KBBPPS

Knowledge Based Bio-based Products' Pre-Standardization

Work package 6

Biodegradability

Deliverable N° 6.1:

Report on current relevant biodegradation and ecotoxicity standards

Public

Version: Final

Ghent, 2013-02-20

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The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013 under grant agreement n° KBBE/FP7EN/312060/"KBBPPS").

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List with abbreviations

ASTM:	American Society for Testing and Materials
BCF:	BioConcentration Factor
BOD:	Biochemical Oxygen Demand
CEN:	European committee for standardisation (Comité Européen de Normalisation)
CFU:	Colony-Forming Units
COD:	Chemical Oxygen Demand
DO:	Dissolved Oxygen
DOC:	Dissolved Organic Carbon
EC:	Effect Concentration
ECB:	Effective Composition to Biodegradation
ECHA:	European Chemicals Agency
EMPA:	Swiss Federal Laboratories for Materials Testing and Research
EU:	European Union
HRT:	Hydraulic Retention Time
IC:	Inorganic Carbon
ISO:	International Organization for Standardization
LC:	Lethal concentration
LOEC:	Lowest Observed Effect Concentration
MATC:	Maximal Acceptable Toxicant Concentration
MITI:	Ministry of International Trade and Industry (Japan)
MHC:	Moisture-Holding Capacity
MSW:	Municipal Solid Waste
NOEC:	No Observed Effect Concentration
OECD:	Organisation for Economic Co-operation and Development
PAH:	Polycyclic aromatic hydrocarbon
PCB:	Polychlorinated biphenyl

QSAR:	Quantitative Structure-Activity Relationship
REACH:	Registration, Evaluation and Authorisation of Chemicals
SCAS:	Semi-Continuous Activated Sludge
SOC:	Soluble Organic Carbon
SRT:	Sludge Retention Time (= sludge age)
ThIC:	Theoretical Inorganic Carbon
ThOD:	Theoretical Oxygen Demand
$ThCO_2$:	Theoretical carbon dioxide
TIC:	Total Inorganic Carbon
TS:	Total Solids
VFA:	Volatile Fatty Acids
WAF:	Water-Accommodated Fraction
WHC:	Water Holding Capacity
WSF:	Water-Soluble Fraction

1 Publishable summary

In this report the current biodegradation test methods in different environments (fresh water, marine environment, anaerobic environment, soil and compost) and the existing test procedures for evaluating environmental safety are reviewed. Existing difficulties and gaps in the current test methods are defined.

This review is focussed on the applicability of the test methods to bio-lubricants and bio-solvents. Consumers consider the “bio”-prefix often as a synonym of good for the environment. It should be avoided that the term “bio” refers to subjective non-measurable criteria (e.g. environmentally friendly, environmentally acceptable, etc.). Objective criteria should be selected with well-defined criteria.

In order to ensure transparent communication, specifications and labelling systems need to be developed. Standardised test methods form the cornerstones for specifications, which encompass well-defined pass criteria with regard to different characteristics (e.g. biodegradation, environmental safety, bio-based content, performance, etc.). These criteria are chosen in function of the objective of the specification. The specifications are designed to form the basic principle for labelling systems. Currently specifications, acceptance criteria and labelling systems are already clearly defined for compostable plastics and packaging, while this is only to a lesser degree the case for other environments (fresh water, marine environment, anaerobic environment and soil) and other products. Existing specifications and labelling systems for plastics, bio-lubricants and bio-solvents are reviewed per environment and difficulties and gaps in the existing specifications are defined.

Freshwater aerobic aqueous environment

Based on the literature review of the different biodegradation test methods in an aqueous aerobic freshwater environment it can be concluded that a sufficiently broad range of measurement techniques already exists. Not each test method is suitable to test bio-lubricants or bio-solvents due to the fact that bio-lubricants are often poorly water soluble and that bio-solvents are often volatile. Therefore specific biodegradation test methods need to be selected taking into account these characteristics. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used. It also needs to be evaluated if the usual water-soluble reference materials (aniline, sodium benzoate, etc.) should be replaced by a poorly water soluble or a volatile alternative.

The review of the freshwater biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others influence of inoculum source (geographical variations, seasonal variations, etc.) on the biodegradability potential and variability of the results, interpretation of variability due to nitrification, necessity of the addition of a nitrification inhibitor, determination of the minimum amount of replicates, etc. should be further investigated.

With regard to environmental safety, it can also be concluded that a sufficiently broad range of testing methods towards freshwater organisms on different trophic levels (bacteria, algae, freshwater aquatic plants, crustacean and fish) already exists. For bio-lubricants and bio-

solvents, additional attention is especially needed towards the addition of poorly water soluble bio-lubricants and volatile bio-solvents to the testing systems as this can influence the test results.

The review revealed that specifications need to be developed towards bio-lubricants and bio-solvents. A technical report with useful recommendations for a specification towards bio-lubricants (CEN/TR 16227) and the specifications for the EU Ecolabel for lubricants can be taken as a guideline in order to develop a specification towards bio-lubricants. This should also be developed for bio-solvents.

A labelling system for bio-lubricants has been developed by different organisations, but no European or international labelling systems especially towards bio-solvents is developed yet. This should be developed taking into account parameters like biodegradability, environmental safety, minimum bio-based content, etc.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Marine aerobic aqueous environment

From the literature review on the biodegradation test methods in a marine aerobic environment, it can be concluded that there exist considerably less biodegradation test methods when compared to a freshwater environment. However, a sufficiently broad range of methods exists in order to determine the biodegradation in a marine environment. Not all methods are suitable in order to evaluate the biodegradability of bio-lubricants and bio-solvents, but a suitable measurement technique can be selected taken into account the specific properties (volatility and/or solubility) of a bio-lubricant or a bio-solvent. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used.

The review of the marine biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others the inoculum (natural seawater versus artificial seawater), the addition of nutrients, the difference between conditions in different parts of the sea (supralittoral, eulittoral, sublittoral benthic, deep sea benthic, pelagic & buried in the sediments), etc. should be further investigated.

With regard to marine environmental safety it can be concluded that less tests were developed when compared to the freshwater environment especially on OECD level. ISO and ASTM are already more progressive as more guidelines towards marine organisms were developed. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

Currently no standard specifications towards more environmentally friendly alternatives for lubricants and solvents used in a marine environment are developed yet. These specifications need to be developed. This can be based on the EU Ecolabel for lubricants, which encompasses already marine applications.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Anaerobic environment

Based on the review on the existing biodegradation standards in an anaerobic environment, it can be concluded that there exists a sufficiently broad range of standards in order to determine the degree of anaerobic biodegradation in aquatic environments, high-solids anaerobic-digestion environments and landfill environments. Suitable methods need to be selected for bio-lubricants and bio-solvents.

Lubricants and solvents can reach anaerobic aquatic environments in wastewater treatment plants, but high-solids anaerobic-digestion environments and anaerobic landfill environments are probably not considered as environments in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

Toxicity towards anaerobic bacteria can be evaluated based on existing methods. These methods can be used for lubricants and solvents. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

For biopolymers, which are degradable in anaerobic digestors, it might be necessary to evaluate if toxic residuals remain present in the produced digestate. Further research is needed in order to determine how this should be done.

Currently no standard specifications nor labelling systems are developed for products which are biodegradable in an anaerobic digester (e.g. biopolymers). This is mainly caused by the fact that there exists a wide variation in the construction and the operation of anaerobic digestion systems. The construction and operation systems can be divided into categories based on two parameters: (1) temperature (mesophilic and thermophilic) and (2) dry solids content (wet systems and dry systems). A standard specification should be developed, which includes criteria per operation system. This standard specification should form the basis for a new labelling system.

The labelling systems for lubricants do not refer to anaerobic environments in order to evaluate biodegradability and environmental safety. However, taken into account that a high percentage of ultimately aerobically biodegradable components needs to be present in the major part of the labelled products, it is expected that the labelled products will already be degraded before they come in contact with anaerobic environments.

Soil environment

A few international norms are available about testing biodegradability in soil. Their current weakness concerns their reliability in the cases of intentional incorporation of biodegradable materials in the soil under real conditions. Such a practice is widely used in agriculture and concerns the vast majority of applications where testing of biodegradation in soil is a key prerequisite with respect to both, environmental and food safety aspects. Considering the existing biodegradable plastics in agriculture and the effective life management of the

plastics in use at the agricultural field, only few norms have suitable tests that could be adapted for testing biodegradability in soil under real field conditions. The standardised criteria, parameters and testing methodologies for the characterization, labelling and validation of the agricultural plastic waste streams with respect to possible biodegradation in soil suggest that some major revisions are needed, before a new (i.e. revised) universal norm and improved standard testing methods become available for testing agricultural plastics for biodegradation under real, and highly variable, soil conditions. Based on the analysis of the different norms and their content it appears necessary to incorporate provisions for transferability of results to different soils, validation of test through a positive reference and set prerequisites for soil media. Furthermore, terminology and technical specifications vary and need to be harmonised. Long term biodegradation in soil prediction is another open issue (*Briassoulis and Dejean, 2010*). It is clarified though that there is no need for new testing method for biodegradation in soil. However, the existing standards for biodegradation in soil have to be improved and adapted in order to take into account the need for transferability of results to different soils under real field conditions. This goal should be achieved in a way that the standard testing method allows for repeatability of results by various laboratories. Another issue raised recently concerns the possibility to measure the possible production of new cell biomass or incorporating it into the humus that is not "measured" through the current testing methods.

An improved revised universal norm should be based on testing method(s) that include a well-documented range of several typical soil types and a well-defined range of conditions bracketing the majority of soil types and prevailing conditions, for a specific region. A basic requirement to characterise a product as "biodegradable product" is the necessary time for biodegradation. This is particularly important for agricultural applications where intentional incorporation in soil is the key motivation for the use of biodegradable products. Such a practice, if biodegradation rate is slow, may result into excessive accumulation of materials in the soil. The time use at the field will depend on the type of crop and on the farmer practices but it is common that the biodegradable plastics should have mineralised in the soil before the soil cultivation practices start for the next year crop. In addition, a range of grades for different biodegradation time under different latitudes or climates can be established, following the analogous procedure established by the standard for ageing of plastic films under different geographic areas (solar irradiance). For example in the French norm a set of grades is adopted to define the required biodegradation time of the different mulching films (*Briassoulis and Dejean, 2010*). In addition all relevant safety (e.g. heavy metals) and ecotoxicity requirements for soil biodegradable products should be met (as for example required by the French Norm, OK biodegradable SOIL).

Concerning bio-based lubricants, solvents etc, there is a need for an appropriate testing method that should be based on proper adaptation of testing methods for biodegradation of bio-based polymers in soil, combined with specifications and labeling analogous to those already available for biodegradable in soil plastics. Work in this direction will be performed in the framework of task 6.2 of KBBPPS and will be based on adaptation of existing standards for biodegradation in soil of biobased plastics.

Industrial composting environment

Many norms concerning testing of compostable plastics have been developed at national and international level. Some are about plastic materials others about products like packaging. The media and conditions of testing cover mainly the conditions designed for industrial composting facilities, and only a few concern home composting conditions (*Briassoulis et al. 2010)*. Also, only a few of the existing norms will be suitable, after appropriate revisions, to be adapted to testing biodegradable/compostable agricultural plastic products under farm composting conditions. Farm composting involves techniques not foreseen by the industrial or home composting methods. Farm composting is particularly relevant to biodegradation of biobased materials used in agriculture and specifications differ depending on the cultivations (e.g. organic farming requirements are more strict according to the relevant legislation).

The terminology and the biodegradability validation criteria under composting conditions, such as the threshold percentages of biodegradation and disintegration, the time and temperature, and the ecotoxicity, differ to some degree for the various norms and standard testing methods. Criteria for the establishment of a new integrative norm for compostable plastics used in agricultural applications need to be defined (*Briassoulis et al. 2010)*. Such a norm may include for example home composting and farm composting or only farm composting test methods and specifications where the last one may be based on the existing test methods adapted to practices and conditions of farm composting.

An industrial composting environment is probably not considered as an environment in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

2 Introduction

In this report the current biodegradation test methods in different environments (fresh water, marine environment, anaerobic environment, soil and compost) and the existing test procedures for evaluating environmental safety are reviewed. Existing difficulties and gaps in the current test methods are defined.

This review is focussed on the applicability of the test methods to bio-lubricants and bio-solvents. Consumers consider the “bio”-prefix often as a synonym of good for the environment. It should be avoided that the term “bio” refers to subjective non-measurable criteria (e.g. environmentally friendly, environmentally acceptable, etc.). Objective criteria should be selected with well-defined criteria. Currently, the prefix “bio” for lubricants and solvents is often associated with biodegradability and/or with the natural origin of the resources, but as long as no clear specifications exist, the term “bio” can be the source of misleading information and confusion for the final consumer (CEN/TR 16227).

In order to ensure transparent communication, specifications and labelling systems need to be developed. Standardised test methods form the cornerstones for specifications, which encompass well-defined pass criteria with regard to different characteristics (e.g. biodegradation, environmental safety, bio-based content, performance, etc.). These criteria are chosen in function of the objective of the specification. The specifications are designed to form the basic principle for labelling systems (Figure 1). Currently specifications, acceptance criteria and labelling systems are already clearly defined for compostable plastics and packaging, while this is only to a lesser degree the case for other environments (fresh water, marine environment, anaerobic environment and soil) and other products. Existing specifications and labelling systems for plastics, bio-lubricants and bio-solvents are reviewed and difficulties and gaps in the existing specifications are defined.

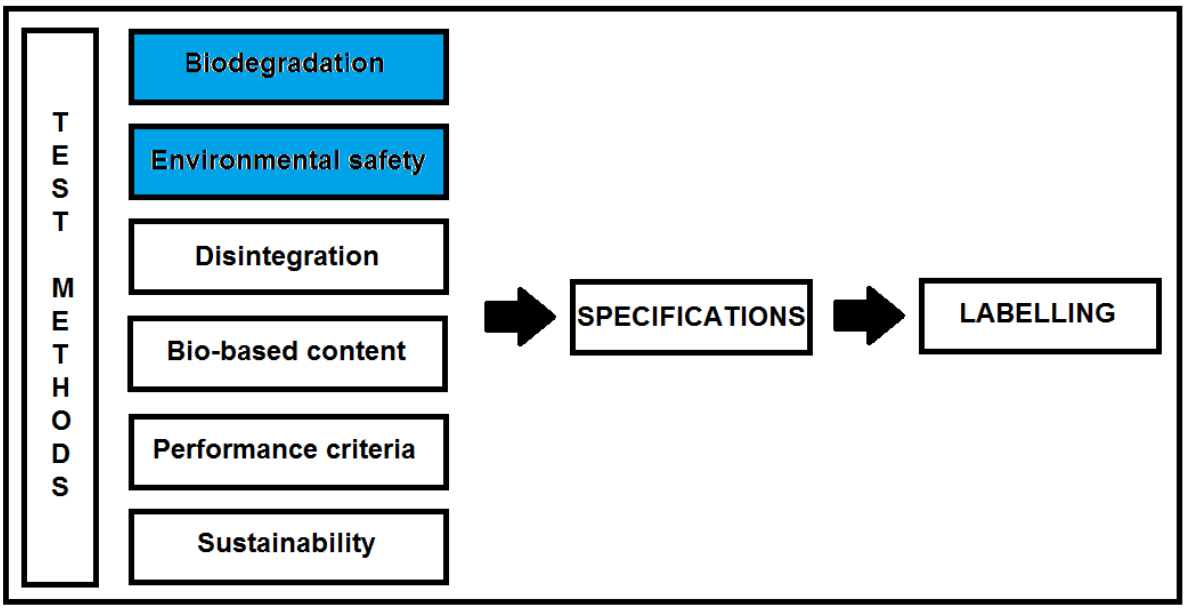


Figure 1. Relation between test methods, specifications and labelling systems.

3 Freshwater aerobic aqueous environment

The review of the freshwater aerobic aqueous environment has been executed by OWS.

3.1 Biodegradation

Biodegradation can be defined as the breakdown or mineralisation of an organic material due to microbial and/or fungal activity. The availability of oxygen determines to which molecules the organic carbon is converted. If complete biodegradation takes place under aerobic conditions the organic carbon of the material will be converted to carbon dioxide and water (Equation 1), while under anaerobic conditions biogas, which is a mixture of carbon dioxide and methane, will be formed (Equation 2). The organic carbon of the material is in both cases also partly converted to new biomass ($C_{biomass}$) and it is possible that some carbon is not converted or remains present under the form of metabolites ($C_{residual}$) (De Wilde (2013)).

$$C_{sample} + O_2 \rightarrow CO_2 + H_2O + C_{biomass} + C_{residual}$$

Equation 1. Biodegradation of carbon under aerobic conditions.

$$C_{sample} \rightarrow CO_2 + CH_4 + C_{biomass} + C_{residual}$$

Equation 2. Biodegradation of carbon under anaerobic conditions.

The biodegradation rate is strongly affected by the environment in which the biodegradation takes place. An environment is determined by the moisture content, the temperature, the type of micro-organisms, the density of micro-organisms, etc. Figure 2 shows a classification with regard to aggressiveness of the aerobic environments.

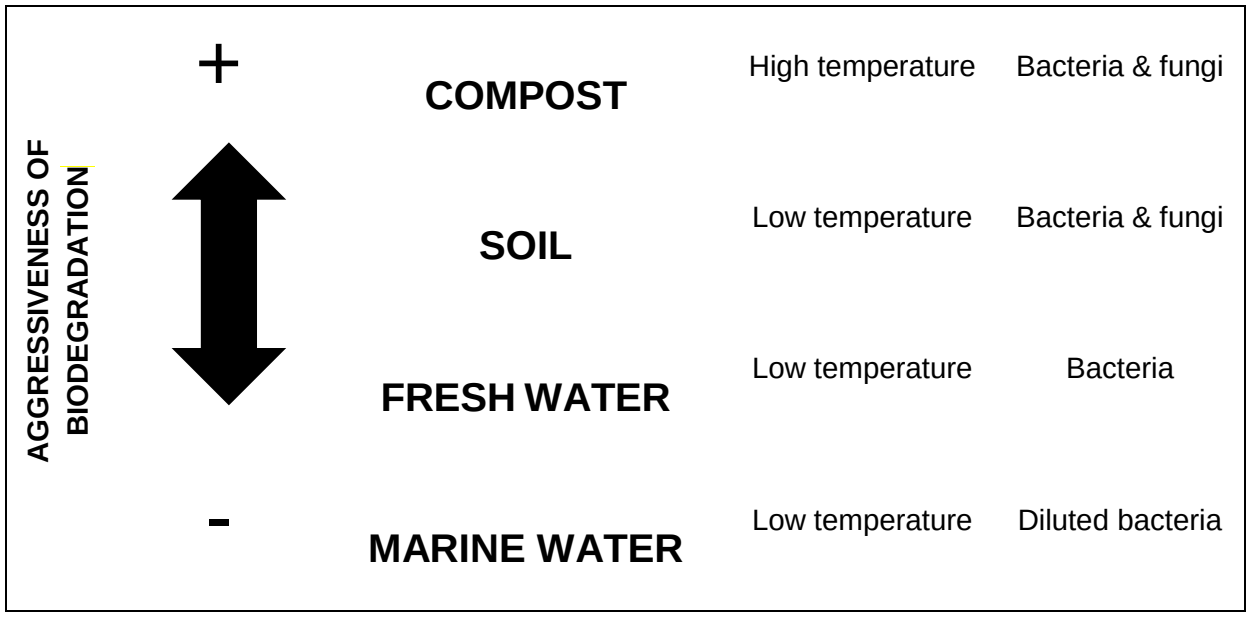


Figure 2. Relation between environment and aggressiveness of biodegradation.

Due to the differences with regard to aggressiveness between the environments and the typical nature of the milieu (solid ↔ aqueous), a biodegradability claim needs to be coupled to a specific well-defined environment. Therefore, test methods, which determine the biodegradability of a material, are always designed for a specific environment (fresh water, marine water, anaerobic conditions, soil or compost).

Biodegradation tests have their roots in the development of degradable synthetic surfactants in the mid-seventies. The first guidelines were developed by the Organization for Economic Cooperation and Development (OECD) in 1981. The OECD is an international organization in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. OECD has a leading role in the promotion of internationally acceptable methods for the testing of chemicals (industrial chemicals, pesticides, food additives, pharmaceuticals, etc.) for regulatory purposes. Afterwards also CEN (European committee for standardisation), ISO (International Organization for Standardization) and ASTM (American Society for Testing and Materials) developed biodegradability guidelines for organic chemicals and also for more complex materials (plastics, etc.). The major part of these standards is based on the principles of the OECD guidelines. Currently a trend towards internationalisation is observed: ISO standards are automatically transformed into national standards and the Vienna treaty guarantees that there exists an agreement between ISO and CEN standards (De Wilde (2005)).

Aerobic aquatic freshwater biodegradation tests form a prediction for the fate of chemicals in an aerobic aqueous environment, especially in the aerobic stages of wastewater treatment as the used inoculum is often activated sludge from a wastewater-treatment plant. Different parameters can be monitored during a test in order to determine the biodegradability of organic products:

- Dissolved Organic Carbon (DOC)
- Dissolved Oxygen (DO)
- Respirometry: carbon dioxide production
- Respirometry: oxygen consumption
- Inorganic Carbon (IC)

The choice of which method should be used for a given chemical depends largely on its physical properties (solubility, volatility and adsorptivity). DOC removal can be influenced by abiotic elimination, such as adsorption onto activated sludge or evaporation. Although the respirometric method based on CO_2 production gives the most direct evidence of oxidation of organic carbon during biodegradation, it can also underestimate the degree of biodegradation due to the fact that some carbon is converted to cell growth. Uptake of oxygen is an indirect measure for assessing biodegradability and such results can be misleading due to oxygen consumption caused by nitrification or caused by chemical reactions (e.g. oxidation of ethanol to acetic acid uses up oxygen but no carbon is removed, decarboxylation reactions cause the loss of carbon without the uptake of oxygen).

The biodegradation percentages based on CO_2 production and oxygen consumption are always lower when compared to biodegradation percentages based on DOC removal because the conversion of carbon to biomass. The percentage carbon used for biomass production varies between species of bacteria and between chemicals. Consequently biodegradation percentages based on CO_2 production and oxygen consumption will vary from test to test and from chemical to chemical (Painter, H.A. (1995)).

The major part of the standards are developed for “chemicals” or “organic compounds”, while a few standards are especially developed towards plastics and lubricants. No specific standards for solvents are currently available. The suitability of the measurement techniques towards the biodegradability determination of lubricants, which are often poorly soluble in water and consist of complex mixtures of chemicals, and solvents, which are often volatile, differs.

Especially methods based on DOC measurements are not suitable for non-soluble and volatile materials. Moreover tests which measure CO_2 production are done frequently in systems that are swept regularly with fresh, CO_2-free air to maintain the aerobic environment. Consequently, materials with a high vapour pressure, which are in the air space of the test medium will be removed (ASTM D 6006 (2011)). Therefore, volatile test compounds can only be tested in closed systems.

3.1.1 OECD guidelines

The OECD has developed several guidelines for testing of chemicals on biodegradation in an aerobic aqueous medium. As these guidelines are developed for chemicals (= a form of matter that has a constant chemical composition and which cannot be separated into components by physical separation methods (= without breaking chemical bonds)), these test methods are not always suitable in order to determine biodegradation of complex materials.

OECD does not provide explicit guidance with regard to biodegradability testing on poorly water-soluble substances (= substances with a water solubility < 100 mg/l). The only critical guidance provided is the applicability of a restricted range of analytical methods (OECD 301 B, C, D (±) and F and OECD 310) and the requirement of additional control vessels where emulsifiers, solvents and carriers are used. Whilst advocating the use of emulsifiers, solvents and carriers, none are specifically identified and no guidance is provided regarding the acceptable level. Consequently, numerous approaches of introducing the test substance can be applied, which make it difficult to identify a set of core acceptable or workable solutions (ECHA (2012)). For volatile substances analytical methods OECD 301 C (±), D and F (±) and OECD 310 are suitable (additional information: see chapter 3.1.1.1.).

OECD 301 and 310 are both designed in order to determine ready biodegradability, while OECD 302 determines inherent biodegradability. OECD 303 and 309 are both guidelines for simulation biodegradation tests in specific freshwater aquatic environments. An overview of the guidelines of OECD with regard to aerobic aqueous biodegradation is given in Table 1.

Table 1. Overview of the OECD guidelines with regard to biodegradation in aerobic aqueous environment.

Guideline	Adopted	Method	Description
OECD 301 Ready Biodegradability	Jul-17-1992	A	DOC Die-Away
		B	CO_2 Evolution (Modified Sturm Test)
		C	Modified MITI[1] Test (I)
		D	Closed Bottle
		E	Modified OECD Screening
		F	Manometric Respirometry
OECD 302 Inherent Biodegradability	May-12-1981	A	Modified SCAS Test
	Jul-17-1992	B	Zahn-Wellens / EMPA[2] Test
	May-12-1981	C	Modified MITI Test (II)
OECD 303 Simulation Biodegradation Test	Jan-22-2001	A	Activated Sludge Units
		B	Biofilms
OECD 309 Simulation Biodegradation Test	Apr-13-2004		Aerobic Mineralisation in Surface Water
OECD 310 Ready Biodegradability	Mar-23-2006		CO_2 in sealed vessels (Headspace Test)

[1] MITI = Ministry of International Trade and Industry, Japan.
[2] EMPA = Swiss Federal Laboratories for Materials Testing and Research.

The term ***"ready biodegradability"*** refers to an arbitrary classification of chemicals, which have passed certain specified screening tests for ultimate biodegradability. It is assumed that such substances will rapidly and completely biodegrade in aquatic environments under aerobic conditions. Ready biodegradability tests are no simulation tests, but tests for potential to biodegrade. Consequently using data from them to assess the performance of a substance within a particular route to the environment would be a misuse of the data and unwarranted extrapolation. The data of such tests state that chemicals passing the test do not offer a serious challenge to the metabolic capability of aerobic aquatic environments (given the presence of bacteria, nutrients, etc.) and that they would be readily degraded in the real environment (Painter, H.A. (1995)).

The duration of these tests is always 28 days, pre-exposure of the inoculum to the chemical is not allowed, the test substance is provided in a rather high concentration (2 to 100 mg/l) as the sole source of carbon for energy and growth and the amount of DOC in the test solution due to the inoculum should be kept as low as possible compared to the amount of DOC due to the test substance. The endogenous activity of the inoculum is corrected by running parallel blank tests with inoculum but without test substance.

The pass levels for ready biodegradability as prescribed by OECD 301 and OECD 310 are:

- 70 % removal of DOC
- Biodegradation > 60 % ThOD
- Biodegradation > 60 % $ThCO_2$
- Biodegradation > 60 % ThIC

The pass level is higher for methods based on the measurement of the residual sample (DOC), while the pass level is lower for methods, which are based on respirometric measurements. This is caused by the fact that the biodegradation percentage based on CO_2 production and oxygen consumption is always less than the percentage determined by DOC removal due to the bacterial metabolism. Some of the organic carbon of the test substance is biochemically oxidized and converted to CO_2, while other fractions are synthesized into new cellular material or into organic metabolic products. These fractions are not oxidised and do not contribute to the CO_2 production. The biomass formation is related to different factors (nature of test substance, bacterial species, etc.).

The pass levels need to be reached within a 10-d window, which starts when the degree of biodegradation has reached 10 % DOC, ThOD, $ThCO_2$ or ThIC and must end before day 28 of the test. If chemicals are classified as readily biodegradable, the need for other ecological parameters (e.g. bioaccumulation and ecotoxicity) may be reduced. As no complete biodegradation needs to be demonstrated, these pass levels (60 % or 70 %) for ready biodegradability are only reasonable for pure chemicals, but not necessarily for chemical mixtures or for chemicals containing significant proportions of impurities.

The term ***"inherent biodegradability"*** is less stringent when compared to ready biodegradability. The test procedures offer a higher chance of detecting biodegradation compared to tests for ready biodegradability. Inherent biodegradability refers to a classification of chemicals for which there is unequivocal evidence of biodegradation (primary

or ultimate) in any test of biodegradability. Such substances are considered not to be persistent and can be assumed to be degraded in the aquatic environment in the medium or long term (in wastewater treatment plants or in other environmental compartments). If an inherent test is negative, this could indicate the potential for persistence in the environment. In these tests the ratio biomass to food is shifted in favour of the biomass and the potential for adaptation is increased significantly.

OECD 302 A prescribes that test chemicals giving a result > 20 % loss of DOC may be regarded as inherently biodegradable, whereas a result > 70 % loss of DOC is evidence of ultimate biodegradability. No recommendations with regard to the interpretation of the results are given in guidelines OECD 302 B and OECD 302 C. The definitions mentioned in Annex B of ISO 7827 (2010) also mention that removal of 20 % DOC, consumption of 20 % ThOD or evolution of 20 % $ThCO_2$ in any degradation test, with or without pre-exposure of the inoculum, indicates that the test substance in question is inherently biodegradable and non-persistent.

A ***simulation test*** reproduces, in the laboratory, defined environmental conditions as well as possible and gives more extensive and better information on the degradation behaviour of substances under simulated environmental conditions. A representative and low concentration of test substance is used in tests designed to determine the biodegradation rate constant whereas higher concentration are for analytical reasons normally used for identification and quantification of major transformation products.

3.1.1.1 Ready biodegradability

A brief overview of the measurement techniques for ready biodegradability is given in Table 2. OECD 301 recommends method D (closed bottle) for volatile compounds, but also methods C (MITI) and F (manometric respirometry) can be suitable. Methods B (CO_2 evolution), C (MITI) and F (manometric respirometry) are recommended for poorly soluble compounds, but also method D (closed bottle) can be suitable. OECD 310 can also be used for poorly soluble test substances on condition that good dispersion can be ensured and for volatile substances with a Henry's law constant up to 50 $Pa.m^3.mol^{-1}$. Methods based on DOC measurements (A and E) can be affected by physico-chemical processes such as adsorption, volatilization, precipitation and hydrolysis. The method based on the determination of evolved CO_2 (B), can be affected by the formation of inorganic carbon.

Table 2. Overview of measurement techniques of ready biodegradability test methods (OECD).

Method	Measurement technique
301 A	Test item (10-40 mg DOC/l) in an inoculated mineral medium is aerated at 22°C ± 2°C. Degradation is followed by DOC analysis. Degree of biodegradation is calculated by expressing the concentration of DOC removed (after correction for blank).
301 B	Test item (10-20 mg DOC/l) in an inoculated mineral medium is aerated with CO_2 free air at 22°C ± 2°C. Degradation is followed by determining the CO_2 produced. The produced CO_2 is trapped in $Ba(OH)_2$ or NaOH and is measured by titration of the residual hydroxide or as inorganic C. Degree of biodegradation is calculated by expressing produced CO_2 (after correction for blank) as a percentage of $ThCO_2$.
301 C	Test item (100 mg/l) in a mineral medium inoculated with specially grown micro-organisms is stirred at 25°C ± 1°C. Evolved CO_2 is absorbed by soda lime and oxygen uptake is measured by an electrolytic BOD meter or respirometer. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD.
301 D	Test item (2-5 mg/l) in a mineral medium inoculated with relatively small number of micro-organisms is kept in full, closed bottles at 22°C ± 2°C. Degradation is followed by analysis of dissolved oxygen. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD.
301 E	Test item (10-40 mg DOC/l) in an inoculated (relatively low concentration of micro-organisms) mineral medium is aerated at 22°C ± 2°C. Degradation is followed by DOC analysis. Degree of biodegradation is calculated by expressing the concentration of DOC removed (after correction for blank).
301 F	Test item (100 mg/l) in an inoculated mineral medium is stirred at 22°C ± 2°C. Evolved CO_2 is absorbed by solution of KOH or other suitable absorbent and oxygen uptake is measured by a suitable respirometer. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD.
310	Test item (20 mg C/l) in an inoculated mineral medium is shaken in a sealed bottle with a headspace of air at 20°C ± 1°C. The CO_2 evolution is determined by measuring the IC produced in the test bottles in excess of that produced in the blank vessels. The IC is measured by (A) acidification to pH < 3 or (B) conversion of CO_2 from the headspace to carbonate. The extend of biodegradation is expressed as a percentage of ThIC (after correction for the blank).

An overview of the different inoculum sources as prescribed by OECD 301 and OECD 310 is given in Table 3. Pre-exposure (= pre-incubation of an inoculum in the presence of the test substance with the aim of enhancing the ability of the inoculum to degrade the test substance) is not allowed in order to determine ready biodegradability. OECD 310 gives more specifications with regard to the amount of colony-forming units (10^2 to 10^5 CFU per ml in the final mixture).

Table 3. Overview of inoculum sources of ready biodegradability test methods (OECD).

Source of inoculum	301 A	301 B	301 C	301 D	301 E	301 F	310
Activated sludge from aeration tank	X	X				X	X
Sewage effluent	X	X		X	X	X	X
Surface water	X	X		X		X	X
Soil extract	X	X				X	X
Mixture of inoculum from at least 10 sites, after at least 1 month in sludge unit			X				
Mixture of sources	X	X				X	X

The prescribed amount of the different series is given in Table 4. No exact amount of replicates is given for OECD 310 as the number of bottles depends on the frequency of analysis and the test duration. It is recommended that triplicate bottles need to be analysed at least weekly and at the end of the test also at least five bottles from the blank series, the reference series and the test series need to be analysed.

Table 4. Amount of replicates as prescribed by ready biodegradability test methods (OECD).

Method	Blank series	Reference series	Test series	Abiotic sterile control[3]	Adsorption control[4]	Toxicity control[5]
301 A	2	1	2	1 (Preferably)	1 (Preferably)	1 (Preferably)
301 B	2	1	2	1 (Preferably)	-	1 (Preferably)
301 C	1	1	3	1	-	-
301 D	10	10	10	-	-	6 (If needed)
301 E	2	1	2	1 (Preferably)	1 (Preferably)	1 (Preferably)
301 F	2	1	2	1 (Preferably)	-	1 (Preferably)
310	Min. 17	Min. 17	Min. 17	Min. 8 (If needed)	-	Min. 8 (If needed)

[3] Series containing test substance and sterilizing agent.
[4] Series containing test substance, inoculum and sterilizing agent.
[5] Series containing test substance, reference compound and inoculum.

In order to check ***the validity of the test procedure***, OECD 301 prescribes that a reference compound, which meets the criteria for ready biodegradability needs to be tested in parallel. OECD 301 proposes aniline, sodium acetate or sodium benzoate as reference materials (Figure 3). OECD 310 refers to aniline, sodium benzoate or ethylene glycol for water-soluble test substances and to 1-octanol for poorly soluble test substances (Figure 4). The validity criteria depend on the used method (Table 5), but OECD 301 prescribes that the test is only considered valid if the difference of extremes of replicate values of removal of test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is < 20 %.

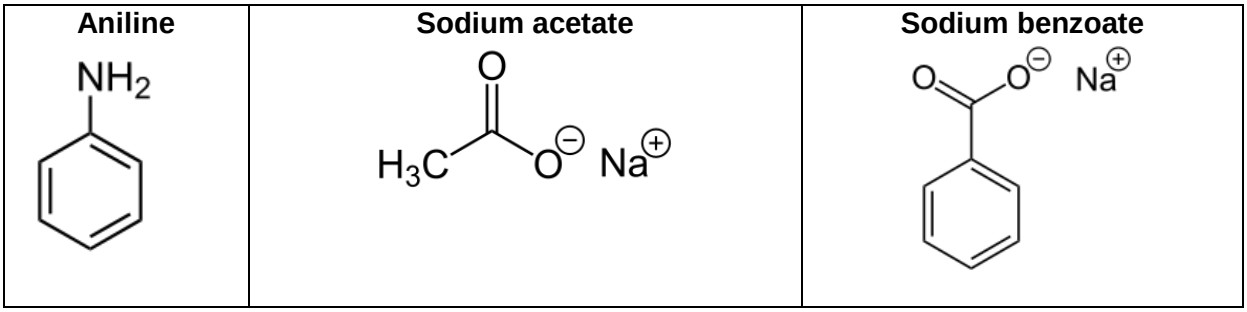


Figure 3. Structural formula of the reference compounds as described in OECD 301.

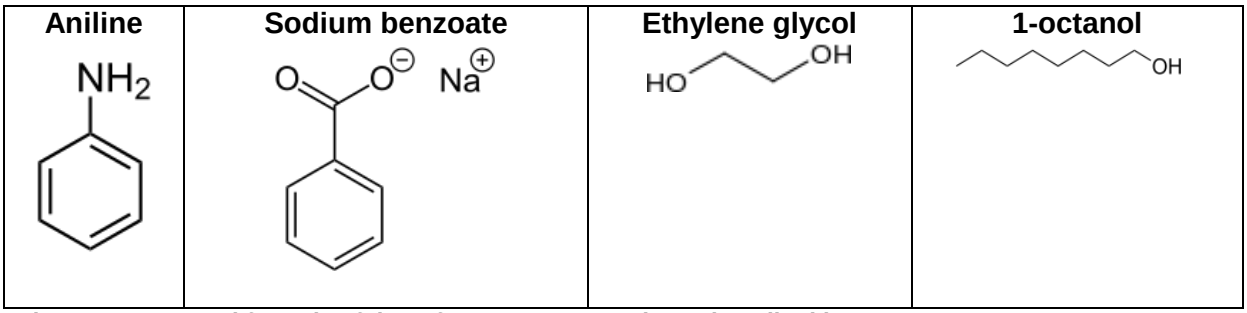


Figure 4. Structural formula of the reference compounds as described in OECD 310.

Table 5. Validity criteria as prescribed by ready biodegradability test methods (OECD).

Method	Validity criteria
301 A	Reference > 70 % DOC removal (after 14 days)
301 B	Reference > 60 % $ThCO_2$ (after 14 days) IC content of test substance suspension < 5 % TC (at start) CO_2 blank inoculum < 40 mg CO_2/l (certainly < 70 mg CO_2/l) (after 28 days)
301 C	Aniline > 40 % ThOD (after 7 days) & > 65 % ThOD (after 14 days) O_2 blank inoculum < 60 mg O_2/l (after 28 days)
301 D	Reference > 60 % ThOD (after 14 days) O_2 depletion in blank < 1.5 mg dissolved O_2/l (after 28 days) Residual O_2 in test bottles > 0.5 mg/l (during entire test)
301 E	Reference > 70 % DOC removal (after 14 days)
301 F	Reference > 60 % ThOD (after 14 days) O_2 blank inoculum < 60 mg O_2/l (after 28 days)
310	Reference > 60 % ThIC (after 14 days) TIC in the blank controls < 3 mg C/l (at end)

3.1.1.2 Inherent biodegradability

A brief overview of the measurements techniques for inherent biodegradability is given in Table 6, while Table 7 shows an overview of the inoculum sources. The minimum amount of replicates is given in Table 8. OECD 302 A "Inherent Biodegradability: Modified SCAS Test" is only applicable to test items, which are soluble in water (≥ 20 mg DOC/l) and have a negligible vapour pressure. Also OECD 302 B "Zahn-Wellens/EMPA Test" is also only applicable to non-volatile soluble organic test items (≥ 50 mg DOC/l). Consequently, these guidelines cannot be used in order to determine the inherent biodegradability of volatile substances or poorly water soluble substances. Test method OECD 302 C "Modified MITI Test (II)" is only applicable for chemicals with a negligible vapour pressure, but this method can be adapted for volatile chemicals using a modified BOD-meter. Moreover, this method can be applied for non-soluble test items.

Table 6.Overview of measurement techniques of inherent biodegradability test methods (OECD 302).

Method	Measurement technique
302 A	Test item (20 mg C/l) is exposed to high concentrations of micro-organisms over a long period (several months) in an aeration unit. Each day aeration is stopped, sludge is allowed to settle and supernatant liquor is removed. Remaining sludge is then again mixed with test item and sewage and the cycle is repeated. Biodegradation is followed by DOC measurements of the supernatant liquor.
302 B	Test item (50-400 mg DOC/l) is agitated and aerated in an inoculated (large amount of activated sludge) mineral medium at 20°C – 25°C. Degradation is followed by DOC analysis. Degree of biodegradation is calculated by expressing the concentration of DOC removed (after correction for blank).
302 C	Test item (30 mg/l) in a mineral medium inoculated with specially grown micro-organisms is stirred at 25°C ± 2°C. Evolved CO_2 is absorbed by soda lime and oxygen uptake is measured (BOD meter). Biodegradability is calculated on the basis of BOD and supplemental chemical analysis (DOC, concentration of residual chemicals, etc.).

Table 7. Overview of inoculum sources of inherent biodegradability test methods (OECD 302).

Source of inoculum	A	B	C
Activated sludge	X	X	
Mix of different sources (waste water treatment plants, soil extracts, river water, etc.)		X	
Mixture of inoculum from at least 10 sites (city sewage plant, industry sewage plant, rivers, lakes, marshes, sea,…), after at least 1 month in sludge unit[6]			X

Table 8. Amount of replicates as prescribed by inherent biodegradability test methods (OECD 302).

Method	Blank series	Reference series	Test series	Additional series
302 A	1	-	1	-
302 B	1 or 2	1	1 or 2	-
302 C	1	1	3	1 (water + chemical)

[6] In the sludge unit the inoculum is regulary fed with synthetic sewage containing glucose, peptones and monopotassium phosphate.

