Methods to test biodegradability:

ASTM D-5338

Aim of the test: This procedure has been developed to permit the determination of the rate and degree of aerobic biodegradability of plastic products when placed in a controlled composting process.

Biodegradation of a plastic within a composting unit is an important phenomenon because it may affect the decomposition of other materials enclosed by the plastic and the resulting quality and appearance of the composted material. Biodegradation of plastics will also allow the safe disposal of these plastics through large, professionally-managed composting plants and well-run residential units, where thermophilic temperatures are achieved.

Principle: This test method determines the degree and rate of aerobic biodegradation of plastic materials on exposure to a controlled-composting environment under laboratory conditions, at thermophilic temperatures. This test method is designed to yield reproducible and repeatable test results under controlled conditions that resemble composting conditions, where thermophilic temperatures are achieved. The test substances are exposed to an inoculum that is derived from compost from municipal solid waste. The aerobic composting takes place in an environment where temperature, aeration and humidity are closely monitored and controlled.

Applicability: This test method is designed to be applicable to all plastic materials, which are intended to be composted in facilities that achieve thermophilic temperatures.

Procedure: The aerobic biodegradation test introduces material to a mixed bacterial and fungal environmental inoculum and uses respirometry to measure biodegradation. Standard testing for ASTM D5338 is a minimum of 90 days in an aerobic and controlled composting environment. As the test samples are exposed to an inoculum derived from a municipal waste stream, the material will (ideally) biodegrade in a representative environment as commonly found in standard compost facilities.

Source:
https://www.astm.org/Standards/D5338
https://allcivilstandard.com/wp-content/uploads/2018/12/D-5338.pdf

OECD 301 B

Aim of the test: This method is used to demonstrate rapid and complete biodegradation under aerobic conditions. The test introduces a material to an inoculum in a closed environment and measures biodegradation of the material by CO2 evolution.

Principle: Respirometry is used to determine the biodegradability of the material by evaluating the production of CO2 over a minimum of 28 days in a liquid environment.

A measured volume of inoculated mineral medium, containing a known concentration of the test substance (10-20 mg DOC or TOC/l) as the nominal sole source of organic carbon is aerated by the passage of carbon dioxide-free air at a controlled rate in the dark or in diffuse light. Degradation is followed over 28 days by determining the carbon dioxide produced. The CO2 is trapped in barium or sodium hydroxide and is measured by titration of the residual hydroxide or as inorganic carbon. The amount of carbon dioxide produced from the test substance (corrected for that derived from the blank inoculum) is expressed as a percentage of ThCO2. The degree of biodegradation may also be calculated from supplemental DOC analysis made at the beginning and end of incubation.

Applicability: The test applies to highly soluble, poorly soluble, and even for materials with certain concentrations known to be insoluble, additionally to adsorbing compounds.

The test applies to the following materials:

- Fuels, lubricant oils, surfactants, personal care products
- Formulations and other solutions

Procedure: A solution, or suspension, of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC in the test solution due to the inoculum should be kept as low as possible compared with the amount of organic carbon due to the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance, although the endogenous activity of cells in the presence of a chemical will not exactly match that in the endogenous control. A reference compound is run in parallel to check the operation of the procedures.

In a typical run, the following flasks are used:

- Flasks1&2 – containing test substance and inoculum (test suspension);
- Flasks3&4 – containing only inoculum (inoculum blank);
- Flask 5 - containing reference compound and inoculum (procedure control); and, preferably and when necessary, also
- Flask 6 – containing test substance and sterilising agent (abiotic sterile control);
- Flask 7 – containing test substance, reference compound and inoculum (toxicity control).

Start the test by bubbling C0 -free air through the suspensions at a rate of 30-100 ml/min.

