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Aquatic ecotoxicity and biodegradability of cracked gas oils

Summary of relevant test data

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ABSTRACT

This report describes the experimental procedures and the results obtained in acute and chronic ecotoxicity tests as well as a biodegradation study on cracked gas oil samples. In a CONCAWE study, three samples were tested for toxicity to the crustacean zooplankter, *Daphnia magna* and the algae, *Pseudokirchneriella subcapitata* (alternatively known as *Selenastrum capricornutum*) using water accommodated fractions. In addition, another sample was tested in a separate API study for toxicity to the fish, *Oncorhynchus mykiss*, the crustacean zooplankter, *Daphnia magna* (acute and chronic) and the algae, *Pseudokirchneriella subcapitata* using water accommodated fractions. The API sample was also tested for ready biodegradability in a manometric respirometry test. All these results assist in determining the environmental hazard posed by cracked gas oils.

KEYWORDS

Ecotoxicity, fish, daphnia, algae, biodegradability, cracked gas oils, OECD guidelines, lethal loading, water accommodated fractions.

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SUMMARY

A series of ecotoxicity tests and a biodegradation test have been performed on four representative cracked gas oils using OECD methods. As the test substances are complex petroleum substances comprising large numbers of poorly water soluble hydrocarbons substances, they were tested in the toxicity tests as water accommodated fractions (WAFs) in sealed test vessels. Test substances were equilibrated with water at each "concentration" or loading rate and the water phase ("WAF") tested for toxicity. The toxicity results for fish, daphnia and algae were expressed as “lethal loading” (LL_{50}), "effective loading (EL_{50})", and effective loading on the growth rate (E_rL_{50}) to cause a 50% response, respectively.

In a CONCAWE study, three cracked gas oil samples were tested for acute toxicity to the crustacean zooplankter, *Daphnia magna* and the algae, *Pseudokirchneriella subcapitata* (alternatively known as *Selenastrum capricornutum*) using water accommodated fractions. In addition, a further sample was tested in a separate API study for toxicity to the fish, *Oncorhynchus mykiss*, the crustacean zooplankter, *Daphnia magna* (both acute and chronic toxicity) and the algae, *Pseudokirchneriella subcapitata* using water accommodated fractions. The API sample was also tested for ready biodegradability in a manometric respirometry test.

In acute toxicity studies of cracked gas oils to daphnia, the 48 hour EL_{50} values obtained (4 samples) range from 0.51 to 5.8 mg/l. The algal 72 hour E_rL_{50} values (4 samples) range from 0.51 to 8.8 mg/l, based on the specific growth rate. In an acute toxicity study of cracked gas oils to fish (1 sample), no toxicity was observed as the loading rate was too low (96 hour LL_{50} value = > 0.30 mg/l). In a 21 day chronic toxicity study with daphnia, EL_{50} values for survival and reproduction were 0.22 mg/l and 0.24 mg/l, respectively. A sample of light catalytically cracked gas oil was shown to be inherently biodegradable (56.3% degradation after 28 days).

1. INTRODUCTION

CONCAWE has recommended that only ecotoxicity data generated using a “water accommodated fraction” (WAF) approach will be suitable for the purposes of classifying and labelling petroleum substances for environmental hazard in accordance with the criteria given in the CLP Regulation [1]. The experimental procedures and methods of presenting results using WAFs have been described [2].

No data on the ecotoxicity of the generic category of petroleum substances known as cracked gas oils were reported in the summary of data for petroleum substances published by CONCAWE in 2001 [3]. Recently, CONCAWE embarked upon a test programme to generate typical acute toxicity data for three cracked gas oils from studies on daphnia and algae. API has also completed a test programme of acute toxicity tests with fish, daphnia and algae, plus a daphnia chronic toxicity study and biodegradation test on a cracked gas oil sample.

2. CRACKED GAS OILS CATEGORY

Cracked gas oils form a group of 10 gas oil streams produced by atmospheric distillation or vacuum distillation of crude oil that are thereafter catalytically or thermally cracked. This group consists predominantly of hydrocarbons with carbon numbers ranging from C_9 to C_{30} that boil over the temperature interval of 150 to 450°C. Cracked gas oils generally have lower cetane numbers than straight-run gas oils and typically occur at lower concentrations in gas oil products. Cracked gas oils can also be used as feedstocks for further refining processes such as hydrogenation, after which they are used as diesel fuel components.

Cracked gas oils are complex petroleum substances consisting of many hundreds of hydrocarbons that each exhibit different properties.