No specific reference substrates are recommended in OECD 302 A, but data on several compounds (4-acetyl amino-benzene sulphonate, tetra propylene benzene sulphonate, 4-nitrophenol, diethylene glycol, aniline and cyclopentane tetra carboxylate) used in ring tests are provided in order to calibrate the method from time to time and in order to permit comparison of results. In order to check the functional capability of the activated sludge, OECD 302 B prescribes that a reference compound of known biodegradability, should be run in parallel with each test series. Following reference materials are recommended: ethylene glycol, diethylene glycol, lauryl sulfonate and aniline (Figure 5). OECD 302 C refers to the same reference materials as prescribed by OECD 301 (aniline, sodium acetate or sodium benzoate) (Figure 3).

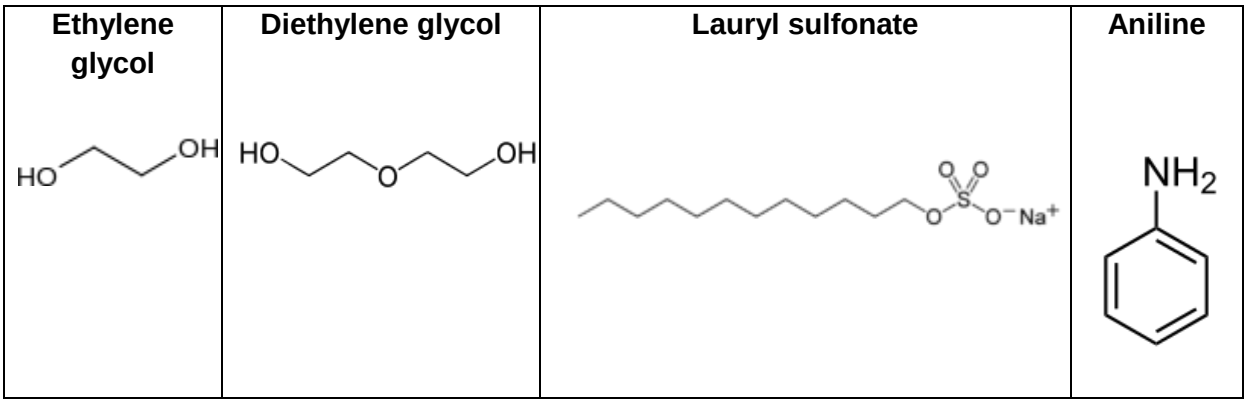


Figure 5. Structural formula of the reference compounds as described in OECD 302 B.

An overview of the validity criteria as prescribed by the OECD 302 guidelines is given in Table 9.

Table 9. Validity criteria as prescribed by inherent biodegradability test methods (OECD 302).

Method	Validity criteria
302 A	-
302 B	Reference > 70 % DOC removal (after 14 days)
302 C	Aniline > 40 % (after 7 days) & > 65 % (after 14 days) Recovery rate of test compounds in the blank tests with water only > 10 %

3.1.1.3 Simulation biodegradation tests

Guideline OECD 303 A simulates an activated sludge unit, while OECD 303 B simulates waste water treatment involving biofilms (percolating or trickling filters, rotation biological contactors, fluidised beds). Guideline OECD 309 simulates the biodegradation in surface water ("pelagic test") or in turbid surface water which, for example, might exist near a water/sediment interface ("suspended sediment test"). A brief overview of the measurements techniques is given in Table 10, while Table 11 shows an overview of the inoculum sources.

Table 10. Overview of measurement techniques of simulation biodegradability test methods (OECD).

Method	Measurement technique
303 A	Test item (10-20 mg DOC/l) is added together with an easily biodegradable organic medium (domestic sewage is recommended) to the influent of a continuously operating test system simulating an activated-sludge process (activated sludge plant model = Husmann apparatus or porous pot) with a mean hydraulic retention time (HRT) of 6 h and a mean sludge retention time (SRT) of 6 d to 10 d at 20°C – 25°C. Effluent samples are analysed for DOC. The difference between DOC values in effluent of test and control unit compared to the influent concentration is used to determine the degree of elimination of the test compound. Duration is 12 weeks.
303 B	Test item (10-20 mg C/l) is added together with an easily biodegradable organic medium (domestic sewage is recommended) to the internal surface of a slowly rotating tubular reactor with a residence time of 125 ± 12.5 sec. for the feed in a clean tube (maximum film: retention time ± 30 min) at 22°C ± 2°C. A layer of micro-organisms is built up on the internal surface. Effluent samples are analysed for DOC. The difference between DOC values in effluent of test and control unit compared to the influent concentration is used to determine the degree of elimination of the test compound. Duration is 12 weeks.
309	Test item (2 different concentration levels, which differ from each other by a factor of 5 to 10 & both < 100 µg/l & lowest concentration < 10 µg/l) is added to stirred natural surface water (= pelagic test) or to surface water with suspended solids/sediment of 0.01 to 1 g/l (= suspended sediment test) at field temperature or at a standard temperature of 20°C - 25°C and serves as secondary substrate (= first order biodegradation kinetics). The degradation is followed by the determination of the residual concentration of the test compound by radiotracer technique (= ^{14}C-labelling of most stable part of the molecule and liquid scintillation counting) or by chemical analysis. A secondary objective of the test is to obtain information on the primary degradation and the formation of major transformation products. Higher test concentrations may be used in this case and less stable parts of the molecule may be labelled. The duration of the test is normally 60 days, but the test may be extended up to 90 days.

Table 11. Overview of inoculum sources of simulation biodegradability test methods (OECD).

Source of inoculum	303 A	303 B	309
Activated sludge	X		
Effluent	X		
Surface water	X		X
Airborne inoculation		X	
Settled sewage		X	
Surface water & sediment			X

The prescribed amount of replicates is given in Table 12.

Table 12. Amount of replicates as prescribed by simulation biodegradability test methods (OECD).

Method	Blank series	Reference series	Test series	Sterile control[7]	Solvent control
303 A	1	1 (occasionally)	1	-	-
303 B	1	1 (occasionally)	1	-	-
309	1	2	(2 ×) (2 ×) 2[8]	1 or 2	2

OECD 303 A and OECD 303 B recommend both that it is useful to test occasionally substances (adipic acid, 2-phenyl phenol, 1-naphthol, diphenic acid, 1-naphthoic acid) (Figure 6) whose behaviour is known simultaneously when test substances are investigated. In order to ensure that the microbial activity of the test water is within certain limits (= water contains an active microbial population), OECD 309 prescribes that a substance, which is easily degraded under aerobic conditions, should be used as reference substrate (aniline or sodium benzoate) (Figure 3).

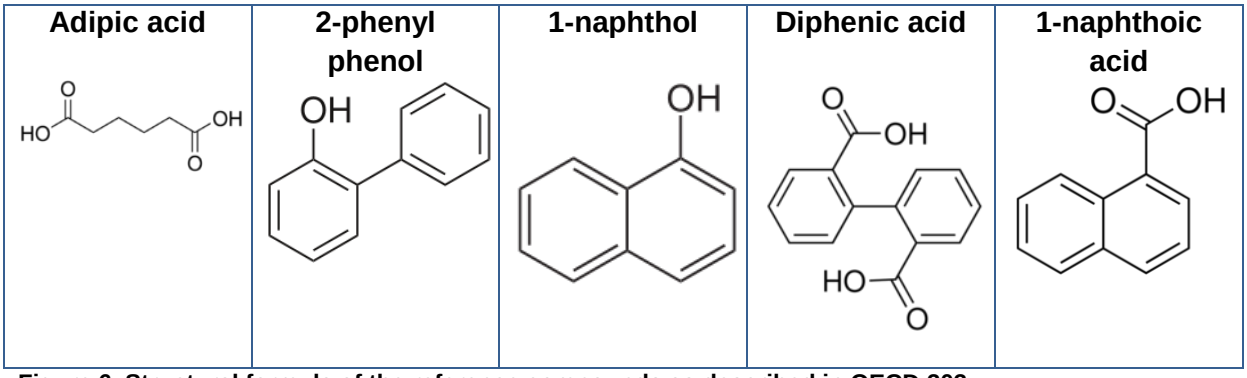


Figure 6. Structural formula of the reference compounds as described in OECD 303.

An overview of the validity criteria is given in Table 13.

Table 13. Validity criteria as prescribed by simulation biodegradability test methods (OECD).

Method	Validity criteria
303 A	DOC (or COD) degradation in control units > 80 % (after 2 weeks) No unusual observations Biodegradation of readily biodegradable reference substances > 90 % Ammonia-N < 1 mg/l & nitrite-N < 2 mg/l (test under nitrifying conditions)
303 B	DOC (or COD) degradation in control units > 80 % (after 2 weeks) No unusual observations Biodegradation of readily biodegradable reference substances > 90 % Difference between duplicate values < 5 %
309	Reference substrate needs to degrade sufficiently Total recovery at end: 90 % - 110 % (radiolabelled test items) Initial recovery at start: 70 % - 110 % (non-labelled test items)

[7] Series containing sterilized test water for examining abiotic degradation.
[8] Two replicates for each test concentration and also two replicates for each concentration for mass balance calculation.

3.1.2 European standards

For organic compounds and plastic materials CEN refers to existing ISO standards. CEN has developed 2 standards for packaging materials. EN 14047 & EN 14048 mention that biodegradability of packaging materials and its constituents must be determined in line with ISO 14852 and ISO 14851, respectively. An overview of the standards is given in Table 14.

Table 14. Overview of the EN standards with regard to biodegradation in aerobic aqueous environment.

Standard	Description
EN ISO 7827 (1995)	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of **organic compounds** – Method by analysis of dissolved organic carbon (DOC) (ISO 7827:1994)
EN ISO 9408 (1999)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium by determination of oxygen demand in a closed respirometer (ISO 9408:1999)
EN ISO 9439 (2000)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Carbon dioxide evolution test (ISO 9439:1999)
EN ISO 9887 (1994)	Water quality – Evaluation of the aerobic biodegradability of **organic compounds** in an aqueous medium – Semi-continuous activated sludge method (SCAS) (ISO 9887:1992)
EN ISO 9888 (1999)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Static test (Zahn-Wellens method) (ISO 9888:1999)
EN ISO 10634 (1995)	Water quality – Guidance for the preparation and treatment of **poorly water-soluble organic compounds** for the subsequent evaluation of their biodegradability in an aqueous medium (ISO 10634:1995)
EN ISO 10707 (1997)	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of **organic compounds** – Method by analysis of biochemical oxygen demand (closed bottle test) (ISO 10707:1994)
EN ISO 11733 (2004)	Water quality – Determination of the elimination and biodegradability of **organic compounds** in an aqueous medium – Activated sludge simulation test (ISO 11733:2004)
EN ISO 14593 (2005)	Water quality – Evaluation of the ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test) (ISO 14593:1999)
EN ISO 14851 (2004)	Determination of the ultimate aerobic biodegradability of **plastic materials** in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer (ISO 14851:1999)
EN ISO 14852 (2004)	Determination of the ultimate aerobic biodegradability of **plastic materials** in an aqueous medium – Method by analysis of evolved carbon dioxide (ISO 14852:1999)
CEN ISO/TR 15462 (2009)	Water quality – Selection of tests for biodegradability (ISO/TR 15462:2006)
EN 14047 (2002)	Packaging – Determination of the ultimate aerobic biodegradability of **packaging materials** in an aqueous medium – Method by analysis of evolved carbon dioxide
EN 14048 (2002)	Packaging – Determination of the ultimate aerobic biodegradability of **packaging materials** in an aqueous medium – Method by measuring oxygen demand in a closed respirometer

3.1.3 International standards

So far ISO has not developed specific standards with regard to the aerobic biodegradation in an aqueous medium for solvents or lubricants. In 1995 ISO concluded that the development of a single method for evaluating the biodegradability of poorly water-soluble organic substances might not be realized in the near future. Therefore, ISO proposed a series of methods where the final selection was based on a judgement of the physic-chemical properties of the test substance (ECHA (2012)). These preparation methods are described in ISO 10634 (1995) "Water quality – Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium". Four preparation techniques are described in this standard:

- Direct addition
    a. Test component is weighted and direct addition
    b. Using an inert support (for example: microscope slides)
    c. Using a volatile solvent, which is removed prior to testing
- Ultrasonic dispersion
- Adsorption on an inert support (for example: silica gel or glass fibre filters)
- Dispersions or emulsions with an emulsifying agent

After the pre-treatment, the biodegradation in an aerobic aqueous medium can be tested using the standard methods, but methods based on DOC measurements are normally not suitable.

ISO has developed different test methods in order to determine the biodegradability of organic compounds and plastics in an aerobic aqueous environment (Table 15). These standards are mainly based on the principles of the OECD guidelines, but the international standards are often more precise and clearer when compared to the OECD guidelines. The major part of the international standards are related to the determination of the biodegradability of organic compounds in an aqueous medium. Only 2 standards are related to the biodegradability of plastic materials (ISO 14851 and ISO 14852). These standards (ISO 14851 and ISO 14852) are based on the same principles as ISO 9439 and ISO 9408, respectively.

The international biodegradability standards are based on the same principles, but due to differences in microbial density, test item concentration and test duration, these standards have not all an equal biodegradation potential.

Test method ISO 14592 is characterised by low microbial densities and very low test item concentrations (< 200 µg/l) as this method is developed in order to evaluate the biodegradation of substances at low environmentally realistic concentrations in the aquatic environment. ISO 14592-1 is a batch test simulating standing water bodies (lakes or ponds), while ISO 14592-2 is a dynamic test simulating flowing waters (rivers).

ISO 10707 (closed bottle test) is also characterised by a rather low microbial density and a low test item concentration (2 mg/l). Moreover these vessels are not stirred nor aerated. Therefore, the biodegradation potential of this method is also rather low. This method is especially developed for volatile and inhibitory test compounds.

Test methods ISO 7827 (DOC), ISO 9408 (oxygen consumption), ISO 9439 (CO_2 production), ISO 10708 (two-phase closed bottle) and ISO 14593 (CO_2 headspace test) are all characterised by a higher biodegradation potential when compared to ISO 14592 and ISO 10707 as the microbial density and the test item concentration are considerably higher. These guidelines are comparable to the OECD guidelines for ready biodegradability (OECD 301 & OECD 310). In spite of the fact that the biodegradation potential of these methods should be rather comparable, it is still possible that different results are obtained. This can be caused by differences in measurement techniques.

Test methods ISO 9887 (SCAS test) and ISO 9888 (Zahn-Wellens test) have a high inoculum concentration and may be extended further than the usual 28 days. The conditions as prescribed in these methods are optimal in order to allow the maximum biodegradation value of a test item. These methods can be used to determine the intrinsic biodegradation of chemicals, which corresponds the OECD guidelines for inherent biodegradability.

ISO 11733 (activated sludge simulation test) is characterised by the highest inoculum concentration, as this continuous test simulates a wastewater treatment plant including nitrification and denitrification techniques.

Table 15. Overview of the ISO standards with regard to biodegradation in aerobic aqueous environment.

Standard	Description
ISO 7827 (2010)	Water quality – Evaluation of the "ready", "ultimate" aerobic biodegradability of **organic compounds** in an aqueous medium – Method by analysis of dissolved organic carbon (DOC)
ISO 9408 (1999)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium by determination of oxygen demand in a closed respirometer
ISO 9439 (1999)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Carbon dioxide evolution test
ISO 9887 (1992)	Water quality – Evaluation of the aerobic biodegradability of **organic compounds** in an aqueous medium – Semi-continuous activated sludge method (SCAS)
ISO 9888 (1999)	Water quality – Evaluation of ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Static test (Zahn-Wellens method)
ISO 10707 (1994)	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of **organic compounds** – Method by analysis of biochemical oxygen demand (closed bottle test)
ISO 10708 (1997)	Water quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of **organic compounds** – Determination of biochemical oxygen demand in a two-phase closed bottle test
ISO 11733 (2004)	Water quality – Determination of the elimination and biodegradability of **organic compounds** in an aqueous medium – Activated sludge simulation test
ISO 14592-1 (2002)	Water quality – Evaluation of the aerobic biodegradability of **organic compounds** at low concentrations – Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions
ISO 14592-2 (2002)	Water quality – Evaluation of the aerobic biodegradability of **organic compounds** at low concentrations – Part 2: Continuous flow river model with attached biomass
ISO 14593 (1999)	Water quality – Evaluation of the ultimate aerobic biodegradability of **organic compounds** in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
ISO 14851 (1999)	Determination of the ultimate aerobic biodegradability of **plastic materials** in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer
ISO 14852 (1999)	Determination of the ultimate aerobic biodegradability of **plastic materials** in an aqueous medium – Method by analysis of evolved carbon dioxide

3.1.3.1 International standards with regard to organic compounds

A brief overview of the measurement techniques is given in Table 16.

Table 16. Overview of measurement techniques of biodegradability test methods for organic compounds (ISO).

ISO method	Measurement technique	Comparable guidelines
7827	Test item (10-40 mg DOC/l) in an inoculated mineral medium is aerated at 22°C ± 2°C. Degradation is followed by DOC analysis. Degree of biodegradation is calculated by expressing the concentration of DOC removed (after correction for blank). Duration is normally 28 days or longer if necessary.	OECD 301 A
9408	Test item (at least 100 mg ThOD/l) in an inoculated mineral medium is stirred at 20°C – 25°C. Evolved CO_2 is absorbed by soda lime or KOH or another suitable absorbent. Oxygen uptake is measured by a suitable respirometer. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD. Duration is normally 28 days or longer if necessary.	OECD 301 F ISO 14851
9439	Test item (10-40 mg organic carbon/l) in an inoculated mineral medium is aerated with CO_2 free air at 20°C – 25°C. Degradation is followed by determining the CO_2 produced. The produced CO_2 is trapped in external vessels and is measured by DIC analyser or by titration after absorption in an alkaline solution. Degree of biodegradation is calculated by expressing produced CO_2 (after correction for blank) as a percentage of $ThCO_2$. Duration is normally 28 days or longer if necessary.	OECD 301 B ISO 14852
9887	Test item (20 mg DOC/l) is exposed to high concentrations of micro-organisms (1 g/l to 4 g/l suspended solids) in an aeration (SCAS) unit at 20°C – 25°C. These conditions are highly favourable for adaptation and extensive biodegradation. At least 3 times per week the aeration is stopped, the sludge is allowed to settle (30 min) and supernatant liquor is removed. Remaining sludge is than again mixed with test compound and sewage and the cycle is repeated. Biodegradation is established by determination of DOC content of the supernatant liquor. Duration varies between 12 weeks to 26 weeks.	OECD 302 A
9888	Test item (50-400 mg DOC/l) in an inoculated (0.2 g suspended solids/l final mixture for 50 mg DOC/l – 1 g suspended solids/l final mixture for 400 mg DOC/l) mineral medium is aerated at 20°C – 25°C. These conditions with a high inoculum concentration are optimal for allowing the maximum value of biodegradation. Degree of biodegradation is calculated by expressing the concentration of DOC removed. Duration is normally 28 days.	OECD 302 B
10707	Test item (2 mg/l) in mineral medium is inoculated with relatively small number of micro-organisms and kept in closed bottles at 20°C - 25°C. The bottles are completely filled. Degradation is followed by analysis of DO. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD. Duration is 28 days.	OECD 301 D ISO 10708

ISO method	Measurement technique	Comparable guidelines
10708	Test item (100 mg ThOD/l) in mineral medium inoculated with stabilised inoculum is stirred in closed bottles, containing known volumes of medium and air, at 20°C - 25°C. Degradation is followed by analysis of DO concentration. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD. Duration is normally 28 days or longer if necessary.	
11733	Test item (10-20 mg DOC/l) is added together with an easily biodegradable organic medium to the influent of a continuously operating test system simulating an activated-sludge process (activated sludge plant = Husmann apparatus or porous pot) with a mean HRT of 6 h and a mean SRT of 6 d to 10 d at 20°C – 25°C. Effluent samples are analysed for DOC. The difference between DOC values in effluent of test and control unit compared to the influent concentration is used to determine the degree of elimination of the test compound. Duration is 12 weeks.	OECD 303A
14592-1	Test item (2 different concentration levels < 100 µg/l) is added to stirred surface water (= pelagic test) or to surface water and sediment at 100 mg/l to 1000 mg/l (= suspended sediment test) at field temperature or at a temperature of 20°C to 25°C and serves as secondary substrate (= first order biodegradation kinetics). The degradation is followed by the determination of the residual concentration of the test compound by radiotracer technique (= ^{14}C-labelling of the most stable part of the molecule and liquid scintillation counting) or by chemical analysis.	OECD 309
14592-2	Test item (< 200 µg/l) is added to tap water or surface water, which might be inoculated at the beginning of the water dosage, in a cascade system, each usually containing 7 trays in the form of an aquatic staircase model. Each tray is filled which glass beads, which serve as an artificial sediment to support biofilm growth. The test system simulates the conditions of a dynamic aerobic surface water system (= river). The test item serves as secondary substrate (= first order biodegradation kinetics). The difference between the inlet and outlet concentrations of the cascade is used to determine the degree of biodegradation. The test item concentration is determined by radiotracer technique (= ^{14}C-labelling of the most stable part of the molecule and liquid scintillation counting) or by chemical analysis. The maximum test duration is 8 weeks.	OECD 303B
14593	Test item (2-40 mg C/l) is incubated in a inoculated mineral medium in a sealed bottle with a headspace of air at 20°C - 25°C. The CO_2 evolution is determined by measuring the IC produced in the test bottles in excess of that produced in the blank vessels. The IC is measured by (A) acidification to pH < 3 or (B) conversion of CO_2 from the headspace to carbonate. The extent of biodegradation is expressed as a percentage of ThIC (after correction for the blank). The duration is normally 28 days or longer if necessary.	OECD 310

An overview of the different inoculum sources as prescribed by the international standards related to aerobic aqueous biodegradation of organic compounds is given in Table 17. ISO prescribes that normally no pre-exposed inoculum should be used, especially in the case of standard tests simulating biodegradation behaviour in natural environments. Depending on the purpose of a test, a pre-exposed inoculum may be used, provided this is clearly stated in the test report (% biodegradation = x % using pre-exposed inocula) and the method of pre-exposure needs to be clarified. ISO 7827, ISO 9408, ISO 9439 and ISO 10707 mention that approximately 10^3 to 10^6 colony-forming units per millilitre should be present in the final mixture. ISO 10708 refers to an inoculum, which gives between 10^4 and 10^8 active cells per millilitre. ISO 14593 recommends 10^2 to 10^5 colony forming units per millilitre in the final mixture. The prescribed amount of replicates for the different test methods is given in Table 18.

Table 17. Overview of inoculum sources of biodegradability test methods for organic compounds (ISO).

Source of inoculum	7827	9408	9439	9887	9888	10707	10708	11733	14592-1	14592-2	14593
Activated sludge from aeration tank	X	X	X	X	X		X	X			X
Waste water (influent or effluent)		X	X								X
Surface water	X	X	X			X	X		X	X	X
Secondary effluent	X					X	X	X		X	
Surface water & sediment									X		
Soil extract										X	
Mixture of sources	X	X	X			X	X			X	X

Table 18. Amount of replicates as prescribed by biodegradability test methods for organic compounds (ISO).

ISO method	Blank series	Reference series	Test series	Abiotic sterile control[9]	Toxicity control[10]
7827	2	1	2	1 (if needed)	1 (if needed)
9408	2	1	2	1 (if needed)	1 (if needed)
9439	2	1	2	1 (if needed)	1 (if needed)
9887	1	1 (optionally)	1	-	-
9888	1	1	1	1 (if needed)	-
10707	10	10	10	-	6 (if needed)
10708	3	3	3	1 (if needed)	1 (if needed)
11733	1	-	1	-	-
14592-1	1	2 (optionally)	(2 ×)[11] 2	1 (optionally)	-
14592-2	-	1 (optionally)	1	-	-
14593[12]	> 9	> 9	> 9	> 6 (if needed)	> 6 (if needed)

[9] Series containing test substance and sterilizing agent.
[10] Series containing test substance, reference compound and inoculum.
[11] Two replicates for each test concentration.
[12] Number of vessels will depend on the frequency of analysis and the confidence limits required for the final extent of biodegradation. At least 5 vessels are required at the end of the test.

In order to check ***the validity of the test procedure***, the most international standards prescribe that a reference compound of known biodegradability needs to be tested in parallel. ISO 9408, ISO 9439 and ISO 14593 propose aniline or sodium benzoate as reference compound, while ISO 7827, ISO 10707 and ISO 10708 propose aniline, sodium acetate or sodium benzoate. ISO 14592-1 and ISO 14592-2 only refer to aniline. No reference compound is mentioned in ISO 9887 and ISO 11733. ISO 9888 refers to diethylene glycol, ethylene glycol, sodium benzoate or aniline as suitable reference materials. The validity criteria depend on the used method (Table 19).

Table 19. Validity criteria as prescribed by biodegradability test methods for organic compounds (ISO).

ISO method	Validity criteria
7827	Difference between degradation values of replicates of test item at end < 20 % DOC removal Reference > 70 % DOC removal (after 14 days) DOC contributed by the inoculum < 10 % contributed by test compound
9408	Reference > 60 % ThOD (after 14 days) BOD blank inoculum < 60 mg O_2/l (after 28 days)
9439	Reference > 60 % ThCO_2 (after 14 days) DIC at start < 5 % organic carbon of the test item CO_2 blank inoculum < 70 mg CO_2/l (at end)
9887	-
9888	Reference > 70 % DOC removal (after 14 days)
10707	O_2 uptake in blank bottles < 1.5 mg O_2/l (after 28 days) Residual oxygen concentration test bottles > 0.5 mg/l (any time) Difference between extremes degradation values at end < 20 % Reference > 60 % ThOD (after 14 days)
10708	Difference between extremes degradation values at end < 20 % Reference > 60 % ThOD (after 14 days) O_2 uptake in blank bottles < 3 mg O_2/l per week (week 1) O_2 uptake in blank bottles < 1 mg O_2/l per week (following weeks)
11733	Degree of DOC (or COD) degradation of the control units > 80% (after 2 weeks) No unusual observations
14592-1	Reference substrate needs to degrade sufficiently within the expected time interval (rate constants aniline: 0.3 d^{-1} – 1.7 d^{-1})
14592-2	Biodegradation reference substrate > 90% (after 14 days)
14593	Reference > 60 % ThIC (after 14 days) TIC in the blank controls at end ≤ 15% organic carbon added initially as test compound

3.1.3.2 International standards with regard to plastics

A brief overview of the measurements techniques is given in Table 20. The main difference with the methods for organic compounds (ISO 9408 and ISO 9439) is (1) the higher test concentration for plastic materials, (2) the longer maximum duration and (3) the possibility to use compost extract as inoculum.

Table 20. Overview of measurement techniques of biodegradability test methods for plastic materials (ISO).

ISO method	Measurement technique	Comparable guidelines
14851	Test item (100 mg/l or 170 mg ThOD/l) in an inoculated mineral medium is stirred at 20°C – 25°C. Evolved CO_2 is absorbed in a suitable absorber. The consumption of oxygen (BOD) is determined by measuring the amount of oxygen required to maintain a constant volume of gas in the respirometer flasks or by measuring the change in volume of pressure in the flasks. Degree of biodegradation is calculated by expressing consumed oxygen (after correction for blank and correction for nitrification) as a percentage of ThOD. Maximum duration is 6 months.	OECD 301 F ISO 9408
14852	Test item (100 mg TOC/l at start) in an inoculated mineral medium is aerated with CO_2 free air at 20°C – 25°C and degradation is followed by the determination of the CO_2 produced. The produced CO_2 is trapped in external vessels and is measured by DIC analyser or by titration after absorption in an alkaline solution. Degree of biodegradation is calculated by expressing produced CO_2 (after correction for blank) as a percentage of ThCO_2. Maximum duration is 6 months.	OECD 301 B ISO 9439

An overview of the different inoculum sources as prescribed by ISO 14851 and ISO 14852 is given in Table 21. ISO prescribes that normally no pre-exposed inoculum should be used, especially in the case of standard tests simulating biodegradation behaviour in natural environments. Depending on the purpose of a test, a pre-exposed inoculum may also be used, provided this is clearly stated in the test report (% biodegradation = x % using pre-exposed inocula) and the method of pre-exposure needs to be clarified. Both standards recommend that the test mixture should preferably contain about 10^3 - 10^6 CFU/ml. The tests are normally executed at a constant temperature between 20°C and 25°C, but it is mentioned that higher temperatures might be appropriate when compost is used as inoculum. Higher test item concentrations (up to 2000 mg/l of organic carbon) can also be tested in both methods on condition that the optimised test medium is used. The optimized medium is highly buffered and contains more inorganic nutrients.

Table 21. Overview of inoculum sources of biodegradability test methods for plastic materials (ISO).

Source of inoculum	ISO 14851	ISO 14852
Activated sludge from aeration tank	X	X
Compost extract	X	X
Soil extract	X	X
Mixture of sources	X	X

The number of replicates in order to execute the test is identical in both standards (Table 22).

Table 22. Amount of replicates as prescribed by biodegradability test methods for plastic materials (ISO).

Method	Blank series	Reference series	Test series	Abiotic sterile control	Negative control	Toxicity control
ISO 14851	2	1	2	1 (optionally)	1 (optionally)	1 (optionally)
ISO 14852	2	1	2	1 (optionally)	1 (optionally)	1 (optionally)

In order to check ***the validity of the test procedure***, ISO 14851 and ISO 14852 prescribe that a reference compound needs to be tested in parallel. These standards propose the organic compound aniline and also the biodegradable polymers microcrystalline cellulose powder, ashless cellulose filters and poly-β-hydroxybutyrate as reference materials (Figure 7). As these standards are both based on respirometric methods, they require that the degree of biodegradation of the reference material is at least 60% at the end of the test.

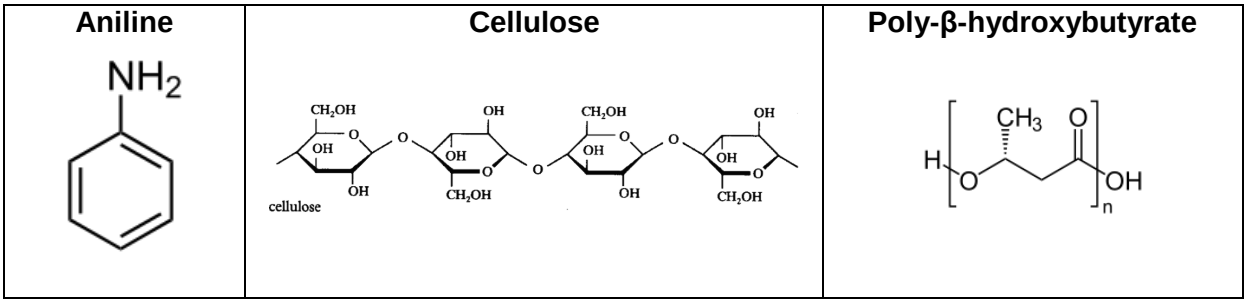


Figure 7. Structural formula of the reference compounds as described in ISO 14851 (1999) and ISO 14852 (1999).

Next to the validation criterion, which is related to the biodegradation of the reference material, also a validation criteria, which is related to the background activity of the blank is given. ISO 14851 prescribes that the BOD of the blank may not exceed 60 mg/l at the end of the test (in case of 30 mg/l dry matter), while ISO 14852 prescribes that the maximum amount of evolved carbon dioxide from the blank may not exceed 90 mg/l (in case of 30 mg/l dry matter).

ISO 14851 and ISO 14852 mention both that the determination of the BOD (inclusive the correction for nitrification) and the determination of the evolved CO_2, respectively, are sometimes not enough in order to characterise the biodegradability of test materials with complex compositions. During a biodegradation test with a long duration the carbon in the test material is partly transformed to biomass but not biochemically oxidized. In this case, the measurements will not reach 100 % of the theoretical values even in case of complete biodegradation of the test material. In order to confirm complete biodegradability, a carbon balance can be determined. The total carbon sum is based on 5 measurements: (1) carbon evolved as CO_2, (2) carbon produced as biomass, (3) carbon transformed into water-soluble organic metabolites, (4) carbon determined as DOC and (5) carbon remaining in the undegraded polymer material.

3.1.4 American standards

ASTM had developed standards for different product categories: (1) lubricants, (2) organic compounds and (3) plastics. No specific standards towards solvents are developed yet.

3.1.4.1 American standards with regard to lubricants

ASTM has developed 3 standard test methods for the determination of the aerobic aquatic biodegradation of lubricants and 1 standard test method for predicting the biodegradability of liquid-based lubricants using a bio-kinetic model (Table 23). Standard test methods ASTM D 5864 and ASTM D 6139 are not designed for volatile lubricants or lubricants components, while standard test method ASTM D 6731 is suitable for evaluating the biodegradation of non-volatile as well as volatile lubricants.

Table 23. Overview of the ASTM standards with regard to biodegradation in aerobic aqueous environment of lubricants.

Standard	Description
D 5864 - 11	Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components
D 6139 - 11	Standard Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants or Their Components Using the Gledhill Shake Flask
D 6731 - 01	Standard Test Method for Determining the Aerobic, Aquatic Biodegradability of Lubricants or Lubricant Components in a Closed Respirometer
D 7373 - 12	Standard Test Method for Predicting Biodegradability of Lubricants Using a Bio-kinetic Model

A brief overview of the different measurements techniques is given in Table 24.

Table 24. Overview of measurement techniques of biodegradability test methods for lubricants (ASTM).

ASTM method	Measurement technique
D 5864	Produced CO_2 is captured in $Ba(OH)_2$ and amount of absorbed CO_2 is determined by titration
D 6139	Produced CO_2 is captured in $Ba(OH)_2$ or other alkaline solution and amount of absorbed CO_2 is determined by titration
D 6731	Oxygen consumption is determined by measuring the amount of O_2 required to maintain a constant gas volume or by measuring the change in volume or pressure

As these methods are the only available biodegradation standard test methods for lubricants, there principles are described in detail below.

Standard test method ***ASTM D 5864*** is composed of (1) a CO_2 scrubbing apparatus, which consists of five 1 l plastic bottles containing 10 M NaOH, an empty Erlenmeyer flask (to prevent liquid carryover), one 1 l Erlenmeyer flask containing 0.0125 M $Ba(OH)_2$ and finally again an empty Erlenmeyer flask, (2) an incubation/biodegradation apparatus, which consists of a 4 l Erlenmeyer flask, and (3) a CO_2 trapping apparatus, which consists of three bottles containing 0.0125 M $Ba(OH)_2$ (Figure 8).

The CO_2 scrubbing apparatus produces CO_2 free air, which is used to aerate continuously the incubation apparatus. The incubation apparatus is filled with mineral medium, which contains ammonium sulphate solution, calcium chloride solution, ferric chloride solution, magnesium sulphate solution and a phosphate buffer, and 1 % activated sludge supernatant to give 30 mg/l suspended solids in the final volume of 3 l. These flasks are agitated with a magnetic stirrer. No additional carbon source is added to the blank reactors, while the reference item and the test item are added in a concentration of 10-20 mg C/l to the control reactors and the test reactors, respectively. The produced CO_2 reacts with $Ba(OH)_2$ and precipitates as $BaCO_3$ in the CO_2 trapping apparatus. The bottles of the CO_2 trapping apparatus are titrated on a regular basis. The amount of CO_2 produced is determined by titrating the remaining $Ba(OH)_2$ with hydrochloric acid.

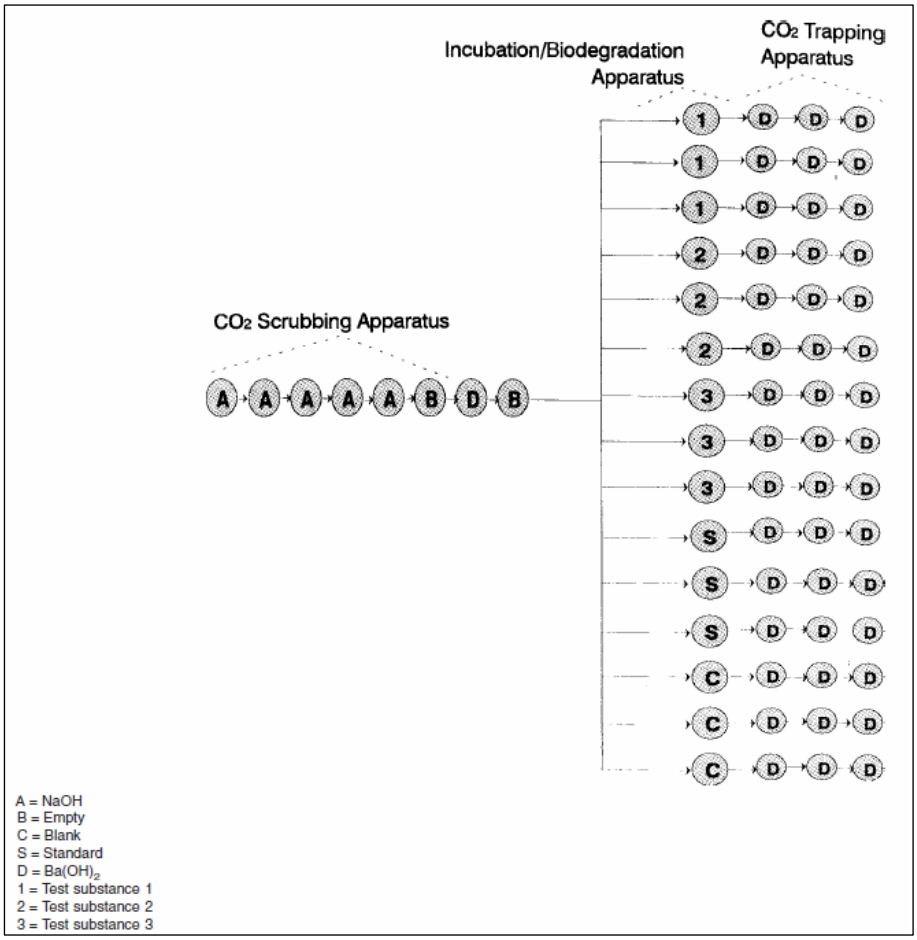


Figure 8. Schematic presentation of the aerobic aquatic biodegradation testing according to ASTM D 5864 (Source: ASTM D 5864-11).

Standard test method ***ASTM D 6139*** is composed of (1) a CO_2 scrubbing apparatus, which consists of a 1 l Erlenmeyer flask containing 10 M NaOH and a 1 l Erlenmeyer flask containing distillated water (optionally also a flask can be added containing 0.1 M $Ba(OH)_2$ to monitor a possible breakthrough of CO_2 and an empty flasks to prevent liquid carryover), (2) an incubation/biodegradation apparatus (= a Gledhill-type Shake Flask Unit with a volume of 2 l), and (3) a CO_2 trapping apparatus, (conical alkaline trap unit containing 10 ml 0.1 M $Ba(OH)_2$) (Figure 9).

The incubation/biodegradation apparatus is filled with a mineral medium, which contains ammonium sulphate solution, calcium chloride solution, ferric chloride solution, magnesium sulphate solution, a phosphate buffer and a trace elements solution, and 1 % activated sludge supernatant to give 30 mg/l suspended solids in the final volume. This mineral medium is identical to the mineral medium of ASTM D 5864 – 11, except for the presence of the trace element solution. Before start-up of the test the aqueous mixture in the Erlenmeyer flasks is aerated with CO_2 free air for at least 1 hour to purge the system of CO_2, after which the system is closed airtight. These flasks are agitated with a magnetic stirrer. No additional carbon source is added to the blank reactors, while the reference item is added to the control reactors (10-20 mg C/l) and the test item is added to the test reactors (10-20 mg C/l). The produced CO_2 reacts with $Ba(OH)_2$ in the conical trap and precipitates as $BaCO_3$. Once significant $BaCO_3$ is evident, $Ba(OH)_2$ solution is removed for titration with hydrochloric acid. Subsequently the trap is refilled with $Ba(OH)_2$, the inlet and outlet tubes of the flask are opened and the content of the flasks is then sparged with CO_2 free air through the inlet tube.

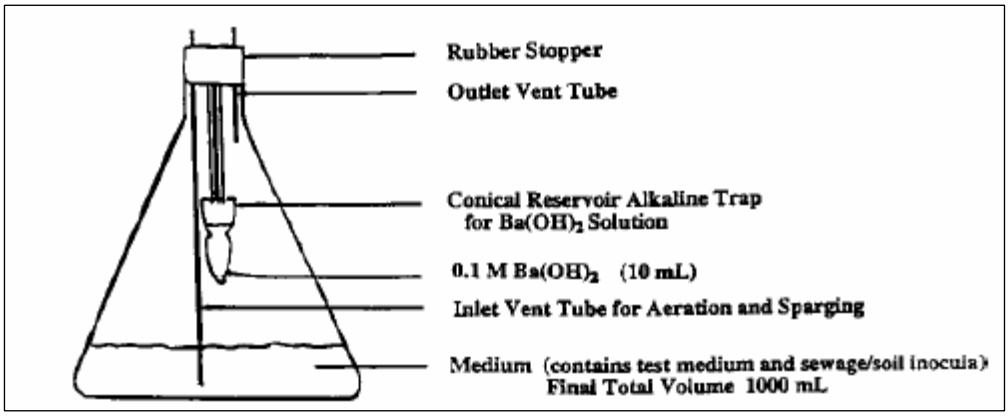


Figure 9. Presentation of the Gledhill shake flask system (Source: ASTM D 6139 - 11).

