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Primary biodegradation of petroleum hydrocarbons in seawater

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ABSTRACT

This report describes primary biodegradation experiments performed to determine the persistence of higher molecular weight petroleum hydrocarbons in seawater. Results from the biodegradation experiments show that the majority of tested petroleum hydrocarbons have half-lives in seawater less than 60 days.

KEYWORDS

Primary biodegradation, seawater, petroleum hydrocarbons, half-life

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SUMMARY

Three separate studies were performed to measure primary biodegradation half-lives ($t_{1/2}$) of a series of pure hydrocarbons in seawater. Primary biodegradation rates of multiple hydrocarbons were measured simultaneously. The compounds tested are representative of the principal higher molecular weight aliphatic and aromatic hydrocarbon classes found in heavier petroleum products and refinery streams. The results of this study were used to ensure that sound environmental decisions are made with regard to the assessments of petroleum substances for their persistent, bioaccumulative and toxic (PBT) properties.

The first study included 34 hydrocarbons (mostly liquid form). The second study included 37 hydrocarbons (mostly solid form). A third study included a series of two and three ring cycloalkanes (naphthenes) plus phenanthrene (n = 10). The studies were conducted with concentrations of the hydrocarbons at or below their limit of solubility. In total, primary biodegradation half-lives in seawater for 74 pure hydrocarbon compounds are reported. Half-lives for seven additional compounds could not be determined, mostly due to lack of sufficient aqueous solubility.

Because of the extremely low water solubility (low parts-per-billion) of the compounds being tested, a novel partition controlled / passive dosing system was used to generate aqueous concentrations. The calculated half-life data for the hydrocarbons are summarized. Most (73%) of the compounds tested and for which primary biodegradation rates could be determined, had measured seawater half-lives of less than 60 days and a majority of these (42%) had half-lives of less than 30 days. Persistent compounds ($t_{1/2}$ >60 days) tended to have one of the following structural characteristics:

- extensive branching (i.e. 2,2,4,4,6,8,8-heptamethylnonane; 2,7-diisopropylnaphthalene; 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-benzene).
- fully or mostly saturated four ring aromatics (i.e. perhydrochrysene; 4,5,9,10-tetrahydropyrene).

1. INTRODUCTION

Petroleum substances are generally complex mixtures of hydrocarbons with variable composition, considered to be substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs). The hydrocarbons comprising petroleum products have different physical-chemical properties, which have an impact on their distribution in the environment and their rate of microbial biodegradation. Few reliable primary biodegradation data are available on hydrocarbons derived from petroleum products, making the persistence assessment for petroleum products uncertain.

The studies reported here were conducted to determine the primary biodegradation half-life in seawater of a large series of higher molecular weight hydrocarbons. The compounds tested in these studies represent the principal aliphatic and aromatic hydrocarbon classes comprising heavier petroleum products and refinery streams. The use of natural seawater provides a convenient means of measuring the primary biodegradation rates of multiple hydrocarbons simultaneously. There are currently no applicable test guidelines for measuring the primary biodegradation rates of multiple compounds with very low aqueous solubility; therefore, a new experimental procedure was designed. The results of these studies were used to ensure that sound decisions are made with regard to the assessments of petroleum substances for their persistent, bioaccumulative and toxic (PBT) properties.

2. MATERIALS AND METHODS

2.1. TEST SUBSTANCES

Three experiments were performed to measure primary degradation of petroleum hydrocarbons in seawater. The test compounds in the three experiments are listed in the tables below.

Table 1 Compounds in liquids study (MRD-08-224)

MRD-08-224 compounds*	CASRN
trans-decalin	91-17-8
2,2,4,6,6-pentamethylheptane	13475-82-6
1,3-dimethyladamantane	702-79-4
2,3-dimethyldecane	17312-44-6
2-methyl-1-undecene	18516-37-5
n-dodecane	112-40-3
2,6-dimethylundecane	17301-23-4
2,2,3-trimethyldecane	62338-09-4
4-methyldodecane	6117-97-1
1-tridecene	2437-56-1
bicyclohexyl	92-51-3
2,2,4,4,6,8,8-heptamethylnonane	04390-04-9
1,3,5-tris(1-methylethyl)-benzene	717-74-8
n-heptylcyclohexane	5617-41-4
2-isopropyldecalin	N/A
2-butyl-1-decene	51655-65-3
2,6,10-trimethyldodecane	3891-98-3
n-octylcyclohexane	1795-15-9
n-octylbenzene	2189-60-8
2,7-diisopropyldecalin	N/A
n-hexadecane	544-76-3
decylbenzene	104-72-3
2,6,10,14-tetramethylpentadecane (pristane)	1921-70-6
2,7-diisopropylnaphthalene	40458-98-8
hexadecahydropyrene	2435-85-0
2-hexyltetralin {MRD-05-452}	66325-09-5
2,6,10,14-tetramethylhexadecane (phytane)	638-36-8
1-methyl-7-(1methylethyl)-hydrophenanthrene (hydrogenated retene) {MRD-07-116}	N/A
4-pentyl-1,1'-biphenyl	7116-96-3
2,3-dimethyl-5-(4methylpentyl)-naphthalene	204256-07-5
dodecahydro-terphenyl (dicyclohexylbenzene) {MRD-08-159}	1087-02-1
3-phenylbicyclohexyl	33460-02-5
dehydroabietine	5323-56-8
hexahydroterphenyl (cyclohexylbiphenyl) {MRD-08-159}	N/A

