



Aquatic hazard and biodegradability of light and middle atmospheric distillate petroleum streams



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HIGHLIGHTS

- Concentrations of dissolved hydrocarbons were stable in sealed test chambers.
- Acute toxicity (LL/EL₅₀) values ranged 0.3–5.5 mg L^{−1} loading rate.
- Chronic NOELR values ranged 0.05–0.64 mg L^{−1} loading rate.
- PETROTOX provided conservative estimates of acute and chronic toxicity.
- Biodegradation reflected extensive microbial oxidation of the test substances.

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ABSTRACT

Light and middle atmospheric distillate petroleum substances are blended to produce fuels used in transportation and heating. These substances represent the majority by volume of crude oil refined products in the United States. The goal of this research was to develop biodegradability and aquatic toxicity data for four substances; heavy, straight-run naphtha (HSRN), hydro-desulfurized kerosene (HDK), hydro-cracked gas oil (HCGO), and catalytic-cracked gas oil (CCGO). Ready biodegradability tests demonstrated rapid and extensive microbial oxidation of these test substances, indicating a lack of persistence in the aquatic environment. Differences in biodegradation patterns reflected compositional differences in the constituent hydrocarbons. Results of aquatic toxicity tests on alga, cladocera, and fish demonstrated that toxicity was greatest for catalytic-cracked gas oil, which contained a high proportion of aromatic hydrocarbons. Aromatic hydrocarbons are more soluble, and hence more bioavailable, resulting in higher toxicity. When expressed on the basis of loading rates, acute toxicity values (LL/EL₅₀) ranged between 0.3 and 5.5 mg L^{−1} for all three species, while chronic no-observed-effect loading rates (NOELR) ranged between 0.05 and 0.64 mg L^{−1}. PETROTOX estimates for acute and chronic toxicity ranged from 0.18 to 2.3 mg L^{−1} and 0.06 to 0.14 mg L^{−1}, respectively, which were generally more conservative than experimental data.

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1. Introduction

This paper reports new data on biodegradability and aquatic toxicity for substances selected from the gasoline/naphtha, kerosene/jet fuel, and gas oils categories which are used to complete the fate and effects profiles for these distillates. Approximately 400 petroleum substances, sponsored by API's Petroleum HPV Testing Group (PHPVTG), were organized into 13 categories to share data and minimize animal testing. These petroleum substances are

transported around the world and fall under one or more statutes for product classification and labeling (United Nations, 2005; OJEU, 2008). Environmental fate and effects data are used to assign hazard ranking under these regulations.

These petroleum substances, listed as Class II (TSCA) chemicals or "Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB)", do not exhibit specific and exact properties, but are characterized by a range for physical-chemical, toxicological, and environmental hazard values. Gasoline, kerosene, and gas oils can be grouped as light or middle atmospheric distillates. Light atmospheric naphtha distillates are low boiling point streams that are a major component in gasoline (US EIA, 2013). Kerosene and gas oils are middle

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atmospheric distillates used to produce jet and diesel fuels. These light and mid-atmospheric distillates have complex and variable compositions of *n*- and *iso*-paraffins, naphthenes, olefins, and aromatics hydrocarbons. Hydrocarbons comprising gasoline and their blending naphthas have approximately 4–12 carbon atoms, those comprising kerosene have 9–16 carbon atoms, and those consisting of gas oils have 9–30 carbon atoms (API, 2008, 2010, 2012). Each test substance was selected to provide new information on the environmental fate and hazards of the types of substances that they represent. Detailed compositional analyses of the kerosene and gas oil samples were used in the PETROTOX model to estimate aquatic toxicity. These results, together with existing data (API, 2008, 2010, 2012) provide a dataset that can be used to characterize the environmental fate and hazard of the broader categories.

2. Materials and methods

2.1. Test substances

HSRN was tested for biodegradability, HDK was tested for chronic aquatic toxicity, and HCGO and CCGO were tested for biodegradability, acute, and chronic aquatic toxicity. Samples of each test substance were characterized for boiling point range, density, and hydrocarbon type using ASTM methods. Carbon number range was determined either from comprehensive gas chromatography-mass spectrometry (GC-MS) analysis (petroleum naphtha) or from two-dimensional gas chromatography/fluorescence ionization detection (2D-GC/FID). High resolution hydrocarbon analysis (2D-GC/FID) for the kerosene and two gas oil test substances was performed following published methods (Blomberg et al., 1997; Edam et al., 2005; Forbes et al., 2006). Data and sample identification are reported in Table 1.

2.2. Aquatic toxicity tests

Testing of green algae (*Pseudokirchnerella subcapitata*), cladocera (*Daphnia magna*), and fish (*Oncorhynchus mykiss*) followed US EPA and OECD standard guidelines (OPPTS 850.5400, 850.1010, 850.1075; OECD 201, 202, 203). Fish tests employed the upper

threshold concentration (UTC) approach in order to reduce the number of test organisms (Jeram et al., 2005; ECVAM, 2006). The UTC approach is based on the observed trend where acute fish toxicity is often less severe than algae and *D. magna* when exposed to a variety of toxicants (Weyers et al., 2000). The UTC was defined as the lower of the two EL_{50} values from the *P. subcapitata* and *D. magna* tests. Fish were tested at the single UTC limit. When less than 50% mortality occurred at the UTC, no further testing was necessary since fish was not the most sensitive organism. *D. magna* 21-day reproduction tests followed OECD 211, and test endpoints were based on the numbers of neonates produced per surviving adult. Test details are summarized in Tables 2 and 3.

