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Published in:
Chemosphere

Link to article, DOI:
[10.1016/j.chemosphere.2017.02.003](https://doi.org/10.1016/j.chemosphere.2017.02.003)

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):

Birch, H., Andersen, H. R., Comber, M., & Mayer, P. (2017). Biodegradation testing of chemicals with high Henry's constants – Separating mass and effective concentration reveals higher rate constants. *Chemosphere*, 174, 716-721. <https://doi.org/10.1016/j.chemosphere.2017.02.003>

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Biodegradation Testing of Chemicals with High Henry's Constants – Separating Mass and Effective Concentration Reveals Higher Rate Constants

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Abstract

During simulation-type biodegradation tests, volatile chemicals will continuously partition between water phase and headspace. This study addressed how (1) this partitioning affects test results and (2) can be accounted for by combining equilibrium partition and dynamic biodegradation models. An aqueous mixture of 9 (semi)volatile chemicals was first generated using passive dosing and then diluted with environmental surface water producing concentrations in the ng/L to $\mu\text{g/L}$ range. After incubation for 2 hours to 4 weeks, automated Headspace Solid Phase Microextraction (HS-SPME) was applied directly on the test systems to measure substrate depletion by biodegradation relatively to abiotic controls. HS-SPME was also applied to determine air to water partitioning ratios. Biodegradation rate constants relating to the chemical in the water phase, k_{water} , were generally a factor 1 to 11 times higher than biodegradation rate constants relating to the total mass of chemical in the test system, k_{system} , with one exceptional factor of 72 times for a long chain alkane. True water phase degradation rate constants were found (i) more appropriate for risk assessment than test system rate constants, (ii) to facilitate extrapolation to other air-water systems and (iii) to be better defined input parameters for aquatic exposure and fate models.

Table of Contents Graphics:

Keywords: Biodegradation test, volatile chemicals, hydrocarbon, rate constant, headspace

1 Introduction

Biodegradation is an important environmental fate process for most organic chemicals, and data describing biodegradation kinetics are thus needed for modelling and risk assessment purposes (Aronson et al., 2006). Outside of the regulatory systems, estimation of kinetic degradation data from screening test data (Aronson et al., 2006) or via quantitative structure relationships has been attempted (Howard et al., 2005), however good quality kinetic data from laboratory based tests or field studies are called for (Aronson et al., 2006; Gouin et al., 2004).

Within the regulatory system biodegradability testing is required under the Registration, Evaluation, Authorisation and Restriction of Chemicals legislation in the European Union (European Parliament, 2006) and Toxic Substances Control Act in the United States (US Public law, 2002). At the screening level of environmental risk assessment or assessment of persistence, bioaccumulation and toxicity, the Ready Biodegradation studies, OECD series 301 (OECD, 1992), are used. They include test methods without a headspace, which are appropriate for volatile chemicals, but are often conducted at very high test substance concentrations. These measure ultimate biodegradation on a pass/fail level. Half-lives or rate constants are assigned based on whether, or not, the chemical is assessed as Readily Biodegradable. Subsequent evaluations may require further assessment through simulation biodegradation studies that can deliver primary biodegradation half-lives or rate constants. Unfortunately, these methods are generally categorized as not applicable to volatile chemicals.

The first step of adapting test systems for volatile chemicals with high air to water partition ratios (i.e. Henry's constants) is a closed test design, which circumvents evaporative losses out of the test system. When testing chemicals at low concentrations, the dissolved oxygen in the

environmental sample is sufficient for the degradation of the test chemicals, however, environmental samples contain natural organic matter which also consumes oxygen, and therefore a headspace can be needed to ensure aerobic conditions. A major fraction of volatile chemicals, will then reside in the headspace. During the degradation phase, partitioning between water phase and headspace will govern the distribution of chemicals in the test system, continuously replenishing the test chemicals degraded in the water phase. For volatile substances, there is then a mismatch between the effective concentration for the biodegradation in the water phase and the total mass distributed between water and headspace. In unsaturated soil, researchers have addressed the importance of the vapor phase as a reservoir and mass-transfer medium for volatile chemicals (Khan et al., 2016). However, in environmental surface water systems, volatilization would mostly act as a sink rather than a buffer for volatile chemicals, and therefore the water phase biodegradation is the relevant parameter when extrapolating to other test or environmental surface water systems.