**This study included a series of individual liquid compounds, some of which are also identified by other MRD numbers, indicating that they were synthesised for this study.*

Table 2 Compounds in solids study (MRD-08-225)

MRD-08-225 compounds*	CASRN
1,2,3,4,5,6,7,8-octahydrophenanthrene	5325-97-3
phenanthrene	85-01-8
anthracene	120-12-7
benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-	1889-67-4
6-n-butyl-2,3-dimethylnaphthalene	N/A
o-terphenyl	84-15-1
1-methylphenanthrene	832-69-9
perhydrochrysene {MRD-08-185}	2090-14-4
1,2,3,10b-tetrahydrofluoranthene	20279-21-4
4,5,9,10-tetrahydropyrene	781-17-9
9-methylanthracene	779-02-2
perhydroterphenyl (tricyclohexyl) {MRD-08-158}	1706-50-9
n-eicosane	112-95-8
isopropylhydrophenanthrene (fichtelite)	2221-95-6
5α(H)-androstane	438-22-2
1,2,3,6,7,8-hexahydropyrene	1732-13-4
fluoranthene	206-44-0
pyrene	129-00-0
m-terphenyl	92-06-8
9-n-butylphenanthrene	10394-57-7
dodecahydrochrysene {MRD-08-183}[1]	1610-22-6
1-methyl-7-(1-methylethyl)-phenanthrene (retene)	483-65-8
benzo(b)fluorene	243-17-4
1-methylpyrene	2381-21-7
hexahydrochrysene {MRD-08-183}	2091-91-0
octahydrochrysene {MRD-08-183}	N/A
tetrahydrochrysene	2091-90-9
Benzo(a)anthracene (1,2-benzanthracene)	56-55-3
chrysene	218-01-9
triphenylene	217-59-4
7-methylbenzo(a)anthracene	2541-69-7
benzo(k)fluoranthene	207-08-9
benzo[a]pyrene	50-32-8
3-methylcholanthene	56-49-5
benzo(ghi)perylene	191-24-2
Dibenzo(a,h)anthracene	53-70-3
indeno(1,2,3-cd)pyrene	193-39-5

**This study included a series of individual solid compounds, some of which are also identified by other MRD numbers, indicating that they were synthesised for this study.*

[1] Substances identified with MRD-08-183 were part of a mixture of hydrogenated chrysenes synthesized in-house

Table 3 Compounds in cycloalkanes study (MRD-09-498). This study included a series of compounds which are also identified by other MRD numbers.

MRD-09-498 compounds*	CASRN
2-isopropyldecalin (MRD-08-396)	1010-74-8
n-propyldecalin (MRD-09-481)	91972-45-1
2,6-dimethyldecalin (MRD-09-495)	1618-22-0
2,3,6-trimethyldecalin (MRD-09-495)	N/A
1,4,6,7-tetramethyldecalin (MRD-09-495)	N/A
perhydro-1-methylfluorene (MRD-09-496)	N/A
perhydrophenanthrene (MRD-09-496)	5743-97-5
perhydro-2-methylanthracene (MRD-09-496)	N/A
perhydro-3,6-dimethylphenanthrene (MRD-09-497)	N/A
Phenanthrene	85-01-8

**This study included a series of individual liquid compounds, identified by other MRD numbers, which were synthesised for this study.*

Hexachlorobenzene (HCB CASRN 118-74-1), dissolved in silicone oil, was used as a negative control.

Neat test substances were stored at room temperature. Both the test substance mixtures and negative control mixture (HCB in silicone oil) were stored at room temperature.

2.2. CHARACTERIZATION OF TEST SUBSTANCES

Records indicating the test substances' identity (chemical name and CASRN, if applicable) and purity (if known) will be maintained by the testing laboratory but not included in this report. A majority of the test substances were purchased from commercial sources and the supplied certificates of analysis serve as documentation of their identity and purity (generally greater than 95%).

