbon components found in gasoline. Detailed hydrocarbon analysis was used to identify the components of this specific gasoline sample. The majority of components in gasoline will partition rapidly to air, where these hydrocarbons will be rapidly

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- oxidized by OH radicals. With the exception of toluene, partitioning to air is > 97% for all components.
- Result** : **Medium** **% distribution**
 Air: 97 to 99.99
 Soil: 0.00 to 1.2
 Water: 0.003 to 2.7
 Sediment <0.001 to 0.02
 Suspended sediment <0.001 to 0.02
- Test substance Conclusion** : Gasoline CONCAWE sample, CWE5, Blend (match to API PS-6)
 : The constituents of this complex petroleum mixture are expected to partition primarily to air. Moderate partitioning to water and soil is predicted for the aromatic components of this mixture
- Reliability** : (2) Valid with restrictions

(13) (24)

3.5 BIODEGRADATION

- Type** : Aerobic
Inoculum : Activated aerobic sludge obtained from an urban wastewater treatment plant.
Deg. product Method : Non-guideline research method using a closed-system shake flask apparatus.
Year : 1999
GLP : No data
Test substance : CAS No. 86290-81-5; a commercial unleaded gasoline topped at 76°C by distillation

- Method** : Aerobic Biodegradability - Evaluation of biodegradability of gasoline in aqueous medium. Method by analysis of disappearance of carbon compounds (gas chromatography with flame ionization detector), kinetics of O₂ consumption (respirometry), and CO₂ production (gas chromatography with thermal conductivity detector).

- Exposure period was 16 or 25 days.
 See test conditions for more details.
- Result** : Biodegradation and Mineralization of Gasoline
 Gasoline was degraded up to 94% under non-limiting conditions after 25 d incubation (500 ml substrate/l medium). The carbon balance of gasoline degradation showed that 61.7% of gasoline was mineralized to CO₂ and that microbial cell production accounted for the remaining carbon of gasoline degraded. Biomass formation and mineralization occurred mainly during the initial fast degradation phase whereas essentially mineralization occurred during the second slow degradation phase. Individual classes of hydrocarbons degraded and carbon balance were shown to be:

Hydrocarbon Class	Amount in Gasoline (mg/g)	Amount in	
		After 2 days	After 25 days
Aromatics	789	88%	99%
Branched alkanes	165	14	74
Linear alkanes	23	17	92
Cyclic alkanes	17	10	99
Alkenes	6	71	99

Carbon balance

Substrate or Products	Initial amount (mg C/l)	Final amount (mg C/l)
Gasoline	357	18
Biomass	39	165
CO ₂	0	204
Total Carbon	396	387

Kinetic Experiments with Gasoline

Two main degradation phases were found, one fast degradation phase (FDP), which started after an 18 h lag period and lasted until the 40th hour. The maximum rate of oxygen consumption during the FDP was 44 mg/l/h and the average rate was 24.5 mg/l/h. The FDP was followed by a slow degradation phase (SDP) where the rate of oxygen consumption slowed steadily from the 40th hour till the 25th day. The average rate was 15 mg/l/d, which was approximately 40 times slower than during the FDP.

Test condition

: Activated sludge containing approximately 3 g/l dry weight was centrifuged at 15000 g for 20 min and re-suspending the biomass in the same volume of nutrient solution. The microbial suspension was used to inoculate nutrient solution at a final concentration of 100 mg dry weight/l. Gasoline (400 mg/l) or individual hydrocarbons (150 mg/l) were added to the medium as the sole carbon source. The nutrient solution was a vitamin-enriched mineral salt medium described by Bouchez et al. Appl. Microbiol. Biotechnol. 43:156-164 (1995).

Biodegradation of Gasoline

The biodegradation tests were performed in 500-ml flasks with sidearms equipped with Mininert® valves. 25 ml of gasoline were added to 50 ml of inoculated nutrient medium (i.e., 500 ml substrate/l medium) through the valve with a syringe. The flasks were incubated for 25 days at 30°C with alternate shaking (70 strokes per min). After the incubation period, 5 ml of CH₂Cl₂ containing 600 mg/ml dodecane as internal standard was introduced to the flasks through the valve, and the remaining hydrocarbon compounds were extracted for 1 h under shaking. The flasks were refrigerated overnight at 4°C before opening. The suspensions were centrifuged at 35000 g for 30 min at 4°C. The CH₂Cl₂ phase of each flask was then analyzed by gas chromatography for carbon compounds. Experiments were performed in duplicate and abiotic controls were prepared similarly to the other treatments with the exception that 1 g/l HgCl₂ were added to the flasks before incubation.

Mineralization of Gasoline

Measurements of CO₂ evolved during the biodegradation of gasoline were conducted in 240-ml flasks closed by Viton® stoppers. 18 ml of inoculated culture medium were added to each flask along with 5 ml of gasoline (i.e., 500 ml substrate/l medium). Flasks were incubated at 30°C for 25 days under alternate shaking. At the end of the incubation period the contents of each flask was acidified with 0.5 ml HNO₃ (68%) and CO₂ was measured by gas chromatography. Endogenous respiration of inoculated medium was measured in flasks without gasoline added.

Kinetic Experiments with Gasoline

Kinetics of O₂ consumption during gasoline biodegradation

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- were determined in duplicate at 30°C over 25 d by respirometry. 500 ml stirred culture flasks contained 250 ml of inoculated nutrient medium and 125 ml of gasoline (i.e., 500 ml substrate/l medium). Control experiments without gasoline were also done. Kinetics of hydrocarbon degradation also was monitored by respirometry. Incubation was stopped at selected times and the remaining hydrocarbons were extracted as described above and analyzed by gas chromatography.
- Kinetic Experiments with Individual Hydrocarbons
Kinetics of CO₂ production during the degradation of individual hydrocarbons was carried out at 30°C over 16 days. Treatments were prepared in 125 ml shaken flasks with 25 ml of nutrient solution containing 70 mg/l of inoculum biomass and 5 ml of hydrocarbon (i.e., 200 ml substrate/l medium). Flasks were closed with Teflon-coated stoppers and sealed. CO₂ was measured at various times by gas chromatography. Endogenous respiration was determined in flasks without hydrocarbon added.
- Test substance** : CAS No. 86290-81-5; a commercial unleaded gasoline topped at 76°C by distillation. It was free of hydrocarbons having less than six carbon atoms and contained no oxygenated compounds.
- Conclusion** : Activated sludge microorganisms were found to biodegrade unleaded commercial gasoline up to 94% within 25 days. For each hydrocarbon class, degradation occurred at different rates. Aromatic compounds were found to be the most readily consumed, although compounds bearing neighboring substituents and those containing longer alkyl groups were consumed at a slower rate than those with no or only one alkyl chain. Likewise, linear alkanes (exception for undecane), alkenes with five to nine carbons, cyclohexane and substituted cyclopentanes were biodegraded. Residual components of gasoline most recalcitrant to biodegradation were found to be branched alkanes, particularly those containing a quaternary carbon and/or alkyl chains on consecutive carbon atoms.
- Reliability** : (2) Valid with restrictions (30)
- Inoculum** : Native soil suspension (NSS) from a spruce forest and microbially-reinforced native soil suspension (MRSS).
- Method** : Non-guideline research method using a closed-system shake flask apparatus
- Year** : 1998
- Test substance** : CAS No. 86290-81-5; artificially-prepared produced gasoline-model mixture (GM23)
- Method** : Soil biodegradation - Evaluation of biodegradability of GM23 by soil microflora; mineralization of GM23; and mineralization of 23 principal components of GM23. Method of analysis of parent mixture, individual components, and CO₂ production made by gas chromatography with flame ionization detector.
- Exposure period: 14, 28 & 34 days
- Result** : See test condition for full description.
: The gasoline model mixture GM23 was degraded about 89% by a native soil suspension, based on GC/FID analysis of the initial and residual individual hydrocarbon concentrations.

Cyclohexane, 2,2,4-trimethylpentane and 2,3,4-trimethylpentane were only slightly biodegraded and 3-methylhexane, 2,4-dimethylhexane, and 1,3,5-trimethylbenzene were incompletely biodegraded. All other compounds were consumed by NSS. Supplementation of NSS with cyclohexane-degrading and 2,2,4-trimethylpentane-degrading microflora led to complete degradation of GM23.

Biodegradation and mineralization of GM23 at 28 days

Mineralization yields were:

MRSS inoculum: 0.58 (58%)

NSS inoculum: 0.45 (45%) .

Kinetic Experiments with GM23

Consumption of each component by NSS did not occur at the same rate. n-alkanes and most of the mono-aromatic compounds were completely biodegraded within the first 14 days, whereas cyclohexane and di- and trimethyl alkanes were not. Methyl alkanes and dimethyl hexanes were slightly consumed over this period of time, but they were nearly totally biodegraded after 28 days. Cyclohexane and trimethyl pentanes showed little biodegradation after 28 days.

Mineralization Experiments with Individual Hydrocarbons

The mineralization yields of individual components of GM23 were high for the aromatics and alkanes with no or few methyl groups. Furthermore, the mineralization yield of GM23 determined with NSS or MRSS agreed with the mineralization data for individual components. The ability of the NSS to mineralize individual hydrocarbons indicated:

Substrate	Mineralization Yield at 34 days
heptane	0.74
octane	0.49
cyclohexane	0
2-methylhexane	0.47
3-methylhexane	0.71
3-methylheptane	0.69
2,4-dimethylhexane	0.56
2,5-dimethylhexane	0.44
2,2,4-trimethylpentane	0.02
2,3,4-trimethylpentane	0
benzene	0.56
toluene	0.63
o-xylene	0.49
m-xylene	0.61
p-xylene	0.48
ethylbenzene	0.48
n-propylbenzene	0.34
2-ethyltoluene	0
3-ethyltoluene	0.38
4-ethyltoluene	0.34
1,2,3-trimethylbenzene	0
1,2,4-trimethylbenzene	0.18
1,3,5-trimethylbenzene	0

Test condition : The soil sample originated from a spruce forest (Foulain, France) and contained mineral and superficial organic matter. The NSS soil suspension was prepared with 20 g of homogenized soil per liter of nutrient solution. The MRSS soil suspension contained a cyclohexane-degrading microflora and a 2,2,4-trimethylpentane-degrading microflora obtained

from gasoline-polluted sites and added at 5% v/v each to the NSS. The nutrient solution contained minerals and vitamins prepared as described in Bouchez et al. Appl. Microbiol. Biotechnol. 43:156-164 (1995).