Water accommodated fractions (WAFs) were used as exposure solutions in all tests (Girling et al., 1992, 1994; ASTM, 2009). All WAFs were independently prepared on the basis of the loading rate (total mg test substance added L^{-1} dilution water). Test substances were added to dilution media in glass mixing bottles using stainless steel and glass syringes. Loading rates were determined from the volumes added and converted to mass per unit volume ($mg L^{-1}$) based on the density of test substance. The solutions were stirred at a rate to maintain a vortex of <10% of the static liquid depth for approximately 24 h, then permitted to settle one hour. The aqueous phase was collected from a bottom port in the mixing vessel, with the initial 75–100 mL solution being discarded. Prior to testing, a WAF equilibration trial was conducted to assess dissolution times and stability of dissolved hydrocarbons.

2.3. Estimation of aquatic toxicity by PETROTOX

PETROTOX (Redman et al., 2012) was used to estimate toxicity of the two gas oils and hydro-desulfurized kerosene. PETROTOX computes toxicity of complex petroleum substances based on the summation of the aqueous-phase concentrations of hydrocarbon blocks that represent each test substance and uses the Target Lipid Model (TLM) to calculate toxicity (Di Toro et al., 2000; CONCAWE, 2007; Redman et al., 2012). Input data for the model were provided by the 2D-GC/FID analyses. PETROTOX modeling was not done for the heavy straight-run naphtha because the aquatic hazard of this substance was previously characterized (API, 2008).

Table 1
Test substances and physiochemical characterizations.

CAS No.	Name	TSCA definition	Carbon number range	ASTM D2887 or D86 boiling point range (°F)			ASTM D4052 density ($g mL^{-1}$)	ASTM D1319 hydrocarbon type (Vol%)		
				Initial	50%	Final		Aromatics	Olefins	Saturates
64741-41-9	Naphtha (petroleum), heavy straight run	A complex combination of hydrocarbons obtained by the fractional distillation of petroleum. This fraction boils in a range of approximately 20–135 °C (58–275 °F)	6–12	210	252	352	0.7535	10	1	89
64742-81-0	Kerosene (petroleum), hydro-desulfurized	A complex combination of hydrocarbons obtained from a petroleum stock by treating with hydrogen to convert organic sulfur to hydrogen sulfide which is then removed. It consists of hydrocarbons having carbon numbers predominantly in the range of C9–C16 and boiling in the range of approximately 150–290 °C (302–554 °F)	6–22	226	408	646	0.8204	17	3	80
64741-77-1	Distillates, (petroleum), light hydro-cracked	A complex combination of hydrocarbons produced by the distillation of products from a hydrocracking process. It consists predominantly of saturated hydrocarbons having carbon numbers predominantly in the range of C10–C18, and boiling in the range of approximately 160–320 °C (320–608 °F)	8–30	274	412	555	0.8238	17	1	82
64741-59-9	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–C25 and boiling in the range of approximately 150–400 °C (302–752 °F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons	6–30	288	522	676	0.9618	75	7	18

Table 2

Summary of measured and estimated acute toxicity endpoints and summary of test design.

Category	<i>P. subcapitata</i> ^a		<i>D. magna</i>		<i>O. mykiss</i>	
	Loading rate 96-h $E_{xL_{50}}$ (mg L ⁻¹)	Measured ^b 96-h $E_{xC_{50}}$ (mg L ⁻¹)	Loading rate 48-h EL_{50} (mg L ⁻¹)	Measured ^b 48-h EC_{50} (mg L ⁻¹)	Loading rate 96-h LL_{50} (mg L ⁻¹)	Measured ^b 96-h LC_{50} (mg L ⁻¹)
<i>Kerosene, hydro-desulfurized (HDK)</i>						
CAS# 64742-81-0	nd		nd		nd	
PETROTOX	0.27		1.3		0.42	
<i>Gas oil, light hydro-cracked (HCGO)</i>						
CAS# 64741-77-1	3.0/5.3	0.51/0.85	5.5	1.0	>2.6	>0.54
PETROTOX	0.91		2.3		0.62	
<i>Gas oil, light catalytic-cracked (CCGO)</i>						
CAS# 64741-59-9	0.31/0.80	0.25/0.70	0.51	0.45	>0.30	>0.21
PETROTOX	0.20		0.35		0.18	
Test parameters: test type	Static		Static		Renewal, 24-h	
System	125-mL flasks; zero headspace; sealed caps		130-mL bottles; zero headspace; sealed caps		8-L bottles; zero headspace; sealed caps	
Replicates/no. of organisms	12/10 ⁴ cells mL ⁻¹ at start		4/5 < 24 h old		1/7 juveniles	
Duration	96 h		48 h		96 h	
Dilution medium	Algal nutrient medium with 400 mg NaHCO ₃ L ⁻¹ supplement		Moderately hard reconstituted		Moderately hard reconstituted	
Other test conditions	Shaken at 100 rpm, 24 ± 2 °C, 4300 lux continuous illumination		20 ± 2 °C under a 16 h light/8 h dark photoperiod		22 ± 1 °C under a 16 h light/8 h dark photoperiod	
Basis of EC_{50}/LC_{50}	Initial measured concentration		Mean measured concentration		Mean measured concentration	

nd = no data, hydro-desulfurized kerosene was not tested for acute toxicity.

^a Algal E_{xL}/C_{50} endpoints are provided first for biomass yield (E_bL/C_{50}) then growth rate (E_rL/C_{50}).^b Measured EC/LC_{50} values were based on mean measured hydrocarbon concentrations in the WAFs.**Table 3**

Summary of measured and estimated chronic effect data.