A number of the compounds tested were synthesised by the ExxonMobil Research and Engineering Corporate Strategic Research laboratory. The synthesised compounds were primarily fully or partially saturated aromatics (naphthenes) produced by catalytic hydrogenation of the corresponding di-aromatic or poly-aromatic hydrocarbon. The synthesized compounds are those listed that include an MRD number identifier in addition to the chemical name. The products of these syntheses typically yielded varying degrees of saturation and isomeric positions, therefore test substances were a mixture of isomers. The identity of specific naphthenic compounds in these products was determined by GC-MS and the characterization data are maintained in the testing facility's compound preparation records. Test characterization data are not reported.

2.3. TEST SUBSTANCE MIXTURE

For the first two studies, separate mixtures containing each of the liquid and solid hydrocarbon test substances were prepared by combining varying amounts of each of the neat compounds. The individual test substance compounds have very low aqueous solubilities. Based on Raoult's law and partition controlled - passive dosing

theory, the mole fraction added for each compound was inversely related to the compounds' expected water solubilities (i.e. the test mixture contained a greater amount of the less water soluble hydrocarbons and lesser amounts of the more water soluble hydrocarbons). The amount of each individual solid hydrocarbon added to the solids mixture in 10 ml silicone oil ranged from approximately 0.01 to 0.3 g. The solids mixture was heated to approximately 100°C and mixed to dissolve as much of the hydrocarbons in the silicone oil as possible. The solids mixture was then permitted to cool to room temperature and centrifuged. The silicone oil supernatant, free of visible solid hydrocarbons, was used. For the third study, a single mixture was prepared containing both the test substances and HCB as a conserved marker. Solid phenanthrene and HCB were first solubilized in silicone oil by heating to approximately 100°C. This mixture was then permitted to cool to room temperature and centrifuged to remove the excess solid crystals. The supernatant was removed and spiked with the remaining liquid cycloalkanes.

2.4. PREPARATION OF PASSIVE DOSING DEVICES

The application of a partition controlled (passive) dosing technique permits the relatively rapid solubilisation in seawater of many very hydrophobic hydrocarbons simultaneously without exceeding the water solubility of any individual compound.

Silicone tubing was used as a carrier (60 cm length, 1.5 mm inner diameter, 0.24 mm wall thickness). Approximately one millilitre of each of the hydrocarbon saturated silicone oil mixtures was loaded into the carrier and the ends of the silicone tubing secured to prevent leakage. For the ‘liquids’ and ‘solids studies’, a separate length of silicone tubing was loaded with approximately one millilitre of silicone oil saturated with HCB and the ends of the silicone tubing secured to prevent leakage. A HCB-saturated silicone oil silicone tubing device was prepared for each of the liquid and solid testing systems. For the ‘cycloalkanes study’, a single length of silicone tubing was utilized containing a mixture of both the test substances and HCB. The passive dosing devices were stored separately in glass-distilled water in small vials with minimal headspace at room temperature prior to use.

2.5. TEST MEDIUM

Natural seawater was obtained from the Atlantic Ocean at Sandy Hook, NJ (Gateway National Recreation Area), USA, a relatively unpolluted site. Seawater was collected within one hour after mean high tide. The collected seawater was stored at laboratory room temperature and continuously aerated. The seawater was not expected to contain any contaminants at levels which would interfere with the studies. Seawater blank samples, filtered and amended with nutrient solution, were analyzed at each interval to measure the extent of potential background concentrations of hydrocarbons relative to the concentrations of the test substances. The seawater was permitted to settle and then coarse-filtered through #4 Whatman filter paper under slight vacuum and aerated prior to use. The filtered seawater was characterized with respect to salinity and dissolved organic carbon (DOC).

The natural microbial population present in the seawater served as the bacterial inoculum. The inoculum was not acclimated nor was it amended with any additional microbial population. No bacterial population counts were performed. Bacto Bushnell-Haas Broth, Dehydrated (Difco Laboratories) was used as a nutrient

solution. It was prepared at a concentration of 3.27 g/l. The solution was not sterilized, it was prepared no more than one day prior to use.

The test medium consisted of ca. 3.5 l coarse-filtered, aerated seawater containing the equivalent of 1% (i.e. 35 ml in 3.5 l) of Bushnell-Haas broth (described above). The test media was analyzed for DOC.

2.6. TEST SUBSTANCE AND NEGATIVE CONTROL IN TEST MEDIA

Two passive dosing devices were placed in the test media for the 'liquid' and 'solids studies', one containing the test hydrocarbon mixture and the second containing HCB (conserved marker) in silicone oil. For the 'cycloalkanes study', a single device containing a mixture with both the test compounds and HCB conserved marker was placed in the test media. Depending on the water solubility of the test substances, the mixtures were stirred from one to three days to permit the hydrocarbons to saturate the test medium at concentrations permitting analytical detection.

