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**Development of a concept for the environmental risk
assessment of biocidal products for authorization purposes
(BIOEXPO)**

- Part 1: Framework and data requirements for environmental compartments -

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16 Abstract The BIOEXPO project is focussed on test data, required for the authorization of biocidal products. The draft EC Biocidal Products Directive distinguishes between a common core data set (Annex II) and an additional data set, with specific data requirements for biocidal product types (Annex III). The BIOEXPO study aims to provide an input to the European discussions on Annex III, by proposing data requirements for 23 biocidal product types. In the project, the life cycles of the 23 biocidal product types were studied, in order to identify the environmental compartments that are exposed due to the application, use and disposal of the biocidal product and treated materials. This is described in another report ("part 2: Release estimation for 23 biocidal product types"). This report describes the criteria for setting data requirements, and data requirements for the environmental compartments fresh surface water, marine surface water, soil, STP and air. For a biocide released to one of these environmental compartments, data requirements are proposed to enable or improve an environmental risk assessment in that compartment. These data items are discussed on their benefits (enable a compartment-specific assessment, enable a chemical-specific assessment or increase the field-relevance of the assessment), on their compatibility (with current risk assessment methods) and their feasibility (are test-protocols available, or do they have to be developed?). As transport from one environmental compartment to another can occur after release, a tiered approach is proposed to identify all relevant environmental compartments.		
17 Keywords Biocidal Products Directive, biocides, data requirements, environmental risk assessment, exposure assessment		
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Preface

The BIOEXPO project (Carried out by TNO, in commission of the German Umweltbundesamt in the period January 1996 to December 1997) aims to identify the test data required for an environmental risk assessment of biocides, in the context of the authorisation of biocidal substances and products (EC Biocidal Products Directive).

The results of the project are described in two reports. The underlying report (CR97/012) contains the principles, starting points and methodology of the project (the framework), together with possible test data for environmental compartments. The second report, “BIOEXPO - Release estimation for 23 biocidal product types” contains release scenario's for 23 groups of biocides. An executive summary of the two reports is available as a separate document.

During the projects, three workshops were organised by the Umweltbundesamt, to discuss intermediate results. These workshops were attended by experts from UBA and international experts on biocides and exposure assessment. The following institutions were represented at one or more workshops:

- Bundesministerium für Umweltschutz, Naturschutz und Reaktorsicherheit, Germany
- College Toelating Bestrijdingsmiddelen (CTB), The Netherlands
- European Chemicals Bureau (ECB), Italy
- European Commission, Belgium
- Finnish Environmental Institute (FEI), Finland
- Ministère de l'environnement, France
- National Chemicals Inspectorate (KEMI), Sweden
- Rijksinstituut voor Volksgezondheid en Milieu (RIVM), The Netherlands
- Robert Koch Institut (Bundesinstitut für Infektionskrankheiten und nicht übertragbare Krankheiten), Germany
- Umweltbundesamt, Germany

1. Introduction

The Biocidal Products Directive (concept directive 97/C69/03), in preparation at this moment (EC, 1997), will complete the European chemicals regulation, i.e. the directives on existing substances (Council regulation 793/93), new substances (Directive 92/32/EC) and agricultural pesticides (Directive 91/414/EC). Biocides include a wide range of product types, such as pest control products, disinfectants, preservatives, and antifouling products. In Annex V of the Biocidal Products Directive, 23 different product types are defined.

The Biocidal Products Directive will regulate the authorisation of biocidal substances and products on the European market. It will harmonise the authorisation schemes for biocidal products, through the use of common principles, with these authorisations being mutually acknowledged in all member states (with exception of the product types 15 (avicides), 17 (piscicides) and 23 (control of other vertebrates)). The granting of authorisations depends on:

- The submission of data in accordance with the data requirements in the common core data set for active substances (Annex II) and products (Annex III);
- The evaluation of data in accordance with the Common Principles (Annex VI);
- The active substance being listed in Annex I.

Three Technical Guidance Documents are being prepared in 1997-1998 to facilitate the implementation of the Biocidal Products Directive:

- Elaboration of the Common Principles for the evaluation of dossiers (Annex VI), performed by the UK;
- Formulation of the criteria to include substances in Annex I (active substances, low-risk substances, commodity substances). Sweden is preparing this Technical Guidance Document;
- Elaboration of data requirements for biocidal products (Annex III). This Technical Guidance Document will be prepared by Finland.

As the scope of the BIOEXPO project shows an overlap with the Technical Guidance Document prepared by Finland, communication was maintained.

The objective of the BIOEXPO project, is to develop specific data requirements for biocidal product types, taking into account:

- Product-specific characteristics, such as formulation and application;
- The life cycle, including the manner of application, the use of treated products, and disposal;
- Exposure assessment, including the identification of the main environmental compartments which are expected to be exposed.

The current project is focused on the data requirements necessary for an environmental risk assessment of biocidal products; occupational exposure and

human health fall outside the scope of the project. Furthermore, the project is focused on chemicals; biocidal products containing fungi, micro-organisms and viruses as active ingredients (data requirements in Annex IV) are not considered.

In this project, the application phase of the life cycle of biocidal products is considered, as well as the disposal of treated materials. The production and formulation phase are not taken into account. The Directive is not conclusive on this aspect. The reason for not including production and formulation is that the project focuses on environmental releases during the use of specific biocidal product types, and they can only be regarded as products after formulation.

The BIOEXPO project has a compartment-specific and a product-specific component. Within the product-specific component (Report “BIOEXPO - Release estimation of 23 biocidal product types”), the life cycles of the 23 product types of Annex V of the Directive are analysed, and the main exposed environmental compartments are identified for each product type. Furthermore, data requirements are identified which are necessary to estimate emissions to these exposed compartments (e.g. leaching rates). In the compartment-specific part (the underlying report), data requirements are identified per environmental compartment. This part lists the data which is required for the environmental risk assessment of a biocide emitted to a specific compartment (e.g. surface water).

In this report, a framework for setting data requirements is presented (chapter 2), as well as data requirements per environmental compartment. The framework describes the rationale for requesting additional data, and describes criteria to evaluate the pro's and con's of additional data. In chapter 3, the contents of Annex II ('common core data set') of the Biocidal Products Directive are discussed. Technical problems are expected with biodegradability tests, as biocides are (often) intended to kill micro-organisms. Biodegradability tests and their applicability to biocides is discussed in a separate chapter (chapter 4). In the remaining part of the report (chapter 5 to 10), data requirements are discussed for the following environmental compartments:

- Fresh surface water (incl. sediments)
- Marine surface water (incl. sediments)
- Soil (incl. groundwater)
- Sewage Treatment Plant (STP)
- Air

The basic question posed is: “What kind of data, additional to the common core data of Annex II, is necessary for a risk assessment of a biocide in a given environmental compartment?”. Data items are proposed when a clear benefit to the risk assessment is evident. These data items are then discussed on their compatibility with existing risk assessment calculation rules, and feasibility in terms of test guidelines. The report is concluded with a discussion on the use of these data requirements (chapter 11).

