Regulator-suggested 'temperature correction' of biodegradation rates leads to overestimation of persistence for hydrocarbon substances

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Since 2017, the European Chemicals Agency (ECHA) has required that all new biodegradation simulation tests be carried out at 12°C, and now also requires that half-life criteria resulting from studies previously undertaken at higher temperatures be 'temperature-corrected' to 12°C using a generic mathematical equation know as the Arrhenius equation. This article outlines why, in Concawe's view, the use of such a generic approach to adjusting biodegradation rates for petroleum substances is not appropriate, and why a more nuanced, hydrocarbon-specific appproach would be justified.

Background

Biodegradability testing indicates whether a chemical will degrade or persist in the environment

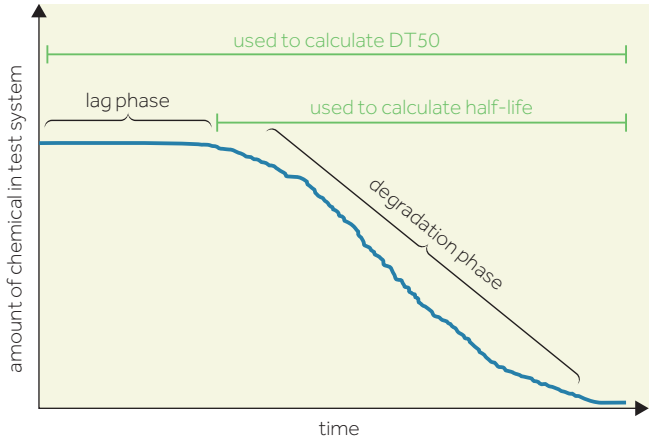
Biodegradation—the breakdown of chemicals by microbes in water, soil and sediment—is a major pathway for the removal of chemicals from the environment. The ubiquity of microbes and their metabolic diversity gives them the collective ability to utilise a stunning array of chemicals as carbon and energy sources. Humans have taken great advantage of the ability of microbes to degrade chemicals to clean waste water in water treatment facilities, to remediate contaminated sites, etc.

Environmental protection requires limiting the accumulation of chemicals in the environment so that they do not reach harmful levels. This potential to accumulate is referred to as the persistence (P) of the chemical and is estimated mostly by the biodegradability of that chemical. A chemical that biodegrades does not accumulate in the environment. However, it is a challenge to establish the biodegradability of a chemical in a clear standardised fashion for regulatory purposes.

Biodegradation testing is required for chemical registration under REACH. These tests involve introducing the chemical into an environmental medium (water, soil or sediment) and observing the microbially-mediated degradation of the chemical. The microbes in the test system are typically taken from environmental samples and consist of a variety of organisms. It is the diversity and density of microbes that affects the probability of an intrinsically biodegradable chemical to have a positive test outcome. For example, if the microbial density is too low, even if there are organisms that can degrade the chemical (known as competent degraders) there would be too few of them to observe biodegradation within the test time frame. Similarly, if there are too few different types of microbes, the diversity of the microbial population would be so low that the chances of a competent degrader being present would also be low.

There are several standardised OECD guideline methods for testing biodegradability of a chemical in different environmental media, with biodegradation simulation tests being used for P assessment. In these simulation tests, a relatively pristine environmental sample with its microbiota is incubated with the test chemical. Biodegradation is typically monitored directly by measuring the chemical in the test system over time (see Figure 1 on page 61). In an OECD simulation test system, there is normally a lag phase where there is no biodegradation and the chemical concentration is stable. During the lag phase, the microbial population adjusts to the presence of an available chemical for consumption, allowing the competent degraders of the test chemical to increase in population. At this point, the competent degraders begin to break down the chemical to a measurable degree. The rate at which the chemical degrades is often reported as a half-life, or the amount of time needed for 50% of the chemical to degrade during the degradation phase shown in Figure 1. An alternative metric is the DT50, which includes the lag phase when calculating the amount of time needed for 50% of the chemical to degrade.

Figure 1: Typical biodegradation curve in a biodegradation test



For regulatory designation of persistence, half-life criteria under REACH have been set for soil, sediment and water, as shown in Table 1. There are specific OECD simulation test methods (OECD 307, 308 and 309) which generate half-life values in these compartments for direct comparison with the criteria.

