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Radiolabelling;

Advantages for Testing

This paper provides an overview of the potential benefits of radiolabelled vs non-radiolabelled test substances in environmental fate and physical chemistry studies

OECD Guidelines referenced: OECD 106; OECD 111; OECD 305; OECD 307; OECD 308; OECD 309; OECD 314; OECD 316.

Summary

The use of radiolabelled test materials to study the environmental fate and physical chemistry studies detailed above is standard practice for good reason. While it is possible to perform the studies without a radiolabelled test substance, the information gained in a non-radiolabelled test system will be limited and less robust than would be obtained using a radiolabel. While the initial costs associated with radiosynthesis can be high, this can be outweighed by the cost of significantly more analytical method development to support non-radiolabelled studies which can often result in equal or higher costs and with less benefit. Running studies without a radiolabel carries a risk that not all endpoints or data requirements will be met.

Overview

Radiolabels or radioisotopes emit an energy that it is possible to track and measure even in complex and solid matrices. By measuring this energy, it is possible to track starting molecules and transformation molecules originating from the starting material. Because the natural abundance of the radioisotopes is so low, any molecule that is measured using these radio-techniques is irrefutably known to have started off as the test material. Tracking this very specific energy allows differentiation between compounds originating in the sample matrix and compounds that started off as the (parent) test substance.

General principal of using radiolabelled test substances

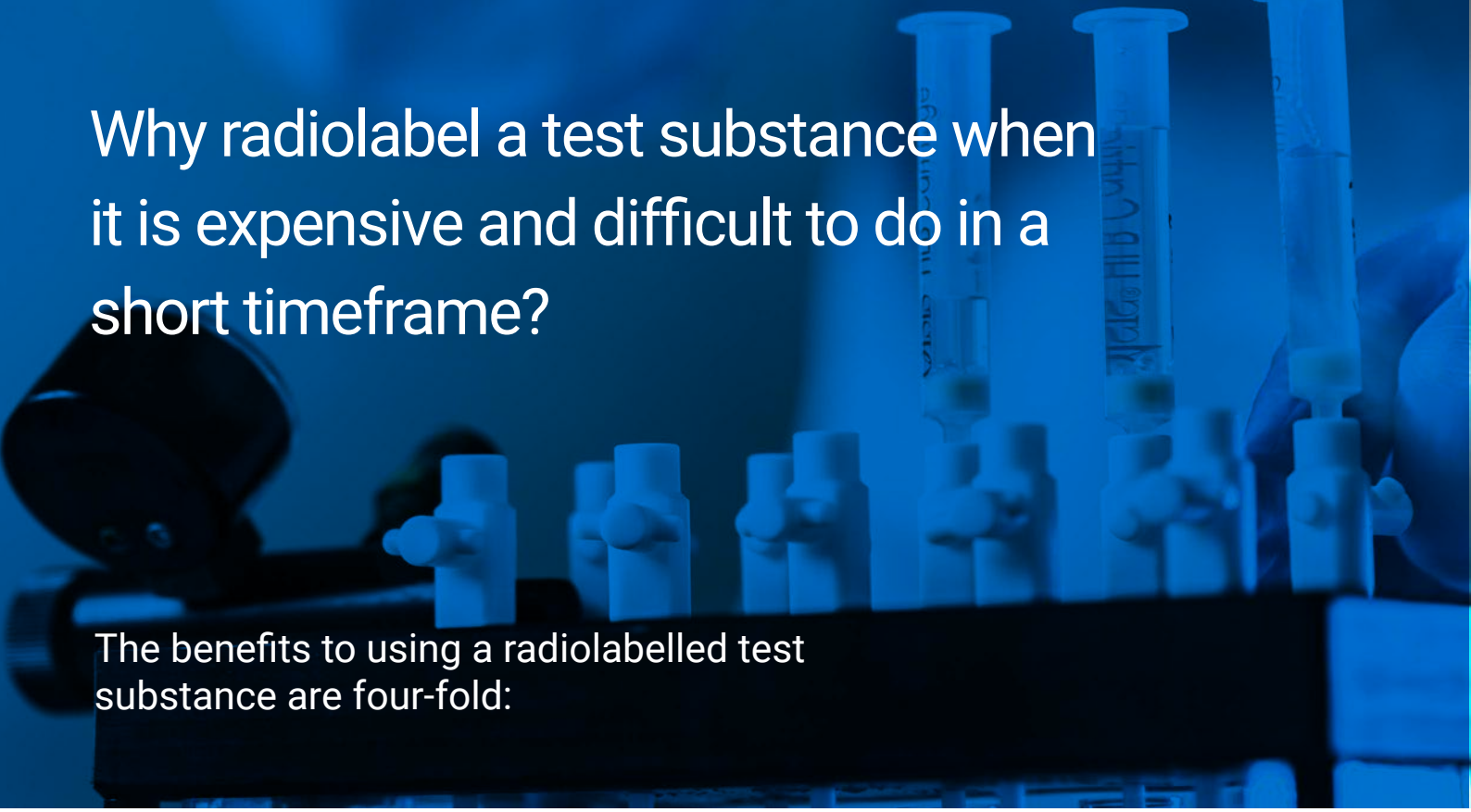
The use of radiolabelled test substances to study the route (and rate) of degradation in environmental fate and physical chemistry studies has been standard practice for decades.