The fact that the dissolved concentration rather than the total mass of test chemical may govern biodegradation rates has been realized earlier, especially for highly sorbing chemicals in test systems including sediments and soils (Reichenberg and Mayer, 2006). Monod kinetic parameters have in some cases been determined using sets of non-linear differential equations describing degradation and the distribution or rates of transfer between the air/water phase and unsaturated soil (Höhener et al., 2003; Ostendorf et al., 2007; Rein et al., 2007; Sleep and Mulcahy, 1998), soil-slurry (Woo et al., 2001), test vessel (Guha and Jaffé, 1996) or microcrystals (Adam et al., 2014). These studies employed quite extensive modeling efforts, which are not usually employed in legislative biodegradation testing.

Schirmer et al. (1999) suggested a simple approach assuming instantaneous partitioning between phases and describing the distribution in the test system by a mass distribution coefficient, defined as the ratio of the total mass in the test system to the bioavailable mass at equilibrium. A mass distribution coefficient of 1.86 was determined for *m*-xylene in their study (Schirmer et al., 1999). Later studies (Comber et al., 2012; Prince et al., 2008, 2007), using more volatile chemicals, did not take distribution to headspace into account, and thus biodegradation rates were likely underestimated.

The present study investigated the primary biodegradation of a mixture of 9 (semi)volatile chemicals in surface water. The chemicals were selected to cover different chemical structures of potential oil constituents (see Table 1), and the testing was conducted at low aqueous concentrations (ng/L - µg/L range) in order to obtain biodegradation results of high environmental relevance. We investigated (1) how partitioning between water phase and headspace affects test results and (2) how partitioning can be accounted for by combining an equilibrium partition model with a first order or logistic biodegradation model. The present study introduces a new experimental framework, where phase partitioning of the test chemicals was applied for the (i) conduct, (ii) analytical measurements and (iii) the assessment of the biodegradation tests: (i) Phase partitioning from a loaded silicone rod was used to generate defined composed mixtures of hydrocarbons at the beginning of the experiments. (ii) Phase partitioning into a thin silicone coating was the basis for the automated sampling directly in the test systems at the end of the experiment. (iii) Finally, the phase partitioning of the test substances between water and headspace was measured and then applied to distinguish the biodegradation kinetics in the test system (water and headspace) and the biodegradation kinetics in the water phase. The experimental and analytical procedure was designed to obtain very

accurate and precise "relative concentrations", as input for fitting the biodegradation kinetic model. This was obtained by incubation of test systems in gas tight 20 mL autosampler vials, which at the end of the experiment were measured directly by automated Headspace Solid Phase Microextraction, and by normalizing the Gas Chromatography (GC) response by measurements of abiotic control vials which had been incubated together with the samples and measured within the same analytical series.

2 Theory

Monod kinetics can be used to describe biomass growth on a single substrate (Simkins and Alexander, 1984). In a system including headspace, we propose to separate the total mass of the test substance in the test system (m_T) from the concentration in the water phase (C_w) determining the biodegradation rate, realizing that degradation takes place in the water phase.

At low substrate concentration (ng/L - µg/L range) and low initial biomass, monod based degradation kinetics can be simplified by the Logistic model (Simkins and Alexander, 1984), shown in Equation 1 for a test system with headspace.

$$(1) \frac{dm_T}{dt} = -a_{water} V_w C_w (C_{w,0} + X_0 - C_w)$$

X_0 is the initial specific degrader population density divided by the yield (i.e. the amount of chemical needed to produce the initial specific degrader population density), a is the logistic rate constant, K_S is the half-saturation constant for growth of the degrading organisms and it is assumed that $C_{w,0} \ll K_S$.