CO2 determinations

It is mandatory to follow the CO2 evolution from the test suspensions and inoculum blanks in parallel and it is advisable to do the same for the other test vessels. During the first ten days it is recommended that analyses of CO2 should be made every second or third day and then at least every fifth day until the 28th day so that the 10-d window period can be identified. On the days of C02 measurement, disconnect the barium hydroxide absorber closest to the test vessel and titrate the hydroxide solution with 0.05 M HCl using phenolphthalein as the indicator. Move the remaining absorbers one place closer to the test vessel and place a new absorber containing 100 ml fresh 0.0125 M barium hydroxide at the far end of the series. Make titrations as needed, for example, when substantial precipitation is seen in the first trap and before any is evident in the second, or at least weekly. Alternatively, with Na0H as absorbent, withdraw a sample of the sodium hydroxide solution from the absorber nearest to the test vessel using a syringe. The sample volume needed will depend on the carbon analyser used,

but sampling should not significantly change the absorbent volume over the test period. Inject the sample into the IC part of the carbon analyser for analysis of evolved carbon dioxide directly. Analyse the contents of the second trap only at the end of the test in order to correct for any carry over of carbon dioxide. On the 28th day withdraw samples, optionally, for DOC and/or specific chemical analysis. Add 1 ml of concentrated hydrochloric acid to each test vessel and aerate them overnight to drive off the carbon dioxide present in the test suspensions. On day 29 make the last analysis of evolved carbon dioxide.

Sources:

https://www.situbiosciences.com/product/oecd-301b-biodegradation-test-co2-evolution/

https://www.oecd-ilibrary.org/environment/test-no-301-ready-biodegradability_9789264070349-en

EN 13432

Aim of the test: Plastic products can prove their compostability by successfully passing the harmonized EN standard EN 13432. The European Packaging Directive 94/62 EC refers to this when meeting recycling requirements

Principle: The maximum material thickness of a plastic is determined for which composting still takes place within the usual practical framework. All tests must be passed successfully, successful individual tests are not sufficient. The test methods described by the standard are based on the scientific definitions of ISO standards 18451, 18452 (aerobic aqueous degradability), 18453 (anaerobic aqueous degradability) and 18455 (aerobic composting). The tests must be carried out by recognized testing laboratories.

Applicability: This test method is designed to be applicable to all plastic materials, which are intended to be composted. 90 % of the polymer mass must be converted into carbon dioxide within 180 days.

Procedure:

- Chemical testing: Disclosure of all ingredients, limit values for heavy metals must be complied with.
- Biodegradability in aqueous medium (oxygen demand and evolution of CO2): It must be demonstrated that at least 90% of the organic material is converted to CO2 in 6 months.
- Disintegration into compost: After 3 months of composting and subsequent screening through a 2 mm sieve, no more than 10% residue relative to the original mass may remain.
- Practical testing of compostability on a pilot plant scale (or a practical plant): there must be no negative effects on the composting process.
- Compost application: investigation of the effect of resulting composts on plant growth (agronomic test), ecotoxicity test.

Sources:

http://www.bioplastics.ch/EN-13432.pdf

ASTM D-6400

Aim of the test: As a product test method, the ASTM 6400 provides for composting conditions as a means to assess biodegradation of finished materials and raw materials or ingredients. 60% of the material must be converted to CO2 in 180 days (six months).

Principle: The test method covers plastics and products made from plastics that are designed to be composted under aerobic conditions in municipal and industrial aerobic composting facilities, where thermophilic conditions are achieved.

Applicability: The test is the standard specification for the biodegradation of solids intended for aerobic composting. It can also be used for other solid materials such as paper products, textiles, foam and food packaging.

Procedure: The series of ASTM D6400 is a four-part biodegradation test for evaluating biodegradability that includes elemental analysis, plant germination (phytotoxicity), and mesh filtration of the resulting particles.

The ASTM D6400 test method uses a set of conditions that favor microorganisms that thrive above 50 degrees centigrade, making the test method somewhat selective for bacterial based biodegradation. This component of the test method does favor bioplastic types of materials, and may not provide for testing parity when comparing the ASTM D6400 to other test methods such as the ISO 16929. In addition, the method is not meant to represent composting conditions in a home compost facility, as the composting temperature requirements are not likely to be achieved in a home facility over an extended period of time.

