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Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of K_{ow} and K_{aw}

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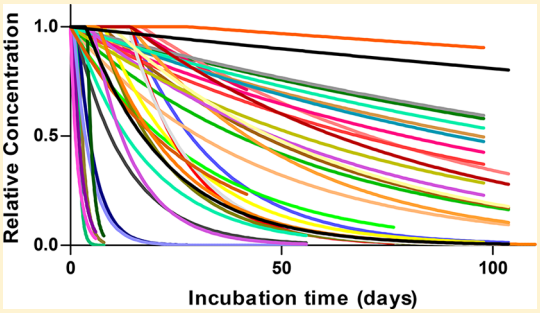
Supporting Information

ABSTRACT: A partitioning-based experimental platform was developed and applied to determine primary biodegradation kinetics of 53 hydrocarbons at ng/L to μg/L concentrations covering C8−C20, 11 structural classes, and several orders of magnitude in hydrophobicity and volatility: (1) Passive dosing from a loaded silicone donor was used to set the concentration of each hydrocarbon in mixture stock solutions; (2) these solutions were combined with environmental water samples in gastight auto sampler vials for 1−100 days incubation, and (3) automated solid phase microextraction (SPME) coupled to GC-MS was applied directly on these test systems for measuring primary biodegradation relative to abiotic controls. First order biodegradation kinetics were obtained for 40 hydrocarbons in activated sludge filtrate, 18 in seawater, and 21 in lake water. Water phase half-lives in seawater and lake water were poorly related to hydrophobicity and volatility but were, with a few exceptions, within a factor of 10 or shorter than BioHCwin predictions. The most persistent hydrocarbons, 1,1,4,4,6-pentamethyldecalin, perhydropyrene, 1,2,3,6,7,8-hexahydropyrene, and 2,2,4,4,6,8,8-heptamethylnonane, showed limited or inconsistent degradation in all three environmental media. This biodegradation approach can cover a large chemical space at low substrate concentrations, which makes it highly suited for optimizing predictive models for environmental biodegradation.



INTRODUCTION

Biodegradation is the most important removal process for many organic chemicals in the environment. The potential for biodegradation of a chemical structure (biodegradability), or lack thereof, is therefore a key element in the regulatory framework for chemical risk assessment in, e.g., Europe and the United States.[1,2] The first tier of regulatory biodegradability testing consists of a qualitative screening test for ready biodegradability.[3] If chemicals fail this test, higher tier simulation tests can be used to obtain biodegradation half-lives under more realistic environmental conditions including low test concentrations and using environmentally native microorganisms for the test. These simulation tests are expensive, and data are much scarcer than from the screening tests.[4]

Although qualitative ready biodegradability test data are appropriate for screening of chemicals, environmental biodegradation kinetics are necessary for environmental fate modeling and risk assessment. Different schemes have been proposed to assign biodegradation half-lives to chemicals based on screening test results,[4] and models have been developed to predict biodegradability based on chemical structure.[5] However, the experimental generation of high quality kinetic data remains crucial because experimental data remain the gold standard in research and regulation, are the basis for building and refining predictive models, and are the ultimate reference for testing such models.[5−7] There is a need for larger data sets because comparability in both the test conditions and inoculum is important for the training set data to build a model with structural generality.[5]

Risk assessment of petroleum products is complicated as these are complex mixtures of varying composition containing thousands of components, each with their own physicochemical and degradation properties.[8] Chemical constituents can be classified according to the chemical space either by physical−chemical properties such as air−water partitioning and octanol−water partitioning or by structural grouping and carbon number classes.[8−10] The constituents of such products are released to the environment as a mixture and will therefore be subject to degradation as a mixture. A biodegradation model, BioHCwin, has been developed to predict environmental half-lives for hydrocarbons.[6] Although field and grab sample tests in water, sediment, and soil were preferred for building the BioHCwin model, screening test data were included for some chemical groups due to lack of data.[6] A number of studies have

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since looked at biodegradation of petroleum product constituents or mixtures at concentrations close to the aqueous solubility of the mixtures or above solubility (dispersions).[11−16] Prosser et al.[16] compared model predictions to the results from these studies and found the model to perform acceptably as a screening tool. Although high substrate concentrations are very relevant for oil spill situations in the proximity of the spill,[18] assessing and predicting biodegradation and persistence in the aquatic environment require data that are obtained at much lower concentrations.[19] Another study has calculated in situ degradation rates for hydrocarbons based on data from the Deepwater Horizon oil spill; however, they did not compare the findings to model predictions.[17]

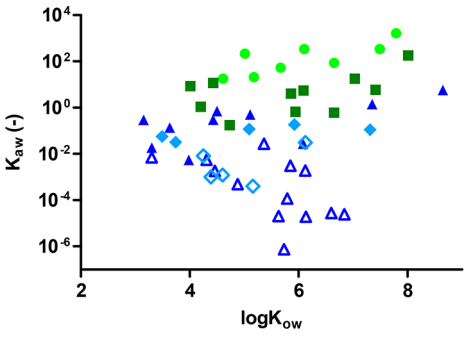
Recently, a new partitioning-based experimental platform was introduced for determining biodegradation kinetics of composed mixtures of hydrophobic organic chemicals at environmentally relevant low concentrations.[20] Important features of this experimental platform are as follows: (1) Passive dosing from a preloaded silicone was used to set concentrations of each constituent in an aqueous mixture without the addition of a cosolvent.[21−23] (2) These solutions were combined with environmental water samples containing native microorganisms for incubation in gastight auto sampler vials for 1−100 days. (3) Finally, automated head space solid phase microextraction (HS-SPME) coupled to GC-MS was applied directly on the test systems for determining primary biodegradation kinetics relative to abiotic controls. This approach has recently been applied to a mixture of nine hydrocarbons with low melting points and high air-to-water partition ratios[20] and was used to study the effect of inoculum origin on biodegradation kinetics of the nine hydrocarbons.[24]

The first aim of the present study was to further develop the applicability domain of this experimental platform to facilitate biodegradation kinetic testing of larger mixtures of hydrocarbons covering a much wider chemical space. For this purpose, the passive dosing technique had to be extended to include different loading principles that in combination are applicable to chemicals with a wider melting point, hydrophobicity, volatility, and chemical class range. Solid phase microextraction (SPME) had to be operated not only in headspace but also direct submersion mode to extend the applicability domain toward the less volatile chemicals and particularly the polyaromatic hydrocarbons (PAHs). The second aim was to apply this new approach for generating a large set of biodegradation kinetic data at environmentally relevant concentrations, covering a substantial part of the chemical space of petroleum hydrocarbons. Fifty-three hydrocarbons were chosen based on (1) ensuring a good coverage of 11 structural classes and carbon numbers from 8 to 20, (2) covering a wide range in hydrophobicity and air−water partitioning, (3) our present capabilities with passive dosing, SPME, and GC-MS, and (4) availability as neat chemicals at a reasonable price. The test chemicals cover a chemical space that is highly relevant for petroleum hydrocarbons, but they were not selected to represent a typical petroleum product composition.