For practical/regulatory purposes, this model is often approximated by a lag phase, t_{lag} , (during which biomass adapts/increases but no degradation takes place) followed by first order degradation (e.g. OECD 309, 2004).

The first order degradation after the lag phase in systems with a headspace is therefore described by equation 2.

$$(2) \frac{dm_T}{dt} = -k_{water} V_w C_w$$

Where k_{water} is the rate constant in the water phase and V_w is the volume of the water phase.

The distribution between water phase and headspace is governed by equilibrium partitioning.

If the partitioning rates are faster than the degradation rate, the ratio m_w/m_T can be assumed constant during the test, and equation 2 can be converted to equation 3 and solved for C_w from $t = 0$ to $t = \infty$ (equation 4).

$$(3) \frac{dC_w}{dt} = -k_{water} \frac{m_{w,0}}{m_{T,0}} C_w$$

$$(4) C_w = C_{w,0} e^{-k_{water} \frac{m_{w,0}}{m_{T,0}} (t - t_{lag})}$$

where $m_{w,0}$ and $C_{w,0}$ is the initial mass and concentration of the test chemical in the water phase and $m_{T,0}$ is the initial total mass of the test chemical.

Fitting a first order model to data without taking distribution in the test system into account yields the test system first order rate constant, k_{system} , which relates to the water phase rate constant by equation 5.

$$(5) k_{system} = k_{water} \frac{m_{w,0}}{m_{T,0}} = k_{water} \cdot \frac{V_w}{V_w + K_{aw} V_h}$$

where V_h is the volume of the headspace, V_w is the volume of the water phase, and K_{aw} is the air water partition ratio for the test chemical. Water phase degradation rate constants for chemicals with high air to water partition ratios are thus higher than test system rate constants, and half-lives are lower in the water phase than in the test system.

For some research purposes, the Monod based logistic model is preferred to the first order degradation model because this model uses descriptors relating to biological processes.

However, the same principle can be used with this model, and the relationship between the test system logistic rate constant (a_{system}) and the water phase logistic rate constant (a_{water}) similarly depends on the fraction of test chemical in the water phase compared to the total mass in the test system (see equation 6 and Supporting Information S1)

$$(6) a_{system} = a_{water} \frac{m_{w,0}}{m_{T,0}} = a_{water} \cdot \frac{V_w}{V_w + K_{aw}V_h}$$

3 Materials and Methods

3.1 Materials

The test chemicals (purity of $\geq 98\%$) were *n*-decane, tetralin, biphenyl, *trans*-decalin, bicyclohexyl, 1,2,4-trimethylbenzene, naphthalene (Sigma-Aldrich, Copenhagen, Denmark), 2,3-dimethylheptane and 1,3,5-trimethylcyclohexane (Tokyo Chemical Industry Europe, Zwijndrecht, Belgium). 1-octanol (Sigma-Aldrich) was used as reference chemical. Translucent silicone rod (diameter 3 mm; custom-made by Altecweb.com, product code 136-8380) was used as partitioning donor for the passive dosing. Its partitioning properties have recently been referenced against other silicones that are used for passive sampling and dosing (Gilbert et al, Anal Chem, 2016). Ethylacetate ($\geq 99.7\%$, Sigma-Aldrich) and ethanol ($> 99.8\%$, VWR chemicals) were used for pre-cleaning of the silicone rods. Ultrapure water was produced on a LaboStarTM 1-DI ultrapure water system from SGwater (Hamburg, Germany).

