



# Is the Arrhenius-correction of biodegradation rates, as recommended through REACH guidance, fit for environmentally relevant conditions? An example from petroleum biodegradation in environmental systems

David M. Brown<sup>a,\*</sup>, Louise Camenzuli<sup>b,d</sup>, Aaron D. Redman<sup>b,d</sup>, Chris Hughes<sup>a</sup>, Neil Wang<sup>c,d</sup>, Eleni Vaiopoulou<sup>d</sup>, David Saunders<sup>d,e</sup>, Alex Villalobos<sup>d,f</sup>, Susannah Linington<sup>d,g</sup>

<sup>a</sup> Ricardo Energy & Environment, Oxon, UK

<sup>b</sup> ExxonMobil Petroleum & Chemical, Machelen, Belgium

<sup>c</sup> Total, Paris, France

<sup>d</sup> Concawe, Brussels, Belgium

<sup>e</sup> Shell International, The Hague, Netherlands

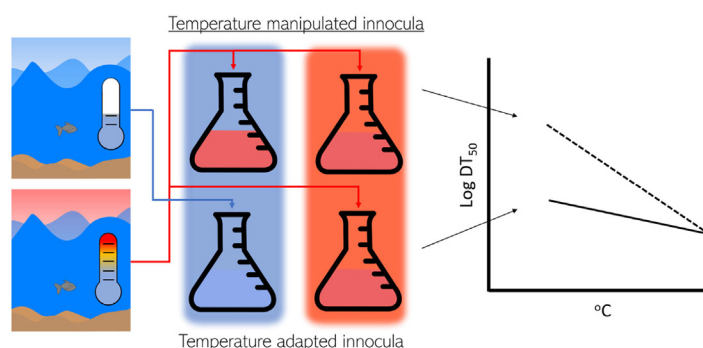
<sup>f</sup> BP, Naperville, USA

<sup>g</sup> BP, Reading, UK

## HIGHLIGHTS

- Persistence is important to the fate of hydrocarbons in the environment.
- Arrhenius has been used to describe the effect of temperature on DT<sub>50</sub>.
- Microbial populations are dynamic and can adapt to ambient environmental temperatures.
- Arrhenius does not describe temperature effects in temperature-adapted microbial populations.
- The use of Arrhenius to temperature correct DT<sub>50</sub> data is not suitable for hydrocarbons.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Biodegradation is a major determinant of chemical persistence in the environment and an important consideration for PBT and environmental risk assessments. It is influenced by several environmental factors including temperature and microbial community structure. According to REACH guidance, a temperature correction based on the Arrhenius equation is recommended for chemical persistence data not performed at the recommended EU mean surface water temperature. Such corrections, however, can lead to overly conservative P/vP assessments. In this paper, the relevance of this temperature correction is assessed for petroleum hydrocarbons, using measured surface water (marine and freshwater) degradation half-time (DT<sub>50</sub>) and degradation half-life (HL) data compiled from relevant literature. Stringent screening criteria were used to specifically select data from biodegradation tests containing indigenous microbes and conducted at temperatures close to their ambient sampling temperature. As a result, ten independent studies were identified, with 993 data points covering 326 hydrocarbon constituents. These data were derived from tests conducted with natural seawater, or freshwater, at temperatures ranging from 5 to 21 °C. Regressions were performed on the full hydrocarbon dataset and on

\* Corresponding author.

E-mail address: [david.brown@ricardo.com](mailto:david.brown@ricardo.com) (D.M. Brown).

several individual hydrocarbons. The results were compared to the trend as predicted by the Arrhenius equation and using the activation energy ( $E_a$ ) as recommend in the REACH Guidance. The comparison shows that the correction recommended in REACH Guidance over predicts the effect of temperature on hydrocarbon biodegradation. These results contrast with temperature manipulated inocula where the test temperature is different from the ambient sampling temperature. In these manipulated systems, the effect of temperature follows the Arrhenius equation more closely. In addition, a more striking effect of temperature on the lag phase was observed with longer lag phases more apparent at lower temperatures. This indicates that the effect of temperature may indeed be even lower when considering hydrocarbon biodegradation without the initial lag phase.

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## 1. Introduction

Biodegradation is a natural process by which organic matter is converted to simpler compounds and ultimately mineralised to inorganic end-products. It is an important environmental fate process that influences the exposure of chemical products and/or substances both to humans and environmental receptors (Di Guardo et al., 2018; MacLeod and Mackay, 2004; Scheringer, 1996). In chemical risk assessments, biodegradation is used together with use and emission patterns to estimate a predicted environmental concentration (PEC) for a chemical. The PEC can then be compared to Predicted No Effect Concentration (PNEC) thresholds to estimate risk. For more site-specific assessments, such as unintended chemical spills or releases, biodegradation is used to predict spatial and temporal variations in chemical exposures which are then used to support time variable toxicity assessments (reviewed in Socolofsky et al., 2019).

Petroleum hydrocarbons and their refined products are used on a global scale and are integral to modern societies. Continued production and use of hydrocarbons may cause inadvertent releases into the environment, where they will be subject to several fate processes including biodegradation. Hydrocarbons are subject to extensive biodegradation under a wide range of environmental conditions in catabolic processes undertaken by microorganisms such as bacteria and fungi (Atlas and Bartha, 1981; Leahy and Colwell, 1990). Microorganisms play a key role in carbon and nutrient cycling and are ubiquitous in the environment (Prince et al., 2010; Cavicchioli et al., 2019). As such, hydrocarbon biodegradation is also known to occur in extreme environments, including the frigid conditions of the Arctic and Antarctica (Delille et al., 1998; Rivkina et al., 2000; Prabakaran et al., 2007; McFarlin et al., 2014; Whelan et al., 2015).

Biodegradation is not solely an inherent property of a substance; it is governed by both biological and chemical processes and is determined by a combination of substance-specific properties and environmental conditions (Boethling et al., 2009). Factors influencing hydrocarbon degradation include chemical concentration (Prince et al., 2017), bio-availability (Bagi et al., 2013; Brakstad et al., 2015; Wang et al., 2016), redox conditions, pH, nutrient availability (Das and Dash, 2014; Hazen et al., 2016), composition (if present as a mixture or crude oil) (Brakstad et al., 2018a), molecular structure (Gros et al., 2014; Prosser et al., 2016), the presence of metabolically capable organisms (Redmond and Valentine, 2012; Ribicic et al., 2018a; Birch et al., 2017), and season (Tremblay et al., 2017). Furthermore, biodegradation of hydrocarbon constituents in oil can also be impacted by the physical state of the oil, which become more viscous at low (<2 °C) temperatures resulting in lower diffusion within the oil phase (Nordam et al., 2020).

Temperature is also a process known to influence degradation kinetics as it affects both chemical and biological processes (Arrhenius, 1889; Leahy and Colwell, 1990; Schulte, 2015). A widely accepted paradigm is that the rate of chemical reactions can be described using the Arrhenius equation (Arrhenius, 1889):

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (1)$$

where  $k$  is the rate of a reaction [ $\text{d}^{-1}$ ],  $A$  is the pre-exponential factor (which is constant at biologically relevant temperatures) [ $\text{d}^{-1}$ ],  $E_a$  is the activation energy of the reaction [ $\text{kJ mol}^{-1}$ ],  $R$  is the gas constant [ $\text{kJ mol}^{-1} \text{K}^{-1}$ ] and  $T$  is the temperature [K]. Under conditions where simple thermodynamic effects dominate the rate of a reaction, the Arrhenius equation predicts an exponential relationship between reaction rate and temperature. The Arrhenius equation was originally applied to describe the temperature dependence of chemical reaction rates in controlled settings, but it has also been used to describe the thermal dependence of biological rates, including enzyme-catalysed reactions and general metabolism (Kooijman, 2000). One issue of using the Arrhenius equation to describe temperature dependence in biological systems is that  $E_a$  is a constant in the standard model, yet it can vary across biologically relevant temperatures (Bagi et al., 2013). It assumes that biological systems are fixed in terms of their catalytic capacity, yet in reality they are dynamic and adaptable (DeLong et al., 2017).