EXPERIMENTAL SECTION

Materials. 1-Octanol, 1,2,4-trimethylbenzene, 1,2-dimethylnaphthalene, 1,3,5-triethylbenzene, 2,2,4,4,6,8,8-heptamethylnonane, 9,10-dihydroanthracene, benzo(*a*)pyrene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, bicyclohexyl, biphenyl, *trans*-decalin, *n*-decane, decylbenzene, isopentylbenzene, naphthalene, phenanthrene, pyrene, tetralin, and *p*-xylene were purchased from Sigma-Aldrich (Copenhagen, Denmark). 1,2,3,10b-Tetrahydrofluoranthene, 1,2,3,4,5,6,7,8-octahydrophenanthrene, 1,2,3,6,7,8-hexahydropyrene, 1,2-dihydronaphthalene, *cis*-1,2-dimethylcyclohexane, 1,3,5-trimethylcyclohexane, 2,3-dimethylheptane, 2,6-diisopropylnaphthalene, 2-methyl-1*H*-cyclopenta(*l*)phenanthrene, 2-methylnonane, 4-methyldodecane, benzo(*a*)anthracene, chrysene, *m*-cymene, decylcyclohexane, *n*-dodecane, dodecylbenzene, fluoranthene, *n*-octylcyclohexane, and *p*-terphenyl were purchased from TCI Chemicals (Zwijndrecht, Belgium). 1,1,4,4,6-Pentamethyldecalin (91.2%), 1,4,6,7-tetramethylnaphthalene, 2,2,5,7-tetramethyltetralin, 2,6,10-trimethyldodecane (96.9%), 2-ethylanthracene, 3-ethylnonane, 5α(*H*)-androstane, butyldecalin, dehydroabietine (96.7%), fichtelite, methylenephenanthrene, perhydrofluorene, and perhydropyrene were purchased from Chiron (Oslo, Norway,). 5,6-Dimethyl-1-(4-methylpentyl)-naphthalene was purchased from Chemsampco via Sigma-Aldrich (Copenhagen, Denmark). Purity of chemicals was at least 97% unless otherwise stated. Translucent silicone rods (custom-made by Altecweb.com, product code 136-8380), ethyl acetate (Sigma-Aldrich, ≥99.7%), ethanol (VWR chemicals, 99.8%), and methanol (Sigma-Aldrich, >99.9%) were used for passive dosing.

Hydrocarbon Mixtures. The 53 hydrocarbons were divided into two groups that were tested in two separate biodegradation experiments. The chemicals had molecular weights between 106 and 252 g/mol and covered 5 orders of magnitude with regard to water solubility and octanol−water partition ratio (K_{ow}) and 9 orders of magnitude regarding air−water partitioning (K_{aw}) (Figure 1). Mixture 1 consisted of 35 hydrocarbons, mostly liquids. The experimental setup was designed for initial concentrations of these chemicals roughly



CN	nP	iP	MN	DN	PN	MA	NMA	DA	NDA	TA	NTA	PA
6-8			1		-	1	-	-	-	-	-	-
9-11	1	3	1	1	-	3	2	1*	-	-	-	-
12-14	1	1	1	2	1	1	2	*1+2*	*1*	*1*	-	-
15-17		2	1	1	1	1		*1*	*2*	*1*	*2*	*1*
18-20					2	1	1	1		*1*		*6*

Figure 1. Chemical space of hydrocarbons included in this study regarding the octanol−water partition ratio, log K_{ow},[25] air−water partitioning, K_{aw},[25] (top), and number of chemicals included in each hydrocarbon block by carbon number (CN) and structural class (bottom). Closed symbols denote liquid mix; open symbols indicate solid mix. nP = *n*-paraffin, iP = *i*-paraffin, MN = mononaphthenic, DN = dinaphthenic, PN = polynaphthenic, MA = monoaromatic, NMA = naphthenic monoaromatic, DA = diaromatic, NDA = naphthenic diaromatic, TA = triaromatic, NTA = napththenic triaromatic, PA= polyaromatic. Mixture 2 is indicated in italics. *Naphthalene was included in both mixtures.

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1000-times below their water solubility (see Supporting Information S1 for initial concentrations). Mixture 2 consisted of 19 solid chemicals (naphthalene was included in both mixtures to compare the two batches of water used for the liquid and solid test). Initial test concentrations were ∼10-times below the solubility for half of these chemicals (lowest solubility) and ∼400-times below solubility for the remaining chemicals (see S1). Test concentrations ranged from 0.004 to 170 μg/L.

Passive Dosing. Passive dosing can produce well-defined low concentrations of hydrophobic organic chemicals in water without addition of cosolvent.[21,22,26] It was in the present study used to set initial concentrations and mixture composition but not to buffer concentrations during the biodegradation experiment as done in previous studies.[27−29]

Silicone rods were used as a passive dosing donor.[23] The rods (3 mm diameter) were washed in the dishwasher without soap and dried using lens cleaning tissue. Then, 20.0 g (length ∼2.6 m) were cut and added to 100 mL of amber Wheaton glass serum bottles with crimp seals and a PTFE-coated silicone septa. The rods were further cleaned by soaking in ethyl acetate for >20 h and in ethanol for >20 h. Ethanol was poured out, and the bottles were heated to 120 °C for 2 h to evaporate the remaining ethanol from the silicone.

Three different loading methods were required to cover the large chemical space of the present study:

Loading of Liquid Substances by Full Absorption. Three passive dosing systems containing 20.0 g of silicone in 100 mL bottles were prepared. An equal mass of 35 hydrocarbons was mixed, dissolving the two solid hydrocarbons (naphthalene and biphenyl) in the liquid hydrocarbons. Then, 400 μL of this mixture was added to each rod. The bottles were rolled for 5 days at ∼40 rpm, after which the majority of the liquid mixture was visually confirmed to be absorbed into the silicone; 65 mL of ultrapure water was added, and the bottles were rolled for 20 h after which the water was discarded. This procedure enabled the full absorption of the hydrocarbons in the silicone.

Loading of Solid Hydrocarbons by Partitioning from Saturated Methanol Solutions. One passive dosing system of 20.0 g of silicone in a 100 mL bottle was prepared. Excess amounts of the least soluble hydrocarbons (pyrene, 1,2,3,6,7,8-hexahydropyrene, *p*-terphenyl, 2-ethylanthracene, 2-methyl-1*H*-cyclopenta(*l*)phenanthrene, benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, and benzo(*a*)-pyrene) were added to 25 mL of methanol, shaken, and left overnight for equilibration and settling of crystals. In four steps, 15 mL of the saturated methanol was transferred to the silicone; the bottle was rolled for >20 h, and the methanol was poured back to the crystals, shaken, and left for settling >4 h before being added back to the silicone.

Loading of Solid Chemicals by Partitioning from Non-saturated Methanol Solution. One passive dosing system of 4.0 g of silicone rod placed in a 20 mL headspace vial was prepared. The loading solution was prepared by adding excess amounts of the solid hydrocarbons (naphthalene, 1,2-dimethylnaphthalene, 1,4,6,7-tetramethylnaphthalene, 9,10-dihydroanthracene, 1,2,3,10b-tetrahydrofluoranthene, phenanthrene, methylenephenanthrene, 2,6-diisopropylnaphthalene, and fluoranthene) to 10 mL of methanol, shaking and leaving overnight for equilibration and settling of crystals. Five milliliters of this solution was then diluted to 25 mL in methanol. In three steps, ∼8 mL of this methanol solution was added to the silicone, rolled for >20 h, and discarded.