Biodegradation Experiments with GM23

The biodegradation tests were performed with NSS and MRSS suspensions (50 ml) in 500-ml flasks with sidearms equipped with Mininert[®] valves. 25 ml of GM23 were added to 50 ml of soil suspension through the Mininert[®] valve with a syringe (500 ml substrate/l medium). After an incubation period of 14 or 28 days at 30°C, 5 ml of CH₂Cl₂, containing dodecane at 600 mg/ml as internal standard, were introduced in the flasks through the valve, and the remaining hydrocarbons were extracted for 1 h with shaking. The flasks were refrigerated overnight at 4°C before opening. The suspension was centrifuged at 4°C and 35000 g for 30 min. The CH₂Cl₂ phase of each flask was then analyzed by gas chromatography. Mineralization yields were estimated at the end of the biodegradation experiment by recovery of CO₂ after acidification of the flask contents. Abiotic treatments were prepared similarly to the other treatments with the exception that 1 g/l HgCl₂ were added to the flasks before incubation.

Kinetic Experiments with GM23

Kinetics of CO₂ production during the degradation of GM23 were studied at 30°C for 28 days. 18 ml of NSS or MRSS were introduced into 240-ml flasks that were sealed by stoppers covered with Teflon film. 8 ml of GM23 were dispensed into the sealed flasks by syringe (444 ml substrate/l medium). A 250-ml gas-tight syringe was used to sample head space gas. Endogenous respiration of NSS and MRSS were determined under the same conditions in flasks incubated without test substance.

Mineralization Experiments with Individual Hydrocarbons

Mineralization yields of the individual hydrocarbon compounds were determined using 125-ml flasks containing 20 ml of soil suspension and 5 ml of each hydrocarbon (250 ml substrate/l medium). Flasks were incubated for 34 days. Two flasks for each compound and six control flasks were used.

Test substance : CAS No. 86290-81-5; artificially-prepared produced gasoline-model mixture (GM23) of main compounds making up a topped gasoline-cut obtained by distillation at 76°C of a French commercial unleaded gasoline. The model mixed gasoline was free of compounds lighter than C6 and contained no oxygenated compounds. Biodegradation also was measured on 23 individual components of the model gasoline.

Conclusion : The use of optimized degradative inoculum (soil microbes pre-exposed to cyclohexane and 2,2,4 TMP) in conjunction with non-acclimated soil organisms enhanced both rate and extent of the more structurally complex hydrocarbons which showed little to minimal degradation in non-acclimated soil systems.
The results of this study indicated that the pattern of gasoline degradation was represented as the sum of the degradation of the individual compounds. No marked occurrence of co-metabolism was observed. Inhibitory effects were observed for 1,3,5 trimethylbenzene, 2-ethyltoluene and 1,2,3 trimethylbenzene at 200 mg/l, but were totally degraded at 35 mg/l by non-acclimated soil suspensions.

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Reliability

- : (2) Valid with restrictions
- (2) Reliable with restrictions: acceptable, well-documented publication which meets basic scientific principles.

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4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : Static
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 : 1995
GLP : Yes
Test substance : Gasoline CAS No. 86290-81-5

Method : LL₅₀ at 96 hr calculated using binomial probability analysis. ASTM Special Technical Publication 634. 1977, pp 65-84
Result : Mortality (no. of deaths/treatment) at 96 hrs:

Treatment (mg/l)	No. of deaths
0	0
0.1	0
1.0	0
5.0	0
10	0
25	15

96-hr LL₅₀ = 16 mg/l, 99% C.I: 10-25 mg/l (as nominal loading rate)
 96-hour No Observed Effect Loading (NOEL) was 10 mg/l, based on mortality, both calculated (Dunnett's Procedure) and observed. Results are quoted in terms of 50% Lethal Loading (LL₅₀), the loading rate of test substance resulting in 50% mortality of the test species exposed to the WAF. At termination, loss of equilibrium was observed in all surviving fish at the 10 mg/l treatment.

Analytical results

Losses of the soluble BTEXN components from the WAF over each 24 hour period ranged from 0 to 8% for the 1.0, 5, 10 and 25 mg/l loadings. Up to 100% loss was observed in the 0.1 mg/l treatment in 24 hrs samples.

Analytical results		Measured BTEXN (mg/l)				
rate (mg/l)		Nominal loading				
Day	Control	0.1	1.	5.0	10	25
0 (new)	ND	0.12	0.31	1.7	3.1	7.7
1 (old)	ND	0.12	0.41	1.6	3.3	7.1
1 (new)	ND	0.16	0.44	1.7	1.9	6.5
2 (old)	ND	0.15	0.45	1.6	2.1	6.8
2 (new)	ND	0.07	0.43	1.6	3.2	NA
3 (old)	ND	0.12	0.43	1.6	3.1	NA
3 (new)	ND	0.16	0.57	1.8	3.3	NA
4 (old)	ND	ND	0.56	1.8	3.5	NA

ND=not detected, NA=not analyzed due to 100% mortality

Guideline/protocol deviations: Body length smaller than recommended range of 4-6 cm; smaller fish used to minimize

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- Test condition** : DO depletion in closed vessel (no-headspace) systems.
: Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.1, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the fish toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 20 liter stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish were approximately five weeks old at test initiation and were obtained from Thomas Fish company, Anderson, CA, Lot 297. Loading of fish body mass to treatment was 0.3 g fish per liter of aqueous solution, mean length at termination was 3.3 cm (sd=0.2), and mean weight was 0.271 g (sd=0.064). Test vessels were 4 liter glass aspirator bottles with foil covered neoprene stoppers. Three replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEXN. Water temperature was 15 °C (0.1sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 609-614 Lux during full daylight periods. Dissolved oxygen measurements ranged from 5.4 to 9.7 ppm, pH values between 6.8 and 8.2.
- Test substance** : Gasoline Sample W94/814, Blend; Detailed hydrocarbon analysis:
N-paraffins: 16% total C4-C8
Iso-paraffins: 25% total C4-C11
Olefins: 12%, C4-C7
Naphthenes: 5% C6-C10
Aromatics: 42% C6-C11
- Reliability** : (1) Valid without restriction
- Type** : Static
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 : 1995
GLP : Yes
Test substance : Gasoline CAS No. 86290-81-5
- Method** : LL₅₀ at 96 hr calculated using Probit procedure (Finney,

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D.J., 1971. Probit Analysis, Third Edition, London: Cambridge University Press, and SAS computer statistics software.

Result : Mortality (no. of deaths/treatment) at 96 hrs:

<u>Treatment</u>	<u>No. of deaths</u>
0	1
1.0	0
5	0
10	7
25	15
50	5

96-hr LL₅₀ = 11 mg/l, 95% C.I: 8.7-16 mg/l (as nominal loading rate)
96-hour No Observed Effect Loading (NOEL) was 5 mg/l, both calculated (Dunnett's Procedure) and observed.
Results are quoted in terms of 50% Lethal Loading (LL₅₀), the loading rate of test substance resulting in 50% mortality of the test species exposed to the WAF. At termination, abnormal behavior/appearance (lethargy, erratic swimming) was observed in all surviving fish at the 10 mg/l treatment. Losses of the soluble components from the WAF over each 24 hour period ranged from 5 to 25% for the 5, 10 and 25 mg/l loadings. Up to 57% loss was observed in the 1.0 mg/l treatment in 24 hrs samples. BTEXN concentrations on 24hour samples of the 50 mg/l treatments due to complete mortality on day 0 were not determined.

Analytical results Measured BTEXN (mg/l)
Nominal loading

rate (mg/l)		1.0	5.0	10	25	50
Day Control						
0 (new) ND		0.54	2.3	4.2	9.5	20
1 (old) ND		0.50	2.3	4.0	10	NA
1 (new) ND		0.47	1.7	4.2	NA	NA
2 (old) ND		0.20	2.1	4.0	NA	NA
2 (new) ND		0.52	2.0	4.1	NA	NA
3 (old) ND		0.25	2.0	4.3	NA	NA
3 (new) ND		0.57	1.6	4.0	NA	NA
4 (old) ND		0.38	1.2	3.2	NA	NA

ND=not detected, NA=not analyzed due to 100% mortality

Guideline/protocol deviations: Body length (2.7cm av.) smaller than recommended range of 4-6 cm; smaller fish used to minimize DO depletion in closed vessel (no-headspace) systems.

Test condition : Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 1, 5, 10, 25 and 50 mg/l were used to prepare test solutions for the fish toxicity tests. Test substance, added volumetrically, was mixed for each

individual treatment in dilution water for 24 hours in 20liter stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. Fish were approximately four weeks old at test initiation and were obtained from Thomas Fish company, Anderson, CA, Lot 297. Loading of fish body mass to treatment was 0.2 g fish per liter of aqueous solution, mean length at termination was 2.7 cm (sd=0.2), and mean weight was 0.136 g (sd=0.034). Test vessels were 4 liter glass aspirator bottles with foil covered neoprene stoppers. Three replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Freshly prepared and old WAF test solutions were analyzed by GC-FID for concentrations of BTEXN. Water temperature was 14.1 °C (0.03sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 619-622 Lux during full daylight periods. Dissolved oxygen measurements ranged from 7.4 to 9.8 ppm, pH values between 7.8 and 8.1.