Microbial communities in the environment are diverse, changing continually and adapted to local conditions (Poursat et al., 2019). Thus, microbes that develop in different temperature regimes can maintain similar rates and extent of metabolic activity (Arnosti et al., 1998; Bentahir et al., 2000). In cold environments psychrophiles display metabolic fluxes close to that of their mesophilic counterparts (Gerday, 2013) and microbes have been found to be metabolically active at temperatures as low as −20 °C indicating adaptations to the cold (Rivkina et al., 2000). Rivkina et al. (1996) studied the relationship between temperature and growth rate in marine bacteria and found no significant difference in cold (≤4 °C) and warm (≥4 °C up to 24 °C) waters, suggesting the growth rates of bacteria from cold and temperate oceans are similar at their respective ambient temperatures. Oil biodegradation studies show comparable degradation rates at different ambient temperatures highlighting the effect of temperature adaptation (Filler et al., 2001; Robador et al., 2010; Whelan et al., 2015). For example, naphthalene biodegradation rate coefficients for temperate and arctic seawater were found to be similar at their ambient temperatures of 7.0 and 1.4 °C, respectively, under non-nutrient limiting conditions (Bagi et al., 2014).

Under the REACH regulation (EC 1907/2006), compartment-specific degradation half-lives are used to define persistence criteria for chemicals. Guidance for persistence assessment under REACH was recently updated to require an EU average environmental reference temperature (ECHA, 2017b). As such there is a requirement for new degradation studies to be carried out at 12 °C, which is considered the mean temperature of European surface waters. For existing test data, the guidance suggests a temperature correction using the Arrhenius equation over a temperature range of 0–30 °C (ECHA, 2017a). This recommendation works under the assumption that biodegradation in the environment varies with temperature according to Arrhenius. Notably, this assumption is based on an analysis of plant protection product biodegradation studies in soils (EFSA, 2007). In this analysis, individual soils were tested across temperature gradients to yield temperature-dependent half-lives of test compounds and results supported an Arrhenius-type relationship of biodegradation with temperature. As a result, the applicability of the REACH guidance suggested Arrhenius equation to predict biodegradation of test compounds in the environment has gained acceptance and application in some chemical regulatory practices. However, there remains a

body of evidence that does not necessarily support this practice (Margesin et al., 2007; Bagi et al., 2013). In a study by Lewis and Prince (2018), the applicability of modelling hydrocarbon biodegradation using Arrhenius was questioned. Observations that growth of many microbial populations demonstrate little dependence to temperature (Rivkina et al., 1996), evolve through natural selection of communities or modify their metabolic apparatus to maintain function (e.g. cold adaptation, Moyer and Morita, 2007), suggest that temperature may not be the only nor the major factor determining hydrocarbon biodegradation rates in the environment (Lewis and Prince, 2018; Poursat et al., 2019).

Rates of biodegradation might be expected to follow Arrhenius where an individual inoculum from soil, sediment or water is tested outside the ambient temperature at which it is adapted. In this scenario, the same community of microorganisms is exposed to the test substance under varying temperature conditions, which can be described as a 'temperature-manipulated' system. A contrasting scenario, which arguably better reflects the conditions under which chemical biodegradation occurs in the environment, is where 'temperature-adapted' systems are used at or close to their ambient temperature conditions. This latter scenario includes inocula that are adapted to perform metabolic functions at the respective temperature and may be a more appropriate means to assess the inherent capacity of environmental microbiome to perform biodegradation. However, due to the already mentioned inherent variability and diversity of factors affecting biodegradation in the environment, a direct assessment of the influence of temperature on biodegradation using temperature-adapted inocula is challenging. It is therefore somewhat unsurprising that efforts to quantify this effect have to date focused on biodegradation in temperature-manipulated systems.

The objective of the present work is to critically evaluate the role of temperature on the biodegradation of hydrocarbons in the environment. More specifically, an assessment was made using data from environmental samples that were tested near their ambient temperatures to determine whether existing assumptions in chemical regulatory practice around the use of the Arrhenius equation to temperature-correct biodegradation half-lives are realistic. Our hypothesis is that temperature-correction using the Arrhenius equation, as described in REACH guidance, is an over-simplification of biological reality due to the overlooked contribution of temperature adaptation of microbial communities in the environment.

## 2. Materials and methods

### 2.1. Biodegradation data compilation

Aerobic biodegradation data for hydrocarbons were identified through a search of peer-reviewed literature. Each retrieved study was screened, and biodegradation data were obtained either directly from the paper or calculated assuming first-order kinetics. The following screening criteria were applied for selection of studies to be included in this assessment:

- (1) Firstly, biodegradation data were derived from naturally occurring environmental microbial communities with no significant pre-exposure to hydrocarbon contamination.
- (2) As hydrocarbons cover a wide range of physical and chemical properties and are subject to varying degrees of equilibrium partitioning in soils and sediments that influence the rate and extent of biodegradation (Redman et al., 2014a), only experiments performed in surface water (fresh, estuarine, marine) were considered.
- (3) To assess the effects of temperature on intrinsic hydrocarbon biodegradation, data were selected from studies in which other confounding factors, known to impact biodegradation, were eliminated or reduced. As such, data were collected only from:
  - a. aerobic biodegradation experiments that contained at least enough O<sub>2</sub> for complete mineralisation of hydrocarbons to

CO<sub>2</sub> based on a theoretical oxygen demand of 3.4 mg O<sub>2</sub> mg<sup>-1</sup> hydrocarbon (Battersby, 2000),

- b. relevant studies where levels of nitrogen and phosphate were comparable to the suggested ideal carbon: nitrogen: phosphate ratio of 100:10:1 (Leys et al., 2005), indicating adequacy of nutrients for hydrocarbon biodegradation,
  - c. studies with hydrocarbon concentrations below their aqueous solubility limit, so as to minimise the effects of reduced bioavailability on biodegradation. Bioavailability is another critical factor known to impact rates of biodegradation (Bagi et al., 2013). The solubility of most hydrocarbons other than the small aromatics such as benzene, toluene, ethylbenzene, and the xylenes is so low that most have a tendency to partition into a separate organic phase and exist as droplets or slicks on water surfaces (Prince et al., 2017). Biodegradation in these circumstances is probably a surface phenomenon, occurring at the oil–water interface and is rate-limited due to limited oil surface area (Prince et al., 2017). This effect of limited bioavailability can be mitigated by reducing oil droplet size through physical or chemical dispersion or dosing hydrocarbons below their saturation concentration.
- (4) Considering the fundamental importance of temperature to the study objectives, a screen for biodegradation data performed at test temperatures close to the ambient temperature of the collected environmental microbial community was undertaken. As such only data derived from experiments performed within  $\pm 5^\circ\text{C}$  of ambient temperatures were used. For some studies, multiple environmental inocula were collected at different temperatures. For these studies, only those data that fell within the  $\pm 5^\circ\text{C}$  criteria were used.
  - (5) Dispersed crude oil at high loadings (>50 mg/L) tend to amalgamate into larger droplets and form slicks which limits oil bioavailability as described previously. Furthermore, modelling based on approaches described by Redman et al. (2014b) indicated these loadings were above LL50 (acute lethal loadings) concentrations and likely inhibitory to the degraders. Therefore, these data were omitted from the database.
  - (6) Experimental designs were assessed to ensure the quality of the reported biodegradation data. All data were derived from studies that used at least three replicates and minimised, or accounted for, abiotic losses of hydrocarbon from the test system. Abiotic losses were minimised using sealed vessels and/or no headspace to limit volatilisation. All data were derived from studies that used sacrificial test vessels for each time point measurement, thus ensuring that hydrocarbon losses through sample processing were not compounded over time. In some cases, abiotic controls were used to measure and adjust for hydrocarbon losses. Variability in most studies was further reduced by normalising data to a persistent biomarker such as 17a(H),21b(H)-hopane.
  - (7) In some cases, studies presented hydrocarbon half-lives as a minimum value because biodegradation had not exceeded 50% within the time frame of the experiment. Only a small number of such examples were found (1% of total data) and were composed exclusively from values that were higher than the values for other substances at that temperature (Table S3). Despite uncertainty regarding the accuracy of these data, they were preserved within the compiled database because removal would lead to the prejudicial reduction of bulk/averaged half-life values.