2. Framework for setting data requirements

2.1 Data requirements in the Biocidal Products Directive

In the draft EC Biocide Product Directive (EC, 1997), principles are outlined for the authorisation of biocidal substances and products for the European market. The Directive prescribes the data (test results), that should be submitted by a company that aims to introduce a new biocide. This data set is extensive, and comprises test results for substances and products. Substances are the active ingredients (in this case, chemicals) whereas products are formulations which contain one or more active ingredients together with solvents, surfactants, builders, etceteras. The required test results are described in two Annexes. Annex II comprises the "common core data set", which is based on that of Directive 67/548/EEC (Annex VIIA). No distinction between product types is made in Annex II. Annex III provides the additional data set, which is additionally required for the authorisation of a biocide. The selection of appropriate Annex III additional data in support of a submission is not definite, and considerable interpretation is required:

"Information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not to be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases a justification, acceptable to the competent authority must be submitted. Such a justification may be the existence of a frame-formulation which the applicant has the right of access to" (Biocidal Products Directive, heading of Annex III).

As the actual contents of a dossier is not clearly defined at the moment, the additional information of Annex III will be further elaborated in a Technical Guidance Document, which will be prepared by Finland in co-operation with other member states. This Technical Guidance Document will list data requirements per product type.

The purpose of the data requirements in the Directive is formulated in Annex VI (Common principles, item 5):

"In order to carry out a risk assessment data are required. These data are detailed in Annexes II, III and IV and, recognising that there are a wide variety of product types, are flexible according to the product type and associated risks. The data required shall be the minimum necessary to carry out an appropriate risk assessment. (...)"

The data in Annex III has to be specified for each individual product type. The data has to resemble the minimal requirements for an "appropriate risk

assessment". Data which is not necessary for an appropriate risk assessment, can be regarded as "not scientifically necessary". An appropriate risk assessment is not clearly defined, but will depend to some extent on the "Common Principles" for the evaluation of dossiers for biocidal products (described in Annex VI, Item 36 to 47). These common principles will be further elaborated in a Technical Guidance Document (to be prepared by England, as stated in the introduction). Data requirements depend on the risk assessment methods. In this project, current state-of-the-art risk assessment methods and the Common Principles are taken as reference for the BIOEXPO project. Risk assessment methods appropriate for non-agricultural biocides are, for example:

- EC Technical Guidance Documents for the risk assessment of new and existing substances (EC 1996a);
- ECETOC guidance on exposure and risk assessment for chemicals (ECETOC 1990a, 1990b, 1992, 1994a, 1994b, 1994c, 1995);
- EUSES model for the risk evaluation of new and existing substances (EC, 1996b);
- CHARM model for the risk evaluation of offshore E&P chemicals, including biocides (Karman *et al.*, 1996a; 1996b)
- Multi-media Mackay-type environmental models (Mackay *et al.*, 1996); such as SIMPLEBOX (Van de Meent, 1993)

In this project, "appropriate risk assessment" is interpreted as the risk assessment as it is laid out in the Common Principles, and performed in practise in the EC Technical Guidance Document, as these concepts deal with the environmental risk assessment of biocides (a.o.) and have an international accepted status.

According to Annex III, an exception in the data requirements can be made when it is not "technically possible" to submit the data. The 'technical possibility' is interpreted in this project as the availability of test guidelines. The availability of test guidelines is not used as an exclusive criterion in this study, but for proposed data items the availability of test guidelines is indicated.

2.2 Criteria for setting data requirements

The minimal contents of a dossier, that needs to be submitted for a new substance or product, is a compromise between needs and restrictions.

The needs are the minimum requirements for an appropriate risk assessment. Risk evaluation procedures are subject to international harmonisation, and are themselves a compromise between demands and possibilities. Data requirements are strongly dependent on the consensus regarding to what kind of risk assessment is appropriate. The ultimate goal is to maintain a high level of human and environmental health and safety in the community.

The restrictions, on the other hand, are the availability of accepted (robust) test guidelines, and the costs for the industry that produces or markets new products or substances.

Any test results, required additional to the core set, must be weighted and evaluated against this spectrum of needs and restrictions. In the current report, the inclusion of additional data is evaluated according to four criteria:

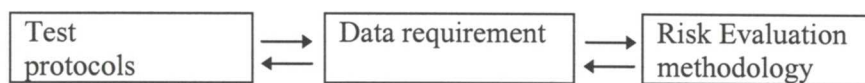
1. **Benefits:** Improvement of the risk characterisation due to the use of additional test data, i.e., the need must be clear. Furthermore, additional data is needed to ensure an ‘appropriate risk assessment’, as laid down in the Common Principles.
2. **Compatibility with risk assessment methods:** Compatibility with generally accepted risk assessment methods (calculation rules): It must be possible to use the requested test results for risk assessment;
3. **Technical feasibility:** The possibility to actually deliver the requested test results; the availability of (international, national, or otherwise appropriate) test guidelines. However, the technical feasibility is not used as a criterion *sensu stricto*, as demands (e.g., for risk assessment purposes) can generate the development of test guidelines.
4. **Minimisation of test animals:** As the EC and OECD aim to reduce the use of test animals, the number of animals required for testing should be kept as low as possible.

The benefits are specified more clearly, to be able to use the “benefits” as a criterion. The improvement of the reliability of the result of the risk assessment (*benefit*) can be based on:

1. **Compartment specific assessment:** When a risk evaluation envisages a compartment-specific assessment of the risks related to the use of a biocide, the requested test data should be sufficient to perform risk assessment for all relevant environmental compartments. This can result in specific test data for compartments.
2. **Enable chemical specific assessment:** The specific behaviour in terms of environmental transport, fate and effects of various chemical groups (e.g. metals, hydrocarbons, acids/bases, surfactants) may require specific tests to be performed to model such factors.
3. **Increase of field relevance:** When the actual environmental fate and effects cannot be adequately predicted from the test results, additional test data, e.g. from mesocosm experiments, biodegradability simulation tests or monitoring studies may be useful. This type of test results can also be required to decrease the uncertainty in the extrapolation from laboratory test data in the risk evaluation, due to over simplification of environmental processes in risk evaluation methods.

A complication is that test protocols, data requirements and risk assessment cannot be separated. There is an continuous interactive feedback between those issues: the

development of risk evaluation methodologies is strongly influenced by availability of data (test protocols and requirements), whereas the development of test protocols is determined by the need of data (risk evaluation and requirements).



It is not coincidental that standard protocols are available for the data of the core data set, and that risk assessment is attuned on these test results. *This circular interaction complicates the justification of additional data.* The demand for additional data should be strongly argued from a perspective of improved risk evaluation (“scientifically necessary”), available test procedures (“technically feasible”), and cost effectiveness. Requirement of additional data implies the extension of risk evaluation methods and implementation of new test protocols, and this should be restricted to current practices and developments in risk evaluation and environmental testing.

Three categories of environmental data can be distinguished as follows:

1. Environmental release
2. Environmental transport and fate: sorption and degradation
3. Environmental effects: toxicity and bioconcentration

For each of these data categories, the data set can be expanded on the basis of an improvement of the risk assessment. In Table 1, potential additional data is presented for each of the three data categories, specified for each of the three 'rationales' for benefit:

Table 1 Examples of additional release, fate and effects data, based on the three aspects of benefit

Benefit				
	<i>Annex II</i>	<i>compartment specific assessment</i>	<i>chemical specific assessment</i>	<i>increase of field relevance</i>
<i>Release</i>	efficacy and use data	--	leaching tests	field studies
<i>Fate</i>				
-sorption	screening 3 soil types	actual matrices	sorption tests for surface active compound	mesocosms/ field monitoring
-degradation	water	marine, sediment or soil tests	specific degradation tests	mesocosms/ field studies
<i>Effects</i>				
-toxicity	algae, fish, <i>Daphnia</i>	marine, sediment or soil tests	concentration tests for surface activate or readily metabolised compounds	mesocosms/ field studies
-bioconcentration	fish	actual biota		field monitoring

increase in scientific performance of risk evaluation →

decrease in technical feasibility of test procedures →

increase of costs for applicant →

It is obvious from this matrix that there is often a conflict between data requirement for a more appropriate risk assessment on the one hand and technical impediments regarding standard or robust data acquisition (test procedures) on the other hand.