2.7. TEST SYSTEMS

Individual test system replicates consisted of amber glass headspace vials with aluminium screw caps and Teflon septum. Each vial contained ca. 15 ml of test media saturated with test substance compounds and the negative control compound. The exact capacity of the headspace vials was 22 ml leaving seven millilitres of headspace in each vial to supply oxygen for the duration of the study. It was expected that based on their respective volatilities, the hydrocarbons would partition between the aqueous and headspace phases. No assessment of this partitioning was performed. The direct immersion SPME (solid-phase microextraction) technique was expected to only extract those compounds dissolved in the aqueous phase. Sufficient test system replicates were prepared to permit analysis of a minimum of nine test system replicates at each sampling interval. The partition - passive dosing technique employed ensured that no individual test substance compound exceeded its aqueous solubility in seawater.

Poisoned control systems were prepared and served to provide standardization at each sampling interval. These were prepared as described above for the test systems except each replicate (vial) contained 100 mg/l mercuric chloride (0.15 ml of a 10,000 mg/l $HgCl_2$ solution). A sufficient number of poisoned control systems were prepared to permit analysis of a minimum of five replicates at each sampling interval.

An approximately 500 ml volume of test media was taken prior to addition of the passive dosing devices and contained no added test substance compounds or negative control compound. The blank test media permitted for analysis of background levels of test substance hydrocarbons and negative control compound.

Systems were identified to differentiate between "test" and "poisoned control" systems. Individual replicates were then selected at random for analysis at each sampling interval.

The test and poisoned control systems were incubated at 20°C ± 1°C and continually agitated at 120 rpm on an orbital shaker. The initial preparation of test substance compounds and negative control compound in test media were done at room temperature.

2.8. ANALYSIS AND QUANTIFICATION OF TEST SUBSTANCES IN TEST SYSTEMS

Test and poisoned control system replicates were analyzed at each sampling interval using an Agilent 6890N Gas Chromatograph with a 5973N Mass Selective Detector (GC-MSD) operated in the selective ion monitoring (SIM) mode. For each compound, a primary ion was used for quantification and a secondary was used as a qualifier to provide confirmation of the compounds' identity. The instrument was equipped with a J&W Scientific DB-5MS capillary column (30 m x 0.20 mm inner diameter x 0.33 µm film thickness).

SPME was performed in the direct immersion mode and sample injection was automated using a CTC Analytics auto sampler. A 30 µm polydimethylsiloxane (PDMS) fibre was used for extraction of the liquid hydrocarbons and cycloalkanes while a 7 µm PDMS fibre was used for the solid hydrocarbons. The fibres were automatically desorbed in the GC inlet for 5 minutes at 275°C (liquid and cycloalkane compounds) or 300°C (solid compounds). The column flow was 0.7 ml/min. The initial oven temperature for the liquids and cycloalkanes analysis was 40°C and 50°C for the solids and programmed to a final oven temperature of 340°C for both analyses.

Prior to analysis of each poisoned control or test sample at the respective sampling intervals, two microliters of an approximately 48 µg/ml semi-volatile internal standard solution in acetone was added to each replicate. This resulted in an equivalent concentration of approximately 6.4 ng/ml, in the same order of magnitude as the concentration of the dissolved test hydrocarbons. The internal standard consisted of the following deuterated compounds: d4-dichlorobenzene, d8-naphthalene, d10-acenaphthene, d10-phenanthrene, d12-chrysene and d12-perylene.

Quantification of absolute concentrations of the individual hydrocarbons was not performed. At each sampling interval, the poisoned controls served as the standards to which the responses of the test samples were compared. Relative response factors were generated by calculating the ratio of the respective internal standard responses to those of the test compounds in the poisoned controls which were each assigned a value of 100%. Mean relative (to a corresponding internal standard compound) response factors (RRF) were determined. A minimum of five poisoned control samples were analyzed at each sampling interval to calculate the RRF. This RRF was then applied to the compounds in each of the individual test samples analyzed at each timed interval. A minimum of nine test replicates were analyzed at each time point. The relative amounts of each hydrocarbon test compound in each sample were then normalized to the response of the HCB conserved marker present in each sample.

3. EXPERIMENTAL PROCEDURE

One to two days prior to the start of the study, ca. 3.5 l of test media was prepared in a 4 l glass aspirator bottle containing a Teflon stir bar. The test medium was aerated and gently stirred for at least one hour. Approximately 150 ml of aerated test media was removed for dissolved oxygen and DOC determinations. Approximately 500 ml of test media (containing no added test substance or negative control compound) was removed and stored at 20°C to be used for blank samples throughout the tests. The passive dosing devices were then added to the approximately 3 l of remaining test media. The aspirator bottle was stoppered with a Teflon screw plug and the contents mixed on a stir plate for one to three days at room temperature.