- The standard ASTM D6400 test series lasts a minimum of 90 days. Testing can last up to 180 days .
- Exposed to an inoculum derived from a municipal waste stream, the material will (ideally) biodegrade in a representative environment as commonly found in standard compost facilities.
- For making compostability claims, ASTM D6400 is the recommended compostability test method for a wide range of industrial components and consumer products .

The biodegradation testing has to be conducted in triplicate on each of the following: 1.) the sample (100g of sample + 600g dry weight of compost), 2.) positive control (100g of cellulose + 600g dry weight of compost), 3.) negative control (100g of polyethylene + 600g dry weight of compost), 4.) blank (600g dry weight of compost). The moisture content of the mixtures is adjusted to 50%, then they are put into the composting vessels as described in "Equipment" above. The composting vessels are placed in the incubator at 58°C ± 2°C. The CO2 free air is then connected and adjusted so that the flow rate is between 150 and 200 ml per minute. The gases exiting the test chambers are plumbed to a solenoid valve which is controlled to divert air for 2 minutes out of every 2 hours. These diverted gases flow into 1 liter adsorption units containing a known volume of 1N sodium hydroxide to adsorb the carbon dioxide being produced in the vessels (for the remainder to the 2 hours the exhaust is simply vented to the room).

The sodium hydroxide is periodically titrated to measure the CO2 production; our standard days for the titration are 3, 7, 14, and every 7 days after that. We titrate to pH 8.5 with 0.5N HCl after adding BaCl2 to precipitate the carbonates formed by the CO2. Fresh 1N sodium hydroxide is placed in the absorption units and the whole process is repeated. The testing is carried out until the CO2 production from both the sample and the positive control have plateaued up to a maximum of 180 days.

Sources:

https://www.worldcentric.com/assets_external/files/TPLA-Utensils-ASTM6400.pdf

https://www.situbiosciences.com/product/astm-d6400-compostable-produc-test-composting/

OECD 302 B

Aim of the test: The original Zahn-Wellens test (1) was adopted in 1981 as OECD Guideline 302 B for determining inherent biodegradability. Later proposals were made by Switzerland and Germany to modify this guideline by merging it with elements contained in a test developed by EMPA (2). The merged version of the test was further changed in respect to the mineral medium used. The medium retained is identical with that which is used in the DOC Die-Away, CO2 Evolution, Mamometric Respirometry and Modified OECD Screening methods of Guideline 301 (adopted 1992) for determining ready biodegradability: ≥70 % mineralisation is set within seven days and degradation must start after three days. The test additionally provides information on the adsorption behaviour of the tested substance.

Principle: A mixture containing the test substance, mineral nutrients and a relatively large amount of activated sludge in aqueous medium is agitated and aerated at 20-25°C in the dark or in diffuse light for up to 28 days. Blank controls, containing activated sludge and mineral nutrients but no test substance, are run in parallel. The biodegradation process is monitored by determination of DOC (or COD) in filtered samples taken at daily or other time intervals. The ratio of eliminated DOC (or COD), corrected for the blank, after each time interval, to the initial DOC value is expressed as the percentage biodegradation at the sampling time. The percentage biodegradation is plotted against time to give the biodegradation curve. Specific analysis of the test substance may be useful in cases where molecular changes, caused by biochemical reactions (primary biodegradation) are to be detected.

Applicability: The method applies to chemicals which are non-volatile and are soluble in water to at least 50 mg DOC/l may be assessed by this method, provided also that they do not significantly adsorb, are not lost by foaming and do not inhibit bacteria at the concentration tested.

Procedure: For practical reasons, do not start the test immediately before a week-end. Run the test, normally for up to 28d, in the dark or in diffuse light at 20-25°C. Aerate the suspensions with purified, humidified air and, if necessary, stir to ensure that sludge does not settle and that the concentration of dissolved oxygen does not fall below 1 mg/l. Check the pH value at regular intervals (e.g. on each day of sampling) and adjust to pH 6.5-8.0 with NaOH (40 g/l) or H2S04 (50 g/l) if necessary.