Based on their perceived benefits, potential additional data is indicated for the cells of the matrix. This proposed data is then evaluated for compatibility with risk assessment procedures, and the availability of test guidelines ('feasibility'). This will result in a list of data from which to choose when registering a biocidal substances or products (inclusion in Annex III).

2.3 Procedure for deriving data requirements

In this section, the procedure to derive data requirements for each biocidal product type is described, using the criteria from section 2.2.

Step 1: Release estimation

Analysis of the application of the biocide, the use of the treated material (e.g. preserved wood, disinfected drinking water, etc.) and the disposal of the treated material, in order to estimate environmental release. The emissions during these phases in the life cycle are inventoried, and relevant emissions are listed. The

release estimation is published in the report “BIOEXPO - Release estimation of 23 biocidal product types”. Knowledge of the life cycle results in the identification of the main environmental compartments to which the biocide is likely to be released in significant quantities.

Step 2: Generic data requirements

The data requirements necessary for environmental transport, fate and effects assessment are discussed on a generic basis in this report (chapters 4 to 9), in reference to criteria described in section 2. The data requirements are specified for environmental compartments, which are linked in turn to biocidal product life cycles. This will result in a list of potential additional data requirements. The benefits, compatibility and feasibility are discussed.

Step 3: Data requirements for product types

Two types of data can be distinguished for the risk assessment of a biocidal product. First, data required for a risk assessment in the environmental compartments to which the biocide is directly emitted (*‘direct exposure compartments’*) are derived from the life cycle. However, after emission to these compartments, transport to other compartments can occur, resulting in further exposures, i.e. *‘indirect exposure compartments’*. Whether this exposure takes place or not, depends on the characteristics of the biocidal product. Therefore, a *tiered approach* is proposed to set data requirements for a specific product type.

In *tier 1*, data requirements for the direct exposure compartments are listed. Then, an assessment has to be made whether indirect exposure compartments are exposed or not.

For this assessment, a combination of techniques, including expert opinion, may be required. A Mackay-type multimedia model is a useful tool to obtain insight in the distribution of a substance over environmental compartments. The data set of Annex II is sufficient to run a Mackay-type model. However, the amount of chemical is not the one important aspect; the sensitivity of the environmental compartments is equally important. The toxicity in compartments other than the aquatic environment can be estimated from aquatic toxicity data (present in Annex II) in this screening phase. Furthermore, Mackay-type models are based on substances, and a product cannot always simply be seen as a collection of substances. Some rules for dealing with mixtures are required.

The use of a multimedia (Mackay-type) model has the advantage that such an approach is in line with risk assessment practise, e.g., the multimedia model SIMPLEBOX is part of the EUSES model.

Tier 2 consists of the listing of data requirements for the risk assessment in relevant indirect exposure compartments.

This tiered approach ensures that only relevant test results are required (see Figure 1).

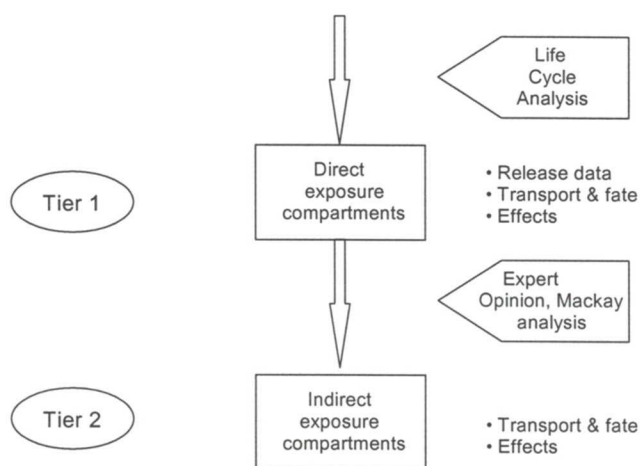


Figure 1 Tiered approach for setting data requirements

Expert opinion will always be necessary in deciding which environmental compartments (and subsequently, which test data) are relevant for risk assessment. A Mackay-type model can provide information about the environmental transport and fate of chemical substances, but the behaviour of products in the environment is also dependent on the application form and technique, as well as natural or environmental conditions (e.g., climate, soil characteristics etceteras). Another consideration to be addressed on a case-to-case basis is whether additional data is required (next to the core data) when an indirect exposure compartment is exposed (or in other words: should indirect exposure compartments be treated on the same level as direct exposure compartments?).

3. Data requirements in Annex II

3.1 Introduction

In Annex II, physico-chemical data, basic ecotoxicity data and biodegradation data of biocidal products and substances is requested. In this chapter, the data items of Annex II regarding release estimation (section 3.2), environmental exposure assessment (3.3) and environmental effects assessment (3.4) are described. The main objective of this chapter is to assess to what extent the issues release estimation, exposure assessment and effects assessment are covered by the data of Annex II.

In this chapter, the data of Annex II is summarised, and the suitability of the available test guidelines as applied to biocides is discussed. Both the absence of data in Annex II (e.g., required for adequate exposure assessment) or the inapplicability of test guidelines for biocides can be a reason to demand additional data in Annex III.

3.2 Release estimation

Sufficient data is required to quantify the input of biocidal substance(-s of a product) into an environmental compartment. In Annex IIA, the purity (item 2.7) of the biocidal *substance* (g.kg^{-1} or g.l^{-1}) and the identity and possible ranges of impurities and additives (item 2.8) are requested. Additionally, the likely tonnage to be placed on the market per year should be submitted (item 5.8).

Annex IIB requires further information on *products* (item 2.2): “Detailed quantitative and qualitative information on the composition of the biocidal product e.g. active substance(s), impurities, adjutants, inert components.” Furthermore, the application rate and, if appropriate, the final concentration of the biocidal product and its active substances in the system in which the preparation is to be used (item 5.3), and the number and timing of applications (item 5.4) is required.

For products that are directly released to the environment (or to a Sewage Treatment Plant), such as disinfectants or some preservatives, this information should be sufficient to calculate the environmental release. However, when the active substances are released slowly (e.g. antifouling biocides or some pest control products) or are inserted in a medium that has to be protected (e.g. wood preservatives), additional information is required, e.g. leaching rates. This data will be introduced during the life cycle descriptions for the relevant product types.