On test day 0, aliquots were dispensed to prepare individual test systems and poisoned control systems. After all replicate systems were prepared, a minimum of nine test and five poisoned control systems were randomly selected for t=0 analyses. The remaining replicate vials were placed on a shaker table where they were agitated at 120 rpm. The shaker table was located in an incubator and maintained at 20°C ± 1°C for the duration of the study. Subsequent sampling and analysis of test, poisoned control and blank replicates occurred after approximately 7, 14, 30, 60, 90 and 180 days. For the cycloalkanes study, sampling occurred only through day 64. At each sampling interval, a minimum of nine test systems, and five poisoned control systems were randomly selected, spiked with the internal standard solution and analysed by SPME GC-MSD.

Calculations

The half-life ($t_{1/2}$) and primary biodegradation rate constant (k) were determined for each test compound using the calculated percentages relative to the internal standard normalized responses for each test compound in the corresponding poisoned controls at each interval. These amounts were then normalized to the amount of HCB measured in each sample. In general, data were included through time intervals in which at least 10% of the initial concentration remained. Results were plotted using Microsoft Office Excel 2003 from which first order decay curves were fitted. Degradation rate constants for each compound were determined using the following equation:

$$y = y_0 e^{(-kt)}$$

where: y = concentration at time

y_0 = initial (day 0) concentration

k = first order primary biodegradation rate (days-1)

t = time (in days)

Half-lives were calculated using k determined above and the following equation:

$t_{(1/2)} = 0.693 / k$

4. RESULTS

Water quality measurements including the salinity and DOC of the collected seawater are listed in **Table 4**.

Table 4 Initial salinity and DOC levels in the biodegradation studies

Sample Type	MRD-08-224		MRD-08-225		MRD-09-498	
	Salinity (ppt)	DOC (mg/l)	Salinity (ppt)	DOC (mg/l)	Salinity (ppt)	DOC (mg/l)
Unfiltered	27 (field)	2.535	30 (field)	4.147	27 (field)	-
Filtered	-	2.806	-	-	-	1.82
Nutrient Amended	31	2.495	29	3.447	28	1.798

Table 5 lists the dissolved oxygen concentrations (DO) and demonstrates that the oxygen concentrations were sufficient to support microbial populations even in the later periods of these studies.

Table 5 Dissolved oxygen levels during the course of the biodegradation studies

MRD-08-224		MRD-08-225		MRD-09-498	
Test day	DO (ppm)	Test day	DO (ppm)	Test day	DO (ppm)
0	7.0	**0**	7.4	**0**	6.45
8	no sample	**7**	6.8	**7**	6.44
14	6.6	**14**	6.6	**14**	6.8
35	6.1	**29**	7.7	**30**	6.67
60	6.7	**61**	6.8	**64**	6.25
90	6.8	**89**	6.1		
191	6.3	**182**	6.1		

The primary biodegradation rate constants and half-lives for all of the compounds tested are listed in **Tables 6, 7** and **8**.

Those compounds with seawater half-lives determined to be approximately 60 days or greater and identified as "persistent" are highlighted in bold. For those compounds with very long half-lives or where no biodegradation was observed, values are reported as "greater than" the duration of the particular study in which they were tested. Most (73%) of the compounds tested and for which primary biodegradation rates could be determined, had measured seawater half-lives of less than 60 days and a majority of these (42%) had half-lives of less than 30 days.

The half-lives for a number of compounds could not be determined for various reasons.

- The aqueous concentrations of the following compounds could not be measured due to their extremely low water solubilities which precluded analytical detection: isopropylhydrophenanthrene (fichtelite), 3-methylcholanthene, benzo(ghi)perylene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene.
- The persistence of n-eicosane and 1-tridecene could not be determined due to their low concentrations relative to background levels determined in corresponding blank test media samples.
- A number of compounds appeared to be persistent, but results from the entire compliment of sampling intervals (approximately 180 days) were not available. This was attributed to a combination of their volatility and inherently low water solubility. Evidence of this was very low and variable responses in the corresponding poisoned controls used for standardization at the later sampling intervals. Compounds which appeared to be persistent yet lacked results for the full time course include: 2,2,4,4,4,6,8,8-heptamethylnonane ($t_{1/2}$ = 100 days after 62 days), trans-decalin ($t_{1/2}$ = 59 days after 35 days) and 1,3-dimethyladamantane ($t_{1/2}$ = 187 days after 62 days).