Test substance : Gasoline Sample W94/813, Blend
Detailed hydrocarbon analysis:
N-paraffins: 20% total C3-C8,
Iso-paraffins: 28% total C4-C9
Olefins: 1%, C5-C7
Naphthenes: 5% C5-C10
Aromatics: 46% C6-C9

Reliability : (1) Valid without restriction

(12)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Acute immobilization test
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1995
GLP : Yes
Test substance : Gasoline CAS No. 86290-81-5

Method : EL₅₀ calculated using the probit procedure (Finney, D.J., 1971. Probit Analysis, 3rd Ed. London: Cambridge Univ. Press)

Result : 48 hr results-number of organisms affected and analytical results

Treatment	Measured		Measured
	Immobilization	BTEXN	BTEXN
		-day 0	-day 2
Control	2	ND	ND
0.1 mg/l	1	0.12	0.20
1.0 mg/l	1	0.31	0.42
5.0 mg/l	1	1.7	1.4
10 mg/l	5	3.1	3.2

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	25 mg/l	20	7.7	7.1
	based upon nominal loading rate 48-hr EL ₅₀ = 12 mg/l (95% C.I. 7.3 to 22 mg/l)			
Test condition	: Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.1, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 4 liter stoppered containers with less than 10% headspace volume. The WAF mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN on day 0 and at termination. Test vessels for daphnid testing were 125 ml glass erlenmeyer flasks with foil covered neoprene stoppers. Four replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. During the study test system solutions: dissolved oxygen concentration range: 7.2 to 9.2; pH ranged from 7.5 to 7.8; temperature was 19 °C (sd:0.2).Daphnia magna were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house.			
Test substance	: Gasoline Sample W94/814, Blend Detailed hydrocarbon analysis: N-paraffins: 16% total C4-C8 Iso-paraffins: 25% total C4-C11 Olefins: 12% C4-C7 Naphthenes: 5% C6-C10 Aromatics: 42% C6-C11			
Reliability	: (2) Valid with restrictions. Three previous attempts to conduct study were invalidated due to excessive (>20%) control mortality			
	(11)			
Type	: Acute immobilization test			
Species	: Daphnia magna (Crustacea)			
Exposure period	: 48 hour(s)			
Unit	: mg/l			
Analytical monitoring	: Yes			
Method	: OECD Guide-line 202			
Year	: 1995			
GLP	: Yes			
Test substance	: Gasoline CAS No. 86290-81-5			
Method	: EL ₅₀ calculated using the probit procedure (Finney, D.J., 1971. Probit Analysis, 3rd Ed. London: Cambridge Univ. Press)			

Result : 48 hr results-number of organisms affected and analytical results

Treatment	Immobilization	Measured	Measured
		BTEXN-day 0	BTEXN-day 2
Control	0	ND	ND
0.5 mg/l	0	0.29	0.10
1.0 mg/l	0	0.28	0.10
5.0 mg/l	3	2.3	1.7
10 mg/l	16	3.9	3.1
25 mg/l	20	8.8	10

Test condition : based upon nominal loading rate 48-hr EL_{50} = 7.6 mg/l (95% C.I. 6.4 to 9.3 mg/l) 48-hr NOEL = 1.0 mg/l

: Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the toxicity tests. Test substance, added volumetrically, was mixed for each individual treatment in dilution water for 24 hours in 4 liter stoppered containers with less than 10% headspace volume. The WAF mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN on day 0 and at termination. Test vessels for daphnid testing were 125 ml glass erlenmeyer flasks with foil covered glass stoppers. Four replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Exposure containers were filled (no headspace volume) and tightly sealed to prevent volatilization. During the study test system solutions: dissolved oxygen concentration range: 7.2 to 9.2; pH ranged from 7.5 to 7.8; temperature was 20 °C. Daphnia magna were supplied by testing laboratory; age < 24 hours old; obtained from culture maintained in-house.

Test substance : Gasoline Sample W94/813, Blend

Detailed hydrocarbon analysis:
 N-paraffins: 20% total C3-C8
 Iso-paraffins: 28% total C4-C9
 Olefins: 1% C5-C7
 Naphthenes: 5% C5-C10
 Aromatics: 46% C6-C9

Reliability : (2) Valid with restrictions. Three previous attempts to conduct study were invalidated due to excessive (>20%) control mortality.

(10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : Growth rate
Exposure period : 96 hour(s)

these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the algal toxicity tests. Test material was added volumetrically to 2.0 liters of sterilized algal nutrient media (enriched with 100 mg/l of sodium bicarbonate) in 2.0 liter aspirator bottles covered with aluminum foil. The mixing vessels were sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks containing ten 4mm glass balls that were completely filled (140 ml) with treatment solution, inoculated with algae and sealed with glass stoppers. Algal cells were obtained from 6 day old laboratory stock cultures maintained in nutrient enriched media, at 24 °C (±2°) C under continuous illumination of 4300(±10%) lux. Original algal cultures (Strain 1648) were provided by the Department of Botany, University of Texas. Cell density of the algal stock culture inoculum was determined prior to study initiation with a Turner filter-fluorometer. Fluorometer readings were converted to cell numbers using a regression formula developed through cell counts. Three replicates were prepared for each treatment level and six replicates were prepared as control systems. The initial algal concentration was approximately 1.0×10^3 cells/ml in each replicate chamber. All test replicates were placed on a shaker table at 150 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 4300 to 4400 Lux as measured daily using a Licor photometric sensor. A sample volume of 3.5 ml was taken daily for density determinations, and an equivalent volume of reserve 24 hour WAF was used to replenish the displaced sample volume. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN at day 0 and 96 hr termination. BTEXN total concentration at termination was at least 80% of the initial concentration for all treatments, with the exception of the 5.0 mg/l exposure, which showed a loss of 73%. This excessive loss compared to the other treatments was determined to be due to sampling technique. Test temperature was 23 °C (sd=0.08)C. The average pH was 7.5 at initiation; and ranged from 9.2 (control) to 7.8 (25 mg/l loading) at termination.

Test substance : Gasoline Sample W94/813, Blend
Detailed hydrocarbon analysis:
N-paraffins: 20% total C3-C8
Iso-paraffins: 28% total C4-C9
Olefins: 1% C5-C7
Naphthenes: 5% C5-C10
Aromatics: 46% C6-C9

Reliability : (1) Valid without restriction

Species : *Selenastrum capricornutum* (Algae)
Endpoint : Growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995

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- GLP** : Yes
Test substance : Gasoline CAS No. 86290-81-5
- Method** : EL₅₀ values were calculated using the inverse extrapolation method of Snedecor and Cochran, Statistical Methods, 8th Ed., 1989, Iowa State University Press/Ames. NOEL values calculated using ANOVA (Duncan D.B., 1975, Biometrics, 31, 339-359).
- Result** : Percent inhibition:
 72 hour EL₅₀ for average growth rate=3.3 mg/l
 (0.24 to >25 mg/l CI @95%)
 72 hour EL₅₀ for area under the growth curve=4.2 mg/l
 (0 to 24 mg/l CI @95%)
 96 hour EL₅₀ for average growth rate=2.5 mg/l
 (0.62 to 14 mg/l CI @95%)
 96 hour EL₅₀ for area under the growth curve=0.25 mg/l
 (0 to 26 mg/l CI @95%)
 72 hour NOEL for average growth rate and area under the growth curve =0.5 mg/l
 96 hour NOEL for average growth rate = 0.5 mg/l
 96 hour NOEL for area under the growth curve =0.5 mg/l

Nominal (mg/l)	% Inhibition					
	Avg cell density (cells/ml)		Avg growth rate		area under growth curve	
	72hr	96hr	72hr	96 hr	72hr	96 hr
Control	9.9E ⁴	3.8 E ⁵	0	0	0	0
0.5	7.7E ⁴	2.6 E ⁵	7.7	7.8	21	27
1.0	5.5E ⁴	1.7 E ⁵	15	17	36	50
5.0	2.5E ⁴	2.2 E ⁴	33	51	54	81
10	3.7E ³	2.0 E ³	76	95	90	97
25	BMDL	BMDL	99	100	98	99

BMDL=below method detection limit

Analytical results

Nominal (mg/l) Measured Concentration (mg/l as BTEXN)

	Day 0	Day 4
Control	none detected	none detected
0.5	0.22	0.23
1.0	0.47	0.51
5.0	1.5	1.3
10	3.5	3.3
25	9.5	7.7

- Test condition** : Individual test treatment solutions were prepared as Water Accommodated Fractions (WAFs). To determine contact time required to achieve maximum solubility between test substance and aqueous solution, samples of WAF test solutions equilibrated for 20, 24, 27 and 49 hours at 100 mg/l loading were analyzed by GC-FID for concentrations of the following: benzene, toluene, ethylbenzene, xylene isomers and naphthalene (BTEXN). Maximum WAF solubility for these components reached equilibrium within 24 hrs of stirring. Nominal loading rates of 0, 0.5, 1, 5, 10, and 25 mg/l were used to prepare test solutions for the algal toxicity tests. Test material was added volumetrically to 2.0 liters of sterilized algal nutrient media (enriched with 100 mg/l of sodium bicarbonate) in 2.0 liter aspirator bottles covered with aluminum foil. The mixing vessels were

sealed with foil covered stoppers and mixed on magnetic stir plates with teflon coated stir bars for approximately 24 hours at room temperature. After mixing the solutions were allowed to settle for one hour and the WAF was removed and used for testing. Test vessels were 125ml glass Erlenmeyer flasks containing ten 4mm glass balls that were completely filled (140 ml) with treatment solution, inoculated with algae and sealed with glass stoppers. Algal cells were obtained from 5 day old laboratory stock cultures maintained in nutrient enriched media, at 24 °C (±2°) under continuous illumination of 4300(±10%) lux. Original algal cultures (Strain 1648) were provided by the Department of Botany, University of Texas. Cell density of the algal stock culture inoculum was determined prior to study initiation with a Turner filter-fluorometer. Fluorometer readings were converted to cell numbers using a regression formula developed through cell counts. Three replicates were prepared for each treatment level and six replicates were prepared as control systems. The initial algal concentration was approximately 1.0×10^3 cells/ml in each replicate chamber. All test replicates were placed on a shaker table at 150 oscillations per minute during the study and exposed to continuous fluorescent light, illumination at 4300 to 4400 Lux as measured daily using a Licor photometric sensor. A sample volume of 3.5 ml was taken daily for density determinations, and an equivalent volume of reserve 24 hour WAF was used to replenish the displaced sample volume. WAF test solutions were analyzed by GC-FID for concentrations of BTEXN at day 0 and 96 hr termination. BTEXN total concentration at termination was at least 80% of the initial concentration. Test temperature was 23 °C (sd=0.08). The average pH was 7.6 at initiation; and ranged from 9.5 (control) to 8.1 (25 mg/l loading) at termination.