3.3 Exposure assessment

In Annex IIA and B, the basic information to predict the environmental transport and fate of a chemical is requested. Essentially, Annex II includes test data necessary to predict the behaviour of an organic substance in an aqueous medium (biotic and abiotic degradation, n-octanol/water partition coefficient, adsorption/desorption). No data however is included to predict the behaviour of a substance in sea water, sediments, soil, air or a sewage water treatment plant. The following items are included in Annex II:

- Molecular and structural formula, molecular mass (IIA, item 2.5)
- Melting point, boiling point, relative density (IIA, item 3.1)
- Vapour pressure (IIA, item 3.2)
- Solubility in water (including effect of pH 5-9, and temperature, when relevant) (IIA, item 3.5)
- Partition coefficient n-octanol/water, including effect of pH 5-9 and temperature (IIA, item 3.6)
- Surface tension (IIA, item 3.10)
- Ready biodegradability and (where appropriate) inherent biodegradability (IIA, item 7.6.1)
- Hydrolysis as a function of pH, and identification of the breakdown products (IIA, item 7.6.2.1)
- Phototransformation in water, including identity of the products of transformation (IIA, item 7.6.2.2)
- Adsorption/ desorption screening test (IIA, item 7.7)
- Exposure data in conformity with Annex VIIA to Directive 92/32/EEC (IIA, item 2.10)
- Foreseeable routes of entry into the environment on basis of the use envisaged (IIB, item 7.1)

3.4 Effects assessment

Most of the required data which can be used for environmental effect calculation are listed in section VII of Annex IIA. The data refer to aquatic toxicity tests with fish (item 7.1), *Daphnia magna* (item 7.2) and micro-algae (item 7.3), in addition to inhibition of microbiological activity (item 7.4) and bioconcentration (item 7.5).

From section VI (toxicological and metabolism data), data is requested that can be used to assess the risk of substances to top predators (e.g. fish eating birds and mammals). Apart from the metabolic studies, the data requirements include the effects resulting from oral uptake by rodents or other mammalian species. Studies include acute (item 6.1), sub-chronic (items 6.3, 6.4), and chronic toxicity (item 6.5), mutagenity (item 6.6), carcinogenicity (item 6.7) and reproductive toxicity (item 6.8). The mammalian toxicity data should be summarised (item 10) and must

include a NOAEL, a NOEL and an overall evaluation with regard to all toxicological data and any other information concerning the active substance.

Furthermore, information on the effects on target organisms (item 5.3) and mode of action (item 5.4) are required (for the effectiveness of the biocide). This information can also be applied in risk assessment.

For biocidal *products* (as opposed to substances), the data requirements are described in Annex IIB. The ecotoxicological information that can be used for environmental effect calculation includes:

- “information on the ecotoxicology of the active substance in the product, where this cannot be extrapolated from the information on the active substance itself” (item 7.2)
- “*available* toxicological information relating to ecotoxicologically relevant non-active substances (item 7.3)

Furthermore, the acute toxicity to one other, non-aquatic, non-target organism (item 7.1) is required. Other required toxicological data are listed in section VI for determination of acute toxicity by oral uptake, and by the likely route of human exposure (item 6.1).

For products, as for substances, the effects on target organisms (item 5.7) and mode of action (item 5.8) are required, where they are not covered by Annex IIA. Observations of undesirable or unintended side-effects (beneficial or other non-target organisms) should be reported (IIA, item 8.6, IIB, item 8.7)

3.5 Applicability of test protocols to biocides

Biocides are developed to control harmful organisms (including micro-organisms). This special feature can cause problems in certain tests. Of the tests mentioned in the sections 3.2 to 3.4, the biodegradation tests in particular require careful interpretation, as the test concentrations required by the respective guidelines are often high in comparison to the toxicity of the test compounds. Therefore, special attention is paid in this report to biodegradation tests, and the applicability to biocides of available test methods (chapter 4).

4. Applicability of biodegradation tests to biocides

4.1 Introduction

Biodegradation tests measure the rate of removal of a substance from a particular environmental compartment, whether it be water, sediment, sediment/water, sewage-sludge, or soil in the presence of a bacterial inoculum or standing bacterial population. A major review of the available methods and their applicability can be found in the OECD “Detailed review paper on biodegradability testing” (Painter, 1995). The purpose of this section is to summarise the international standards and where possible draw conclusions as to their suitability in the testing of biocides, many of which were designed to kill micro-organisms. In Appendix I to this report, the most common international biodegradation test protocols are summarised.

Measuring biodegradation.

Biodegradation tests usually measure integral processes, such as the production of CO₂, the consumption of O₂ (BOD), or the reduction of the dissolved organic carbon concentration during the degradation process. Chemical specific analyses may also be used to determine the disappearance or ‘die-away’ of the parent compound and the appearance and subsequent disappearance of any significant metabolites.

In practise, tests which measure die-away of parent compound and primary metabolites as well as an integral parameter may be the most effective. The parent substance and metabolite die-away pattern can provide accurate half-life data for risk analysis, while the integral end-points provide assurance of ultimate biodegradation. The fate of the parent compound can be followed by chemical analysis under most of the currently available international guidelines as an optional extra.

Ultimate vs. Primary biodegradation.

Ultimate aerobic and anaerobic biodegradation can be defined as the breakdown of an organic chemical compound by micro-organisms in the presence or absence of oxygen to respectively produce carbon dioxide and water, or alternatively methane. In both cases, mineral salts or any other elements present (mineralisation) and new biomass may be produced.

On the other hand, primary biodegradation is the structural change (transformation) of an organic chemical compound by micro-organisms resulting in the loss of a specific property, regardless under aerobic or anaerobic conditions.

End-points vs. rate constants.

Classic biodegradation tests were designed to measure the percentage

biodegradation as an end-point following a set period of time, e.g. 28 days. Although each guideline specifies a varying number of measurement points, in aquatic ready tests, these are usually intended to assess the “10d window” (see section 4.2) and are not necessarily suited for determining rate constants. For the purposes of risk analysis, the measurement regime needs to describe more accurately the die-away rate for the compound at the concentration(s) tested.

4.2 Aquatic, aerobic biodegradation tests

Variability of the inoculum.

The strength and composition of the microbial inoculum used has been considered to be critical for the accuracy of biodegradation tests. However, the implementation of strict quality standards is difficult. It should be borne in mind, that while the nature of the start inoculum is only loosely determined in the guideline of ready biodegradation tests, adaptation to the test compound will take place during the test in any case. For a ready biodegradation test, an inoculum is usually sampled from the secondary effluent of a sewage treatment plant receiving “predominantly” domestic sewage. However, such a source will be at least adapted to domestic cleaning products, etc. Activated sludge may be maintained in the laboratory through the use of simple sludge reactors, e.g. a porous pot.

Marine tests such as OECD 306 rely on a natural population of marine bacteria from coastal waters, which may also be chemically adapted to some extent. A recent ring-test comparison of four marine ready biodegradability methods showed very similar performance of all four tests using seawater from a variety of locations around Europe as the source of inocula (Hanstveit, pers comm.).

Ready vs. Inherent biodegradability.

The OECD (1992) defines ready biodegradability tests as:

“Stringent tests which provide limited opportunity for biodegradation and acclimatisation to occur. It may be assumed that a chemical giving a positive result in a test of this type will rapidly degrade in the environment and therefore may be classified as readily biodegradable.”

Specifically the term refers to compounds which are rapidly biodegraded in tests using non-adapted inocula as opposed to “inherent biodegradability” tests in which micro-organisms are pre-adapted to chemicals in general or to one type of chemical in particular. The term inherent may also be applied to soil tests.

The EU New and Existing Chemicals and the Pesticide directives rely heavily on ready biodegradability data for the purposes of hazard classification in relation to transport and supply & use. The validity criteria applied to tests under the OECD 301 A, B, D, E and F guidelines (but not to C) are 70% removal of DOC and 60%

of theoretical oxygen demand or theoretical CO₂ production (respirometric methods). These pass values have to be reached within a 10 days window starting when biodegradation has reached 10% and ending before 28d, i.e. the end of the test, so adding a further conservative element to the tests.