The types of cracked gas oils discussed in this dossier are:

- **Distillates (petroleum), light catalytic cracked**: A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C_9 through C_{25} and boiling in the range of approximately 150°C to 400°C (302°F to 752°F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons.

- **Distillates (petroleum), light thermal cracked**: A complex combination of hydrocarbons from the distillation of the products from a thermal cracking process. It consists predominantly of unsaturated hydrocarbons having carbon numbers predominantly in the range of C_{10} through C_{22} and boiling in the range of approximately 160°C to 370°C (320°F to 698°F).

The complex and variable composition of such UVCB (Unknown or Variable compositions, Complex reaction products and Biological materials) substances, means that it is not possible to define precisely their physico-chemical and environmental properties, but they will fall into a range, defined by the properties and concentrations of the individual hydrocarbons present. An overview of the physical-chemical properties of cracked gas oils is reviewed in **Table 1**.

Table 1 Physical-chemical properties of cracked gas oils

Test Type	Method[1]	Results	Reference
Pour point	ASTM 1999	<-20°C	4
Boiling range	ASTM D86 EN ISO 3405	150 - 411°C	4
Density absolute	EN ISO 3675EN ISO 12185ASTM D1298	0.816 - 0.993 g/m^3	4
Flash point	ASTM D93 EN ISO 2719	56 - 154°C	4
Viscosity	ISO 3104ASTM D445	1.1 - 4.5 cSt at 40°C 6.0 - 8.1 cSt at 20°C	4

[1]Commonly accepted method guidelines set by American Society for Testing and Materials (ASTM) and International Organization for Standardization (ISO).

3. CHARACTERISATION OF TEST SUBSTANCES

Three cracked gas oils samples were obtained via CONCAWE from various European refineries as well as another cracked gas oil sample obtained via API (**Table 2**). These test substances are representative of some of the cracked gas oils types (**Appendix 1**).

Three samples (a light thermal cracked gas oil and two light catalytic cracked gas oils) were characterised [5] and tested by ExxonMobil Biomedical Sciences, Inc. (EMBSI) in 2010 [6-11] as part of a CONCAWE programme. The API sample (a light catalytic cracked gas oil) was characterised [5] and tested by EMBSI as part of an API test programme [12-16] (**Tables 3** and **4**).

Table 2 Cracked gas oil samples

Descriptor	Substance	EINECS No.	CAS No.	lab. Code No.
light cracked gas oil	Distillates (petroleum), light catalytic cracked	265-060-4	64741-59-9	MRD-10-576
light cracked diesel oil	Distillates (petroleum), light catalytic cracked	265-060-4	64741-59-9	MRD-10-578
light cracked gas oil	Distillates (petroleum), light catalytic cracked	265-060-4	64741-59-9	MRD-10-595
Thermally cracked gas oil	Distillates (petroleum), light thermal cracked	265-084-5	64741-82-8	MRD-10-604

Detailed compositional analyses of the four samples have been carried out using a high-resolution approach involving comprehensive two-dimensional gas chromatography (GCxGC). In addition, detailed PAH analysis using a capillary GC-MS method based on that described in U.S. EPA SW-846 8270C has been conducted.

A summary of the GCxGC compositional analysis is provided in **Table 3**. GCxGC has previously been employed for the detailed characterisation of complex middle distillate fuels [17-19]. The upper volatility range of GCxGC currently limits its application to analysis of the lighter ($<C_{35}$) petroleum products. However, this technique should be able to provide a detailed description of the hydrocarbon components by structure/class and carbon number present in cracked gas oils, which typically cover the $C_9 – C_{30}$ range [2].

Table 3 Compositional analysis of the cracked gas oil samples

Analysis (%ww)	**light catalytic cracked gas oil MRD-10-576**	**light catalytic cracked gas oil MRD-10-578**	**light catalytic cracked gas oil MRD-10-595**	**light thermal cracked gas oil MRD-10-604**
n-Alkanes	5.16	5.41	9.06	17.65
iso-Alkanes	4.86	14.46	15.09	14.87
Naphthenics	5.22	9.81	12.12	31.19
Aromatics	60.65	41.62	36.40	17.02
Naphthenic Aromatics	24.12	28.69	27.32	19.25
Sum of components $<C_{30}$	100.01	99.99	99.99	99.98

A study of the GCxGC data above confirms it was possible to analyse 100% of the hydrocarbon components (i.e. < C_{30}) for these cracked gas oil samples. A compositional comparison of these samples is provided in **Table 4** with further detailed compositional PAH analyses shown in **Table 5.**