Filter the samples of sludge suspensions (test, blank and procedure control) as soon as they are taken, discarding the first 5 ml of filtrate. Use either carefully washed paper filters or membrane filters, which are suitable if they neither release nor adsorb organic compounds. Otherwise wash the membranes three times in deionised or distilled water at about 60°C, and store them in water. Separate sludges which are difficult to filter by centrifugation or by other suitable separation techniques.

Determine the DOC or COD in duplicate in the filtered or centrifuged samples by any suitable methods e.g. (4) (5). If primary biodegradation is to be followed, use specific analyses, e.g. UV spectroscopy, in addition to DOC or COD. If the filtrates cannot be analysed on the day of sampling, store at 2-4°C for a maximum of 48h, or at -18 C for longer periods. Storage for long periods however is not recommended.

Calculation of the percentage degradation at time t from:

$$D_t = \left[1 - \frac{C_t - C_B}{C_A - C_{BA}}\right] \times 100$$

Dt = percentage degradation at time t;
CA = concentration (mg/l) of DOC or COD in the test suspension measured after 3h ± 30 min of incubation;
Ct = mean concentration (mg/l) of DOC or COD in the test suspension at time t;
CBA = mean concentration (mg/l) of DOC or COD in the blanks measured after 3h ± 30 min of incubation;
CB = mean concentration (mg/l) of DOC or COD in the blanks at time t.

Sources:

https://www.oecd-ilibrary.org/environment/test-no-302b-inherent-biodegradability-zahn-wellens-evpa-test_9789264070387-en

OECD 302 C

Aim of the test: The purpose of the test is the measurement of the Biochemical Oxygen Demand (BOD) and the analysis of residual chemicals in order to valuate the inherent biodegradability of chemical substances which have been found by the Modified MITI Method to indicate low degradability. ≥70 % mineralisation is set within seven days and degradation must start after three days.

Principle: The test method is based on the following conditions:

- Test chemicals as solo organic carbon sources
- No adaptation of micro-organisms to test chemicals

Applicability: The method is only applicable to those organic test materials which, at the concentration used in the test, have negligible vapour pressure; are not inhibitory to bacteria, and do not reach and react with the CO2 adsorbent.

Procedure: The test item is exposed to activated sludge from the aeration tank of a domestic waste water treatment plant for normally 28 days. The biodegradation is followed by the oxygen uptake of the microorganisms during exposure. The percentage of degradation is determined by correction for the blank and under consideration of the theoretical oxygen demand of the test item. For the assessment of primary degradation, the test item concentration is determined in aqueous phase as well as the adsorption on the sludge. The grade of primary degradation is assessed by measurement of the test item concentration in the test vessels by a substance-specific analysis.

Test conditions:

- Concentration of test chemicals: 30 ppm (W/V)
- Concentration of activated sludge 100 ppm (W/V)
- Test temperature: 25°C ± 2°C
- Period: 14 to 28 days
- Perform in darkness. The temperature and the change in colour of the contents of the culturing vessels should be checked every day.
- Stir vigorously with mechanical stirrer

After the 14 to 28 days of testing, pH, residual chemicals and intermediates in the testing vessels are analyzed. The testing vessels in the testing vessel without activated sludge are also analyzed in order to determine whether there is any change in the test chemical during the testing period, or any loss of the original test chemical by evaporation or by adsorption by the walls of the vessels.

If the test compound is soluble water, the residual amount of total organic carbon is also determined.

1. Where a total organic carbon analyser is ued: 10 ml of the tested solution is sampled from the vessel and centrifuged at 3000 g for five minutes. The residual amount of the total organic carbon in the supernatant is determined on a total organic carbon analyser.

2. Where other analysers are used: the total content of a testing vessel is extracted with a solvent suitable for the test compound and, after proper pretreatment such as concentration, the residual amount of the test compound is determined on an analysing instrument.

In the case of volatile chemicals, the temperature control bath of the BOD-meter should be cooled to 10°C and this temperature held for at least 30 minutes, in order to prevent evaporation. The analytical procedures mentioned above (1. And 2.) should then be started.