It can be concluded that the principle of methods ASTM D 5864 and ASTM D 6139 is identical (the produced CO_2 is captured in $Ba(OH)_2$ and the amount of CO_2 is determined by the titration of the remaining $Ba(OH)_2$). The main difference between these two methods is the aeration: test method ASTM D 5864 prescribes that the incubation apparatus is continuously aerated with CO_2 free air, while test method ASTM D6139 is a closed system (without continuous aeration).

Standard test method ***ASTM D 6731*** is composed of a closed respirometer (Figure 10), which contains a CO_2-absorber compartment. The closed respirometer is filled with a mineral medium, which contains calcium chloride solution, ferric chloride solution, magnesium sulphate solution and a phosphate buffer, and 1 % activated sludge supernatant to give 30 mg/l suspended solids in the final volume. In contrast to test methods ASTM D 5864 and ASTM D 6139 no ammonium sulphate solution (40 g/l) is added. The only source of nitrogen in the mineral medium is present in the phosphate buffer (containing 0.5 g NH_4Cl/l). Moreover, the presence of NH_4Cl in the phosphate buffers of test methods ASTM D 5864 and ASTM D 6139 is also higher (1.7 g/l). The composition of this mineral medium is identical to the mineral medium as described in the international test methods ISO 9408 (1999) and ISO 14851 (1999), which are also based on oxygen consumption. The flasks are agitated with a magnetic stirrer. No additional carbon source is added to the blank reactors, while the reference item is added to the reference reactors (50-200 mg ThOD/l) and the test item is added to the test reactors (50-200 mg ThOD/l). Alkaline solution (10 M NaOH or 10 M KOH) is added to the CO_2-absorber compartment. The produced CO_2 reacts with an absorbent solution. Consequently the total pressure in the flask decreases. The pressure drop is detected by a manometer, which produces a signal that results in the electrolytic generation of oxygen. The signal is stopped when the original pressure is re-established and the quantity of the electricity is measured. The amount of electricity used in proportional to the amount of the consumed oxygen.

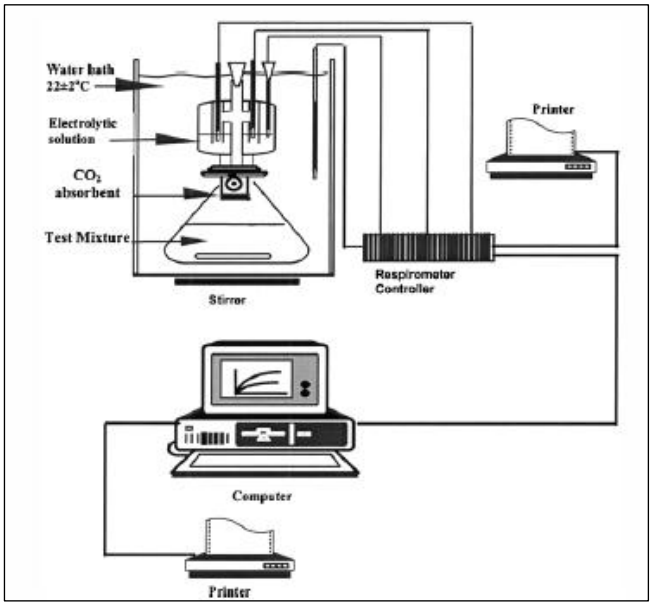


Figure 10. Principle of a closed respirometer (Source: ASTM D 6731 - 01 (2011)).

The source of inoculum as prescribed by the American test methods is given in Table 25. Pre-adaptation of the inoculum to a test material is allowed in these test methods.

Table 25. Overview of inoculum sources of biodegradability test methods for lubricants (ASTM).

Source of inoculum	D 5864	D 6139	D 6731
Wastewater-treatment plant: activated sludge	X	X	X
Secondary effluent			X
Surface water	X	X	X
Soil extract	X	X	X
Mixture of sources	X	X	X

In order to ***verify the activity of the inoculum*** a reference or control sample known to biodegrade needs to be tested in parallel with the test item. Test is regarded invalid if the reference does not demonstrate > 60 % of the $ThCO_2$ (ASTM D 5864 and ASTM D 6139) or > 60 % of the ThOD (ASTM D 6731) within 28 days. The test methods suggest using sodium benzoate or aniline for water soluble test materials and low erucic acid rapeseed oil (for example Canada oil) for water insoluble test materials.

Besides the validity criteria based on the biodegradation rate of the reference material, also a ***validation criteria with regard to the blank*** is given in test methods ASTM D 5864 and ASTM D 6731. ASTM D 5864 prescribes that a total CO_2 evolution in the blank at the end of the test > 75 mg CO_2 per 3 l of medium shall be considered as invalidating the test, while ASTM D6731 prescribes that a total oxygen utilization in the blank at the end of the test > 60 mg O_2/l invalidates the test. ASTM D 6139 has no validation criteria with regard to the blank series.

Moreover, also a criterion with regard to ***the quantity of micro-organisms*** in the inoculum is given. An overview of the required range of colony-forming units is given in Table 26.

Table 26. Amount of colony-forming units per millilitre as prescribed by biodegradability test methods for lubricants (ASTM).

ASTM method	Colony-forming units per millilitre
D 5864	$10^6 – 20 \times 10^6$
D 6139	$10^6 – 10^8$
D 6731	$10^6 – 10^7$

Test method ASTM D 5864 is run at 20 to 25°C, while methods ASTM D 6139 and ASTM D 6731 are both run at 22°C ± 2°C. Test method ASTM D 5864 needs to be executed in triplicate, while methods ASTM D 6139 and ASTM D 6731 may be executed in duplicate (although triplicates are preferred).

The minimum duration of these test methods is 28 days, but the test can be extended until the CO_2 production has reached a plateau.

The standard test method ***ASTM D 7373*** predicts the biodegradability of liquid-based lubricants within 1 day using a bio-kinetic model without dealing with micro-organisms. This test method is based on the determination of the content of nonaromatics, non-polar aromatics, ester fraction and polar aromatics using a glass chromatographic column packed with activated bauxite and silica gel. The different fractions are determined using specific eluents:

- Nonaromatics: n-pentane
- Non-polar aromatics: Toluene and n-pentane
- Ester fraction: Diethyl ether
- Polar aromatics: Chloroform and ethyl alcohol

The effective composition to biodegradation (ECB) is calculated only taken into account the nonaromatics and the esters. Based on the ECB the percentage of biodegradation is determined using a bio-kinetic model. According to ASTM D 7373 excellent correlation is established between the results based on the bio-kinetic model and the conventional biodegradation tests.

3.1.4.2 American standards with regard to organic compounds

An overview of the American standards with regard to aerobic biodegradation of organic compounds in aqueous aerobic environments is given in Table 27. As these guidelines are comparable to guidelines described in earlier chapters, no detailed description of the measurements techniques is given.

Table 27. Overview of the ASTM standards with regard to biodegradation in aerobic aqueous environment of organic compounds.

Standard	Description	Comparable guidelines
E1279 – 89 (2008)	Standard Test Method for Biodegradation By a Shake Flask Die Away Method	OECD 309, ISO 14592-1
E1625 – 94 (2008)	Standard Test Method for Determining Biodegradability of Organic Chemicals in Semi Continuous Activated Sludge (SCAS)	OECD 302 A, ISO 9887
E1720 – 01 (2008)	Standard Test Method for Determining Ready, Ultimate, Biodegradability of Organic Chemicals in a Sealed Vessel CO_2 Production Test	OECD 301 B, ISO 9439
E1798 – 96 (2008)	Standard Test Method for Assessing Treatability or Biodegradability, or Both, of Organic Chemicals in Porous Pots	OECD 303 A, ISO 11733

3.1.4.3 American standards with regard to plastic materials

An overview of the American standards with regard to aerobic biodegradation of plastic materials in aqueous aerobic environments is given in Table 28. Standard test method ASTM D 6340 can be used in order to determine biodegradation in aqueous and in compost environment. Only the aqueous environment is discussed in this chapter. A brief overview of the measurements techniques of the different test methods is given in Table 29.

Table 28. Overview of the ASTM standards with regard to biodegradation in aerobic aqueous environment of plastic materials.

Standard	Description
D 5271-02	Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials in an Activated-Sludge-Wastewater-Treatment System
D 6340-98	Standard Test Methods for Determining Aerobic Biodegradation of Radiolabeled Plastic Materials in an Aqueous or Compost Environment

Table 29. Overview of measurement techniques of biodegradability test methods for plastic materials (ASTM).

Method	Measurement technique	Comparable guidelines
D 5271	Test item (at least 60 mg/l) in an inoculated mineral medium is stirred at 23°C ± 2°C. Evolved CO_2 is absorbed in KOH absorber with phenolphthalein indicator. The consumption of oxygen (BOD) is determined by measuring the amount of oxygen required to maintain a constant volume of gas in the respirometer flasks. Moreover, also the soluble-organic-carbon (at start and at end), nitrite/nitrate (at start and at end) and insoluble plastic (at end) are determined in order to determine the biodegradation percentage. Correction needs to be made for oxygen update due to nitrification. Maximum duration is 6 months.	OECD 301 F ISO 9408 ISO 14851
D 6340	A radiolabeled plastic (50-100 mg per 80 ml medium) in an inoculated limited basal media (BAM broth minus glucose) is aerated at 58°C ± 5°C. The produced gasses pass through an acid trap and consequently though a CO_2 absorption column containing cooled methoxyethyl amine. The $^{14}CO_2$ absorbed in the methoxyethyl amine is determined in a liquid scintillation counter, which measures the beta radiation of ^{14}C.	

An overview of the different inoculum sources is given in Table 30. ASTM D 5271 mentions that adaptation of the biomass may be desirable to provide increased reproducibility.

Table 30. Overview of the different inoculum sources as prescribed by ASTM.

Source of inoculum	D 5271	D 6340
Activated sludge from aeration tank	X	X
Soil extract	X	
Compost extract	X	X
Other environmental sources		X
Waste water		X
Mixture of sources	X	X

The prescribed amount of the different series is given in Table 31.

Table 31. Amount of replicates as prescribed by biodegradability test methods for plastic materials (ASTM).

ASTM method	Blank series	Reference series	Test series	Negative control
D 5271	3	3	3	3 (optional)
D 6340			1	

In order to check ***the activity of the inoculum***, ASTM D 5271 mentions analytical-grade cellulose and starch as suitable reference material. The validation criteria depend on the used method (Table 32). No reference material, nor validation criteria is mentioned in ASTM D 6340.

Table 32. Validity criteria as prescribed by biodegradability test methods for plastic materials (ASTM).

ASTM method	Validity criteria
D 5271	Reference > 60 % ThOD (at end) BOD blank inoculum < 60 mg O_2/l (at end)
D 6340	-

3.2 Toxicity

Aquatic toxicity refers to the intrinsic property of a substance to be detrimental to an organism in short-term and/or long-term exposure to that substance. In general, it is assumed that the aquatic toxicity is mainly related to waterborne exposure of a substance and expressed as external concentration of that substance in test water, but there may be cases where food uptake is the predominant route of exposure (i.e. for very hydrophobic or very sorptive substances). It is believed that substances dissolved in water and taken up by organisms may accumulate to a certain internal concentration, which may then cause adverse effects. Therefore factors that influence bioconcentration (molecular weight, water solubility and log K_{ow}) influence also toxicity to aquatic species.

There exist 2 types of aquatic toxicity tests: (1) acute toxicity tests and (2) chronic toxicity tests (ECHA (2012)).

Acute toxicity related to waterborne exposure is generally expressed in terms of a concentration which is lethal to 50 % of the test organisms (lethal concentration, LC_{50}), causes a measurable adverse effect to 50 % of the test organisms (e.g. immobilization of daphnids), or leads to a 50 % reduction in test (treated) organism responses from control (untreated) organism responses (e.g. growth rate in algae) following an exposure in the range of hours to days. This is expressed as effective concentration (EC_{50}).

Chronic toxicity related to waterborne exposure refers to the potential or actual properties of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life-cycle of the organism. Such chronic effects usually include a range of sublethal endpoints and are generally expressed in terms of NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration), EC_x or MATC (Maximal Acceptable Toxicant Concentration). Observable endpoints in chronic studies typically include survival, growth and/or reproduction.

There exist different types of information relevant for assessing aquatic toxicity. This includes “in vivo” testing methods and non-testing methods (QSAR, etc.). Currently no EU / OECD guidelines for “in vitro” tests relevant to aquatic toxicity are available.

The OECD guidelines for aquatic toxicity testing comprise internationally agreed testing methods for environmental effects. Tests undertaken following the OECD guidelines are useful for both risk assessment and classification purposes. The obtained data are covered by the principle of mutual acceptance of data. Therefore, the OECD guidelines related to aquatic toxicity are described in detail in chapter 3.2.2, while only an overview is given of the other existing standard test methods (international standards and American standards). Chapter 3.2.1 describes useful preparation techniques in order to evaluate aquatic toxicity of poorly water soluble and volatile substances.

3.2.1 Preparation of the test items

ASTM has developed a specific standard with regard to the sample preparation and the results interpretation of aquatic toxicity tests on lubricants: ***ASTM D 6081 – 98 (Reapproved 2009) "Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation"***. As most lubricants and lubricant components are mixtures of chemical compounds with varying and usually poor solubility in water, they are difficult to evaluate in toxicity tests. Consequently the toxicity of mixtures of poorly soluble components cannot be expressed in terms of lethal concentration, effect concentration or inhibition concentration because the mixtures may not be completely soluble at treat levels that lead to toxic effects. This standard practice prescribes that toxicity results of poorly soluble materials should better be expressed as loading rate.

The lubricants or lubricant components should not be added directly to aquatic systems for toxicity testing because the addition procedure will affect the results of the toxicity tests. Therefore ASTM D 6081 – 98 describes 3 different methods for material preparation of lubricants or lubricant components: (1) preparation of Water-Accommodated Fraction (WAF), (2) preparation of Water-Soluble Fraction (WSF) and (3) preparation of a mechanical dispersion. The disadvantage of last technique is that it cannot be used for poorly swimming organisms (due to the fact that this technique generates turbulence).

WAF refers to components dissolved in the water phase or entrained as stable droplets in the water phase, while WSF only refers to components dissolved in the water phase. The WAF is prepared by adding the test item in the desired nominal exposure load to water. The content of the vessel is stirred (vortex depth: 10 – 35 % of test solution height) for 20 to 24 hours. This is followed by a subsequent settling period of 1 to 4 hours. After the settling period the aqueous solution is decanted (= WAF). The WSF is prepared by the filtration of the WAF through a 0.45 µm cellulose-acetate filter or by centrifugation of the WAF to remove the undissolved material.

The mechanical dispersion technique is based on the presence of a motor-driven propeller assembly to move the test material and the dilution water in the test vessel during the study. A schematic presentation of this technique is given in Figure 11.

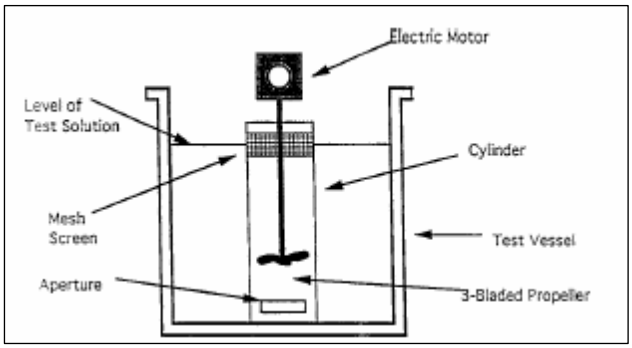


Figure 11. Schematic presentation of the mechanical dispersion technique (Source: ASTM D 6081).

Also ***OECD*** has developed a guidance document on aquatic toxicity testing of difficult substances and mixtures (Guidance document on aquatic toxicity testing of difficult substances and mixtures). When the concentration of a test substance declines significantly (> 20 %) during a test due to physical, chemical or biological processes, modifications to media preparation and exposure systems can be required. When no data are available in order to identify the process responsible for the decline, a preliminary study is required in order to assess the stability of the test substance. The goal of a preliminary study is to determine if a substance is stable, volatile, sorbed onto glass, photodegraded, hydrolytically unstable, oxidizable or biodegradable. The OECD guidance documents gives specific recommendations with regard to different categories of difficult substances and mixtures, but in the scope of this study only poorly water-soluble substances, volatile substances, biodegradable substances, hydrophobic substances and multi-component substances (= complex mix of individual substances with different solubility and physical-chemical properties) are described in detail.

For poorly water-soluble substances 4 methods of test media preparation are described: (1) direct addition, (2) water-miscible solvents (acetone, ethanol, methanol, tertiary-butyl alcohol, etc.), (3) generator systems (chemically inert matrix which has been coated with the substance) and (4) dispersions and emulsions.

Volatile substances can be lost by evaporation to the atmosphere. Losses of volatile substances during preparation and exposure can be minimised using relatively straightforward modifications to procedures. The guideline mentions that the vessels should be sealed during preparation and the headspace should be kept to a minimum if possible. For algal tests a sealed exposure system will result in culture growth being limited by CO_2 depletion and increasing pH. Consequently it is necessary to perform the test in a CO_2 enriched headspace or the test can be executed in a system without headspace in which gaseous CO_2 is replaced with $NaHCO_3$ and the pH is adjusted with HCl.

Readily biodegradable substances are likely to be degraded in aquatic test systems once competent bacterial populations become established. The maintenance of exposure concentrations is consequently dependent upon preventing the development of significant microbial populations. Therefore a strict test vessel hygiene will decay and limit, but not prevent the development of bacteria. Moreover, a flow-through exposure regime with sufficient volume renewal and a high concentration stock solution maintained under nitrogen has been shown to prevent aerobic biodegradation, minimise the concentration of breakdown products and maintain exposure concentration of the parent substance.

Hydrophobic substances (log K_{ow} > 4 or BCF > 500) can be partitioned into or onto test organism biomass and onto food or other organic detritus in the test system. In order to maintain the exposure concentrations (1) the ratio test organism biomass to test medium volume can be reduced, (2) the excess food and detritus can be removed, (3) a semi-static renewal or flow-through exposure regime can be prefered, (4) the dissolved total organic carbon concentrations need to be maintained at or below 2 mg/l, (5) feeding can be executed a few hours before test medium renewal and (6) the system surfaces can be saturated with the compound.

The toxicity of multi-components substances, which are only partly soluble in water, can be determined by preparing water-accommodated fractions (WAF's).

ISO has developed a comparable document with regard to poorly soluble materials, volatile compounds, metals and wastewater, but only related to algal growth inhibition tests: ***ISO 14442 (2006) "Water quality – Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water"***.

ISO 14442 prescribes that poorly soluble mixtures of organic substances (= homogenous aggregates of a number of compounds with different physic-chemical and/or chemical properties, which cannot be easily separated into their component parts by physical means and formulated products) should be tested by preparing the water-accommodated fractions (WAF's) by stirring and phase separation. Consequently, the results need to be expressed as loading rates instead of the usual concentrations. The preparation of the WAF is identical to the preparation method as prescribed by ASTM D 6081.

According to ISO 14442 volatile substances (H > 1 Pa.m^3/mol) should be tested in closed systems. However, as optimum algal growth and the maintenance of a stable pH require both a sufficient supply of CO_2, the headspace needs to be enriched with CO_2 or the dissolved CO_2 needs to be increased by the addition of $NaHCO_3$.

3.2.2 Test methods

3.2.2.1 OECD guidelines

An overview of the guidelines with regard to aquatic toxicity as developed by OECD is given in Table 33. These test methods are all designed to evaluate the aquatic toxicity of chemicals. Different types of freshwater aquatic species can be evaluated: (1) algae, (2) aquatic plants, (3) invertebrates and (4) fish. OECD 229 is the only guideline, which is developed in order to test potential endocrine disrupting chemicals.

A short description of the test method of these aquatic toxicity tests is given in Table 34. The validation criteria of the test methods is given in Table 35.

Table 33. Overview of the OECD guidelines with regard to freshwater aquatic toxicity.

Guideline	Adopted	Description
OECD 201	Mar-23-2006	Freshwater Alga and Cyanobacteria, Growth Inhibition Test
OECD 202	Apr-13-2004	*Daphnia* sp., Acute Immobilisation Test
OECD 203	Jul-17-1992	Fish, Acute Toxicity Test
OECD 204	Apr-04-1984	Fish, Prolonged Toxicity Test: 14-day Study
OECD 209	Jul-22-2010	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)
OECD 210	Jul-17-1992	Fish, Early-life Stage Toxicity Test
OECD 211	Oct-02-2012	*Daphnia magna* Reproduction Test
OECD 212	Sep-21-1998	Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages
OECD 215	Jan-21-2000	Fish, Juvenile Growth Test
OECD 221	Mar-23-2006	*Lemna sp.* Growth Inhibition Test
OECD 229	Oct-02-2012	Fish Short Term Reproduction Assay

Table 34. Description of the OECD test methods for aquatic toxicity tests.

Guideline	Description
OECD 201	Effect of a substance on the growth of freshwater microalgae and/or cyanobacteria is evaluated over a period of normally 72 hours at a temperature of 21°C to 24°C. The system response is the reduction of growth (= algal biomass as a function of time) in a series of algal cultures exposed to various test concentrations. The concentration bringing about a specified x % inhibition of growth rate is determined and expressed as the E_rC_x.
OECD 202	Effect of a substance on the immobilization of daphnids with an age < 24 hours (at least 20 daphnids per test series) is evaluated over a period of 48 hours at a temperature within the range of 18°C and 22°C. Immobilization is recorded after 24 hours and after 48 hours. The results are analysed in order to calculate the EC_{50}.
OECD 203	Effect of a substance on the survival of fish (at least 7 fish per test series) is evaluated over a period of 96 hours in a static, a semi-static or a flow-through procedure at a temperature, which is adapted to the used fish species. Mortalities and other observations are recorded at 24, 48, 72 and 96 hours. The concentration, which kills 50 % of the fish (LC_{50}) is determined when possible.
OECD 204	Effect of a substance on the survival of fish (at least 10 fish per test series) is evaluated over a period of at least 14 days in a semi-static or a flow-through procedure at a temperature, which is adapted to the used fish species. Mortalities and other observations are recorded minimum three times per week. Moreover, fish should be measured and weighted before the start-up and at the termination of the test. Threshold levels of lethal and other observed effects and NOEC are determined.
OECD 209	Effect of a substance on the respiration rate of activated sludge fed with synthetic sewage is determined after a contact time of 3 hours at 20°C ±2°C. Respiration rate is determined by measuring the concentration of dissolved oxygen. Three different oxygen uptakes may be determined: total, heterotrophic only and that due to nitrification. The EC_x and/or the NOEC are determined.
OECD 210	Define the lethal and sub-lethal effects of a test substance on the early-life stages of fish (at least 60 fertilised eggs, divided equally between at least 2 replicate test chambers) in a semi-static or a flow-through procedure at a temperature, which is adapted to the used fish species. The test is continued at least until all the control fish are pre-feeding. The LOEC and the NOEC are determined.
OECD 211	Effect of a substance on the reproductive output of young female Daphnia magna with an age < 24 hours is evaluated over a period of 21 days at a temperature within the range of 18°C and 22°C. The test can be executed in a semi-static procedure (10 animals individually held per concentration) or a flow-through procedure (40 animals divided into 4 groups of 10 animals at each test concentration). At the end of the test the total amount of living offspring produced is assessed. The toxic effect of the test substance on reproductive output is expressed as EC_x or alternatively as NOEC/LOEC value.

Guideline	Description
OECD 212	Define the lethal and to a limited extent sub-lethal effects of a test substance on fish embryo and sac-fry stages (at least 30 fertilised eggs, divided equally between at least 3 replicate test chambers) in a semi-static or a flow-through procedure at a temperature, which is adapted to the used fish species. The test is terminated just before the yolk-sac of any larvae in any of the test chambers has been completely absorbed or before mortalities by starvation start in the controls. The LOEC and the NOEC are determined.
OECD 215	Effect of a substance in a range of at least 5 sublethal concentrations on the growth of juvenile fish is evaluated over a period of 28 days in a semi-static or a flow-through procedure at a temperature, which is adapted to the used fish species. The juvenile fish are weighted at start, optionally after 14 days and at the end of the test. Effects on growth rates are analysed using a regression model in order to estimate the concentration that would cause a x % variation in growth rate (EC_x). In addition LOEC and NOEC can be determined.
OECD 221	Effect of a substance in a range of at least 5 test concentrations on the vegetative growth of freshwater aquatic plants of the genus Lemna (duckweed) is evaluated over a period of 7 days in a static, a semi-static or a flow-through procedure at 24°C ± 2°C. Frond number is the primary measurement variable. At least one other measurement variable (total frond area, dry weight of fresh weight) is also measured. Concentrations bringing about a specified x % inhibition of growth (= average specific growth rate) and a x % inhibition of yield are determined and expressed as the E_rC_x and E_YC_x, respectively. In addition LOEC and NOEC can be determined.
OECD 229	Detect endocrine active substances by exposing sexually mature male and spawning female fish (zebrafish, fathead minnow or Japanese medaka) to three chemical exposure concentrations and a water control during 21 days. The amount of replicates and the amount of fish per replicate are in function of the fish species. Quantitative fecundity is monitored daily, while at the end of the test secondary sex characteristics are measured (only for fathead minnow or Japanese medaka), vitellogenin is determined and gonads are fixed for potential histopathological evaluation.

Table 35. Validity criteria of the OECD test methods for aquatic toxicity tests.

Guideline	Validity criteria
OECD 201	Biomass control cultures: exponential increase ≥ factor 16 within 72 hours Mean coefficient of variation for section-by-section growth rates in control cultures < 35 % Coefficient of variation of average growth rates in replicate control cultures < 7 % (Pseudokirchneriella subcapitata & Desmodesmus subspicatus) or < 10 % (other species)
OECD 202	Immobilization in the control series < 10 % after 48 hours Dissolved oxygen concentration at the end of the test ≥ 3 mg/l
OECD 203	Mortality in the control series < 10 % (at end) Constant conditions should be maintained Dissolved oxygen concentration > 60 % of air saturation value (complete test) Evidence that the concentration of the substance is maintained (at least 80% of nominal concentration)

Guideline	Validity criteria
OECD 204	Mortality in the control series < 10 % (at end) Dissolved oxygen concentration > 60 % of air saturation value (complete test) Evidence that the concentration of the substance is maintained (at least 80% of nominal concentration)
OECD 209	Oxygen uptake rate of blank controls > 20 mg O_2 / g activated sludge (dry weight of suspended solids) in an hour Coefficient of variation of O_2 uptake rate in control replicates < 30% at the end Range EC_{50} 3,5-DCP: 2 – 25 mg/L (total respiration), 5 – 40 mg/L (heterotrophic respiration) & 0.1 – 10 mg/L (nitrification respiration) Range EC_{50} copper (II) sulphate pentahydrate: 53 – 155 mg/L (total respiration)
OECD 210	Overall survival > limit values (which are defined per species) Dissolved oxygen concentration > 60 % of air saturation value (complete test) Variation water temperature < 1.5°C between test chambers or successive days Evidence that the concentration of the substance is maintained (within ± 20% of the mean measured values)
OECD 211	Mortality of the parent animals < 20 % (at end) Mean number of living offspring produced per surviving parent animal $\geq$ 60
OECD 212	Overall survival > limit values (which are defined per species) Dissolved oxygen concentration > 60 % of air saturation value (complete test) Variation water temperature < 1.5°C between test chambers or successive days
OECD 215	Mortality in the control series < 10 % (at end) Mean weight of fish in the control(s) must have increased enough to permit the detection of the minimum variation of growth rate considered as significant. Dissolved oxygen concentration > 60 % of air saturation value (complete test) Variation water temperature < 1°C between test chambers and within a range of 2°C within the temperature ranges specified for the test species
OECD 221	Doubling time of frond number in the control < 2.5 days (60 hours)
OECD 229	Mortality in the controls < 10% (at end) Dissolved oxygen concentration > 60 % of air saturation value (complete test) Variation water temperature < 1.5°C between test chambers and within a range of 2°C within the temperature ranges specified for the test species Evidence that the concentration of the substance is maintained (within ± 20% of the mean measured values) Evidence that fish are actively spawning in all replicates prior to initiating chemical exposure

3.2.2.2 International standards

An overview of the international standards with regard to freshwater aquatic toxicity is given in Table 36. It is noticed that in addition to Daphnia sp., also chronic toxicity tests were developed towards Ceriodaphnia dubia (water flea occurring in freshwater lakes, ponds and marshes) and towards Brachionus calyciflorus (planktonic rotifer occurring in freshwater).

Table 36. Overview of the international standards with regard to freshwater aquatic toxicity.

ISO standard	Description
6341 (2012)	Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) – Acute toxicity test
7346-1 (1996)	Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 1: Static method
7346-2 (1996)	Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 1: Semi-static method
7346-3 (1996)	Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 1: Flow-through method
8192 (2007)	Water quality – Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation
8692 (2012)	Water quality – Fresh water algal growth inhibition test with unicellular green algae
10229 (1994)	Water quality – Determination of the prolonged toxicity of substances to freshwater fish – Method for evaluation the effects of substances on the growth rate of rainbow trout (Oncorhynchus mykiss Walbaum (Teleostei, Salmonidae))
10706 (2000)	Water quality – Determination of long term toxicity of substances to Daphnia magna Straus (Cladocero, Crustacea)
12890 (1999)	Water quality – Determination of toxicity to embryos and larvae of freshwater fish – Semi-static method
20665 (2008)	Water quality – Determination of chronic toxicity to Ceriodaphnia dubia
20666 (2008)	Water quality – Determination of chronic toxicity to Brachionus calyciflorus in 48 h

3.2.2.3 American standards

An overview of the American standards with regard to aquatic toxicity is given in Table 37. In addition to the species tested in the OECD guidelines also standards towards other species were developed (amphibians, Rotifer Brachionus, freshwater mussels, etc.).

Table 37. Overview of the American standards with regard to freshwater aquatic toxicity.

ASTM standard	Description
E 729 – 96 (2007)	Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians
E 1193 – 97 (2004)	Standard Guide for Conducting Daphnia magna Life Cycle Toxicity Tests
E 1218 – 04e1	Standard Guide for Conducting Static Toxicity Tests with Microalgae
E 1241 – 05	Standard Guide for Conducting Early Life Stage Toxicity Tests with Fishes
E 1366 – 11	Standard Practice for Standardized Aquatic Microcosms: Fresh Water
E 1415 – 91 (2004)e1	Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3
E 1440 – 91 (2004)	Standard Guide for Acute Toxicity Test with the Rotifer Brachionus
E 1604 – 94 (2007)	Standard Guide for Behavioral Testing in Aquatic Toxicology
E 1706 – 05 (2010)	Standard Test Method for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates
E 1711 – 95 (2008)	Standard Guide for Measurement of Behaviour During Fish Toxicity Tests
E 1850 – 04	Standard Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests
E 2455 – 06	Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels
E 2591 – 07	Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians

3.3 Standard specifications

3.3.1 European specifications

Currently, no European specification is developed yet defining the criteria for bio-lubricants and bio-solvents, but CEN has already developed a technical report with recommendations (no specifications) for terminology and characterisation of bio-lubricants and bio-based lubricants: ***CEN/TR 16227 (2011) "Liquid petroleum products – Bio-lubricants – Recommendation for terminology and characterisation of bio-lubricants and bio-based lubricants"***. This technical report recommends that the claim "bio" should refer to an international or at least European agreed standard. In order to avoid market hindering confusion, following minimum requirements are recommended in this technical report for bio-lubricants:

- Minimum content of renewable raw material: 25 %
  - Method: ASTM D 6866 or equivalent CEN version (to be developed)
- Minimum biodegradation percentage: 60 % (oils) or 50 % (lubricating greases)
  - Method: OECD 301 B, C, D or F or adequate ISO or EN standards
- Not labelled as "Dangerous to the environment" (Symbol N)
  - $EC_{50}/LC_{50}/IC_{50}$ fully formulated product > 100 mg/l
  - Method: OECD 201/202/203
- Minimum performance criteria
- Any lubricant according to the present criteria of the EU Ecolabel for Lubricants is per definition a bio-lubricant

The technical report also gives an overview of the biodegradation and ecotoxicity test methods which are of importance for lubricants:

- Biodegradation
  - OECD 301 B, C, D & F
  - ISO 10708
  - EN ISO 14593
- Ecotoxicity
  - Bacteria test: EN ISO 8192
  - Algae toxicity test: OECD 201
  - Daphnia test: OECD 202
  - Fish toxicity: OECD 203
  - Fish toxicity: OECD 204 (incorporated in "Blue Angel" ecolabel)
  - Plant growth method: OECD 208 (incorporated in "Blue Angel" ecolabel)

The technical report mentions that also the EC Dangerous Preparations Directive (DPD) needs to be taken into account. The DPD identifies and labels dangerous formulations in finished products. Based on the amount of Dangerous for the Environment classified components, the final lubricant might be classified as "Dangerous for the Environment" and might carry the "dead fish/dead tree" hazard symbol. The health and environmental hazards

of a preparation can be evaluated by either a conventional method (with limits for single components) or by testing the preparation using standard testing guidelines.

In order to test the preparation, all test species (algae: OECD 201, daphnia: OECD 202 and fish: OECD 203) need to be tested and the IC/EC/LC_{50} needs to be > 100 mg/l for each trophic level. When this testing was successful, a preparation is not related to a symbol, even if the calculation scheme would demand labelling. Only if a preparation contains more than the specified amount of a component, which is classified as a carcinogen, mutagen or reproductive toxicant, the conventional test method (with limits for single components) shall be applied.

For plastic material a specification is developed towards disposability of plastics in waste water treatment systems: ***EN 14987 (2006) "Plastics – Evaluation of disposability in waste water treatment plant – Test scheme for final acceptance and specifications"***. This test scheme specifies test methods and criteria which are applied in order to verify if a solid plastic material can be considered as disposable in waste water treatment systems. Materials which are shown to be in compliance with this standard can be eventually disposed of in municipal or industrial waste water treatment plants, through the sewage.

A distinction is made between cold and hot "water soluble/dispersible plastics". The hot water soluble/dispersible plastics can only be disposed of through the sewage after exposition to hot water. Moreover, also a distinction is made between soluble and dispersible plastics. The dispersible plastics are not suitable for application where the final solution shall pass through small diameter pipes or orifices (e.g. laundry bags for washing machines). An overview of the specific requirements is given in Table 38.

Table 38. Overview of the requirements for plastics disposable in waste water treatment systems as prescribed by EN 14987 (2006).

Parameter	Requirement as prescribed by EN 14987 (2006)
Biodegradation	Minimum mineralization degree of 90 % (absolute) or 90 % of the mineralization degree reached in the same time by a reference material (soluble starch or microcrystalline cellulose), tested in parallel (relative), within 56 days. (EN ISO 14851 / EN ISO 14852 at 20°C – 25°C & soil or compost extracts are not allowed)
Water dispersibility or water solubility	Plastic material must be water dispersible (dispersible fraction D ≥ 0.9 after dissolution in cold or hot water) or water soluble (soluble fraction S ≥ 0.9 after dissolution in cold or hot water)

3.3.2 International specifications

There exists an international standard, which gives specifications for environmentally acceptable hydraulic fluids (HE): ***ISO 15380 (2011) "Lubricants, industrial oils and related products (class L) – Family H (Hydraulic systems) – Specifications for categories HETG, HEPG, HEES and HEPR"***. The specification is related towards ready biodegradability (in aerobic aqueous environment) and (aquatic) ecotoxicity. Only criteria are given for 4 categories: (1) HETG (triglycerides), (2) HEPG (polyglycols), (3) HEES (synthetic esters) and (4) HEPR (polyalphaolefins and related hydrocarbons). The hydraulic fluids, which meet the specifications of this standard are designed to minimize the impact upon the environment in the event of a leak or a spill. An overview of the specifications is given in Table 39.

Table 39. Overview of the requirements for environmentally acceptable hydraulic fluids as prescribed by ISO 15380 (2011).

Parameter	Requirement as prescribed by ISO 15380 (2011)
Biodegradation	Min. 60 % (ISO 14593 or ISO 9439)
Toxicity	
Acute fish toxicity, 96 h, LC_{50}	Min. 100 mg/l (ISO 7346-2)
Acute Daphnia toxicity, 48 h, EC_{50}	Min. 100 mg/l (ISO 6341)
Bacterial inhibition, 3 h, EC_{50}	Min. 100 mg/l (ISO 8192)

3.4 Labelling

The objective of ecolabelling is to provide clear information to customers to allow them to select products that are the least harmful to the environment. This should stimulate environmental concern in product development. These labelling systems have defined and established methods to measure the properties of a substance that would qualify it as being environmentally acceptable.

3.4.1 Der Blaue Engel / Blue Angel

The first national labelling scheme for lubricants was the German Blue Angel label, developed in 1988 (Figure 12). This label indicates that the impact of the product on the environment is lower when compared to impact of a comparable product. Three biodegradable lubricant categories can be certified under the Blue Angel Eco-label. An overview of these categories is given in Table 40.

Figure 12. Logo of Der Blaue Engel ecolabel.

Table 40. Overview of the lubricant categories as prescribed by the Blue Angel labelling system.

Designation	
RAL-UZ 48	Readily Biodegradable Chain Lubricants for Power Saws
RAL-UZ 64	Readily Biodegradable Lubricants & Forming oils
RAL-UZ 79	Readily Biodegradable Hydraulic Fluids

The Blue Angel eco-labels are mainly focused on biodegradability, low toxicity to aquatic organisms, non-bioaccumulative, no dangerous components (such as carcinogens or toxic substances) and technical specifications. However, no criteria with regard to bio-based content are mentioned. Consequently, lubricants comprised completely of petroleum-sourced components can receive Blue Angel certification.

A significant amount of product are already certified according to the Blue Angel label:

- RAL-UZ 48: > 70 vendors & > 90 products
- RAL-UZ 64: > 25 vendors & > 50 products
- RAL-UZ 79: > 20 vendors & > 60 products

The biodegradability and eco-toxicity requirements as described in the Blue Angel ecolabel are briefly described below.

- Each basic substance (= ingredient in a concentration > 5 %) need to be biodegradable by at least 70 % (OECD 301 B, C, D & F, ISO 14593 or ISO 10708) and if it is suspected that basic substances have a toxicity < 100 mg/l following eco-toxicity tests must be conducted: OECD 202, 203, 201, 202, 204, 208, 209, DIN 38418-6.
- The content of non-biodegradable or only potentially biodegradable (OECD 302 B or C: ≥ 20 % biodegration) ingredients must not exceed 5 weight % and there shall not be eco-toxicological doubts. Ecotoxicological doubts exist if one of following items is fulfilled:
    - Highly toxic to aquatic organisms
        - Acute: $EC_{50}/LC_{50} \leq 1$ mg/l OR
        - Chronic: NOEC ≤ 0.01 mg/l
    - Negative impact on aquatic organisms and bioaccumulative potential
        - Acute: $EC_{50}/LC_{50} \leq 100$ mg/l OR
        - Chronic: NOEC ≤ 1 mg/l
        - Log $P_{ow} \geq 3.0$ OR
        - BCF > 100 OR
        - Surface active
    - Toxic effect on vascular plants
        - $EC_{50} \leq 100$ mg/kg
    - Inhibitory to bacterial growth
        - $IC_{50} < 100$ mg/l
- If additives are non-biodegradable polymers, following items need to be fulfilled:
    - Proof of immobility
    - Water solubility < 1 mg/l
    - Components with molecular weight ≤ 1000 g/mol < 1 %
    - No ecotoxicological doubts

3.4.2 Swedish labelling scheme

Also Sweden has developed a national labelling system for lubricants. Two standards were developed:

- SS 155434: Hydraulic fluids – Requirements and test methods
- SS 155470: Lubricating grease – Requirements and test methods

The evaluation of the lubricant involves testing for biodegradability, aquatic toxicity and sensitizing properties of a lubricant formulation and its components. Depending upon class also requirements with regard to renewable resources content are defined.

3.4.3 Nordic Swan

The first international labelling program for environmentally acceptable lubricants was the Nordic Swan program (Figure 13). The Nordic Swan is the official Ecolabel for the Nordic Countries (Sweden, Norway, Finland, Iceland and Denmark). The secretariats in the participating countries are responsible for implementing the scheme on national level. It imposes stringent environmental and climate criteria towards 63 product groups. The objective of Nordic Ecolabelling is to offer consumers guidance on the selection of products within a particular group which are considered to have the least potential adverse impact on health and the environment during their lifecycle.

Figure 13. Logo of the Nordic Swan Ecolabel.

In 2000 the Nordic Swan had developed a criteria document for lubricating oils. However, due to the fact that there was no sufficient interest in this product category, the category of lubricants has been cancelled on 31 January 2012. The low interest is probably caused by the requirements for renewability, which are the highest of all the labelling programs.

The criteria of the withdrawn criteria document for ecolabelling of lubricants are described below in order to illustrate why this labelling program was too stringent.