Test substance : Gasoline Sample W94/814, Blend
Detailed hydrocarbon analysis:
N-paraffins: 16% total C4-C8
Iso-paraffins: 25% total C4-C11
Olefins: 12% C4-C7
Naphthenes: 5% C6-C10
Aromatics: 42% C6-C11

Reliability : (1) Valid without restriction

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4.9 ADDITIONAL REMARKS

Memo : Ecotoxicity

Remark : Experimental studies with fish, invertebrates and algae tested in closed systems with minimal head-space were performed on WAFS of low boiling point naphthas prepared at maximum loadings of 50 mg/l or less. Results show that acute aquatic toxicity lethal loading (LL), effective loading (EL) or inhibition of growth rate values (IrL) affecting 50% of the organism population are greater than 1 mg/l and mostly in the range 1-100 mg/l. Summarized CONCAWE test data indicating the extent of aquatic toxicity are as follows, and 95% confidence intervals are included in parentheses:

PARAFFINIC NAPHTHA CAS 64741-70-4, CONCAWE sample W94/810
Fish (*Oncorhynchus mykiss*) LL₅₀, 96h 10 mg/l (5-23)
Invertebrate (*Daphnia magna*) EL₅₀, 48h 10 mg/l (8.5-13)
Algae (*Selenastum capricornutum*) IrL₅₀, 72h >50 mg/l (not calculable)

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OLEFINIC NAPHTHA CAS . 64741-54-4, CONCAWEe sample W94/811
Fish (Oncorhynchus mykiss) LL₅₀, 96h 15 mg/l (10-23)
Invertebrate (Daphnia magna) EL₅₀, 48h 13 mg/l (12-15)
Algae (Selenastum capricornutum) IrL₅₀, 72h 3.1 mg/l (3.6-14)

NAPHTHENIC NAPHTHA, CAS 64741-46-4, CONCAWE sample W94/809
Fish (Oncorhynchus mykiss) LL₅₀, 96h 18 mg/l (15-20)
Invertebrate (Daphnia magna) EL₅₀, 48h 4.5 mg/l (not calculable)
Algae (Selenastum capricornutum) IrL₅₀, 72h 4.1 mg/l (not calculable)

AROMATIC NAPHTHA, CAS 64741-63-5, CONCAWE sample W94/812
Fish (Oncorhynchus mykiss) LL₅₀, 96h 12 mg/l (9-16)
Invertebrate (Daphnia magna) EL₅₀, 48h 8.4 mg/l (6.7-11)
Algae (Selenastum capricornutum) IrL₅₀, 72h 6.4 mg/l (1-280)

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5.1.1 ACUTE ORAL TOXICITY

Type : LD₅₀
Value : = 18.75 ml/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 10
Vehicle : Undiluted
Year : 1980
GLP : Yes
Test substance : API PS-6

Method : Groups of 10 fasted rats (five male and five female) were given API PS-6 at doses of 10, 15, 17.5, 20 and 25 ml/kg as a single oral dose. The animals were then allowed food and water ad libitum and were observed hourly for clinical signs for the first 6 hours after dosing. Observation was twice daily thereafter for 14 days. Body weights were recorded at 7 and 14 days after administration of test material. At the end of the study, the animals were killed and subjected to a gross necropsy and any abnormalities were recorded. [In addition 2 extra males and one female were given 15 ml/kg because 3 of the original animals died soon after dosing and this was believed to be due to dosing injury. However, at necropsy, no evidence of injury was found and therefore all animals were included in the calculations for an LD₅₀]

Result : Toxic signs were the same in all dose groups, increasing in severity with increasing dose. There were oily urine stains, but most of the test material seemed to be excreted via the feces. The area around the anus became very irritated. Diarrhea was common in each dose level and blood was commonly seen around the eyes, nose and mouth. Observations at necropsy were similar for all dose groups. Animals surviving to 14 days had very few abnormalities and these were usually of a minor nature such as enlarged Peyer's patches on the intestines. There were numerous instances of lung involvement in both surviving animals and those dying before 14 days. These changes consisted of mild irritation and congestion, to fluid filled abscesses. Almost all animals that died before 14 days had intestinal damage. The intestines, and often the stomach, became hemorrhagic and sometimes blood was observed in the intestine or stomach. The intestine wall became thin and there was an increased amount of gas in the gastro intestinal tract. The heart was enlarged or irregularly shaped in some rats.

Mortality and body weight changes are summarized in the following table.

Dose group (ml/kg)	Mortality dying/dosed	Weight change (over 14days)
<u>Males</u>		
10	0/5	weight gain
15	2/7	weight loss
17.5	3/5	weight loss
20	4/5	weight loss
25	5/5	weight loss

Females

10	0/5	weight gain
15	1/6	weight gain
17.5	3/5	weight loss
20	0/5	weight gain*
25	4/5	weight loss

* one animal had a weight loss over the 14 day period.

The oral LD₅₀ was determined to be 18.75 ml/kg. The 95% confidence limits were 16.3 to 21.6 ml/kg.

Reliability : (1) Valid without restriction

(5)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD₅₀
Value : > 5 ml/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 4
Vehicle : Undiluted
Year : 1979
GLP : Yes
Test substance : API PS-6

Method : The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. A single dose of undiluted test material (5 ml/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. 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 and the animals were observed for a total of 14 days post-dosing. At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.

Result : When the patches were removed following dosing dark red to almost purple skin was seen in all animals. Slight erythema and dry skin was observed in all rabbits during the study. With the exception of one animal all animals weighed more at the end of the study than they did at study commencement. One female rabbit died on day 6 of the 14 day study and the gross necropsy revealed slightly congested lungs, no food in the stomach and white areas in the liver. At necropsy of the surviving animals four rabbits had congested lungs, one had pale kidneys, one had an irritated stomach lining and one had enlarged Peyer's patches on the jejunum. These observations were considered to be normal and not dose-related.

Reliability : (1) Valid without restriction

(5)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Remark : Not appropriate

5.2.1 SKIN IRRITATION

Species : Rabbit
 Concentration : Undiluted
 Exposure : Occlusive
 Exposure time : 24 hour(s)
 Number of animals : 6
 PDII : .98
 Result : Slightly irritating
 Method : Draize Test
 Year : 1979
 GLP : Yes
 Test substance : API PS-6

Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each of 3 male and 3 female 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 and again at 96 hours, 7 and 14 days. Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : A summary of the dermal irritation scores is given below.

	Exposure time (hours)	Average value of all animals
<u>Erythema</u>		
Intact skin	24	0
	72	0.92
Abraded skin	24	0
	72	1.0
<u>Edema</u>		
Intact skin	24	0.5
	72	0.5
Abraded skin	24	0.5
	72	0.5
		3.92
Primary irritation score =Total/4=		0.98

Edema but no erythema was noted at 24 hours, although the test area was whiter than the surrounding skin.

At 72 hours erythema and edema were observed.

By 7 days almost all erythema had cleared but some edema was still present and the test site was dry and flaky.

By day 14 all edema and erythema had cleared but there was no hair growth at this time.

Reliability : (1) Valid without restriction

(5)

5.2.2 EYE IRRITATION

Species	: Rabbit
Concentration	: Undiluted
Dose	: 0.1 ml
Comment	: Rinsed after (see exposure time)
Number of animals	: 9
Vehicle	: None
Result	: Not irritating
Method	: Draize Test
Year	: 1979
GLP	: Yes
Test substance	: API PS-6
Method	: 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits (4 male, 5 female), the other eye was untreated and served as control. After 20 to 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	: No irritation was observed in any animal at any of the three observation times. Animals whose eyes had been irrigated following instillation of test material were no different from those whose eyes had not been washed.
Reliability	: (1) Valid without restriction

(5)

5.3 SENSITIZATION

Type	: Buehler Test
Species	: Guinea pig
Concentration	: 1 st : Induction 50 % occlusive epicutaneous 2 nd : Challenge 50 % occlusive epicutaneous
Number of animals	: 10
Vehicle	: Mineral oil
Result	: Not sensitizing
Year	: 1979
GLP	: Yes
Test substance	: API PS-6
Method	: 0.5 ml of undiluted test material was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application the dressings were removed and the skin wiped to remove residues of test material. After the first application, irritation was sufficiently severe that for further dosing a 50% dilution in mineral oil was used. The animals received one application 3 times each week for 3 weeks. The same application site was used each time. 2 weeks following the third application a challenge dose (0.5 ml of a 50% dilution in mineral oil) 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.

Result : Positive control (0.05% 2,4-dinitrochlorobenzene in ethanol), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups.
: On a subjective basis, the challenge treatment did not appear to be more reactive than the sensitizing treatments. The average scores for erythema and edema following induction and challenge are summarized below.

Average scores	PS-6 gasoline		Positive control	
	Erythema	Edema	Erythema	Edema
Induction	0.9	0.3	1.3	0.3
Challenge	0.1	0	1.9	1.7

Reliability : The authors concluded that the test material was not sensitizing.
: (2) Valid with restrictions. Although the study was conducted to GLP, there was no vehicle control and the results from the positive control were not convincing

(5)

5.4 REPEATED DOSE TOXICITY

Species : Rats and monkeys
Sex : Male/female
Strain : Sprague Dawley rats and squirrel monkeys
Route of admin. : Inhalation
Exposure period : 6 hours each day
Frequency of treatm. : 5 days a week for 13 weeks
Doses : Leaded gasoline: 0, 100 & 400 ppm. Unleaded gasoline: 0, 400 & 1500 ppm
Control group : Yes, concurrent no treatment
Year : 1984
GLP : No data
Test substance : Leaded and unleaded gasoline

Method : This study was conducted as a preliminary range finding study prior to conducting a two year study on the same test materials.

20 rats and 4 monkeys of each sex were housed in 1m³ glass and stainless steel exposure chambers 24 hours a day and were only removed for cleaning purposes. Target exposure vapor concentrations of the test materials were:

Unleaded gasoline: 400 and 1500 ppm

Leaded gasoline: 100 and 400 ppm

A control group of 20 rats and 4 monkeys of each sex were exposed to air only.

Exposures were for 6 hours each day, 5 days each week for 13 weeks.

Blood was taken from 10 rats of each sex at the end of the study from the highest dose groups only for hematological

evaluation. Blood was taken from all monkeys in the highest dose group at 1.5, and 3 months.

Urine samples were analyzed for all animals at 1.5 and 3 months for levels of protein, glucose, ketones, bilirubin, blood and lead.

CNS evaluations were conducted on the monkeys in the control and high level dose groups at before exposure and at 3 months. The CNS evaluations consisted of recording simultaneous and evoked responses and this was accomplished using electrodes that had been implanted permanently in the visual cortex.

Pulmonary function tests similar to those reported by Alarie were conducted on all monkeys prior to exposure and at 1.5 and 3 months on the control and high level unleaded groups. All animals that died or were sacrificed at termination of the study were subjected to a gross necropsy. Organ weights were recorded and lungs, kidneys, spleen, heart, brain and bone marrow from the control and high dose groups were evaluated for histopathology.