	<i>D. magna</i> 21-day reproduction tests ^a					
	Loading rate endpoints			Concentration endpoints		
	EL_{50} (mg L ⁻¹)	LOELR (mg L ⁻¹)	NOELR (mg L ⁻¹)	EC_{50} (mg L ⁻¹)	LOEC (mg L ⁻¹)	NOEC (mg L ⁻¹)
<i>Kerosene, hydro-desulfurized (HDK)</i>						
CAS# 64742-81-0	>0.48, <1.2	1.2 ^b	0.48	>0.092, <0.23	0.23 ^b	0.092
PETROTOX			0.12			
<i>Gas oil, light hydro-cracked (HCGO)</i>						
CAS# 64741-77-1	>0.64	– ^c	0.64	>0.13	– ^c	0.13
PETROTOX			0.14			
<i>Gas oil, light catalytic-cracked (CCGO)</i>						
CAS# 64741-59-9	0.24	0.10 ^b	0.05	0.18	0.075 ^b	0.038
PETROTOX			0.06			
Test parameters: test type	Static renewal, every 48 h					
System	130-mL bottles; zero headspace; sealed caps					
Replicates/no of organisms	10/1 < 24 h old neonates					
Duration	21 d					
Dilution medium	Moderately hard reconstituted water					
Feeding	<i>P. subcapitata</i> equivalent to 0.13 mg C daphnid ⁻¹ day ⁻¹ (Days 0–7) and 0.20 mg C daphnid ⁻¹ day ⁻¹ (Days 8–21); Vita-chem added during renewals at 25 µL L ⁻¹					
Other test conditions	20 ± 2 °C under a 16 h light/8 h dark photoperiod					
Basis for EC_{50} , LOEC, NOEC	Mean measured concentration					

^a Endpoints based on numbers of young produced per surviving adult.^b Statistically significant, $p < 0.05$.^c The LOELR and LOEC were not determined in this test.

2.4. Biodegradability studies

Ready biodegradability tests were run using a manometric respirometer (Co-ordinated Environmental Services, Ltd., Kent, England) according to OECD 301F. Oxygen consumption was measured during 28 or 47 days. The amount of oxygen taken up by the

microbial population over time was expressed as a percentage of the theoretical oxygen demand (ThOD) of each test substance.

Concentrations of 50 mg L⁻¹ were tested for naphtha, gas oils, the positive control sodium benzoate, a test substance toxicity control, abiotic control, and mineral salts medium blanks. Inoculum was prepared using fresh activated sludge from a sewage

treatment facility that received predominantly domestic sewage. The microbial activity of the inoculum was 10^5 colony-forming-units mL^{-1} in each test. Respirometry flasks were incubated at 20–22 °C.

2.5. Analytical methods

Dissolved hydrocarbon concentrations in the WAFs were analyzed using automated static headspace gas chromatography with flame ionization detection (HS GC-FID). A Perkin Elmer Autosystem XL gas chromatograph was used together with a 30 m \times 0.53 mm id, 1.5 μm film DB-5 (J&W Scientific) analytical column. Test substance standards and o-xylene internal standard solutions were prepared in acetone. Data were acquired and processed using Perkin Elmer TotalChrom Workstation software (Version 6.3.1). The total peak areas of chromatograms between 5.2 and 7.6 min (kerosene), 3.9 and 7.3 min (CCGO), and 3.5 and 6.3 min (HCGO) for eluted hydrocarbon components were summed for quantification of total solubilized hydrocarbons. The resulting concentrations of hydrocarbon components in the WAFs differed from the parent standards owing to the differing solubilities of individual gas oil hydrocarbon components. Due to the complex nature of the test substance, no attempt was made to identify and quantify specific hydrocarbon components solubilized in the WAFs.

2.6. Data analysis

The 50% lethal or effect loading rate (LL/EL₅₀) was defined as the loading rate of the test substance calculated to produce 50% effect in the test population for a given exposure period. The No Observed Effect Loading Rate (NOELR) was defined as the highest loading rate that did not exhibit a significant difference from the control.

The Lowest Observed Effect Loading Rate (LOELR) was the lowest loading rate that resulted in a significant difference in reproductive output from the control group. These calculations were derived for *D. magna* reproductive effects (fecundity, growth) and inhibition of growth rate and biomass yield in *P. subcapitata*. Measurements of total hydrocarbons in the WAFs were used to derive concentration-effect endpoints (LC/EC₅₀; NOEC/LOEC). The calculations were performed using standard statistical methods and computer software (Hamilton et al., 1977; SAS, 2008; Gulley and WEST, 1994; US EPA, 2001). Statistical tests were performed at the 5% significance level. Percent biodegradation values were calculated following the methods in the test guideline using Microsoft Excel® and the respirometer software.

3. Results

3.1. Test substance characterization data

Table 1 provides carbon number range, boiling point range, density, and hydrocarbon types for each of the test substances used in biodegradation and/or aquatic toxicity testing. Carbon numbers and distillation ranges reflect the refining processes these substances undergo. Saturated hydrocarbons made up the largest proportion of hydrocarbon types in the HSRN (89%), HDK (80%), and HCGO (82%). CCGO contained comparatively low saturate content (18%), but was correspondingly high in aromatics (75%).