Table 4 Compositional comparison of the cracked gas oil samples

Sample description	Sample ID	Total Saturate Fraction (wt%)	Total Aromatic Fraction (wt%)	Total (wt%)	Sum of 16 Priority PAHs (ppm)
Distillates (petroleum), light catalytic cracked (CAS 64741-59-9)	MRD-10-576	15.24	84.77	100.01	17329
Distillates (petroleum), light catalytic cracked (CAS 64741-59-9)	MRD-10-578	29.68	70.31	99.99	12450
Distillates (petroleum), light catalytic cracked (CAS 64741-59-9)	MRD-10-595	26.27	63.72	99.99	7986
Distillates (petroleum), light thermal cracked (CAS 64741-82-8)	MRD-10-604	63.71	36.27	99.98	1392

Table 5 Detailed PAH analyses of the cracked gas oil samples

PAH analytes (ppm)	light catalytic cracked gas oil MRD-10-576	light catalytic cracked gas oil MRD-10-578	light catalytic cracked gas oil MRD-10-595	light thermal cracked gas oil MRD-10-604
Naphthalene	2420	9450	2500	336
Acenaphthylene	84	43	16	13
Acenaphthene	2170	1330	872	110
Fluorene	2410	1030	938	104
Anthracene	491	56	367	123
Phenanthrene	9740	539	2820	321
Fluoranthene	2	<1	74	56
Pyrene	12	2	328	250
Benz[a]anthracene	ND	ND	26	26
Chryene/Triphenylene	ND	ND	43	53
Benzo[b]fluoranthene	ND	ND	1	<1
Benzo[k]fluoranthene	ND	ND	<1	ND
Benzo[a]pyrene	ND	ND	1	<1
Indeno[1,2,3-cd]pyrene	ND	ND	ND	ND
Dibenzo[a,h]anthracene	ND	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	<1	ND
Sum of 16 priority PAHs	17329	12450	7986	1392

ND = Not Detected

4. TEST METHODS

4.1. GENERAL APPROACH

Mixtures of poorly water soluble, complex chemicals, e.g. petroleum products, present special problems with regard to preparing aqueous solutions for toxicity testing. With soluble chemicals, the amount of chemical dissolved in water is varied in incremental steps to produce a range of toxic responses, from which a "dose - response" relationship and the associated median lethal concentration (LC_{50}) may be derived. With mixtures of poorly soluble complex chemicals, un-dissolved material appears as soon as the least soluble component reaches water saturation. Thereafter, the relative composition of the water phase varies in a non-linear fashion from the composition of the "neat" substance [20]. This does not apply to pure substances where the concentration will, if sufficient time is provided, equal the solubility limit when excess is added, regardless of the amount of excess. For poorly water soluble, complex chemicals, it has become a standard practice to test toxicity at substance additions far in excess of the amount that will dissolve, resulting in a two phase system.

There are, however, many divergent procedures for establishing and maintaining equilibrium between water and un-dissolved substance [21]. A recognised guideline [22] for testing mixtures of poorly water soluble substances has been developed by the Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP). This method involves stirring various amounts (loading ratios) of test substance with water for a sufficient time to reach equilibrium, followed by separation of the water phase ("water accommodated fraction" or "WAF"). Toxicity testing of the WAFs generated in this manner allows the determination of the amount of the substance equilibrated with water which will cause 50% mortality. This end-point has been termed LL_{50} (lethal loading) to distinguish it from the LC_{50} [23]. (The LC_{50} is determined by completely dissolving the chemical in water and then making a dilution series to obtain a relationship between concentration and lethality). The LL_{50} procedure has also been described in a CONCAWE report [1]. It is also the approach specified by MARPOL for the marine pollution testing of poorly soluble mixtures [24] and by OECD for the aquatic toxicity testing of difficult substances and mixtures [25].

A further complication for the testing of hydrocarbon liquids is their volatility, particularly from aqueous solution. Although it may be environmentally unrealistic, it is necessary to prevent volatilization of the substance in order to maintain constant concentrations and, by doing so, to determine its inherent toxicity. This necessitates using closed test systems. In preparing WAFs, some headspace in the test vessel is necessary to achieve adequate interfacial area and mixing. In each test, measured amounts of test substance are added to measured volumes of the appropriate test medium (for daphnia and algae). The vessels containing the medium and the test substance are then sealed leaving only a small headspace, and the contents stirred with a 1-2 cm vortex depth for a period of time shown to be sufficient for the aqueous and test substance phases to equilibrate. After stirring, the contents of the vessels are left to stand to allow any un-dissolved material to separate out. The aqueous phases – the WAFs – are then drawn off for use in the tests. Control media are subject to the same regime but do not contain the test substance. It is important that mixing is sufficient to ensure that the aqueous phase is in equilibrium with the un-dissolved hydrocarbon phase. Mixing needs to be slow enough not to cause dispersion or emulsification of the un-dissolved hydrocarbon, yet vigorous enough