All male and female animals from the control and high exposure groups were also evaluated for the presence of IgG in the renal glomerulus and lungs.

A lead analysis was also made on rat brain, kidney, liver, urine and blood from both the leaded dose groups and controls.

Result : The mean exposure concentrations were found to be as follows:

Group	Gasoline vapor exposure concentration		Alkyl lead µg Pb/l ±SD
	Mg/l ±SD	ppm	
Control	-	-	-
Unleaded 1500 ppm	6.35±0.44	1552	-
Unleaded 400 ppm	1.57±0.15	384	-
Leaded 400 ppm	1.53±0.23	374	0.72±0.1
Leaded 100 ppm	0.42±0.04	103	0.19±0.04

Three rats at different dose levels and three monkeys also at different dose levels died during the study. These deaths were not considered to be treatment-related.

Two female monkeys in each of the high dose groups exhibited emesis, 13 and 17 days after commencing exposure for the 1500 ppm unleaded and 400 ppm leaded groups respectively. Although there was a reduction in body weights in males in the lowest dose group of each of the test materials but by the end of the study they were demonstrating increased weights. No differences were observed in any of the other treated groups.

The hematological values for the monkeys exposed to either test material at either dose level were similar to those for the control animals. In the rats the only changes observed were:

unleaded (1500 ppm males) 64% increase in thrombocytes
unleaded (1500 ppm females) 150% increase in reticulocytes

leaded (400 ppm males) 4% decrease in MCHC
leaded (400 ppm females) 10% increase in hematocrit
leaded (400 ppm females) 11% increase in MCV

leaded (400 ppm females) decrease in WBC

Mean flash-evoked response time for the monkeys was measured prior to exposure and was unaffected by exposure.

The results of the mean pulmonary function data are summarised in the following table. Only increases (+%) or decreases (-%) compared to controls are shown in the table. All other parameters were similar for treated and control animals.

	Pre-exposure	42 days	90 days
Respiratory rate			
Unleaded 1500 ppm F	-	-	-
Unleaded 1500 ppm M	-30%	-	-
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	-
Tidal volume			
Unleaded 1500 ppm F	-	-	-22%
Unleaded 1500 ppm M	-	-	-
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	-
Minute volume			
Unleaded 1500 ppm F	-	-	-
Unleaded 1500 ppm M	-	-	+36%
Leaded 400 ppm F	-	-	-
Leaded 400 ppm M	-	-	+53%

There were no effects on airway resistance, dynamic compliance or breaths to 1% nitrogen.

Urinalysis showed no differences between treated and control animals in either species.

There was no evidence of IgG deposition in the kidneys of rats or monkeys of either sex following exposure to the test materials for 90 days.

Group mean lead levels in the rat tissues were as follows:

		Control	Leaded 400 ppm	Unleaded 100 ppm
Brain	M	1.26	9.49	7.23
	F	1.44	5.39	2.32
Kidney	M	1.71	12.4	7.06
	F	2.97	9.57	13
Liver	M	0.71	17.9	6.51
	F	1.21	19.7	8.41
Blood	M	0.61	6.1	0.77
	F	0.24	1.32	0.46
Urine	M	0.17	0.21	0.19
	F	0.31	0.18	0.25

No actual values are given on organ weights or organ/body weight ratios but the following effects are reported:

Rats

	Liver wt	Kidney wt
Unleaded 400 ppm M	increased	
Unleaded 400 ppm F		
Unleaded 1500 ppm M		
Unleaded 1500 ppm F		

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Leaded 400 ppm M
 Leaded 400 ppm F decreased
 Leaded 100 ppm M increased
 Leaded 100 ppm F increased

Monkeys

	Thyroid	Kidney
Unleaded 400 ppm M	increased	
Unleaded 400 ppm F		
Unleaded 1500 ppm M	increased	
Unleaded 1500 ppm F		
Leaded 400 ppm M		decreased
Leaded 400 ppm F		
Leaded 100 ppm M		
Leaded 100 ppm F		

Organ weights were also expressed as % of body weight and the following effects were recorded:

Rats:
 Decreased heart weight in both male leaded groups
 Decreased brain weight in both male unleaded groups
 Decreased liver weight in 400 ppm female leaded group
 Decreased adrenal weight in 1500 ppm female unleaded group.
 Monkeys:
 Decreased kidney weight in 400 ppm male unleaded group.

No evidence of treatment-related histopathology was observed in either rats or monkeys, with the exception of lesions noted in the kidneys of all male rats. The lesions were characterized by subtle but discernible increases in the incidence and severity of regenerative epithelium and dilated tubules. The latter were seen to contain protein in their lumens.

Test condition

: The gasoline samples were piped to an atomizer to which nitrogen heated to 105 °C was also fed at a pressure of 10 psig. The atomized gasoline was then carried to the exposure chamber with air. Exposure chamber atmospheres were analyzed for gasoline vapor concentration twice daily. The mean exposure concentrations for the two gasoline samples were as follows:

Target concentration	Gasoline vapor exposure concentration		Alkyl lead µg Pb/l ±SD
	mg/liter ±SD	ppm	
0 ppm (control)			
Unleaded gasoline			
1500 ppm	6.35 ± 0.44	1552	-
400 ppm	1.57 ± 0.15	384	-
Leaded gasoline			
400 ppm	1.53 ± 0.23	374	0.72 ± 0.10
100 ppm	0.42 ± 0.04	103	0.19 ± 0.04

Test substance

: An unleaded EPA reference fuel and a commercially available leaded gasoline were tested. The compositional properties of the two fuels were as follows:

	<u>Unleaded fuel</u>	<u>Leaded fuel</u>
Calculated data		
Research octane No.	93	87
Motor octane No.	88	86
Reid vapor pressure (PSIA)	6.9	6.3
Distillation °F (ASTM D-86)		
Initial boiling point	80	80
10%	135	160
50%	210	217
90%	275	295
100%	345	340
FIA analysis		
% aromatics	30.1	27.4
% olefins	8.2	7.8
% saturates	61.7	64.8
Experimental data		
API gravity at 60°F	57.0	58.4
Sulfur, ppm	240	75
Lead, g/gallon	<0.005	1.94
Benzene, LV%	0.2	0.4
Toluene, LV%	16.7	11.4
n-Butane, LV%	1.0	0.4
Isopentane, LV%	5.4	5.5
n-Pentane, LV%	4.8	4.0
Reliability	: (2) Valid with restrictions	

(21)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	: Mouse lymphoma assay
System of testing	: Forward mutation assay using cell line L5178Y TK+/-
Metabolic activation	: With and without
Result	: Negative
Year	: 1977
GLP	: No data
Test substance	: Unleaded gasoline

Method : The test material was dissolved in acetone for this assay. The positive control substances were Ethyl methane sulphonate (EMS) and Dimethylnitrosamine (DMN).

A cytotoxicity study was carried out prior to the mutagenicity assay.

For the mutation assay the lymphoma cells were exposed for 5 hours to test material at concentrations ranging from 0.065 to 1.04 µl/ml for both the activation and non-activation assays. Metabolic activation was accomplished using Araclor-induced rat liver S-9 suspension. After exposure to the test material, the cells were allowed to recover for 3 days and then cultures were selected for cloning and mutant selection. Surviving cell populations were determined by plating diluted aliquots in non-selective growth medium.

A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and

from positive and negative controls.

A compound is considered mutagenic if:

A dose response relationship is observed over 3 of the 4 dose levels employed.

The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value.

The solvent control data are within the normal range of the spontaneous background for the TK locus.

Result : Little toxicity was observed with the test material. Positive control values exhibited significant responses over the negative controls, and the negative controls were within the normal range. All results for the test material from the non-activation assay were negative. The results from the activation assay were also considered to be negative. There was an increase in the number of mutants at the 0.52 µl/ml concentration but this appeared to result from a slight increase in the number of viable clones. There was no trend indicating a dose-related response and, therefore, the increases were not believed to be compound related. The results are summarized below.

Dose (µl/ml)	Rel. susp.	Mutant clones	Viable clones	% Rel. growth	Mutant frequency
<u>Non-activation</u>					
0.065	121.8	76	159	139.3	0.478
0.13	103.7	29	215	160.4	0.1349
0.26	114.6	44	211	174	0.2085
0.52	141.8	66	161	164.3	0.4099
1.04	107.5	58	270	208.9	0.2148
Solvent	100	14	139	100	0.1007
Negative	129.9	41	140	130.8	0.2929
EMS	58.7	227	67	28.3	3.3881

<u>Activation</u>					
0.065	120.6	66	87	79.5	0.7586
0.13	108.6	46	126	103.7	0.3651
0.26	106	70	130	104.4	0.5385
0.52	112.4	92	108	92	0.8519
1.04	68.9	21	193	100.8	0.1088
Solvent	100	30	132	100	0.2273
Negative	92.1	41	150	104.7	0.2733
DMN	16.7	91	7	0.9	13

Reliability : (1) Valid without restriction

(2)

Type : Ames test
System of testing : Microbial mutation assay in Salmonella typhimurium and Saccharomyces cerevisiae
Metabolic activation : With and without
Result : Negative
Year : 1977
GLP : No data
Test substance : Unleaded gasoline

Method

- : The solubility, toxicity and dose levels for the test material were determined prior to the mutagenicity screening.
DMSO was used as solvent.
Based on the preliminary studies the following concentrations of test material were used in the mutagenicity assays:

Test doses	% Concentration	
	Bacteria	Yeast
1/8 50% survival	0.375	0.625
1/4 50% survival	0.75	1.25
1/2 50% survival	1.5	2.5
50% survival	3	5

Plate tests

For non-activation assays cells in broth were exposed to the test material at the concentrations shown above. The contents of the tubes of broth plus test material were poured over selective agar plates which were then incubated. The test was conducted with and without Araclor-induced rat liver S-9 metabolic activation. Positive control substances (see results section) were also run in the same assay.

The following evaluation criteria were used in this plate test.

Strains TA1535, 1537 and 1538

If the solvent control value is within the normal range a chemical which produces a positive response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

Strains TA98, 100 and D4

If the solvent control value is within the normal range, a chemical which produces a positive response over three concentrations with the highest increase equal to twice the solvent control value for TA100 and two to three times the solvent control value for strains TA98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

Pattern

Because TA1535 and TA100 were both derived from the same parental strain (G-46) and because TA1538 and TA98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain responds to a mutagen in non-activation tests it will generally do so in activation tests, but the converse of this is not anticipated.