3.2 Preparation of stock solution by passive dosing

Pre-cleaned silicone rods were loaded by addition of an equi-mass mixture (1:1:1...) of nine neat test chemicals that were quantitatively absorbed by the rods. An aqueous stock solution was then generated by passive dosing from these loaded rods. The stock solution was subsequently diluted 10 fold resulting in initial test substance concentrations 2-4 orders of magnitude below

the solubility of each test substance. Later measurements of concentrations produced by the silicone rods revealed initial test concentration levels of approximately 0.03 µg/L bicyclohexyl, 30 µg/L biphenyl, 0.5 µg/L decalin, 0.2 µg/L *n*-decane, 0.2 µg/L 2,3-dimethylheptane, 70 µg/L naphthalene, 30 µg/L tetralin, 60 µg/L 1,2,4-trimethylbenzene and 0.5 µg/L 1,3,5-trimethylcyclohexane. All transfer of stock solution was done using gas tight syringes. See Supporting Information S2 for details.

3.3 Surface water inoculum

A surface water grab sample was taken from a small brook (Fønstrup Brook) in northern Sealand, Denmark, which runs through forest, and is not pre-adapted to petroleum hydrocarbons by stormwater or wastewater discharges. The samples contained no measurable background concentrations of the test chemicals. Heterotrophic Plate Count measurements yielded a bacterial count of $1.2 \cdot 10^4$ colony forming units/mL.

3.4 Biodegradation test method

Biodegradation test systems were prepared in 20 mL headspace vials with PTFE faced silicone septa compatible with the GC autosampler. 13.5 mL of unfiltered surface water, spiked with 30 µg/L 1-octanol, was added to all biotic test system vials. 1.5 mL stock solution was then added, bringing the total water volume to 15 mL, and the vials were closed immediately. Abiotic test systems were prepared in the same way using ultrapure water instead of surface water. Test systems were incubated at 20 °C on a roller mixer (~ 30 rpm). On day 0, 1, 2, 5, 6, 8, 10, 14, 20 and 27, three biotic and three abiotic test systems were harvested for analysis.

3.5 Phase distribution tests

Varying the headspace to water phase ratio in test systems, can reveal the air to water partition ratio (Mayer et al., 2000). Stock solution was added to 20 mL vials in volumes of 2.5, 5, 15, 17

and 19 mL in triplicate. After equilibration by 5 minutes shaking on a vibrax orbital shaker at 1000 rpm, 2 mL of the water phase was transferred to 20 mL vials. 2 mL of the stock solution was also transferred directly to three 20 mL vials.

3.6 Analytical method

Analysis was done by Gas Chromatography - Mass Spectrometry using fully automated Headspace Solid Phase Micro Extraction directly in test vials (See Supporting Information S3 for details).

3.7 Data treatment

The analytical response in the test systems (Supporting Information S7) were first normalized by the response in the abiotic controls measured within the same analytical series, and then plotted as a function of incubation time. GraphPad Prism 5.00 was used to fit a first order degradation model with lag phase (excluded for chemicals partly degraded after one day) to the data. 95 % confidence limits were estimated by substituting k_{system} for $10^{(log(k_{system}))}$ and treating $log(k_{system})$ as the variable, since degradation rate constants are constrained to positive values and lognormal distribution of biodegradation rate constants were seen for hydrocarbons in a parallel study (data not included). In two test vials the response for 2,3-dimethylheptane and in three test vials the response for 1,3,5-trimethylcyclohexane were > 150% of the response in the abiotic controls. These five data points (of a total of ~300 datapoints) were removed as outliers. This correction resulted in < 15% change in the fitted degradation rate constant (See Supporting Information S4). In order to illustrate that the approach works equally well for both types of models, a logistic model was also fitted to the data (see equations in Supporting information S1 and discussion in S5). a_{water} and k_{water} were calculated from a_{system} and k_{system} using equation 4 and 5.

In the phase distribution experiment, the distribution between headspace and water phase was described by equation 6.

$$(6) \frac{m_w}{m_T} = \frac{C_w}{C_{stock}} = \frac{1}{1 + K_{aw} \frac{V_h}{V_w}}$$

Where C_{stock} is the concentration of the test chemicals in the stock solution. GraphPad Prism was used to plot C_w/C_{stock} against V_h/V_w , fit equation 6 to the data and estimate K_{aw} . 95 % confidence limits of K_{aw} were estimated using lognormal transformation of K_{aw} as described above. The quality criteria, $R^2 > 0.8$, was used for the measured K_{aw} .