### 2.2. Biodegradation database construction

An overview of the studies and data used in the final database is provided in Table 1. Studies reviewed but not meeting the selection criteria are summarised in Section 1 of the Supplementary information. This

**Table 1**

Summary information of biodegradation studies that passed data screening criteria.

Ref.	Test substance	Total hydrocarbon/oil load	Environmental inocula	Ambient inocula temp	Test temp	Dosing method	Experimental quality control	Biodegradation kinetics calculation	Observations
(Birch et al., 2018)	Mixture of 53 hydrocarbons	0.3–0.44 mg/L (estimate). Below aqueous saturation limit	Seawater: North Sea W of Esbjerg; lake water: Maglesø Lake, Sealand, Denmark	Lake: 8.4–17 °C Seawater: 15.5–19 °C	20 °C	Passive. Aqueous stock solution generated via silicon rod passive dosing, and diluted 10-fold	Abiotic losses minimised by sealing vessels with PTFE caps immediately after addition of test substance and inocula. Averaged replicate data ( $n = 3$ ) normalised to losses observed in abiotic controls. Sacrificial vessels used at each time point measurement.	Lag phase + first order	Test temperature greater than estimated ambient inocula temperature. Partial data included.
(Brakstad et al., 2018a)	5 Norwegian crude oils (unweathered)	2.0 mg/L	The seawater was collected Trodheimsfjord via a pipeline system. The source is considered pristine	6–8 °C	5 °C	Dispersed. Oil droplet generator <sup>1</sup> used to make oil dispersion in the seawater	Abiotic losses minimised by sealing vessels and excluding headspace or air bubbles. Averaged replicate data ( $n = 3$ ) normalised to conservative internal marker 17a(H),21b (H)-hopane. Sacrificial vessels used at each time point measurement.	Pseudo first order	Test temperature within 3 °C of ambient inocula temperature
(Brakstad et al., 2018b)	Norwegian crude oil (unweathered)	2.0 mg/L		Not described but assigned 6–8°C based on Brakstad et al., 2018a	13 °C	Dispersed. Two methods: 1) fresh oil: oil droplet generator <sup>1</sup> used to make oil dispersion in the seawater	Abiotic losses minimised by sealing vessels and excluding headspace or air bubbles. Averaged replicate data ( $n = 3$ ) normalised to conservative internal marker 17a(H),21b (H)-hopane. Sacrificial vessels used at each time point measurement.	Pseudo first order	Test temperature within 5 °C of ambient inocula temperature
(CONCAWE, 2012)	Mixture of 34 liquid hydrocarbons; mixture of 36 solids hydrocarbons; mixture of 10 cycloalkanes	Not reported but expect to be below aqueous saturation limit	Natural seawater collected from Atlantic Ocean at Sandy Hook, NJ, USA.	18 °C	20 °C	Passive. HC saturated silicon oil loaded into silicon tubing added to 3.5 l medium and stirred from 1 to 3 days. 15 ml aliquots dispensed into incubation vials	Abiotic losses minimised by sealing vessels with PTFE caps immediately after addition of test substance and inocula. Averaged replicate data ( $n = 9$ ) normalised to losses observed in killed abiotic controls. Hexachlorobenzene also used as internal conservative marker. Sacrificial vessels used at each time point measurement.	Pseudo first order	Test temperature within 3 °C of estimated ambient inocula temperature
(Prince et al., 2007)	Unleaded, unoxxygenated regular gasoline (US)	70 ppm Vol. Equivalent to 52 g/mL based on density of 0.75 g/cm <sup>3</sup>	Natural seawater collected from Atlantic Ocean at Sandy Hook, NJ, USA.	20–23 °C	21 °C	Direct addition. No slick formed due to high aqueous solubility of product and portioning into headspace.	Abiotic losses minimised by sealing vessels with PTFE caps immediately after addition of test substance and inocula. Averaged replicate data ( $n = 9$ ) normalised to conservative internal marker 2,2,4-trimethylpentane, iso-octane 1,1,3-trimethylcyclohexane as conserved internal standards. Sacrificial vessels used at	Pseudo first order	Test temperature within 3 °C of estimated ambient inocula temperature. Partial dat. Alkenes not included

(Prince et al., 2013)	Alaska North Slope Crude (unweathered)	2.5 ppm vol. equivalent to 2.25 mg/L based on oil density of 0.9 g/cm <sup>3</sup>	Natural seawater collected from Atlantic Ocean at Sandy Hook, NJ, USA (winter conditions, temperature = 8 °C)	8 °C	8 °C	Dispersed. 10 µl crude oil (with/without Corexit 9500 dispersant) added to 4000 mL seawater.	each time point measurement Abiotic losses reduced by capping vessels but they were not airtight (this allowed for ambient O <sub>2</sub> to diffuse into the system). Biodegradation was normalised to 17a (H), 21b (H)-hopane as a conserved internal marker. Sacrificial vessels used at each time point measurement	Pseudo first order	Test temperature within 3 °C of ambient inocula temperature
(Prince et al., 2017)	European crude oil (unweathered)	2.5–250 ppm vol. equivalent to 2.25–225 mg/L based on oil density of 0.9 g/cm <sup>3</sup>	Natural seawater collected from Atlantic Ocean at Sandy Hook, NJ, USA, June 2015	20 °C	21 °C	Dispersed with Corexit 9500. 10 µl crude oil (with/without Corexit 9500 dispersant) added to 4000, 400 and 40 mL of seawater.	Abiotic losses reduced by capping vessels but they were not airtight (this allowed for ambient O <sub>2</sub> to diffuse into the system). Biodegradation was normalised to 17a (H), 21b (H)-hopane as a conserved internal marker. Sacrificial vessels used at each time point measurement	Pseudo first order	Test temperature within 3 °C of ambient inocula temperature. Partial data included. Higher loadings (225 and 2250 mg/L) due to toxicity and formations of slicks (see materials and methods).
(Prosser et al., 2016)	Mixture of 78 hydrocarbons	Not reported but expect to be below aqueous saturation limit	Natural seawater collected from Atlantic Ocean at Sandy Hook, NJ, USA	19–21 °C	20 °C	Passive. Silicon tube passive dosing suspended in 3.5 l env medium for 3 days. 15 ml aliquots dispensed into incubation vials	Abiotic losses minimised by sealing vessels with PTFE caps immediately after addition of test substance and inocula. Averaged replicate data (n = 9) normalised to losses observed in abiotic controls. Sacrificial vessels used at each time point measurement.	Pseudo first order	Test temperature within 3 °C of ambient inocula temperature. Partial data included
(Ribicic et al., 2018a)	Grane and Troll crudes (unweathered)	2–3 mg/L	The seawater was collected from Trodheimsfjord via a pipeline system. The source is considered pristine	5.9 °C	5 °C and 13 °C	Dispersed. Oil droplet generator <sup>1</sup> used to make oil dispersion in the seawater	Abiotic losses minimised by sealing vessels and excluding headspace or air bubbles. Averaged replicate data (n = 3) normalised to conservative internal marker 17a (H), 21b (H)-hopane. Sacrificial vessels used at each time point measurement.	Lag phase + first order	5 °C test temperature within 3 °C of ambient inocula temperature. 13 °C test temperature within 7 °C of ambient inocula temperature Partial data included
(Ribicic et al., 2018b)	Fresh paraffinic oil (Statfjord crude)	2 mg/L		Not described but assigned 5.9 °C based on Ribicic et al., 2018a	5 °C	Dispersed. Oil droplet generator <sup>1</sup> used to make oil dispersion in the seawater	Abiotic losses minimised by sealing vessels and excluding headspace or air bubbles. Averaged replicate data (n = 3) normalised to conservative internal marker 17a (H), 21b (H)-hopane. Sacrificial vessels used at each time point measurement.	Lag phase + first order	5 °C test temperature within 3 °C of ambient inocula temperature



included the BioHCWin hydrocarbon biodegradation prediction model, described by Howard et al. (2005), derived from over 2800 experimental biodegradation datapoints. This database represents one of the largest hydrocarbon biodegradation datasets in existence and was a potential source of biodegradation data for this study. A review of this database identified a relatively small number of appropriate surface-water based data for further consideration (255 datapoints). Further evaluation of these datasets using the selection criteria described above, indicated no appropriate data were available for inclusion in the biodegradation database of this study.