Prescribed test concentrations vs. environmentally realistic concentrations.

The doses of chemicals prescribed by the current crop of aquatic test guidelines are generally high in relation to what can be expected in terms of expected environmental concentrations following some usage patterns. Of the ready tests, OECD 301 D (ISO 10707) and OECD 306 have the lowest test concentrations of 2 to 10 mg.l⁻¹ dissolved organic carbon. OECD 301 A (ISO 7827), B (ISO 9439) and E require concentrations of 10-40 mg.l⁻¹, while OECD 301C and F require 100 mg.l⁻¹. In some practical situations, these levels may be similar to those found for chemicals in waste water treatment influents. Whether they are generally appropriate for continuous low discharges or agricultural run-off seems unlikely.

A large proportion of biocides are designed to be toxic to microbes and in such cases, the use of the prescribed high test concentrations is almost certain to provide a 0% biodegradation result in most ready tests. As shown in Annex I, the majority of the currently available guidelines are not suitable for microbiocidal compounds. This is a fundamental problem in the application of current ready and inherent international guidelines. OECD (1992) & Reynolds et al. (1987) recommend that the toxicity of the substance to the inoculum used in the biodegradation test should be tested beforehand in an OECD 209 bacterial inhibition test. They further recommend that:

“If inhibition due to toxicity is to be avoided, the test substance concentrations used in ready biodegradability testing should be less than 1/10 of the EC₅₀ values (or less than EC₂₀ values) obtained in toxicity testing..... EC₅₀ values of less than 20 mg.l⁻¹ are likely to pose serious problems for the subsequent testing. Low test concentrations should be employed, necessitating the use of the stringent and sensitive Closed Bottle test (OECD 301D/ISO 10707, and OECD 306 marine) or the use of ¹⁴C labelled material. Alternatively, an inoculum previously exposed to the test substance may permit higher test substance concentrations to be used. In the latter case, however, the specific criterion of the ready biodegradability test is lost”.

In this regard, the recently drafted ISO 14592 guideline measuring the ‘die-away’ of low parent compound concentrations (in water less than 100µg.l⁻¹, or in a sediment suspension, slightly higher) may prove exceptionally useful in testing biocides. Designed as either a shake-flask batch test, or a more complex continuous-flow river model, this guideline brings the higher discrimination power of chemical analysis and radio-isotope scintillation counting (relative to that of

dissolved organic carbon, CO₂ or O₂ measurements) to bear on the problem of biocide biodegradation.

In practise, where integral measurements of CO₂ or DOC are required, instead of, or in addition to parent compound and metabolite die-away, there may be little option but to use radio-labelled compounds. In practise, testing at two or more chemical concentrations, each separated by an order of magnitude, may be the only way to provide accurate rates of biodegradation at lower, more 'environmentally realistic' concentrations (where possible, in addition to those prescribed by the guidelines).

Simulation tests.

Waste water treatment.

Flow-through or semi-static simulation tests such as OECD 302A (ISO 9887) and 303A (ISO 11733) better reflect the conditions of a waste water treatment plant and provide a detailed model of biodegradation rates. Again, the use of environmentally realistic test concentrations and ¹⁴C labelling should be considered. As the activated sludge is generally pre-adapted to the test compound beforehand, these are tests of inherent biodegradability.

The OECD detailed review paper on biodegradation (1995) suggests that biodegradation simulation tests require further development, to include methods such as the porous pot on its own, or in combination with Husmann coupled units (EC method). It is also suggested that more attention should be paid to insolubles and volatiles, biofilters, and most importantly for the purposes of biocide testing, to modifications for low concentrations.

Sediment-water tests.

Guidelines for two compartment tests with sediment and water are issued by the German UBA and the Dutch Pesticides Bureau. This type of test which employs several soil types and measures the die-away of ¹⁴C labelled compound and metabolites plus the evolution of ¹⁴CO₂ in water and sediment was developed primarily for pesticides. It may be of considerable use in testing biocides, as it provides information on partitioning to sediment as well as degradation in both water and soil phases. Scaled down versions of the BBA guideline may be used for screening groups of compounds together.

4.3 Aquatic anaerobic biodegradation tests

Only one test guideline was reviewed, i.e. the ISO 11734 anaerobic biogas production test. While this appears to be suitable for poorly soluble compounds, the test is not recommended for compounds which are toxic to the inoculum at the

test concentrations of 20-100 mg.l⁻¹ dissolved organic carbon. The comments made above for aerobic aquatic tests probably apply equally here.

4.4 Soil biodegradation tests

The OECD 304A test of inherent biodegradability in soil is probably the best known of many related tests. This comprises aerobic and/or anaerobic methods to evaluate the mineralisation rate of ¹⁴C labelled test compound in soil by trapping ¹⁴CO₂ or ¹⁴CH₄ respectively, followed by scintillation counting. The test has been widely applied in pesticide research. The anaerobic variant can be used to assess the PEC in saturated sub-soil, while the aerobic test is representative of aerobic top-soils. If desired, the test can provide information on the quantity and nature of bound residues.

4.5 Conclusions

1. Without substantial amendment, many of the current aquatic biodegradation test guidelines are unsuited for testing biocides. In many cases amendment may render the tests invalid in a regulatory sense, although valuable scientific data may be produced.
2. Test substance concentrations which produce accurately measurable CO₂ evolution, dissolved organic carbon die-away or O₂ consumption may prove to be toxic to the inoculum. Possible exceptions are the Closed bottle type tests, including the Two-phased model (ISO 10708).
3. Pre-adaptation of the inoculum may reduce toxicity in the currently available guidelines; this may prove useful providing that inherent data is appropriate to the risk evaluation.
4. Die-away tests (e.g. ISO 14592 and sediment water type tests) using chemical analysis of parent compound and metabolites provide the most useful solution in testing biocides. The use of ¹⁴C labelling produces the most comprehensive data in this respect, as both parent compound die-away and ¹⁴CO₂ evolution can be accurately measured.
5. Testing at more than one concentration in the expected environmental range for the usage pattern in question may provide more reliable data.
6. Where detailed waste water treatment data is required, simulation tests provide the best opportunity (e.g. OECD 302A/ISO 9887, OECD 303A/ISO 11733). The usefulness of the cheaper, static OECD 302B/ISO 9888 for testing biocides is uncertain.
7. Generally, the test sampling regimes of most tests need to be adapted to suit the statistics of half-life rate constants as well as the traditional end of test data.

5. Generic data requirements for fresh surface water and sediment

5.1 Exposure assessment

Current situation

The data set specified in Annex II of the Directive is sufficient for assessing the partitioning and fate of a substance in fresh surface water compartment, by applying calculation rules from the EC Technical Guidance Document. However, the data set of Annex II does not require test data for fresh water sediments.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing (1) a compartment specific assessment, (2) by allowing a chemical-specific assessment, or (3) by increasing the field relevance of the assessment.

Compartment specific assessment

In Annex II of the Directive, a ready and inherent biodegradability test is demanded. As stated in chapter 4, the establishment of biodegradation rates for biocides can be erroneous, and improved degradation tests may be required. Degradation rate in sediments is not required in the common core data set (Annex II). In the EC Technical Guidance Document, biodegradation in sediments can be estimated from biodegradation in water. However, as microbial communities in sediments differ from communities in the water column, with higher densities of micro-organisms and a greater variety, a sediment-water biodegradation test is relevant. According to ECETOC (1990a), the mentioned differences and problems such as limiting conditions (e.g., oxygen) in sediments can cause readily biodegradable substances (in aquatic tests) to be persistent in sediments. As many sediments are mainly anaerobic (except for the top layer), the anaerobic biodegradability is important.