While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

Reproducibility

If a chemical produces a response in a single test which cannot be repeated in one or more additional runs, the initial positive test data loses significance.

The above criteria are not absolute and other extenuating factors may enter into a final evaluation decision.

Suspension tests

Bacteria and yeast cultures were grown in complete broth. The cells were removed, washed and exposed to the test material at the concentrations shown in the results section. For the yeast cells exposure to the test material was for 4 hours whereas for the bacterial cells exposure was for 1 hour. Aliquots of the cells were plated onto the appropriate complete media. After suitable incubation periods, the number of revertant colonies were counted. This assay was also conducted with and without metabolic activation and positive control substances were also included.

The following criteria were used in the suspension assay.

Surviving population counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests show very low (<25%) survival compared to the tissue controls. Data of this type are generally unacceptable and these experiments are repeated at a lower dose level.

Total mutant counts

For non mutagens, the ratio of mutant to surviving population should be roughly equivalent for each test point in a given experiment. A mutagenic chemical will produce an altered mutant/surviving population ratio. An attempt is made to keep the surviving population of cells high and to look for positive responses that show increases in both numbers of mutants and mutation frequencies.

Dose-response

Dose-related increases in mutants and mutation frequencies are the most convincing data when assessing mutagenic activity. To ensure a proper dose response, dose levels are kept within a relatively low range

Result

: Plate test

There was no increase in revertants caused by exposure to the test material at any concentration. The results in this assay were negative both with and without metabolic activation.

Suspension test

The mutation frequencies are summarized in the following table for assays with and without metabolic activation.

Non activation assay

Dose level	Salmonella strains					Yeast
	TA100	TA1535	TA1537	TA1538	TA98*	D4**
-ve control	5.48	3.59	6.15	7.1	41.99	23.69
+ve control	125.51	185.65	161.54	84.75	100	66.29
1 (low)	18.18	2.26	12.54	27.78	233.33	9.52

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2	2.9	2.15	8.97	11.76	63.04	36.99
3	3.1	2.98	7.19	10	9.56	30.02
4 (high)	4.13	2.66	9.68	3.21	35.74	32.38

* Assay repeated for negative control and lowest 2 doses.
Results were 54.59 for -ve control
10.84 for lowest dose
14.11 for next highest dose

** Assay repeated at all dose levels
Results were: -ve control 4.66
+ve control 97.73
dose level 1 1.3
dose level 2 8.33
dose level 4 12.65

Slight increases are observed at the high dose levels with TA100, TA1537 and TA1538. However the responses are not adequate enough to be considered positive. The increases with TA98 could not be reproduced.

With activation

Dose level	Salmonella strains					Yeast D4**
	TA100	TA1535	TA1537	TA1538	TA98*	
-ve controls*						
A+C	17.08	5.25	6.01	4.8	21.01	52.66
A-C	17.29	8.77	9.29	8.25	62.02	7.96
AL1	17.34	7.32	3.99	6.48	45.03	30.06
+ve control	25.51	89.92	0.22	1253.4	555.35	115.3
1 (low)	22.97	41.67	100	71.43	100	
2	15.64	7.21	0	300	30.66	27.22
3	17.26	9.57	20	15.38	83.33	27.03
4	22.31	7.21	5.43	6.93	60.13	29.04

* Controls were

A+C	No activation system but including positive control
A-C	Solvent control, no test chemical or activation system
AL1	Liver homogenate control plus solvent

Scattered increases were found at one or more dose levels (see table above). All apparent positive effects were repeated and were not reproducible indicating problems associated with the initial runs. When the raw data were inspected it was observed that the increases were due to anomalous reductions in viable cell counts. The results of this assay were therefore considered to be negative.

Reliability : (2) Valid with restrictions due to poor quality of initial assay.

(2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male
Route of admin. : i.p.
Doses : Acute study: 0.024, 0.08 & 0.24 ml/rat; subacute study: 0.01, 0.03 & 0.1 ml/rat
Result : Negative
Year : 1977
GLP : No data

5. Toxicity

Id Gasoline
Date 03.12.2002

- Test substance** : API PS-6 unleaded gasoline
- Method** : Two studies were conducted viz an acute and a subacute study. The test material was administered to the animals intraperitoneally in acetone.
- Acute study
Groups of 15 rats were given either acetone (0.1 ml/rat), or test material at doses of 0.024, 0.80 or 0.24 ml/rat. An additional group of 5 rats were given Triethylenemelamine(TEM) at a dose of 0.3 mg/kg. 6, 24 and 48 hours after administration of the test material 5 animals in each dose group were killed. For the TEM group, all five animals were killed 24 hours after administration of the substance.
Two hours prior to being killed, cells were arrested in metaphase by the administration of a single i.p. dose of colchicine (4 mg/kg).
Bone marrow was aspirated from the femurs and tibias of the lower limbs of the animals after they had been killed. The marrow plug was washed and then fixed. Slides of the cells were prepared and stained with Giemsa for microscopic examination. Fifty spreads were located for each animal and when of suitable quality, the chromosomes were counted and evaluated for the presence of abnormalities.
- Subchronic study
18 animals were used in this study. They were dosed with three levels of test compound (0.01, 0.03 & 0.1 ml/rat) once each day for 5 days. All animals were killed 6 hours after administration of the last dose and 2 hours prior to being killed they were treated with colchicine in the same way as the animals in the acute study.
Slides were prepared and examined as for the acute study. A negative and positive control group were also included, again the same as for the acute study.
- Remark** : A subsequent study (API ref 26-60099) was also carried out and this supported the negative conclusion of the original study. The second study is not summarized here.
- Result** : The results of the acute and repeat dose studies are summarized in the following table:

Material & dose (ml/rat)	Time after dose	No of rats	Total No of cells	% cells with aber*	Mitotic index
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Acute study

Acetone (0.1)	6	3	100	1	3.4	
	24	3	100	0	4.3	
	48	3	150	0	3.8	
TEM (0.3 mg/kg)	24	5	200	30	3.8	
	PS-6 (0.024)	6	5	250	6	3.9
		24	5	250	1	4.9
48		5	250	3	4.7	
(0.08)	6	5	250	1	5.6	
	24	5	200	3	4.7	
	48	5	100	5	2.7	
(0.24)	6	5	187	2	3.2	
	24	3	100	0	4.1	
	48	5	200	3	4.5	

Subacute study

Acetone (0.1)	5 days	3	150	1	5.8
PS-6 (0.01)	5 days	5	200	1	3.5
(0.03)	5 days	4	159	2	2.9
(0.1)	5 days	5	174	2	3.2

* = aberrations

The results of the acute study were considered to be negative. There was an increase in aberrations at the 48 hour sacrifice period of the intermediate dose. The increases of 5% was significant, but did not fit into a trend suggestive of a compound-related mutagenic response. No other increases were observed at any dose level or sacrifice time.

The results of the subacute study were considered to be negative. There was no indication of an increased number of cells with aberrations.

Reliability : (1) Valid without restriction

(2) (3)

Type : Dominant lethal assay
Species : Mouse
Sex : Male/female
Strain : CD-1
Route of admin. : Inhalation
Exposure period : 6 hours/day, 5 days/week for 8 weeks
Doses : 400 & 1600 ppm
Result : Negative
Year : 1980
GLP : Yes
Test substance : API PS-6 unleaded gasoline

Method : Groups of 10 male mice were exposed to either filtered air (negative controls) or test material at concentrations of 400 or 1600 ppm. Generation of test atmospheres was accomplished by bubbling air through the test material. Exposures were for 6 hours a day, 5 days each week for 8 weeks. On the final day of exposure a positive control group of 10 male mice were given Triethylenemelamine (TEM) intraperitoneally as a single i.p. dose, at a dose level of 0.3 mg/kg. The dose volume was 0.1 ml/mouse and the TEM was dissolved in 0.9% saline. Chamber concentrations were monitored at least hourly during the exposure periods. After 2 days rest following termination of exposures, each male was caged with 2 unexposed virgin female mice. At the end of 5 days, the females were removed. This weekly mating sequence was continued for 2 weeks. Each pair of mated females were transferred to a fresh cage and after 14 days after the midweek of being caged with the male were sacrificed. The uterine contents of the females were examined and scored for the numbers of dead and living implants and total implants.

Evaluation Criteria

Dominant lethality was determined from a) a mutation index derived from the ratio of total to dead implants; or b) the number of dead implants per pregnant female. If true dominant lethality is observed then a significant

increase in the number of dead implants per pregnant female should be accompanied by a significant decrease in the number of living implants per pregnant female. The two ratios are compared with both concurrent and comparable historical control values. Dose-related trends are also looked for. Any statistically significant differences must also be strongly evaluated for their biological significance.

In this study the following parameters were determined:

- Fertility index ie. Proportion of pregnant females.
- Average No of implants/pregnant female.
- Average No. of dead implants/pregnant female.
- Proportion of females with one or more dead implants.
- Proportion of females with two or more dead implants.

Result : During the exposure phase actual chamber concentrations were found to be 0, 396.4 and 1524.6 ppm. One male died in the 1600 ppm group and another animal in the same group exhibited excessive lacrimation in the seventh week but this cleared in the final week.

The data for each of the parameters determined are as follows for untreated control, historical control, positive control and the two groups exposed to test material.

Week	Hist. -ve	-ve	+ve	400 ppm	1600 ppm
Fertility index					
1	22/24	21/23	19/24	17/20	21/22
2	16/24	19/24	13/24	18/19	16/22
Av. No. of implants/pregnant female					
1	267/22	240/21	140/19	203/17	214/21
2	193/16	220/19	91/13	219/18	183/16
Av. No. of dead implants/pregnant female					
1	12/22	14/21	83/19	9/17	9/21
2	13/16	5/19	66/13	9/18	12/16
Proportion of females with one or more dead implants					
1	11/22	9/21	19/19	6/17	8/21
2	9/16	4/19	13/13	8/18	7/16
Proportion of females with two or more dead implants					
1	1/22	3/21	17/19	3/17	1/21
2	2/16	1/19	13/13	1/18	3/16
No of dead implants/total implants					
1	12/267	14/240	83/140	9/203	9/214
2	13/193	5/220	66/91	9/219	12/183

Interpretation of the results:
The test material did not cause any significant reduction in the fertility index.
The test material had no effect on the average number of implants per pregnant female.
With respect to the number of dead implants per pregnant female, the test material showed no significant differences from the values of the concurrent as well as the negative

controls.
The results support the conclusion that the test material did not cause increases in post-implantation deaths.