The two passive dosing systems for solids were cleaned by washing 10 times with pure water (1 min vigorous shaking) to remove methanol and any possible crystals from the loaded silicone rods. The last fill was rolled overnight and discarded.

Aqueous stock solution was then prepared by adding 65 mL of ultrapure water to the 100 mL passive dosing systems and 13 mL of ultrapure water to the 20 mL passive dosing systems and rolling at ∼40 rpm for >30 min. Stock solution was transferred to test systems using gastight syringes.

Environmental Inocula. The inocula for the biodegradation experiments originated from three types of surface water: wastewater treatment plant-activated sludge filtrate, seawater, and lake water.

A sample of activated sludge was taken at the Lynetten wastewater treatment plant (WWTP) in Copenhagen (Denmark) on the 19th of April 2016 (for liquid hydrocarbon test) and fourth of October 2016 (for solid hydrocarbon test). Lynetten is the largest WWTP in Denmark. The samples were filtered through Whatman 114 V filters (retention 20 μm) to prepare the activated sludge filtrate.

A surface seawater sample was taken in the North Sea west of Esbjerg, Denmark, on the 18th of May 2016 (ETRS89 UTM32N: 451415; 6143811) (for liquid hydrocarbon test) and 27th of September 2016 (ETRS89 UTM32N: 449694; 6142556) (for solid hydrocarbon test) in the open sea, 5−8 km off the coast, 1−2 km from the main sailing route.

A surface sample of lake water was taken from Maglesø Lake (Zealand, Denmark) on the fifth of April 2016 (for liquid hydrocarbon test) and fifth of October 2016 (for solid hydrocarbon test) 6−7 m from the shore. This is a clean lake with a very small catchment, without major point sources, receiving no direct runoff discharge from roads, and situated in rural surroundings (forest/fields). This lake had the slowest biodegradation for nine hydrocarbons in an initial study including two lake and three stream samples,[24] and it was therefore selected as the most conservative choice of inoculum in the present study.

All water samples were used within 24 h of sampling. Background characterization of the samples is included in the Supporting Information (S2). The samples showed quite similar characteristics at the same site between the two sampling dates, although temperatures were higher in the fall than in the spring. The activated sludge filtrate sample taken in the fall showed a higher degree of treatment (lower nutrients and dissolved organic carbon) than the sample taken in the spring. Culturable bacterial densities, measured by the heterotrophic plate count, HPC, were similar in the lake and seawater (∼1−2 × 10^3 CFU/mL) and 2 orders of magnitude lower than in the activated sludge filtrates (2.5 × 10^5 and 4.8 × 10^5 CFU/mL).

Biodegradation Tests. Biodegradation and final chemical analysis both took place in autosampler vials, maximizing the number of replicate test systems and minimizing test substance losses between the experiment and the analysis. A large number of biotic and abiotic test systems were prepared by combining 13.5 mL of environmental water containing the inoculum spiked with 30 μg/L of 1-octanol (positive control substance) with 1.5 mL of aqueous stock solution containing the test chemicals in 20 mL amber glass vials with screw caps and PTFE-coated silicone septa. For the solid chemicals, 1.3 mL stock solution was added from the 100 mL passive dosing system and 0.2 mL from the 20 mL passive dosing system. Abiotic controls were prepared using ultrapure water instead of

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environmental samples. For liquid chemicals in seawater, abiotic controls of seawater salinity were prepared by adding 35 g/L of NaCl to ultrapure water. For the solid chemicals, ultrapure water was used without adding salts. The vials were closed immediately and incubated at 20 °C on a benchtop laboratory roller at ∼30 rpm. At time points from 2 h to ∼100 days, three biotic and three abiotic test systems were taken for chemical analysis (destructive sampling). Although no systematic differences were seen between test vials prepared from the three replicate silicone rods loaded with liquids, a balanced test design was chosen where one biotic and abiotic test system were analyzed from each rod at each time point. Test durations were 77 or 85 days for the activated sludge test, 56 or 111 days for the seawater, and 98 or 104 days for the lake water with the liquid and solid mixtures, respectively.

Chemical Analysis. Automated solid phase microextraction (SPME) coupled to gas chromatography mass spectrometry (GC-MS) (Agilent Technologies 7890B/5877A GC/MSD) was applied directly on the test systems. A CTC PAL RSI 85 autosampler (CTC Analytics, Zwingen, Switzerland) was used for SPME sampling and subsequent thermal desorption of the SPME fiber. Headspace-SPME with 100 μm polydimethylsiloxane (PDMS) coating was used for the liquid chemicals, and water phase SPME with 7 μm PDMS coating was used for the solid chemicals. Analytical details are included in the Supporting Information (S3).

Quality Assurance. Blank test systems were coincubated and measured with each sampling point. For 10 of the 2246 data points, elevated blank responses for a chemical resulted in exclusion from the data set. Two vials (1 of 246 biotic and 1 of 246 abiotic test systems) showed signs of being leaky (selective loss of volatile chemicals compared to nonvolatile) and were excluded.

Background concentrations of the test chemicals in the environmental media were evaluated by comparing triplicate measurements of each environmental media with the abiotic controls from the same GC-MS run. In the lake and seawater, background concentrations were <1% of the test concentrations. In the activated sludge filtrate, slightly higher background response levels were observed, and levels above 10% were observed for 2,6,10-trimethyldodecane (15%), 2,2,4,4,6,8,8-heptamethylnonane (18%), and 2,6-diisopropylnaphthalene (135%). 2,6-Diisopropylnaphthalene was therefore excluded from the data for activated sludge filtrate.

In four cases, the GC peak areas of the biotic test systems were more than twice as high as for the abiotic controls. The data series for 2,2,4,4,6,8,8-heptamethylnonane and 1,2,3,4,5,6,7,8-octahydrophenanthrene in activated sludge filtrate as well as 5-α(H)-androstane and Fichtelite in lake water were therefore removed from the data set.

Data Analysis. For each time point, the relative concentration, $C_{relative}$, was determined as the ratio between the peak areas in the biotic test systems, A_{biotic}, relative to the peak areas in the abiotic test systems, $A_{abiotic}$. Because data from the abiotic and biotic test systems were not paired, and there is uncertainty associated with both the abiotic and biotic test systems, the uncertainty related to the ratio between these two variables was calculated using Taylor series approximations of the mean, $\mu_{C_{relative}}$, and variance, $\sigma^2_{C_{relative}}$, of a ratio according to eqs 1 and 2.[30]

$$\mu_{C_{relative}} \approx \frac{\mu_{A_{biotic}}}{\mu_{A_{abiotic}}} - \frac{Cov(A_{biotic}, A_{abiotic})}{(\mu_{A_{abiotic}})^2} + \frac{\sigma^2_{A_{abiotic}}\mu_{A_{biotic}}}{(\mu_{A_{abiotic}})^3} \quad (1)$$

$$\sigma^2_{C_{relative}} \approx \frac{(\mu_{A_{biotic}})^2}{(\mu_{A_{abiotic}})^2}\left[\frac{\sigma^2_{A_{biotic}}}{(\mu_{A_{biotic}})^2} - 2\frac{Cov(A_{biotic}, A_{abiotic})}{\mu_{A_{biotic}}\mu_{A_{abiotic}}} + \frac{\sigma^2_{A_{abiotic}}}{(\mu_{A_{abiotic}})^2}\right] \quad (2)$$

where $\mu_{A_{abiotic}}$ and $\mu_{A_{biotic}}$ are the means of the area of response in the abiotic and biotic test systems, $\sigma^2_{A_{abiotic}}$ and $\sigma^2_{A_{biotic}}$ are the variances of the area of response in the abiotic and biotic test systems, and $Cov(A_{biotic},A_{abiotic})$ is the covariance between the area of response in the biotic and abiotic test systems, respectively.