A more comprehensive representation of the hydrocarbon composition of kerosene and the two gas oils was provided by the 2D-GC/FID data. Detailed analysis was not done for HSRN because no aquatic toxicity testing was conducted on this sample. Fig. 1A–C presents the 2D-GC/FID plots for HDK (A), HCGO (B), and

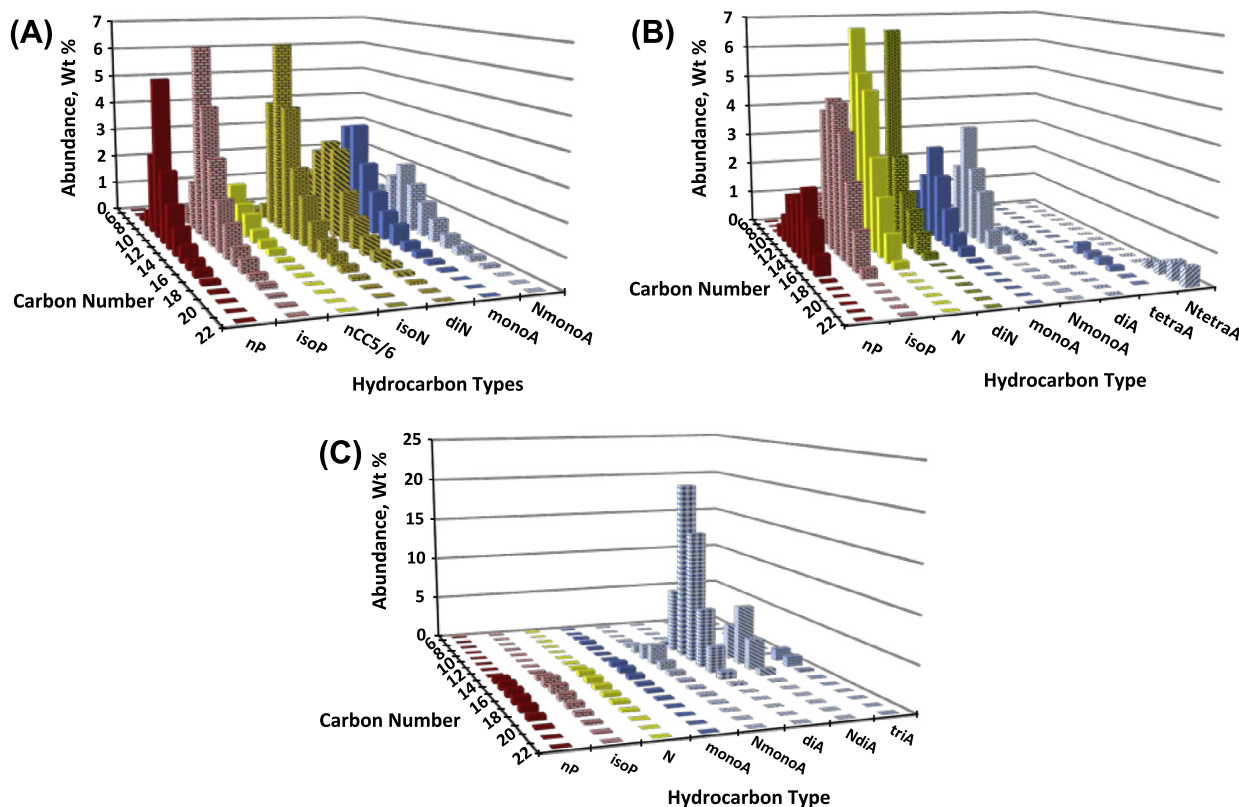


Fig. 1. Two-dimensional GCxGC plots for (A) hydro-desulfurized kerosene (CAS# 64742-81-0), (B) light hydro-cracked gas oil (CAS# 64741-77-1), and (C) light catalytic-cracked gas oil (CAS# 64741-59-9). Hydrocarbon types: np = normal paraffins; isop = iso-paraffins; N = naphthenes; nCC5/6 = substituted cyclopentanes and cyclohexanes; isoN = iso-naphthenes; diN = di-naphthenes; monoA = mono-aromatics; NmonoA = naphtheno-mono-aromatics; diA = di-aromatics; NdiA = naphtheno-di-aromatics; triA = tri-aromatics; tetraA = tetra-aromatics; NtetraA = naphtheno-tetra-aromatics.

CCGO (C). The saturate component of HDK consisted of *n*- and *iso*-paraffins, *iso*-naphthenes, di-naphthenes, and smaller proportions of substituted cyclopentanes and cyclohexanes (Fig. 1A). The aromatic fraction was composed of monoaromatic and naphtho-monoaromatic compounds. Polyaromatic constituents were essentially absent from the hydro-desulfurized kerosene. Analysis of HCGO showed saturates to be composed of *n*- and *iso*-paraffins, naphthenes and di-naphthenes; aromatics consisted of monoaromatic and naphtho-aromatic compounds (Fig. 1B). Trace amounts of polyaromatic compounds also were present. The hydrocarbon fingerprint of CCGO differed substantially from the HCGO sample. Saturated hydrocarbons in CCGO comprised only a small fraction when compared to the aromatic constituents (Fig. 1C). The aromatic fraction was dominated by di-aromatic compounds with lesser amounts of naphtho-di-aromatic compounds.

3.2. WAF equilibration and stability

A 24-h WAF equilibration was selected since no increase in total WAF hydrocarbons was measured beyond this equilibration time (Fig. 2A–C). Total hydrocarbon concentrations in the WAFs tended to remain steady in the sealed test chambers, and the presence of *Daphnia* food in the WAFs also did not substantially alter the

dissolved hydrocarbon concentrations (Fig. 2E and F). Analysis of hydrocarbon concentrations in WAFs as a percentage of the loading rates showed 60–88% of CCGO dissolved, compared to 15–31% of HDK, and 18–33% of HCGO (Fig. 3).