Separate requirements were imposed to chain oil, mould oil, hydraulic oil, 2-stroke oil, lubricating grease, metal cutting fluid and transmission-/gear fluid. The products must not present an undue hazard to environment or health, must be conform technical performance criteria and must have a high content of renewable resources. The requirements were set with the aim that maximum 1/3 of the products on the Nordic market should be able to fulfil the criteria.

For chain oils, mould oils, hydraulic oils, 2-stroke oils and lubricating greases requirements were imposed towards the minimum quantity of renewable raw materials (min. 85 % for chain oils and mould oils, min. 65 % for hydraulic oils and lubricating greases and min. 50 % for 2-stroke oils) and strict environmental requirements are imposed to the base oil and the additives used.

For metal cutting fluids and transmission-/gear fluids it was possible to choose between using renewable raw materials or rerefined oil (min. 65 %). Additionally the metal cutting fluid must fulfil environmental requirements as to the base oil and the additives, while

environmental requirements are not imposed to transmission/gear fluids since requirements relating to toxicity, degradability and bioaccumulation are not compatible with the technical performance criteria.

The environmental criteria encompass that the components of the lubricant should have a high level and rate of biological degradability, have a low toxicity to waterborne organisms and should not have bioaccumulative potential (log P_{ow} < 3 or BCF < 100). Specific criteria are determined for the base oil and for the additives:

- Base oil
  - Not classified with a N phrase (R50, R50/53, R51/53, R52/53 or R53)
  - Not classified as carcinogenic
  - Ready biodegradable (OECD 301 B, F, ISO 14593, ISO 9439 or ISO 9408)
  - Aquatic toxicity towards algae (OECD 201) and daphnia (OECD 202)
- Additives
  - If risk phrase R50 or R50/53
    - < 2 %: hydraulic oil & metal cutting fluid
    - < 1 %: chain oil, mould oil, 2-stroke oil & lubricating grease
  - If risk phrase R51/53
    - < 2 %: metal cutting fluid
    - < 1 %: chain oil, mould oil, hydraulic oil, 2-stroke oil & lubricating grease
  - If risk phrase R52/53 or R53
    - < 3 %: chain oil, mould oil & hydraulic oil
    - < 17%: lubricating grease
    - < 5 %: metal cutting fluid
    - < 15 %: 2-stroke oil
  - Aquatic toxicity towards algae (OECD 201) and daphnia (OECD 202)
  - No presence of endocrine disrupters
  - Aerosol products may not contain halogen hydrocarbon

In contrast to the other labelling systems, no fish toxicity is required. It is mentioned in the criteria document that fish toxicity is not required as fish have proved to be less sensitive than algae and Daphnia.

3.4.4 EU Ecolabel

The European Union has developed a label for products and services that have a reduced environmental impact throughout their complete life cycle, from the extraction of the raw material through production, use and disposal. The EU Ecolabel specifies criteria that reduce their environmental impact per product category. These criteria have been developed and agreed upon scientists, NGOs and stakeholders. The Ecolabel is considered to be the first major advancement towards creating a single international standard and it is becoming the most generally accepted label.

In order to obtain the EU Ecolabel, every product or service is carefully checked by independent experts. The scheme is voluntary, but currently already hundreds of companies across Europe have joined up. The logo of the EU Ecolabel is represented by a flower (Figure 14).

Figure 14. Logo of the EU Ecolabel.

The criteria in order to obtain the Ecolabel are specified per product category or per service category. There exists a specific category for lubricants. No specific category for solvents is described, but there exist product categories (for example “Indoor Paints & Varnishes”, “Outdoor Paints & Varnishes”, “Detergents” etc.), which will certainly contain solvents. Within the framework of this literature review also the criteria for outdoor paints and varnishes are described in order to obtain information with regard to solvents.

3.4.4.1 EU Ecolabel for lubricants

The aim of the EU Ecolabel for lubricants is promoting products that have a reduced impact on water and soil during use and contain a large fraction of bio-based material. The ecological criteria for the award of the EU Ecolabel to lubricants is described in the commission decision of 24 June 2011 (2011/381/EU).

The product group “lubricants” is subdivided into 5 categories:

I. Hydraulic fluids and tractor transmission oils
II. Greases and stern tube greases
III. Chainsaw oils, concrete release agents, wire rope lubricants, stern tube oils and other total loss lubricants
IV. Two-stroke oils
V. Industrial and marine gear oils

Already a few products are certified according to the Ecolabel:

- Hydraulic fluids: 11 products
- Greases: 1 product
- Chainsaw oils: 5 products
- Two-stroke oils: 4 products
- Total loss lubricants: 4 products

The lubricants have to meet requirements for performance, show limited toxicity to aquatic organisms, have high biodegradability and a low potential for bioaccumulation and contain a high fraction of renewable raw materials. In order to obtain the EU Ecolabel for lubricants 7 criteria need to be fulfilled.

Criteria 1: Excluded or limited substances and mixtures (R-phrases)

The product or any part of the product may not contain substances meeting the criteria with the hazard statements or risk phrases (R-phrases) as described in the commission decision of 24 June 2011 (2011/381/EU). These include qualities such as carcinogenic potential, potential to cause birth defects, toxicity to aquatic life, hazards to ozone layer, etc. Moreover, it may not contain substances referred to in Article 57 of Regulation (EC) No 1907/2006. Concentration limits for substances meeting criteria of Article 57 (a), (b) or (c) of EC No 1907/2006 shall not exceed 0.010 % (w/w) and if specific concentration limits are referred to, they should remain below one tenth of the lowest specific concentration value unless this value falls below 0.010 % (w/w).

Criteria 2: Exclusion of specific substances

Substances, which are present in a concentration > 0.010 % (w/w) in the final product, may not be mentioned on the Union list of priority substances in the field of water policy. Moreover, no organic halogen compounds, nitrite compounds, metals and metallic compounds may be present in a concentration > 0.010 % (w/w). An exemption is made for sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) and for thickeners containing lithium (Li) or aluminium (Al) up to a certain concentration.

Criteria 3: Additional aquatic toxicity requirements

The aquatic toxicity needs to be evaluated for (1) the lubricant and its main components (= substances accounting for more than 5 % by weight of the lubricant) or for (2) each stated substance present above 0.10 % (w/w). Experience has learned that in nearly all current applications the second option is used.

In case that the lubricant and its main components are evaluated, acute aquatic toxicity data shall be provided for the lubricant on 3 trophic levels (algae, daphnia & fish), while for the main components data shall be provided on 2 trophic levels (algae & fish). Only (72hr)E_rC_{50} for algae (OECD 201, ISO 10253 or C.3 of EC No 440/2008[13]), (48hr)EC_{50} for daphnia (OECD 202, ISO TC 147/SC5/WG2 or C.2 of EC No 440/2008) and (96hr)LC_{50} for fish (OECD 203 or C.1 of EC No 440/2008) are accepted. The critical concentration for the acute aquatic toxicity for the lubricant is at least 100 mg/l for categories I or V or at least 1000 mg/l for categories II, III or IV. The critical concentration for the main components is at least 100 mg/l.

In case that each stated substance present above 0.10 % (w/w) is evaluated, chronic toxicity test results in the form of NOEC data shall be provided on 2 trophic levels (daphnia (C.20 of EC No 440/2008) and fish (C.14 of EC No 440/2008)). In case chronic toxicity results are missing, acute aquatic toxicity data shall be provided on 2 trophic levels (algae and daphnia). Only (72hr)E_rC_{50} for algae (OECD 201, ISO 10253 or C.3 of EC No 440/2008) and (48hr)EC_{50} for daphnia (OECD 202, ISO TC 147/SC5/WG2 or C.2 of EC No 440/2008) are accepted. The maximum cumulative mass concentration of substances exhibiting a certain degree of toxicity is given in Table 41.

If the substance is already stated on the Lubricant Substance Classification list or if a valid letter of a competent body can be submitted, no aquatic toxicity study needs to be conducted. Moreover, an aquatic toxicity is also not required if (1) the molecular weight is > 800 g/mol, (2) the molecular diameter is > 1.5 nm, (3) molecular weight fraction < 1000 g/mol of a polymer is less than 1 % or (4) substance is highly insoluble in water (< 10 µg/l) as such substances are not regarded as toxic for algae and daphnia in the aquatic system.

[13] Council regulation EC No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation No 1907/2006 of the European Parliament and the Council of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

Table 41. Cumulative mass percentages (%w/w) of substances in the different lubricant categories with regard to aquatic toxicity.

Aquatic toxicity		Category 1	2	3	4	5
Not toxic	Acute toxicity > 100 mg/l OR NOEC > 10 mg/l			Not limited		
Harmful	10 mg/l < Acute toxicity ≤ 100 mg/l OR 1 mg/l < NOEC ≤ 10 mg/l	≤ 20	≤ 25	≤ 5	≤ 25	≤ 20
Toxic	1 mg/l < Acute toxicity ≤ 10 mg/l OR 0.1 mg/l < NOEC ≤ 1 mg/l	≤ 5	≤ 1	≤ 0.5	≤ 1	≤ 5
Very toxic	Acute toxicity ≤ 1 mg/l OR NOEC ≤ 0.1 mg/l	≤ 0.1 (M*)	≤ 0.1 (M*)	≤ 0.1 (M*)	≤ 0.1 (M*)	≤ 1 (M*)

* M is the multiplication factor of 10 for very toxic substances.

Criteria 4: Biodegradability and bioaccumulative potential

Only substances present in a concentration above 0.10 % (w/w) need to fulfill the requirements with regard to biodegradability and bioaccumulative potential. The minimum cumulative mass percentage of ultimately aerobically biodegradable substances and the maximum cumulative mass percentage of inherently aerobically biodegradable substances, non-biodegradable and non-bioaccumulative substances and non-biodegradable and bioaccumulative substances is given in Table 42.

Table 42. Cumulative mass percentages (%w/w) of substances in the different lubricant categories with regard to biodegradability and bioaccumulation.

Biodegradation and bioaccumulation	Category 1	2	3	4	5
Ultimately aerobically biodegradable	> 90	> 75	> 90	> 75	> 90
Inherently aerobically biodegradable	≤ 5	≤ 25	≤ 5	≤ 20	≤ 5
Non-biodegradable and non-bioaccumulative	≤ 5		≤ 5	≤ 10	≤ 5
Non-biodegradable and bioaccumlative	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1

Test substances are considered ultimately aerobic biodegradable if at least 70 % biodegradation is reached based on dissolved organic carbon measurements within 28 days or if at least 60 % biodegradation is reached based on oxygen depletion or carbon dioxide

generation within 28 days (C.4 of EC No 440/2008 (OECD 301 A-F, OECD 306 or OECD 310) or if the BOD_5/ThOD or BOD_5/COD ratio is $\geq$ 0.5 (C.5 and C.6 of EC No 440/2008). For the evaluation of the biodegradation the principle of the 10-day window is not required.

Test substances are considered inherently biodegradable if a least 70 % biodegradation is reached in a test for inherently biodegradability (C.9 of EC No 440/2008 or OECD 302 C) or if a biodegradation percentage between 20 % and 60 % is obtained based on oxygen depletion or carbon dioxide generation within 28 days (C.4 of EC No 440/2008, OECD 306 or OECD 310).

A biodegradation test is not required if the substance, base fluid or additive is already stated on the Lubricant Substance Classification List or if a substance is not-biodegradable. Moreover, also read-across data may be used to estimate the biodegradability of a substance.

Bioaccumulation data are only required for those substances that are not ultimately nor inherently biodegradable. Test substances are considered not potential bioaccumulative if (1) the molecular weight is > 800 g/mol, (2) the molecular diameter is > 1.5 nm, (3) log K_{ow} value of < 3 or > 7, (4) BCF $\leq$ 100 l/kg or (5) molecular weight fraction < 1000 g/mol of a polymer is less than 1 %.

Criteria 5: Renewable raw materials

The minimum carbon content derived from renewable raw materials is determined per product category. The minimum carbon content varies between 45 % (Category 2) and 70 % (Category 3) (Table 43).

Table 43. Minimum renewable content in the different lubricant categories.

Renewability	Category				
	1	2	3	4	5
Based on carbon	≥ 50 %	≥ 45 %	≥ 70 %	≥ 50 %	≥ 50 %

Criteria 6: Minimum technical performance

The minimum technical performances are determined per lubricant type.

Criteria 7: Information appearing on the EU Ecolabel

On the product an optional label may be used, which mentions that the product has a reduced harm for water and soil during use and that the product contains a large fraction of biobased material.

3.4.4.2 EU Ecolabel for outdoor paints and varnishes

The aim of the EU Ecolabel for outdoor paints and varnishes is to promote outdoor paints and varnishes, which are characterised by efficient use, a minimum of waste, a reduced environmental risk and other risks (by reducing the solvent emissions) and a reduced discharge of toxic or polluting substances into water. The ecological criteria for the award of

the EU Ecolabel to outdoor paints and varnishes is described in the commission decision of 13 August 2008 (2009/543/EC).

These criteria are related towards the white pigment content, the emissions and discharges of wastes from the production of any titanium dioxide pigment, a maximum volatile organic compounds (VOC) content, a maximum volatile aromatic hydrocarbon (VAH) content, heavy metals, restrictions towards the presence of dangerous substances and performance of the outdoor paints and varnishes.

The criteria towards dangerous substances are divided into different categories. The product may not be classified as very toxic, toxic, dangerous to the environment, carcinogenic, toxic for reproduction, harmful, corrosive, mutagenic or irritant. Moreover no ingredient classified as very toxic, toxic, carcinogenic, mutagenic and toxic for reproduction may be used according to a list of risk phrases or based on a list of categories of the Globally Harmonised System (GHS) of classification.

Moreover the use of alkylphenolethoxylates (APEOs), isothiazolinone compounds, perfluorinated alkyl sulfonates (PFAS), perfluorinated carboxylic acids (PFCA), formaldehyde, halogenated organic solvents and phthalates is forbidden or restricted.

Also the use of ingredients dangerous for the environment (defined with risk phrases ("dangerous for the environment") or on the GHS of classification) is restricted. GHS is the global (= worldwide) system for classification and labelling of chemical substances based on intrinsic hazardous properties. This system is developed by the United Nations. On European level, the CLP (= Classification, Labelling and Packaging of substances and mixtures) regulation (EC No 1272/2008 of 16 December 2008) modifies the existing European legislation is line with the regulation of GHS. This CLP regulations replaces the existing Dangerous substances directive (67/548/EG) and the Dangerous preparations directive (1999/45/EG). Especially with regard to the labelling significant modifications will be implemented: (1) signal words "Danger" and "Warning" will be implemented, (2) the R-phrases and the S-phrases will be replaced by H (= Hazard) statements and P (= Precaution) statements and (3) a new pictogram system will be applied. An overview of the existing risk phrases and categories based on the GHS of classification is given in Table 44.

Table 44. Overview of the existing classifications for environmental hazards.

Risk phrase	Description	GHS classification
N R50	Very toxic to aquatic organisms	Acute I
N R51	Toxic to aquatic organisms	Acute II
N R52	Harmful to aquatic organisms	Acute III
N R50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment	Chronic I
N R51-53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment	Chronic II
N R52-53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment	Chronic III
N R53	May cause long-term adverse effects in the aquatic environment	Chronic IV

The basic elements used for classification of aquatic environmental hazards in both systems are based on acute aquatic toxicity, degradation, biodegradation and chronic aquatic toxicity. In Table 45 an overview is given of the criteria for the different GHS categories for substances.

Table 45. Overview of the criteria of the GHS classification system for environmental toxicity of substances.

GHS classification		
Acute I	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants)	≤ 1 mg/l and/or ≤ 1 mg/l and/or ≤ 1 mg/l
Acute II	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants)	>1 - ≤ 10 mg/l and/or >1 - ≤ 10 mg/l and/or >1 - ≤ 10 mg/l
Acute III	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants)	>10 - ≤ 100 mg/l and/or >10 - ≤ 100 mg/l and/or >10 - ≤ 100 mg/l
Chronic I	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants) AND Not rapidly degradable and/or Log K_{ow}	≤ 1 mg/l and/or ≤ 1 mg/l and/or ≤ 1 mg/l ≥ 4 (unless the BCF < 500)
Chronic II	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants) AND Not rapidly degradable and/or Log K_{ow} Unless NOEC	>1 - ≤ 10 mg/l and/or >1 - ≤ 10 mg/l and/or >1 - ≤ 10 mg/l ≥ 4 (unless the BCF < 500) > 1 mg/l
Chronic III	96 hr LC_{50} (for fish) 48 hr EC_{50} (for crustacea) 72 or 96 hr ErC_{50} (for algae / other aquatic plants) AND Not rapidly degradable and/or Log K_{ow} Unless NOEC	>10 - ≤ 100 mg/l and/or >10 - ≤ 100 mg/l and/or >10 - ≤ 100 mg/l ≥ 4 (unless the BCF < 500) > 1 mg/l
Chronic IV	Poorly water soluble substances: No acute toxicity at levels up to water solubility AND Not rapidly degradable AND Log K_{ow} UNLESS BCF or NOEC	 > 4 < 500 > 1 mg/l

The approach for mixtures differs from the approach for substances. There exist different possibilities in order to classify mixtures.

(1) When a mixture has been tested as a whole, it can be classified according to the criteria for substances for acute toxicity. It is not possible to classify a mixture for chronic classification because data from biodegradability and bioaccumulation of mixtures are only meaningful for single substances. If acute toxicity data are available for the mixture as a whole, these data and classification of the components for chronic hazard shall be used.
(2) If there are no data available for the complete mixture, bridging principles can be used.
(3) Classification can also be executed when data are available for all components or only for some components of the mixture. In this case the classification is based on the summation of the classification of its components taken into account the multiplication factor.

3.4.5 OK biodegradable WATER (Vinçotte)

The Belgian certification institute Vinçotte has developed a conformity mark for products, which are biodegradable in water (Figure 15).

Figure 15. OK biodegradable WATER logo (Vinçotte).

The criteria are partly based on the European specification EN 14987 (2006) towards plastics, which are disposable in waste water treatment systems. In order to obtain the OK biodegradable WATER conformity mark, the product must meet several criteria, which are related to biodegradability, solubility or dispersibility, ecotoxicity and chemical characteristics.

A product can only be approved on the condition that it is biodegradable (90% absolute or relative biodegradation) in water at ambient temperatures (20°C – 25°C) within 56 days (ISO 14851 or ISO 14852). Moreover, the product needs to be cold water soluble or cold water dispersible (EN 14987) and the heavy metals content and fluorine content must fulfil the requirements as prescribed by EN 13432. Furthermore, the product may not contain constituents that appear on the (candidate) list of Substances of Very High Concern.

3.5 Discussion and critical review

Based on the literature review of the different ***biodegradation*** test methods in an aqueous aerobic freshwater environment it can be concluded that a sufficiently broad range of measurement techniques already exists. The biodegradation rate can be determined based on the measurement of dissolved organic carbon, dissolved oxygen, CO_2 production, oxygen consumption or inorganic carbon. Not each test method is suitable to test bio-lubricants or bio-solvents due to the fact that bio-lubricants are often poorly water soluble and that bio-solvents are often volatile. Therefore specific biodegradation test method need to be selected taken into account these characteristics.

ASTM is the only organisation, that has developed specific biodegradation testing methods in an aqueous aerobic environment towards bio-lubricants. These methods are based on CO_2 production (ASTM D 5864 & ASTM D 6139) and oxygen consumption (ASTM D 6731). However, also OECD methods, international methods and European methods based on CO_2 production (OECD 301 B, ISO 9439 & EN ISO 9439), oxygen consumption (OECD 301 C, OECD 301 F, ISO 9408 & EN ISO 9408), dissolved oxygen (OECD 301 D, ISO 10707, EN ISO 10707 & ISO 10708) and inorganic carbon (OECD 310, ISO 14593 & EN ISO 14593) are appropriate to measure the biodegradation of bio-lubricants. Methods based on dissolved organic carbon are not suitable.

No specific standards were developed towards bio-solvents, but it is possible to use the already developed methods based on oxygen consumption (OECD 301 C, OECD 301 F, ISO 9408 & EN ISO 9408), dissolved oxygen (OECD 301 D, ISO 10707, EN ISO 10707 & ISO 10708) and inorganic carbon (OECD 310, ISO 14593 & EN ISO 14593) on condition that these methods are - if necessary - slightly adapted (= lower headspace) in order to minimize volatilisation. Methods based on dissolved organic carbon and CO_2 production (especially if the produced CO_2 is purged out of the system) are not appropriate for bio-solvents.

When testing poorly water soluble test items the addition of the test item to the test system needs to be adapted when compared to the addition of water soluble test items. Therefore the addition method as prescribed in the previous mentioned OECD, ISO and EN methods need to be modified. Such modifications are already described in ISO 10634, which was especially developed towards poorly water soluble test items.

The suitability of the proposed reference materials in the above recommended guidelines towards the testing of bio-lubricants and bio-solvents can be questioned. Reference materials are necessary in order to verify the activity of the inoculum. OECD 301 and the international standards for organic compounds propose aniline, sodium acetate and sodium benzoate as suitable reference materials. The main disadvantage of these materials is that they are very easily to degrade and even degraded when no inoculum is deliberately added (Aniline: Figure 16 & Sodium benzoate: Figure 17). Since the media in biodegradation tests are not sterilized, the distilled water contains also micro-organisms and these are often sufficient to degrade the proposed reference materials.

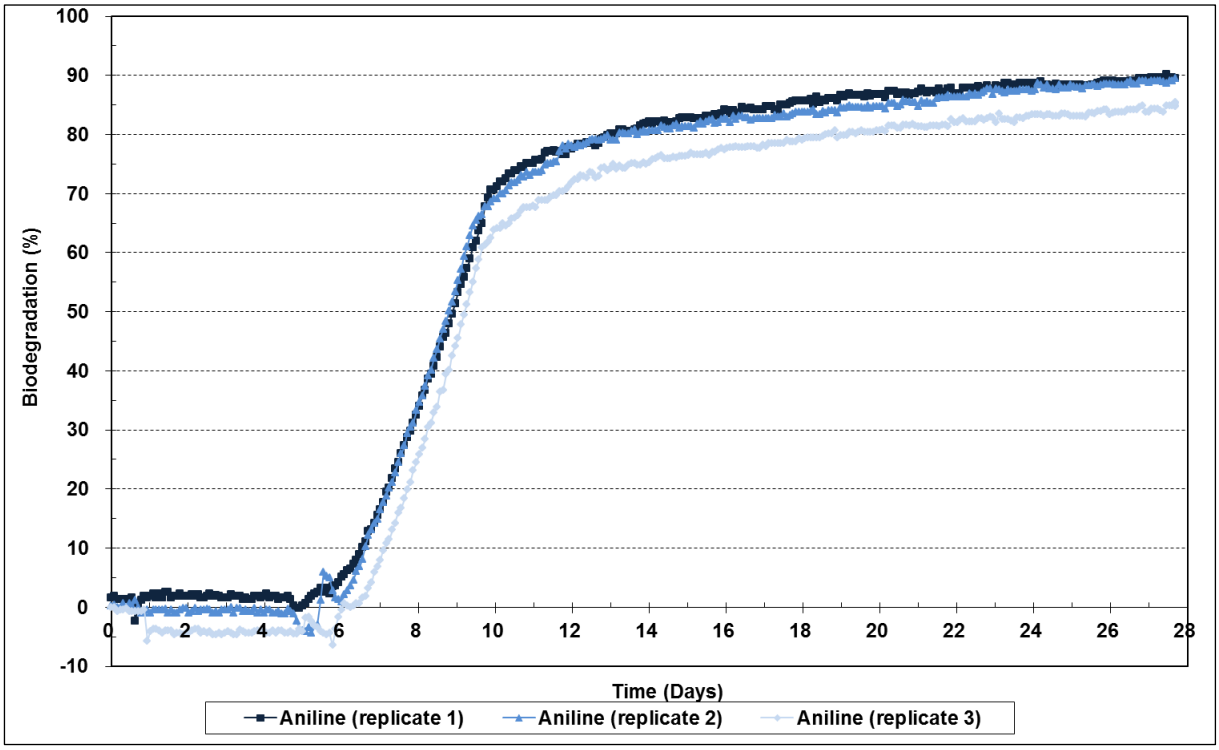


Figure 16. Evolution of the biodegradation percentage of 3 replicates of aniline (test system without inoculum based on the measurement of the oxygen consumption).

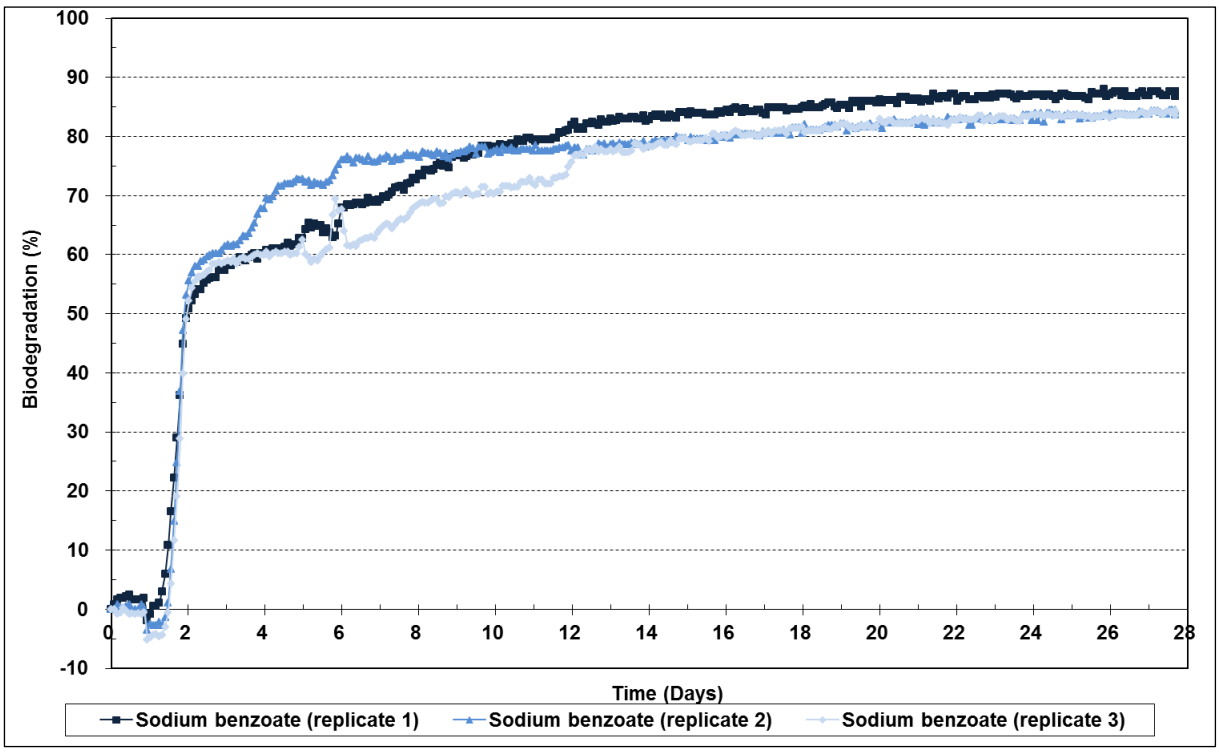


Figure 17. Evolution of the biodegradation percentage of 3 replicates of sodium benzoate (test system without inoculum based on the measurement of the oxygen consumption).

Consequently, objections can raise towards the use of these reference materials as they are not suitable in order to check the viability of the used inoculum. Therefore, it was suggested by OECD 301 to search another reference compound, which should be readily biodegradable but requires the addition of an inoculum. OECD investigated the suitability of potassium hydrogen phthalate (Figure 18) as ready biodegradable reference material, but at this moment no sufficient evidence is available.

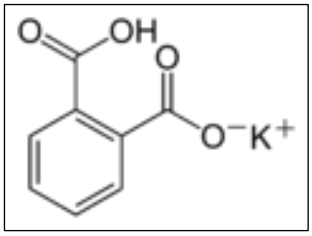


Figure 18. Structural formula of potassium hydrogen phthalate.

Another disadvantage of OECD 301 and the international standards towards organic compounds, is that they only refer to easy water-soluble chemicals as reference materials (Table 46).

Table 46. Solubility of reference materials in water (at a temperature of 20°C).

Reference material	Solubility
Aniline	34 g/l (Assink Chemie)
Sodium acetate	1190 g/l (Fisher Scientific)
Sodium benzoate	660 g/l (Chemical Book)

This can be considered as a deficit in the guidelines especially when testing bio-lubricants. OECD 310, which is a more recent guideline with regard to ready biodegradability of chemicals (2006), makes already a distinction between water-soluble (identical/comparable to OECD 301) and water-insoluble reference materials (1-octanol). However, it was demonstrated that 1-octanol also degrades without the presence of an inoculum (Figure 19). Also the American standards with regard to aerobic aqueous biodegradability of lubricants make a clear distinction between water-soluble (sodium benzoate and aniline) and water-insoluble (low erucic acid rapeseed oil) reference materials. The guidance document ECHA-12-G-22-EN for the implementation of REACH also mentions that the normal positive reference substances offer little support in the assessment of poorly soluble substances other than demonstrate that the inoculum is active (and even this can be questioned). It is suggested that in order to “bench mark” methods to assess poorly soluble substances common poorly soluble reference substances should be used (diisooctylphthalate or anthroquinone) (Figure 20) (ECHA (2012)).

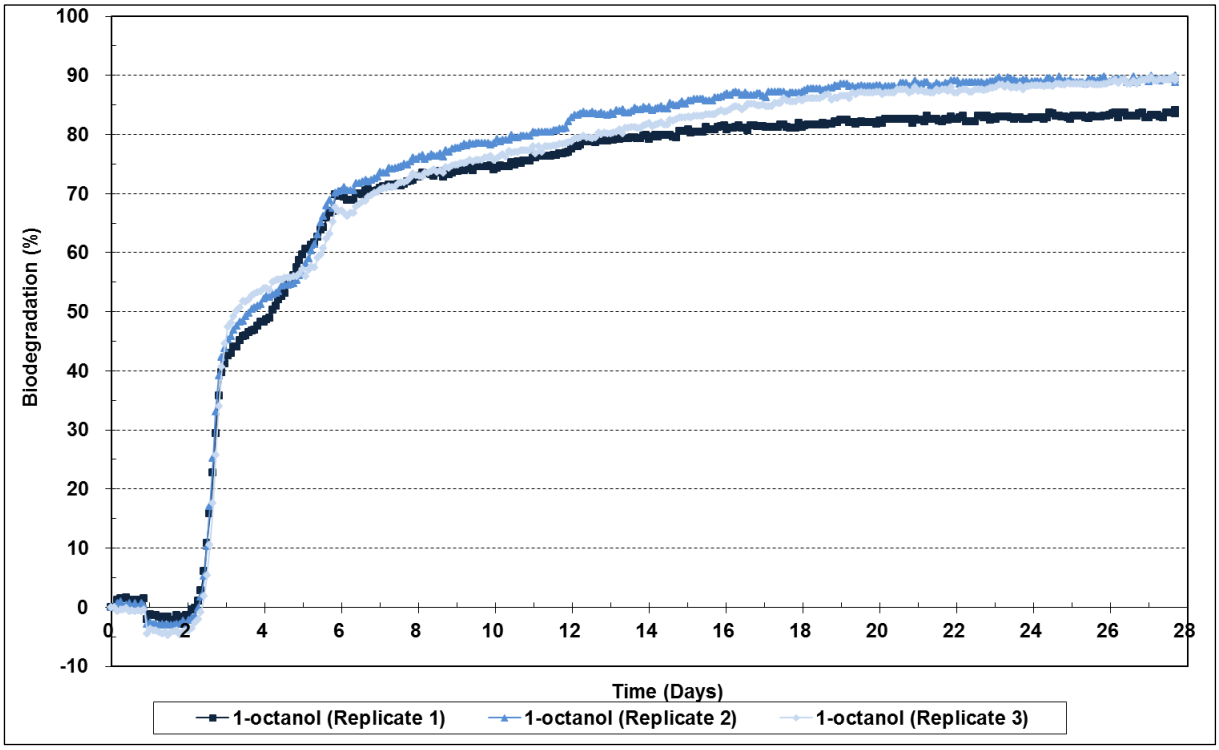


Figure 19. Evolution of the biodegradation percentage of 3 replicates of 1-octanol (test system without inoculum based on the measurement of the oxygen consumption).

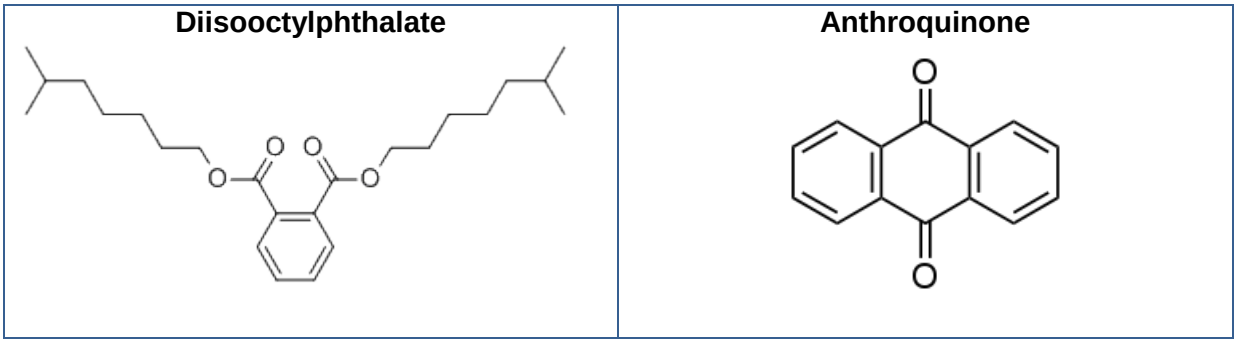


Figure 20. Structural formula of diisooctylphthalate or anthroquinone.

When testing bio-lubricants and bio-solvents, more appropriate reference materials should be chosen when compared to the currently proposed water soluble and very easily degradable reference materials as mentioned in the guidelines. A comparable system is already applied in biodegradation standards for plastics (ISO 14851 and ISO 14852). These standards still refer to a pure chemical (aniline) as reference material, but also more appropriate and challenging complex polymers (microcrystalline cellulose powder, ashless cellulose filters and poly-β-hydroxybutyrate) are mentioned as reference materials.

The biodegradation activity of the inoculum will be determined by various factors (geographical location of sampling, season of sampling, etc.). The major part of the standards refer to activated sludge, secondary effluent, surface water and soil as a suitable inoculum. It is expected that the diversity of the microbial flora and as such the

biodegradation potential will increase if more different sources are mixed in order to prepare the inoculum. Test methods OECD 301 C and OECD 302 C even prescribe that the micro-organisms need to be derived from at least 10 places (city sewage plants, industry sewage plants, rivers, lakes or sea). This will certainly increase the diversity of the bacterial community. However, after collecting the different sources, the inoculum is fed in the laboratory on a regular basis with synthetic sewage, which contains glucose, peptones and monopotassium phosphate. In spite of the fact that the microbial diversity at start is large, it is expected that the population of micro-organisms will lose their diversity due to the fact that they are always fed with the same food source.

The influence of the inoculum source on the biodegradation rate was evaluated in a study, which made a comparison between the biodegradation rate of cellulose, a biodegradable synthetic polyester (Mater-Bi NF01U) and poly(ε-caprolactone) in three different inoculum sources (sludge from a municipal wastewater-treatment plant in Novara, sludge from a municipal wastewater-treatment plant in Pero-Milan and sludge from an industrial membrane bioreactor treating wastewater from a chemical-pharmaceutical site). This study revealed that the origin of the inoculum is an important source of variability which affects the evaluation of the degree of mineralization. Sludges from different wastewater-treatment plants can be composed of different microbial populations and they can be characterized by different metabolic activities. Therefore, it was advised to define a “standard” inoculum, formed by mixing specific microbial strains conserved in microbial collections and known to possess, all together, all the relevant biochemical properties (Mezzanotte et al. (2005)).

Standardised inocula are already commercially available. A standardised inoculum could be a solution in order to increase reproducibility of test methods and this could reduce the dependence on seasonal and geographical variability. However, it still needs to be evaluated if they are divers enough in order to degrade difficult products. The diversity of standardised inocula should be compared to the diversity of natural inocula.

Another difficulty in the existing aerobic aqueous biodegradation test is related towards the high variation, which is sometimes observed for materials that require an adaptation phase. Test materials, which are either easily or poorly biodegradable, normally produce rather similar test results. On the other hand it is often noticed that the reproducibility of aqueous aerobic biodegradation tests on partly or moderately biodegradable substances is often very low, even if tests had been carried out in the same laboratory, by the same technicians, with the same test procedure and with the same batches of the same materials. Such substances often need special consortia of bacteria and long adaptation periods. More investigation is required in order to minimise the variation rate in aqueous aerobic freshwater biodegradation tests.

The variation in tests based on the oxygen consumption can be caused by nitrification. Nitrification takes place if ammonium salts and nitrogen-containing test compounds are oxidized to nitrite and nitrate (Equation 3 and Equation 4).

$$NH_3 + {}^3/_2\ O_2 \rightarrow NO_2^- + H^+ + H_2O$$

Equation 3. Nitrification reaction (conversion of ammonium to nitrite).

$$NO_2^- + {}^1/_2\ O_2 \rightarrow NO_3^-$$

Equation 4. Nitrification reaction (conversion of nitrite to nitrate).

This reaction might cause interference in biodegradability assessments by reducing the pH value, which might inhibit bacterial action, and by taking up oxygen unpredictably, which can lead to problems in interpreting results of biodegradation tests based on oxygen consumption (OECD 301 C, OECD 301 F, ISO 9408, ISO 14851,…). If nitrification has occurred, the BOD values need to be corrected for interference by nitrification. In theory, it is expected that the nitrification process in the blank reactors, the reference reactors and the reactors with test item would be similar in the case the test item does not contain nitrogen. The international standards even mention that errors in case of nitrogen-free substances are normally negligible, because the oxidation of the ammonium in the medium is taken into account by the subtraction of the blank. In practice, however, it is often seen that this process shows large variations between the different reactors. This sporadic behaviour is typical for a reaction from micro-organisms which are relatively slow-growing and which are only present in a small minority in a population. Consequently, biodegradation curves based on oxygen consumption are often no correct reproduction of the biodegradation rate of a test item.

The nitrification process can be inhibited by the addition of specific inhibitors (e.g. allylthiourea, 2-chloro-6-trichloromethyl)pyridine, …). ISO 9408 and ISO 14851 mention that allylthiourea can only inhibit nitrification during short incubation periods, as it is biodegradable. Therefore addition of allylthiourea to prevent nitrification is not recommended. It is mentioned that with low inoculum concentrations nitrification will not occur, even during long incubation periods without inhibitor. From the experience of OWS, it is known that nitrification often forms a problem for aerobic aqueous biodegradation tests based on oxygen consumption. Another option is to lower the ammonium concentration in the medium or to substitute it completely by nitrate. However, interferences cannot be completely eliminated (Painter, H.A. (1995)). Further research is required with regard to the suitability of tests based on oxygen consumption especially towards biodegradation tests with a long duration.

Taken into account that a large variation can be observed in aerobic freshwater tests, it would be better to test each series at least in triplicate (as suggested by the American aquatic biodegradability standards for lubricants) instead of the duplicates as recommended by OECD and ISO. Moreover, in order to ensure that the tests are reproducible, a validation criterion with regard to a maximum standard deviation between the replicates should be implemented in each test method (currently this is only the case for OECD guidelines).

ISO 15462 (2006) mentions that the criteria for readily biodegradability as described by OECD 301 seem to be too stringent for certain chemicals. Studies showed that products, which failed the criteria for ready biodegradability were, nevertheless, found to be adequately removed in simulation tests and in the real environment. Therefore, a recent European Regulation for testing of detergents no longer stipulates the 10-days window. The limit values (> 70 % for measurements based on DOC or > 60 % based on respirometric measurements)

should only be obtained within the 28 days period. This is also the case in the European Ecolabel for lubricants.

No specific criteria is given in ASTM D 5864, ASTM D 6139 and ASTM D 6731. These test methods state that if a test material achieves a high degree of biodegradation, it may be assumed that the test material easily biodegrades in many aerobic aquatic environments. However, no specific pass level ("high" degree: > 60 %, > 70 %, > 90 %?) nor a maximum duration are given. This should be clearly defined in order to avoid miscommunications.

Based on the literature review of the available standards with regard to ***environmental safety***, it can be concluded that a sufficiently broad range of testing methods towards freshwater organisms on different trophic levels (bacteria, algae, freshwater aquatic plants, crustacean and fish) already exists. For bio-lubricants and bio-solvents, additional attention is especially needed towards the addition of poorly water soluble bio-lubricants and volatile bio-solvents to the testing systems as this can influence the test results. There exist already documents with specific guidance toward the sample preparation and the interpretation of the results for difficult substances (OECD), poorly water soluble substances (ISO 14442) and lubricants (ASTM D 6081). These guidelines should be taken into account when testing bio-lubricants and bio-solvents.