Table 6 Measured primary biodegradation rate constants and half-lives in seawater for study MRD-08-224

CASRN	Compound	Class	Carbon Number	Results Through Day	Primary Biodegradation Rate Constant (%/day)	Half-life (days)
112-40-3	n-dodecane	n-P	12	7	0.1505	4.6
544-76-3	n-hexadecane	n-P	16	14	0.1523	4.6
13475-82-6	2,2,4,6,6-pentamethylheptane	i-P	12	14	0.0253	27
17312-44-6	2,3-dimethyldecane	i-P	12	35	0.1011	6.9
17301-23-4	2,6-dimethylundecane	i-P	13	14	0.2961	2.3
62338-09-4	2,2,3-trimethyldecane	i-P	13	14	0.3575	1.9
6117-97-1	4-methyldodecane	i-P	13	14	0.2079	3.3
04390-04-9	**2,2,4,4,6,8,8-heptamethylnonane**	**i-P**	**16**	**62**	**0.0116**	**60**
3891-98-3	2,6,10-trimethyldodecane	i-P	15	14	0.1993	3.5
1921-70-6	2,6,10,14-tetramethylpentadecane (pristane)	i-P	19	62	0.0338	21
638-36-8	2,6,10,14-tetramethylhexadecane (phytane)	i-P	20	62	0.0246	28
5617-41-4	n-heptylcyclohexane	MN	13	14	0.1663	3.7
1795-15-9	n-octylcyclohexane	MN	14	14	0.3082	2.2
91-17-8	**trans-decalin**	**DN**	**10**	**35**	**0.0105**	**66**
92-51-3	bicyclohexyl	DN	12	90	0.0789	8.8
N/A	2-isopropyldecalin	DN	13	64	0.0344	20
N/A	**2,7-diisopropyldecalin**	**DN**	**16**	**191**	**0.001**	**>191**
702-79-4	**1,3-dimethyladamantane**	**PN**	**12**	**35**	**0.0011**	**187**
2435-85-0	**hexadecahydropyrene**	**PN**	**16**	**191**	**no degradation**	**>191**
N/A	**1-methyl-7-(1methylethyl)-hydrophenanthrene (hydrogenated retene) {MRD-07-116}**	**PN**	**10**	**191**	**0.004**	**>191**

CASRN	Compound	Class	Carbon Number	Results Through Day	Primary Biodegradation Rate Constant (%/day)	Half-life (days)
66325-09-5	2-hexyltetralin {MRD-05-452}	NMAH	16	14	0.3592	1.9
33460-02-5	3-phenylbicyclohexyl	NMAH	18	62	0.0303	23
5323-56-8	dehydroabietine	NMAH	19	191	0.0183	38
1087021	dodecahydro-terphenyl (dicyclohexylbenzene) {MRD-08-159}	NMAH	18	90	0.03	23
N/A	**hexahydroterphenyl (cyclohexylbiphenyl) {MRD-08-159}**	**NDAH**	**18**	**191**	**0.0103**	**67**
717-74-8	1,3,5-tris(1-methylethyl)-benzene	MAH	15	62	0.0129	54
2189-60-8	n-octylbenzene	MAH	15	62	0.0129	54
104-72-3	decylbenzene	MAH	16	14	0.2312	3
40458-98-8	**2,7-diisopropylnaphthalene**	**DAH**	**16**	**191**	**0.0057**	**122**
7116-96-3	4-pentyl-1,1'-biphenyl	DAH	17	14	0.2326	3
204256-07-5	**2,3-dimethyl-5-(4methylpentyl)-naphthalene**	**DAH**	**18**	**191**	**0.0107**	**65**
18516-37-5	2-methyl-1-undecene	i-O	12	90	0.0789	8.8
2437-56-1	1-tridecene	i-O	13	not determined due to background levels		
51655-65-3	2-butyl-1-decene	i-O	14	14	0.3025	2.3

Table 7 Measured primary biodegradation rate constants and half-lives in seawater for study MRD-08-225