The process of radiolabelling involves synthesising a molecule containing a radioactive atom (or more than one), typically ^{3}H (tritium) or ^{14}C.

As these radioisotopes decay, they emit an energy which can be quantified.

The level of these radioisotopes in nature is extremely low, therefore, in simulation studies the detectable radioactivity can be used to determine the quantity of radioisotopes in different sample types (for example in sample extracts, bound to solid material and metabolised to carbon dioxide).

This ability to measure the level of radioisotope in different sample types allows differentiation between loss of the test substance through degradation and irreversible binding to solid phases (e.g. soil/sediment), which is important for understanding its environmental profile.



Why radiolabel a test substance when it is expensive and difficult to do in a short timeframe?

The benefits to using a radiolabelled test substance are four-fold:

1) It enables tracking of the route of degradation.

Using chromatographic techniques coupled to radio-detection techniques it is possible to separate the different components that have been extracted from the sample and profile the radiolabelled substances. The detection of the energy released by the decay of the radionuclide is (effectively) independent of the nature of the molecule it is part of, meaning measurement of response using a radio-detector directly translates into relative amount. All isolated components that are detected can be said, with a high level of certainty, to have originated from the starting molecule. When used in conjunction with high resolution mass spectrometry these isolated compounds can be identified to allow elucidation of the route of degradation/transformation. An additional benefit of radiolabelling when seeking to identify metabolites is the ratio of masses that is a signature of the abundance of radiolabelling which helps to identify a mass originating from a compound related to the parent substance.

2) It allows measurement of ultimate biodegradation.

In the case of ^{14}C, ultimate biodegradation (aerobic metabolism of the molecule) will yield $^{14}CO_2$ which can be trapped and measured. Under anaerobic conditions ultimate biodegradation will yield $^{14}CH_4$ which can be oxidised (see point 3) to form $^{14}CO_2$ which can be trapped and measured. This measurement allows determination of the ultimate biodegradation of a test substance.

3) It allows determination of mass balance.

In addition to measuring all molecules containing the radioisotope in solutions (e.g. soil extracts), solid materials such as soil and sediment can be oxidised at high temperature which causes breakdown of all carbon to CO_2. This can be trapped and quantified to measure the amount of radionuclide bound to the solid phase. By combining the amount of radioactivity measured in solution(s) (water/ sample extracts), in $^{14}CO_2$ traps and bound to any solid material a mass balance can be obtained for the sample.

4) Minimising analytical difficulties in samples that can contain a high amount of complex matrix (soil and sediment in particular).

This is perhaps the most often overlooked advantage of using a radiolabel but can have a big impact on the quality of the data obtained from a study. This is especially true for studies using soil, sediment and sewage sludge, where high levels of complex sample matrix can hamper the quality of non-radiolabelled analysis. Analytical difficulties can, of course, still be encountered, but the ability of the molecules of interest to emit a signature energy can greatly improve accuracy and sensitivity. The radiochemical techniques used enable a robust LOD and LOQ for typically employed levels of radiolabelling, ensuring the analysis can support studies dosed at environmentally relevant concentrations.

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Why can any of the above benefits not be achieved using a non-radiolabelled test substance?

1) Without a radiolabel there is nothing to distinguish any degradation/transformation products from other compounds in the sample matrix. When testing natural water, soil, sediment and sewage plant systems the level of the matrix is many orders of magnitude above the concentration of the test substance or its metabolites. It's like looking for an unknown number and identity of needles in a swimming pool full of other needles. Accurate quantitation of non-radiolabelled compounds usually relies on external calibration, where a range of known concentration solutions of the substance are prepared and analysed alongside the unknown concentration solution. Comparison of the responses is back calculated to a concentration. If the metabolic pathway for the substance is known going into the study, and analytical standards for each of the metabolites are available, the route of degradation can be confirmed, and the rate determined without a radiolabel.