The literature review on the ***specifications*** revealed that currently no clear specification towards bio-lubricants and bio-solvents exists on European level. Therefore, there is an urgent need as standardisation can play an important role in the uptake of bio-based lubricants and bio-based solvents and consequently can help to increase market transparency by providing reference methods and criteria.

A technical report with useful recommendations for a specification towards bio-lubricants is already developed (CEN/TR 16227). This document and the specifications for the EU Ecolabel for lubricants can be taken as a guideline in order to develop a specification towards bio-lubricants. The document CEN/TR 16227 recommends that the minimum biodegradation percentage for oils is 60 % and the minimum biodegradation percentage for lubricating greases is 50 %. The biodegradation criterion as prescribed in the Ecolabel for lubricants is more progressive as it requires that the biodegradation of each component in a concentration > 0.1 % needs to be evaluated and the minimum biodegradation requirements (75 % or 90 %) refer to a minimum cumulative mass percentage that is ultimately biodegradable. This method prevents that a significant amount of non-biodegradable and potentially bioaccumulative substances are present in the lubricant. If only the final lubricant is evaluated for biodegradability and even if a biodegradation percentage of 60 % is obtained, it is still possible that a non-biodegradable and bioaccumulative component is present in the lubricant. This certainly needs to be taken into account when developing standard specifications for bio-lubricants. The system to evaluate biodegradation as mentioned by the Ecolabel can be considered as a good system to avoid that non-biodegradable and bioaccumulative substances are used in bio-lubricants. In the recommendation document and the specifications of the EU Ecolabel for lubricants no criteria are mentioned towards biodegradability and environmental safety in soil in spite of the fact that lubricants are often accidentally spilled in soil. Biodegradation and toxicity in a

soil environment are probably not required in order to avoid that it becomes too difficult for a product to fulfil the requirements. However, it might be possible that the biodegradation of some products proceeds slower in a soil environment when compared to a freshwater environment and it is possible that the lubricant concentration in soil is considerably higher than in water due less rapid dilution. Therefore, differences between biodegradation in soil and freshwater should be further investigated in order to be sure that this does not form a deficit in the standard specifications.

On international level a specification is developed towards environmentally acceptable hydraulic fluids (ISO 15380). The specification combines requirements with regard to readily biodegradability and aquatic environmental safety, but no criteria with regard to a minimum bio-based content nor biodegradation and/or toxicity in the soil environment are given. Moreover, the minimum biodegradation percentage (60 %) refers to the total lubricant and it is not mentioned that the biodegradability and the bioaccumulative potential of each component needs to be evaluated. As mentioned in the previous paragraph, this might allow the presence of non-biodegradable and bioaccumulative components in lubricants.

The review of the existing ***labelling systems*** revealed that there exist already different types of labelling systems towards bio-lubricants (EU Ecolabel for lubricants, German Der Blaue Engel ecolabel for chain lubricants for power saws (RAL-UZ 48), for lubricants and forming oils (RAL-UZ 64) and for hydraulic fluids (RAL-UZ 79)). The EU Ecolabel for lubricants is more progressive when compared to the German ecolabel as the EU Ecolabel has also specific criteria with regard to a minimum bio-based content. Although it must be noticed that the criteria with regard to renewability should not be too stringent as otherwise the industry will not be interested and the labelling system will not be used sufficiently (cfr. Nordic Swan ecolabel). It is remarkable that – in spite of the fact that bio-lubricants often contaminate soil – no biodegradation tests in soil nor toxicity tests on terrestrial organisms (earthworms or plants) are required.

No European or international labelling systems especially towards bio-solvents is developed yet. Following factors can be taken into account when developing a labelling system for bio-solvents:

- Biodegradability
- Source: bio-based
- Low environmental and human toxicity
- Performance
- No ozone depleting chemicals
- No global warming compounds
- No hazardous air pollutants
- Low or no volatile organic compounds
- Low vapour pressure
- High flash point

It is advised that a European or international labelling system towards bio-solvents should be developed in order to increase the market transparency.

4 Marine aerobic aqueous environment

The review of the marine aerobic aqueous environment has been executed by OWS.

4.1 Biodegradation

Initially biodegradation test methods in an aqueous medium were developed towards freshwater (Chapter 3.1). However, due to the growing awareness of the need to protect the marine environment against the increasing loads of chemicals, biodegradation methods were also developed for the marine environment. Biodegradation in a marine aerobic environment differs from biodegradation in a freshwater aerobic environment due to differences with regard to (1) the microbial population and (2) the chemical parameters of the water (salt content, nutrient content, etc.). Test conditions of marine environments are generally considered to be less favourable for biodegradation than those of limnic test systems.

Currently biodegradation test methods for a marine environment are developed on OECD level, ISO level and ASTM level. No European test method has been developed yet.

4.1.1 OECD guidelines

One OECD guideline with regard to the evaluation of the biodegradation of chemicals in seawater is developed yet: OECD 306 “Biodegradability in Seawater” (Adopted 17 July 1992). The results of this test give a first impression of biodegradability in seawater. If the results are positive (> 70 % DOC removal or > 60 % ThOD), it may be concluded that there is a potential for biodegradation in a marine environment. Although it must be noticed that this guideline is no simulation test as nutrients are added and the test concentration of the substance is much higher than the concentration that would be present in the sea. If a more definitive value would be required for the degree of biodegradation in seawater, other methods need to be used (e.g. simulation test in seawater using a test item concentration closer to the likely environmental concentration).

In this guideline two test methods are described: (1) the shake flask method and (2) closed bottle method. As the shake flask method is based on DOC measurements, this method is not very suitable in order to evaluate the biodegradation of bio-lubricants and bio-solvents. The closed bottle method, which is based on dissolved oxygen measurements, is more suitable for these substances.

An overview of the main parameters of these methods is given in Table 47, while the amount of replicates as prescribed by OECD 306 is given in Table 48.

Table 47. Overview of the main parameters as described in the shake flask method and the closed bottle method (OECD 306).

Parameter	Shake flask method	Closed bottle method
Suitable test items	Min. solubility: 25-40 mg C/l Not volatile No adsorption onto glass	Min. solubility: 2 mg/l (less soluble can also be tested)
Inoculum	Natural seawater (after filtration) to which nutrients are added (phosphate buffer, $CaCl_2$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$) $DOC_{seawater}$ < 20% $DOC_{test\ mixture}$	Natural seawater (after filtration) to which nutrients are added (phosphate buffer, $CaCl_2$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$)
Temperature	15-20°C	
Reference material	Sodium benzoate, sodium acetate or aniline	
Measurement technique	DOC	Dissolved oxygen
Amount of test item	5-40 mg DOC/l	2-10 mg test substance/l
Duration	60 days Can be extended	28 days Can be extended on condition that the blank BOD values remain within the 30 % limit of the O_2 in the test vessel (if this is not the case, results are not reliable due to interferences as wall growth and nitrification)
Validity	Reference substrate: comparable to results of ring test	Biodegradation reference substrate ≥ 60 % (short time span) & comparable to results of ring test Blank respiration < 30 % O_2 test vessel

Table 48. Amount of replicates as prescribed by OECD 306.

Method	Blank series	Reference series	Test series	Abiotic sterile control	Toxicity control
Shake flask method	2	1	2	1 (optionally)	1 (optionally)
Closed bottle method	> 8	> 8	> 8	2 (optionally)	6

4.1.2 International standards

One ISO standard with regard to biodegradation of organic compounds in a marine environment is developed: ISO 16221 (2001) "Water quality – Guidance for determination of biodegradability in the marine environment". This standard is based on OECD 306, but a few modifications are made with regard to the measurement techniques and the inoculum. The measurement techniques are based on established aerobic freshwater tests. The main parameters of the test method are given in Table 49. The amount of replicates is in function of the used measurement technique (Table 50). For methods in which vessels have to be sacrificed for measurements more vessels are required.

Table 49. Overview of the main parameters as described in ISO 16221.

Parameter	Description
Inoculum	Natural seawater (after filtration) (bacterial concentration: ± 10^5 cells/ml) with nutrients (phosphate buffer & $FeCl_3.6H_2O$) or artificial seawater
Temperature	15-25°C
Reference material	Sodium benzoate or aniline
Measurement technique	DOC die-away test (ISO 7827) (DOC measurements) Closed bottle test (ISO 10707) (BOD measurements) Two-phase closed bottle test (ISO 10708) (BOD measurements) CO_2 evolution test (ISO 9439) (CO_2 measurements) CO_2 headspace test (ISO 14593) (TIC measurements)
Amount of test item	5-40 mg DOC/l (ISO 7827) 2-10 mg substance/l (ISO 10707) 100 mg ThOD/l (ISO 10708) 20 mg TOC/l (ISO 9439) 20-40 mg TOC/l (ISO 14593)
Duration	60 days
Validity	Biodegradation reference material > 60 % (respirometric measurements) or > 70 % (DOC measurements) after 14 days

Table 50. Minimum amount of replicates as prescribed by ISO 16221.

Method	Blank series	Reference series	Test series	Abiotic sterile control	Toxicity control
ISO 16221	2	1	2	1 (optionally)	1 (optionally)

At this moment ISO is developing a new method in order to determine the aerobic biodegradation of plastics sunk at the sea water / sandy sediment interface. This method will simulate a habitat found in the benthic zone where sunlight reaches the ocean floor (= photic zone = sublittoral zone). The proposed test set-up consists of a solid phase (= sandy sediment) and a liquid phase (= synthetic seawater) in a closed, unstirred respirometer incubated at 20°C – 28°C. The film sample, as a disk, is put on the sediment and covered by a coverslip. Biodegradation is measured by oxygen consumption. This test method is based on the article written by Tosin et al. (2012).

4.1.3 American standards

An overview of the American standards with regard to biodegradation and weight attrition in a marine environment is given in Table 51. ASTM D 6691 and ASTM D 7473 are both referring to plastics, but ASTM D 6691 can be described as a Tier 1 test, while ASTM D 7473 is a Tier 2 test, closer to real-life conditions. In ASTM D 6691 the sample is cryogenically milled to increase the surface area and biodegradability (CO_2 production) is determined, while plastics are tested as such in ASTM D 7473 and weight loss is measured. As weight loss (= disintegration = physically fallen apart into smaller pieces) is measured, this standard cannot be used for demonstrating biodegradation (= complete mineralisation to H_2O, CO_2 and biomass). ASTM D 6692 is designed in order to determine the degree of aerobic biodegradability of polymeric compounds utilized in plastic materials by determining the level of respiration of such radiolabeled carbon compounds to radiolabeled carbon dioxide.

No specific American standards with regard to the biodegradation of lubricants or solvents are available.

Table 51. Overview of the ASTM standards with regard to biodegradation in aerobic marine environment.

Standard	Description
D 6691 - 09	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum
D 6692 - 01	Standard Test Method for Determining the Biodegradability of Radiolabeled Polymeric Plastic Materials in Seawater
D 7473 - 12	Standard Test Method for Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations

The main principles of the American standard test methods D 6691 and D 6692, which determine the biodegradability of plastic materials in marine environments, are given in Table 52.

Table 52. Overview of the main parameters as described in ASTM D 6691 – 09 and ASTM D 6692- 01.

Parameter	ASTM D 6691 - 09	ASTM D 6692 - 01
Inoculum	Synthetic seawater with pre-grown population of at least 10 aerobic marine micro-organisms Natural seawater with inorganic nutrients (0.5 g/l NH_4Cl & 0.1 g/l of $KH_2(PO_4)$)	Natural sea water with inorganic nutrients (0.5 g/l NH_4Cl & 0.1 g/l of $KH_2(PO_4)$) Marine sediment can be added to increase the microbial diversity
Temperature	30 ± 2°C	Constant temperature (no specific temperature is mentioned)
Reference material	Cellulose, chitin or Kraft paper (control for activity of the inoculum) Sodium bicarbonate and sodium sulfite (control for CO_2 sensors)	Glucose or starch (uniformly labeled by ^{14}C)
Measurement technique	Respirometer to measure the CO_2 production	Measurement of amount of radioactive polymer that had been mineralized to $^{14}CO_2$ at various time points. Bottles are sacrificed at measurement. Before measuring the produced CO_2, the pH of the samples is brought to 2.5-3 followed by 6 hours shaking. During this period the $^{14}CO_2$ is trapped in a filter paper wick with an appropriate CO_2 trapping agent.
Sample bottle	125 ml bottles	120 ml serum bottles
Amount of test item	20 mg per bottle	5-10 mg uniform ^{14}C radiolabeled polymer per bottle (Specific activity > 0.1 µCi/mg and < 5-10 µCi/mg)
Duration	Normally 10 – 90 days	Several days to several weeks
Amount of replicates	Triplicate	At least 6 bottles per series
Validity	Reference > 70 % biodegradation	No validation criteria

In spite of the fact that ASTM D 7473 is no standard test method with regard to biodegradability, the main principles of this standard is discussed in this chapter due to the relationship with ASTM D 6691. ASTM D 7473 is used to measure the weight loss as a function of time for non-floating plastic materials under continuous flow (open system) aquarium conditions. The conditions as simulated in this test are representative for aquatic environments near the coasts and near the bottom of a water body in absence of sunlight. Aquarium testing is considered as a more realistic approach of a marine environment when compared to a closed flask test as an aquarium test allows flushing, exposure to a diverse population of microbes, removal of metabolic end products, re-supply of oxygen, exposure to anoxic conditions in sediment and exposure to seasonal temperature variation of incoming seawater and natural concentration of macro- and micronutrients.

This test method can only be applied on materials, which achieve at least 30 % mineralization in test method ASTM D 6691. If a test material does not reach 30 % mineralization according to ASTM D 6691, it shall be considered non-biodegradable in the marine environment. The main parameters of test method ASTM D 7473 are given in Table 53.

Table 53. Overview of the main parameters as described in ASTM D 7473 - 12.

Parameter	ASTM D 7473 - 12
Inoculum	(1) Continuous fresh supply of natural seawater (= oxygenated seawater) (2) Continuous fresh supply of natural seawater (= oxygenated seawater) & surface marine sediment (anaerobic processes can play a role for films placed on the sediment)
Temperature	Temperature of the natural seawater is recorded at zero time and at each sampling point. Seasonal temperature fluctuations and mesophilic and psychrophilic microbes will play a role.
Reference material	-
Measurement technique	At selected time intervals, samples (triplicate) are removed from the aquarium box. The samples are rinsed and the weight of the rinsed samples is determined after drying to constant weight (35-40°C). The samples are also inspected visually (for example: blackening of the undersides of the sample). Correction is made for soluble components. Weight loss is also calculated per unit area of film.
Sample bottle	Plastic boxes
Amount of test item	0.5 by 0.5 inch pieces
Duration	180 days
Amount of replicates	3 replicates per weight determination / 5 weight determinations per test
Validity	-

4.2 Toxicity

4.2.1 Preparation of the test items

The preparation of poorly soluble or volatile substances is in detail described in chapter 3.2.1.

4.2.2 Test methods

4.2.2.1 OECD guidelines

OECD has developed several guidelines for freshwater species (chapter 3.2.2.1). The major part of the fish toxicity tests were also developed towards freshwater fish species, but in OECD 210 also a marine fish species is recommended in order to execute the test (Sheepshead minnow (Cyprinodon variegatus)).

4.2.2.2 International standards

ISO has already developed growth inhibition tests towards marine algal species (Skeletonema costatum, Phaeodactylum tricornutum and Ceramium tenuicorne) (Table 54). The principle of these tests are comparable to the growth inhibition test on freshwater alga as prescribed in OECD 201, but these marine toxicity tests need to be executed in natural or synthetic seawater to which nutrients are added.

Table 54. Overview of the international standards with regard to marine aquatic toxicity.

ISO standard	Description
10253 (2006)	Water quality – Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum
10710 (2010)	Water quality – Growth inhibition test with the marine and the brackish water macroalga Ceramium tenuicorne

4.2.2.3 American standards

ASTM has developed a broad range of toxicity tests on different marine species (luminescent marine bacterium, saltwater bivalve molluscs, saltwater mysids, estuarine and marine invertebrates, polychaetous annelids, echinoid, bioluminescent dinoflagellates, meiobenthic copepod). An overview of the available standards is given in Table 55.

Table 55. Overview of the ASTM standards with regard to marine aquatic toxicity.

Standard	Description
D 5660 – 96 (2009)	Standard Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with Luminescent Marine Bacterium
E 724 – 98 (2004)	Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs
E 1191 – 03a (2008)	Standard Guide for Conducting Life Cycle Toxicity Tests with Saltwater Mysids
E 1367 – 03 (2008)	Standard Test Method for Measuring the Toxicity of Sediment Associated Contaminants with Estuarine and Marine Invertebrates

Standard	Description
E 1463 – 92 (2004)	Standard Guide for Conducting Static and Flow Through Acute Toxicity Tests With Mysids from the West Coast of the United States
E 1562 – 00 (2006)	Standard Guide for Conducting Acute, Chronic and Life Cycle Aquatic Toxicity Tests with Polychaetous Annelids
E 1563 – 98 (2004)	Standard Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos
E 1611 – 00 (2007)	Standard Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids
E 1924 – 97 (2004)	Standard Guide for Conducting Toxicity Tests with Bioluminescent Dinoflagellates
E 2317 – 04	Standard Guide for Conducting Renewal Microplate Based Life Cycle Toxicity Tests with a Marine Meiobenthic Copepod

4.3 Standard specifications

Standard specifications especially towards bio-lubricants or bio-solvents are not yet developed for a marine environment.

The ***Convention for the Protection of the Marine Environment of the North-East Atlantic*** (OSPAR Convention) is the current legal instrument guiding international cooperation on the protection of the marine environment of the North-East Atlantic. The OSPAR Commission is made up by representatives of 15 contracting Governments and the European Commission.

Offshore chemicals that are identified by one of following criteria shall be substituted if a less hazardous (or preferably non-hazardous) substitute is available:

- Listed in the OSPAR List of Chemicals for Priority Action
- Substances considered by the authority to be of equivalent concern for the marine environment as the above mentioned category
- Inorganic combined with high toxicity
- Persistent
- Meet two of following criteria:
    - Not readily biodegradable
    - High bioaccumulation potential
    - High toxicity

The OSPAR guidelines for environmental compliance require component level testing of chemicals released to the marine environment for biodegradation, bioaccumulation and toxicity. These standards are considered to be the most appropriate for measuring the overall impact of a substance in the marine environment (United States Environmental Protection Agency, 2011). Following criteria need to be evaluated for each component:

- Persistence (biodegradation in seawater according to OECD 306)
- Bioaccumulation (K_{ow})
- Marine toxicity
    - Growth inhibition test using the marine alga *Skeletonema costatum*
    - Acute toxicity test using the marine copepod *Acartia tonsa*
    - A sediment bioassay using an amphipod *Corophium sp*
    - A fish acute toxicity test (recommendation: turbot juvenile)

ASTM D 7081 is a standard specification, which encompasses criteria (including disintegration, biodegradation and environmental impacts with regard to aquatic toxicity, metals and other toxic substances) for non-floating plastics that are designed to be biodegradable under the marine environmental conditions of aerobic marine waters or anaerobic marine sediments. This standard specification is intended to establish the requirements for labelling materials and products, as "biodegradable in marine waters and sediments" or "marine disposable". An overview of the specific requirements is given in Table 56.

Table 56. Overview of the requirements for biodegradable plastics in marine waters and sediments as prescribed by ASTM D 7081 - 05.

Parameter	Requirement as prescribed by ASTM D 7081 - 05
Density	Minimum 1.05 g/cm³
Disintegration	Monomaterial: Maximum 30 % remains in the > 2 mm fraction after 12 weeks (ASTM D 6691 under mesophilic or psychrophilic conditions)
Biodegradation	Minimum 30 % relative biodegradation (ASTM D 6691) within 180 days at 30 ± 2°C Minimum 90 % biodegradation in an active environment (compost) (ASTM D 5338) Satisfactory rate of biodegradation in test method ASTM D 5338 within 180 days or 365 days (for radiolabeled materials): 1. Homopolymer: minimum 60 % relative biodegradation OR 2. Other polymers and substrates: minimum 90 % relative biodegradation OR 3. Products (> 1 polymer): each polymer in a concentration > 1 %: 60 % relative biodegradation Remark: Plastics used as coating or binder, need to be tested separately and need to reach 90 % biodegradation
Toxicity	Products shall not adversely impact on the survival of marine organisms nor adversely affect the ecosystem using one of following methods: 1. Polytox (microbial oxygen absorption) 2. Microtox (microbial bioluminescence) 3. Fish acute toxicity (OPPTS 850.1075) 4. Daphnia acute toxicity (OPPTS 850.1010) 5. Static algal toxicity (OPPTS 850.5400) Heavy metals < 25 % of those prescribed in the country where the product is sold
Others	Compliance to ASTM D 6400

4.4 Labelling

The product group “lubricants” as described in the chapter with regard to labelling systems related to freshwater aerobic aqueous environments (Chapter 3.4) often contains applications for the shipping industry. For example:

- EU Ecolabel for lubricants
  - Category 2: Stern tube greases
  - Category 3: Stern tube oils
  - Category 5: Marine gear oils

In the EU Ecolabel for lubricants it is not specified that a marine biodegradation test or environmental safety towards marine species must be evaluated if the product is used in a marine environment. However, the commission decree specifies that besides a freshwater biodegradation test (Part C.4 of the Annex to Regulation (EC) No 440/2008 (= DOC Die-Away, Modified OECD Screening DOC Die-Away, CO_2 Evolution, Manometric Respirometry, Closed Bottle or MITI) or OECD 310) a marine biodegradation test (OECD 306) can be used in order to determine if a substance is ultimate biodegradable or inherently biodegradable.

In the European Ecolabel application pack for lubricants it is mentioned that the applicant will select the most appropriate test method according to the intended use for the candidate lubricant, marine or freshwater applications (e.g. in a marine environment the biodegradation is established in marine water). It is also mentioned in the European Ecolabel application pack for lubricants that both sea and freshwater toxicity tests are allowed.

Consequently, it is possible to evaluate biodegradability and aquatic toxicity in a marine environment for marine applications.

4.5 Discussion and critical review

From the literature review on the ***biodegradation*** test methods in a marine aerobic environment, it can be concluded that there exist considerably less biodegradation test methods when compared to a freshwater aerobic environment. However, it can still be concluded that a sufficiently broad range of methods exists in order to determine the biodegradation in a marine environment.

Not all methods are suitable in order to evaluate the biodegradability of bio-lubricants and bio-solvents, but a suitable measurement technique can be selected taken into account the properties (volatility and/or solubility) of the bio-lubricant or the bio-solvent. Following methods are normally appropriate:

- OECD 306 (closed bottle test)
- ISO 16221 (measure method based on ISO 10707, ISO 10708, ISO 9439 (only for bio-lubricants) or ISO 14593)

Comparable to the freshwater biodegradation tests, the addition method of the poorly soluble and volatile substances to the test reactors needs to be adapted when compared to the addition of water soluble test items. Such modifications are already described in ISO 10634, which was especially developed towards poorly water soluble test items.

In spite of the fact that all methods measure biodegradation in a marine environment, there exist differences with regard to the preparation of the seawater inoculum. OECD 306 only refers to natural seawater, while ISO 16221 allows the use of natural seawater and artificial seawater. If natural seawater is used as inoculum, seasonal and geographic variations will influence the biodegradation activity. Moreover, it is noticed that the addition of inorganic nutrients to the natural seawater also varies between the different methods. OECD 306 prescribes that a phosphate buffer, $CaCl_2$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$ is added to the seawater, while only the inorganic nutrients phosphate buffer and $FeCl_3.6H_2O$ are allowed by ISO 16221. The nutrient availability can also influence the biodegradation results. Further research should be executed in order to reduce inoculum variability.

The developed biodegradability test methods simulate aerobic environments in seawater. Tosin M. et al. (2012) suggests that a test methodology with regard to biodegradability of bioplastics in a marine environment should be developed per different habitat (supralittoral, eulittoral, sublittoral benthic, deep sea benthic, pelagic & buried in the sediments) as there exist large differences with regard to light, oxygen concentration, microbial community, friction and nutrient concentrations between these environments. Further research should be executed in this area.

From the review of the standards with regard to ***marine environmental safety*** it can be concluded that less tests were developed when compared to freshwater environment safety especially on OECD level. ISO and ASTM are already more progressive as more guidelines towards marine organisms were developed. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances (OECD), poorly water soluble substances (ISO 14442) and lubricants (ASTM D 6081) should be taken into account.

Currently no ***standard specifications*** towards more environmentally friendly alternatives for lubricants and solvents used in a marine environment are developed yet.

The ***EU Ecolabel system*** for lubricants described in the chapter with regard to the freshwater environment (Chapter 3.4.4.1) also covers applications in the shipping industry in a marine environment (stern tube lubricants, etc.). The European Ecolabel application pack for lubricants mentions that the applicant needs to select the most appropriate test method according to the intended use for the candidate lubricant (e.g. in a marine environment the biodegradation is established in marine water). Due to differences between a freshwater and a marine environment, it is indeed better to execute the tests in a marine environment. This should also be recommended in the commission decision of 24 June 2011 (2011/381/EU). If it is allowed that both freshwater and marine tests can be used in order to obtain an Ecolabel for marine applications, more research should be executed, which compares results of freshwater tests with results of marine tests.

The application of the marine test environment for organic substances, which might be accidentally spilled in the sea, is already clearly defined in the regulation of the OSPAR Convention, which requires that both biodegradation and environmental safety are tested using marine biodegradation and marine toxicity methods, and in the American standard specification for non-floating biodegradable plastics in a marine environment (ASTM D 7081), which requires that biodegradation needs to be tested in a marine environment (ASTM D 6691). ASTM D 7081 requires that a high biodegradation percentage (60 % for homopolymers or 90 % for other polymers) needs to be obtained in a biodegradation test in a compost environment, but the biodegradation criterion in a marine environment (only 30 %) as prescribed by this standard specification is very low. For example: a plastic material composed of 50 % component A, which is only biodegradable at high temperatures, and 50 % component B, which is biodegradable at high and low temperatures, will easily reach the 90 % biodegradation criterion in a controlled composting environment at 58°C and will also easily reach 30 % biodegradation criterion in a marine environment due to the degradation of component B. However, as component A needs a thermal trigger in order to biodegrade, component A will not be degraded in a marine environment. Such materials are already on the market. This criterion should better be revised. For the evaluation of the toxicity, it is not required that the proposed test are executed using marine species.

5 Anaerobic environment

The review of the anaerobic environment has been executed by OWS.

5.1 Biodegradation

Anaerobic biodegradation tests in an aquatic environment are especially suitable for substances, which adsorb onto activated sludge, and which enter in this way in anaerobic digesters in wastewater treatment plants. No methods were developed especially towards lubricants and solvents.

Besides anaerobic biodegradation tests in the aquatic environment, also methods were developed in order to determine the anaerobic biodegradation of materials (especially biopolymers) under high-solids anaerobic-digester conditions or under landfill conditions.

5.1.1 OECD guidelines

There exists one OECD guideline with regard to the evaluation of the biodegradation of organic compounds in digested sludge: OECD 311 “Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production” (Adopted 23 March 2006). This guideline is based on ISO 11734 (1995) “Water quality – Evaluation of the “ultimate” anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production”. This international standard is described in chapter 5.1.3. An overview of the main parameters of OECD 311 is given in Table 57.

Table 57. Overview of the main parameters as described in OECD 311.

Parameter	OECD 311
Inoculum	Washed digested sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 10 mg/l). Medium contains also resazurin.
Temperature	35°C ± 2 °C
Reference material	Sodium benzoate, phenol or polyethyleneglycol 400 (Figure 21)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure meter connected to a syringe needle during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.
Bottle volume	0.1 l – 1 l
Amount of test item	20-100 mg organic C/l
Replicates	Triplicate for specimen bottles, blanks, reference compound and inhibition control
Duration	60 days (can be extended)
Validity	Anaerobic biodegradation reference > 60 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.

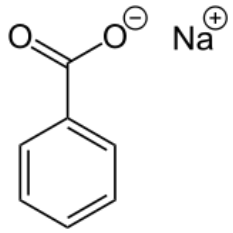
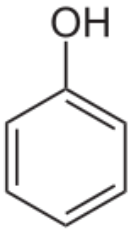
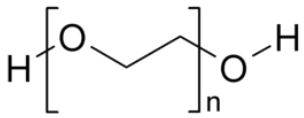
Sodium benzoate	Phenol	Polyethyleneglycol 400
		

Figure 21. Structural formula of the reference compounds as described by OECD 311.

5.1.2 European standards

The European committee for standardisation refers to the standard developed on international level (see chapter 5.1.3). An overview is given in Table 58.

Table 58. Overview of the European test method for the determination of anaerobic biodegradation of materials.

Standard	Description
EN ISO 11734 (1998)	Water quality – Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production (ISO 11734:1995)

5.1.3 International standards

ISO has developed 2 standards with regard to the anaerobic biodegradation in digested sludge (Table 59). ISO 11734 is developed in order to evaluate the anaerobic biodegradation of organic compounds, while ISO 14853 is developed for more complex products (plastic materials). An overview of the main parameters of these two methods is given in Table 60.

ISO has also developed a standard referring to anaerobic conditions under high-solids anaerobic-digestion conditions (ISO 15985). This method is an optimized simulation of an intensive anaerobic digestion process in which biodegradation and disintegration of a plastic material can be determined. An overview of the main parameters of this method is given in Table 61.

Table 59. Overview of the different international test methods for the determination of anaerobic biodegradation of materials.

Standard	Description
ISO 11734 (1995)	Water quality – Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production
ISO 14853 (2005)	Plastics – Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system – Method by measurement of biogas production
ISO 15985 (2004)	Plastics – Determination of the ultimate anaerobic biodegradation and disintegration under high-solids anaerobic-digestion conditions – Method by analysis of released biogas

Table 60. Overview of the main parameters as described in ISO 11734 (1995) and ISO 14853 (2005).

Parameter	ISO 11734 (1995)	ISO 14853 (2005)
Inoculum	Washed sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 10 mg/l). Medium contains also resazurin.	Washed sludge (preferably anaerobically digested for 5 days) from a sewage treatment plant treating predominantly domestic sewage is added to a mineral medium in order to obtain a total solids concentration of 1 g/l to 3 g/l in the vessels (IC test solution < 20 mg/l). Medium contains also resazurin.
Temperature	35°C ± 2 °C	35°C ± 2 °C
Reference material	Sodium benzoate, phenol or polyethyleneglycol 400	Poly-β-hydroxybutyrate, cellulose or polyethyleneglycol 400 (Figure 22)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure meter connected to a syringe needle during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.	Manometric or volumetric measurement of gas production (CH_4 & CO_2) during the test and measurement of inorganic carbon (= evolved CO_2 which is transformed to hydrogen carbonate or carbonate) at the end of the test.
Bottle volume	0.1 l – 1 l	0.1 l – 1 l
Amount of test item	20 – 100 mg organic C/l	20 – 200 mg organic C/l
Replicates	Triplicate for specimen bottles & blanks and singular for reference compound and inhibition control	Triplicate for specimen bottles & blanks and singular for reference compound and inhibition control
Duration	60 days	Normal duration: 60 days Maximum duration: 90 days
Validity	Anaerobic biodegradation reference > 60 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.	Anaerobic biodegradation reference > 70 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded. Gas production in inhibition vessels shall be at least equal to that in the vessel containing only reference substrate.

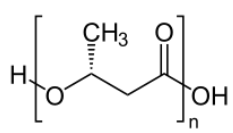
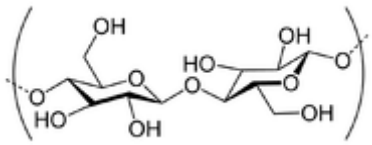
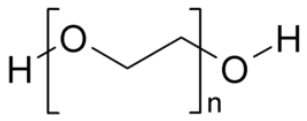
Poly-β-hydroxybutyrate	Cellulose	Polyethyleneglycol 400
		

Figure 22. Structural formula of the reference compounds as described in ISO 14853 (2005).

Table 61. Overview of the main parameters as described in ISO 15985 (2004).

Parameter	ISO 15985 (2004)
Inoculum	Methanogenic inoculum (> 20 % TS) from anaerobic digesters (preferable: digester under dry conditions, although it is also acceptable to derive it from wet fermentation on condition that the sludge is dewatered) operating only on pre-treated household waste. Digester shall have a maximum retention time of 30 days under thermophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 30 days. Inoculum must undergo a post fermentation (7 days). Inoculum is added as such (no dilution). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg
Temperature	52°C (± 2°C) (thermophilic conditions)
Conditions	Static non-mixed
Reference material	Cellulose (particle size < 20 µm)
Measurement technique	Measurement of total carbon in gas (CO_2 and CH_4): produced gas volume is measured and optionally CO_2 and CH_4 concentration in the produced gas is measured.
Bottle volume	Minimum 750 ml
Amount of test item	15-20 g volatile solids test item per 1000 g wet weight inoculum (dry solids inoculum > 20 %) For evaluation of disintegration: maximum surface area = 2 cm × 2 cm
Replicates	Triplicate for all series
Duration	15 days or longer until a plateau in biodegradation has been reached
Validity	Cellulose > 70 % biodegradation (after 15 days) Difference between % biodegradation of the reference material in the different vessels is < 20 % of the mean value

5.1.4 American standards

ASTM has developed 2 standards with regard to the anaerobic biodegradation in municipal sewage sludge (Table 62). These standards are representative for the anaerobic part of a wastewater plant. ASTM E 1196 is developed in order to evaluate the anaerobic biodegradation of organic chemicals, while ASTM D 5210 is developed for more complex products (synthetic plastic materials including formulation additives). An overview of the main parameters of these two methods is given in Table 63. The measurement techniques as prescribed by ASTM D 5210 are more progressive when compared to ASTM E 1196.

The other methods (ASTM D 5511 and ASTM D 5526) are referring to anaerobic degradation under dryer conditions. ASTM D 5511 has been developed to permit the determination of the rate and degree of anaerobic biodegradability of plastic products when placed in a high-solids anaerobic digester for the production of digestate from municipal solid waste. Biodegradation of plastic materials is an important phenomenon as if affects the decomposition of other waste materials enclosed by the plastic and it influences the quality and the appearance of the digestate. ASTM D 5526 refers to anaerobic biodegradation of plastic materials in an accelerated-landfill test environment. This method resembles landfills in which the generated gas is recovered and/or actively promoted (by inoculation, moisture control and temperature control). An overview of the main parameters of these methods is given in Table 64.

Table 62. Overview of the different American test methods for the determination of anaerobic biodegradation of materials.

Standard	Description
E 1196 – 92	Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals
D 5210 – 92	Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge
D 5511 – 12	Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High Solids Anaerobic Digestion Conditions
D 5526 – 12	Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions

Table 63. Overview of the main parameters as described in ASTM E 1196 - 92 and ASTM D 5210 - 92.

Parameter	ASTM E 1196 – 92	ASTM D 5210 - 92
Inoculum	Sieved sludge (fresh sludge is recommended) from an anaerobic sludge digestor (total organic solids: 1 to 2 %) is added in a 10 % concentration to a mineral medium, which contains also resazurin (= oxidation / reduction indicator).	Sieved sludge (anaerobically digested for 7-14 days in order to reduce the background activity) from an anaerobic sludge digestor (total organic solids: 1 to 2 %) is added in a 10 % concentration to a mineral medium, which contains also resazurin (= oxidation / reduction indicator).
Temperature	35°C ± 2 °C	
Reference material	Ethanol (Figure 23)	Cellulose or starch (Figure 23)
Measurement technique	Measurement of gas production (CH_4 & CO_2) with a pressure transducer or syringe	Measurement of gas production (CH_4 & CO_2) with a pressure transducer or syringe, SOC and residual polymer weight at the end of the test
Bottle volume	160 ml	
Amount of test item	50 mg organic C/l	Not specified
Replicates	Triplicate (specimen bottles, blanks and controls)	
Duration	56 days	Until gas evolution of test compound has stopped (= two weeks without significant gas production in excess of that in the blank).
Validity	Anaerobic biodegradation reference > 50 % of the theoretical maximum. Bottles containing oxidized (pink) resazurin should be discarded.	Anaerobic biodegradation reference > 70 % (on basis of CO_2 and CH_4). Bottles containing oxidized (pink) resazurin should be discarded.

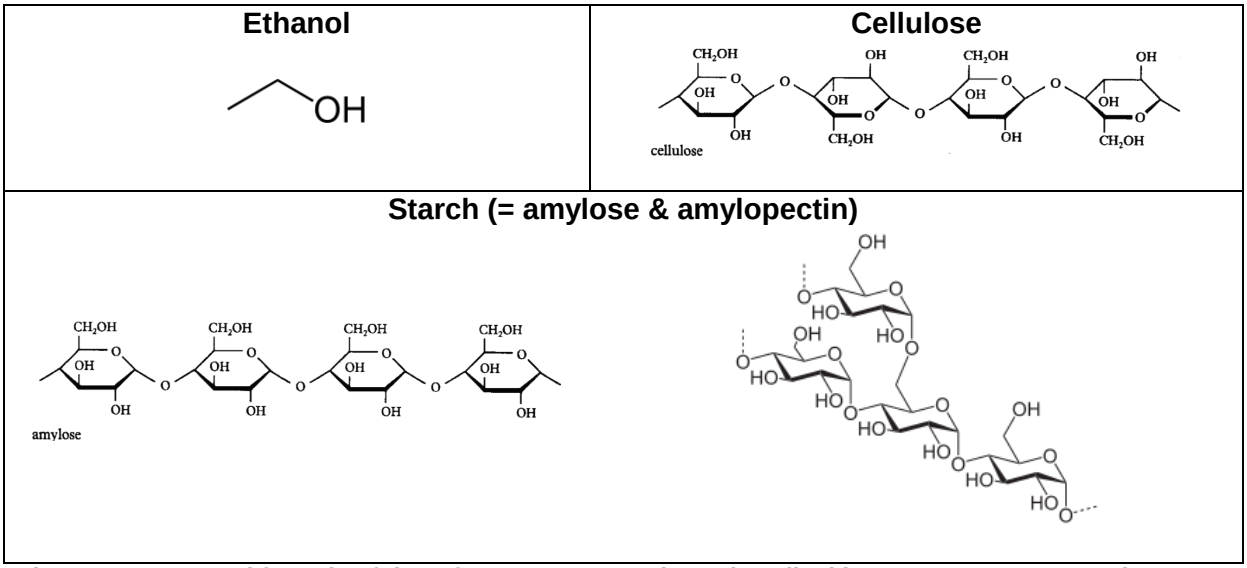


Figure 23. Structural formula of the reference compounds as described in ASTM E 1196 – 02 and ASTM D 5210 - 92.

Table 64. Overview of the main parameters as described in ASTM D 5511 - 12 and ASTM D 5526 - 12.

Parameter	ASTM D 5511 -12	ASTM D 5526 - 12
Inoculum	Methanogenic inoculum (> 20 % TS) from anaerobic digesters (preferable: digester under dry conditions, although it is also acceptable to derive it from wet fermentation on condition that the sludge is dewatered) operating only on pre-treated household waste. Digester shall have a maximum retention time of 30 days under thermophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 30 days. Inoculum must undergo a post fermentation (7 days). Inoculum is added as such (no dilution). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg	Methanogenic inoculum (> 30 % TS) from anaerobic digesters operating only on pre-treated household waste. Digester shall have a max. retention time of 30 days under mesophilic conditions and a minimum gas-production yield of at least 15 ml/g dry solids/day for at least 7 days or batch digester with a gas production > 1 l/kg waste/day and a CH_4 concentration > 60 %. Inoculum must undergo a post fermentation (7 days). Inoculum is mixed with pretreated-household waste (aerobically stabilised over 2-4 weeks by aeration and maintaining a dry-matter content of 50 ± 5% and a temperature of 55 ± 10°C). Requirements: pH: 7.5 – 8.5, VFA < 1 g/kg, NH_4^+-N: 0.5 – 2 g/kg
Temperature	37°C (± 2°C) (mesophilic conditions) 52°C (± 2°C) (thermophilic conditions)	35°C (± 2°C)
Conditions	Static non-mixed	
Reference material	Cellulose Polyethylene (optional as a negative control)	
Measurement technique	Measurement of total carbon in gas (CO_2 and CH_4): produced gas volume is measured and CO_2 and CH_4 concentration in the produced gas is measured.	Measurement of total carbon in gas (CO_2 and CH_4): produced gas is measured (based on pressure) and CO_2 and CH_4 concentration in the produced gas is measured.
Bottle volume	2 l wide mouth Erlenmeyer flask	Glass vessel (4 l – 6 l)
Amount of test item	15-100 g volatile solids test item per 1000 g wet weight inoculum (dry solids inoculum > 20 %)	60-100 g dry matter test item per 600 g dry matter pretreated household waste and 100 g dry weight mesophilic anaerobic inoculum or 150 g dry weight anaerobic inoculum from batch digester at 3 dry matter contents (35 % - 45 % and 60 %)
Replicates	Triplicate	
Duration	15-30 days	Until no significant gas production in excess of the blank during 1 week
Validity	Cellulose > 70 % biodegradation (after 30 days) Deviation among cellulose replicates < 20 % of the mean	Cellulose > 70 % biodegradation

5.2 Toxicity

When substances are accidentally spilled in the environment, they can also reach anaerobic environments. In a wastewater treatment system anaerobic sludge is present in the secondary settlement tank and in the natural environment chemicals can reach anaerobic sediments in bays, estuaries and the sea. Due to their physical properties (low solubility in water, high adsorption on suspended solids, etc.) some chemicals will preferably reach such anaerobic zones.