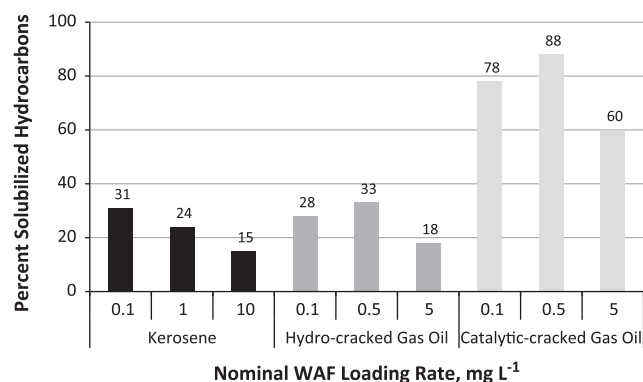


Fig. 3. Measured solubilized hydrocarbons in the WAFs as a percent of the loading rates.

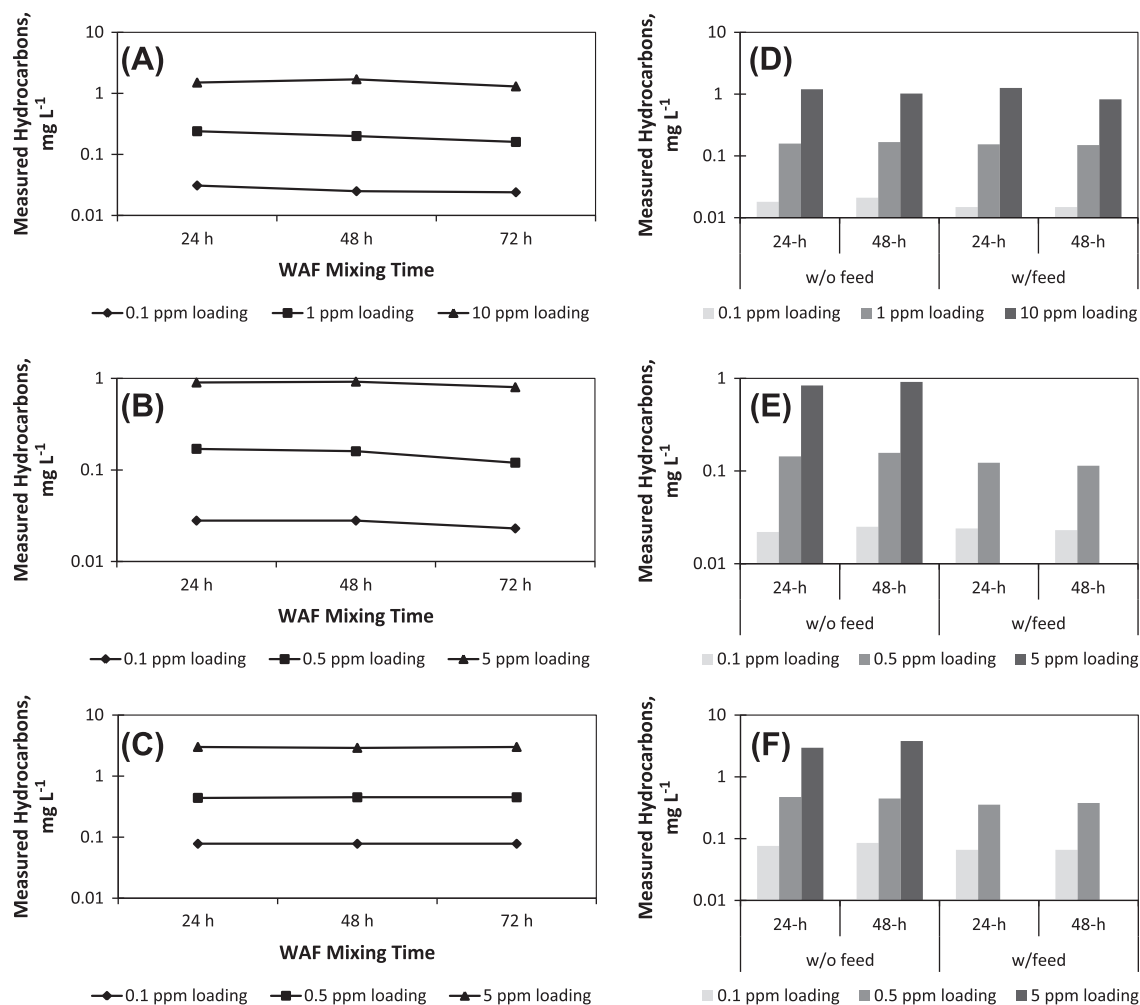


Fig. 2. Results of the equilibration trial showing dissolved hydrocarbon concentrations in WAFs of HDK (A), HCGO (B), and CCGO (C), after 24, 48, and 72-h stirring and concentrations of dissolved hydrocarbons in WAFs of HDK (D), HCGO (E), and CCGO (F) in sealed test vessels after 24-h and 48-h. No data were available for the 5 ppm HCGO and CCGO for treatments with *Daphnia* food present.