Sources:

https://www.oecd-ilibrary.org/environment/test-no-302c-inherent-biodegradability-modified-miti-test-ii_9789264070400-en

EN ISO 14852:2018

Aim of the test: This method is carried out by measuring the amount of carbon dioxide evolved, for the determination of the degree of aerobic biodegradability of plastic materials, including those containing formulation additives. The test material is exposed in a synthetic medium under standardized laboratory conditions to an inoculum from activated sludge, mature compost or soil under aerobic, mesophilic conditions.

Principle: If an unadapted activated sludge is used as the inoculum, the test result can be used to assess the aerobic biodegradation processes which occur in a waste water treatment plant environment. If a mixed or pre-exposed inoculum is used, the method can be used to investigate the potential biodegradability of a test material.

Applicability: The method applies to the following materials:

- natural and/or synthetic polymers, copolymers or mixtures thereof;
- plastic materials which contain additives such as plasticizers, colorants or other compounds;
- water-soluble polymers;
- materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see, for example, ISO 8192[1]). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum can be used.

It does not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but this test method is designed to measure the biodegradation of plastic materials and give an indication of their potential biodegradability.

Procedure: The test material shall be of known mass and contain sufficient carbon to yield CO2 in a quantity that can be adequately measured by the analytical system used. Calculate the TOC from the chemical formula or determine it by a suitable analytical technique and calculate the ThCO2. Use concentration of a test material such that the TOC content is at least 30 mg/l preferably 100 mg/l-. The maximum amount of test aterial is limited by the oxygen supply to the test system and the test medium used. When using higher amount of test material, the optimized test medium should be used and, in any case the test-material concentration shall be such the TOC does not exceed about 2000 mg/l. If higher concentrations are to be tested, increase the nitrogen amount in the test medium.
Provide a number of flasks, so that the test includes at least the following:

- 3 test flasks for the test material
- 3 flasks for the blank
- 3 flasks for checking the inculum activity using a reference material

Connect the flasks to the CO2-free-air production system. Incubate at the desired test temperature and areate the flasks for 24 h to purge carbon dioxide from the system.Agitate throughout the test with a magnetic stirrer. If excessive foaming is observed, replace the air purge by overhead aeriation with stirring. Now connect the air exit of each flask to the carbon dioxide trapping.
Add the test material, the reference material and the material for the negative control to the respective flasks indicated and start the test by bubbling CO2-free air through the flasks to ensure a sufficient quantity of oxygen throughout the test. A rate of 50 ml/min to 100 ml/min is usually suitable.
When a constant level of CO2 release is attained and no further biodegradation is expected, the test is considered to be completed. The maximum test period s 6 months. On the last day measure pH, acidify all the bottles with 1 ml of concentrated hydrochloric acid in order to decompose the carbonates and bicarbonates, and purge to remove the CO2. Continue aeration for 24 h and measure the amount of CO2 evolved in each of the deries of flasks.

Sources:

https://www.iso.org/standard/72051.html

http://www.bpcinstruments.com.cn/wp-content/uploads/2020/06/ISO-14852-Determination-of-the-ultimate-aerobic-biodegradability-of-plastic-materials-in-an-aqueous-medium-—-Method-by-analysis-of-evolved-carbon-dioxide.pdf

EN ISO 17556:2012

Aim of the test: This method is used for determining the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a closed respirometer or the amount of carbon dioxide evolved. The method is designed to yield an optimum degree of biodegradation by adjusting the humidity of the test soil.

Principle: If a non-adapted soil is used as an inoculum, the test simulates the biodegradation processes which take place in a natural environment; if a pre-exposed soil is used, the method can be used to investigate the potential biodegradability of a test material.

Applicability: The test applies to the following materials:

- natural and/or synthetic polymers, copolymers or mixtures of these;
- plastic materials which contain additives such as plasticizers or colorants;
- water-soluble polymers.

It does not necessarily apply to materials which, under the test conditions, inhibit the activity of the microorganisms present in the soil. Inhibitory effects can be measured using an inhibition control or by another suitable method. If the test material inhibits the microorganisms in the soil, a lower test material concentration, another type of soil or a pre-exposed soil can be used.

