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Persistence assessments in the 21st century

In the first of a three-part series, Marian Ponte, senior scientific advisor at EAG Laboratories, explains the updated process for persistence testing in the EU and the US

Both EU REACH and the amended Toxic Substances Control Act (TSCA) in the US signal important changes to the regulatory processes governing chemical substances, including persistent, bioaccumulative and toxic substances (PBTs). This series of three articles will provide a high-level explanation of that updated process, helping manufacturers, importers, distributors and users navigate the new regulatory environment.

Where is an assessment needed?

Under REACH, a PBT assessment is required for substances manufactured or imported in volumes of 10 tonnes/year or more. Under TSCA, it is required for any substance prioritised for a chemical safety assessment (CSA). More chemicals are being added to that list every year.

In either case, this assessment applies to all activities carried out with the chemical in question along its likely route of exposure to humans and other living organisms. Only chemicals containing an organic moiety are subject to assessment; inorganic substances are excluded. If you have a chemical that fulfills these criteria, it is your responsibility to determine:

- persistence: the chemical's resistance to degradation;
- bioaccumulation: its tendency to accumulate in a living organism; and
- toxicity: the degree to which it can harm all or part of a living organism.

What is persistence?

Persistence is a key parameter in predicting a chemical's risk to living organisms through long-term exposure. Persistence tests are designed to measure a chemical's resistance to environmental degradation, either through a biological process, in which it is metabolised by naturally occurring organisms, or an abiotic process involving chemical or photolytic reactions.

The results of these tests help environmental agencies identify a chemical substance as persistent, non-persistent or very persistent. The following sections will walk through these tests, starting where all persistence testing must start: screening.

First step: screening

The screening step determines whether or not a chemical must proceed into persistence testing based on its intrinsic physical properties.

If you are the registrant, you must provide detailed information about your chemical, including its solubility, melting and boiling point, vapour pressure and other physical-chemical properties. You can determine these

properties through experimentation; when that is not possible, you can supplement it with modelling software (such as the US EPA's EPI Suite).

Many chemicals are ruled non-persistent at this stage. Chemical substances with the potential for persistence must proceed to the second step.

Second step: biodegradability testing

If you have reached this step because you could not rule out persistence based on your chemical's intrinsic physical properties. These tests are designed to simulate an aquatic environment, where wastewater and sewage treatment microorganisms may contribute to its degradation.

To run these tests, you must know the purity (or, in the case of a mixture, the composition) of your chemical substance. With that benchmark, you can monitor degradation by measuring the decline of dissolved organic carbon (DOC), the biochemical oxygen demand (BOD) or the production of CO2 in the aqueous environment.

You can predict your chemical's biodegradability using computer models, such as wastewater treatment simulators (BioWin 2, 3 and 6), EPA PBT profiler software, or quantitative structure-activity relationship (Qsar) models.

These models may determine that your chemical is non-persistent, meaning no more tests are required. If that determination cannot be made, you will need to proceed with one or more of the following tests. Your chemical's physical properties will determine which tests are required.

First is the ready biodegradability test (OECD 301 A-F and 310) in an aerobic aqueous medium. Tests 301 A, B and E apply if the chemical is water-soluble (solubility >100 mg/L), non-volatile and non-adsorptive; 301 C, D and F apply if it is insoluble and/or volatile. Test 310 applies for volatile substances, regardless of water-solubility. A chemical is considered non-persistent if results show:

- >70% biodegradation (tests 301A and E); or
- >60% biodegradation (tests 301B, C, D, F and Test 310).

If tests do not meet this threshold for biodegradability, you must proceed with test OECD 302 A-C (inherent biodegradability in an aqueous medium with activated sludge), in which a low concentration of chemical is exposed to a high concentration of microorganisms in activated sludge over a prolonged time period.

Tests 302A (semi-continuous activated sludge test) and 302B (Zahn-Wellens test) apply to non-volatile, water soluble chemicals; 302C (Modified MITI II test) applies to substances with some volatility and low solubility in water.

Your chemical is considered non-persistent if results show >70% mineralisation within seven days (302B) or 14 days (302C). In both cases, positive results must have a lag phase of less than three days. If your tests do not meet this threshold, your chemical cannot yet be ruled non-persistent and you must proceed with the higher tier tests described below.

Third step: abiotic degradation and higher tier tests

If your chemical substance does not pass the biodegradability tests, you must determine if it can degrade by abiotic processes, such as hydrolysis, oxidation or photolysis, or by chemical or biochemical degradation in natural environments.