3.3. Aquatic toxicity testing and modeled data

3.3.1. Acute toxicity data

Table 2 presents acute toxicity test results of HCGO and CCGO and PETROTOX toxicity estimates for HDK using 2D-GC FID hydrocarbon composition. Toxicity endpoints were calculated on the basis of loading rates (EL/LL values) and measured hydrocarbon concentrations (EC/LC values). Lethal and other effect endpoints based on concentration measurements should not be interpreted as being equivalent to concentrations of the whole test substance. The distribution and percentage of hydrocarbon constituents measured in the WAFs differed from the parent test substance owing to the various water solubility values of the individual hydrocarbons. Therefore, measured concentrations do not represent all or the same proportions of hydrocarbons constituting the test substance. A comparison of toxicity results on a loading rate basis showed CCGO to be approximately ten times more toxic to *P. subcapitata* and *D. magna* than HCGO. *P. subcapitata* biomass (50% reduction) effects for the two gas oils occurred at 3.0 (HCGO) and 0.31 mg L⁻¹ (CCGO) loading, while 50% immobilization in *D. magna* was observed at 5.5 versus 0.51 mg L⁻¹, respectively. Algal endpoints based on growth rate showed less of a difference in effects between the gas oils than when compared to cell yields. Application of the UTC method (Jeram et al., 2005) for vertebrate testing proved advantageous in limiting the numbers of fish used in evaluating the toxicity of gas oils. The UTC was defined by the algal tests of HCGO (3.0 mg L⁻¹) and CCGO (0.31 mg L⁻¹). Fish tested at the UTC loading limit for both gas oils (2.6 mg L⁻¹: HCGO and 0.3 mg L⁻¹: CCGO) showed no mortality or adverse effects, thus no further testing of fish was necessary.

PETROTOX estimates of LL₅₀/EL₅₀ values for HDK were 0.27 mg L⁻¹ (*P. subcapitata*), 1.3 mg L⁻¹ (*D. magna*), and 0.42 mg L⁻¹ (*O. mykiss*). PETROTOX predicted CCGO was more toxic to all species than HCGO (Table 2), and this finding is consistent with the experimental data. Differences in predicted toxicity endpoints were approximately two to six times lower than the observed effects for the CCGO. For HCGO and CCGO, *O. mykiss* was predicted to be the more sensitive organism, but this was not corroborated by the test data.

Acute EC/LC₅₀ values determined from measured hydrocarbon concentrations in the WAFs are included in Table 2. The 96-h EC₅₀ values for the toxicity of HCGO to *P. subcapitata* were 0.51 mg L⁻¹ (biomass) and 0.85 mg L⁻¹ (growth rate), while those for CCGO were 0.25 mg L⁻¹ (biomass) and 0.70 mg L⁻¹ (growth rate). For *D. magna*, acute 48-h EC₅₀ values were 1.0 mg L⁻¹ for HCGO and 0.45 mg L⁻¹ for CCGO. For *O. mykiss*, the acute 96-h LC₅₀ values were >0.54 mg L⁻¹ (HCGO) and >0.21 mg L⁻¹ (CCGO).

3.3.2. Chronic toxicity data

Reproductive toxicity test data and PETROTOX estimates of reproductive NOELRs are presented in Table 3 for HDK, HCGO, and CCGO. Trends in chronic toxicity followed a similar pattern as that shown for acute toxicity. CCGO demonstrated the greatest reproductive toxicity to daphnids, followed by HDK and HCGO. The 21-day reproductive EL₅₀ for HDK fell between the 0.48 and 1.2 mg L⁻¹ exposure levels. The NOELR for HDK was 0.48 mg L⁻¹ based on a lack of statistically significant effects on reproduction and adult growth, while the LOELR of 1.2 mg L⁻¹ caused 90% mortality in the parent animals. Therefore the reproductive EL₅₀ lay between these two loading rates. No adverse effects on reproduction were evident at any of the HCGO loading rates used in the test. The reproductive NOELR and EL₅₀ were 0.64 mg L⁻¹ and >0.64 mg L⁻¹, respectively. The chronic reproductive EL₅₀, LOELR, and NOELR values for CCGO were 0.24 mg L⁻¹, 0.10 mg L⁻¹, and 0.05 mg L⁻¹, respectively. Corresponding endpoints determined for measured hydrocarbon concentrations are shown in Table 3.

PETROTOX estimates of chronic toxicity for HDK, HCGO, and CCGO showed reasonably good agreement to the endpoints defined in the tests. Differences can be attributed to variability in species sensitivity or loss of hydrocarbons during manipulations of the WAFs. These modeled estimates complement the test data for describing the chronic toxicity hazards for the categories.

3.4. Biodegradation

The biodegradation plots for HSRN, HCGO, CCGO, and sodium benzoate illustrate the rapid consumption of these petroleum substances and reference chemical by the activated sludge inoculum (Fig. 3). HSRN and HCGO reached 77% and 64% biodegradability at 28 days, respectively, and met the 60% ready biodegradability criteria. CCGO degraded by 56%, and did not meet the criteria. By 47 days, CCGO degraded to 61% and HCGO reached 74% degradation, although the majority of both substances had been consumed within the first 28 days. These data show substantial ultimate biodegradability since the remaining fraction (e.g., 30–40%) is assumed to be assimilated as microbial biomass. Although not shown, the patterns of biodegradation of the toxicity control treatments approximated those for each test substance alone, indicating no inhibitory effect of the test substances on biodegradation.

4. Discussion

4.1. Loading rates and WAF equilibration

Petroleum substances have long been recognized as being 'difficult to test' in aquatic toxicity studies due to the variable water solubility and volatility characteristics of the hydrocarbon constituents (Bennett et al., 1990; ECETOC, 1996; OECD, 2000). Insoluble oil retained in the exposure solutions creates gross exposure hazards such as physical entrapment, loss of buoyancy, and fouling of gill surfaces from the bulk oil product. To distinguish these effects from true chemical toxicity during aquatic toxicity testing and to avoid masking the chemical toxicity by physical effects of the oil on the test organism, it is necessary to eliminate insoluble oil substance in the exposure vessels (Girling et al., 1992, 1994).

The dissolution of the hydrocarbons of HDK, HCGO, and CCGO in the WAFs were examined (Fig. 2A–C). All test substances achieved equilibrium between the oil product and aqueous phases after 24 h of stirring, regardless of loading rate. Once WAFs were placed in sealed test chambers with no headspace, little or no loss of dissolved hydrocarbons occurred (Fig. 2E and F).