4 Results and discussion

The measured air water partition ratios for the five test chemicals meeting the quality criteria were in the same order of magnitude as literature data (Table 1).

Table 1: Measured air water partition ratio with 95% confidence limits in brackets and literature values. The values used for calculations in the manuscript indicated in bold, predicted values indicated in italic.

<i>Compound</i>	<i>Measured K_{aw} (L/L)</i>	<i>R²</i>	<i>Literature K_{aw} (L/L) at 25°C</i>
Bicyclohexyl	ND ^a		4^b
Biphenyl	ND ^a		0.013^c ; 0.005-0.17 ^d
Decalin	14 (12-16)	0.96	19 ^b ; 4.8 ^e
<i>n</i> -Decane	ND ^a		210 ; 130-280 ^d
2,3-Dimethylheptane	31 (25-37)	0.88	17; 281; 163 ^f
Naphthalene	ND ^a		0.006 ^g ; 0.018^c -0.030 ^d
Tetralin	0.049 (0.034-0.056)	0.83	0.056 ^b ; 0.076 ^e
1,2,4-Trimethylbenzene	0.15 (0.13-0.19)	0.93	0.25 ^h
1,3,5-Trimethylcyclohexane	33 (28-38)	0.93	39; 24; 11 ^f

^aND Not determined as R² was less than 0.8. ^b(from National Food Institute DTU, 2015) ^c(Shiu and Mackay, 1997) ^d(Mackay et al., 2006) ^e(Ashworth et al., 1988) ^fQSAR estimates from EPISuite using the vapor pressure/water solubility method, Group method and Bond method respectively (from National Food Institute DTU, 2015) ^g(Lee et al., 2012) ^h(Sanemasa et al., 1982).

In Table 2, the best fit for the lag phases, first order rate constants and logistic rate constants are shown. Most of the chemicals showed a fast degradation after the lag phase, as illustrated in Figure 1.

240 **Table 2:** Lag phase, t_{lag} , test system first order degradation rate constants, k_{system} , water phase
 241 first order rate constants, k_{water} , test system logistic degradation rate constants, a_{system} , water phase
 242 logistic rate constants, a_{water} , and conversion factor, k_{water}/k_{system} , between test system and water
 243 phase rate constants. 95 % confidence intervals are indicated in brackets.

	t_{lag} [d]	k_{system} [d ⁻¹]	k_{water} [d ⁻¹]	a_{system} [d ⁻¹ % ⁻¹]	a_{water} [d ⁻¹ % ⁻¹]	k_{water} / k_{system}
Bicyclohexyl	< 1	0.28 (0.25-0.32)	0.65	0.002 (0.0008-0.006)	0.005	2.3
Biphenyl	5.4 (5.1-5.6)	0.8 (0.5-1.4)	0.8	0.017 (0.010-0.030)	0.017	1.0
Decalin	5.7 (4.9-6.6)	0.23 (0.16-0.33)	1.3	0.009 (0.006-0.013)	0.051	5.7
n-Decane	< 1	1.4 (1.1-1.8)	96	0.02 (0.007-0.06)	1.5	71
2,3-Dimethylheptane	5.6 (4.5-6.6)	0.5 (0.3-0.9)	6	0.020 (0.016-0.025)	0.22	11
Naphthalene	5.8 (5.7-6.0)	1.1 (0.7-1.7)	1.1	0.020 (0.015-0.027)	0.020	1.0
Tetralin	7.7 (7.5-8.0)	0.8 (0.5-1.4)	0.8	0.015 (0.010-0.023)	0.015	1.0
1,2,4-Trimethylbenzene	5.5 (5.2-5.8)	0.42 (0.31-0.58)	0.44	0.013 (0.008-0.019)	0.013	1.1
1,3,5-Trimethyl-cyclohexane	No Biodegradation					

244

Figure 1. Degradation data for the test chemicals (A and B) with standard error on mean (n=3) and fitted degradation curves using the logistic model (full line) and the first order model including lag phase (broken line).