It is desirable that chemicals, which might enter in the environment, are biodegradable under both aerobic and anaerobic environments and it is essential that such chemicals do not inhibit the activity of the microorganisms in either zone. OECD and ISO have developed guidelines in order to measure the inhibition on the gas production of anaerobic bacteria.

5.2.1 OECD guidelines

Currently OECD has developed a guideline for testing of chemicals towards anaerobic bacteria: OECD 224 “Determination of the inhibition of the activity of anaerobic bacteria – reduction of gas production from anaerobically digesting (sewage) sludge” (Adopted: 8 January 2007). This guideline provides useful information for predicting the effect of a substance on gas production in anaerobic digesters. However, it must be noticed that only longer tests can indicate whether adaptation of the microorganisms to the test substance can occur or whether substances, which are adsorbed onto the sludge, can build up to a toxic concentration.

In this test a mixture of anaerobically digesting sludge (20 g/l to 40 g/l total solids) and a degradable substrate solution (= nutrient broth, yeast extract and D-glucose) is incubated alone and simultaneously with a range of concentrations of the test substance (500 mg/l, 250 mg/l, 125 mg/l, 62.5 mg/l, 31.2 mg/l and 15.6 mg/l) in sealed vessels up to 3 days at 35°C ± 2°C. The gas production is measured by monitoring the pressure increase in the bottles. The percentage gas inhibition is calculated from the amounts of produced gas in the control bottles and the test bottles (EC_{50}).

This guideline also allows the use of inocula from other anaerobic sites (muds, saturated soils and sediments). A few modifications are required when using these inocula (e.g. incubation temperature can be adapted at the temperature of the sample site in order to minimise the disturbance of the methane-producing consortia of bacteria, etc.).

5.2.2 International standards

Two ISO standards were developed towards the effect of substances on anaerobic bacteria: ISO 13641-1 (2003) “Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 1: General test” and ISO 13641-2 (2003) “Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 2: Test for low biomass concentrations”. The first method uses undiluted sludge and the second method uses one hundredth diluted sludge to represent muds and sediments having low bacterial populations. The principles of these tests are comparable OECD 224.

5.3 Standard specifications

No standard specifications were found for products, which may occur in an anaerobic environment due to accidental spills. Also no standard specifications are developed towards biodegradable biopolymers, which might be disposed in an anaerobic digester.

5.4 Labelling

No labelling systems were found for products, which may occur in an anaerobic environment due to accidental spills. Also no labelling systems are developed towards biodegradable biopolymers, which might be disposed in an anaerobic digester.

5.5 Discussion and critical review

Based on the review on the existing ***biodegradation standards*** in an anaerobic environment, it can be concluded that there exists a sufficiently broad range of standards in order to determine the anaerobic biodegradation in aquatic environments, high-solids anaerobic-digestion environments and landfill environments.

Anaerobic aquatic environments (in wastewater treatment plants) can indeed be suitable in order to evaluate anaerobic biodegradability of lubricants and solvents, but high-solids anaerobic-digestion environments and anaerobic landfill environments are normally not considered as environments in which lubricants and/or solvents are disposed. These standards are more suitable for biopolymers.

The methods developed in order to determine anaerobic biodegradability of organic compounds in an aquatic anaerobic environment (OECD 311, ISO 11734 and ASTM E 1196) can be used in order to evaluate the anaerobic biodegradability for lubricants and solvents.

The American standard (ASTM D 1196) is mainly based on respirometric techniques. However, as part of the produced CO_2 dissolves in the test medium, the anaerobic biodegradation will be underestimated if only the gas production is taken into account. In order to calculate the anaerobic biodegradation more correctly, the recent guidelines (OECD 311 and ISO 11734) are all based on biogas production and inorganic carbon determination. These test methods are more appropriate in order to determine the biodegradation percentage.

Not all standards give guidance with regard to the interpretation of the results of the anaerobic biodegradation tests in aquatic environments. According to ASTM E 1196 a “high” biodegradability result in this test method is a good evidence that the test substance will be biodegradable in wastewater treatment plants, anaerobic digesters and in many natural anaerobic environments (e.g. sediments, swamps,…). No further specifications are given with regard to the interpretation of a “high” biodegradability result. OECD 311 has filled this gap by stating that complete anaerobic biodegradation can be assumed to occur if 75 % to 80 % of theoretical gas production is achieved.

One useful method was developed in order to determine ***toxicity*** towards anaerobic bacteria (OECD 224 or ISO 13641). This standard can be used for lubricants and solvents. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances (OECD), poorly water soluble substances (ISO 14442) and lubricants (ASTM D 6081) should be taken into account.

For biopolymers, which are degradable in anaerobic digestors, it might be necessary to evaluate if toxic residuals remain present in the produced digestate. This could be evaluated after an aerobic stabilisation period of the digestate (= composting phase). The produced compost can be checked on toxicity using plants or earthworms. Further research is needed in order to determine how this should be done.

Currently no ***standard specifications nor labelling systems*** are developed for products which are biodegradable in an anaerobic digester (e.g. biopolymers). This is mainly caused

by the fact that there exists a wide variation in the construction and the operation of anaerobic digestion systems. The construction and operation systems can be divided into categories based on two parameters: (1) temperature (mesophilic and thermophilic) and (2) dry solids content (wet systems and dry systems). This makes it difficult to develop one set of criteria. A standard specification should be developed, which includes criteria per operation system. Such specifications should encompass criteria with regard to anaerobic biodegradation, but also environmental safety of the produced digestate should be checked. Further investigation is required in order to develop such standard specification. This standard specification should form the basis for a new labelling system.

The labelling systems for lubricants (Chapter 3.4) do not refer to anaerobic environments in order to evaluate biodegradability and environmental safety. However, taken into account that a high percentage of ultimately aerobically biodegradable components need to be present in the major part of the labelled products, it is expected that the labelled products will already be degraded before they come in contact with anaerobic environments.

6 Soil environment

The review of the soil environment has been executed by the Agricultural University of Athens.

Biodegradable in soil plastics are defined those degradable plastics in which the degradation results from the action of naturally (i.e. in real soil conditions) occurring micro-organisms such as bacteria, fungi and algae. Most of the available international standards for biodegradable materials are designed for testing biodegradation under various conditions in a variety of media (including composting conditions), but not for testing specifically biodegradation under real soil conditions, especially in agricultural soil which is used for the production of food. This topic remains a highly controversial issue.

Organic chemicals may be introduced into the soil both intentionally and accidentally, after which they may, or may not, degrade biologically. For chemicals which do degrade, the rate of degradation can vary considerably, depending not only on the molecular structure of the chemical, but also on soil conditions such as temperature, water and oxygen availability which influence microbial activity. The activity of microorganisms often plays a major role in degradation processes. It is necessary to have laboratory tests available to estimate the rate and extent of biodegradation and thereby the persistence of organic chemicals in soil. Numerous laboratory methods are available for the estimation of aerobic biodegradation based on different specific circumstances, for example, soil type, temperature and exposure times.

6.1 Biodegradation

In this section the currently available norms and standards or, non-standardised testing methods, on testing plastics for biodegradation in soil are analysed with the aim to identify and clarify the constraints, gaps and the limitations of existing relevant testing methods, and propose needed modifications especially with regard to simulating soil conditions. This updates the relevant information presented earlier by *Briassoulis and Dejean, 2010.*

Table 65 presents an overview of the most important standard test methods for assessing biodegradation of plastics and chemicals in soil up to the year 2012.

Table 65 Overview of Standard testing methods for determining biodegradability of materials in soil.

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D 5988-12	***ASTM D 5988-96/ 2003**	Standard test method for determining aerobic biodegradation of plastic materials in soil *(Previous title: Standard test method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting)*
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 17556-2012	***ISO 17556-2003**	Plastics--determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved
ISO 11266-1994		Soil quality-Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions
French Normalisation Organisation (AFNOR)		
NF U52-001 February 2005		Biodegradable materials for use in agriculture and horticulture-mulching products-Requirements and test methods
OECD Guidelines		
304A		Inherent Biodegradability in Soil
307		Aerobic and Anaerobic Transformation in Soil

*Standard superseded by the 2012 version

6.1.1 ASTM Standards for testing biodegradability of plastics in soil

There is only one ASTM standard testing method (ASTM D 5988-12) for testing biodegradability of plastic materials in soil. No relevant ASTM standard exists for organic chemicals.

ASTM D 5988 (2003) *"Standard test method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting"* refered to the determination under laboratory conditions of the degree and rate of aerobic biodegradation of plastic materials, including formulation additives that may be biodegradable, in contact with soil, or a mixture of soil and mature compost. This test method was designed to measure the biodegradability of plastic materials relative to a reference material (e.g. cellulose or starch) in an aerobic environment. In the revised version of this specification - ASTM D 5988-12 - the title has been altered into *"Standard test method for determining aerobic biodegradation of plastic materials in soil"* and the following changes have been made with regard to the provisions of the previous version:

- In the used apparatus, the case of using vessels for testing a compost containing residual plastic material, has been erased and in the darkened chamber or cabinet, a range of allowed temperature is described between (20-28)^{o}C±2^{o}C instead of the 21±2^{o}C of the previous method.
- The soil used in the new method should be natural and fertile collected from the surface layers of fields and forests. A laboratory mixture is made of equal parts (by weight) of soil samples obtained from at least 3 diverse locations (for example, an agricultural field, a forest, and a pasture or meadow). It is advisable to avoid soil that has been exposed to pollutants that cause significant perturbations of the microbial population. The soils are preferably used fresh from the field to assure active microbiota. Air-dried or frozen soils must be reactivated before use in this test. It is preferable to use fertile soil classified as "sandy loam" in accordance with USDA classification, or "silty sand" in accordance with the German DIN classification.
- It is also acceptable in the revised version of the test that the test matrix is a mixture of natural soil and mature compost such as obtained at the end of Test Method D 5338 (ratio 1 g compost to 25 g soil).
- Validation criteria in the revised version have been expanded and are analytically described in the corresponding paragraph that follows.

Technical characteristics

- Measuring the evolved carbon dioxide as a function of time of exposure.
- Technical specifications: room temperature (e.g. 20-28˚C±2˚C); soil medium conditions: pH 6-8, moisture content to 80 to 100% of the moisture-holding capacity (MHC) of the soil (if the MHC is determined in accordance with Test Method D425; if in accordance with Test Method D2980 then 50 to 70% MHC is appropriate), C:N ratio (assuming it refers to the test-sample) is adjusted to a value between 10:1 and

20:1 by weight (e.g. with ammonium phosphate solution). The soil is sieved so that soil particle size is less than 2 mm.

Validation criteria:

- A control substance known to biodegrade (starch or cellulose) has also to be tested, in order to check the activity of the soil. If after six months less than 70% theoretical CO2 evolution is observed for the control substance, the test has to be regarded as invalid and should be repeated using fresh soil.
- The amounts of carbon dioxide evolved from the blanks (or the BOD values for the alternative measurement of oxygen consumption) shall be within 20% of the mean at the plateau phase or at the end of the test. If not, the test must be regarded as invalid and must be repeated using fresh soil.

Applicability:

- All plastic materials that are not inhibitory to the bacteria and fungi present in soil.

Equivalence:

- This test method is equivalent to ISO 17556.

6.1.2 ISO Standards for testing biodegradability of plastics and chemicals in soil

- There is one ISO Standard testing method (ISO 17556:2012) for testing biodegradability of plastic materials in soil and another one (ISO 11266:1994) for testing biodegradability of organic chemicals in soil.

ISO 17556:2012

ISO 17556:2003 with the title *"Plastics -- Determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved"* (see for details Briassoulis and Dejean, 2010) was used to determine the optimum degree of biodegradation of plastics by adjusting the humidity of the test soil. The new version of the standard is the ISO 17556:2012. This second edition cancels and replaces the first edition (ISO 17556:2003), which has been technically revised. Main technical changes include:

- A definition of the term "total organic carbon" has been added.
- The temperature of the test environment has been changed: constant to within ± 2°C in the range between 20°C and 28°C, preferably 25°C (instead of; constant temperature to within 1°C, preferably between 20°C and 25°C).
- The specifications for the analytical instrument for determining the amount of carbon dioxide evolved have been revised: according to the standard, this equipment consists of any suitable apparatus with sufficient accuracy, e.g. a carbon dioxide IR analyser or DIC analyser or apparatus for titrimetric determination after complete absorption in a basic solution or apparatus for the gravimetric determination of carbon

dioxide in accordance with ISO 14855-2 (the previous version of the standard presented only the use of carbon dioxide or DIC analyser or apparatus for Titrimetric determination after complete absorption in a basic solution).

- The description of the preparation of the test material has been revised. More specifically, this text has been rephrased and additionally it has been recommended that the test samples may be reduced in size by means of cryogenic milling.
- The description of the collection and sieving of soil has been revised. Some points of the text have been rephrased and the appropriate size of the particles of the sieved soil has been changed into 5 mm giving a preference of a size less than 2 mm (in the previous version, it was recommended particles of the soil to have size less than 2mm).
- The use of a standard soil is permitted as an alternative to natural soil. More analytically, the method describes a standard soil that constitutes of industrial quartz sand, clay, natural soil and mature compost. According to the standard, the use of this soil is very useful in determining the biodegradability of plastic materials in bulky soils (loamy or clayey soils), reducing handling and aeration problems. Also, specific salts are added to the soil preferably when adjusting the water content.
- The description of the start-up and execution of the test has been revised.
- A new annex giving examples of long-term tests has been added.
- A new annex giving the results of round-robin testing has been added.

Technical characteristics

- Measuring the oxygen demand in a closed respirometer or the amount of carbon dioxide evolved.
- The test period should typically not exceed six months. If significant biodegradation is still observed and the plateau phase has not been reached after this length of time the test may be extended up to 24 months.
- The amount of test material shall be sufficient to outweigh any variations in the background oxygen consumption or any carbon dioxide evolved from the test soil: 100-300 mg of test material to 100-300 g of soil is usually adequate. The maximum amount of test material is limited by the oxygen supply to the test system. The use of 200 mg of test material with 200 g of soil is recommended unless the soil contains an excessively large amount of organic matter.
- room temperature: constant to within ± 2°C in the range between 20°C and 28°C, preferably 25°C.
- ratio C / N: 40 / 1 for organic C of test or reference material to nitrogen in the soil.
- optimum water content of soil between 40 – 60 % of total water-holding capacity .
- pH: 6 - 8.

Validation criteria:

- The degree of biodegradation of the reference material (microcrystalline-cellulose powder, ashless cellulose filters or poly(-hydroxybutyrate)) is more than 60 % at the plateau phase or at the end of the test.
- The BOD values of, or amounts of carbon dioxide evolved from the three blanks are within 20 % of the mean at the plateau phase or at the end of the test.

If these criteria are not fulfilled, the test must be repeated using another pre-conditioned or pre-exposed soil.

Applicability:

- 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.

Equivalence:

- This test method is equivalent to ASTM D 5988.

Interpretation of the results:

- Information on the toxicity of the test material may be useful in the interpretation of test results showing a low biodegradability.

ISO 11266-94

Correspondingly, ISO 11266-1994 provides guidance on the selection and conduct of appropriate test method for the determination of biodegradation of organic chemicals in aerobic soils. Usually, during the laboratory testing, a radiolabelled compound is used allowing the determination of the rate of disappearance of the test compound and the formation of metabolites, carbon dioxide, other volatiles and non-extractable residue. The metabolites should be identified using appropriated analytical methods. The disappearance of the test compound can also be followed by specific analysis.

Technical characteristics

- If practicable, soil selected should come directly from the site where chemical contact is anticipated. However, if it is not possible to obtain samples owing to contamination which has already been introduced, the soil selected should have comparable properties. Also, the field history of the soil should be considered and recent amendments, such as tillage practices and pesticide applications should be noted.
- Substances to be tested should be pure compounds (chemical purity > 98 %). The influence of any carriers or formulation ingredients should also be considered.

- The test chemical may be added in water (depending on the solubility in water), in organic solvents, or directly as a solid (e.g. mixed in quartz sand).
- The incubation is usually carried out in the dark. However, if the contribution of algae to biodegradation needs to be considered appropriate lighting conditions should be selected.
- Incubation temperature: 25-35^{o}C is the range of maximum microbial activity in soil. For soils from temperate zones temperature between 10-25^{o}C is adequate and more representative of natural conditions.
- The water content of the soil should be appropriate for the specific goals of the study. It is usually expressed as pore-water pressure. Generally, microbial activity in soil is optimal at between -0.01MPa and -0.031MPa (measurement based on ISO 11274). Alternatively, waterholding capacity (WHC) may be used although it does not give comparable measurements between different soil samples. Maximum microbial activity is found between 40-60 % of the WHC.
- Test duration: there is no recommended minimum length of a test but, as microbial activity in soil decreases during long incubation periods, it is recommended that tests should not be continued for longer than 120 days.
- If no biodegradation is observed, the likely reasons may be:
    - the test substance is toxic
    - the test substance does not biodegrade
    - the microbial activity of the soil is zero

6.1.3 Testing method based on French norm for testing biodegradability of agricultural films in soil

The French Norm NF U52-001 (2005) determines the biodegradability of agricultural films in soil (Briassoulis and Dejean, 2010). It includes the description of the testing method, specifications and labelling of the tested film that meets the requirements set. In this section the NF U52-001 (2005) testing method for biodegradability in soil is presented.

Technical characteristics:

- C:N should be adjusted to 10:1 up to 20:1 of organic carbon in the sample to total N in the soil (by addition of monohydrate of ammonium phosphate to the soil).
- pH: 6-8; water content at 80 % of saturation; natural soil, sieved < 2 mm with organic C < 2%.
- Sample containing between 200 mg - 1g of organic carbon in 500 g of soil substrate; sample is added as fragments (with a length of 1-2 cm) or as powder.

Validation criteria for soil biodegradability testing:

- Degree of biodegradation of microcrystalline cellulose (reference material) in the soil is more than 70 % at plateau phase or at the end of a six month period.
- Replicate between the tests of the same material should not present more than 20 % relative variation.

Applicability:

- Biodegradable mulching films for agriculture and horticulture

6.1.4 OECD guidelines for testing biodegradability in soil

OECD guideline No 307 describes a method designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed by using ^{14}C-labelled material to determine the rate of transformation of the test substance, and the nature and rates of formation and decline of transformation products to which plants and soil organisms may be exposed. Such studies are required for chemicals which are directly applied to soil or which are likely to reach the soil environment.

Another method is presented in OECD Guideline No 304A where the evaluation of the mineralisation rate of a ^{14}C-labelled compound in soil is done. The method is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms.

Both test methods are described in detail in the following tables:

Table 66. Description of the OECD test methods for the measurement of the biodegradability and the transformation rate of chemicals in soil.

Guideline	Description
OECD 307	Soil samples are treated with the test substance and incubated in the dark under controlled laboratory conditions (at constant temperature 20 ± 2 °C for exposure in temperate climates, or 10 ± 2 °C in the case of colder climates and soil moisture of between 2.0 and 2.5 pF*). 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 ^{14}C-labelled material, the various mineralisation rates of the test substance can be measured by trapping evolved $^{14}CO_2$ and a mass balance, including the formation of soil bound residues, can be established. The rate and pathway studies should normally not exceed 120 days. 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.

Guideline	Description
OECD 304A	*Basic test:* A small sample of soil is treated with the ^{14}C-labelled test chemical in a biometer flask apparatus in temperature 22°C ± 2°C. Release of $^{14}CO_2$ from the test chemical is measured by means of alkali absorption and liquid scintillation counting. *Optional experiments include the following tests:* *Evaporation test*: When testing chemicals of a vapour pressure higher than 0.0133 Pa, a polyurethane foam plug is placed into the biometer flask apparatus to absorb the labelled volatile part of the parent compound and volatile metabolites for liquid scintillation counting. *Residue test*: At the point of 50 % mineralisation, the test soil may be extracted. The extractable portion of the compound, and its metabolites remaining in the soil, may be determined by liquid scintillation counting. Furthermore, data on the bound residue part may be obtained by measuring the $^{14}CO_2$ released after combustion of the soil.

* pF expresses the force with which soil particles hold water: It is a function of volumetric moisture content.

Table 67. Validity criteria of the test methods for the measurement of the biodegradability and the transformation rate of chemicals in soil.

Guideline	Validity criteria
OECD 307	*Recovery* Extraction and analysis of, at least, duplicate soil samples immediately after the addition of the test substance gives a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances. Recoveries should range from 90% to 110% for labelled chemicals and from 70 % to 110 % for non-labelled chemicals.
OECD 304A	-

6.2 Toxicity

6.2.1 OECD guidelines

An overview of the guidelines referring to the ecotoxicity of chemicals in soil medium as developed by OECD is given in Table 68.

Table 68. Overview of the OECD guidelines with regard to ecotoxicity of chemicals in soil.

Guideline	Adopted	Description
OECD 207	4 April 1984	Earthworm, Acute Toxicity Tests
OECD 208	19 July 2006	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
OECD 222	13 April 2004	Earthworm Reproduction Test (*Eisenia fetida/Eisenia Andrei*)
OECD 317	22 July 2010	Bioaccumulation in Terrestrial Oligochaetes

The principle of each ecotoxicity test presented in the above table as well as a brief summary of the test procedure is recorded in Table 67. Correspondingly, Table 68 gives the conditions for the validity of each method.

Table 69. Description of the OECD test methods for the measurement of the ecotoxicity of chemicals in soil.

Guideline	Description
OECD 207	It includes two kinds of tests for the determination of the toxicity of chemicals to earthworms: *a paper contact toxicity test (optional)*: indicates those substances likely to be toxic to earthworms in soil and requires further testing in an artificial soil. Exposure of the earthworms to test substances on moist filter paper to identify potentially toxic chemicals to earthworms in soil. Test temperature: 20°±2°C. Tests are done in the dark and for a period of 48 hours with a further optional mortality assessment after 72 hours. *artificial soil test:* gives toxicity data more representative of natural exposure of earthworms to chemicals. Earthworms are kept in samples of a precisely defined artificial soil to which a range of concentrations of the test substance has been applied. Mortality is assessed 7 and 14 days after application. In both tests, one concentration resulting in no mortality and one resulting in total mortality should be used. The mortality in the controls should not exceed 10 per cent at the end of either test.
OECD 208	Assessment of the effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil. Seeds are evaluated for effects following usually 14 to 21 days after 50 % emergence of the seedlings in the control group. Endpoint: Visual assessments of seedling emergence, dry or fresh shoot weight or height, visible detrimental effects on parts of the plant. Comparison to those of untreated control plants. The emerging plants should be maintained in controlled environment chambers, phytotrons, or greenhouses. Recommended conditions for greenhouse testing: temperature: 22 ^{o}C ± 10 ^{o}C, humidity: 70 % ± 25 %, photoperiod: minimum 16 hour light, light intensity: 350 ± 50 µE/m^2/s.
OECD 222	Assessment of the effects of chemicals in soil on the reproductive output (and other sub-lethal end points) of the earthworm species Eisinia fetida. Adult worms are exposed to a range of concentrations of the test substance. The range of concentrations is selected to cause both sub-lethal and lethal effects within 8 weeks. Mortality and growth effects on the adult worms are determined after 4 weeks of exposure. The adults are then removed from the soil and effects on reproduction assessed after a further 4 weeks by counting the number of offspring present in the soil. The reproductive output of the worms exposed to the test substance is compared to that of the control(s) by using a regression model.

Guideline	Description
OECD 317	Measurement of the bioaccumulation of a substance in terrestrial oligochaetes. Applicable to stable, neutral organic chemicals, which tend to adsorb to soils, to metallo-organic compounds, metals and other trace elements. Two phases: *uptake (exposure) phase*: replicated groups of worms are exposed to soil containing the test substance. Groups of control worms are also held under identical conditions without the test substance. The dry weight and lipid content of the test organisms are measured. Measurements are made at sampling times up to 14 days (enchytraeids) or 21 days (earthworms) until the steady-state. *elimination (post-exposure) phase:* the worms are transferred to a soil free of the test substance. This phase is always required unless uptake of the test substance during the exposure phase has been insignificant. An elimination phase provides information on the rate at which the test substance is excreted by the test organisms. Measurements are made at sampling times during 14 days (enchytraeids) or 21 days (earthworms) unless earlier analytical determination showed 90% reduction of the test substance residues in worms. The concentration of the test substance in/on the worms is monitored throughout both phases of the test.

Table 70. Validity criteria of the test methods for the measurement of the ecotoxicity of chemicals in soil.

Guideline	Validity criteria
OECD 207	The mortality in the controls series should not exceed 10 per cent at the end of the paper contact toxicity as well as the artificial soil test
OECD 208	For the control series: - the seedling emergence ≥ 70 % - the seedlings should not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants should exhibit only normal variation in growth and morphology for that particular species - the mean survival of emerged control seedlings ≥ 90 % for the duration of the study - environmental conditions for a particular species must be identical and growing media should contain the same amount of soil matrix, support media, or substrate from the same source.
OECD 222	For the control series: - each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test - the coefficient of variation of reproduction to be ≤ 30 % - adult mortality over the initial 4 weeks of the test to be ≤ 10 %. Where a test fails to meet the above validity criteria the test should be terminated unless a justification for proceeding with the test can be provided. The justification should be included in the report.
OECD 317	For both controls and treatments: - At the end of the test, the overall mortality during uptake and elimination phase should not exceed 10 % (earthworms) or 20 % (enchytraeids) of the total number of the introduced worms. - For *Eisenia fetida* and *Eisenia andrei*, the mean mass loss as measured at the end of the uptake and at the end of the elimination phase should not exceed 20% compared to the initial fresh weight at start of each phase.

6.2.2 International standards

Table 71 presents the currently available international standards that are relative to the measurement of the ecotoxicity of chemicals in soil media:

Table 71. Overview of the international standards with regard to ecotoxicity of chemicals in soil.

Standard	Description
ISO 11269-2:2012	Soil quality -- Determination of the effects of pollutants on soil flora -- Part 2: Effects of chemicals on the emergence and growth of higher plants
ISO 11268-1:2012	Soil quality -- Effects of pollutants on earthworms (Eisenia fetida) -- Part 1: Determination of acute toxicity using artificial soil substrate
ISO 22030:2005	Soil quality -- Biological methods -- Chronic toxicity in higher plants

ISO 11269-2:2012 describes a method that is applicable to the determination of possible toxic effects of solid or liquid chemicals incorporated in soil on the emergence and early stages of growth and development of a variety of terrestrial plants. It does not give an indication of damage resulting from direct contact of seedlings with the chemical in the vapour or liquid phase outside the soil environment. The method is also applicable to the comparison of soils of known and unknown quality.

ISO 11268-1:2012 is based on placing adult earthworms in a defined substrate containing the test substance in different concentrations and determining the percent mortality after 7 days and 14 days. It is not applicable to volatile substances, i.e. substances for which Henry's constant or the air/water partition coefficient is greater than 1, or for which the vapor pressure exceeds 0,0133 Pa at 25°C. It does not take into account the possible degradation of the test substance.

ISO 22030:2005 describes a method for determining the inhibition of the growth and reproductive capability of higher plants by soils under controlled conditions. Two species are recommended: a rapid-cycling variant of turnip rape and oat. The duration of test should be sufficient to include chronic endpoints that demonstrate the reproductive capability of the test plants.

By using natural test soils, e.g. from contaminated sites or remediated soils, and by comparing the development of the test plants in these soils with reference or standard control soils, the test is applicable to assess soil quality, especially the function of the soil as a habitat for plants. This method can be modified to allow use of the chronic plant assay for the testing of chemicals incorporated into soil. By preparing a dilution series of a test substance in standard control soils, the same endpoints can be measured to assess the chronic toxicity of chemicals.

6.2.3 American standards

The American standard ASTM E 1676 – 04 “Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm Eisenia Fetida and the Enchytraeid Potworm Enchytraeus albidus” covers procedures for obtaining laboratory data to evaluate the adverse effects of contaminants (for example, chemicals or biomolecules) associated with soil to earthworms (Family Lumbricidae) and potworms (Family Enchytraeidae). The methods are designed to assess lethal or sublethal toxic effects on earthworms or bioaccumulation of contaminants in short-term tests (7 to 28 days) or on potworms in short to long-term tests (14 to 42 days) in terrestrial systems.

6.3 Standard specifications

Table 72 presents an overview of standard specifications for plastics, which are biodegradable in soil. No standard specifications were developed on European or on international level.

Table 72. Overview of specifications standards for determining biodegradability in soil.

American Society for Testing and Materials International (ASTM)	
ASTM WK29802 (under development)	Standard Specification for Aerobically Biodegradable Plastics in Soil Environment
French Normalisation Organisation (AFNOR)	
NF U52-001 February 2005	Biodegradable materials for use in agriculture and horticulture - Mulching products-Requirements and test methods
Other Specifications	
Belgium Royal decree (9/09/2008) effective in July 2009	Decree specifying the norms that the products should meet to be compostable or biodegradable

6.3.1 ASTM Standards specifications for aerobically biodegradable plastics in soil

The ASTM specifications for biodegradability of plastics in soil are under development (since 2010; http://www.astm.org/DATABASE.CART/WORKITEMS/WK29802.htm). This specification covers plastics and products made from plastics that are designed to biodegrade aerobically when in contact with soil with no adverse impact on the environment by the plastics or its degradation products. Also plastics, which need the pre-treatment of light or heat to facilitate biodegradation are within the scope of this new standard specification.

The properties in this specification are those required to determine if products (including packaging) will biodegrade to predetermined acceptable limits under controlled test conditions. Further, the properties in the specification are required to assure that biodegradation of these materials will not diminish the value or utility of the soil resulting from the biodegradation process (= environmental safety).

As stated in the scope of this draft standard, although results may indicate that the tested plastic material will biodegrade under the after-mentioned test conditions at a certain rate, it is cautioned that the results of any laboratory exposure in this specification cannot be directly extrapolated to soil environments at the actual site of use or disposal since soil properties, local temperatures, and humidity ranges shall be considered as they vary widely with geography. Real-world testing is required to establish a correlation with laboratory methods.

As this draft Standard is not public yet, no specifications may be provided. Currently, the testing method ASTM D 5988-12 is used in combination with the specifications for biodegradability of plastics in soil set by other organisations of labelling schemes (see chapter 6.4: VINÇOTTE Certest Products - OK biodegradable SOIL - Initial acceptance tests).

6.3.2 Specifications defined based on French norm for biodegradable agricultural films in soil

In the framework of this specification, a classification of the products is defined based on their expected life time. Moreover, requirements with regard to biodegradability and environmental safety were developed.

Biodegradability testing requirements & criteria:

- The tests can be done in three media: water, soil, compost
- Minimum biodegradation percentage (%): 90 (water), 60 (soil), 90 (compost)
- Time (months): 6 (water), 12 (soil), 6 (compost)
- Rate of biodegradation should be reached at a minimum for two of the three media for validation of the biodegradability of the mulching film. It is not required though that the soil medium is necessarily one of the two media to be tested for the validation of its biodegradability in soil.

Environmental safety testing requirements & criteria:

- Threshold limits in heavy metals, fluorine, PCB (polychlorinated biphenyl) and PAH (polycyclic aromatic hydrocarbon) contents
- Ecotoxicity tests:
    - Emergence and growth of 1 mono and 1 dicotyledonous plants (ISO 11269-2)
    - Acute earthworm toxicity test ((FD X 31-251)
    - Growth inhibition test with Pseudokirchneriella subcapitata (NF T 90-375)

Requirements with regard to labeling:

On the Packaging label should be indicated:

- Product conforms to NF U 52-001
- Name and address of producer of the product
- Commercial name or reference of the product
- Composition: Families of the three principle components
- Length
- Width
- Thickness
- Apparent density
- Class of material: A, B, C, D, E
- Final disposal: burying / composting
- Fabrication date and lot No
- Storage condition in original packaging (temperature, humidity, light etc)

NOTE: The expiration date of the product under optimal storage conditions and in the original packaging (month, year) may be mentioned

On the Mulching Film:

The commercial name or the reference number of the material should be printed on the seam if possible otherwise it should be printed on the tube around which it is rolled.

NOTE: The expiration date of the product under optimal storage conditions (month, year) may be mentioned

6.3.3 Belgian Royal Decree for Acceptance of Compostable and Biodegradable Plastic Materials

As analytically reported in the work of Briassoulis et al. 2010, in Belgium a royal decree became effective in July 2009 that defines three properties of a product depending on its end-of-life management option: compostable, home compostable and biodegradable. This Belgium decree determines the requirements and standards that have to be fulfilled by each category of product.

Biodegradability testing requirements & criteria:

The materials should conform to the French specification NF U 52-001 for biodegradability of agricultural films in soil as described later in section. Also, the biodegradation should be at minimum 90 % absolute or relative (reference material: microcrystalline cellulose) within 24 months.

Environmental safety testing requirements & criteria:

The following ecotoxicity test is required: OECD 208 test (*Terrestrial Plant Test 208: Seedling Emergence and Seedling Growth Test)* also in combination with the ecotoxicity tests described in EN 13432 norm.

6.4 Labelling

6.4.1 VINÇOTTE Certest Products - OK biodegradable SOIL - Initial acceptance tests

The Belgian certification institute *AIB-VINÇOTTE International S.A./N.V. has established a certificate for* awarding and use of the OK biodegradable SOIL conformity mark on bioproducts. A number of normative references have been used and specific requirements have been set evaluating the biodegradation and the environmental safety of the bioproducts under test.

Biodegradability testing requirements and criteria:

- Biodegradability: 90 % biodegradation (absolute or relative to a suitable reference substrate) within a maximum period of 2 years.
- Test method: ISO 17556, ISO 11266 or ASTM D 5988.
- Biodegradability must be determined for the complete product/material or for each organic constituent present in more than 1 % of dry weight of the material.
- The total proportion of organic constituents, not tested on biodegradability, may not exceed 5 %.
- Materials of natural origin are exempted as specified in EN 13432.
- All constituents in the maximum concentrations as specified on the positive list (TS-OK-10) are regarded as fulfilling the biodegradation requirements.

Environmental safety testing requirements and criteria:

- The concentration of the test material (constituent) must be tested in soil is always at least one hundredth of the concentration in which the constituent is added to the final product (soil) on wet mass basis. No assessment of ecotoxicity is necessary for constituents accounting for less than 0.1% of the dry weight of the material of product provided that the total percentage of these constituents does not exceed 0.5% of the dry weight of this material or product. All food additive approved ingredients are regarded as fulfilling the compost quality requirements. Constituents that appear on the (candidate) list of substances of very high concern are not accepted. This must be verified for all constituents that are not tested for ecotoxicity, do not appear on the positive list and are not food additive approved ingredients.

Applicability:

- All raw materials
- All components and constituents also known as intermediate products
- All finished products
- The use of the OK biodegradable SOIL conformity mark is only allowed on finished products for horticultural and agricultural application that have a function in the same environment (soil) where they are meant to biodegrade.

6.5 Discussion and critical review

Despite that several different international and national norms and standards have been developed for testing the biodegradability of plastic materials or plastic products, only a few of them are applicable to testing biodegradability in soil and only a national one for testing biodegradability of agricultural plastic films in soil (*Briassoulis and Dejean, 2010*).

According to the few norms, testing methods, specifications and certification schemes identified for soil medium the biodegradability level of the plastic material or product should be:

- 60 to 90 %.
- obtained in a range of temperatures from 20°C to 28°C
- in a time period from 6 to 12 months (long term biodegradation: 90 % reached in 24 months)

The chosen by all norms parameter recorded to quantify the biodegradability is the evolved carbon dioxide. The lowest accepted rate of biodegradation is 60 %. The compliance requirements of the few standards dedicated to testing biodegradability of polymers in soil, including the corresponding “validity of test” requirements, adapted from (Briassoulis and Dejean, 2010), are summarised in Table 73.

Table 73. Standards Compliance Requirements for biodegradability of plastic and chemicals in soil

Standard Test Method								Standard Specification
Standard Test Method	**Method**	**Test Validity**	**Temperature (°C)**	**C:N Ratio (by weight)**	**pH of soil**	**Sample quantity Water Content (% MHC)**	**Soil Quality**	**Biodegradation Requirement/ mineralisation (%) time frame to achieve biodegradation requirements (months)**
ASTM D 5988-12	Analysis of evolved carbon dioxide; successive titrations	- more than 70% theoretical CO_2 evolution is observed for a reference material (starch or cellulose) - CO_2 evolved from the blanks (or	(**20 - 28**) ± 2	**10:1** **up to** **20:1** Amend the soil with nitrogen to give a C:N of between 10:1 and 20:1 (by	**6-8** (pH measured on a 5 parts of soil in 1part distilled water)	*Sample Quantity:* - 200-1000 mg C for 500 g soil *Water content:* - 80-100 % of moisture-holding	-Natural, fertile collected from the surface layers of fields and forests. -Laboratory mixture: made of equal parts (by weight) of	This test method is used in association with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts: Biodegradation 90 %

		BOD values) shall be within 20% of the mean at the plateau phase or at the end of the test		weight) to the added carbon in the test specimen by adding the appropriate volume of ammonium phosphate solution.		capacity (MHC) of the soil (by D425) or - 50 to 70 % (by D2980)	soil samples obtained from at least 3 diverse locations (e.g. an agricultural field, a forest, and a pasture or meadow). -Avoid soil exposed to pollutants - Soil particle size less than 2 mm	absolute or 90 % relative (EN 13432) in 24 mo)
ISO/17556-2012	Oxygen demand or evolved carbon dioxide.	*a)* Degree of biodegradation is more than 60% of reference material at the plateau phase or at the end of the test & b) Amounts	(**20 - 28**) ± 2 (Preference in 25°C)	**40:1** Organic C of test or reference material to Nitrogen in the soil (N in soil adjusted with	**6-8** pH adjusted between 6-8 (not specified how)	*<u>Sample Quantity:</u>* - 100-300 mg of test material to 100-300 g of soil is usually adequate. - 200 mg of test material	-“Standard” bulky soil , which is made of a predefined mixture containing industrial quartz sand, clay, natural	This test method is used in association with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts:

		of carbon dioxide evolved or oxygen consumed from the three blanks are within 20% of the mean at the plateau phase or at the end of the test Test duration: 6 - 24 [2]		ammonium chloride or other from a list) N should be similarly adjusted in reference material. N adjustment for soil used in blank is not mentioned		with 200 g of soil recommended *Sample conditioning:* Sample should be pulverised or small size (size affects rate) *Water content:* 40-60% of the total water holding capacity of the soil	soil (16%) and mature compost. - Natural soil collected from the surface layers of fields and forests. is sieved with particles<5mm - preferably less than 2 mm	Biodegradation 90 % absolute or 90 % relative (EN 13432) in 24 mo)
ISO 11266-1994	Determination of oxygen demand in a	*Test duration:* There is no recommende	Max microbial activity: 25-35°C (±2°C), for soils from	Properties found in natural soil	pH found in natural soil	Test substance: concentration depends	If practicable, soils selected for testing should be	This test method is used in association

	closed respirometer (based on ISO 9408). Use of radiolabelled compound to determine rate of test compound disappearance and formation of metabolites, CO_2 other volatiles, non-extractable residue. Metabolites identified using analytical methods. Test compound disappearanc	d min length for a test but, as microbial activity in soil decreases during long incubation periods, it is recommended that tests should not be continued for longer than 120d.	temperate zones: 10-25 ^{o}C ±2^{o}C)			on the experimental objectives. Water content of the soil: Pore-water pressure: microbial activity in soil is optimal between -0,01MPa & -0,031 MPa. WHC: optimal water content between 40-60% of the WHC	natural coming from the site where chemical contact is anticipated, or the soil selected should have comparable properties	with specifications for biodegradability in soil (e.g. Vinçotte Certest roducts: Biodegradation 90 % absolute or 90 % relative (EN 13432) in 24 mo)

	e can also be followed by specific analysis							
NF U52-001 February 2005 **(Part on testing method for biodegradation in soil)**	Analysis of evolved carbon dioxide.	Degree of biodegradation of microcrystline cellulose (reference material) in the soil is more than 70% at plateau phase or at the end of a six month period Replicate between the tests of the same material should not present more than 20% relative	**28**±2 (biodegradation in soil)	**10:1** up to **20:1** of organic carbon in the sample to total N in the soil. (soil N adjusted by addition of monohydrate of ammonium phosphate) Same soil should be used in the	**6-8** (measured one part of soil in 5 parts of water)	*Sample Quantity:* Sample containing between 200 mg -1 g of organic carbon in 500 g of dry soil substrate *Sample conditioning:* Sample film cut to pieces 1-2 cm or powder	- Natural soil: - Extraneous mater removed (leaves, wood, stones) sieved < 2mm - organic C < 2%	This Standard includes specifications: Biodegradation in soil **≥ 60 %** in **12** mo Reach the threshold rate of biodegradation in at least two out of three media: a) at least 60% of the maximum degradation of cellulose in soil b) at least 90%of maximum degradation of

		variation.		reference and blank test		*<u>Water content:</u>* - 80% of saturation		cellulose in compost or in water medium
Vinçotte Certest Products	ISO 17556, ISO 11266 or ASTM D5988-96	*a)* Degree of biodegradation is more than 70% (ASTM D5988) or 60% (ISO 17556) of reference material at the plateau phase or at the end of the test & b) Amounts of carbon dioxide evolved or oxygen consumed from the three blanks are within	(**20 - 28**) ± 2	According to the corresponding standard	**6-8**	According to the corresponding standard	According to the corresponding standard	This certification scheme includes specifications: Biodegradation **90 %** absolute or **90 %** relative (EN 13432) **in 24** mo

		20% of the mean at the plateau phase or at the end of the test						
Belgium Royal decree (July 2009)	Oxygen demand or evolved carbon dioxide (ISO 17566, ISO 14851, ISO 14852 or ISO 14855)	According to the correspondin g standard	**20–30**	According to the correspondin g standard	According to the correspondin g standard	According to the correspondin g standard	According to the correspondin g standard	This certification scheme includes specifications: **90** % absolute or **90** % relative Or 60 % absolute within 12 months at 20°C – 30°C & 90% absolute or relative within 6 months at 58°C in **24** mo
OECD 304A	Evaluation of the mineralisatio n rate of a ^{14}C-labelled		22°C±2°C	Range of values depending on the type	Range of values depending on the type	*Sample Quantity:* 50 g of soil and 100 ml	Several types of soil used	N/A

	compound in soil			of soil	of soil	of the radioactive test solution *Water content:* 40 % maximum water capacity.		
OECD 307	Evaluation of aerobic and anaerobic transformation of chemicals in soil using ^{14}C-labelled material	Recoveries should range from 90 % to 110 % for labelled chemicals and from 70 % to 110 % for non-labelled chemicals	20°C±2°C for temperate climates and 10°C±2°C	Organic carbon content of 0.5 - 2.5 % and a microbial biomass of at least 1 % of total organic carbon recommended	pH of 5.5-8.0	*Sample Quantity:* 50-200 g of soil (substance can be dissolved in water or, when necessary, in minimum amounts of acetone or other organic solvents in which the test substance is sufficiently	A sandy loam or silty loam or loam or loamy sand	N/A

						soluble and stable) *<u>Water content:</u>* pF between 2.0 and 2.5		

([1]) ASTM WK29802 is under development

([2]) The test period should typically not exceed six months. If significant biodegradation is still observed and the plateau phase has not been reached after this length of time the test may be extended up to 24 months.