The mean and uncertainty of $C_{relative}$ for each time point were used as input to GraphPad Prism 5.00 for fitting the first order degradation model with lag phase in eq 3. T_{lag} was constrained to positive values; no weighting of the data was used, and the number and scatter among replicates were accounted for in the fit. Confidence limits for k_{system} were obtained assuming log-normal distribution of k_{system}.

$$C_{relative} = \begin{cases} 1 & \text{for } t < t_{lag} \\ e^{-k_{system}(t-t_{lag})} & \text{for } t \geq t_{lag} \end{cases} \quad (3)$$

Test system half-lives, $T_{1/2,system}$, were obtained by eq 4.

$$T_{\frac{1}{2},system} = \ln(2)/k_{system} \quad (4)$$

Degradation half-times in the system DT_{50} were obtained by summing the lag-phase and half-life.

The biodegradation experiments were performed with a headspace in the test systems to ensure aerobic conditions throughout the incubation (necessary for the activated sludge filtrate). Test system half-lives of the volatile chemicals were thus corrected for headspace partitioning to obtain water phase half-lives ($T_{1/2,water}$) as described by Birch et al.[20] This correction did not account for the hydrocarbon binding to third phases such as dissolved organic matter.

When degradation proceeds, the concentrations in biotic test systems will at some point reach a level below detection. This is important information, but the low concentration measurements should not be given too much weight in the fitting of the degradation curve. In the present study, three times the peak area of the blank response was used as the limit of detection, and for each degradation curve, a maximum of one data point was included below this limit. Furthermore, a maximum of three data points of $C_{relative}$ < 0.01, were included in each time series.

The degradation curves were evaluated based on the coefficient of determination of the fit (R^2) and visual inspection and were subsequently divided into six categories:

(1.1) High quality model fit with a goodness of fit of R^2 > 0.8. For this category, the degradation rate constant, lag-phase, half-life, and half-times were determined.

(1.2) Goodness of fit was R^2 < 0.8, but after initial scatter, a clear degradation curve was observed and described by at least 4 data points. For this category, the degradation rate constant, lag-phase, half-life, and half-times were determined.

(1.3) Data showed clear evidence of degradation; however, a high variation among replicates and/or no clearly defined first order degradation curve was seen. For this category, only half-times were determined.

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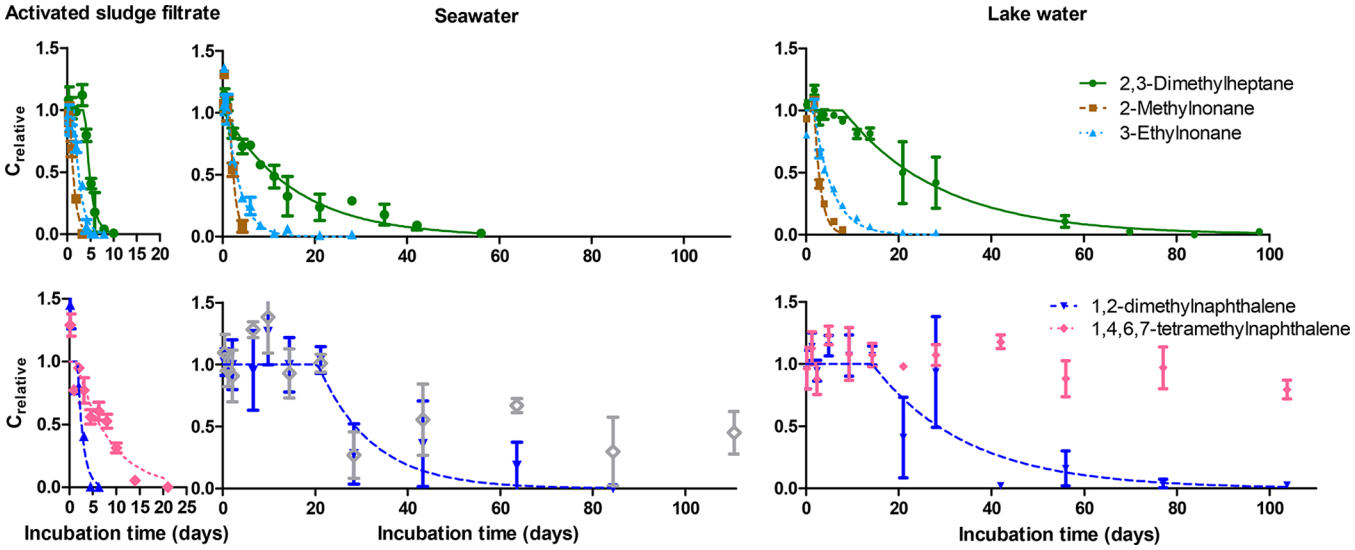

Figure 2. Relative concentrations for three C9−C11 iso-paraffins (top) and for two C12−C14 diaromatics (bottom) and first order degradation curves. 1,4,6,7-Tetramethylnaphthalene in seawater is shown in gray open symbols because it was discarded based on inconsistent data (category 2). Error bars show standard error of mean (n = 3) based on three replicate biotic and three replicate abiotic test systems for each time point.

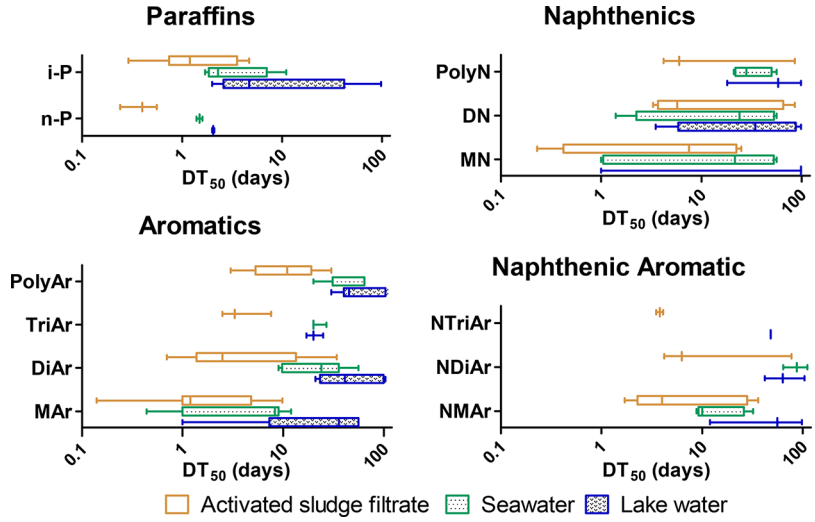

Figure 3. Half-times for hydrocarbon groups in activated sludge filtrate, seawater, and lake water. Median, 25th, and 75th percentile whiskers show minimum to maximum range. For hydrocarbon group abbreviations, see Figure 1.