The conversion factor, $k_{water}/k_{system} (=a_{water}/a_{system})$, (see Table 2) can be viewed as the factor by which degradation rates are underestimated if headspace is not considered for volatile chemicals, and demonstrate the degree of conservatism currently applied to results of biodegradation tests. The conservatism is not only applied to volatile chemicals, but also to hydrophobic chemicals when sorbed to the test vessel, sorbed to suspended matter or partly undissolved. For a correction to be appropriate, some requirements need to be fulfilled. First of all, the approach requires that the transfer between the headspace and the water phase is faster than the degradation in order to maintain equilibrium in the test system. Secondly, the correction should be larger than the uncertainty of the original value and thirdly, the correction factor should have a reasonably low uncertainty. In stirred or agitated systems, the phase transfer is expected to be fast compared to

the biodegradation. For the test chemicals with an air water partition ratio < 0.4 L/L (or Henry's laws constant $< 10^{-2}$ atm m³/mol or 10^3 Pa m³/mol), the calculated k_{water} and a_{water} were within the 95 % confidence intervals of k_{system} and a_{system} , respectively, and these chemicals did therefore not require attention to the distribution in the test system. For the test chemicals with an air water partition ratio ≥ 4 L/L (or Henry's laws constant $> 10^{-1}$ atm m³/mol or 10^4 Pa m³/mol) the water phase rate constants were significantly higher than the test system rate constants. For two of these chemicals the distribution in the test system could be determined relatively precisely, and for these chemicals the correction therefore improves the determination of the degradation rate constant. For decalin the first order degradation rate constant corresponded to a half-life of 3 days in the test system and 0.5 days in the water phase.

Decane has a very high volatility from water, and determination of reliable air to water partition ratios are a challenge and has yielded highly varying results (Sedlbauer et al., 2002). The conversion factor (72) for decane should therefore be treated with care. The rates corresponded to a half-life of 0.5 days in the test system and a half-life of 10 minutes in the water phase. These data indicate that the degradation is so fast that it can compete with volatilization processes in the environment. However, until more reliable measurements of the air water partitioning are produced, the results for decane are subjected to a large uncertainty.

There are different aims and reasons for doing biodegradation tests. It may be argued that a correction should not be done in order to keep the estimate conservative for regulatory purposes. However, we find it unreasonable to apply conservatism that applies selectively to certain types of chemicals (in this case volatile chemicals). Furthermore test results are not only used in a regulatory context. Comparison of biodegradation kinetics between different biodegradation tests is only appropriate based on headspace-corrected rate constants, and the corrected rate constants

are better defined input parameters for aquatic exposure and fate models. It may also be argued that the variation between degradation rate constants measured in water/inoculum from different sites can be higher than the correction factors used here and correction is therefore not necessary. However, since the correction affects most or all of the measurements, it shifts the complete population of rate constants.

We here supply researchers, regulators and contract laboratories with an approach, easily adoptable into the regulatory framework, to correct for artifact due to the difference between test system and water phase degradation rates and suggest the use of this conversion method when reporting degradation kinetics for chemicals with an air water partition ratio ≥ 4 L/L (or Henry's laws constant of $\sim 10^{-1}$ atm m³/mol or 10^4 Pa m³/mol). For chemicals with lower air water partition ratio but higher hydrophobicity, the dominating process will be the partitioning between water and suspended matter, sediment and dissolved organic matter or the dissolution from the pure phase. The proposed framework of separating effective concentration and total mass for the calculation of water phase degradation rates can then still be applied as long as degradation rather than phase partitioning is rate limiting.