Reliability : (1) Valid without restriction (6)

5.7 CARCINOGENICITY

Species : Rat and mouse
Sex : Male/female
Strain : F 344 rat B6C3F mouse
Route of admin. : Inhalation
Exposure period : Up to 113 weeks
Frequency of treatm. : 6 hours/day, 5 days/week
Doses : 50, 275 & 1500 ppm nominal concentration
Control group : Yes
Method : Similar to NCI guidelines
Year : 1984
GLP : Yes
Test substance : API PS-6 gasoline

Method : Groups of 100 rats of each sex and 100 mice of each sex were exposed to wholly vaporized gasoline at nominal concentrations of 50, 275 and 1500 ppm. 100 mice and 100 rats of each sex were exposed to air only and served as controls. Whole body exposures were in 16 m³ glass and stainless steel chambers. Exposures were for 6 hours a day, 5 days each week for up to 113 weeks.

All animals were individually housed and were allowed free access to food and water except during the exposure periods. Any animals that died during the first 10 days of exposure were replaced but thereafter no replacements were made. All animals were observed twice daily, once before and once after the exposure period. Animals found moribund were removed from the study and sacrificed. All animals were examined once per month for clinical signs and palpable tissue masses. Body weights were recorded monthly for the first 17 months and bi-weekly thereafter.

After approximately 18 and 24 months exposure 7 male and 7 female rats from each dose group were selected and hematological and clinical evaluations were conducted on these.

After 3, 6, 12 and 18 months exposure 10 rats and 10 mice of each sex from each dose group were sacrificed and underwent complete post mortem examinations.

At study termination all surviving animals were sacrificed. Body weight were recorded and after gross examination a wide range of organs/tissues were removed, weighed and fixed for subsequent histopathological examination.

Result : Monitoring of the exposure chamber concentrations established that actual concentrations for the study were: 0, 67, 292 and 2056 ppm.

Results of study in rats

There were very few pharmacotoxic signs that occurred in only a few animals and insufficiently frequently to be considered treatment-related. Mortality rates were also unaffected by exposure to gasoline vapor. Male rats in the highest dose group had lower body weights

than controls from week 5 throughout the study. The difference amounted to 33 g at week 44 and this remained throughout. Females at the highest dose also weighed less than controls. A difference of 30 g had occurred by week 66 and this remained throughout the study.

The few differences in hematological data between controls and several treatment groups were within the normal range for rats of similar age and not considered to be treatment-related.

Similarly, small changes in a few clinical chemical parameters were not considered to be treatment-related.

At gross necropsy at the 3, 6 12 and 18 month sacrifice the only significant macroscopic findings were in the kidneys of the high dose group male rats and these consisted of tan color, foci, mottling, discolored and granular surface. Although the incidence was small it was considered significant in the light of the histopathology findings. Additionally, masses or nodules were observed in mid (3 masses/nodules) and high (5 masses/nodules) dose male kidneys that died between 18 months and study termination. There were no other gross findings.

Microscopic pathology examination revealed an increase in the incidence of renal disease with tubular degeneration and regeneration or cystic dilatation in the mid and high dose males from 3 months onwards. At 24 months primary renal neoplasms were observed in the following incidence:

Dose group	Neoplasm	Males	Females
0 ppm		0	0
50 ppm	renal carcinoma	1	0
275 ppm	renal adenoma	2	0
	renal carcinoma	2	0
	renal sarcoma	1	1
2056 ppm	renal carcinoma	6	0
	renal adenoma	1*	

* Occured at 18 months

With the exception of one renal sarcoma all other tumors occurred in males.

Results of study in mice

There were no consistent signs of toxicity attributable to treatment and mortality rates were considered to be unaffected by treatment. Growth rates were similar for treated and control groups up until approximately week 70 after which the highest dose group males and females had lower body weights than controls. The difference amounted to

approximately 2.3 to 4.4g on a body weight of 35g for males and 2 to 3g on a weight of 33g for females. The investigators considered this reduced body weight to be attributable to treatment. Organ weights were unaffected by treatment.

There was an increased incidence of liver nodules and masses in treated females in the high dose group that died on the study from 18 months to termination and which were terminally sacrificed. The incidence is tabulated as

follows:

	Dose group (ppm)			
	0	67	292	2056
MALES				
Dead on study				
plus 18mth group	3/5	2/7	3/14	4/8
Terminal sacrifice	14/46	12/35	13/30	21/46
Total	17/51	14/42	16/44	25/54
FEMALES				
Dead on study				
plus 18mth group	4/16	4/19	7/20	8/14
Terminal sacrifice	5/41	6/33	8/37	18/42
Total	9/57	10/52	15/57	26/56

There was a possible reduction in the incidence of cystic or enlarged uteri for female mice.

The incidences were

0 ppm 38/41

67ppm 26/33

292ppm 19/37

2056ppm 12/42

There were no other treatment-related findings at necropsy.

Microscopic examination of the tissues of animals up to and including the 18 month sacrifice did not reveal any compound-related effects.

At 24 months, however, there was an increased incidence of hepatocellular tumors in the high dose group females when compared to controls. The actual incidence of liver tumors is shown in the following table.

	Dose group (ppm)			
	0	67	292	2056
Number examined	57	52	57	56
Hepatocellular adenoma	1	4	4	8
Hepatocellular csarcoma	7	6	9	20
Animals with hepatocellular tumors*	8	10	12	27

* Some animals had more than 1 tumor.

No other compound-related lesion were observed.

(19) (20) (22)

5.8.1 TOXICITY TO FERTILITY

Type	:	Two generation study
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	6 hours
Frequency of treatm.	:	Daily
Premating exposure period		
Male	:	10 weeks
Female	:	10 weeks

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Duration of test : Two generations
No. of generation studies :
Doses : 5000, 10 000 & 20 000 mg/m³
Control group : Yes
NOAEL parental : = 20000 mg/m³
NOAEL F1 offspring : = 20000 mg/m³
Method : OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year : 2000
GLP : Yes
Test substance : Volatile fraction of gasoline

Method : Groups of 30 male and 30 female Sprague Dawley rats were exposed 6 hours/day, seven days/week to volatilized test material at target concentrations of 5000, 10 000 and 20 000 mg/m³.
Singly housed animals were exposed for 10 weeks prior to mating. There was then a 3 week mating period and mating was confirmed by either presence of sperm in a vaginal rinse or by the presence of a vaginal plug.
Exposure of females was continued until gestation day 20. Exposure was then suspended until post partum day 5 to avoid unduly stressing the dams during birth and was then re-commenced and continued until sacrifice of parental females after weaning.
The pups were culled on a random basis to approximately 5/sex/litter. At weaning on postnatal day 28, the F1 pups were selected for the second generation. Among the pups not selected, 3/sex/litter were sacrificed and examined for internal abnormalities. The remainder were examined for external abnormalities, sacrificed and discarded.

The pups selected for F1 were exposed for a 13 week pre-mating period and then for a 3 week mating period as described above.

The males were sacrificed at this time and the females continued to be exposed until gestation day 20. As described above exposures were resumed on post partum day 5 and was continued until weaning, when all remaining animals were sacrificed. Other than during the period from gestation day 20 until post partum day 5, all F1 offspring were exposed from conception to sacrifice.

All animals were examined regularly for viability and clinical observations. Body weights and food intakes were also recorded regularly throughout the study.

All pups were counted and examined externally on a daily basis and weighed at regular intervals until post natal day 21. F1 pups were examined regularly between post natal days 21 to 28 and were weighed on days 28 and 35.

All surviving F1 and F2 pups were examined for developmental landmarks, including pinna detachment, hair growth, incisor eruption, eye opening and the development of the surface righting reflex. Surviving F1 female offspring were monitored for vaginal opening and males were examined for preputial separation.

Reproductive parameters evaluated included: male and female fertility indices, male mating index, female fecundity and gestational indices, mean litter size, mean days of gestation, female estrous cycle length and number of females cycling normally. Live birth index, survival index, survival

indices (post partum days 1, 4, 7, 14 and 21), viability index at weaning, mean live and dead offspring on day 0, sex ratio at day 0, offspring in-life observations, offspring body weight and offspring gross postmortem findings were also assessed.

All animals dying or sacrificed in a moribund condition were necropsied.

Culled pups were examined externally but were only necropsied if external evidence warranted it. Randomly selected pups were necropsied and the weight of the following organs was determined: ovaries, liver, adrenals, testes, kidneys, spleen and brain. Additionally a wide range of tissues were taken for histology.

Similar evaluations were also carried out on all adults surviving to scheduled sacrifice. Tissues taken from the high dose group and controls were evaluated histologically and since there were no untoward findings, tissues from the lower dose groups were not examined.

Samples of sperm from the left distal cauda epididymis were collected from all males at terminal sacrifice for evaluation of sperm parameters. These included assessments of total caudal epididymal sperm numbers, % progressively motile sperm and homogenization resistant spermatid count, % morphologically normal sperm and % sperm with an identified abnormality. An ovarian examination was carried out in the females that included confirmation of growing follicles and corpora lutea and quantification of primordial oocytes. This was done in the high dose and control groups and since there were no abnormal findings other groups were not evaluated.

Result

- : There were no treatment-related clinical signs, or effects on mortality, body weight or food intake in either parents or pups. Furthermore there were no treatment-related post mortem findings.

There were no significant differences in absolute organ weights in either males or females from the first parental generation. In the second parental generation, however, there were some statistically significant increases in absolute organ weights, including liver, kidneys and testis in the males and lungs in the females, but none of the differences between controls and the high dose group were statistically significant. In the absence of a clear dose-response relationship the significance of the result was unclear. When expressed as organ/body weight ratios, the only significant difference was seen in male kidney weights in the lowest dose group of the first parental generation and an increase in the highest dose group of the second parental generation. Although this latter may have been treatment related it was not considered to be of clinical importance.