and long enough to attain equilibrium. In the current studies, mixing was done with a magnetic stirring bar set to develop a vortex at the surface of about 10% of the water height. Preliminary studies showed that this mixing condition was sufficient to reach equilibrium within 24 or 48 hours. After mixing for this period, solutions were allowed to stand for 1 hour before use in order to facilitate phase separation. The mixing vessel was fitted with a stopcock at the bottom of the vessel for siphoning off the water phase, without contamination by the surface layer of un-dissolved hydrocarbon.

All the studies were conducted in a single laboratory ExxonMobil Biomedical Sciences, Inc., Annandale, New Jersey, USA (EMBSI) in accordance with the principles of Good Laboratory Practice (GLP).

4.2. FISH ACUTE TOXICITY STUDY

This tst was carried out in accordance with OECD Guideline 203, Part I (equivalent to EC methods for the determination of ecotoxicity, C1 – Acute toxicity for fish) and USEPA ecological effects test guidelines OPPTS 850.1075. It was conducted to evaluate the acute toxicity of the WAFs of a light catalytic cracked gas oil to the rainbow trout, *Oncorhynchus mykiss*. Details of the husbandry and selection of test organisms are provided in the laboratory report [12]. This study was performed as a 96-hour static-renewal test.

The definitive test design generally conformed to the Upper Threshold Concentration (UTC) tiered testing strategy [26]. The loading rate for this study was the UTC, defined as the lowest EL_{50} value generated from either the daphnia acute immobilization study [13] or the algal growth inhibition test [14]. The loading rate for this study was based on the EL_{50} value (yield) from the algal test [14].

A single treatment WAF was prepared by adding the appropriate amount of test substance to dilution water in a 20 l glass aspirator bottle and stirring on a magnetic stir plate with a vortex of approximately 10% of the static liquid depth for approximately 24 hours. A 24 hour mixing period was sufficient to achieve dissolution of the soluble components in the test substance in the WAF solutions. Approximately one hour after stirring termination, the aqueous portion of the WAF solution was removed for testing. A fresh WAF was prepared daily for test solution renewals.

One test chamber was prepared for the treatment group and control. Each test chamber contained seven rainbow trout. The test chambers were 8 l glass aspirator bottles containing approximately 8500 ml of solution (no headspace) and closed with foil covered stoppers. Water quality (temperature, pH, and dissolved oxygen) measurements of each new and old solution were measured. Observations for mortality and abnormal behavior or appearance were performed at 6, 24, 48, 72 and 96 ±1 hour intervals after the beginning of the test.

Concentrations of the test substance hydrocarbon components were quantified against gas oil standards using automated static headspace gas chromatography with flame ionization detection (HS GC-FID) analysis. The total peak area for eluted hydrocarbon components from WAF headspace analysis was summed for quantification. The distribution and percentage of gas oil components measured in the WAFs differed from the parent gas oil standards owing to the differing solubilities of individual gas oil hydrocarbons. Therefore, measured concentrations do not

represent all hydrocarbons constituting the test substance. Due to the complex nature of the test substance, no attempt was made to identify and quantify specific hydrocarbons solubilized in the WAFs.

Acute toxicity results are expressed as percent mortality. The 50% lethal loading (LL_{50}) is the calculated loading rate of the test substance which would cause 50% mortality in a population of test organisms over a specified exposure period.

4.3. DAPHNIA ACUTE TOXICITY STUDIES

These tests were carried out in accordance with OECD Guideline 202, Part I (equivalent to EC methods for the determination of ecotoxicity, C2 – Acute toxicity for Daphnia) and USEPA ecological effects test guidelines OPPTS 850.1010 (for the API sample). The test species was *Daphnia magna*, a fresh water invertebrate commonly used for ecotoxicity testing. Details of the husbandry and selection of test organisms are provided in the laboratory reports [6,8,10,13]. The organisms used for testing were less than 24 hour old neonates, from parents ranging from 12 – 19 days. For definitive studies, four replicates, each involving 5 organisms were tested at each loading rate. The exposure period was 48 hours.