2) In a non-radiolabelled test system, it is not possible to distinguish between CO_2 from the atmosphere, CO_2 from aerobic metabolism of 'other' substrates and CO_2 from aerobic metabolism of the test substance or its metabolites. Each CO_2 is identical.

3) In addition to point 2 it is not possible to measure the amount of non-extracted compound.

Creating an extraction method that works well for freshly exposed samples using the compound of interest is often possible, but once samples have aged and transformation has occurred it isn't clear if extraction efficacy has fallen or if transformation has occurred.

Which studies benefit from using a radiolabelled substance?

For the reasons previously mentioned, we highly recommend using radiolabelled test substances for any study that seeks to determine the route of degradation/transformation.

These studies include:

OECD Guideline	Title	Aims
106	Adsorption – desorption using the batch equilibrium method	Determine adsorption coefficient

The OECD 106 study can be performed non-radiolabelled, however, it is highly recommended to, wherever possible, conduct it using a radiolabelled test substance. One of the key requirements of the study is that the test substance remains stable over the exposure period. Using a radiolabelled material allows much clearer demonstration of stability during this test. Other advantages of using a radiolabelled test substance are the improved detection it allows (test concentrations in this study can be very low), mitigation of high levels of matrix interference, and differentiation between formation of bound residues and degradation. Developing and validating a non-radiolabelled method to support these requirements can be very difficult, time consuming and expensive.

OECD Guideline	Title	Aims
111	Hydrolysis as a function of pH	Hydrolysis route and rate

Tiers 1 and 2 of this study can be conducted using a non-radiolabelled material (rate of hydrolysis), but tier 3 requires a radiolabel or analytical standards for the hydrolysis products. Advantages of a radiolabelled test substance include the ability to easily differentiate between binding to surfaces and hydrolysis, determination of mass balance and the ability to identify and accurately quantify hydrolysis products (tier 3).

OECD Guideline	Title	Aims
305	Bioaccumulation in fish	Mass balance with identification and quantification of metabolites
307	Aerobic and anaerobic transformation in soil	Route and rate of transformation
308	Aerobic and anaerobic transformation in aquatic sediment systems	Route and rate of transformation
309	Aerobic transformation in surface water – simulation biodegradation test	Route and rate of transformation
314	Simulation tests to assess the biodegradability of chemical discharged in wastewater	Route and rate of biodegradation
316	Photo transformation of chemicals in water – direct photolysis	Route and rate of transformation

As discussed in detail above, when looking at transformation of natural systems the rate of loss can be performed non-radiolabelled, but the route of transformation, the level of ultimate degradation and the determination of mass balance cannot.

Disadvantages of using a radiolabelled material

The main disadvantage of using a radiolabelled material for the above detailed studies is the initial cost of synthesising the material, and any difficulties associated with obtaining the starting materials for synthesis labs, which may lead to longer study initiation lead times.

Not all test materials are suitable for radiolabelling, but our expertise allows for quick decisions to be made on the feasibility of labelling to help manage testing strategies and regulatory expectations.

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At Scymaris, our foundation is a heritage of over 75 years' experience in serving the global pharmaceutical, agrochemical and chemical industries. Our modern, world class facility and state of the art laboratories as well as unique access to marine species in Brixham, Devon, means we can provide a turnkey solution for GLP and non-GLP Environmental Risk Assessment requirements.

We provide services in:

- Ecotoxicology (freshwater and marine), Environmental Fate/Metabolism & Biodegradation, and Analytical Chemistry.
- Endocrine disruptor testing, including FSTRA Daphnia repro/FSDT/FFLC/FELs/AMA. As well as validating LAGDA/XETA/ZEOGRT/MEOGRT.
- Services in lower and higher tier Environmental Fate studies with radiolabel expertise
- State of the art analytical equipment for substance characterisation, Metabolite ID, and complex compounds analysis.
- Regulatory testing according to GLP, OCSPP, ISO, OSPAR and licensed to work with radiochemicals (^{14}C & 3-H)

As a dedicated environmental testing laboratory, we bring together expertise across all disciplines to ensure that our testing strategies are adapted to the challenges of today's new chemistries. This includes poorly soluble, UVCB, volatile or unstable, natural products, and difficult to analyse substances.

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