In total, 993 datapoints were collected. The chemical name, carbon number, degradation half-time (DT50), data source, hydrocarbon class, temperature and dosing strategy were identified for each datapoint and assembled within a spreadsheet (Table S3). These data were then used in all subsequent analysis.

### 2.3. Biodegradation kinetics

The degradation half-time (DT50) was the primary evaluation metric for the present work. This was described either as pseudo first-order kinetics, a 'one-phase decay model from a plateau' as presented by Birch et al. (2018) or an apparent half-life calculation described in Prince et al. (2007). Each approach deals with the lag phase differently. Pseudo first-order kinetics is based on first-order degradation rate and summarised by:

$$\frac{\ln(2)}{k} \quad (2)$$

where  $k$  is degradation rate constant ( $d^{-1}$ ). In this approach, the initial lag phase is part of the overall kinetic calculation. One phase decay model from a plateau calculates the initial lag and degrading phases as distinct and separate kinetic components and is described as:

$$X_0 + \frac{\ln(2)}{k} \quad (3)$$

where  $X_0$  is the lag time after which biodegradation begins. The apparent half-life calculation, described by Prince et al. (2007), is also based on first order kinetics:

$$\ln(2) \cdot \left( \frac{-t}{\ln A} \right) \quad (4)$$

where  $t$  is time and  $A$  is the remaining fraction of hydrocarbon. Apparent half-lives are calculated at multiple time points where there was >10% loss of the hydrocarbon. Multiple calculations are determined for each experiment and an average is calculated. Like pseudo-first order, the initial lag phase is part of the overall kinetic calculation.

These models are considered distinct to half-life (HL) which indicates the time required to reduce the concentration by 50% during the period in which degradation kinetics are first-order, i.e. after any apparent lag phase (see Section 4 of the Supplementary information for more information). Based on this difference a decision was made to present the data as DT50 (ensuring that both the Lag phase and HL were included in data calculated from 'one-phase decay model from a plateau or apparent half-life kinetics'). Approximately one third of the biodegradation database was derived from studies that separated lag phase and HL, a third were from pseudo-first order kinetics and third from apparent half-life calculations (Table S5).

### 2.4. Temperature dependence

The  $Q_{10}$  coefficient is a measure of the temperature sensitivity of biodegradation as a consequence of altering the temperature by 10 °C. It has been extensively used in temperature-controlled experiments where HCs are subject to biodegradation at several incubation

temperatures (Bagi et al., 2014). In this study  $Q_{10}$  was calculated for comparison to other literature values based on:

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_1 - T_2)} \quad (5)$$

where  $R$  is the rate and  $T$  is the temperature. In all cases, 20 °C was used as the reference temperature. For comparison, the Arrhenius equation was converted to a  $Q_{10}$  using the generic EFSA (2007)  $E_a$  of 65.4 kJ mol<sup>-1</sup> according to Eq. (6) where  $T$  is temperature and  $R$  is the universal gas constant.

$$Q_{10} = e^{\left( \frac{E_a \cdot 10}{R \cdot T \cdot (T + 10)} \right)} \quad (6)$$

### 2.5. Data transformation

The complete biodegradation dataset approximately followed a log-normal distribution. Consequently, log transformation was undertaken to linearise and reduce skewness before statistical assessment. The resulting dataset was assessed for normality through visual inspection of Q-Q plots, skewness and kurtosis Z-scores based on the approaches described by Bulmer (1979) and Kim (2012, 2013). Further descriptions and results of data transformation are provided in Section 2 of the Supplementary information.

### 2.6. Simple linear regression analysis

Linear regressions were performed to analyse the impact of temperature on biodegradation of total hydrocarbons and single hydrocarbon constituents. The determinants included carbon number, chemical class (e.g., aromatic, aliphatic), temperature and several other factors described in Section 3.5. To reduce bias from any single study and ensure adequate coverage of the data in the analysis of individual hydrocarbons, only constituents that had measured DT50s spanning at least 3 temperatures and included data from multiple studies at 5 °C and 20 °C and/or 21 °C were selected for this trend analysis. All data manipulation treatments, correction and statistical analysis were performed in Microsoft Excel®.

### 2.7. Multilinear regression analysis

Multilinear regression was performed using the simultaneous (a.k.a. enter) method; all independent variables were entered into the equation at the same time, which is appropriate when dealing with independent variables of unknown significance. Categorical predictors, such as hydrocarbon class and biodegradation kinetic equation, were assessed using dummy variables. Standardised coefficients ( $\beta$ ) were generated from a regression of standardised variables in order to assess the relative importance of each variable in the multilinear regression models. Standardised variables were calculated and then rescaled using the Z-score formula:

$$\frac{x_i - \mu}{\sigma} \quad (7)$$

where:  $x_i$  is the residual value,  $\mu$  is the sample mean and  $\sigma$  is the standard deviation of the total variable dataset. The data analysis was carried out by using SPSS Statistics (Version 26.0, IBM Corp. Armonk, NY, USA).

### 3. Results and discussion

#### 3.1. Biodegradation database

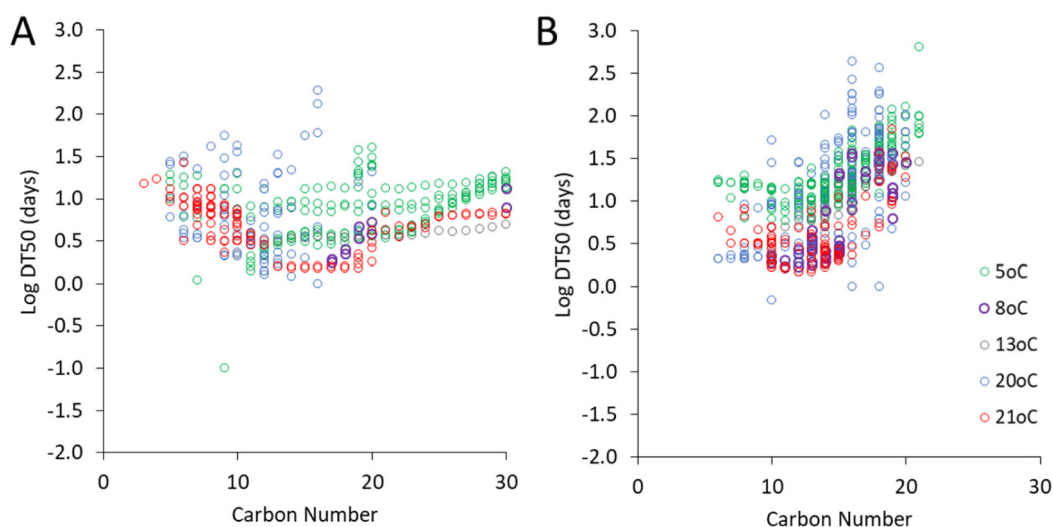
The biodegradation database for this analysis is compiled from 10 independent studies that fulfilled the criteria listed in Section 2.1 of the Materials and methods and is captured in Table 1. Studies that were screened, and failed to meet the selection criteria, are listed in Table S1 of the Supplementary information. In total 993 data points, covering 326 hydrocarbon constituents, were collected from studies performed at temperatures between 5 °C and 21 °C. This dataset was judged to be normally distributed based on visual inspection of the Q-Q plots showing good linearity ( $R^2 = 0.99$ ) and skewness and kurtosis Z-scores (2.29 and  $-2.62$  respectively) indicating normality (Fig. S2 and Table S4). Distribution for the 326 hydrocarbon constituents suggests the data broadly covers the chemical space of hydrocarbon substances (Fig. S2-B). Aliphatic, aromatic and cyclo-aromatic hydrocarbons are all represented in the total dataset, although normal (*n*)-paraffins (nP) and polyaromatic hydrocarbons (PAH) are the major classes of hydrocarbon present in the dataset. Data for the various classes of hydrocarbon are not equally distributed between separate test temperatures (Fig. S2-C). For example, data at 5 °C and 13 °C are mainly composed of linear and branched alkanes and PAHs, whereas 20 °C and 21 °C data are composed of a greater variety of hydrocarbon classes. Minor differences are also observed in the carbon number distribution of the temperature datasets but most data fall within the C<sub>10</sub> to C<sub>20</sub> range (Fig. S2-D).