Unlike the biodegradability tests, these higher tier tests are not designed assuming terminal degradation (that is, the formation of carbon dioxide). Instead, they measure how much of your chemical is present in different environments over time, if it forms any products during degradation, and how much. Depending on their magnitude, those products may need to undergo testing of their own.

These tests involve analytical methods specific to a chemical. If such methods do not exist, they need to be developed. This can be a complex process, particularly because you must identify and measure your chemical and its products in a crowded environment. How will you know which materials are the result of your chemical's degradation, and which are not?

The answer is a technique called radiolabelling. The technique works by tagging your chemical with a radioactive marker (usually 14C of 3H), which acts like a beacon to differentiate your chemical from the environment around it. You will need to choose your radiolabel and its position carefully in order to get measurable, high-quality results.

Most higher tier tests require a radiolabel. Even when not required, a radiolabel is helpful in gathering information about your chemical. The main objective is to determine the degradation rate of your chemical and identify any significant degradates formed during the tests described below:

- hydrolysis (OECD 111), which measures your chemical's degradation in water as a function of pH. Your first step is to conduct a short experiment to determine the potential of hydrolysis at basic, neutral and acidic pH conditions. If more than 10% of the substance degrades during this test, you must undertake the definitive test, which is conducted at the pH condition or conditions at which it is hydrolysable;

- aqueous photolysis (OECD 316) to test if the chemical is photolabile – that is, it degrades when exposed to sunlight, particularly in natural bodies of water. You must conduct this test using distilled water or a buffer solution and expose it to artificial sunlight for up to 30 days;

- aerobic mineralisation (OECD 309), which picks up where your earlier biodegradation tests left off by exposing your chemical to very diluted natural water, sometimes with the addition of sediment, for up to 60 days. This will determine if your chemical undergoes mineralisation (complete degradation to carbon dioxide) over a longer time period;

- transformation in sediment water systems (OECD 308), which studies how your chemical will behave when exposed to a combination of water and sediment, such as a lake or river, over a long period and under both aerobic and anaerobic conditions (such as a stagnant pond or in marine water with sediment). The test lasts up to 100 days – sometimes longer, if 100 days pass without any significant degradation; and

- transformation in soil (OECD 307), which is required regardless of the intended use, if there is a chance it will be used terrestrially or may end up in contact with soil. For instance, sludge obtained from a wastewater treatment plant and used as a fertiliser in agriculture is a candidate for this test, as it may contain chemicals that were not intended for use as fertiliser. To conduct this test, you must expose your chemical substance to four different types of soil, representative of the soils most commonly used in agriculture, for up to 120 days. You may extend this test depending on how your chemical behaves.

Final step: agency assessment

If you have proceeded as far as these higher tier tests, your final step is to submit a dossier of results to a regulatory agency. Agency officials may require additional tests at their discretion. Figure 1 below shows the criteria used to determine persistence in the EU and US. Countries outside of these jurisdictions may have different criteria, of course.

	Persistence (P) criteria	Very Persistent (vP) criteria
REACH	$T_{1/2}$ > 60 days in marine water $T_{1/2}$ > 40 days in fresh or estuarine water $T_{1/2}$ >180 days in marine sediment $T_{1/2}$ > 120 days in fresh or estuarine sediment $T_{1/2}$ > 120 days in soil	$T_{1/2}$ > 60 days in marine, fresh or estuarine water $T_{1/2}$ > 180 days in marine or fresh or estuarine sediment $T_{1/2}$ > 180 days in soil
US EPA	$T_{1/2}$ > 60 days in water, soil or sediment $T_{1/2}$ in air > 2 days	$T_{1/2}$ > 180 days in water, soil or sediment

Figure 1 – Persistence assessment criteria

Conclusion

Analytical chemistry never stands still. What satisfied regulators 20 years ago is no longer enough. Meanwhile, advances in technology, a deeper understanding of our environment and shifting standards for its protection are all contributing to the constant evolution of testing methodology.

Navigating that methodology is increasingly difficult for many registrants. Not only must they understand their chemical substance and its behavioural potential, they are also expected to anticipate which tests will likely be required, which new and emerging technology applies to those tests, and how to compile and interpret the results.

That is a lot for any registrant to manage, particularly for companies that do not have an internal regulatory department. For this reason, seeking help from a reputable independent consultant or laboratory is a good idea. Such organisations have the deep experience and insight needed to work smoothly with the regulatory agencies as you undergo your PBT assessment.

Marian Ponte **is a senior scientific advisor, environmental fate and metabolism, life sciences division at EAG Laboratories**

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