Reconstituted water was used for the daphnia studies. Fresh WAFs were prepared for each treatment in the same manner using the same equipment and analyses as the fish studies, but were not renewed daily (i.e. static tests). The WAFs were siphoned into sealed flasks, typically 130 ml, without headspace, and the daphnia were introduced. The light duration was 16 hours at 108 - 215 lux. No reductions in dissolved oxygen concentration (range 8.0 – 9.3 mg/l) or pH (range 7.4 - 8.8) were seen at the end of the 48 hour exposure period. Observations were made for immobilization at 24 and 48 hours. The daphnids were not fed during the exposure periods. HS GC-FID data are available on the analysis of WAFs for each loading at the beginning and end of the exposure.

4.4. ALGAL GROWTH INHIBITION STUDIES

The algal growth studies were conducted in accordance with OECD guideline 201 (equivalent to EC methods for the determination of ecotoxicity, C3 – Algal Inhibition Test) and USEPA ecological effects test guidelines OPPTS 850.5400 (for the API sample). The test species was *Pseudokirchneriella subcapitata* (alternatively known as *Selenastrum capricornutum)*. Details of the culture methods are provided in the laboratory reports [7,9,11,14]. The algae used were taken from 4 day old stock cultures in the log phase of growth. Initial concentrations were approximately 1.0 x 10^4 cells/ml in each replicate test chamber. The exposure period was 72 hours (and also 96 hours for the API sample).

WAFs were prepared in algal growth medium. WAFs were prepared in the same manner using the same equipment and analyses as for the fish and Daphnia studies, but were not renewed daily (i.e. static tests). WAFs were analyzed for each loading at the beginning of the test period, and again on a composite from test flasks after 72 hours. Test vessels, typically 125 ml, were filled completely with inoculated WAF and then closed with screw caps. Nine replicate vessels were set up for each treatment and the control to facilitate daily algal cell counting and pH measurements. The flasks were incubated at 22.4 – 23.7°C on an orbital shaker, at 100 cycles/min or rpm. Lighting was continuous and in the range of 8200 to 9500 lux. Cell counts were determined at 24, 48, and 72 hours using a haemacytometer

and microscope. The pH values during these studies were within the range 8.1 – 9.4.

4.5. DAPHNIA CHRONIC TOXICITY STUDY

This test was carried out as a 21-day semi-static renewal test in accordance with OECD Guideline 211 and USEPA ecological effects test guidelines OPPTS 850.1300. The test species was *Daphnia magna*, a fresh water invertebrate commonly used for toxicity testing. Details of the husbandry and selection of test organisms are provided in the laboratory report [15]. Individual treatments were prepared by adding the appropriate amount of test substance to dilution water in glass aspirator bottles and stirring on magnetic stir plates with a vortex of approximately 10% of the static liquid depth for approximately 24 hours. Approximately one hour after terminating the stirring, the aqueous portion of each WAF solution was removed for testing. The control and treatment WAFs were prepared every other day. Five treatments were prepared at loading rates in the range ~0.04 - 0.65 mg/l.

Ten replicate test chambers were prepared for each of the test substance loading rates and the control. Each replicate test chamber contained one daphnid. Replicate chambers were 130 ml glass bottles containing approximately 130 ml of solution (no headspace) closed with PTFE-lined screw top caps. Water quality parameters (temperature, pH, dissolved oxygen, and hardness) were measured once or twice a week in each new (i.e. at the start of each 24 hour period) and old (i.e. at the end of each 24 hour period) solution for each treatment and the control. Water quality parameters were within acceptable limits throughout the testing period. Adult daphnids were observed daily for immobilization, reproduction, and abnormal behavior/appearance. Any offspring were counted and observed for immobilization at each renewal period and at the end of the test.

Concentrations of the test substance hydrocarbon components were quantified against gas oil standards, prepared in acetone, spiked directly into water for automated static headspace gas chromatography with flame ionization detection (HS GC-FID) analysis. The total peak area for eluted hydrocarbon components from WAF headspace analysis was summed for quantification. The distribution and percentage of gas oil components measured in the WAFs differed from the parent gas oil standards owing to the differing solubilities of individual gas oil hydrocarbons. Therefore, measured concentrations do not represent all hydrocarbons constituting the test substance. Due to the complex nature of the test substance, no attempt was made to identify and quantify specific hydrocarbons solubilized in the WAFs. Old test solutions ranged from 75 to 98% of the measured hydrocarbon concentrations in new test solutions.