6.5.1 Biopolymers

The major category of commercial biodegradable in soil materials concerns agricultural applications. A major consideration for biodegradable agricultural plastics, mainly biodegradable mulching films, is the fact that the end of the life management of these products will be done at the farm to reduce the management of the waste and the associated cost. So, at the present time it seems that two streams are possible for biodegradation at the farm: biodegradation in soil and biodegradation in farm yard composting. In both cases it is important that the tests should be run in media simulating the conditions of the end of life management of these materials, that is to say in soil or in farm yard composting and with a time duration corresponding to a year, maximum. If this frame of parameters and media can be achieved at the laboratory, the corresponding tests will approach the reality of the practices at the farm.

The applicability of ISO 17556:2012, as well as of ASTM D 5988-2012 and NF U52-001-2005, for biodegradable plastics in real soil conditions (e.g. agricultural biodegradable bio-based films) remains questionable because of several concerns:

- Transferability of results: there is no way to predict same results in different soils
- Validation of test (positive reference)
- Prerequisites for soil

According to De Wilde B. (2002), as far as acceptance of biodegradable plastics in soil is concerned, two main categories of testing requirements concern:

1) Biodegradation:
- 90 %
- Duration: depending on the application (under mesophilic conditions the material shall be biodegraded to at least 90 % during the time of maximum 24 months. If the material fails to reach 90 % but reaches 60 % (absolute) this is considered to be a proof that the material is potentially biodegradable).

2) Soil quality:
- Chemical: heavy metals
- Ecotoxicity

These proposed specifications for biodegradation in soil are analogous, to some extent, with those of the French Norm NF U52-001 (2005) (apart for the optional use of the soil medium by NF U52-001), extended over a longer reference period to determine long-term effects. As mentioned by *Briassoulis and Dejean, 2010* analogous provision has been adopted by SP Technical Research Institute of Sweden concerning the requirements and associated test methods to certify polymeric materials and products: the ultimate aerobic biodegradability in soil (by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved) is determined by the requirement of biodegradation ≥ 90 % within 24 months. The time frame and biodegradation rate requirements of NF U52-001 do not guarantee the achievement of the long term biodegradation in soil behaviour as proposed by De Wilde B. (2002).

Furthermore, as pointed out in (*Briassoulis and Dejean, 2010*), it should be required that the soil medium is necessarily one of the two media to be tested for the validation of the biodegradability of a biodegradable bio-based product in soil. This requirement is missing in the present version of the norm NF U52-001, and should be added, and explicitly specified as such, in a future revised version of this norm.

6.5.2 Lubricants and solvents

The only standard test method for testing biodegradability of organic chemicals (pure compounds (chemical purity > 98 %) in aerobic soil is, ISO 11266-1994.

OECD guidelines 304A and 307 describe special test methods for the determination of the mineralisation rate of a ^{14}C-labelled compound in soil and the degree of aerobic and anaerobic transformation of chemicals in soil correspondingly. However, these guidelines are very specialized and not easily applicable as they require highly specialised laboratories and the use of specially produced radiolabeled samples of the substances to be tested.

There are no standard test methods or specifications for biodegradation of bio-lubricants and bio-solvents in soil available up-to-date.

6.5.3 Major discrepancies in the biodegradation test methods

Concerning the three main standard testing methods for biodegradation in soil, ISO 17566, ISO 11266 and ASTM D5988, some major discrepancies are identified:

6.5.3.1 Soil Medium

ASTM D 5988 standard

- Soil should be natural and fertile collected from the surface layers of fields and forests.
- Laboratory mixture made of equal parts (by weight) of soil samples obtained from at least 3 diverse locations
- A mixture of natural soil and mature compost is also acceptable
- Soil pH should be 6-8. No suggestion about the case when pH is measured outside this range

ISO 17566 standard

- Recommends that the test samples may be reduced in size by means of cryogenic milling.
- The use of a “standard soil” is permitted as an alternative to natural soil that constitutes of industrial quartz sand, clay, natural soil and mature compost. This soil is considered very useful in determining the biodegradability of plastic materials in loamy or clayey soils, reducing handling and aeration problems.
- Specific salts may be added in the soil preferably when adjusting the water content.
- Soil pH should be 6-8; pH should be adjusted to meet this requirement.

ISO 11266 standard

- If practicable, soils selected for testing should be natural, coming from the site where chemical contact is anticipated, or the soil selected should have comparable properties.
- pH value is the pH found in the natural soil.

NF U52-001 standard

- Recommends that the test samples film is cut to pieces 1-2 cm or powder
- Biodegradation in soil using natural soil with organic C < 2%
- Soil pH should be 6-8; pH should be adjusted to meet this requirement

The ISO procedures may enhance or alter biodegradation of polymers drastically, but they lead to a rather controlled biodegradation process in an artificial soil that in many cases may be not representative of biodegradation of non-pulverised polymers under real soil conditions. In contrast the ASTM standard leads to biodegradation results closer to real soil conditions, which however may still be very different from some specific categories of soil types and real soil conditions. As this standard allows the test specimens to be in the form of films, pieces, fragments, powder or formed articles, biodegradability of the same material will be affected by the test specimens form. If not tested in its real form, measured biodegradability will not be realistic. Also, since the size (surface area exposed to the soil) of the sample affects the rate of biodegradation and ASTM D 5988-12 requires evolution of 70 % of the $ThCO_2$ in 6 months for the reference material, the biodegradability validation criteria may be affected by the surface area of the sample.

6.5.3.2 Ratio C/N:

ASTM D5988 standard

- C:N ratio adjusted to a value between 10:1 and 20:1 *by weight to the added carbon in the test specimen* (note defined explicitly; presumably for the C:N of test sample; needs clarification though).

ISO 17566 standard

- The ratio C:N is at least 40:1 for the sample organic C to the soil N

ISO 11266 standard

- Value as found in the natural soil

NF U52-001 standard

- The ratio C:N 10:1 up to 20:1 for the sample organic C to the soil N

Average C:N ratios of soils vary from region to region depending on the predominant soil type and the prevailing conditions (e.g. farming, climate etc). However, as a C:N ratio value between 8 and 17 is typical (Alistair F. Pitty, Geography and soil properties, Taylor & Francis, 1979), the recommendation of the ASTM standard is in better agreement with the real soil

conditions in that respect provided that the sample organic C to N (if this provision interpreted correctly) remains in the same range and so it will not affect significantly this ratio. The provision of NF U52-001 defines this ratio in the same range but for organic C over the total sample-soil N. On the other hand, the provision of the ISO standard for a ratio of the organic C of the sample to the soil N of 40:1 "*so as to ensure good biodegradation*" follows the general direction of this method for enhancing artificially biodegradation in soil. It may also be noticed that N is necessary for the biodegradation of C. Therefore high C:N may not ensure good biodegradation.

6.5.3.3 Round Robin Tests:

The most recent version of the standard ISO 17556 – 2012 provides in its Annex G the results of a Round Robin Test, which was organized among six laboratories in 2009. The objective of this test was to prove the suitability of a "standard" soil proposed in the standard for tests of biodegradation in soil. However, the presented data also demonstrate the difficulties involved in this testing method. Throughout this RR test, a test material (starch/poly(butylene adipate-co-butylene terephthalate) blend) and a reference (microcrystalline cellulose) were used following the procedure of the standard. Not all trials reached the plateau phase. Two different testing methods were considered, namely the free airflow method and the method using flasks. Only one lab used the flask option, while six labs used the free airflow method.

In order to reduce variability, only the results obtained by the free airflow method were considered in the following analysis. The results show that the degree of biodegradation of the reference material is approximately equal to 72 % with a standard deviation 18. Figure 24 shows that the results concerning the biodegradation of the reference material exhibit a typical experimental variability which follows the normal distribution (green columns).

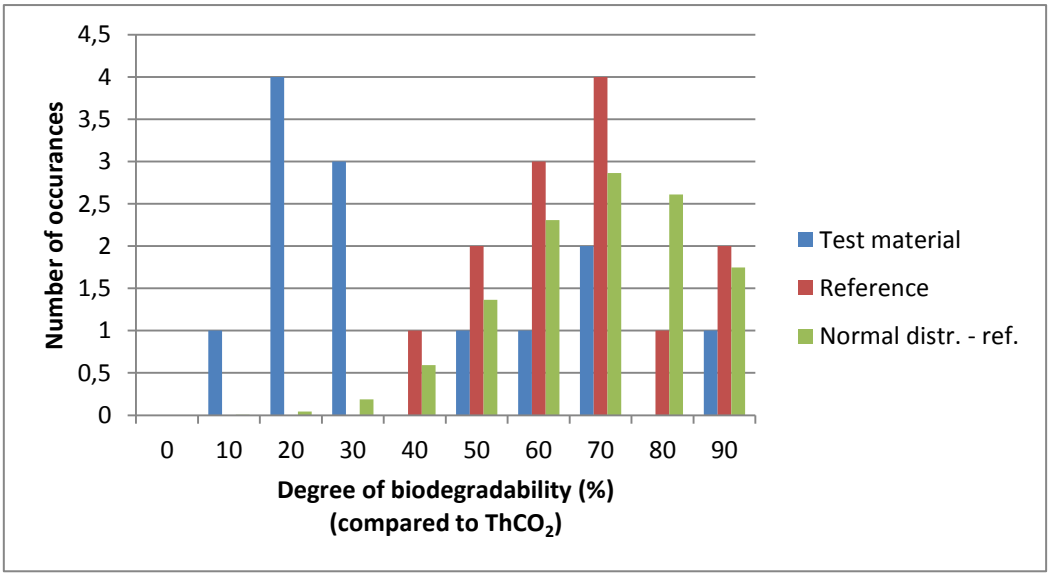


Figure 24. Statistical analysis of results obtained in six different labs for the biodegradation in soil of a test material using the Standard ISO 17556.

On the contrary, the values obtained for the test material present an erratic, almost random behavior. No explanation was offered for this high variability. Biological or morphological characteristics of the different types of soil used in this RR test may be responsible for this discrepancy. These results indicate that the current test method provides results with variable quality depending on the tested material. Therefore, further research is necessary for assessing the validity and the possible reasons of failure of the currently used testing method for a wide range of test materials.

Figure 24 presents all the data obtained through the RR test including cases where the plateau phase was not reached. However, the same statistically erratic data also appear if the results are restricted to the cases where the plateau phase was reached.

In Annex G of ISO 17556:2012 it is concluded that standard soil can help in standardizing the test procedure, as it makes use of a standard matrix, with a standard texture and particle size.

7 Industrial composting environment

The review of the industrial composting environment has been executed by the Agricultural University of Athens.

Nowadays, the terms “biodegradation”, “biodegradable materials”, “compostability” etc. are very common but frequently misused and source of misunderstanding Compostability is not only related to biodegradation, but 4 characteristics need to be taken into account (Figure 25).

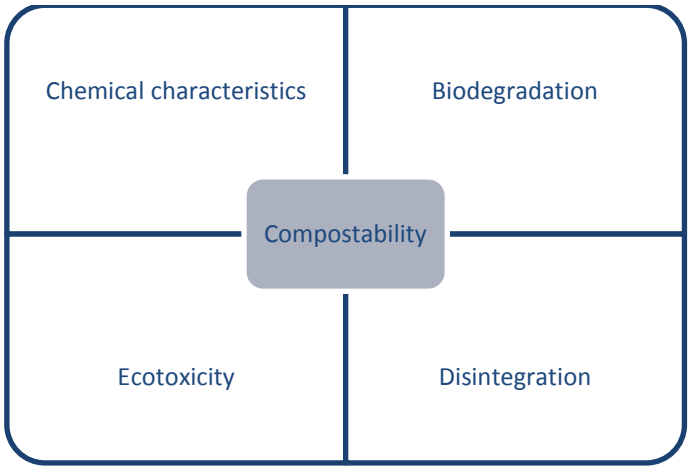


Figure 25. Overview of the main characteristics required for industrial compostability.

Two main characteristics are related to environmental safety (= compost quality): chemical characteristics and ecotoxicity. The two other characteristics are related to degradation: biodegradation and disintegration. Disintegration is the physical falling apart of the plastic material, or more precisely of the product that has been made from it, into fine visually indistinguishable fragments (dimensions < 2 mm) at the end of a typical composting cycle, while biodegradation is the complete breakdown to mineral end products (De Wilde (2002)).

Existing norms and standards on testing plastics with regard to biodegradability in industrial composting environments and compostability are reviewed in this chapter. The disintegration methods will not be discussed in detail in this study. This updates the relevant information presented earlier by Briassoulis et al. 2010.

7.1 Biodegradation

Table 74 presents a synthesis of the most important tests for assessing biodegradability in industrial composting environments and compostability of plastics up the year 2012. The norms presented in this table are all about plastic materials or packaging.

Table 74. Overview of standard testing methods for determining biodegradability of materials in compost

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D5929-96 (Reapproved 2009)		Standard Test Method for Determining Biodegradability of Materials Exposed to Municipal Solid Waste Composting Conditions by Compost Respirometry
ASTM D5338-11	***ASTM D5338-98 (2003)**	Standard test method for determining aerobic biodegradation of plastic materials under controlled composting conditions Incorporating thermophilic temperatures
ASTM D6340-98 (2007)		Standard test method for determining aerobic biodegradation of radiolabeled plastic materials in an aqueous or compost environment.
	****ASTM D5509-96**	Standard practice for exposing plastics to a simulated compost environment
	****ASTM D5512-96**	Standard practice for exposing plastics to a simulated compost environment using an externally heated reactor
	****ASTM D5951-96 (2002)**	Standard practice for preparing residual solids obtained after biodegradability standard methods for toxicity and compost quality testing—fate & effect testing
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 14855-1:2012	***ISO 14855-1:2005**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide—Part 1: General method
ISO 14855-2:2007/Cor 1:2009	**ISO 14855-2:2007**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide—Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test

European committee for standardization (CEN)		
Current versions of standards	**Previous versions of standards**	**Title**
EN 14046:2003		Packaging—Evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions—Method by analysis of released carbon dioxide.
EN ISO 14855-1:2007 + AC: 1:2009 (to be replaced by FprEN ISO 14855-1-2012)	***EN ISO 14855:2004**	Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide Part 1: General method: identical ISO 14855-1:2005/Cor 1:2009 Identical (ISO 14855-1:2005 + Cor 1:2009)
EN ISO 14855-2:2009	***EN ISO 14855:2004**	Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory scale test (ISO 14855-2:2007, including Cor 1:2009)
Deutsches Institut fur Normung (DIN)		
DIN V 54900-2		Testing of compostability of plastics—Part 2: testing of the complete biodegradability of plastics in laboratory tests.
Japanese industrial standard		
***JIS K 6953-2000**	**JIS K 6953-2011**	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions—Method by analysis of evolved carbon dioxide

*Standard superseded by its updated version

** Withdrawn standard, no replacement

7.1.1 ASTM Standards for testing biodegradability of biodegradable materials under municipal and industrial composting conditions

There are several ASTM Standard testing methods for testing biodegradability of plastic materials under industrial composting conditions. No relevant ASTM standard exists for organic chemicals.

ASTM D 5929-09

The ASTM D 5929-96 (2009) standard covers the biodegradation properties of a material by reproducibly exposing materials to conditions typical of municipal solid waste (MSW) composting. A material is composted under controlled conditions using a synthetic compost matrix and determining the acclimation time, cumulative oxygen uptake, cumulative carbon dioxide production and percent of theoretical biodegradation over the period of the test.

Technical characteristics:

- The reactors are operated for a period of 45 days; maintenance of temperature at 40^{o}C. The nitrogen content of the synthetic MSW should be adjusted if the C/N ratio is greater than 40:1.
- At the end of the run of the experiment, the total weight of the compost material and the dry solids concentration is determined. Also, the pH is measured and if it is below 7, the volatile fatty acids (VFA) content of the compost is determined.
- The total oxygen uptake and carbon dioxide produced are compared with the theoretical values obtained from the elemental analysis, and a percentage of biodegradation is generated. Possible negative effects of the material are evaluated by observing the acclimation time of the synthetic MSW and evaluating the oxygen uptake rate.

Validation criteria:

- If the VFA is > 2 g/kg, the reactors have soured and the results are invalid.
- To ensure an active and viable inoculum, the total oxygen uptake for the control reactors should exceed 80 g. If this is not observed over the 45 days then the test must be regarded as invalid and should be repeated with new inoculum.

Applicability:

This test method is applicable to any material that is designated to be disposed in municipal solid waste composting facilities

ASTM D 5338-03

The ASTM D 5338-98 (2003) standard that determines the degree and rate of aerobic biodegradation of plastic materials on exposure to a controlled-composting environment under laboratory conditions, at thermophilic temperatures, was updated into ASTM D5338-11. This latest version clarifies that the thermophilic temperatures are most readily achieved in large-scale, professionally-managed facilities. However, these temperatures may also be

reached in smaller residential composting units, frequently referred to as “backyard” or “home” composting. This test method is designed to yield reproducible and repeatable test results under controlled conditions that resemble composting conditions, where thermophilic temperatures are achieved.

Technical characteristics:

- Controlled-composting environment under laboratory conditions, at thermophilic temperatures of 58^{o}C±2^{o}C.
- Test substances are exposed to an inoculum that is derived from compost from municipal solid waste (2 – 4 months old).
- The aerobic composting takes place in an environment where temperature, aeration and humidity are closely monitored and controlled.
- Biodegradation percentage of the test item is obtained by determining the percentage of carbon in the test substance that is converted to CO_2 during the duration of the test.The net production of CO_2 is recorded relative to a control containing only mature compost.

Validation criteria:

- If more than 2 g of volatile fatty acids per kilogram of dry matter in the composting vessel is formed, the test must be regarded as invalid.
- If sufficient biodegradation - a minimum of 70 % for cellulose within 45 days - is not observed with the positive reference, the test must be regarded as invalid and should be repeated, using new inoculum.
- If the deviation of the percentage of biodegradation of the positive reference is greater than or equal to 20 % at the end of the test, then the test shall be regarded as invalid.

Applicability:

- All plastic materials, which are intended to be composted in facilities that achieve thermophilic temperatures.

Equivalence:

This test method is equivalent to ISO 14855.

ASTM D 6340-07

The ASTM D 6340-98 (2007) standard reported in Table 74 that is relevant to testing the biodegradability of plastic materials in several compost environments is briefly presented in the research work of Briassoulis et al. 2010. ASTM D 6340-98 (2007) determines the rate and degree of biological oxidation of carbon in radio-labelled plastic materials when placed in a composting environment containing simulated municipal solid waste or an aqueous environment under laboratory conditions.

Technical characteristics:

- For the case of using of compost environment: Compost for this testing may be made up with either municipal solid waste (MSW) or woody compost, or obtained from an active municipal solid waste or yard waste composting center. So, the compost, designed to stimulate MSW organic matter should contain the biochemical ingredients found in MSW (lignified cellulose, protein, natural inoculum, soluble carbohydrates and buffering capacity sufficient to maintain a neutral to slightly basic pH).
- The particle size of the components of the compost mix are sized to pass through a 6 mm screen. Moisture content should be adjusted to 55-60 % for testing.
- Addition of 200-300 mg of plastic (in pieces of ~2 cm^2) in approximately 300 g of compost.
- Controlled-composting environment in the testing chamber: temperature of $58^oC \pm 5^oC$.
- Requirement: the target component of the plastic material be synthesized using the radioactive isotope carbon-14. Depending upon the objective, either a portion of the components of the plastic or all of the carbon can be uniformly labeled with carbon-14. The test method will determine how that labeled portion will be metabolized and biologically oxidized by the microorganisms in the system tested.

Validation criteria:

- $^{14}CO_2$ measured in this test method is a direct indication of the oxidation of the sample. However, the extent and the rate of oxidation are related to the compost mixture made for that individual test and the form of the sample. Although different batches of compost can produce different results, the compost formula for the simulation of MSW in this test method will generally give repeatable results. This is due to the selection of common feed ingredients that are standardized in the trade and tend to have a consistent composition.
- Depending upon the objectives of the test, it is generally wise to include within each test series a standard preparation of known degradation rates and to make test comparisons.
- Early days of the composting process: monitoring of the viscosity of methoxyethyl amine by observing the bubble flow.
- Occasionally, two phases develop due to larger quantities of water trapped in the methoxyethyl amine, and these 2 phases can be blended into one by addition of several ml of methanol. During prolonged composting trials (over 20 days) the sampling interval can be extended depending upon the objectives of the experiments.
- In order to test whether the oxidation of the carbon in the plastic could occur chemically under the composting conditions, it may be necessary to use a sterile control. This is not necessary if the chemistry of the compound being tested is well documented and it is known that chemical oxidation does not occur under these composting conditions.

Applicability:

The test method applies to plastics the biodegradation rate of which is slow and requires test periods of as long as 365 days.

7.1.2 ISO Standards for testing biodegradability of plastics under municipal and industrial composting conditions

There are several ISO standard testing methods for testing biodegradability of plastic materials under aerobic industrial composting conditions.

ISO 14855-1:2012

ISO 14855-1:2005 that is analytically described in the work of Briassoulis et al. 2012, was revised into ISO 14855-1:2012 and specifies a method for the determination of the ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions by measurement of the amount of carbon dioxide evolved and the degree of disintegration of the plastic at the end of the test. The method is designed to simulate typical aerobic composting conditions for the organic fraction of solid mixed municipal waste.

Technical characteristics:

- The test material is exposed to an inoculum which consists of stabilized mature compost derived, if possible, from composting the organic fraction of solid municipal waste.
- Temperature, aeration and humidity are closely monitored and controlled for a test period not exceeding 6 months.
- Testing enclosure or room is maintained at a constant temperature 58°C±2°C and is kept free from vapours inhibitory to microorganisms. Monitored and controlled humidity should be kept at around 50 %, and proper air circulation to maintain the oxygen concentration above a minimum of 6 %.
- The C:N ratio for the test mixture should preferably be between 10 and 40.
- The biodegradation percentage is given by the ratio of the carbon dioxide produced from the test material to the maximum theoretical amount of carbon dioxide that can be produced from the test material. The biodegradation percentage does not include the amount of carbon converted to new cell biomass which is not metabolized in turn to carbon dioxide. Additionally the degree of disintegration and the loss in mass of the test material can be determined at the end of the test.

Alternative method:

The use of mature compost can lead to some difficulties in interpreting the results: (1) “priming effect”: the polymer-induced degradation of the organic matter present in large amounts in the mature compost, affects the measurement of the biodegradability, (2) biomass determination is not possible and (3) quantification of residual polymeric material left in the bed.

Therefore a variant was developed. This alterantive uses a mineral bed (vermiculite) inoculated with thermophilic microorganisms obtained from compost with a specific activation phase instead of mature compost in order to eliminate the difficulties associated with the use of mature compost. This variant can be used to measure the biodegradation in terms of CO_2 evolution and the rate of conversion, to quantify and analyse the biomass and the residues of polymeric material left in the solid bed at the end of the test, and to perform a complete carbon balance. This variant is not sensibly affected by the priming effect and can, therefore, be used to assess materials known to cause this problem with mature compost.

Validation criteria:

The test is considered as valid if:

- The degree of biodegradation of the reference material is more than 70 % after 45 days.
- The difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % at the end of the test.
- The inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean values) after 10 days of incubation.

Applicability:

This part of ISO 14855 specifies a method that is designed to determine ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions

Equivalence:

This test method is equivalent to EN 14046.

ISO 14855-2:2007/Cor 1:2009

ISO 14855-2:2007 standard testing method presents a method for determining the ultimate aerobic biodegradability of plastic materials under controlled composting conditions (humidity, aeration ratio and temperature) by gravimetric measurement of the amount of carbon dioxide evolved. The degradation rate is periodically measured by determining the mass of the evolved carbon dioxide using an absorption column filled with soda lime and soda talc on an electronic balance. The standard was revised into ISO 14855-2:2007/Cor 1:2009 with a technical Corrigendum 1.

The test material is mixed with an inoculum derived from mature compost and with an inert material such as sea sand. The sea sand plays an active part by acting as a holding body for humidity and micro-organisms.

Technical characteristics:

- It is recommended to adjust the compost to a C/N ratio of 15 and a C/P (carbon/phosphorous) ratio of 30. Water content equal to 65 %. The three previous parameters may also be adjusted to other values, determined by experience,

depending on seasonal variations and climatic differences. pH adjustment between 7 and 9.

- The relation between the total dry solids of the inoculum and the total dry solids of the test material should preferably be about 6:1. If added, inert material is not considered in this relationship. The test mixture should have the same water content as the inoculum. The water content of the test mixture should be set at 80 % to 90 % of the water-holding capacity (WHC) of the test mixture. The same amount of inoculum by total dry solids should be placed in each test vessel.

Validation criteria:

- The degree of biodegradation of the reference material is more than 70 % after 45 days.
- The difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % at the end of the test.
- The pH should be measured at regular intervals, as at the start of the test. If the pH is found less than 7, biodegradation could be inhibited due to acidification of the compost by the rapid degradation of an easily degradable test material. In this case, measurement of the volatile fatty acid spectrum is recommended to check for souring of the contents of the composting vessel. If more than 2 g of volatile fatty acids per kilogram of total dry solids has been formed, then the test shall be regarded as invalid due to acidification and the resultant inhibition of microbial activity. To prevent acidification, more compost should be added to all the test vessels or the test should be repeated using, for example, less test material, more compost or pre-exposed compost.

If these criteria are not fulfilled, the test must be repeated using pre-conditioned or pre-exposed compost.

Applicability:

- Natural and/or synthetic polymers and copolymers, and mixtures of these
- Plastic materials that contain additives such as plasticizers or colorants
- Water-soluble polymers
- Materials that, under the test conditions, do not inhibit the activity of micro-organisms present in the inoculum

Equivalence:

This test method is equivalent to EN 14046 that describes correspondingly a method for the evaluation of the ultimate aerobic biodegradability of packaging materials based on organic compounds under controlled composting conditions by measurement of released carbon dioxide at the end of the test. This method is designed to resemble typical aerobic composting conditions for the organic fraction of mixed municipal solid waste. The test method is designed to yield a percentage and rate of conversion of carbon of the test material to released carbon dioxide.

7.1.3 European Norms for testing biodegradability of plastics under municipal and industrial composting conditions

There are several EN standard testing methods for testing biodegradability of plastic materials under aerobic industrial composting conditions which are equivalent to the corresponding ISO standard test methods.

EN 14046:2003

The standard EN 14046:2003 *'Packaging—evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions—Method by analysis of released carbon dioxide'* was developed under the directive area of ''Packaging and packaging waste 94/62/EC'', by CEN/TC 261. This standard is equivalent to the standard ISO 14855 described in the previous section (EN ISO 14855:2004; *Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—method by analysis of evolved carbon dioxide*).

EN ISO 14855-2:2009, EN ISO 14855-1:2010, EN ISO 20200:2005

- EN ISO 14855-2:2009 is identical to ISO 14855-2:2007 incorporating Corrigendum 1:2009
- EN ISO 14855-1:2007 + AC:2009 is identical ISO 14855-1:2005/Cor 1:2009

Further analysis and critical review of these standards can be found in Briassoulis et al. 2010.

7.1.4 Other Norms for testing biodegradability of plastics under municipal and industrial composting conditions

Some national standards are also presented in Table 74 for illustrative purposes.

7.2 Toxicity

The compost obtained at the end of the composting process can be adversely influenced by the addition of compostable plastics or packaging at start of the composting process. In order to verify if this is the case, ecotoxicological tests with compost with and without test material can be executed.

As mature compost is used in order to increase the soil quality, the residuals of the compostable materials will finally reach the soil environment. Consequently, compost quality can be checked by mixing the compost with artificial soil. This mixture can then be used in order to executed toxicity tests. The toxicity in the test mixture can be compared with the toxicity in the blank mixture. To assess toxicity associated with compost applications, plastics can be tested on both plant and animal species, as required by the corresponding norms and/or chosen by the interested parties (*Briassoulis et al, 2010*). Rudnik et al, 2007 mentions that the methods for the evaluation of the ecotoxicity of compostable polymer materials are mainly based on the use of plants, soil fauna (earthworms), aquatic fauna (Daphnia), algae (green algae), microbes (luminescent bacteria).

With regard to plant phytotoxicity testing, while a product may not negatively impact plant growth in the short term, over time it could become phytotoxic along plant development due to the build-up of inorganic materials within determined parts of the plant, which could potentially lead to a reduction in soil productivity (*Biodegradable Plastics, 2002*). To test for such a potential effect plant phytotoxicity testing can be used on the finished compost that contains degraded polymers. However, in order to simulate the accumulation a test item during several years, the test item should be added in a high concentration at start of the pilot-scale composting test. Both acute plant toxicity and chronic plant toxicity tests can be executed on the obtained compost/soil mixtures.

Animal testing is generally carried out using earthworms (soil organisms) and Daphnia (aquatic organisms) (*Biodegradable Plastics, 2002)*. The Daphnia toxicity test is used to establish whether degradation products present in liquids pose any problem to surface water bodies. Earthworms are used because they feed on soil and they are very sensitive to toxicants. Earthworms are exposed to several mixture ratios of compost and soil mixtures. Following 14 days of exposure, the number of surviving earthworms is counted and weighed and the survival rate is calculated (test of acute toxicity within 14 days (mortality) (OECD 222); chronic toxicity (reproduction) 56 days (OECD 207)). Compost worms (Eisenia fetida) are used for testing the toxicity of biodegradable plastic residues. These worms are very sensitive to metals such as tin, zinc, heavy metals and to high acidity (Rudnik, 2008)]. For this test worms are cleaned and accurately weighed at intervals over 28 days.

An overview of toxicity tests in water and soil is given in Chapter 3.2 and Chapter 6.2, respectively.

Concentrations of heavy metals of the compostable material must be below the limits set by the standards (*Briassoulis et al, 2010)*. The corresponding provisions differ between the various standards as presented in Table 75 (adapted from *(Briassoulis et al, 2010))*.

Table 75. Maximum heavy metal content for compostable materials according to various standards

Element (mg/kg d.w.)	US ASTM D 6400 ASTM D 6868	Canada BNQ P 9011-911-5	Europe/Australia EN 13432 AS 4736	Japan (Biodegradable plastic Society)
Zn	1400	925	150	150
Cu	750		50	60
Ni	210	90	25	30
Cd	17	10	0.5	0.5
Pb	150	250	50	10
Hg	8.5	2.5	0.5	0.2
Cr			50	50
Mo		10	1	
Se	50	7	0.75	
As	20.5	37.5	5	5
F			100	
Co	75	75		

7.3 Standard specifications

Table 76 presents the standard specifications for the compostability under municipal and industrial aerobic conditions for different product categories (plastics, packaging, etc.). These standards on all based on the same criteria (biodegradation, disintegration and environmental safety including ecotoxicity tests and chemical characterization).

Table 76. Overview of specifications standards for determining compostability of plastics.

American Society for Testing and Materials International (ASTM)		
Current versions of standards	**Previous versions of standards**	**Title**
ASTM D 6400-12	***ASTM D 6400-04**	Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (Previous title: Standard specification for compostable plastics)
	****ASTM D 6002-96 (2002)**	Standard guide for assessing the compostability of environmentally degradable plastics
ASTM D 6868-11		Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities
International Organization for Standardization (ISO)		
Current versions of standards	**Previous versions of standards**	**Title**
ISO 17088:2012	***ISO 17088:2008**	Specifications for compostable plastics
ISO 18606:2013		Packaging and the environment - Organic recycling
European committee for standardization (CEN)		
EN 13432-2000		Packaging—Requirements for packaging recoverable through composting and biodegradation—Test scheme and evaluation criteria for the final acceptance of packaging
EN 14995-2006		Plastics—Evaluation of compostability—Test scheme and specifications
Deutsches Institut fur Normung (DIN)		
DIN EN 13432-2000		Requirements for packaging recoverable through composting and biodegradation—Test scheme and evaluation criteria for the final acceptance of packaging.

DIN EN 14995-2007	Plastics—Evaluation of compostability—Test scheme and specifications.
DIN V 54900-1998	Testing of the compostability of plastics
Italian Norm (Italian Unification Agency) (UNI)	
UNI EN 14995-2006	Plastics—Evaluation of compostability—Test scheme and specifications
Other Specifications	
Belgium Royal decree (9/09/2008) effective in July 2009	Decree specifying the norms that the products should meet to be compostable or biodegradable
Australian standard	
AS 4736-2006	Biodegradable plastics—Biodegradable plastics suitable for composting and other microbial treatment

*Standard superseded by its updated version

** Withdrawn standard, no replacement

7.3.1 ASTM Standard specifications for characterising compostable plastic products and materials

There are several ASTM standard specifications for defining compostability of plastics under municipal and industrial composting conditions.

ASTM D 6400-04

ASTM D 6400-04 establishes the requirements for identifying plastics and products made from plastics that will compost satisfactorily in industrial and municipal aerobic composting facilities. During 2012, the standard was expanded into and superseded by the new version ASTM D 6400-12, which covers plastics and products made from plastics that are designed to be composted under aerobic conditions in municipal and industrial composting facilities, where thermophilic conditions are achieved. The properties in this specification are those required to determine if end items (including packaging), which use plastics and polymers as coatings or binders, will compost satisfactorily, in large scale aerobic municipal or industrial composting facilities. The standard establishes the requirements for labeling of materials and products, including packaging made from plastics, as “compostable in aerobic municipal and industrial composting facilities”. This later version also highlights that maximum throughput is a high priority to composters, and the intermediate stages of plastic disintegration and biodegradation should not be visible to the end user for aesthetic reasons.

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety.

Biodegradation testing requirements & criteria:

- Method for the measurement of biodegradation: the test is carried out using the test methods ASTM D 5338, ISO 14855-1 or ISO 14855-2.

- A plastic product must demonstrate a satisfactory rate of biodegradation by achieving the following ratio of conversion to carbon dioxide within 180 days:
    - 90 % of the organic carbon in the whole item or for each organic constituent, which is present in the material at a concentration of more than 1 % (by dry mass), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute.
    - Organic constituents present at levels between 1 to 10 % shall be tested individually.
    - Organic constituents which are present at concentrations less than 1 % do not need to demonstrate biodegradability. However, the sum of such unproven constituents shall not exceed 5 %.

Disintegration testing requirements & criteria:

- Method for the measurement of disintegration during composting: the test is carried out in accordance with ISO 16929 with a minimum vessel volume of 35 l, or ISO 20200 under thermophilic aerobic composting conditions.
- A plastic product is considered to have demonstrated satisfactory disintegration if after twelve weeks (84 days) in a controlled composting test, no more than 10 % of its original dry weight remains after sieving on a 2.0 mm sieve.

Environmental safety testing requirements & criteria:

- The plastic material shall have concentrations of regulated metals less than 50 % of those prescribed for sludges or composts in the country where the product is sold. In the case of United States and Canada there are specific regulated metal concentrations.
- The germination rate and the plant biomass of the sample composts shall be no less than 90 % that of the corresponding blank composts for two different plant species following OECD Guideline 208 with specific modifications found in Annex E of EN 13432. Sample composts generated in accordance with ISO 20200 should not be used for ecotoxicity testing unless the concentration of the test items at the start of testing is in accordance with the requirements of ISO 16929.

Applicability:

- 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.

Equivalence:

- This test method is equivalent to ISO 17088-2012

ASTM D 6868-11

Correspondingly, ASTM D 6868-11 specification covers end items that include plastics or polymers where plastic film/sheet or polymers are incorporated (either through lamination,

extrusion or mixing) to paper and other substrates and the entire end item is designed to be composted under aerobic conditions in municipal and industrial composting facilities, where thermophilic temperatures are achieved.

It is intended to establish the specifications for labeling of end items which use plastics or polymers as coatings or binders, as “compostable in aerobic municipal and industrial composting facilities”.

The properties in this specification are those required to determine if end items (including packaging) which use plastics and polymers as coatings or binders will compost satisfactorily, in large scale aerobic municipal or industrial composting where maximum throughput is a high priority and where intermediate stages of plastic biodegradation should not be visible to the end user for aesthetic reasons.

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety.

Biodegradation testing requirements & criteria:

- A level of biodegradation for the plastic coatings and additives shall be established by tests under controlled conditions.
- An end item having a plastic coating or additives are considered to have achieved a satisfactory level of biodegradation if the following criteria are met or exceeded:
    - The plastic coating or polymeric additives must meet a satisfactory rate of biodegradation by achieving the following ratio of conversion to carbon dioxide within 180 days (ASTM D 6400):
        - 90 % of the organic carbon in the whole item or for each organic constituent, which is present in the material at a concentration of more than 1 % (by dry mass), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute.
        - Organic constituents present at levels between 1 to 10 % shall be tested individually.
        - Organic constituents which are present at concentrations less than 1 % do not need to demonstrate biodegradability. However, the sum of such unproven constituents shall not exceed 5 %.
    - Substrates used in the end item must individually satisfy the following requirements:
        - 90 % of the organic carbon is converted to CO_2 using Test Method D 5338 within 180 days at 58±2°C when compared to the positive control (alternative test methods: ISO 14851:1999, ISO 14852:1999, ISO 14855:1999)
        - End items made of ligno-cellulosic substrates are assumed to be biodegradable by showing that over 95% of their carbon comes from biobased resources using D 6866.
        - Polymers or additives derived from biobased resources that are blended with ligno-cellulosic substrates shall separately demonstrate

that they meet the requirements of subsection 6.3 of specification D 6400, if they are more than 1 % of the dry weight of the end item.

- Any organic constituent present in more than 1 % of the dry weight of the end item shall fulfill the biodegradation requirements of subsection 6.3 of specification D 6400.
- The total portion of organic constituents or additives that do not fulfill the requirements of subsection 6.3 of specification D 6400 shall not exceed 5 % of the end item by weight.

Disintegration testing requirements & criteria:

- An end item will disintegrate during composting such that any remaining residuals (plastic, polymer, or substrate) are not readily distinguishable from the other organic materials in the finished product. Additionally, the material or product must not be found in significant quantities during screening prior to final distribution of the compost. An end item is considered to have demonstrated satisfactory disintegration if after 12 weeks in a controlled composting test, no more than 10% of its original dry weight remains after sieving on a 2mm-sieve.

Environmental safety testing requirements & criteria:

- The end item shall have concentrations of heavy metals less than 50% of those prescribed in the Government Standard "*40 CFR Part 503.13: Standards for the Use or Disposal of Sewage Sludge*".

- The germination rate and the plant biomass of the sample composts shall be no less than 90 % that of the corresponding blank composts for 2 different plant species following OECD guideline 208 with the modifications found in Annex E of EN 13432.

7.3.2 ISO Standard specifications for characterising compostable materials

ISO 17088:2012

ISO 17088 is a standard specification for identification of plastic products and materials as compostable under municipal and industrial composting conditions. The specification ISO 17088:2008 is intended to establish the requirements for the identification and labelling of plastic products and materials, including packaging made from plastics, as "compostable" or "compostable in municipal and industrial composting facilities" or "biodegradable during aerobic composting" (Briassoulis et al. 2010). The labelling will, in addition, have to be conform to all international, regional, national or local regulations (e.g. European Directive 94/62/EC). It has been revised into the ISO 17088:2012. This second edition cancels and replaces the first edition (ISO 17088:2008).