(2) Inconclusive data. More than one data point showed signs of degradation with $C_{relative}$ < 0.5, but subsequent data points did not show degradation. No data were determined for this category.

(3.1) Limited degradation. All time points had $C_{relative}$ > 0.5. For this category, the test duration was used to assign the minimum half-time (e.g., half-time > test duration).

(3.2) Limited degradation. One time point had $C_{relative}$ < 0.5. For this category, the time of the last data point before the data point below 0.5 was used to assign a minimum half-time.

The threshold of 50% reduction relative to abiotic controls for positive identification of degradation in categories 2, 3.1, and 3.2 was chosen as a common criterion for all chemicals and degradation curves. It was based on typical variability between replicates at each data point seen for chemicals with slow degradation.

RESULTS AND DISCUSSION

Biodegradation Kinetics. Degradation curves and the associated biodegradation kinetic parameters such as lag phase, first order rate constant, half-times, test system half-lives, and water phase half-lives are listed in the Supporting Information (S4 and S5). 1-Octanol was degraded within 2 days in both batches for all three water types, confirming the biological activity of all samples. Examples of degradation curves fitted to experimental data are shown for five hydrocarbons in Figure 2. The three iso-paraffins in Figure 2 are examples of a category 1.1 fit in all three water types, 1,2-Dimethylnaphthalene is an example of a category 1.2 fit in the activated sludge filtrate and 1.3 fit in the seawater and lake water. 1,4,6,7-Tetramethylnaphthalene is a category 1.1 fit in the activated sludge filtrate, a category 2 fit in the seawater, and a category 3.1 fit in the lake water. The number of hydrocarbons within each of the degradation curve categories is shown in the Supporting Information (S6).

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Biodegradation Differences between Water Types. Of 53 hydrocarbons, first order degradation kinetics (category 1.1 + 1.2) were obtained for 40 hydrocarbons in the activated sludge filtrate, 18 hydrocarbons in the seawater, and 21 hydrocarbons in the lake water. Half-lives in activated sludge filtrate were shorter than in lake water for all hydrocarbons and shorter than in seawater for most hydrocarbons. A slightly lower number of hydrocarbons were degraded in the seawater compared to the lake water; however, when degradation was initiated, half-lives were, with few exceptions, similar or slightly shorter in the seawater. Although the heterotrophic plate counts were similar in the sea and lake water samples, the seawater samples were taken in the vicinity of a trafficked shipping port, which likely implied pre-exposure of the natural bacterial consortia to petroleum hydrocarbons. The lake was, on the contrary, located in a rural area without direct runoff discharge from roads.

In a previous study inoculum from the lake was found to give the slowest hydrocarbon biodegradation among two lakes and three streams, and seven out of nine hydrocarbons were degraded even slower in the present study compared to those in the previous study (e.g., DT_{50} for trans-decalin was >56 days in this study but only 35 days in the previous study).[24] The aromatic chemicals in the liquid mixture test in lake water showed highly varying results with no clear first order degradation curves, and naphthalene was only degraded in lake water in the experiment including the solid hydrocarbons. Although theoretically the difference in degradation of naphthalene between the two batches of lake water could be caused by the difference in the constituents of the two mixtures, we hypothesize that these differences and inconsistencies in biodegradation kinetics for the lake water in this experiment were caused by an insufficient number of competent aromatic degraders in the 15 mL test systems prepared from the sample taken on the first sampling date. This explanation is in line with a recent study showing that higher inoculum concentrations in screening tests can increase reproducibility of results because it reduces the risk of excluding specific degraders in the test volume.[31]

Hydrocarbon Groups. Figure 3 shows the range of DT_{50} for the tested chemicals in each hydrocarbon group in activated sludge filtrate, seawater, and lake water. Note that a different number of chemicals were tested within the different groups (see Figure 1), and for some chemicals (category 2), a DT_{50} was not determined as described above.

The linear paraffins in this test were degraded faster than the branched paraffins. For aromatic hydrocarbons, a trend was seen of longer half-lives with increasing number of rings. The sequence of degradation based on median DT_{50} for each group is in agreement with earlier observations of hydrocarbon group susceptibility to biodegradation (*n*-paraffin < *i*-paraffin < low molecular weight aromatic < naphthenic/high molecular weight aromatic).[32−34] Large differences in half-times were observed within some of these groups, however, and the span of degradation half-times overlapped for all hydrocarbon groups.

Chemical Space. DT_{50} and $T_{1/2,water}$ for the test chemicals in seawater were grouped into ranges and plotted within the chemical space in terms of their air−water (K_{aw}) and octanol−water (log K_{ow}) partition ratio (Figure 4). Ranges were chosen to represent very fast degradation (<1 day), fast degradation (1−10 days), medium degradation (10−40 days), and slow/no degradation (>40 days).

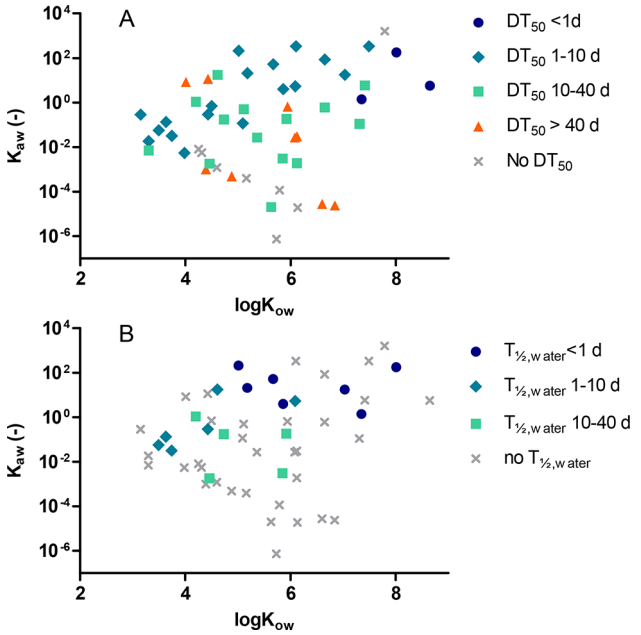

Figure 4. Biodegradation of hydrocarbons in terms of (A) half times (DT_{50}) and (B) half-lives ($T_{1/2,water}$) in seawater within the chemical space regarding octanol−water partitioning and air−water partitioning.

Among the structures that were susceptible to degradation and showed first order biodegradation, there was a slight tendency of lower $T_{1/2,water}$ and DT_{50} with higher K_{aw} but no relationship between $T_{1/2,water}$ or DT_{50} and K_{ow}. K_{aw}, however, ranged twice as many orders of magnitude as K_{ow}. The fast degradation rates for some of the volatile chemicals were not caused by losses from the test system; abiotic losses were corrected for using the abiotic controls, and for many chemicals, clear lag phases were seen, which is a strong indicator for biodegradation. Two factors determine the difference between the DT_{50} and the $T_{1/2,water}$, the inclusion of lag phase in the DT_{50} and the headspace correction for the $T_{1/2,water}$. The lag phase affects DT_{50} for all chemicals and was less than 11 days for most chemicals where $T_{1/2,water}$ was determined with one exception of 26 days. The headspace correction affected the volatile chemicals in the upper part of Figure 4 and was the main reason for a number of chemicals going from a fast degradation in terms of DT_{50} (between 1 and 10 days) to a very fast degradation in terms of $T_{1/2,water}$ (<1 day). This difference is not very important in a screening perspective, where these chemicals would be categorized as biodegradable no matter what endpoint was considered. However, it may have implications in modeling of biodegradation where rate constants are used.