Measured primary DT50s for the individual data points range from 0.1 to >100 days with the bulk (86%) of the data ranging between 1 and 30 days (Fig. 1A and B). The limited range of measured DT50 is notable considering the differences in study protocols, hydrocarbons and petroleum substances tested, and sources of inoculum used to collect these data. DT50s for aliphatics, including linear and branched alkanes and cyclic hydrocarbons, mostly ranged between 1.3 and 25 days (0.1 and 1.4 log days respectively) (Fig. 1A), with little dependency of DT50 of carbon number. Slightly longer DT50 are observed at both boundaries of the carbon number range. In contrast, DT50s for aromatics were typically higher than the corresponding aliphatic carbon number, with an overall range of 1.6 and 125 days (0.2 to 2.1 log days). A carbon number dependency is apparent for the aromatics (Fig. 1B). This dichotomy reflects in part the known differences in biodegradability of aliphatics and aromatics (Das and Chandran, 2011; Leahy and Colwell, 1990). It may also highlight greater levels of heterogeneity in chemical structure typically observed of aromatic hydrocarbons (Marshall and Rodgers, 2008).

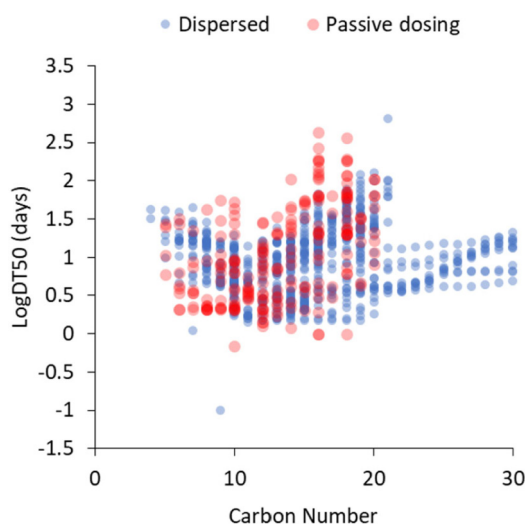
The compiled biodegradation database is derived from studies using two distinct hydrocarbon dosing systems; dispersion and passive dosing. In these systems, hydrocarbon exists either as dispersed droplets or as fully dissolved constituents, respectively (Table 1). Biodegradation of dispersed droplets is expected to be a surface phenomenon, occurring at cell surfaces and oil-water interfaces (Prince et al., 2017). This contrasts with passive dosing, where dissolved hydrocarbons are degraded via uptake from the aqueous phase (Hua and Wang, 2014). This difference presents an opportunity to introduce experimental bias into the data set. To further understand the potential impact of dosing, data points from both systems are plotted against carbon number (Fig. 2). The range of DT50s is similar for both datasets sharing the same carbon number, suggesting differences to be marginal. Thus, the effect of dosing system selection is independent of the substance biodegradability as long as bioavailability of the substance is maximised. Mean DT50 values of dispersed and passively dosed hydrocarbons are 10 and 11 days respectively and *t*-test analysis indicates no significant difference between the groups ( $p > 0.05$ ) (Fig. S5-A). Additionally, plots of passive and dispersed dosing data of individual hydrocarbon constituents showed no obvious preference for either dosing system (Fig. S7-B). Based on this analysis, the effects of experimental dosing system appear to be limited and consequently all data are considered comparable for this assessment.

#### 3.2. Assessment of temperature dependency

The effect of temperature on hydrocarbon degradation half-times (DT50s) was assessed using box and whisker plots (Fig. 3). Mean DT50 values vary between 0.76 and 1.12 log days (6–13 days respectively) over a temperature range from 5 °C to 21 °C; which corresponds to a change of 7 days in the DT50 across a four-fold (16 °C) temperature differential (Fig. 3). Variability within temperature datasets was typically larger than differences between them. Interquartile ranges were >7 days (unlogged) for all temperatures except 13 °C (6 days, unlogged). As discussed in Section 3.1, this spread is mainly a function of differences between hydrocarbon constituents and experimental approaches used to generate the data. Simple regression analysis of this data, using temperature as the only independent variable, yielded a statistically significant ( $p = 1.5E-19$ ) but weak correlation (Pearson correlation coefficient:  $R = -0.3$ ;  $R^2 = 0.1$ ) with DT50. The slope of the regression trend was  $-0.018$  (Fig. 3) and indicates an approximate two-fold increase in measured DT50 values with a four-fold reduction in temperature – specifically from 6 days at 21 °C to 13 days at 5 °C. According to REACH guidance (ECHA R.7b, 2017a), the recommendation

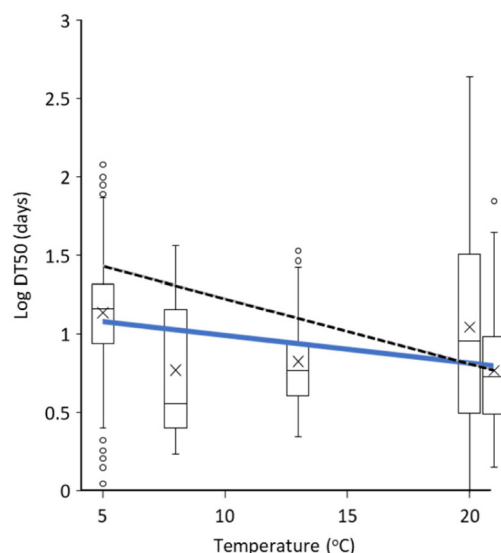


**Fig. 1.** Log DT50 (days) against carbon number for aliphatic (A) and aromatic (B) hydrocarbons. The test temperature is indicated according to the respective colour. 5 °C is green, 8 °C is purple, 13 °C is grey, 20 °C is blue and 21 °C is red.



**Fig. 2.** Log DT50 (days) against carbon number for experiments using dispersed petroleum product (blue) and passively dosed experiments (red). Total datasets for each dosing system presented ( $n = 773$ , dispersed;  $n = 220$ , passive dosing).

for industrial chemicals is for a temperature correction based on the Arrhenius equation, using an  $E_a$  of  $65.4 \text{ kJ mol}^{-1}$  (EFSA, 2007). The relationship as determined by the Arrhenius trend, as recommended by REACH guidance, is shown in Fig. 3 as a dashed line. A comparison of both relationships revealed it to be substantially greater (slope =  $-0.042$ ) than the trend produced in this study (slope =  $-0.018$ ) (Fig. 3). As such, DT50 at  $5^\circ\text{C}$  for the REACH Arrhenius trend was 2.4-fold greater than what was observed in this study (31 and 13 days respectively). Based on this comparison, the REACH recommended adjustment over-predicts the effects of temperature on hydrocarbon biodegradation and consequently its suitability in this field is uncertain. The  $E_a$  used for the Arrhenius trend, as recommended by REACH guidance, was derived from the median value of 99 experimental datasets



**Fig. 3.** Box plot of log DT50 (days) measured at different temperatures for all hydrocarbons available in the data set. The box plot includes median, inner quartiles, min, max and outliers at different temperatures. The crosses represent mean values. The blue line shows the result of the simple linear regression ( $y = -0.018x + 1.2$ ). The dashed grey line is the Arrhenius temperature dependency ( $y = -0.042x + 1.7$ ) based using  $E_a = 65.4 \text{ kJ mol}^{-1}$  (EFSA, 2007).