The composting specifications refer to requirements and criteria towards biodegradability, disintegration and environmental safety (negative effects on the finished compost & maximum concentration of regulated metals in compost).

Biodegradation testing requirements & criteria:

- The aerobic biodegradation test should be carried out in accordance with ISO 14855-1, ISO 14855-2 or ASTM D 5338.
- The test period shall be no longer than 180 days.
- The aerobic biodegradability shall be determined for the whole material or for each organic constituent. For organic constituents which are present in the material in a concentration between 1 % and 10 % (by dry weight), the level of biodegradation shall be determined separately. Constituents which are present in a concentration < 1 % do not need to demonstrate biodegradability. The sum of such constituents shall not exceed 5 %.
- The plastic product under test is considered to have demonstrated a satisfactory rate and level of biodegradation if 90 % of the organic carbon (relative to a positive-control reference material) shall have been converted to CO_2 by the end of the test period. Both the positive control and the test sample shall be composted for the same length of time and the results compared at the same point in time after the activity of both has reached a plateau. As an alternative, 90 % (in absolute terms) of the organic carbon shall have been converted to carbon dioxide by the end of the test period.

Disintegration testing requirements & criteria:

- The disintegration test should be carried out in accordance with ISO 16929, ISO 20200, ISO 14855-1 or ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment.
- A plastic product is considered to have demonstrated satisfactory disintegration if, after 84 days in a controlled composting test, no more than 10 % of its original dry mass remains after sieving through a 2.0 mm sieve.

Environmental safety testing requirements & criteria:

- The concentrations of regulated metals and other toxic substances in the plastic product or material shall be less than 50 % of those prescribed for sludges, fertilizers and composts in the country where the final product will be placed on the market or disposed of.
- The plastic product or material shall contain a minimum of 50 % of volatile solids.
- The seedling germination rate and the plant biomass in the test compost shall be no less than 90 % of that of corresponding blank compost to which no test or reference material was added at the start of testing, determined in accordance with OECD Guideline 208 with the modifications specified in Annex E of EN 13432:2000.

Applicability:

- Plastics and products made from plastics that are suitable for recovery through aerobic composting.

Equivalence:

This test method is equivalent to ASTM D 6400-2012

Need for revision of this standard:

Although the biodegradation test includes the conversion of the polymers into biomass and humic substances in addition to carbon dioxide, no recognized standard test methods or specifications exist for the quantification of these conversion products. When such tests and specifications become available, this International standard might be revised.

ISO 18606:2013

In January 2013 another ISO standard was published: ISO 18606 “Packaging and the Environment-Organic Recycling”. While ISO 17088 only mentions specifications for plastics, ISO 18606 covers a broader range of products as it encompasses criteria for packaging (= plastic packaging, paper packaging, food packaging, packaging from bagasse, etc.). Packaging is considered as recoverable by organic recycling only if all the individual components meet the requirements. Therefore, packaging is not considered recoverable by organic recycling if only some of the components meet the requirements of ISO 18606:2013. However, if the components can be easily, physically separated before disposal, then the physically separated components can be individually considered for organic recycling.

ISO 18606:2013 is applicable to organic recycling of used packaging but does not address regulations regarding the recoverability of any residual packaged goods.

ISO 18606:2013 does not provide information on requirements for the biodegradability of used packaging which ends up in the soil environment as litter, because littering is not considered as a recovery option. It is also not applicable to biological treatment undertaken in small installations by householders.

For each of the packaging components the following four aspects are addressed: (1) biodegradation, (2) disintegration during biological waste treatment process (i.e. composting), (3) negative effects on the biological process and (4) negative effects on the quality of the resulting compost, including the presence of high levels of regulated metals and other substances hazardous to the environment.

Biodegradation testing requirements & criteria:

- Chemically unmodified packaging materials and constituents of natural origin, such as wood, wood fibre, cotton fibre, starch, paper pulp, bagasse or jute shall be accepted as biodegradable without testing.
- The aerobic biodegradation test should be carried out in accordance with ISO 14855-1 or ISO 14855-2. (As an alternative also ISO 14851 and ISO 14852 are allowed.)
- The test period shall be no longer than 180 days.
- The aerobic biodegradability shall be determined for the whole material or for each organic constituent. For organic constituents which are present in the material in a concentration between 1 % and 10 % (by dry weight), the level of biodegradation shall be determined separately. Constituents which are present in a concentration < 1 % do not need to demonstrate biodegradability. The sum of such constituents shall not exceed 5 %.

- A constituent or the material is considered to have demonstrated a satisfactory rate and level of biodegradation if 90 % of the organic carbon (relative to a positive-control reference material) shall have been converted to CO_2 by the end of the test period. Both the positive control and the test sample shall be composted for the same length of time and the results compared at the same point in time after the activity of both has reached a plateau. As an alternative, 90 % (in absolute terms) of the organic carbon shall have been converted to carbon dioxide by the end of the test period.

Disintegration testing requirements & criteria:

- The disintegration test should be carried out in accordance with ISO 16929. Alternatively also a lab-scale test ISO 20200 can be used. In case of differing results, ISO 16929 shall prevail. Also a full-scale industrial composting testing can be used as long as it is well defined and uses equivalent test duration, sample concentration and analytical evaluation of disintegration. In contrast to ISO 17088, ISO 14855-1 and ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment are not allowed anymore in order to determine the disintegration.
- Packaging is considered to have demonstrated satisfactory disintegration if, after 84 days in a controlled composting test, no more than 10 % of its original dry mass remains after sieving through a 2.0 mm sieve. The particles or pieces which do not differ from the compost for colour, structure, dimension, moisture feeling and brightness/gloss are considered to be compost.

Environmental safety testing requirements & criteria:

- The concentrations of regulated metals and other substances hazardous to the environment in the packaging shall not exceed the limits specific to the country where the final product will be placed on the market or disposed (US = 50 % of those prescribed in 40 CFR 503.13, Canada = BNQ 9011-911-I/2007, EU = EN 13432, etc.).
- The packaging or packaging components shall contain a minimum of 50 % of volatile solids.
- The seedling germination rate and the plant biomass in the test compost shall be no less than 90 % of that of corresponding blank compost to which no test material was added at the start of testing, determined in accordance with OECD Guideline 208 with certain modifications. Compost to be used for plant toxicity tests shall be prepared according to ISO 16929 using a 10 % sample input concentration.

The procedure for applying ISO 18606:2013 is contained in ISO 18601. The relevance of this recently presented standard to the biodegradation and compostability of packaging will become clear during the next years.

7.3.3 European Norms specifications for characterising compostable packaging, plastic products and materials

There are two European norms available for identification of plastic products and packaging materials as compostable under municipal and industrial composting conditions.

EN 13432:2000, EN 14995-2006

The European norm EN 13432:2000 with the title *"Packaging—requirements for packaging recoverable through composting and biodegradation—test scheme and evaluation criteria for the final acceptance of packaging"* defines the characteristics a material must own in order to be claimed as "compostable" and, therefore, recycled through composting or organic solid waste.

The European norm EN 13432:2000 is a reference point for all European producers, authorities, facility managers and consumers. Unlike the ASTM standards, this standard can be applied to any packaging or packaging component, and is not limited to plastic materials. Correspondingly, EN 14995-2006 also refers to the evaluation of the compostability of plastics.

The scope of testing materials under EN 13432-2000 and EN 14995-2006 is to ensure that a compostable packaging or plastic material, respectively, must have the following characteristics:

Biodegradation testing requirements & criteria:

- Biodegradability tested according to ISO 14855
- Percentage of biodegradation shall be at least 90 % in total or 90 % of the maximum degradation of reference material after a plateau has been reached for both test and reference materials over a period of 6 month, when biodegradation is defined according to Specifications Standard EN 13432.

Disintegration testing requirements & criteria

- Tested in a controlled pilot-scale test or in a full-scale treatment facility
- The test material is degraded, together with organic waste, for 3 months. After this time, the compost is sieved with a 2 mm sieve. The residues of the tested material with dimensions higher than 2 mm are considered as not having disintegrated. This fraction must be less than 10 % of the initial mass.

Environmental safety testing requirements & criteria:

- Low levels of heavy metals and other toxic and hazardous substances and a minimum of 50 % of volatile solids.
- No negative influence on the composting process is permitted. Chemical-physical parameters by which the compost quality shall be defined are: volumetric weight (density), pH, salinity, total dry solids, volatile solids, total N, ammonium N, P, Mg and K.
- Moreover, ecotoxic effects on 2 higher plants shall be determined by comparing compost produced with and without addition of test material (OECD test 208, modified). The germination rate and plant biomass of the sample composts of both plant species should be more than 90 % of those from the corresponding blank compost.

Need for revision of this standards:

There are different opinions on this and suggestions to amend the EN 14046 standard to 90 % biodegradation within 90 days; A proposal was made also to amend the EN 13432 standard to 90 % disintegration within a maximum of 12 months. As a compromise, extension of the timescale could be accompanied by a reduction of the temperature at which the tests are carried out – from 58°C to 38°C [Perchard D (2005) CEEES workshop, Biodegradable Polymers – Where are the Limits, 3 November 2005; CEEES-Confederation of the environmental engineering societies http://www.ceees.org/auxiliary/biopolymer051103.pdf Accessed 16 April 2009].

7.3.4 Belgian Royal Decree for Acceptance of Compostable and Biodegradable Plastic Materials

As analytically reported in the work of Briassoulis et al. 2010, in Belgium a royal decree became effective in July 2009 that defines three properties of a product depending on its end-of-life management option: compostable, home compostable and biodegradable. This Belgium decree determines the requirements and standards that have to be fulfilled by each category of product:

Biodegradation testing requirements & criteria:

- Not chemically modified materials from natural origin do not need to be tested for biodegradation.
- Not significant organic components on condition that their cumulative percentage is < 5 % and their individual share is < 1 % do not need to be tested for biodegradation.
- Test methods ISO 14588, ISO 14851, ISO 14852 or ISO 17566 shall be used.
- Pursuant to the EN 13432 standard, at least 90 % absolute or 90 % relative (referring to microcrystalline cellulose) material have to be broken down by biological action within 6 months

Disintegration testing requirements & criteria:

- Test method ISO 16929 or a test in a full-scale treatment facility shall be used.
- Less than 10 % of the plastic remains on a 2 mm screen within 84 days is required.

Environmental safety testing requirements & criteria:

- Minimum volatile solids content: 50 % on dry weight basis.
- Restrictions with regard to heavy metals (Zn, Cu, Ni, Cd, Pb, Hg, Cr, Mo, Se & As) and fluorine.
- Substances, which are dangerous for humans or the environment (KB 17/7/2002), may not be used in the product.
- No negative influence on the composting process is permitted. Chemical-physical parameters by which the compost quality shall be defined are: volumetric weight (density), pH, salinity, total dry solids, volatile solids, total N, ammonium N, P, Mg and K.

- Moreover, ecotoxic effects on 2 higher plants shall be determined by comparing compost produced with and without addition of test material (OECD test 208, modified according to EN 13432). The germination rate and plant biomass of the sample composts of both plant species should be more than 90 % of those from the corresponding blank compost.

7.3.5 Australian Standard AS 4736:2006

According to the Australian Standard AS 4736:2006 with the title *"Biodegradable plastics-Biodegradable plastics suitable for composting and other microbial treatment*", it is demonstrated that compostable plastics must meet the following criteria:

General requirements:

- Minimum volatile solids content: 50 %
- Heavy metals content < limit values

Biodegradability testing requirements & criteria:

- Test methods ISO 14855, ISO 14851 or ISO 14852 shall be used.
- The test material shall degrade at least 90 % in total or of the maximum degradation of a suitable reference material within 180 days (after a plateau has been reached).

Disintegration testing requirements & criteria:

- ISO 16929 shall be used.
- Less than 10% of the plastic remains on a 2 mm screen within 12 weeks is required.

Environmental safety testing requirements & criteria:

- Ecotoxic effects on two higher plants shall be determined by EN 13432 Appendix E.
- Germination rate and plant germination of the plants in the test composts shall be more than 90 % of those from the corresponding blank compost.
- Ecotoxic effect on earthworms shall be determined by ASTM E1676.
- The difference in the morbidity or mean weight of surviving earthworms between the test compost and the control compost shall not be larger than 10 %.

7.3.6 Other national specifications for characterising compostable packaging, plastic products and materials

There are several national specifications established for the identification of plastic products and materials as compostable under municipal and industrial composting conditions. Some illustrative examples are briefly presented.

In Italy a previous standard (UNI 10785), concerning the compostability of plastics, was replaced by its European version: UNI EN 14995-2006: plastics - evaluation of compostability - test scheme and specifications (Briassoulis et al. 2010).

Also a German standard with regard to compostability (DIN V 54900-1998) was developed.

7.4 Labelling

Labeling of qualified products is an important communication and promotional tool, e.g. from producers to other involved parties such as the consumers or municipal staff. Table 77 lists some of currently available labels, their certifying bodies, and the required standards. The OK COMPOST labeling system of AIB Vinçotte in described in detail in this chapter.

Table 77. Overview of certification schemes and labels for environmentally degradable polymeric materials and plastics.

Logo	Organization	Norm	Symbol
Seedling logo	European Bioplastics	EN 13432, ASTM D 6400, EN 14995 and ISO 17088	
OK compost logo	Vinçotte	EN 13432	
DIN-Geprüft Compostable logo	DIN CERTCO	EN 13432, ASTM D 6400, EN 14995, ISO 17088 and AS 4736	
Compostable logo	Biodegradable Products Institute (BPI)	ASTM D 6400 and ASTM D 6868	
Cedar Grove Composting logo	Cedar Grove	based on ASTM D6400 and ASTM D6868 with additionally mandatory full-scale test	
GreenPla logo	Japan Bioplastics Association (JBPA)	Green PLA certification scheme	

Logo	Organization	Norm	Symbol
Australian seedling logo	Australasian Bioplastics Association (ABA)	AS 4736	
National logo in Italy	Consorzio Italiano Compostatori (CIC)	based on EN 13432 with additionally mandatory full-scale test	
National logo in Finland	Jätelaitosyhdistys	EN 13432	
National logo in Sweden	SP Technical Research Institute of Sweden	SPCR 141	
National logo in Spain (Catalonia)	Departament de Medi Ambient i Habitatge	EN 13432 and EN 14995	

7.4.1 VINÇOTTE Certest Products - OK compost - Initial acceptance tests

AIB-VINÇOTTE International S.A./N.V. has established a set of specifications for identifying the compostability of products and materials under municipal or industrial composting conditions. These specifications are based on the European standard specification with regard to compostability EN 13432 (2000). To be eligible for “OK Compost” certification, materials or products shall obtain the following properties:

Biodegradation testing requirements & criteria:

- At least 90 % of the test material has to be biodegraded within 6 months.

Disintegration testing requirements & criteria:

- At least 90 % of the product should be able to pass through a 2 × 2 mm mesh sieve after 12 weeks.

Environmental safety testing requirements & criteria:

- Criteria of EN 13432 need to be fulfilled (heavy metals, fluorine, volatile solids and plant toxicity), but some clarifications are mentioned in the document of VINÇOTTE:
  - The concentration of test material to be added to the compost must be 10% on wet mass basis (of which 9% as powder or granulates) according to Standard ISO 16929 or Standard EN 14045.
  - An assessment of the negative effects (ecotoxicity) of constituents accounting for less than 0.1 % of the dry weight of a material or product does not have to be checked provided the total percentage of these constituents does not exceed 0.5 % of the dry weight of this material or product.
  - All constituents and their maximum concentrations as specified on the positive list (TS-OK-10) are regarded as fulfilling the compost quality requirements.
  - All food additive approved ingredients are regarded as fulfilling the compost quality requirements
  - Constituents that appear on the (candidate) list of substances of very high concern are not accepted. This must be verified for all constituents that are not tested for ecotoxicity, do not appear on the positive list and are not food additive approved ingredients.

7.5 Discussion and critical review

ASTM D 6400 (2012) is comparable or in harmony with standards in Europe, Japan, Korea, China, and Taiwan. The specifications set by the ASTM D 6400 (2012) standard along with the three standards to which it is referred to, are comparable (but not the same) to what has been developed by the European Committee for Standardization (CEN) in Europe, and in harmony with the relevant ISO standards for compostable plastics, moving the industry closer to global standards.

7.5.1 Biodegradability

The key specification of EN 13432:2000 is the requirement of at least 90 % biodegradation in total or at least 90 % biodegradation of the maximum degradation of the reference material after a plateau stage for both reference and test materials has been reached , as measured by ISO 14855-2012 (controlled composting) test method. The biodegradation test described in ISO 14855-1-2012 is similar to the test method in ASTM D 5338-2011, with a few differences. Thus, ISO 14855-1-2012:

- Does not require the negative control vessels; therefore, only 9 vessels are required instead of 12.
- Includes the determination of percentage of biodegradation based on weight loss as an optional result to support the value determined from carbon dioxide evolution.

Biodegradability is determined by measuring the amount of CO_2 produced over a certain period by the test material. The main point of differentiation between the various international standards is the percentage of biodegradation required for compliance. ASTM D 6400-12 and ISO 17088:2012 require both that 90 % absolute or relative biodegradation of the whole item or for each organic constituent, which is present in the material at a concentration of more than 1% (by dry mass) within 180 days. Moreover, all organic constituents present at levels between 1 % and 10 % shall also meet the 90 % biodegradation criteria. EN 13432 (2000) is less strict as organic constituents present at levels between 1 % and 10 % do not need to be tested separately. Furthermore there are on-going discussions on revising the European Norm EN 13432-2000 (and EN 14046-2003) so that it requires 90 % biodegradation within 90 days instead of 180 days (Perchard D, 2005). This is an important issue that is under discussion at ISO level. The compliance requirements for the key standards, adapted from (*Briassoulis et al, 2010*), are shown in Table 78.

Table 78. Standards Compliance Requirements under controlled aerobic composting conditions.

Standard Test Method							Standard Specification
Standard Test Method	**Method**	**Test Validity**	**Temperature (°C)**	**C:N Ratio (by weight)**	**Sample quantity and media**	**Water Content (% weight of water/dry weight)**	**Composting Requirement -Time frame to achieve biodegradation requirements (months)**
ASTM D5929-96 (Reapproved 2009)	Cumulative carbon dioxide evolved and/or oxygen consumed	-The total oxygen uptake for the control reactors should exceed 80g. If this is not observed over the 45 days then the test must be regarded as invalid and should be repeated with new inoculum. -After 45 days take 10 g of solids and dilute it in 50 ml of water. If pH<7 and VFA (volatile fatty acids method D2908) >2 g/kg dry sample then test is invalid	**40°** Compost temperature not to exceed 65° C. (If exceeded increase the compost aeration rate through higher recirculation)	**≤ 40:1** Synthetic MSW (simulated municipal waste media inoculated with active MSW compost) should be adjusted with urea not to exceed a C:N ratio of 40:1 if 35 g of C are added to this mixture	12g inoculum and quantity of test material required to obtain 50g of ThO_2 <u>Sample size</u> <3x3x12 cm <u>Sample per vessel:</u> Quantity of sample to contain 50 g of theoretical	Water is added through the buffer solution to reach: 102.8 % wgt/wgt of water to dry solid MSW media.	***May use applicable Specification Standards*** ***(ASTM D6400, D6868, ISO 17088, Vincotte, EN 13432)***

				(Organic C of test sample plus MSW; Total N of test mixture)	O_2 uptake Compost media per vessel: 269.37 g synthetic MSW, 12 g inoculum from active MSW unit, 276.92 ml buffered water		
ASTM D5338-11	Analysis of evolved carbon dioxide.	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at the end of the test;	**58**±2	**<40** Organic C for both the inoculum and test substance combined Total nitrogen content of the test mixture (if needed add ammonium chloride)	600 g dry solids of inoculum with 100 g of dry solids coming from the sample Inoculum quality: -Ash content <70%	Addition of distilled water so that the dry solids content in the mixture is ~ 50% of wet solids	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10% of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90% of the organic carbon (absolute or relative) shall be converted to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the*

					- pH 7-8.2 - Total dry solids: 50 % - 55 % -To produced more than 50 mg but less than 150 mg of CO_2 per gram of volatile solids (mean values) after 10 days of incubation.		*materials have to be broken down by biological action within 6 months* *Time frame to achive biodegradation*: 45 days, but incubation time may be extended if significant biodegradation of the test substance is still being observed
ASTM D6340-07	Determination of rate and degree of biological oxidation of carbon in radiolabelled plastic materials in	$^{14}CO_2$ measured in this test method is a direct indication of the oxidation of the sample. The compost formula for the simulation of MSW in this test	**58**±5	Compost, designed to stimulate MSW organic matter Should contain the biochemical	200-300 mg of plastc in ~300 g compost	Moisture content should be adjusted to 55-60%	***May use applicable Specification Standards*** ***(ASTM D 6400, D 6868, ISO 17088, Vincotte, EN 13432)***

	composting or aqueous environment	method will generally give repeatable results. Depending upon the objectives of the test, include within each test series a standard preparation of known degradation rates and make test comparisons.		ingredients found in MSW (lignified cellulose, protein, natural inoculum, soluble carbohydrates and buffering capacity sufficient to maintain a neutral to slightly basic pH)			
ISO 14855-1:2012 **and** **EN 14046:2003**	Analysis of evolved CO_2.	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at	58±2	10-40 Organic C for both the inoculum and test substance combined Total nitrogen content of the test mixture (if needed	Two alternatives: <u>Inoculum:</u> Dry mass of inoculum: dry mass of test material = 6:1 <u>Activated Vermiculite:</u>	Test mixture shall have the same water content as the inoculum	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10% of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90% of the organic carbon (absolute or relative) shall be converted

		the end of the test; - the inoculum in the blank has produced more than 50 mg but less than 150 mg of CO_2 per gram of volatile solids (mean values) after 10 days of incubation.		addurea)	Dry mass of activated vermiculite: dry mass of test material = 4:1 <u>Inoculum quality</u>: -Volatile solids > 15 % of the wet solids -Total dry solids shall be between 50 % and 55 % of the wet solids -To produced more than 50 mg but less than 150 mg of CO_2 per gram of		to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the materials have to be broken down by biological action within 6 months* *Time frame to achieve biodegradation:* 6* months

					volatile solids (mean values) after 10 days of incubation.		
ISO 14855-2:2007	Gravimetric measurement of the amount of CO_2 evolved	- ≥70 % biodegradation of the reference material after 45 days; - the difference between the % biodegradation of the reference material in the different vessels is less than 20 % at the end of the test; - if more than 2g volatile fatty acids per kg of total dry solides has been formed, the test is regarded as invalid	58±2	15	Total dry solids of the inoculum and the total dry solids of the test material should preferably be about 6:1.	Test mixture: 80-90 % of the WHC of the test mixture	***Based on ASTM D6400, D6868, ISO 17088*** *Disintegration:* after 84 days <10 % of the original weight of a plastic product remains after sieving on a 2.0 mm sieve *Biodegradation:* 90 % of the organic carbon (absolute or relative) shall be converted to CO_2 after 180 days ***Vincotte, EN 13432*** *At least 90% of the materials have to be broken down by biological action within 6 months*

(*) There are different opinions on this and suggestions to amend the EN 14046 standard to 90 % biodegradation within 90 days; A proposal was made also to amend the EN 13432 standard to 90% disintegration within a maximum of 12 months. As a compromise, extension of the timescale could be accompanied by a reduction of the temperature at which the tests are carried out – from 58°C to 38°C.

In both ISO 14855-1-2012 and ASTM D5338-2011 methods, the amount of CO_2 evolved due to biodegradation can be measured using acid–base titration, or by using a direct measurement such as infrared or gas chromatography. ISO 14855-2-2009, which measures mineralization of a polymer by a gravimetric method, is similar to 14855-1-2012 except for the method of CO_2 measurement: In 14855-2 method the system is capable of determining carbon dioxide directly from the change in mass of a carbon dioxide trap. The carbon dioxide trap consists of columns filled with soda lime, soda talc and anhydrous calcium chloride. An ammonia trap (dilute sulfuric acid) and a water trap (silica gel and calcium chloride) are required between the composting vessel and the carbon-dioxide-absorbing column.

In ISO14855-1 specification, the apparatus for the determination of carbon dioxide is designed to determine carbon dioxide directly or by complete absorption in a basic solution and determination of the dissolved inorganic carbon (DIC). If the carbon dioxide in the exhaust air is measured directly, for example with a continuous infrared analyser or a gas chromatograph, exact control or measurement of the air-flow rate is required.

In addition, inert materials such as sea sand or vermiculite can be used with the compost for providing better aeration and retention of moisture content. The mixture of compost and sea sand or vermiculite is periodically taken out from the closed system to turn or agitate to prevent channelling of air in the biodegradation vessel.

Furthermore, while the temperature profile is continuously at 58°C and the maximum test duration is 6 months in ISO 14855-1-2012, ISO 14855-2-2007, the CEN test procedures and ASTM D 5338-11 , a lower temperature of 40°C and a much shorter period of 45 days is applied.in the case of ASTM D 5929-09

7.5.2 Disintegration

Materials are tested for disintegration in the form in which they will be ultimately used. Either a controlled pilot-scale test or a test in a full-scale aerobic composting treatment facility can be used (*Briassoulis et al, 2010)*. The disintegration of the test material is evaluated on the basis of the total dry solids by comparing the retrieved fractions of the test material > 2 mm and the amount tested. Less than 10% in weight should remain on a 2 mm screen for most standards at the end of the test period. Disintegration tests cannot differentiate between biodegradation related disintegration and abiotic disintegration[14], but instead demonstrate that sufficient disintegration of the test materials has been achieved within the specified testing time (*Briassoulis et al, 2010)*. For disintegration, the European norm EN 13432 suggests testing the materials in controlled pilot-scale or full-scale tests, while the international standard ISO 17088 (2012) refers to ISO 16929, ISO 20200, ISO 14855-1 and ASTM D 5338 under thermophilic composting conditions without the CO_2-trapping equipment. ISO 18606 only refers to ISO 16929, ISO 20200 or a full-scale industrial composting test. ASTM D 6400-12 refers to ISO 16929 and ISO 20200 under thermophilic

[14] Abiotic degradation may be induced by UV or thermal in dry or humid conditions, water, salt solution etc. The ASTM D6954, Tier 1, BS 8472 and ISO 4611, ISO 4892-2 Standards define the requirements for abiotic degradations as follows:
Average molecular, weight Mw < 10 000, gel fraction < 5 %, elongation at break ≤ 5 % of the original value

aerobic composting conditions. But the rest is similar, i.e., the final compost is screened with a 2 mm sieve, and the material needs to pass the disintegration criterion (i.e., no more than 10% of the original dry weight is recovered after 12 weeks of composting according).

7.5.3 Industrial compostability

According to the review of *Briassoulis et al, 2010* ASTM and ISO standard guidelines are limited to the compostability evaluation (biodegradation, disintegration, compost quality) of plastic materials or a plastic material from a package; however, the standards EN 13432-2000 and EN 14995-2006 developed by the European Committee for Standardization (CEN) provide detailed guidelines for evaluation of biodegradability and compostability of packaging and packaging components, and plastics, respectively, based on their characterization, biodegradability, disintegration, and compost quality/ecotoxicity. For the compost quality or ecotoxicity test, physical and chemical parameters such as density, total dry and volatile solids, salt content, and pH, have to be determined to show that the tested packaging does or does not have negative effects on the compost quality. Only the plant growth test, based on OECD guideline 208, is included in EN 13432-2000 for ecotoxicity. The results (germination numbers and plant biomass) of the compost with the tested material and the blank compost are compared.

7.5.4 Home compostability

In spite of some debate on the value of home composting – some saying it is a valuable and sustainable way of waste reduction and management, others saying it is an important source of greenhouse gases if improperly managed while also hygienic aspects could be a concern – it is an important waste management option in several countries. The major difference with industrial composting is the temperature profile: although heat generation is the same, heat losses are much bigger because of smaller volume and maximum temperatures reached are much lower. For some biodegradable polymers, which need a thermal trigger to start hydrolysing, this makes a big difference.

So far, no international standards exist which regard to specifications for home compostability. Only in Australia a norm was published: AS 5810-2010 “Biodegradable plastics – Biodegradable plastics suitable for home composting”. This norm is largely inspired by the OK compost HOME programme of Vinçotte which was published earlier. In essence, the requirements are largely the same as for industrial compostability with as major difference the necessity to determine biodegradation at ambient temperature as well as disintegration. For the latter a qualitative or visual evaluation of disintegration is sufficient and a quantitative determination with calculation of a mass balance after sieving and retrieval is not needed if such information is already available for industrial composting.

8 Relation towards REACH

The regulation (EC) No 1907/2006 of the European parliament and of the council of 18 December 2006 describes an integrated system for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). This regulation is based on the principle that it is the task for the manufacturers, importers and downstream users to ensure that they manufacture, place on the market or use substances that do not adversely affect human health or the environment.

Chemical substances will be subject to registration followed by a defined procedure that will be applied by the European Chemicals Agency (ECHA) in Helsinki. This agency is responsible for the accepting or rejecting new chemical substances.

Registration is the essence of REACH: no data = no market. All substances, which are produced or imported in a quantity of 1 tonne or more per year shall be submitted for registration to the Agency. For some product groups a registration duty does not exist. The required information includes:

(1) General registrant information
(2) Identification of the substance
(3) Information on manufacture and use(s) of substance
(4) Classification and labelling
(5) Guidance on safe use
(6) Information on exposure
(7) Physicochemical properties of the substance
(8) Toxicological information
(9) Ecotoxicological information

The amount of required information depends on the production volumes. Standard requirements are outlined for the lowest tonnage level (1-10 tonnes) and every time a new tonnage level (10-100 tonnes, 100-1000 tonnes, > 1000 tonnes) is obtained, additional requirements are added.

As an illustration more information is given with regard to the required ecotoxicological information required per tonnage level in Table 79.

Table 79. Overview of the required ecotoxicological information per tonnage level.

Tonnage level	Required ecotoxicological information
1 – 10	Aquatic toxicity: Short-term toxicity testing on invertebrates Aquatic toxicity: Growth inhibition study with aquatic plants Biotic degradation: Ready biodegradability
10 – 100	Aquatic toxicity: Short-term toxicity testing on fish Aquatic toxicity: Activated sludge respiration inhibition testing Abiotic degradation: hydrolysis as a function of pH Fate and behaviour in the environment: adsorption/desorption

Tonnage level	Required ecotoxicological information
100 – 1000	Aquatic toxicity: Long-term toxicity testing on invertebrates Aquatic toxicity: Long-term toxicity testing on fish Biotic degradation: Simulation testing on ultimate degradation in surface water Biotic degradation: Soil simulation testing (substances with high potential for adsorption to soil) Biotic degradation: Sediment simulation testing (substances with high potential for adsorption to sediment) Biotic degradation: Identification of degradation products Fate and behaviour in the environment: Bioaccumulation in aquatic species Fate and behaviour in the environment: Further information on adsorption/desorption Effect on terrestrial organisms: Short-term toxicity to invertebrates Effect on terrestrial organisms: Effects on soil micro-organisms Effect on terrestrial organisms: Short-term toxicity to plants
> 1000	Effect on terrestrial organisms: Long-term toxicity testing on invertebrates Effect on terrestrial organisms: Long-term toxicity testing on plants Long-term toxicity to sediment organisms Long-term or reproductive toxicity to birds

The next step is the evaluation. There are two types of assessment: dossier evaluation and substance evaluation.

The final step is authorisation. For substances of very high concern a permission of the Commission is required. Substances of very high concern include substances with CMR (carcinogenic, mutagenic or toxic for reproduction) properties, PTB-substances (Persistent, Bioaccumulative and Toxic), vPvB-substances (Very Persistent and Very Bioaccumulative) and other substances, which have serious and irreversible consequences towards human health and the environment. Only if the risks can be controlled, a permission will be given. If this is not the case, the Commission will evaluate if the use of the substance has a high social and economic importance and if there exist alternatives. Based on these criteria, the Commission will decide if a permission is given.

9 Conclusion

Freshwater aerobic aqueous environment

Based on the literature review of the different biodegradation test methods in an aqueous aerobic freshwater environment it can be concluded that a sufficiently broad range of measurement techniques already exists. Not each test method is suitable to test bio-lubricants or bio-solvents due to the fact that bio-lubricants are often poorly water soluble and that bio-solvents are often volatile. Therefore specific biodegradation test methods need to be selected taking into account these characteristics. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used. It also needs to be evaluated if the usual water-soluble reference materials (aniline, sodium benzoate, etc.) should be replaced by a poorly water soluble or a volatile alternative.

The review of the freshwater biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others influence of inoculum source (geographical variations, seasonal variations, etc.) on the biodegradability potential and variability of the results, interpretation of variability due to nitrification, necessity of the addition of a nitrification inhibitor, determination of the minimum amount of replicates, etc. should be further investigated.

With regard to environmental safety, it can also be concluded that a sufficiently broad range of testing methods towards freshwater organisms on different trophic levels (bacteria, algae, freshwater aquatic plants, crustacean and fish) already exists. For bio-lubricants and bio-solvents, additional attention is especially needed towards the addition of poorly water soluble bio-lubricants and volatile bio-solvents to the testing systems as this can influence the test results.

The review revealed that specifications need to be developed towards bio-lubricants and bio-solvents. A technical report with useful recommendations for a specification towards bio-lubricants (CEN/TR 16227) and the specifications for the EU Ecolabel for lubricants can be taken as a guideline in order to develop a specification towards bio-lubricants. This should also be developed for bio-solvents.

A labelling system for bio-lubricants has been developed by different organisations, but no European or international labelling systems especially towards bio-solvents is developed yet. This should be developed taking into account parameters like biodegradability, environmental safety, minimum bio-based content, etc.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Marine aerobic aqueous environment

From the literature review on the biodegradation test methods in a marine aerobic environment, it can be concluded that there exist considerably less biodegradation test methods when compared to a freshwater environment. However, a sufficiently broad range of methods exists in order to determine the biodegradation in a marine environment. Not all

methods are suitable in order to evaluate the biodegradability of bio-lubricants and bio-solvents, but a suitable measurement technique can be selected taken into account the specific properties (volatility and/or solubility) of a bio-lubricant or a bio-solvent. Special care should be given towards the addition of these substances (poorly water soluble or volatile) to the testing system and if required special addition techniques should be used.

The review of the marine biodegradation test methods also revealed a few items, which should be further investigated in order to optimize the test methods. Among others the inoculum (natural seawater versus artificial seawater), the addition of nutrients, the difference between conditions in different parts of the sea (supralittoral, eulittoral, sublittoral benthic, deep sea benthic, pelagic & buried in the sediments), etc. should be further investigated.

With regard to marine environmental safety it can be concluded that less tests were developed when compared to the freshwater environment especially on OECD level. ISO and ASTM are already more progressive as more guidelines towards marine organisms were developed. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

Currently no standard specifications towards more environmentally friendly alternatives for lubricants and solvents used in a marine environment are developed yet. These specifications need to be developed. This can be based on the EU Ecolabel for lubricants, which encompasses already marine applications.

Work with regard to the above mentioned aspects will be performed in the framework of task 6.2 of KBBPPS.

Anaerobic environment

Based on the review on the existing biodegradation standards in an anaerobic environment, it can be concluded that there exists a sufficiently broad range of standards in order to determine the degree of anaerobic biodegradation in aquatic environments, high-solids anaerobic-digestion environments and landfill environments. Suitable methods need to be selected for bio-lubricants and bio-solvents.

Lubricants and solvents can reach anaerobic aquatic environments in wastewater treatment plants, but high-solids anaerobic-digestion environments and anaerobic landfill environments are probably not considered as environments in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

Toxicity towards anaerobic bacteria can be evaluated based on existing methods. These methods can be used for lubricants and solvents. The guidance documents towards the sample preparation and the interpretation of the results of toxicity tests for difficult substances, poorly water soluble substances and lubricants should be taken into account.

For biopolymers, which are degradable in anaerobic digestors, it might be necessary to evaluate if toxic residuals remain present in the produced digestate. Further research is needed in order to determine how this should be done.

Currently no standard specifications nor labelling systems are developed for products which are biodegradable in an anaerobic digester (e.g. biopolymers). This is mainly caused by the fact that there exists a wide variation in the construction and the operation of anaerobic digestion systems. The construction and operation systems can be divided into categories based on two parameters: (1) temperature (mesophilic and thermophilic) and (2) dry solids content (wet systems and dry systems). A standard specification should be developed, which includes criteria per operation system. This standard specification should form the basis for a new labelling system.

The labelling systems for lubricants do not refer to anaerobic environments in order to evaluate biodegradability and environmental safety. However, taken into account that a high percentage of ultimately aerobically biodegradable components needs to be present in the major part of the labelled products, it is expected that the labelled products will already be degraded before they come in contact with anaerobic environments.

Soil environment

A few international norms are available about testing biodegradability in soil. Their current weakness concerns their reliability in the cases of intentional incorporation of biodegradable materials in the soil under real conditions. Such a practice is widely used in agriculture and concerns the vast majority of applications where testing of biodegradation in soil is a key prerequisite with respect to both, environmental and food safety aspects. Considering the existing biodegradable plastics in agriculture and the effective life management of the plastics in use at the agricultural field, only few norms have suitable tests that could be adapted for testing biodegradability in soil under real field conditions. The standardised criteria, parameters and testing methodologies for the characterization, labelling and validation of the agricultural plastic waste streams with respect to possible biodegradation in soil suggest that some major revisions are needed, before a new (i.e. revised) universal norm and improved standard testing methods become available for testing agricultural plastics for biodegradation under real, and highly variable, soil conditions. Based on the analysis of the different norms and their content it appears necessary to incorporate provisions for transferability of results to different soils, validation of test through a positive reference and set prerequisites for soil media. Furthermore, terminology and technical specifications vary and need to be harmonised. Long term biodegradation in soil prediction is another open issue (*Briassoulis and Dejean, 2010*). It is clarified though that there is no need for new testing method for biodegradation in soil. However, the existing standards for biodegradation in soil have to be improved and adapted in order to take into account the need for transferability of results to different soils under real field conditions. This goal should be achieved in a way that the standard testing method allows for repeatability of results by various laboratories. Another issue raised recently concerns the possibility to measure the possible production of new cell biomass or incorporating it into the humus that is not “measured” through the current testing methods.

An improved revised universal norm should be based on testing method(s) that include a well-documented range of several typical soil types and a well-defined range of conditions bracketing the majority of soil types and prevailing conditions, for a specific region. A basic requirement to characterise a product as “biodegradable product” is the necessary time for

biodegradation. This is particularly important for agricultural applications where intentional incorporation in soil is the key motivation for the use of biodegradable products. Such a practice, if biodegradation rate is slow, may result into excessive accumulation of materials in the soil. The time use at the field will depend on the type of crop and on the farmer practices but it is common that the biodegradable plastics should have mineralised in the soil before the soil cultivation practices start for the next year crop. In addition, a range of grades for different biodegradation time under different latitudes or climates can be established, following the analogous procedure established by the standard for ageing of plastic films under different geographic areas (solar irradiance). For example in the French norm a set of grades is adopted to define the required biodegradation time of the different mulching films (*Briassoulis and Dejean, 2010*). In addition all relevant safety (e.g. heavy metals) and ecotoxicity requirements for soil biodegradable products should be met (as for example required by the French Norm, OK biodegradable SOIL).

Concerning bio-based lubricants, solvents etc, there is a need for an appropriate testing method that should be based on proper adaptation of testing methods for biodegradation of bio-based polymers in soil, combined with specifications and labeling analogous to those already available for biodegradable in soil plastics. Work in this direction will be performed in the framework of task 6.2 of KBBPPS and will be based on adaptation of existing standards for biodegradation in soil of biobased plastics.

Industrial composting environment

Many norms concerning testing of compostable plastics have been developed at national and international level. Some are about plastic materials others about products like packaging. The media and conditions of testing cover mainly the conditions designed for industrial composting facilities, and only a few concern home composting conditions (*Briassoulis et al. 2010)*. Also, only a few of the existing norms will be suitable, after appropriate revisions, to be adapted to testing biodegradable/compostable agricultural plastic products under farm composting conditions. Farm composting involves techniques not foreseen by the industrial or home composting methods. Farm composting is particularly relevant to biodegradation of biobased materials used in agriculture and specifications differ depending on the cultivations (e.g. organic farming requirements are more strict according to the relevant legislation).

The terminology and the biodegradability validation criteria under composting conditions, such as the threshold percentages of biodegradation and disintegration, the time and temperature, and the ecotoxicity, differ to some degree for the various norms and standard testing methods. Criteria for the establishment of a new integrative norm for compostable plastics used in agricultural applications need to be defined (*Briassoulis et al. 2010)*. Such a norm may include for example home composting and farm composting or only farm composting test methods and specifications where the last one may be based on the existing test methods adapted to practices and conditions of farm composting.

An industrial composting environment is probably not considered as an environment in which lubricants and/or solvents are often spilled or disposed. These standards are more suitable for biopolymers.

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