There were no compound-related microscopic changes in any of the reproductive tissues or in the upper or lower respiratory

tract from any of the P1 or P2 rats exposed to 20 000 mg/m³. The only microscopic changes seen were in the kidneys of males of both generations. There was an exposure related increase in the amount and size of hyaline droplets. In three male rats of the high exposure group from both P1 and P2 animals granular casts were observed in the medullary

tubules of the kidneys. These kidney changes and the accompanying weight increases are regarded as a sex and species specific effect and of no relevance for man.

In the first generation there were no differences in mating index, fecundity, pregnancy or length of gestation. Among the offspring there were no differences in litter size, fraction of live births or sex ratio.

Results in the second generation were similar.

There were no differences in survival of offspring through weaning in the first generation and in the second generation early survival was slightly higher among the offspring from the exposed dams.

There were no differences in the weight of the offspring through weaning in either generation.

There were no unusual post mortem observations.

The sperm analysis carried out on both P1 and P2 (F1) males revealed no effects on sperm count, progressive motility or gross appearance.

No effects were found on the estrous cycle length, quantification of primordial oocytes or % females with abnormal cycles in the P1 or P2 generations.

There were no significant differences in incisor eruption, pinna detachment, or surface righting reflex in the F1 or F2 offspring. Hair growth was delayed by just less than one day in males only of the F1 pups and in both sexes of the lowest dose group (approx half day) for the F2 pups. Eye opening was advanced by approximately one-half day for the high dose males of the F2 offspring.

Test substance

: The test material was a condensate of gasoline vapor that had been collected from a vapor recovery unit during normal operations. This test material was selected since it was representative of the exposures that normally occur for the general public during self-service refueling. Analytical studies were conducted on the condensate and the results compared with exposure studies that had been carried out during refueling operations. The results confirmed that the vapor recovery condensate was similar in composition to the vapors to which the public are exposed during refueling.

Test atmospheres for the inhalation study were generated by fully volatilizing the condensate and diluting with air to achieve target concentrations of 5000, 10 000 and 20 000 mg/m³. The highest concentration was approximately 50% of the lower explosive limit and several orders of magnitude greater than the concentrations to which the public are exposed..

Chamber analyses of the test atmospheres confirmed the actual concentrations to be: 5076, 10 274 and 20 241 mg/m³.

Analysis of the vapor recovery condensate gave the following results:

Component	Vol %
Non aromatics	
C3	1.0
C4	51.7

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C5	37.2
C6	8.3
C7	0.4
C8	0.2
C9	-
C9+	-
Total saturates (vol%)	-
Total olefins (vol%)	-
Aromatics	
Benzene	0.7
Toluene	0.7
C8	-
C9	-
C9+	-
Total aromatics (vol%)	-

Reliability : NB - denotes no data available.
(1) Valid without restriction

(26)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours each day
Frequency of treatm. : Daily
Duration of test : Days 6 through 15 of gestation
Doses : 400 and 1600 ppm
Control group : Yes
NOAEL maternal tox. : = 1600 ppm
NOAEL teratogen. : = 1600 ppm
Year : 1978
GLP : No data
Test substance : Unleaded gasoline as described in section 1.1.1. above

Method : Female rats were mated with sexually mature males of the same strain. The females were examined daily for evidence of a copulatory plug and when this was observed it was designated day 0 of gestation. The mated female rats were assigned sequentially into three groups of 25 animals for the 0, 400 and 1600 ppm dose groups and were caged individually. The animals were subjected to whole body exposure to gasoline vapors at the concentrations shown above for 6 hours each day from day 6 through day 15 of gestation. Mated females were weighed on days 0, 6, 15 and 20 of gestation. Food consumption was recorded daily during the periods 0-6, 6-15 and 15-20 days of gestation. Observations were made daily for clinical signs. On day 20 of gestation the female rats were anesthetized and their visceral and thoracic organs were examined. The uterus was removed and opened and the number of implantation sites, their placement in the uterine horns, live and dead fetuses and resorption sites recorded. The fetuses were removed, examined externally for abnormalities and weighed. One third of the fetuses from each litter were fixed in Bouin's and examined later for

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Result

changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses in each litter were stained with Alizarin Red S and examined for skeletal abnormalities.

The uterus and ovaries from the adult females were preserved for possible future examination.

: Chamber concentrations were found to be:

Nominal (ppm)	Actual (ppm)
0	0
400	442 ± 42
1600	1573 ± 80

There were no deaths during the study and all animals appeared normal throughout. There were no treatment-related effects on body weight or food consumption.

There were no treatment related effects on any of the reproductive parameters recorded. These data are summarized as follows:

	Historical control	0 ppm	400 ppm	1600 ppm
Pregnancy ratio (pregnant/bred)	-	20/22	22/22	20/21
Live litters	99%	20	22	20
Implantation sites (left /right horn)	46/54%	123/145	149/158	143/152
Resorptions	252	16	22	15
Litters with resorptions	50%	65%	41%	55%
Dead fetuses	1	0	0	0
Litters with dead fetuses	1	0	0	0
Live fetuses/ Implantation site	92%	95%	93%	95%
Mean live litter size	12.2	13	13	14
Average fetal wt. (g)	3.5	3.8	3.7	3.6

No treatment related effects were observed during the examination for soft tissue changes in the fetuses.

Results of the skeletal examination of the stained fetuses are summarized below:

Dose (ppm)	Fetuses examined	Fetuses normal	Fetuses commonly encountered changes only	Fetuses with Unusual skeletal variations
0	177 (20)*	112	60 (18)	5 (5)
400	197** (22)	128	55 (16)	14 (4)
1600	196 (20)	131	47*** (14)	18*** (7)

* Average No. of litters in parenthesis

** Two specimens of one litter lost on processing

*** p<0.05

The unusual changes were mainly related to retarded ossification and were not considered as malformations.

Statistical analysis of data on a pup basis revealed a significant difference between the 1600 and 0 ppm groups. However when analyzed on a litter basis no statistically significant differences were found.

Conclusion

: Exposure of pregnant rats to vapors of unleaded gasoline at concentrations of 400 or 1600 ppm did not cause effects on pregnant dams. There was no evidence of variation in sex ratio, embryo toxicity, inhibition of fetal growth or development or teratogenic potential.

Reliability

: (1) Valid without restriction

(4)

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Gasoline Concawe sample, CWE5, Blend (match to API PS-6)	%	Physical Chemical Elements		Environmental Fate and Elements	
		CAS No. 86290-81-5	Partition Coefficient- Log P	OH rate constant (cm ³ /molecule-sec)	Photodeg half life (12-hr day; 1.5E6 OH/cm ³)
Paraffin					
n-butane	1.088	2.89	2.6322 E-12	4.064 Days	
n-heptane	0.774	4.5	6.8713 E-12	1.557 Days	
n-hexane	1.44	4	5.4583 E-12	1.960 Days	
n-pentane	1.616	3.45	4.0452 E-12	2.644 Days	
isopentane	7.024	3.11	0.6691 E-12	15.985 Days	
2,2 dimethyl butane	0.769	3.82	1.8179 E-12	5.884 Days	
2,3 dimethyl butane	1.518	3.84	1.8179 E-12	5.884 Days	
2,4 dimethyl pentane	0.82	3.63	6.8546 E-12	1.560 Days	
2,3 dimethyl pentane	1.7	3.63	7.1392 E-12	1.498 Days	
2 methyl hexane	1.331	3.71	6.8630 E-12	1.559 Days	
3 methyl hexane	1.358	3.71	6.8630 E-12	1.559 Days	
2 methyl pentane	4.128	3.21	5.4499 E-12	1.963 Days	
3 methyl pentane	2.8	3.6	5.7345 E-12	1.865 Days	
2,2,4 trimethyl pentane	5.566	4.09	4.6356 E-12	2.307 Days	
2,3,4 trimethyl pentane	2.445	4.05	8.5438 E-12	1.252 Days	
2,3,3 trimethyl pentane	2.92	4.09	4.3714 E-12	2.447 Days	
trans-2-pentene	0.924	2.58	65.2373 E-12	1.967 Hrs	
2-methyl-1-butene	1.493	2.72	52.6373 E-12	0.203 Days	
methyl cyclopentane	1.444	3.37	5.6631 E-12	1.889 Days	
benzene	5.936	2.13	1.9498 E-12	5.486 Days	
toluene	9.246	2.73	5.2263 E-12	2.047 Days	
ethylbenzene	1.815	3.15	5.9463 E-12	1.799 Days	
m-xylene (1,3 dimethylbenzene)	3.564	3.20	13.5606 E-12	0.789 Days	
p-xylene (1,4 dimethylbenzene)	1.151	3.15	6.5119 E-12	1.643 Days	
o-xylene (1,2 dimethylbenzene)	2.194	3.12	6.5119 E-12	1.643 Days	

Environmental Fate and Pathway Elements

radiation		FUGACITY / PERCENT DISTRIBUTION (Mackay, EQC Model Le				
O3 rx rate constant, cm ³ /molecule-sec	half life (days, 7E11O ₃ /cm ³)	AIR	WATER	SOIL	SEDIMENT	SUSPENDED SED
		99.99	0.01	0.00	0.00	0.00
		99.89	0.02	0.09	0.00	0.00
		99.95	0.02	0.03	0.00	0.00
		99.97	0.02	0.01	0.00	0.00
		99.98	0.01	0.01	0.00	0.00
		99.96	0.02	0.02	0.00	0.00
		99.96	0.02	0.02	0.00	0.00
		0.038	0.00	0.00	0.00	
		99.92	0.02	0.06	0.00	0.00
		99.93	0.01	0.06	0.00	0.00
		99.93	0.01	0.06	0.00	0.00
		99.97	0.01	0.02	0.00	0.00
		99.97	0.01	0.02	0.00	0.00
		100	0.003	0.039	0.00	0.00
		100	0.003	0.031	0.00	0.00
		99.9	0.011	0.123	0.00	0.00
20 E-17	1.37 Hrs	99.97	0.03	0.00	0.00	0.00
1.20E-17	22.920 Hrs	99.98	0.02	0.00	0.00	0.00
		99.95	0.02	0.03	0.00	0.00
		98.89	1.00	0.11	0.00	0.00
		96.5	2.7	0.8	0.0	0.00
		98.13	0.82	1.03	0.02	0.00
		97.91	0.86	1.20	0.03	0.00
		97.97	0.89	1.11	0.03	0.00
		97.72	1.04	1.21	0.03	0.00

QC Model Level 1)

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