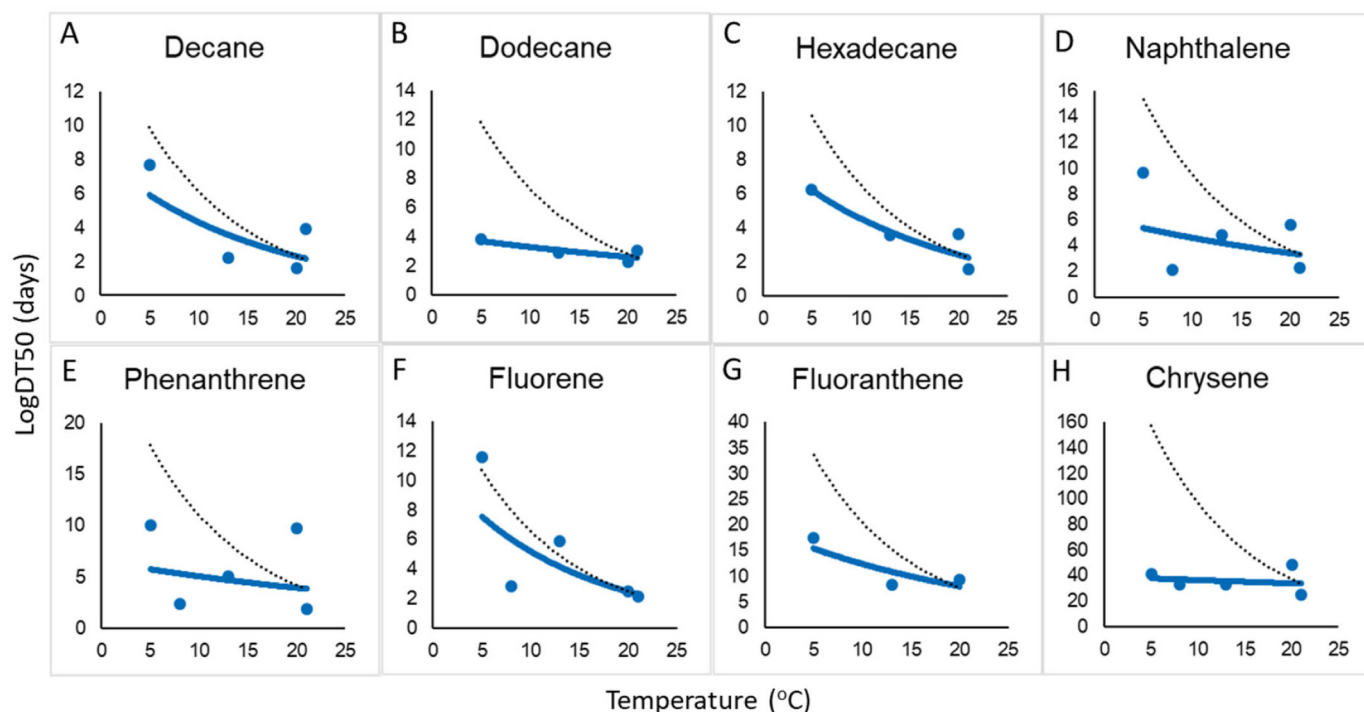
on plant protection products, using single soil sources across a range of temperatures (EFSA, 2007). Although studies with soil are typically performed over longer time-frames, which presents a possible opportunity for temperature adaptation, these experiments would not have fulfilled screening criterion #4 for this study (see Section 2.1), since the temperature of the soil system was manipulated across a temperature range. A more in-depth assessment of this criterion is given in Section 3.4 below.

The relationship between measured degradation half-times and temperature was further evaluated for individual constituent hydrocarbons. From the hydrocarbons available in this data set, only 19 constituents fulfilled the criteria necessary for an individual constituent evaluation. The mean DT50 (days) measured at the respective temperature for 8 hydrocarbon constituents is shown in Fig. 4. Similar patterns are observed for the remaining constituents (Table S7). The degree of temperature effect on DT50 varied amongst the constituents. No effect of temperature was observed for chrysene, with a mean DT50 ranging from 34 to 38 days, over the available temperature range (Fig. 4H). The largest temperature effect was seen for fluorene (Fig. 4F), with a mean DT50 ranging from 3 to 8 days over the same temperature range. The magnitudes of the temperature effect (slope gradient) varied between constituents, however no correlation with hydrocarbon class or chemical characteristics (octanol water coefficient or molecular weight, etc), was observed (data not shown). It should also be noted that for some constituents, there is a high scatter in the data, indicating that temperature may not be the dominant factor resulting in changes in the mean DT50. Also included in each panel in Fig. 4 is the temperature correction according to the Arrhenius equation from REACH guidance (dashed line). It is clear that for individual constituents, the effect of temperature on DT50 generally appears to be lower than that predicted by the Arrhenius trend recommended in REACH guidance. The result for the individual constituents supports the results from the full data set that indicate the Arrhenius correction does not accurately predict the temperature effects of hydrocarbon biodegradation.

### 3.2.1. Influence of temperature on lag phase

In aqueous biodegradation experiments, a lag phase is often observed between the time-point that the microorganisms are introduced to the substrate and the onset of detectable microbial degradation (Birch et al., 2018; Ribicic et al., 2018a; Ribicic et al., 2018b). However, the lag phase is not always captured due to test design with long sampling intervals, and often it is not captured in the calculations due to the kinetics applied (Section 4 of the Supplementary information). From the studies included in this paper, a lag phase has been captured in Birch et al. (2018), Ribicic et al. (2018a) and Ribicic et al. (2018b). For these studies, the influence of temperature on lag phase was assessed. The mean lag phase for this subset of hydrocarbon data set was found to be significantly ( $p < 0.05$ ) longer at  $5^\circ\text{C}$  compared to  $20^\circ\text{C}$  (12 and 6 days respectively) (Fig. 5A). In Fig. 5B and C, the DT50 (days) is plotted against the biodegradation half-life (days) - the DT50 minus the lag phase - and the contribution of lag phase to the DT50 for individual hydrocarbons is demonstrated. In this analysis, data to the left of the central line ( $y = x$ ) have longer lag phases. The tendency to move away from the central line is more pronounced at  $5^\circ\text{C}$  relative to  $20^\circ\text{C}$ , indicating that lower temperatures induce longer lag phases prior to the onset of biodegradation. As DT50s incorporate both the lag phase and the biodegradation half-life, this temperature influence on lag phase may obscure the assessment of temperature influence on half-lives, when considering only DT50 data. Increasing lag phases with decreasing temperatures have been demonstrated previously for hydrocarbons (Atlas and Bartha, 1972; Bagi et al., 2013) and are regarded as a period of acclimation required for the development of an optimum degrading community before vigorous degradation of the introduced substance can occur (Poursat et al., 2019). How temperature affects lag phase is unclear. It may impact microbial metabolic activity during acclimation or have a broader effect on microbial cell density.



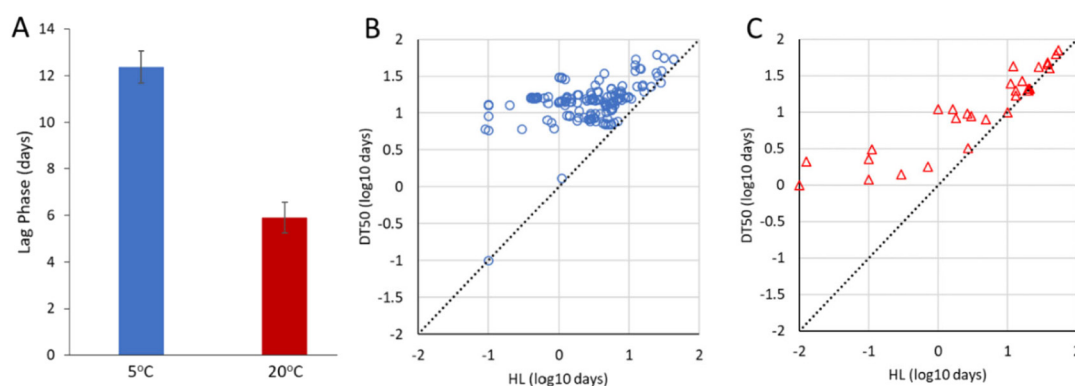


**Fig. 4.** Mean DT50 (days) vs temperature for (a) decane, (b) dodecane, (c) hexadecane, (d) naphthalene, (e) phenanthrene, (f) fluorene, (g) fluoranthene and (h) chrysene. The solid blue line represents the trend produced by an exponential curve based on  $y = ae^{bx}$ . The black dotted line is the trend derived from the Arrhenius equation using an  $E_a = 65.4 \text{ kJ mol}^{-1}$  as described by EFSA (2007). Analysis of hydrocarbons constituents was based on the presence of data across at least three temperatures and at least three datapoints present at 5 °C and two datapoints at 20 °C and/or 21 °C.