The most persistent hydrocarbons in the seawater (DT_{50} > 40 d) covered 3 orders of magnitude in log K_{ow} and 5 orders of magnitude in K_{aw} without a clear grouping in any region of the two-dimensional space shown in Figure 4 (orange triangles). Although the two physicochemical properties were useful to describe the chemical space of the tested hydrocarbons, they seem poorly related to the biodegradation end points of the study. This observation is specific to biodegradation testing in water because increases in sorption with increasing hydrophobicity can induce strong relationships between hydrophobicity and biodegradation half-lives in soils and sediments.[35] Whereas sorption may reduce the bioavailable fraction and thus reduce biodegradation rates when sediments are included in

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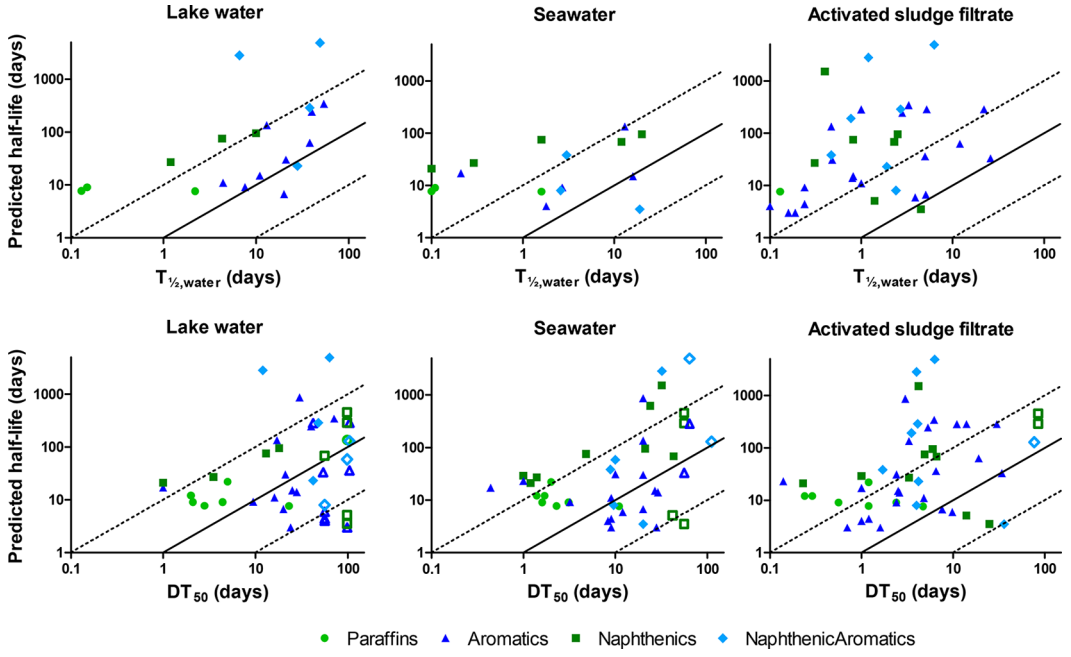

Figure 5. BioHCwin predicted half-lives vs experimental water phase half-lives (top row) in lake water, seawater, and activated sludge filtrate. BioHCwin predicted half-lives vs test system half-times (bottom row). Open symbols indicate minimum half-times of hydrocarbons with limited degradation during the test. A 1:1 ratio is indicated as a solid line, and 10-times under and overprediction are indicated as dotted lines.

tests, sediments have also been observed to increase biodegradation rates because of the increase in sediment-associated bacteria.[36,37] The most persistent hydrocarbons were also distributed widely between hydrocarbon classes and carbon number groups, again without a clear trend and grouping. Although the carbon block approach again is very useful to describe the chemical space of hydrocarbons, there are clearly additional structural features beyond the groups that determine the biodegradability. For example, a structural factor that resulted in fast primary degradation in all waters was the inclusion of a long (>C4) linear alkyl chain. Higher methyl substitution resulted in slower degradation for naphthalene, 1,2-dimethylnaphthalene, and 1,4,6,7-tetramethylnaphtalene, and aromatic ring structures such as naphthalene and pyrene were degraded faster than their naphthenic analogues in the activated sludge filtrate and in some cases in the sea and lake water.

Comparing the Experimental Data to the BioHCwin Model. A comparison between BioHCwin predicted half-lives and $T_{1/2,water}$ or DT_{50} from this study is shown in Figure 5. Both end-points (first order half-lives and degradation half-times) are relevant in an environmental context. Half-lives are relevant for biodegradation of diffuse ongoing emissions and is the end-point used from simulation tests (such as OECD 309) to compare to persistency criteria. Degradation half-times are more relevant to spill scenarios and are used in screening studies.

Because biodegradation is not an inherent property of the chemical, and environmental factors such as sorption and microbial activity are important for biodegradation rates, half-lives can easily vary by a factor of 10 between studies with different environmental conditions.[6] Even within batches of water from the same site, variations occur, as seen for naphthalene in the two mixtures. The BioHCwin model was developed to predict degradation in "different environmental media (e.g., water, soil, and sediment)",[6] and more than half of the input data was from sediment and soil studies. It is unclear whether the included studies were detailed enough to determine the lag-phase and report true first order degradation rates. Our $T_{1/2,water}$ data set targets degradation in the water phase only and is thus not necessarily directly comparable to the BioHCwin model.

Activated sludge filtrate has a higher bacterial density and contains better adapted bacteria than surface water and seawater, and the use of this type of data was limited in the BioHCwin model development. BioHCwin predictions for environmental half-lives were therefore similar or longer than all experimental water phase half-lives ($T_{1/2,water}$) in activated sludge filtrate and higher or within a factor 10 of the DT_{50}'s.

Generally, the predicted half-lives were within a factor 10 or higher than $T_{1/2,water}$ and DT_{50} in sea and lake water (Figure 5). The higher half-lives could be explained by the inclusion of sediment and soil studies in the training set for the BioHCwin model or if the bacteria in the current seawater sample were more preadapted to petroleum hydrocarbons than the inoculum in studies used to develop the BioHCwin model. A study by Prosser et al.[16] also reported higher half-life predictions by BioHCwin compared to biodegradation data summarized from the literature in seawater and stormwater pond water in which there were no sediment or soil present. Three additional explanations for the generally faster degradation in the present study compared to BioHCwin predictions are as follows: (1) Biodegradation testing in gastight vials allowed the determination of water-phase first order half-lives.[20] (2) Biodegradation testing at lower and more environmentally relevant substrate concentrations can lead to higher biodegradation rate constants.[27] (3) The use of passive dosing for setting initial concentrations circumvents the testing of dispersed microdroplets of pure hydrocarbons. This ensured that biodegradation did not become rate limited by the dissolution of the nondissolved phase. A comparison between the seawater half-lives in the present study and calculated well adapted in situ half-lives from the Deepwater Horizon oil spill[17]

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showed similar half-lives for 1,2,4-trimethylbenzene (1.8 days in the present study and 0.8 days in situ), whereas phenanthrene had a longer half-life in this study (16 days) compared to the in situ-calculated half-life (1.6 days).