Sediment-water partitioning is not included in the core data set. The K_{oc} for soil/water, included in Annex II (adsorption/ desorption screening test) might also be applicable to sediments.

In certain cases, a leaching rate is necessary for assessing environmental concentrations. This is the case for specific biocidal applications (such as antifouling biocides) and formulations. This topic will be elaborated in the release scenario's.

Chemical specific assessment

Risk assessment according the EC Technical Guidance Document is especially suited for non-polar, non-ionic organic compounds. The behaviour of these

compounds is predicted on the basis of the P_{ow} , from which many partitioning coefficients are extrapolated. For non-organic compounds, additional partitioning data may be required.

For surfactants, the concept of P_{ow} is not entirely valid, as surfactants tend to accumulate at the interface between the water phase and the octanol phase, due to their surface-active properties. A risk assessment using equilibrium partitioning based on a P_{ow} is then not realistic. A second problem in the exposure assessment of surfactants is the formation of micelles, when a critical micelle concentration (CMC) is exceeded. Specific tests for assessing the environmental fate of surfactants must be developed.

Increase of field relevance

Experimental ecosystems (outdoor microcosms and mesocosms) allow long term studies on the fate of chemicals and their impact on selected biota (multispecies communities) under controlled, but realistic (semi-natural) conditions. The measurement of the fate in experimental ecosystems can be performed when the behaviour of a substance is not fully understood (e.g. in the case of surfactants). Mesocosm studies have a high field relevance and can therefore be considered as additional data requirements.

Table 2 Potential data requirements for exposure assessment (PEC calculation) of substances in the compartment fresh surface water.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Die-away biodegradation test with specific chemical analyses or using a radiolabel at environmentally realistic biocide concentrations.	- Where testing with Annex II recommended ready/inherent tests is hindered due to toxicity to the inoculum, e.g. biodegradation is low or zero, further testing is essential. - Can be used with river water, or with suspended sediment to simulate a riverine environment.	Provides data suited to risk analysis at relevant environmental concentrations.	Recent guidelines available: Draft ISO 14592, ASTM E-1279, or other suitable methods which can be used to test a range of test concentrations (including $\mu\text{g.l}^{-1}$ levels of test compound). Note: OECD 301 D cannot be used below 0.5-0.8 mg.l^{-1} test substance.
Aerobic/anaerobic biodegradation in freshwater sediment/water systems, with specific chemical analyses or using a ^{14}C radiolabel.	- Allows a compartment specific assessment, particularly for chemicals which are liable to adsorb strongly to sediment. - Anaerobic and aerobic flasks can be run in parallel. - Can provide data on the quantity and nature of soluble metabolites as well as bound residues.	Provides data suited to risk analysis at relevant environmental concentrations.	Various National sediment/water test guidelines are available and routinely used, e.g. German UBA, Dutch CTB. International standards can be expected in the future.
Biodegradation in fresh water microcosms or mesocosms, i.e. field simulation tests.	- Field relevance is increased - A partial mass balance approach can be taken with several environmental compartments. - Can provide some validation of laboratory data.	Provides data suited to risk analysis	No international guidelines available. Many laboratories have their own unpublished Standard Operating Procedures. ASTM 1624 and a recent SETAC guidance document provide guidelines
Sediment-water partitioning coefficient (fresh or marine)	- Allows compartment specific assessment (instead of using K_{oc} soil-water). - Relevant for non-organics	Possibly more appropriate risk parameter. This data can possibly be derived from a sediment/water degradation test.	Adaptation of OECD 106 soil adsorption/desorption possible.
Leaching rate (from treated material to fresh surface water)	- Required to estimate environmental emissions - Only relevant for some application types, such as antifoulings and wood preservatives	Needs to be known prior to risk assessment	Available for some product types, such as ASTM D5108-90 for antifoulings. Will be elaborated in relevant release scenario's

5.2 Effects assessment

Present situation

In the Biocidal Products Directive, the following fresh water toxicity data is required in the common core data set (Annex II):

- 7.1 Acute toxicity to fish
- 7.2 Acute toxicity to *Daphnia magna*
- 7.3 Acute toxicity to algae (growth inhibition)

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing (1) a compartment specific assessment, (2) by allowing a chemical-specific assessment, or (3) by increasing the field relevance of the assessment.

Compartment specific assessment

The core data set is sufficient for a risk assessment for pelagic and epibenthic species. However, no sediment toxicity tests are required. The EC Technical Guidance Document provides in procedures to estimate the $PNEC_{\text{sediment}}$ from the $PNEC_{\text{water}}$ using equilibrium partitioning (based on P_{ow}). This method has considerable limitations, as it is only valid for non-polar, non-organic substances, and uptake is assumed via the interstitial water. Therefore, there is a need for sediment toxicity tests.

Secondary poisoning can occur when substances are accumulated in the foodchain (bioconcentration, biomagnification). In the EC Technical Guidance Document, the following foodchain is considered to be representative for assessing the secondary poisoning from the aquatic compartment:

water $\Rightarrow$ fish $\Rightarrow$ fish-eating bird or mammal

To assess the concentration in fish (food organisms for fish-eating birds and mammals), the BCF required in Annex II is sufficient. When secondary poisoning is likely, BCF's for other potential food organisms (having a different exposure to water than fish, such as mussels or other bivalves) can be considered.

Test results are required to assess the effects of the consumption of contaminated fish to fish-eating birds and mammals. EU policy is to reduce the number of (vertebrate) test animals used for toxicity testing, and therefore care should be taken regarding the requirement of specific food chain toxicity data. Annex II requires extensive toxicity data on vertebrates, and some of these tests (such as avian dietary tests) can be used to assess secondary poisoning.

Chemical specific assessment

Sediment toxicity tests are especially needed for substances which might partition

to, and form bound residues in, sediments (e.g., heavy metals and hydrophobic organic compounds).

The core data set of the Directive demands non-specific baseline toxicity data (with mortality, reproduction or growth as endpoint). Many biocides, however, rely on specific toxic action, that might be unestimated in standard toxicity tests. An illustrative example is the environmental risk of tributyltin (TBT), which is primarily related to malformations of the reproductive system of marine snails (IMPOSEX). Recently, the problems associated with endocrine disrupters (xeno-oestrogens) have come into attention. Mutagenity, surface activity, metabolic responses and radical formation are other factors that receive attention in the environmental risk evaluation. Although specific biomarkers are available to test specific toxic actions, no such data are required yet. It seems most practical to demand biomarker tests only in those cases that there are strong indications of specific toxic action.

Increase of field relevance

Additional data for fresh water species will increase the field relevance of the assessment, and lower assessment factors can be used. Species from other taxonomical groups, e.g. insects or molluscs, are preferred. Mesocosm studies can provide results with a strong predictive value for actual 'field' results. Both direct effects on populations and indirect effects on ecosystem functioning can be observed, and related to the exposure concentration of the substance tested. The indirect effects resulting from shifts in the community due to sensitivity differences may be more easily determined than direct effects, as indirect effects give an integral estimate of the effects. Next to this, also the toxicity of more toxic metabolites or degradation compounds may be traced. Data comparisons have shown that the direct effects observed in mesocosm studies are generally in agreement with toxicity levels derived from risk assessment models using laboratory data.