In the latter case, lower numbers of competent degraders would require a longer lag phase to generate cell biomass before the onset of biodegradation (Ott et al., 2020). This process may be important for intermittent releases of chemicals, such as unintended hydrocarbon spills, but is not relevant to environmental persistence assessment under REACH regulation, which intends to assess degradation of chemicals in the environment under environmentally relevant conditions (ECHA, 2017a). Under these conditions of long-term, diffuse and lower-level exposure the experimental lag phase ceases to be relevant to the concentration of a chemical in the environment and its subsequent removal. Furthermore, the focus of persistence assessments is emphatically on environmental degradation half-lives based on kinetic rates of removal (Boethling et al., 2009; ECHA, 2017a; Matthies et al., 2016; see also REACH, PPP and BPR regulations). The standard biodegradation simulation test in surface waters requires the first order degradation rate and half-life to be calculated and specifically excludes any observed lag

period (OECD, 2004). It is therefore important to recognise that the DT50 data used in this analysis represent a conservative surrogate for measured half-lives, and that the incorporated lag phase is a distinct process that is not relevant to the persistence assessment but is itself influenced by temperature.

DT50 values used in this study are based on three kinetic calculations, two of which ignore lag as a distinct phase and assimilate the lag phase into first order biodegradation calculations, as explained in Section 2.3 of the Materials and methods. The remaining approach defines both the lag and the degrading phase separately and combines both values to produce a DT50 (Fig. S4). Interestingly, a lag phase can affect the results of each of these calculations differently (Tables S4 & S5). In a test using hypothetical data, DT50 values produced by the three approaches varied between 1.1 and 2.8 days based on the same set of data, with the calculation method that separated both the lag and the degrading phase resulting in the longest DT50 (Table S4). This is



**Fig. 5.** The effect of temperature on lag phase. Mean lag phases of total hydrocarbons at 5 °C and 20 °C (A) shown. Data used in this comparison were based on experiments where lag phase and HL were specifically distinguished. Error bars indicate 95% confidence intervals ( $n = 188$ , 5 °C and  $n = 31$ , 20 °C). Means found to be significant ( $p < 0.01$ ) by  $t$ -test: Two-Sample Assuming Unequal Variances. (B) and (C) are scatterplots of HL vs DT50 of individual hydrocarbons at 5 °C and 20 °C respectively. 1:1 shown as dotted black line indicates parity between HL and DT50. Points to the left of the 1:1 line indicate longer lags with the assumption that this increase is caused by a lag phase.

noteworthy because the proportion of data derived from different kinetic calculations varies across temperature datasets (Table S5). For example, almost half (46%) of the dataset at 5 °C is derived from calculations that are expected to produce relatively longer DT50 values. This contrasts with data at 20 °C–21 °C which is produced mainly (86%) from calculations that assimilate lag into first order biodegradation calculations and are expected to produce relatively shorter DT50 values. How these differences ultimately affect the temperature dependency of hydrocarbon biodegradation in this study is difficult to quantify, but an effort was made in subsequent analyses to understand their significance.

### 3.3. Manipulated vs temperature adapted inocula systems

This study set out to understand the impact of temperature on hydrocarbon biodegradation using temperature adapted environmental inocula. As such, stringent criteria were established to select only data from studies that assessed biodegradation close to the ambient temperature of collection. This differs to manipulated systems, where testing and ambient inocula temperatures are substantially different ( $>\pm 5$  °C). To understand the effects of this distinction a comparison was made between manipulated and temperature-adapted systems. Data for the manipulated system came from a study by Ribicic et al. (2018a) in which the ambient inocula temperature was 6 °C and the test temperatures 5 °C and 13 °C. The temperature adapted system data was derived from separate studies by Prince et al. (2013, 2017) performed at two temperatures (8 °C and 21 °C) very close ( $\pm 1$  °C) to ambient temperature. A comparison between these data was chosen because of similarities in the hydrocarbons assessed, initial loading concentrations, the dispersed nature of the oil used in the experiments, consistency in the inoculums used and similar DT50 calculation method. These considerations were put into place to minimise inherent variability in the data so that the opportunity to discern differences caused by temperature would be maximised. The comparison of both systems is shown in Fig. 6 with the DT50 measured with the temperature-adapted inocula (indicated in blue) and DT50 measured the

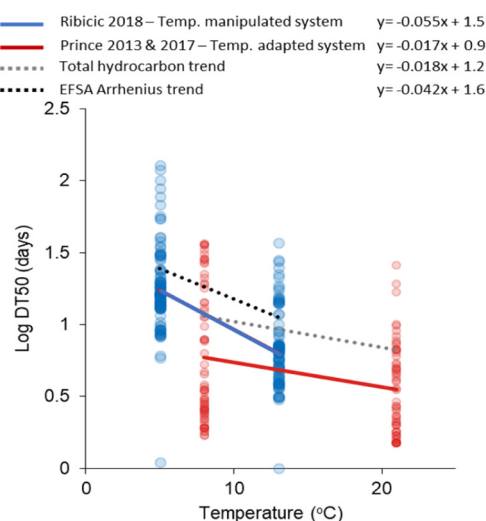
manipulated inocula (indicated in red). The comparison indicates differences in DT50 response according to the system. The manipulated system showed substantially more temperature dependency compared to the adapted system (slopes =  $-0.055$  and  $-0.017$  respectively). Included in Fig. 6 for reference, is the expected gradient according to the Arrhenius trend recommended in EFSA (2007), shown as a dashed line. Interestingly the manipulated system correlation was similar in gradient to the Arrhenius trend recommended by EFSA, whilst the temperature adapted system was comparable to the temperature dependency derived from the whole hydrocarbon dataset. These distinctions highlight the effects different inoculum communities can have on the effect of temperature during hydrocarbon biodegradation. The EFSA study was based on experiments using temperature-manipulated soil systems. Each temperature dependency was produced from experiments using a single source of soil performed at different temperatures. Therefore, it is not surprising to observe a similar temperature dependency to the Ribicic et al. (2018a) study which is also based on a manipulated temperature system.

### 3.4. Multiple linear regression

Multiple linear regression (MLR) analysis was used to test if temperature and other independent variables significantly predicted hydrocarbon DT50s. In this analysis, hydrocarbon class, carbon number, initial hydrocarbon concentration and nitrogen availability were used alongside temperature as quantitative variables. The effect of hydrocarbon class and different DT50 calculation methods were also tested as qualitative (categorical) variables. In total 10 sub-groups were assessed for hydrocarbon class (Fig. S2-B) and three DT50 calculation subgroups, corresponding to the biodegradation kinetic models described in Section 2.3, were used. The results of the MLR are provided in Table 2 and indicate that the chosen variables explained 49.7% of the variance ( $R^2 = 0.50$ ,  $F(15,977) = 114$ ,  $p = 2E-132$ ). Furthermore, the coefficient of multiple correlation ( $R = 0.7$ ), indicating a strong direct relationship between the predicted data and the observed data. In this model, all independent variables were significant to at least  $p < 0.01$ . In order to compare the relative importance of each regression coefficient, standardised beta coefficients ( $\beta$ ) were calculated (Table 2). Carbon number and initial hydrocarbon loading were amongst the most important predictor variables in this model and both correlated with increases in DT50. Positive correlations were also observed for all the hydrocarbon class variables used in the model, demonstrating that they contribute to larger DT50s when *n*-alkanes were used as the reference for the dummy variables in MLR. The different biodegradation calculations exhibited a contrasting effect with 'One phase decay model from a plateau' and 'Apparent HL' calculations contributing to an increase or decrease in predicted DT50s respectively. Interestingly, this observation supports the hypothetical calculations made earlier that demonstrated variability in DT50 values as a result of using different approaches to calculate DT50 (Table S4). Although temperature was observed to be a relatively minor component of this model, it was found to be statistically significant ( $p < 0.01$ ). An approximate 1.8-fold increase in DT50 values with a four-fold reduction in temperature is predicted by the model, which is similar to the temperature effect observed with simple linear regression (Fig. 3).