A detailed look at the results showed that BioHCwin highly overpredicted the half-lives of a few chemicals: Dehydroabietine and 1,2,3,10b-tetrahydrofluoranthene, for example, had predicted half-lives of 2819 and 4908 days and observed half-times of 12 and 63 days in lake water, respectively. These predicted half-lives are not reliable as they are outside of the input data range for the model calibration and probably result from a lack of data for naphthenic di- and triaromatic hydrocarbons.[6,16]

Underprediction of half-lives is more problematic than overprediction because it can lead to environmental risks being overlooked. The two cycloalkanes *cis*-1,2-dimethylcyclohexane and 1,3,5-trimethylcyclohexane had BioHCwin predicted half-lives of 5.1 and 3.5 days but were not degraded in the sea or lake water. In activated sludge filtrate, they were degraded with $T_{1/2,water}$ (1.4 and 4.5 days) close to the BioHCwin predictions. Lack of or slow degradation was similarly seen for 1,3,5-trimethylcyclohexane in a number of surface water samples in earlier similar experiments,[24] but faster degradation was observed in other studies in seawater and rainwater retention pond water using higher initial chemical concentrations.[16] Two other mononaphthenic structures included here had long linear alkyl chains, and primary degradation was presumably driven by this chain rather than their naphthenic structure. It is however noteworthy that the di- and trinaphthenic structures decalin, bicyclohexyl, and perhydrofluorene, which did not include any alkyl chains, were degraded in the seawater.

The most persistent of the hydrocarbons included in this study were 1,1,4,4,6-pentamethyldecalin, perhydropyrene, and 1,2,3,6,7,8-hexahydropyrene, which had limited degradation in all three types of water. Furthermore, 2,2,4,4,6,8,8-heptamethylnonane showed inconsistent degradation in the activated sludge filtrate and seawater and did not degrade in the lake water. In line with observations from Comber et al.,[15] these hydrocarbons were either highly branched with quarternary carbons or highly cyclic structures and had β-substituted terminal carbons (see S7), preventing β-oxidation as a primary transformation step.[38] BioHCwin identified these structures as slow to degrade with half-life predictions of 129−451 days, which is longer than the test duration in this study.

The application of passive dosing for setting initial hydrocarbon concentrations in combination with the very close alignment of test system and SPME-GC-MS analysis provided new possibilities for biodegradation testing. The chosen test volume of 15 mL was a compromise between the 100−1000 mL test systems often used in regulatory biodegradation studies[3,39] and high-throughput miniaturized systems.[40,41] This reduced test volume was very practical and appeared sufficient for the biodegradation testing with the activated sludge filtrate and seawater. However, for the specific lake water with limited biodegradation activity, we observed larger deviations between replicate test systems and a higher frequency of inconsistent data, which might indicate an insufficient test volume for such samples and asks for further studies. The parallel biodegradation testing of up to 34 test chemicals was shown to be a very time- and cost-efficient approach for the generation of a large and consistent data set of biodegradation kinetic data while minimizing the effect of confounding factors. However, further research is needed for determining possible cosubstrate effects on the biodegradation kinetics at low concentrations. The use of pure water for abiotic controls instead of poisoned controls was considered appropriate for these aquatic tests with surface water or activated sludge filtrate but might require adjustments when increasing the amount of suspended particles. In case of concern, poisoned controls can be used instead. Overall, the new approach has several advantages, but of course also limitations. The advantages of this approach are mainly (1) the potential to generate large data sets for chemicals covering a large and relevant chemical space, (2) the possibility of conducting degradation studies at very low environmentally relevant concentrations while avoiding dispersions of pure phase, and (3) the minimization of experimental steps that facilitates biodegradation testing with native microorganisms and rather volatile test substances. The main limitations of the approach are that it is based on substrate depletion, which limits it to the study and quantification of primary biodegradation, and that it presently is limited to biodegradation testing in aqueous media.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05624.

Initial concentrations, characterization of environmental samples, details of GC-MS analysis, lag phases, first order rate constants, half-times, test system half-lives, water phase half-lives, degradation curves, and structure of chemicals with limited degradation in all waters (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) European Parliament. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006.

(2) US Public Law. *Toxic Substances Control Act*; U.S. Senate: 15 US Code, Chapter 53, 2002.

(3) OECD 301. OECD Guideline for Testing of Chemicals. Ready Biodegradability, 1992; pp 1−62.

(4) Aronson, D.; Boethling, R.; Howard, P.; Stiteler, W. Estimating biodegradation half-lives for use in chemical screening. *Chemosphere* **2006**, *63* (11), 1953−1960.

(5) Rücker, C.; Kümmerer, K. Modeling and predicting aquatic aerobic biodegradation − a review from a user's perspective. *Green Chem.* **2012**, *14* (0), 875.

(6) Howard, P.; Meylan, W.; Aronson, D.; Stiteler, W.; Tunkel, J.; Comber, M.; Parkerton, T. F. A new biodegradation prediction model