Table 3 Potential data requirements for effects assessment (PNEC calculation) of substances in the compartment fresh surface water.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Chronic fresh water aquatic toxicity tests	Chronic NOECs improve the risk assessment, and allow a lower assessment factor than the acute EC ₅₀ s of Annex II	Test results (EC ₅₀ , NOEC) can be used for standard PNEC calculation methods	Guidelines for chronic tests are available, e.g. OECD 202 (chronic <i>Daphnia</i> test), 204, 210. OECD draft guideline (based on ex-BBA draft) for <i>Chironomus riparius</i> available.
Fresh water mesocosm (semi-field scale) toxicity tests	Increase of field relevance; can provide some validation over laboratory data; reduction of assessment factors in PNEC calculation	NOECs produced in mesocosm tests can be used to calculate PNECs, but proper assessment factor that should be applied is not clear.	No international guidelines available. Many laboratories have their own unpublished Standard Operating Procedures. ASTM 1624 and a recent SETAC guidance document provide guidelines
Specific toxic action tests (biomarkers) (fresh or marine)	Account for specific effects of certain chemicals in risk assessment (e.g. endocrine disruptors)	Not compatible, as tests do not yield a NOEC, LOEC or EC _x . Effect at population level and field validation usually lacking.	No harmonised guidelines available. Methods diverse
Toxicity test for hazardous degradation products (fresh or marine)	Required for risk assessment for degradation products. Benefits not clear: degradation products are often bound to the sediment, and show reduced toxicity due to primary degradation. Often low biological availability ("bound residues").	Separate risk evaluation can be performed for degradation products	When degradation product is isolated, standard tests can be executed. No standard procedure available.
Toxicity test sediment-dwelling species (fresh water)	For compartment specific calculation of PNEC _{sediment} instead of extrapolation from aquatic toxicity data.	Test results (EC ₅₀ , NOEC) can be used for standard PNEC calculation methods	Test guidelines available: <i>Hyatella azteca</i> (ASTM 1383); combined <i>Daphnia</i> - chironomids test (Dutch RIZA). OECD draft (former BBA guideline) available
Bioconcentration factor for other aquatic organisms than fish (fresh or marine)	- Required when exposure of important food organisms is different from fish - When there is a high risk of secondary poisoning.	Test results can be used in risk assessments.	No standard test guidelines; possibly adaptation from toxicity tests.
Toxicity test for fish-eating predators (fresh or marine)	- Required for assessment of secondary poisoning. - Only in case of strong evidence of secondary poisoning, to reduce number of test animals	Data suited for risk assessment	Dietary avian test available.

6. Generic data requirements for marine water and sediment

6.1 Exposure assessment

Current situation

No specific data for environmental transport and fate assessment in marine environments is requested in Annex II. The EC Technical Guidance Document does not address marine waters specifically.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing (1) a compartment specific assessment, (2) by allowing a chemical-specific assessment, or (3) by increasing the field relevance of the assessment.

Compartment specific assessment

Degradation data from the core data set refers to fresh water. Biodegradation rates in the water column can differ between fresh water and marine waters, due to differences in microbial communities, and differences in the number of micro-organisms between fresh water and salt water. A systematic difference between degradation in fresh water and sea water is however not conclusively reported. In a three year research project by Aquateam, the biodegradation rates show the following order: fresh water with inoculum > sea water with inoculum > sea water without inoculum (Aquateam, unpublished data.). ECETOC concludes in a literature review (ECETOC, 1993) that a chemical which is readily biodegradable in fresh water, will also be readily biodegradable in marine environments. According to ECETOC, this is partly due to the fact that the nutrient enriched (natural) sea water in the OECD salt water biodegradation test (OECD 306), and the high concentration of chemicals, mask the specific conditions of the marine environment (low concentrations of organic substrates, low essential nutrient concentrations, and low concentrations of micro-organisms). Furthermore, ECETOC concludes that indications exist that degradation is slower in the marine environment than in fresh waters. No comparisons have been performed for inherent biodegradation in fresh and marine environments. In conclusion, consensus about differences in degradation rates between marine water and fresh water has not yet been reached. Additional research on this subject is required. For degradation in marine sediments, the same arguments are valid as for fresh water sediments. It is not clear whether the degradation rate differs between fresh and marine sediments, although the arguments for differences in degradation rate between fresh water and sea water may also hold true for sediments. Therefore, unless proven different, specific seawater biodegradation tests are required.

Chemical specific assessment

See fresh water

Increase of field relevance

See fresh water.

Table 4 Potential data requirements for exposure assessment (PEC calculation) of substances in the compartment seawater.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Seawater die-away biodegradation test with specific chemical analyses or using a radiolabel at environmentally realistic biocide concentrations.	- Where testing with Annex II recommended (freshwater or marine) ready/inherent tests is hindered due to toxicity to the inoculum, e.g. biodegradation is low or zero, further testing is essential. - Can be used with seawater, or with suspended sediment to simulate a riverine environment.	Provides data suited to risk analysis at relevant environmental concentrations.	Only freshwater guidelines are available, but may be adapted: Draft ISO 14592, ASTM E-1279, or other suitable methods which can be used to test $\mu\text{g.l}^{-1}$ levels of test compound. Note: OECD 306 cannot be used below 0.5-0.8 mg.l^{-1} test substance.
Aerobic/anaerobic biodegradation in marine sediment/water systems, with specific chemical analyses or using a ^{14}C radiolabel.	- Allows a compartment specific assessment, particularly for chemicals which are liable to adsorb strongly to sediment. - Anaerobic and aerobic flasks can be run in parallel. - Can provide data on the quantity and nature of soluble metabolites as well as bound residues.	Provides data suited to risk analysis at relevant environmental concentrations.	Only freshwater sediment/water test guidelines are available, although some laboratories may have unpublished standard operating procedures. This is an area requiring attention in relation to several groups of compounds, including biocides and veterinary medicines used in aquaculture.
Biodegradation in marine microcosms, i.e. field simulation tests.	- Field relevance is increased - A mass balance approach can be taken with several environmental compartments. - Can provide some validation of laboratory data.	Provides data suited to risk analysis	No international guidelines available. Many laboratories have their own unpublished Standard Operating Procedures. ASTM 1624 and a recent SETAC guidance document provide guidelines
Sediment-water partitioning coefficient (fresh or marine)	- Allows compartment specific assessment (instead of using K_{oc} soil-water). - Relevant for non-organics	Possibly more appropriate risk parameter. This data can possibly be derived from a sediment/water degradation test.	Adaptation of OECD 106 soil adsorption/desorption possible.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Leaching rate (from treated material to fresh surface water)	– Required to estimate environmental emissions – Only relevant for some application types, such as antifoulings and wood preservatives	Needs to be known prior to risk assessment	Available for some product types, such as ASTM D5108-90 for antifoulings. Will be elaborated in relevant release scenario's

6.2 Effects assessment

Present situation

No toxicity data for marine species (aquatic or sediment-dwelling) are required in Annex II.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing (1) a compartment specific assessment, (2) by allowing a chemical-specific assessment, or (3) by increasing the field relevance of the assessment.

Compartment specific assessment

Toxicity data for marine species, i.e. algal species, a crustacean (*Acartia tonsa*) and a fish species may in some cases result in a more relevant toxicity estimate for substances entering the marine environment.