ASSOCIATED CONTENT

Supporting Information. Details of passive dosing format and analytical method, logistic model equations, degradation data for 2,3-Dimethylheptane without outlier removal, comment on model choice in relation to distribution conversion and fit of data from the phase distribution test. This material is available free of charge.

ACKNOWLEDGMENT

305 The authors thank Concawe for financial support and Hanne Bøggild for technical assistance in
306 the laboratory.

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308 REFERENCES

- 309 Adam, I.K.U., Rein, A., Miltner, A., Fulgêncio, A.C.D., Trapp, S., Kästner, M., 2014.
 310 Experimental results and integrated modeling of bacterial growth on an insoluble
 311 hydrophobic substrate (phenanthrene). *Environ. Sci. Technol.* 48, 8717–8726.
 312 doi:10.1021/es500004z
- 313 Aronson, D., Boethling, R., Howard, P., Stiteler, W., 2006. Estimating biodegradation half-lives
 314 for use in chemical screening. *Chemosphere* 63, 1953–1960.
 315 doi:10.1016/j.chemosphere.2005.09.044
- 316 Ashworth, R.A., Howe, G.B., Mullins, M.E., Rogers, T.N., 1988. Air-Water Partitioning
 317 Coefficients of Organics in Dilute Aqueous Solutions. *J. Hazard. Mater.* 18, 25–36.
- 318 Comber, M.I.H., den Haan, K.H., Djemel, N., Eadsforth, C.V., King, D., León Paumen, M.,
 319 Parkerton, T., Dmytrasz, B., 2012. Primary Biodegradation of Petroleum Hydrocarbons in
 320 Seawater. Concawe, Brussels. doi:ISBN 978-2-87567-012-0
- 321 European Parliament, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of
 322 the Council of 18 December 2006.
- 323 Gouin, T., Cousins, I., Mackay, D., 2004. Comparison of two methods for obtaining degradation
 324 half-lives. *Chemosphere* 56, 531–5. doi:10.1016/j.chemosphere.2004.04.018
- 325 Guha, S., Jaffé, P.R., 1996. Determination of monod kinetic coefficients for volatile hydrophobic
 326 organic compounds. *Biotechnol. Bioeng.* 50, 693–9. doi:10.1002/(SICI)1097-
 327 0290(19960620)50:6<693::AID-BIT10>3.0.CO;2-G
- 328 Höhener, P., Duwig, C., Pasteris, G., Kaufmann, K., Dakhel, N., Harms, H., 2003.

329 Biodegradation of petroleum hydrocarbon vapors: Laboratory studies on rates and kinetics
 330 in unsaturated alluvial sand. *J. Contam. Hydrol.* 66, 93–115. doi:10.1016/S0169-
 331 7722(03)00005-6

332 Howard, P., Meylan, W., Aronson, D., Stiteler, W., Tunkel, J., Comber, M., Parkerton, T.F.,
 333 2005. A new biodegradation prediction model specific to petroleum hydrocarbons. *Environ.*
 334 *Toxicol. Chem.* 24, 1847–1860. doi:10.1897/04-453R.1

335 Khan, A.M., Wick, L.Y., Harms, H., Thullner, M., 2016. Biodegradation of vapor-phase toluene
 336 in unsaturated porous media: Column experiments. *Environ. Pollut.* 211, 325–331.
 337 doi:10.1016/j.envpol.2016.01.013

338 Lee, H., Kim, H., Kwon, J., 2012. Determination of Henry's Law Constant Using Diffusion in
 339 Air and Water Boundary Layers.

340 Mackay, D., Shiu, W.Y., Ma, K.-C., Lee, S.C., 2006. Handbook of Physical-Chemical Properties
 341 and Environmental Fate for Organic Chemicals, Second Ed. ed. CRC Press, Taylor &
 342 Francis, London.