DOI: 10.1021/acs.est.7b05624
Environ. Sci. Technol. 2018, 52, 2143−2151

specific to petroleum hydrocarbons. *Environ. Toxicol. Chem.* **2005**, *24* (8), 1847−1860.
(7) Rorije, E.; Verbruggen, E. M. J.; De Knecht, J. A. Framework Contract No ECHA/2008. Service Request on a critical review of the environmental and physicochemical methodologies commonly employed in the environmental risk assessment of petroleum substances in the context of REACH registrations. 2012. http://echa.europa.eu/documents/10162/13628/review_environmental_physicochemical_methodol_en.pdf.
(8) Quann, R. J. Modeling the chemistry of complex petroleum mixtures. *Environ. Health Perspect.* **1998**, *106*, 1441−1448.
(9) van de Meent, D.; Hollander, A.; Comber, M.; Parkerton, T. Environmental Fate Factors and Human Intake Fractions for Risk Assessment of Petroleum Products. *Integr. Environ. Assess. Manage.* **2009**, *6* (1), 135−144.
(10) King, D. J.; Lyne, R. L.; Girling, A.; Peterson, D. R.; Stephenson, R.; Short, D. *Environmental risk assessment of petroleum substances: the hydrocarbon block method*; Concawe: Brussels, Report No. 52/96, 1996; Vol. 52.
(11) Prince, R. C.; Parkerton, T.; Lee, C. The primary aerobic biodegradation of gasoline hydrocarbons. *Environ. Sci. Technol.* **2007**, *41*, 3316−3321.
(12) Prince, R. C.; Haitmanek, C.; Lee, C. C. The primary aerobic biodegradation of biodiesel B20. *Chemosphere* **2008**, *71* (8), 1446−1451.
(13) Prince, R. C.; McFarlin, K. M.; Butler, J. D.; Febbo, E. J.; Wang, F. C. Y.; Nedwed, T. J. The primary biodegradation of dispersed crude oil in the sea. *Chemosphere* **2013**, *90* (2), 521−526.
(14) Brakstad, O. G.; Nordtug, T.; Throne-Holst, M. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Mar. Pollut. Bull.* **2015**, *93* (1−2), 144−152.
(15) Comber, M. I. H.; den Haan, K. H.; Djemel, N.; Eadsforth, C. V.; King, D.; León Paumen, M.; Parkerton, T.; Dmytrasz, B. *Primary Biodegradation of Petroleum Hydrocarbons in Seawater*; Concawe: Brussels, Report No. 10/12, 2012.
(16) Prosser, C. M.; Redman, A. D.; Prince, R. C.; Paumen, M. L.; Letinski, D. J.; Butler, J. D. Evaluating persistence of petroleum hydrocarbons in aerobic aqueous media. *Chemosphere* **2016**, *155*, 542−549.
(17) Thessen, A. E.; North, E. W. Calculating in situ degradation rates of hydrocarbon compounds in deep waters of the Gulf of Mexico. *Mar. Pollut. Bull.* **2017**, *122* (1−2), 77−84.
(18) Prince, R. C.; Butler, J. D.; Redman, A. D. The rate of crude oil biodegradation in the sea. *Environ. Sci. Technol.* **2017**, *51* (3), 1278−1284.
(19) Lee, K.; Nedwed, T.; Prince, R. C.; Palandro, D. Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea. *Mar. Pollut. Bull.* **2013**, *73*, 314−318.
(20) Birch, H.; Andersen, H. R.; Comber, M.; Mayer, P. Biodegradation testing of chemicals with high Henry's constants − Separating mass and effective concentration reveals higher rate constants. *Chemosphere* **2017**, *174*, 716−721.
(21) Birch, H.; Gouliarmou, V.; Lützhoft, H. C. H.; Mikkelsen, P. S.; Mayer, P. Passive dosing to determine the speciation of hydrophobic organic chemicals in aqueous samples. *Anal. Chem.* **2010**, *82* (3), 1142−1146.
(22) Gouliarmou, V.; Smith, K. E. C.; De Jonge, L. W.; Mayer, P. Measuring binding and speciation of hydrophobic organic chemicals at controlled freely dissolved concentrations and without phase separation. *Anal. Chem.* **2012**, *84*, 1601−1608.
(23) van der Heijden, S. a.; Hermens, J. L. M.; Sinnige, T. L.; Mayer, P.; Gilbert, D.; Jonker, M. T. O. Determining High-Quality Critical Body Residues for Multiple Species and Chemicals by Applying Improved Experimental Design and Data Interpretation Concepts. *Environ. Sci. Technol.* **2015**, *49*, 1879−1887.
(24) Birch, H.; Hammershøj, R.; Comber, M.; Mayer, P. Biodegradation of hydrocarbon mixtures in surface waters at environmentally relevant levels - Effect of inoculum origin on kinetics and sequence of degradation. *Chemosphere* **2017**, *184*, 400−407.
(25) National Food Institute DTU. Danish (Q)SAR Database. National Food Institute; Technical University of Denmark. http://qsardb.food.dtu.dk/database/index.html (accessed Jun 30, 2017).
(26) Smith, K. E. C.; Dom, N.; Blust, R.; Mayer, P. Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing. *Aquat. Toxicol.* **2010**, *98* (1), 15−24.
(27) Smith, K. E. C.; Rein, A.; Trapp, S.; Mayer, P.; Karlson, U. G. Dynamic passive dosing for studying the biotransformation of hydrophobic organic chemicals: Microbial degradation as an example. *Environ. Sci. Technol.* **2012**, *46* (9), 4852−4860.
(28) Tejeda-Agredano, M. C.; Mayer, P.; Ortega-Calvo, J. J. The effect of humic acids on biodegradation of polycyclic aromatic hydrocarbons depends on the exposure regime. *Environ. Pollut.* **2014**, *184*, 435−442.
(29) Lee, Y. S.; Lee, D. H. Y.; Delafoulhouze, M.; Otton, S. V.; Moore, M. M.; Kennedy, C. J.; Gobas, F. A. P. C. In vitro biotransformation rates in fish liver S9: Effect of dosing techniques. *Environ. Toxicol. Chem.* **2014**, *33* (8), 1885−1893.
(30) Seltman, H. Approximations for Mean and Variance of a Ratio. http://www.stat.cmu.edu/~hseltman/files/ratio.pdf (accessed Jun 29, 2017).
(31) Martin, T. J.; Snape, J. R.; Bartram, A.; Robson, A.; Acharya, K.; Davenport, R. J. Environmentally Relevant Inoculum Concentrations Improve the Reliability of Persistent Assessments in Biodegradation Screening Tests. *Environ. Sci. Technol.* **2017**, *51* (5), 3065−3073.
(32) Leahy, J. G.; Colwell, R. R. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* **1990**, *54* (3), 305−315.
(33) Gros, J.; Reddy, C. M.; Aeppli, C.; Nelson, R. K.; Carmichael, C. A.; Arey, J. S. Resolving biodegradation patterns of persistent saturated hydrocarbons in weathered oil samples from the Deepwater Horizon disaster. *Environ. Sci. Technol.* **2014**, *48* (3), 1628−1637.
(34) Head, I. M.; Jones, D. M.; Röling, W. F. M. Marine microorganisms make a meal of oil. *Nat. Rev. Microbiol.* **2006**, *4* (3), 173−182.
(35) Rein, A.; Fernqvist, M. M.; Mayer, P.; Trapp, S.; Bittens, M.; Karlson, U. G. Degradation of PCB congeners by bacterial strains. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 469−481.
(36) Shrestha, P.; Junker, T.; Fenner, K.; Hahn, S.; Honti, M.; Bakkour, R.; Diaz, C.; Hennecke, D. Simulation Studies to Explore Biodegradation in Water-Sediment Systems: From OECD 308 to OECD 309. *Environ. Sci. Technol.* **2016**, *50* (13), 6856−6864.
(37) Xia, X. H.; Yu, H.; Yang, Z. F.; Huang, G. H. Biodegradation of polycyclic aromatic hydrocarbons in the natural waters of the Yellow River: Effects of high sediment content on biodegradation. *Chemosphere* **2006**, *65* (3), 457−466.
(38) Alexander, M. Nonbiodegradable and other recalcitrant molecules. *Biotechnol. Bioeng.* **1973**, *15*, 611−647.
(39) OECD 309. OECD Guideline for the testing of Chemicals. Aerobic Mineralisation in Surface Water − Simulation Biodegradation Test; OECD, 2004.
(40) Cregut, M.; Jouanneau, S.; Brillet, F.; Durand, M. J.; Sweetlove, C.; Chenèble, J. C.; L'Haridon, J.; Thouand, G. High throughput and miniaturised systems for biodegradability assessments. *Environ. Sci. Pollut. Res.* **2014**, *21* (16), 9545−9552.
(41) Martin, T. J.; Goodhead, A. K.; Acharya, K.; Head, I. M.; Snape, J. R.; Davenport, R. J. High Throughput Biodegradation-Screening Test To Prioritize and Evaluate Chemical Biodegradability. *Environ. Sci. Technol.* **2017**, *51* (12), 7236−7244.

DOI: 10.1021/acs.est.7b05624
Environ. Sci. Technol. 2018, 52, 2143−2151