Procedure: The plastic material, which is the sole source of carbon and energy, is mixed with the soil. The mixture is allowed to stand in a flask over a period of time during which the amount of oxygen consumed (BOD) or the amount of carbon dioxide evolved is determined. Provided the CO_2 evolved is absorbed, the BOD can be determined, for example, by measuring the amount of oxygen required to maintain a constant gas volume in a respirometer flask, or by measuring either automatically or manually the change in volume or pressure (or a combination of the two). The amount of carbon dioxide evolved is measured at intervals dependent on the biodegradation kinetics of the test substance by passing carbon dioxide free air over the soil and then determining the carbon dioxide content of the air by a suitable method. The level of biodegradation, expressed as a percentage, is determined by comparing the BOD with the theoretical oxygen demand (ThOD) or by comparing the amount of carbon dioxide evolved with the theoretical amount ($ThCO_2$). The influence of possible nitrification processes on the BOD has to be considered. The test is terminated when a constant level of biodegradation has been attained or, at least, after six months.

Unlike ISO 11266, which is used for a variety of organic compounds, this international standard is specially designed to determine the biodegradability of plastic material.

Sources:

https://www.iso.org/standard/56089.html

https://www.sis.se/api/document/preview/915073

OECD TP 307

Aim of the test: The method is designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine

(i) the rate of transformation of the test substance, and

(ii) the nature and rates of formation and decline of transformation products to which plants and soil organisms may be exposed.

Principle: Soil samples are treated with the test substance and incubated in the dark in biometer-type flasks or in flow-through systems under controlled laboratory conditions (at constant temperature and soil moisture). After appropriate time intervals, soil samples are extracted and analysed for the parent substance and for transformation products. Volatile products are also collected for analysis using appropriate adsorption devices. Using 14C-labelled material, the various mineralisation rates of the test substance can be measured by trapping evolved $14CO_2$ and a mass balance, including the formation of soil bound residues, can be established.

Applicability: The method is applicable to all chemical substances (non-labelled or radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or water-insoluble compounds. The test should not be applied to chemicals which are highly volatile from soil (e.g. fumigants, organic solvents) and thus cannot be kept in soil under the experimental conditions of this test.

Procedure: For addition to and distribution in soil, the test substance can be dissolved in water (deionised or distilled) or, when necessary, in minimum amounts of acetone or other organic solvents in which the test substance is sufficiently soluble and stable. However, the amount of solvent selected should not have a significant influence on soil microbial activity. The use of solvents which inhibit microbial activity, such as chloroform, dichloromethane and other halogenated solvents, should be avoided. 20. The test substance can also be added as a solid, e.g. mixed in quartz sand or in a small subsample of the test soil which has been air-dried and sterilised. If the test substance is added using a solvent the solvent should be allowed to evaporate before the spiked sub-sample is added to the original non-sterile soil sample. For general chemicals, whose major route of entry into soil is through sewage sludge/farming application, the test substance should be first added to sludge which is then introduced into the soil sample. The use of formulated products is not routinely recommended. However, e.g. for poorly soluble test substances, the use of formulated material may be an appropriate alternative

To determine the transformation pathway, a representative soil can be used; a sandy loam or silty loam or loam or loamy sand [according to FAO and USDA classification] with a pH of 5.5-8.0, an organic carbon content of 0.5 - 2.5% and a microbial biomass of at least 1% of total organic carbon is recommended. For transformation rate studies at least three additional soils should be used representing a range of relevant soils. Those soils should vary in their organic carbon content, pH, clay content and microbial biomass. All soils should be characterised, at least, for texture (% sand, % silt, % clay) [according to FAO and USDA classification], pH, cation exchange capacity, organic carbon, bulk density, water retention characteristics and microbial biomass (for aerobic studies only). Additional information on soil properties may be useful in interpreting the results. Microbial biomass should be determined by using the substrate-induced respiration (SIR) method or alternative methods.