Solubilization of hydrocarbon type was influenced both by loading rate and hydrocarbon composition of the test substance. Mono and di-aromatic constituents, present at higher percentages in petroleum streams such as CCGO have greater solubility than saturate hydrocarbon constituents, as in HCGO and HDK, which are present at a lower percentage in the dissolved phase. This was illustrated by the comparison of the percentage of the loading rates that dissolved into the WAFs for the two gas oils (Fig. 3). The ratio of dissolved total hydrocarbons to loading rate generally declines as loading rate increases. The percentages of the solubilized hydrocarbons at the 0.1 mg L⁻¹ loading rate for HCGO and CCGO did not follow this pattern, but this was likely due to analytical limitations at that loading rate. As more petroleum product is added to water, only the more soluble components continue to dissolve, until maximum solubility is reached for each constituent. This means that the ratios of the dissolved hydrocarbon constituents in WAFs will not be equal to their proportions in the test substance (Shiu et al., 1990). Therefore, toxicity endpoints determined on the basis of dissolved concentrations, i.e., LC/EC₅₀, should not be considered equivalent to concentrations of the whole test substance.

Loading rate is a necessary element in the description of toxicity test results, as it provides a unifying concept for expressing the results of toxicity tests of poorly-soluble, multi-constituent petroleum substances. The concept and rationale for loading rates have previously been described (Girling et al., 1992, 1994), and their use has found acceptance in scientific and regulatory bodies (GESAMP, 2002; OJEU, 2005; ASTM, 2009; ECHA, 2012).

4.2. Acute toxicity

Loading rate endpoints for the two gas oils are similar to those reported for other gas oil samples. Measured EL/LL₅₀ values reported by Redman et al. (2012) ranged between 0.28 and 25 mg L⁻¹ for *P. subcapitata* (*n* = 8), 0.32–42.2 mg L⁻¹ for *D. magna* (*n* = 15), and 6.6–65 mg L⁻¹ for *O. mykiss* (*n* = 3). EL/LL₅₀ values from our tests fell at the low end of those ranges. The absence of any response by *O. mykiss* at the UTC for either gas oil prevented comparison to fish values in Redman et al. (2012).

When EL₅₀ values for *P. subcapitata* and *D. magna* are considered on the basis of loading rates, there was roughly a ten-fold difference between the high saturate gas oil (HCGO) and the high aromatic gas oil (CCGO) (Table 2). On the basis of dissolved hydrocarbon concentration, the EC₅₀ values differed by about a factor of two. The “higher effective loading rate” for HCGO is due to the lower proportion of water soluble constituents as compared to the high aromatic gas oil. CCGO showed greater toxicity than HCGO on a loading rate basis due to the higher proportion of soluble aromatics, and hence, more bioavailable hydrocarbon constituents.

Bioavailability of the hydrocarbon types is also dependent on aqueous solubility cut-off values for saturated and aromatic hydrocarbons. As chain length and/or molecular weight increases, aqueous solubility decreases to a concentration where no biotic effect occurs. Adema and van den Bos Bakker (1987) demonstrated this point occurs at C10 for paraffins (i.e., no acute toxicity was observed in a saturated solution of *n*-decane and higher molecular weight paraffins). For a series of alkylbenzenes, the water solubility cut-off was reported to be C14 (Adema, 1991). The test substances used in this study are not exclusively composed of *n*-paraffins or mono-aromatics, but the solubility behaviors of other saturated and aromatic hydrocarbons for structures of equal or near equal molecular weights are expected to follow a similar relationship. In addition to the effect of hydrocarbon type on solubility, CCGO has constituents of lower molecular weight than HCGO. This was evident by the carbon number ranges of C8–30 and C6–C30 for HCGO and CCGO, respectively. The C6 and C7 fraction of CCGO would have included constituents of water soluble aromatic and saturated compounds.

Both alkyl and aryl hydrocarbons are believed to exert their toxicity primarily via a common nonpolar narcosis mode of action, and all hydrocarbons in solution are expected to contribute to toxicity (Peterson, 1994). Toxicity is manifested in the organism once a critical body burden is attained, thus the organism–water partitioning of the dissolved constituents is an important aspect of the process (van Wezel and Opperhuizen, 1995). Individual hydrocarbons have different partition coefficients that vary with molecular weight and isomeric structure. The endpoint variability on a dissolved hydrocarbon basis shown by the two gas oil tests may be explained by differences in target organ partitioning behavior of the hydrocarbon types (McCarty and Mackay, 1993; Peterson, 1994; van Wezel and Opperhuizen, 1995).

The estimated EL/LL₅₀ endpoints derived using PETROTOX support the relative toxicity of the two gas oils determined by the test data. Predicted endpoints for the three test species were all lower than test results. Compared to estimates of Redman et al. (2012), our EL/LL₅₀ estimates proved similar for gas oils but fell slightly

lower than for kerosene. Endpoint values for several gas oils reported by Redman et al. (2012) for *P. subcapitata* and *D. magna* ranged 0.2–0.92 mg L⁻¹ (*n* = 5) and 0.35–2.30 mg L⁻¹ (*n* = 5), respectively. No PETROTOX estimates for *O. mykiss* were reported. Ranges reported for kerosene were 1.03–1.31 mg L⁻¹ (*P. subcapitata*, *n* = 3), 2.52–3.81 mg L⁻¹ (*D. magna*, *n* = 4), and 1.72–2.24 mg L⁻¹ (*O. mykiss*, *n* = 3) (Redman et al., 2012).