Chronic toxicity results are expressed as the Effect Loading 20 and 50 (EL_{20} and EL_{50}), which are the loading rates of test substance in dilution water calculated to result in a 20% and a 50% reduction in reproductive output relative to the control group for the test. The No Observed Effect Loading Rate (NOELR) was the highest loading rate that did not exhibit a statistical difference in reproductive output from the control group. The Lowest Observed Effect Loading Rate (LOELR) was the lowest loading rate that resulted in a statistical difference in reproductive output from the control group. The Maximum Acceptable Toxicant Loading Rate (MATLR) is the geometric mean of the NOELR and LOELR values. These endpoints were calculated for adult growth and survival where possible.

4.6. BIODEGRADABILITY STUDIES

The aerobic biodegradation of cracked gas oil samples was measured in an OECD 301F test (manometric respirometry). A positive control treatment (sodium benzoate), a toxicity control treatment (test compound in combination with the positive control substance), and an abiotic control treatment (test compound with mercuric chloride) were included in the study design [16]. The respirometer was operated within a temperature range of 21-23ºC. Biodegradation was determined by measuring oxygen consumption in a test medium containing trace nutrients and inoculated with activated sludge supernatant. The cracked gas oil, the positive control substance, the toxicity control and the abiotic control were evaluated at mean concentration close to 50, 50, 100 and 50 mg/l, respectively.

5. RESULTS

A summary of the ecotoxicity and biodegradation data from studies of cracked gas oils samples on daphnia and algae generated using WAFs has been summarised in **Table 6**.

Table 6 Summary of all the cracked gas oils ecotoxicity acute data for fish, Daphnia and algae

Sample details	Fish 96h LL_{50} (mg/l)	Daphnia 48h EL_{50} (mg/l)	Algae 72h E_rL_{50} (96h E_rL_{50}) (mg/l)	Daphnia 21 day EL_{50} (mg/l)	Biodeg	Refs
light catalytic cracked gas oil (CAS 64741-59-9) MRD-10-576	>0.30	0.51	0.53 (0.80)	0.22 (survival) 0.24 (repro) NC (growth)	56.3% after 28 days 61.2% after 47 days	12-16
light catalytic cracked gas oil (CAS 64741-59-9) MRD-10-578	NT	0.70	0.93	NT	NT	6-7
light catalytic cracked gas oil (CAS 64741-59-9) MRD-10-595	NT	0.32	0.51	NT	NT	8-9
light thermal cracked gas oil (CAS 64741-82-8) MRD-10-604	NT	5.80	8.82	NT	NT	10-11

NT = Not tested ; NC = Not calculable

5.1. FISH ACUTE TOXICITY DATA

This study met the acceptability criteria for control mortality, abnormal behaviour and dissolved oxygen concentration. None of the control fish died or exhibited abnormal behaviour. Dissolved oxygen remained above 60% of the air saturation value at the exposure temperature of 15 ±1°C.

The test was performed at a single "UTC" (upper threshold concentration). No mortality or sub-lethal effects were observed. As such, the result is reported as a "greater than" value.

Old test solutions ranged from 63 to 78% of the measured hydrocarbon concentrations in new test solutions. Analytical results confirm that the WAFs were effectively dosed in line with their loading rates, and there was no significant loss of hydrocarbon components during the experiment.

5.2. DAPHNIA ACUTE TOXICITY DATA

These studies met the acceptability criteria for control immobilization and dissolved oxygen concentration. In the controls, no *Daphnia* were immobilized or trapped at the surface of the water. Dissolved oxygen remained above 60% of the air saturation value at the exposure temperature of 20°C.

All test solutions retained ≥ 80% of the initial measured hydrocarbon concentrations after the 48 hour exposure period.

Daphnids were observed daily for immobilization, behavior and appearance during the 48 hour exposure. No observation of test substance insolubility (surface slicks, precipitates, and adherence to the test chamber) was noted during the time of organism observations.

5.3. ALGAL GROWTH INHIBITION DATA

At WAF stirring initiation and termination, all treatments appeared clear with clear test substance floating at the surface. No un-dissolved test substance was observed in the test chambers during the study. No unusual cell shapes, color differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers, or aggregation of algal cells were observed.

Based on the results of the study, all guideline validity criteria were met in this study. Control cell density increased by more than a factor of 16 within 72 hours. The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 22%, which is below the guideline value of 35%. The coefficient of variation of average specific growth rates during the 72-hour period in replicate control cultures was 3% and did not exceed the guideline value of 7%.

5.4. DAPHNIA CHRONIC TOXICITY DATA

This study met the acceptability criteria for mortality (not to exceed 20%) and mean number of live offspring produced (≥ 60) in the control group at the end of test. The coefficient of variation around the mean number of living offspring produced per adult in the control was below 25%.