Detailed information on the history of the field site from where the test soil is collected should be available. Details include exact location, vegetation cover, treatments with chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or other contamination. If soils have 2 Water retention characteristics of a soil can be measured as field capacity, as water holding capacity or as water suction tension (pF). It should be reported in the test report whether water retention characteristics and bulk density of soils were determined in undisturbed field samples or in disturbed (processed) samples. OECD/OCDE 307 been treated with the test substance or its structural analogues within the previous four years, these should not be used for transformation studies.

The soil should be freshly collected from the field (from the A horizon or top 20 cm layer) with a soil water content which facilitates sieving. For soils other than those from paddy fields, sampling should be avoided during or immediately following long periods (> 30 days) of drought, freezing or flooding. Samples should be transported in a manner which minimises changes in soil water content and should be kept in the dark with free

access of air, as much as possible. A loosely-tied polyethylene bag is generally adequate for this purpose. The soil should be processed as soon as possible after sampling. Vegetation, larger soil fauna and stones should be removed prior to passing the soil through a 2 mm sieve which removes small stones, fauna and plant debris. Extensive drying and crushing of the soil before sieving should be avoided. When sampling in the field is difficult in winter (soil frozen or covered by layers of snow), it may be taken from a batch of soil stored in the greenhouse under plant cover (e.g. grass or grass-clover mixtures). Studies with soils freshly collected from the field are strongly preferred, but if the collected and processed soil has to be stored prior to the start of the study storage conditions must be adequate and for a limited time only (4 ± 2°C for a maximum of three months) to maintain microbial activity. Before the processed soil is used for this test, it should be pre-incubated to allow germination and removal of seeds, and to re-establish equilibrium of microbial metabolism following the change from sampling or storage conditions to incubation conditions. A pre-incubation period between 2 and 28 days approximating the temperature and moisture conditions of the actual test is generally adequate. Storage and pre-incubation time together should not exceed three months.

The rate and pathway studies should normally not exceed 120 days, because thereafter a decrease of the soil microbial activity with time would be expected in an artificial laboratory system isolated from natural replenishment. Where necessary to characterise the decline of the test substance and the formation and decline of major transformation products, studies can be continued for longer periods (e.g. 6 or 12 months). Longer incubation periods should be justified in the test report and accompanied by biomass measurements during and at the end of these periods.

Sources:

https://www.oecd-ilibrary.org/docserver/9789264070509-en.pdf?expires=1620033690&id=id&accname=guest&checksum=0BA9042A54744AC90F87A6AE9C457DB7

OECD TP 308

Aim of the test: This method aims to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems. The method permits the measurement of

(i) the transformation rate of the test substance in a water-sediment system and in the sediment

(ii) the mineralisation rate of the test substance and/or its transformation products,

(iii) the distribution of the test substance and its transformation products between the two phases during a period of incubation in the dark, at constant temperature, and

(iv) the identification and quantification of transformation products in water and sediment phases including mass balance.

To gain information on the transformation of active ingredients in the environment, long-term studies like transformation in aquatic water/sediment systems according to OECD guideline 308 are required for the environmental risk assessment for human active pharmaceutical ingredients.

Principle: At least two sediments different with respect to organic carbon content and texture are used. Ideally the test substance (one concentration) should be applied as an aqueous solution into the water phase. The duration of the experiment should normally not exceed 100 days, and should continue until the degradation pathway and water/sediment distribution pattern are established or when 90 % of the test substance has been removed by transformation and/or volatilisation. The number of sampling times should be at least six. The study includes: concentration in the water and sediment of the test substance and the transformation products at every sampling time; results from gases/volatiles trapping systems at each sampling time; mineralisation rates; and non-extractable residues in sediment at each sampling point. Half-lives, DT50, DT75 and DT90 values are determined where the data warrant.

Applicability: The OECD 308 guideline states that it should not be used for chemicals which are highly volatile from water. It is considered applicable to slightly volatile chemicals, although the criteria for defining such compounds are not specified. For testing slightly volatile chemicals, a biometer-type (closed) test setup is recommended, but a detailed description of the system geometries and construction of the test setup is lacking. It has been pointed out the influence of varying system geometries and sediment:water (S:W) ratios on the partitioning and degradation of non-volatile chemicals in such tests. These variables alter the headspace volume in the test setup, which is expected to affect the extent to which volatile chemicals will partition into the headspace and hence be unavailable for degradation.