Some conservatism in the predicted endpoints may be expected as the PETROTOX model cannot account for all potential losses of hydrocarbon constituents from WAFs, particularly for fish tests (McGrath et al., 2005; Redman et al., 2012). Manipulations of WAFs during preparation and distribution to test chambers can result in loss of hydrocarbon components from the WAFs. The PETROTOX model does not account for these potential losses, and the reduced exposure in toxicity tests would result in higher endpoint values than what the model predicts.

4.3. Chronic toxicity

No 21-d reproduction studies with *D. magna* using WAFs or PETROTOX chronic endpoint estimates have been previously reported in the literature for kerosene or gas oil substances. The chronic toxicity endpoints from the reproduction studies on the two gas oils (Table 3) showed a similar relationship as demonstrated for acute toxicity. Based on a reduction in the numbers of young produced per surviving adult, the high aromatic CCGO demonstrated the greatest chronic toxicity of the three test substances. No significant adverse effects on reproduction (*p* > 0.05) were measured for the HCGO WAFs prepared at any loading rates. Comparison of the chronic data endpoints for the two gas oils clearly demonstrates that composition influences bioavailability and toxicity of these substances. The test of HDK achieved both effect and no-effect levels. While the 0.48 mg L⁻¹ loading rate caused no adverse effect on reproduction, the next higher loading rate of 1.2 mg L⁻¹ caused significant mortality and reduced neonate production. These data show that when steps are taken to control the loss of dissolved constituents in the exposure WAFs, these middle atmospheric distillates can show chronic toxicity below one part per million as loading rate and dissolved hydrocarbon constituents.

PETROTOX estimates of chronic NOELRs were in good agreement with test NOELR for CCGO, but more conservative than those obtained for HCGO and kerosene. Loss of volatile components during handling of the HCGO and kerosene WAFs may have played a role in reducing the exposure concentrations.

4.4. Biodegradation

Biodegradation testing of petroleum substances presents a challenge due to water solubility limits and volatilization, so the appropriate method is important to establish the capacity of these substances to degrade. The respirometry method (OECD 301F) makes use of sealed incubation vessels and zero headspace to prevent volatile loss and enhance solubility.

Biodegradation results demonstrated rapid utilization of the petroleum substances by the microbial inoculum (Fig. 4). The relative differences in patterns of biodegradation of these substances can be explained by molecular weight and hydrocarbon type characteristics. Low molecular weight components degrade relatively easily, while higher molecular weight components require more time to mineralize (Prince, 2002; Seo et al., 2009). Molecular structure also affects microbial degradation. Highly branched alkanes and multi-ring cycloalkanes are slower to degrade than their straight-chain analogs (Schaeffer et al., 1979; Solano-Serena et al., 1999; Prince, 2002).

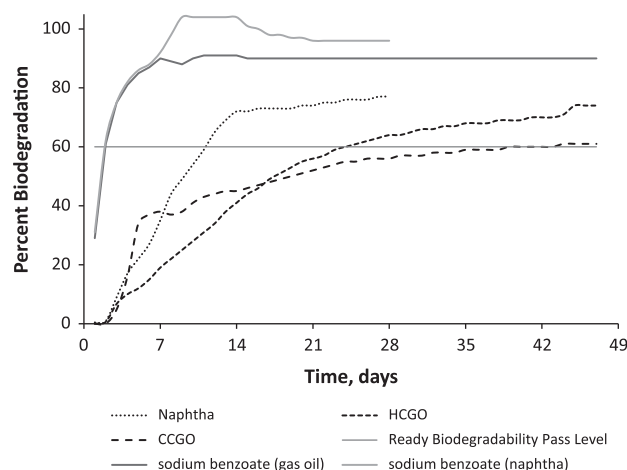


Fig. 4. Percent biodegradation plot for heavy straight-run naphtha, hydro-cracked gas oil, and catalytic-cracked gas oil.

With a relatively low and narrow boiling range, and high alkyl composition, HSRN proved the most amenable to biodegradation. It showed the greatest extent and rate of biodegradation of the three substances, easily meeting the 28-d criteria for ready biodegradability. HCGO was composed of a similar proportion of saturated components, but included higher molecular weight compounds that required more time to degrade. The HCGO oil achieved similar biodegradation as HSRN (74% biodegradation) by day 47. Biodegradation of CCGO showed an initial rapid phase that likely represented simple paraffins and monoaromatic compounds.

5. Conclusions

Acute and chronic aquatic toxicity tests of the two gas oils together with chronic aquatic toxicity of hydro-desulfurized kerosene illustrates the impact that hydrocarbon composition of a petroleum substance has on bioavailability. Test substances with the larger proportion of aromatic constituents showed greater toxicity, due to the greater bioavailability of aromatic hydrocarbons, when compared to saturated hydrocarbons on an equivalent molecular weight basis. The reported acute and chronic aquatic toxicity results are new data for HDK, HCGO, and CCGO.

PETROTOX estimates of toxicity endpoints generally overestimated toxicity, but in lieu of testing many individual samples, PETROTOX can provide conservative estimates of aquatic toxicity.

Biodegradation experiments showed these light and middle atmospheric distillate substances biodegrade at a relatively rapid rate. The differences in biodegradation patterns for each test substance reflect the molecular weight and compositional differences in the constituent hydrocarbons.

Testing WAFs is the most reliable method to assess aquatic effects of hydrocarbon UVCB substances. The loading rate method provides a consistent basis for aquatic testing of UVCB hydrocarbons, and has found acceptance in scientific and regulatory bodies (GESAMP, 2002; OJEU, 2005; ASTM, 2009; ECHA, 2012).

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