### 3.5. Comparison of temperature dependences

A  $Q_{10}$  value, based on a 10 °C (20 °C – 10 °C) temperature difference, was calculated using the temperature coefficient determined in the previous MLR analysis. This value, 1.4 (SE = 0.06) was found to be substantially lower than the EFSA-based  $Q_{10}$ , recommended in ECHA guidance (2017a), which results in a relative fold change of 2.6 (SE = 0.1) between 20 °C to 10 °C. This difference highlights an apparent uncertainty in the estimation of temperature dependency and strongly indicates that correction based on the Arrhenius equation, as suggested by ECHA (2017a), is unsuitable for temperature adapted biodegradation



**Fig. 6.** Log DT50 (days) vs temperature (°C) for hydrocarbons tested with manipulated (blue) and temperature adapted (red) inocula systems. Data for the temperature manipulated system are obtained from Ribicic et al. (2018a), whilst temperature adapted system are derived from two studies by Prince et al. (2013, 2017). Data used for comparison at different temperatures is identical for the Ribicic et al. (2018a) study, whilst 88% of the same hydrocarbon constituents are used in the Prince et al. (2013, 2017) comparison. For comparison, temperature trends from total hydrocarbon dataset of this study and the trend derived from the Arrhenius equation using an  $E_a = 65.4 \text{ kJ mol}^{-1}$  (EFSA, 2007) are provided as a solid and dashed lines respectively.

**Table 2**  
Regression coefficients of MLR models predicting DT50 values for hydrocarbons.

	Coefficients (B)	Standard error	Standardised coefficients (β)	P-value	Lower 95%	Upper 95%
Intercept	0.261	0.058		8E-05	0.147	0.375
Total loading (mg/L)	0.011	0.001	0.365	6E-19	0.008	0.013
Carbon number	0.029	0.002	0.417	1E-36	0.025	0.036
Test temperature (°C)	−0.016	0.003	−0.242	3E-08	−0.021	−0.010
Nitrogen/loading ratio	0.013	0.003	0.171	9E-05	0.007	0.020
Biodeg. calc. one phase decay model from a plateau <sup>a</sup>	0.170	0.032	0.166	1E-07	0.107	0.232
Biodeg. calc. apparent HL <sup>a</sup>	−0.389	0.043	−0.375	3E-19	−0.472	−0.305
HC class – iso-alkanes <sup>b</sup>	0.344	0.048	0.215	1E-12	0.250	0.437
HC class – monoaromatics <sup>b</sup>	0.227	0.052	0.132	1E-05	0.125	0.328
HC class – mononaphthenics <sup>b</sup>	0.455	0.064	0.201	2E-12	0.329	0.581
HC class – mononaphtho-monoaromatics <sup>b</sup>	0.264	0.064	0.105	4E-05	0.138	0.390
HC class – di-aromatics <sup>b</sup>	0.337	0.049	0.192	7E-12	0.242	0.433
HC class – di-naphthenics <sup>b</sup>	0.691	0.096	0.177	1E-12	0.503	0.878
HC class – mononaphtho-di-aromatics <sup>b</sup>	0.526	0.042	0.349	1E-33	0.444	0.609
HC class – polyaromatics <sup>b</sup>	0.688	0.034	0.563	1E-74	0.620	0.755
HC class – mononaphtho-polyaromatics <sup>b</sup>	0.709	0.054	0.323	2E-36	0.603	0.815

<sup>a</sup> Categorical variables representing biodegradation kinetic calculation subgroups with Pseudo first-order calculation used as the reference group.

<sup>b</sup> Categorical variables representing hydrocarbon class subgroups with *n*-alkanes used as the reference group.

systems. It should also be noted that the MLR-derived  $Q_{10}$  value is based on the total hydrocarbon dataset, and that individual substance- and class-specific differences may exist. In previous work by [Bagi et al. \(2013\)](#) large variation was shown amongst  $Q_{10}$  values from experiments on crude oils with different physico-chemical properties. They report that temperature can influence the physical state of the oil and at low temperatures, reductions in bioavailability caused by decreased solubility and increased viscosity, can reduce biodegradation rates. Thus, changes to the physical state of the oil or hydrocarbons are likely to influence the derivation of  $Q_{10}$  values. Understanding the contribution of temperature-induced physical changes to hydrocarbon biodegradation was not within the scope of this study. Recent work by [Nordam et al. \(2020\)](#) suggests that the effect of temperature is relatively more pronounced in heavier hydrocarbon constituents and that limitations in the diffusive transport of these components to where biodegradation occurs in oil droplets may be limited. In our study a lack of apparent temperature correlation with hydrocarbon class or its chemical characteristics was observed. This might be a result of combining data from studies with different dosing strategies or using different crude oils and petroleum products with a range of physico-chemical properties. Regardless of the cause, it is apparent that further investigation is needed to ensure that temperature dependencies accurately reflect the specific hydrocarbon or oil of interest.

Considering the universal requirement to efficiently outcompete other microbes in the same environment ([Good et al., 2018](#)), it is likely that temperature adaptation is an important process in all environmental compartments. Therefore, conclusions on the inappropriateness of the temperature correction recommended by REACH guidance is unlikely to be limited to surface water systems and is probably also applicable to other environmental compartments, such as sediments soils and groundwater, and other chemicals of concern. Assessment of temperature dependency in these compartments will likely require further considerations in addition to the stringent screening criteria of data used this study. For example, multiphase partitioning between aqueous and solids in these compartments will likely influence chemical bioavailability and overall rates of biodegradation ([Scow et al., 1995](#)). The tendency of a chemical to partition will be dependent on both the characteristics of the solid phase (organic carbon content, particle size and type, etc) and geometry of the experimental setup ([Honti and Fenner, 2015](#)). Furthermore, in groundwater an understanding of the prevailing redox conditions which can influence biodegradation would be required ([Barbieri et al., 2011](#); [Greskowiak et al., 2017](#)). Ensuring that the effect of these factors on biodegradation is understood will be important before discerning temperature dependencies in these environmental compartments.

### 3.6. Regulatory implication of results

An understanding of temperature dependence is important for determining persistence of chemicals in both hazard and risk assessments under REACH. The differences of degradation potential between temperature-manipulated and temperature-adapted systems highlight the effect of microbial adaptation to test temperature. These distinct systems are likely to be useful for different purposes in the assessment of a chemical's risk in the environment. In long-term exposure scenarios, sudden temperature changes are rare and usually occur gradually as measured by season or by geographical location. In these circumstances, where temperature variations are smooth, the use of temperature-adapted systems is more appropriate to assess chemical persistency. The current REACH guidance ([ECHA, 2017a](#)) recommends an Arrhenius-based correction of any persistency data produced at temperatures different to the European Continental average temperature (12 °C), using a generic energy activation value. The present study demonstrates that this approach is not applicable to hydrocarbon biodegradation assessed in temperature-adapted systems and that the use of specific  $Q_{10}$  values maybe more appropriate. Further research is required to better understand the relationship between temperature and chemical persistency in settings that better reflect ambient environmental conditions. More information is required from chemical specific data rather than relying on the use of a generic and possibly inaccurate temperature corrections. This can then be used to better assess the risk of chemicals and improve predictions of their fate in the environment. It is recommended that, by default, any degradation simulation study on any chemical should use test systems adapted to the envisaged test temperature.

### CRedit authorship contribution statement

**David M. Brown:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Louise Camenzuli:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Aaron D. Redman:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Chris Hughes:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Neil Wang:** Methodology, Formal analysis, Writing - review & editing. **Eleni Vaiopoulou:** Methodology, Formal analysis, Writing - review & editing. **David Saunders:** Formal analysis, Writing - review & editing. **Alex Villalobos:** Formal analysis, Writing - review & editing. **Susannah Linington:** Formal analysis, Writing - review & editing.



## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.139293>.

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