All old test solutions ranged from 75 to 98% of the initial measured hydrocarbon concentrations.

At WAF stirring initiation and termination, all treatments appeared transparent with test substance visible on the surface. No observation of test substance insolubility (surface slicks, precipitates, and adherence to the test chamber) was noted during the time of organism observations. No immobilization or abnormal appearance was observed in the control group.

No aborted eggs were observed in any treatment throughout the entire exposure. At test termination, all surviving adults were measured for body length (excluding anal spine) to determine growth effects.

5.5. BIODEGRADABILITY DATA

The average percent biodegradation of triplicate test systems of the light catalytic cracked gas oil (MRD-10-576) was determined to be 56.32% over a 28 day testing period. The testing period was extended to see if the test substance had the ability to further degrade. The test substance biodegraded to 61.23% on day 47. Based on the OECD guideline, the sample of light catalytic cracked gas oil can be considered inherently biodegradable.

The toxicity control systems for the test substance exceeded 25% on day 3, which suggests that the test substance was not inhibitory at the tested concentration of approximately 50 mg/l. The abiotic control systems maintained 0% biodegradation throughout the study.

The validity requirements of the OECD 301F guideline are that the difference between extremes of replicate biodegradation values should be less than 20% at the end of test and the positive control should achieve >60% biodegradation by day 14. Further, the oxygen consumption of the inoculum blank should not exceed 60 mg/l in 28 days. This test passed all validity criteria set forth in the OECD guideline.

6. DISCUSSION

No reliable experimental data on the aquatic effects of cracked gas oils were reported in the summary of data for petroleum substances published by CONCAWE in 2001 [3]. Subsequently, ecotoxicity data have been generated on three cracked gas oil samples under a recent CONCAWE test programme. Ecotoxicity and biodegradation data on a further cracked gas oil sample have also been generated from an API programme. These data were made available and have been included in this report. All ecotoxicity data were generated using the water accommodated fraction (WAF) methodology at the same testing laboratory.

When preparing a WAF of a mixture which contains sparingly soluble components, two phases are present in the mixing system. Consequently, the individual components do not dissolve at their maximum water solubility, but equilibrate (partition) between the hydrocarbon and water phases. For this reason, the composition of the water phase varies for each component with the loading rate [20]. Petroleum products such as cracked gas oils will show toxicity at those loadings where the combined toxicities of the components in solution equal or exceed threshold levels. Although in these tests great care was taken to prevent volatilization losses during exposure, the mixing system, of necessity, had some headspace. It is important to standardise this aspect of test protocols, since for all hydrocarbon mixtures containing volatile components, the toxic constituents are likely to partition significantly to air. Accordingly, in conducting acute toxicity studies with volatile hydrocarbons, the headspace in the vessels should be kept as low as is practicable. In the current studies, closed vessels were used.

Detailed compositional analyses of the four cracked gas oil samples was carried out using comprehensive two-dimensional gas chromatography (GCxGC). Although the upper volatility range of GCxGC limits its application to analysis of lighter ($<C_{35}$) petroleum products, this technique was able to provide a comprehensive description of all the components present in cracked gas oils, which typically cover the $C_9 – C_{30}$ range [2].

Analytical results in these studies confirm that the WAFs were effectively dosed in line with their loading rates, and there was no significant loss of hydrocarbon components during the experiments.

Comparable ecotoxicity data for daphnia and algae are available for all four samples of cracked gas oils. The three light cracked gas oil samples (all of CAS number 64741-59-9) show similar toxicity to daphnia (48h EL_{50} range 0.32 - 0.70 mg/l) and algae (72h E_rL_{50} range 0.51 – 0.93 mg/l). The light thermal cracked gas oil (CAS number 64741-82-8) is less toxic to daphnia (48h EL_{50} = 5.80 mg/l) and algae (72h E_rL_{50} = 8.82 mg/l).

It is noticeable from the GCxGC analytical data and also the specific PAH analysis for these four samples that the higher toxicity of the cracked gas oil samples (CAS number 64741-59-9) vs. the light thermal cracked gas oil (CAS number 64741-82-8) could be associated, in part, with their higher aromatic content (range 64 – 85% vs. 36%) and in particular the specific PAH content (range 7986 – 17329 ppm vs. 1392 ppm). However, it is difficult to draw any substantive conclusions from these few studies. On the basis of the available data set, daphnia appear to be slightly more sensitive than algae to cracked gas oils.