Procedure: The test involves incubation of a (radio-labelled) test substance, e.g. a pharmaceutical AI, in a test vessel filled with sediment and water at a ratio of 1:3 to 1:4 sampled from the same location from either a river or a lake. The test setup according to the guideline consists of four different systems, two aerobic and two anaerobic systems. For pharmaceuticals, information on anaerobic systems is usually not received, therefore, only aerobic studies were evaluated. The test sediments have to fulfil specific criteria concerning their organic carbon content and texture, and the test system has to be characterised concerning pH, particle size distribution, TOC, microbial biomass and redox potential. The test substance is added to the water phase of the system, and incubation is started. Parameters measured are the partitioning of the test substance (and TP) between water and sediment, mineralisation of the test substance (only if radio labelled), non-extractable residues in the sediment (only if radio labelled), the amount of test substance and TP at different sampling time points. A screening for the formation of TP has to be included, and detected TP have to be quantified. TP present at or above 10 % of the initially applied dose or those that do not show a clearly decreasing behaviour in the end of the study have to be identified. Additionally, by fitting an appropriate kinetic model to the data points, DTx (usually DT50 and DT90) for the parent or the TP should be derived (FOCUS 2006).

Sources:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4133017/

https://www.oecd-ilibrary.org/environment/test-no-308-aerobic-and-anaerobic-transformation-in-aquatic-sediment-systems_9789264070523-en

https://www.sciencedirect.com/science/article/pii/S0045653519317400#bib15

OECD TP 309

Aim of the test: The purpose of this test is to measure the time course of biodegradation of a test substance at low concentration in aerobic natural water and to quantify the observations in the form of kinetic rate expressions. Degradation of the test substance will be followed for the parent substance and its potential (known) transformation products. Degradation rate, mass balancing and transformation product pattern will be determined for the test substance. Degradation kinetics will also be followed for any major transformation product, if possible.

Principle: Typically, the test will be carried out with 14C-labelled test substance. Incubation in surface water ("pelagic test") will be accomplished in the dark at a temperature of 20°C under aerobic conditions and agitation (stirring). The test will be conducted using multiple flask design where each flask is connected to a flow-through system. A moderate stream of air will be used as carrier gas to collect CO2 and other volatiles in distinct traps consisting of different solutions.

Applicability: The test is applicable for test substances at low concentration in aerobic natural water.

Procedure: Two concentrations of the test substance differing by a factor of 5 to 10 will be used for the test. One concentration will be ≤ 10 µg/L (concentration I) and one ≤ 100 µg/L (concentration II).
Microbial activity of the surface water will be verified by using 14C-labelled benzoic acid (control samples). For examining possible abiotic degradation or other non-biological removal of the test substance (e.g. hydrolysis or adsorption to the test vessel), sterile samples will be prepared.
The test will run for a maximum of 60 days. Apart from samples taken directly after application, at least 7 sampling time points will be included. Time intervals will be chosen in such a way that the pattern of decline of the test substance and possible transformation products can be established.

In case of the 14C-labelled test substance the analytical methods will base on LSC and HPLC coupled with UV and radio detection. LC-MS/MS can be carried out as additional analytical method. The analytical method and sample preparation will be established to allow a limit of quantification of ≤ 5% of the applied radioactivity.

Non-labelled test substance can also be used for the test to investigate the degradation kinetics (e.g. DT50) of the test substance. In order to provide a minimum of mineral and nutrient source for the microbial population, a small concentration of suspended solids, in the form of sediment, can be added to the surface water (≤ 0.01 g/L).In general, the test samples will be agitated by stirring. Depending on the costumer needs, a setup for shaking the test samples is also available.

Sources:

https://www.ibacon.com/your-study-type/environmental-fate/oecd-309-aerobic-mineralisation-surface-water-simulation

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