CASRN	Compound	Class	Carbon number	Results Through Day	Primary Biodegradation Rate Constant (%/day)	Half-life (days)
5325-97-3	1,2,3,4,5,6,7,8-octahydrophenanthrene	NMAH	14	14	0.1773	3.9
85-01-8	phenanthrene	PAH	14	29	0.1377	5
120-12-7	anthracene	PAH	14	14	0.253	2.7
1889-67-4	**benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-**	**DAH**	**18**	**182**	**0.0021**	**>182**
N/A	6-n-butyl-2,3-dimethylnaphthalene	DAH	16	29	0.1839	3.8
84-15-1	**o-terphenyl**	**PAH**	**18**	**182**	**0.0008**	**>182**
832-69-9	1-methylphenanthrene	PAH	19	14	0.2485	2.9
2090-14-4	**perhydrochrysene {MRD-08-185}**	**PN**	**18**	**182**	**0.0059**	**117**
20279-21-4	1,2,3,10b-tetrahydrofluoranthene	NDAH	16	14	0.0317	22
781-17-9	**4,5,9,10-tetrahydropyrene**	**NDAH**	**16**	**182**	**0.0016**	**>182**
779-02-2	9-methylanthracene	PAH	15	29	0.1146	6
1706-50-9	perhydroterphenyl (tricyclohexyl) {MRD-08-158}	PN	18	29	0.113	6.1
112-95-8	n-eicosane	n-P	20	not determined due to background levels		
2221-95-6	isopropylhydrophenanthrene (fichtelite)	PN	12	not determined due to background levels		
438-22-2	5α(H)-androstane	PN	19	182	0.0201	34
1732-13-4	**1,2,3,6,7,8-hexahydropyrene**	**NDAH**	**16**	**182**	**0.0018**	**>182**
206-44-0	fluoranthene	PAH	16	61	0.0752	9.2
129-00-0	**pyrene**	**PAH**	**16**	**182**	**0.0046**	**151**
92-06-8	m-terphenyl	PAH	18	99	0.0471	15
10394-57-7	**9-n-butylphenanthrene**	**PAH**	**18**	**182**	**0.0096**	**72**

CASRN	Compound	Class	Carbon number	Results Through Day	Primary Biodegradation Rate Constant (%/day)	Half-life (days)
1610-22-6	**dodecahydrochrysene {MRD-08-183}[1]**	**NMAH**	**18**	**182**	**0.0073**	**95**
483-65-8	1-methyl-7-(1-methylethyl)-phenanthrene (retene)	PAH	18	99	0.0352	20
243-17-4	benzo(b)fluorene	PAH	17	29	0.1646	4.2
2381-21-7	**1-methylpyrene**	**PAH**	**17**	**182**	**0.00108**	**64**
2091-91-0	hexahydrochrysene {MRD-08-183}	NDAH	18	61	0.0603	11
N/A	octahydrochrysene {MRD-08-183}	NDAH	18	89	0.0345	20
2091-90-9	tetrahydrochrysene	PAH	18	29	0.0937	7.4
56-55-3	**Benzo(a)anthracene (1,2-benzanthracene)**	**PAH**	**18**	**182**	**no degradation**	**> 182**
218-01-9	chrysene					
217-59-4	triphenylene					
2541-69-7	7-methylbenzo(a)anthracene	PAH	19	29	0.149	4.7
207-08-9	benzo(k)fluoranthene	PAH	20	61	0.00609	11
50-32-8	benzo[a]pyrene	PAH	20	61	0.0509	11
56-49-5	3-methylcholanthene	PAH	21	not determined due to background levels		
191-24-2	benzo(ghi)perylene	PAH	22	not determined due to background levels		
53-70-3	Dibenzo(a,h)anthracene	PAH	22	not determined due to background levels		
193-39-5	indeno(1,2,3-cd)pyrene	PAH	22	not determined due to background levels		

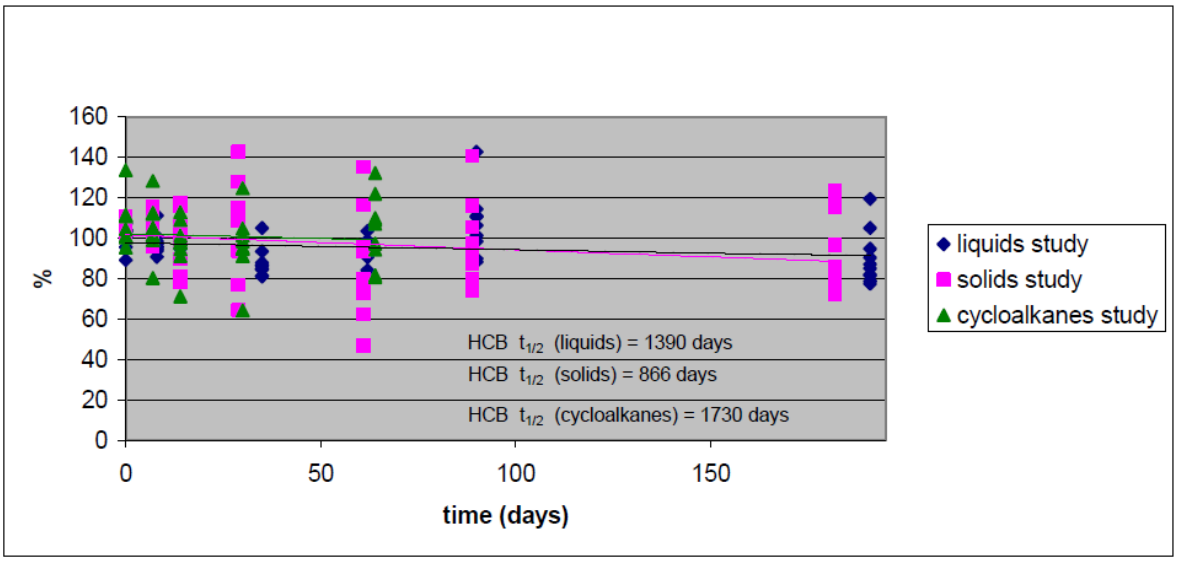
[1] Substances identified with MRD-08-183 were part of a mixture of hydrogenated chrysenes synthesized in-house