Table 1: Persistence criteria under REACH

Environmental compartments	Persistent (half life, days)	Very persistent (half life, days)
Marine water	60	60
Fresh or estuarine water	40	60
Marine sediment	180	180
Fresh or estuarine sediment	120	180
Soil	120	180

Biodegradation is a function of both the nature of the test substance (physicochemical properties, bonding, etc.) and the environmental parameters in which it is found (temperature, organic loading, etc.). The set-up of a simulation test can, therefore, greatly alter the perceived biodegradability of the test substance. The rate of biodegradation, or the half-life, will vary depending on the microbes involved and the environmental parameters. If the environment is unsuitable for the competent degraders, for example too saline, too hot or too cold, biodegradation will be slower than under optimal conditions. As mentioned above, the parameters of a biodegradation test should reflect common environmental circumstances under which the guidelines were developed. For convenience, OECD simulation and other similar tests have, historically, been performed largely at room temperature (20–25°C), and so the half-life criteria in Table 1 to designate persistent chemicals were based on experimental data also generated at this temperature range.

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The change

New standard from ECHA to change the temperature of biodegradability testing to reflect typical temperatures in Europe

Starting in 2013, ECHA began requesting biodegradation simulation testing at 12°C, and then in 2017 ECHA altered its guidance so that it required all new simulation testing to be performed at 12°C, which is the average temperature of European waters.[1] This is consistent with REACH Annex XIII requirements that biodegradation testing reflects relevant environmental parameters. However, there are some practical issues associated with this change. Testing laboratories will need to have appropriate incubators and protocols, since testing will no longer take place at room temperature. A more pressing concern is that the persistence criteria in Table 1 have been established based on data at 20–25°C. If the temperature at which biodegradation data are being generated is changed, the persistence criteria would also need to be adjusted to values appropriate at 12°C. Finally, it has been repeatedly shown in literature that changing the temperature of the microbial inoculum, i.e. temperature manipulation, will change its behaviour. For example, a river water sample taken at 5°C will not have the same microbial profile or activity if it is incubated at 20°C and vice versa. Thus the goal of having an environmentally-representative biodegradation test is thwarted if the temperature of the inoculum is greatly altered from its source. The guidance issued by ECHA should be clearer on the way the inoculum is gathered and used.

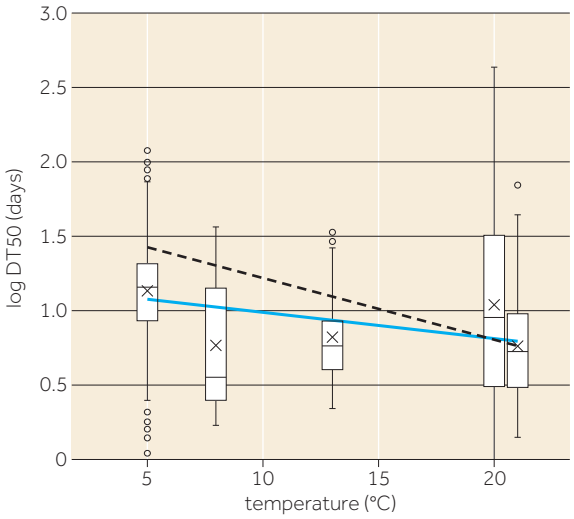
ECHA now also requires that biodegradation half-lives from any studies performed at higher temperatures be 'temperature corrected' to 12°C using a specific mathematical equation known as the Arrhenius equation. The Arrhenius equation shows an exponential relationship between chemical reaction rates and temperature (lower temperature = slower reaction rate, and so in this case longer half-lives and DT50s). The specific Arrhenius equation recommended by ECHA is derived from degradation data on pesticides, with the intention to adjust half-life data for exposure assessment. In Concawe's view, the use of the generic Arrhenius equation offered by ECHA is not appropriate for adjusting biodegradation rates for petroleum substances. The guidance allows for the use of chemical-specific corrections. A petroleum hydrocarbon-specific approach is justified in a Concawe article published in 2020 in the peer-reviewed journal *Science of the Total Environment*, entitled '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' (Brown *et al.*, 2020).[2]

The issue

Use of the default Arrhenius equation to 'temperature correct' biodegradation half-lives greatly overestimates persistence for petroleum hydrocarbons