343 Mayer, P., Vaes, W.H.J., Hermens, J.L.M., Oomen, A.G., Tolls, J., 2000. Absorption of
 344 hydrophobic compounds into the poly(dimethylsiloxane) coating of solid-phase
 345 microextraction fibers: high partition coefficients and fluorescence microscopy images.
 346 *Anal. Chem.* 72, 459–64. doi:10.1021/ac990948f

347 National Food Institute DTU, 2015. EPISuite data accessed via Danish (Q)SAR Database.
 348 [WWW Document]. URL <http://qsardb.food.dtu.dk/database/index.html> (accessed 3.8.16).

349 OECD, 301, 1992. Test No. 301: Ready Biodegradability. OECD Guidel. Test. Chem. 1–62.

doi:10.1787/9789264070349-en

OECD 309, 2004. OECD Guideline for the testing of Chemicals. Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test. doi:10.1787/9789264070547-en

Ostendorf, D.W., Schoenberg, T.H., Hinlein, E.S., Long, S.C., 2007. Monod Kinetics for Aerobic Biodegradation of Petroleum Hydrocarbons in Unsaturated Soil Microcosms. *Environmenal Sci. Technol.* 41, 1–11.

Prince, R.C., Haitmanek, C., Lee, C.C., 2008. The primary aerobic biodegradation of biodiesel B20. *Chemosphere* 71, 1446–1451. doi:10.1016/j.chemosphere.2007.12.010

Prince, R.C., Parkerton, T., Lee, C., 2007. The primary aerobic biodegradation of gasoline hydrocarbons. *Environ. Sci. Technol.* 41, 3316–3321. doi:10.1016/j.chemosphere.2007.12.010

Reichenberg, F., Mayer, P., 2006. Two complementary sides of bioavailability: accessibility and chemical activity of organic contaminants in sediments and soils. *Environ. Toxicol. Chem.* 25, 1239–1245. doi:10.1897/05-458R.1

Rein, A., Fernqvist, M.M., Mayer, P., Trapp, S., Bittens, M., Karlson, U.G., 2007. Degradation of PCB congeners by bacterial strains. *Appl. Microbiol. Biotechnol.* 77, 469–481. doi:10.1007/s00253-007-1175-6

Sanemasa, I., Araki, M., Deouchi, T., Nagai, H., 1982. Solubility Measurements of Benzene and the Alkylbenzenes in Water by Making Use of Solute Vapor. *Bull. Chem. Soc. Japan* 55, 1054–1062.

Schirmer, M., Butler, B.J., Roy, J.W., Frind, E.O., Barker, J.F., 1999. A relative-least-squares

371 technique to determine unique Monod kinetic parameters of BTEX compounds using batch
372 experiments. *J. Contam. Hydrol.* 37, 69–86. doi:10.1016/S0169-7722(98)00159-4

373 Sedlbauer, J., Bergin, G., Majer, V., 2002. Group Contribution Method for Henry ' s Law
374 Constant of Aqueous Hydrocarbons. *AIChE J.* 48, 2936–2959.

375 Shiu, W.Y., Mackay, D., 1997. Henry's Law Constants of Selected Aromatic Hydrocarbons,
376 Alcohols, and Ketones. *J. Chem. Eng. Data* 42, 27–30. doi:10.1021/je960218u

377 Simkins, S., Alexander, M., 1984. Models for mineralization kinetics with the variables of
378 substrate concentration and population density. *Appl. Environ. Microbiol.* 47, 1299–1306.

379 Sleep, B.E., Mulcahy, L.J., 1998. Estimation of Biokinetic Parameters for Unsaturated Soils. *J.*
380 *Environ. Eng.* 124, 959–969. doi:10.1061/(ASCE)0733-9372(1998)124:10(959)

381 US Public law, 2002. Toxic substances control act.

382 Woo, S.H., Park, J.M., Rittmann, B.E., 2001. Evaluation of the interaction between
383 biodegradation and sorption of phenanthrene in soil-slurry systems. *Biotechnol. Bioeng.* 73,
384 12–24.

385