Table 8 Measured primary biodegradation rate constants and half-lives in seawater for study MRD-09-498

CASRN	Compound	Class	Carbon Number	Results Through Day	Primary Biodegradation Rate constant (%/day)	Half-life (days)
1010-74-8	**2-isopropyldecalin (MRD-08-396)**	**DN**	**13**	**35**	**0.0067**	**103**
91972-45-1	n-propyldecalin (MRD-09-481)	DN	13	64	0.0351	20
1618-22-0	2,6-dimethyldecalin (MRD-09-495)	DN	12	64	0.0695	10
N/A	2,3,6-trimethyldecalin (MRD-09-495)	DN	13	64	0.0208	33
N/A	1,4,6,7-tetramethyldecalin (MRD-09-495)	DN	14	64	0.0322	22
N/A	perhydro-1-methylfluorene (MRD-09-496)	PN	14	64	0.0152	46
5743-97-5	perhydrophenanthrene (MRD-09-496)	PN	14	64	0.0219	32
N/A	perhydro-2-methylanthracene (MRD-09-496)	PN	15	64	0.0209	33
N/A	perhydro-3,6-dimethylphenanthrene (MRD-09-497)	PN	16	64	0.0137	51
85-01-8	phenanthrene	PAH	14	29	0.1377	5

Figure 1 is a plot of the HCB recovery from test samples over the duration of each study. In all cases, measured half-lives greatly exceeded the duration of the respective studies indicating that HCB satisfied the requirement of a persistent, conserved marker to which the test compounds could be compared.

Figure 1 HCB Recovery from test system samples



5. CONCLUSIONS

Three separate experiments were performed to measure primary biodegradation of high molecular weight petroleum hydrocarbons in seawater. The compounds tested in these studies represent the principal aliphatic and aromatic hydrocarbon classes comprising heavier petroleum products and refinery streams. It can be concluded that:

- The novel passive dosing methodology presented here allows the simultaneous measurement of primary biodegradation rate constants of several hydrocarbons at their solubility limit.
- The use of direct immersion SPME allows extracting only those compounds dissolved in the aqueous phase.
- The passive dosing methodology worked well for most tested hydrocarbons. Half-lives for a limited number of compounds could not be determined for various reasons, e,g, extremely low water solubility which precluded analytical detection, or volatilisation during the duration of the test.
- Most (73%) of the compounds tested for which primary biodegradation rates could be determined had measured seawater half-lives of less than 60 days and a majority of these (42%) had half-lives of less than 30 days.
- Persistent compounds ($t_{1/2}$ > 60 days) tended to be extensively branched (i.e. 2,2,4,4,6,8,8-heptamethylnonane; 2,7-diisopropylnaphthalene; 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-benzene) or be formed by fully or mostly saturated four ring aromatics (i.e. perhydrochrysene; 4,5,9,10-tetrahydropyrene).

6. REFERENCES

1. EMBSI (2009) Primary biodegradation in seawater study. Study numbers 0822469, 0822569, 0949869. Report 09TP 26. Annandale NJ: ExxonMobil Biomedical Sciences Inc.

7. GLOSSARY

CASRN	Chemical Abstract Service Registry Number
DAH	Di-aromatic hydrocarbon
DN	Di-naphthenic
DO	Dissolved oxygen
DOC	Dissolved organic carbon
GC	Gas chromatograph
GC-MSD	Gas chromatograph with mass selective detector
HCB	Hexachlorobenzene
i-O	Iso-olefin
i-P	Iso-paraffin
MAH	Mono-aromatic hydrocarbon
MN	Mono-naphthenic
MRD	Medical Research Division
NDAH	Naphthenic di-aromatic hydrocarbon
NMAH	Naphthenic mono-aromatic hydrocarbon
n-P	Normal paraffin
PAH	Poly-aromatic hydrocarbon
PBT	Persistent, bioaccumulative, toxic
PDMS	Polydimethylsiloxane
PN	Poly-naphthenic
RRF	Relative response factor
PPT	Part per trillion
SIM	Selective ion monitoring
SPME	Solid-phase microextraction
UVCB	Substances of Unknown or Variable composition, Complex reaction products or Biological materials

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