The goal of the Brown *et al.* paper was to determine the relationship between temperature and biodegradation rates for petroleum hydrocarbons from available biodegradation test data. Another publication[3] had already demonstrated in 2018 that the Arrhenius approach does not apply to the biodegradation of petroleum at low temperatures in seawater. Indeed, the biodegradation rates observed in that study are remarkably similar at -1.7, -1 and 5°C. In the Brown *et al.* paper, thanks to the large volume of petroleum hydrocarbon biodegradation data available in the literature, 993 data points on 326 hydrocarbon constituents across a temperature range of 5–21°C were available for consideration. The data were from tests in which the microbial inoculum was incubated within 5°C of their source temperature, meaning that they were 'temperature-adapted' and not 'temperature-manipulated'. The results (Figure 2) show that there is a correlation between temperature and DT50 when looking at 5–21°C, although the data are quite scattered. It would seem that the 5°C points are driving the correlation, such that if the 5°C data are removed, there is little correlation between DT50 and temperature. Still, the overall correlation (blue solid line) shows a lower effect of temperature on DT50 than ECHA's Arrhenius equation would predict (dashed black line). Thus, it is inaccurate to use the Arrhenius equation as described in the ECHA guidance to 'correct' DT50s for petroleum substances, as it would result in an overestimation of the DT50 (slower biodegradation rate). Furthermore, for the substances where a half-life instead of DT50 could be calculated, there was a poorer correlation with the Arrhenius prediction. This result truly undermines the use of the Arrhenius equation since half-lives are the metric for the persistence criteria under REACH. The direct impact of using the generic temperature correction method for petroleum substances is likely a higher number of hydrocarbons being concluded as 'persistent' when they would have been 'not persistent' if tested at 12°C.

Figure 2: Box plot of log DT50 (days) measured at different temperatures for all hydrocarbons available in the data set



Notes:

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 black line is the Arrhenius temperature dependency ($y = -0.042x + 1.7$) based using Ea = 65.4 kJ mol−1.[4]

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It is not only petroleum substances for which the Arrhenius relationship has been shown to be inappropriate. Another recent publication looked at micropollutants and similarly concluded that the classic Arrhenius equation does not capture the effect of temperature on biodegradation rates with a temperature-manipulated system.[5] The authors explain that Arrhenius does not account for multiple enzyme systems that could have different temperature ranges existing in the same microbial community.

The explanation

Using the Arrhenius equation to 'temperature correct' biodegradation rates ignores the biological complexity of microbial systems

In the OECD simulation tests, many species make up the microbial community naturally found in environmental media. These microbial communities are adapted to their ambient temperatures. Different geographical locations with different temperatures may have different species (and thus different biodegradation capabilities) that are adapted to their ambient temperatures. When a microbial inoculum with its inherent microbial community is shifted to a different temperature from that of the source (temperature-manipulated), the relative populations of the microbial species in those communities shift. For example, those microbes that are more cold-tolerant may increase in relative density at a colder temperature. No new microbes are introduced. Since it is the same microbes (and same set of biodegradation capabilities) in this case, an Arrhenius-type relationship is expected. A soon-to-be-published study by the Danish Technical University sponsored by Concawe affirms that there is a reduction in biodegradation rate with temperature if one microbial community is used. Such a temperature-manipulated system is, however, of less environmental relevance, since it implies changing the temperature from which the microbial community comes. In the environment, the degradation process will take place at the same temperature to which the microbial community is adapted.

Competent degraders in an inoculum would normally have temperature optima that are in the range of their ambient temperature. Practically, this means that a microbial community adapted to a low temperature may perform as well as another microbial community at a higher temperature, as has been seen in the above-mentioned literature. This is particularly the case for hydrocarbons, which are ubiquitous in the environment, because many different organisms are capable of biodegrading them (not just one organism that performs well at one temperature).

Conclusions

Temperature adjustment of petroleum substance biodegradation data should be specific for petroleum substances

Concawe concludes that biodegradation rates for petroleum substances do not follow the generic Arrhenius relationship in ECHA's guidance. Based on the data analysed by Brown *et al.* the relationship between temperature and biodegradation rate for petroleum hydrocarbons is variable and weaker than predicted by the generic Arrhenius relationship. Substance-specific data for petroleum hydrocarbons should be used to avoid an erroneously long half-life calculation.

With ECHA's PBT (persistence, bioaccumulation and toxicity) guidance to either 'correct' biodegradation half-lives using the Arrhenius equation or to perform testing at 12°C regardless of the temperature at the inoculum source, biodegradation assessments lose their environmental relevance. Adjustment using the generic Arrhenius equation from ECHA would result in incorrect half-lives, which would be overly conservative. It will (and has) resulted in chemicals that are biodegradable in the environment being erroneously flagged as persistent and listed as Substances of Very High Concern (SVHC).[6] This is exacerbated by the lack of adjustment of the persistence criteria that were established at 20°C. Substances on the SVHC list can be subject to authorisation or restriction, greatly impacting the sale and use of those chemicals.

Through this article and further stakeholder engagement, Concawe is seeking to highlight the technical drawbacks and regulatory repercussions of ECHA's 'temperature correction' guidance. While Concawe agrees with the need for more accurate persistence assessment, a blanket 'temperature correction' does not solve the problem. As advocated in the Brown *et al.* paper, a nuanced approach to adjusting biodegradation results based on the inoculum source and the type of chemical would be more appropriate.

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