7. GLOSSARY

API	American Petroleum Institute
ASTM	American Society for Testing of Materials
CAS no	Chemical Abstracts Service (Registry) Number
EC	European Council
EINECS	European Inventory of Existing Commercial Chemical Substances
EL	Effective Loading
EL_{50}	Loading Rate of Test Substance (in dilution water) which causes adverse effects in 50% of the exposed population
E_rL_{50}	Loading Rate of Test Substance (in dilution water) which causes 50% reduction in algal growth rate
GC-FID	Gas Chromatography with Flame Ionisation Detection
GC-MS	Gas Chromatography with Mass Spectrometry Detection
GCxGC	Two-Dimensional Gas Chromatography
GESAMP	Group of Experts on the Scientific Aspects of Marine Pollution
GLP	Good Laboratory Practice
HS	Head Space
LC_{50}	Concentration of Test Substance in test media which causes lethal effects in 50% of the exposed organisms
LL	Lethal Loading
LL_{50}	Loading Rate of Test Substance (in dilution water) which causes lethal effects in 50% of the exposed population
LOELR	Lowest Observed Effect Loading Rate
MARPOL	Maritime Pollution
MATLR	Maximum Acceptable Toxicant Loading Rate
mg/l	Milligram per litre
Ml	Millilitre
NOELR	No Observed Effect Loading Rate
OECD	Organisation for Economic Co-operation and Development

PAH	Polycyclic Aromatic Hydrocarbon
PTFE	Polytetrafluoroethylene
USEPA	United States Environmental Protection Agency
UTC	Upper Threshold Concentration
UVCB	Substance of Unknown or Variable Composition, Complex Reaction Products and Biological Materials
WAF	Water Accommodated Fraction

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APPENDIX 1: CRACKED GAS OILS

CAS#	EINECS #	Substance Name	Substance Description
64741-59-9	265-060-4	Distillates (petroleum), light catalytic cracked	A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150°C to 400°C (302°F to 752°F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons.
64741-60-2	265-062-5	Distillates (petroleum), intermediate catalytic cracked	A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C30 and boiling in the range of approximately 205°C to 450°C (401°F to 842°F). It contains a relatively large proportion of tricyclic aromatic hydrocarbons.
64741-82-8	265-084-5	Distillates (petroleum), light thermal cracked	A complex combination of hydrocarbons from the distillation of the products from a thermal cracking process. It consists predominantly of unsaturated hydrocarbons having carbon numbers predominantly in the range of C10 through C22 and boiling in the range of approximately 160°C to 370°C (320°F to 698°F).
68333-25-5	269-781-5	Distillates (petroleum), hydrodesulfurized light catalytic cracked	A complex combination of hydrocarbons obtained by treating light catalytic cracked distillates with hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150°C to 400°C (302°F to 752°F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons.
68921-07-3	272-930-7	Distillates (petroleum), hydrotreated light catalytic cracked	A complex combination of hydrocarbons obtained by treating a petroleum fraction with hydrogen in the presence of a catalyst.
85116-53-6	285-505-6	Distillates (petroleum), hydrodesulfurized thermal cracked middle	A complex combination of hydrocarbons obtained by fractionation from hydrodesulfurized thermal cracker distillate stocks. It consists predominantly of hydrocarbons having carbon numbers predominantly in the range of C11 to C25 and boiling in the range of approximately 205° C to 400°C (401°F to 752°F).
92045-29-9	295-411-7	Gas oils (petroleum), thermal-cracked, hydrodesulfurized	No EC number description available in ESIS
92201-60-0	295-991-1	Distillates (petroleum), light catalytic cracked, thermally degraded	A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process which has been used as a heat transfer fluid. It consists predominantly of hydrocarbons boiling in the range of approximately 190°C to 340°C (374°F to 644°F). This stream is likely to contain organic sulfur compounds.

97926-59-5	308-278-8	Gas oils (petroleum), light vacuum, thermal-cracked hydrodesulfurized	A complex combination of hydrocarbons obtained by catalytic dehydrosulfurization of thermal-cracked light vacuum petroleum. It consists predominantly of hydrocarbons having carbon numbers predominantly in the range of C14 through C20 and boiling in the range of approximately 270°C to 370°C (518°F to 698°F).
101316-59-0	309-865-1	Distillates (petroleum), hydrodesulfurized middle coker	A complex combination of hydrocarbons obtained by fractionation from hydrodesulphurised coker distillate stocks. It consists of hydrocarbons having carbon numbers predominantly in the range of C12 through C21 and boiling in the range of approximately 200°C to 360°C (392°F to 680°F).

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