With respects to secondary poisoning, no differences between the assessment in fresh water and marine waters are foreseen that require specific test results.

Chemical specific assessment

See fresh water

Increase of field relevance

See fresh water

Table 5 Potential data requirements for the effects assessment (PNEC calculation) of substances in the compartment sea water.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Toxicity test for marine fish, algae, crustacean	Compartment specific PNEC calculation (instead of estimating from freshwater data). Rationale (difference freshwater - marine water) not clear.	Test results can be used in PNEC calculation procedures instead of freshwater data	Test guidelines for marine species available, e.g. <i>Skeletonema</i> and <i>Acartia</i> (ISO); <i>Cyprinodon</i> (OECD) bivalves (ASTM 1022)
Toxicity test sediment-dwelling species (marine)	For compartment specific calculation of $PNEC_{\text{sediment}}$ instead of extrapolation from aquatic toxicity data.	Test results (EC_{50} , NOEC) can be used for standard PNEC calculation methods	Test guidelines available, e.g. <i>Corophium</i> (ASTM/PARCOM), bivalves (ASTM 1022), polychaetous annelids (ASTM 1611)
Specific toxic action tests (biomarkers) (fresh water or marine)	Account for specific effects of certain chemicals in risk assessment (e.g. endocrine disruptors, or imposex on molluscs)	Not compatible, as tests do not yield a NOEC, LOEC or EC_x Effect at population level and field validation usually lacking.	No harmonised guidelines available. Methods diverse
Chronic marine toxicity tests	Chronic NOECs improve the risk assessment, and allow a lower assessment factor than the acute EC_{50} s of Annex II	Test results (EC_{50} , NOEC) can be used for standard PNEC calculation methods	OECD 210 (Early Life Stage) includes marine fish species. Algae tests can be used as acute (EC_{50}) and chronic (NOEC) data. Chronic crustacean test guideline not available.
Marine mesocosm (semi-field scale) toxicity tests	Increase of field relevance; can provide some validation over laboratory data; reduction of assessment factors in PNEC calculation	NOECs produced in mesocosm tests can be used to calculate PNECs, but proper assessment factor that should be applied is not clear.	Few comprehensive guidelines available. Many laboratories have their own unpublished SOP's. Recent SETAC document can be of some help. Expensive.
Toxicity test for hazardous degradation products (fresh or marine)	Required for risk assessment for degradation products. Benefits not clear: degradation products are often bound to the sediment, and show reduced toxicity due to primary degradation. Often low biological availability ("bound residues").	Separate risk evaluation can be performed for degradation products	When degradation product is isolated, standard tests can be executed. No standard procedure available.
Bioconcentration factor for other aquatic organisms than fish (fresh or marine)	- Required when exposure of important food organisms is different from fish - When there is a high risk of secondary poisoning.	Test results can be used in risk assessments.	No standard test guidelines; possibly adaptation from toxicity tests.
Toxicity test for fish-eating predators (fresh or marine)	- Required for assessment of secondary poisoning. - Only in case of strong evidence of secondary poisoning, to reduce number of test animals	Data suited for risk assessment	Dietary avian test available.

7. Generic data requirements for soil and groundwater

7.1 Exposure assessment

Current situation

In current EC Technical Guidance Document, the soil compartment is taken into consideration as an environmental compartment through which terrestrial (soil dwelling) biota is exposed, and indirectly top predators, humans, animals and wildlife. In the core data set, the adsorption/desorption screening test is included, yielding a K_{oc} for soil-water partitioning. Biodegradation tests for soil are not included in the core data set.

In the EC Technical Guidance Document, the (local) concentration in soil ($PEC_{local,soil}$) is calculated with a simple box model. This model requires a first order removal rate in soil as input, which is calculated from the volatilization rate, the leaching rate and the biodegradation rate. These rate constants can either be measured (test results) or estimation procedures which are provided in the Technical Guidance Document can be used.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing a compartment specific assessment, by allowing a chemical-specific assessment, or by increasing the field relevance of the assessment.

Compartment specific assessment

The estimation of soil biodegradation rates from the aquatic degradation rate (e.g., according to Struijs & Van den Berg, 1992) is inaccurate, and demanding a specific soil biodegradation test is therefore strongly recommended. These test typically yield the half-life (DT_{50}) of a substance in soil, which has to be translated into a rate constant for risk assessment purposes. The use of ^{14}C -labelled substances can give helpful information on metabolites, mineralisation rate and soil-bound residues, but is expensive. Anaerobic conditions are not likely to occur in the terrestrial soil environment, except in (water-saturated) subsoil. At present, no standardised tests are available to investigate degradation in subsoil.

For specific biocidal products, a leaching rate from treated material (e.g., preserved wood) to the soil is required to estimate emissions to the soil. This is only relevant for some product types. The leaching rate will be elaborated in the release scenario's.

Chemical specific assessment

No additional test data is foreseen for chemical-specific assessments.

Increase of field relevance

The field relevance of biodegradation rates can be improved by performing lysimeter studies instead of standard (batch) biodegradation tests. Leaching rates can be measured instead of using model calculation results. These studies should be carried out in the case of a high risk for groundwater contamination. Several leaching tests are available, with increasing field relevance: disturbed soil columns, undisturbed soil columns, and lysimeter studies. The lysimeter studies can be combined with biodegradation studies.

Table 6 Potential data requirements for exposure assessment (PEC calculation) of substances in the compartment soil.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Aerobic biodegradation in soil, using specific chemical analysis or a ¹⁴ C label	- Enables compartment specific assessment. - Practical considerations, such as toxicity to the inoculum may prevent the use of aquatic ready/inherent data, making this test essential. - Nature and quantity of bound residues can be evaluated in the same test (only with ¹⁴C-labelled substances).	Produces comprehensive data, ideal for purposes of risk analysis	Well tried international standard (OECD 304A) available, or BBA Guideline (IV,4-1: "Fate of plant protection agents in soil; degradation, transformation and metabolism").
Leaching rate in disturbed or undisturbed soil columns.	- Allows measured leaching rate instead of model calculations - Increased field relevance (undisturbed columns)	Produces data suitable for risk analysis	No standard guidelines. Laboratories have their own SOPs, and experiments are described in literature.
Biodegradation and leaching rate in lysimeter studies	- High field relevance, as undisturbed field soil sample is used - Combination with ¹⁴C labelling provides comprehensive overview of behaviour of a substance in soil.	Produces data suitable for risk analysis	OECD draft guideline available ("Performance of out-door lysimeter studies", Nov. 1996)
Leaching rate (from treated material to soil)	- Required to estimate environmental emissions - Only relevant for some application types	Needs to be known prior to risk assessment	Depends on treated material and product (formulation). Will be elaborated in relevant release scenario's

7.2 Effects assessment

Present situation

No specific test data for effects assessment in the soil compartment is required in Annex II of the Biocidal Products Directive.

In the EC Technical Guidance Document, guidance is given to calculate a PNEC for the soil compartment. The PNEC is based on toxicity data (ideally for a producer, a consumer and a decomposer) and extrapolation factors, conform the PNEC for the aquatic compartment. However, as the Technical Guidance Document identifies the low availability of soil toxicity test results, calculation

rules are provided to assess the $PNEC_{soil}$ from the $PNEC_{water}$ (equilibrium-partitioning, based on the P_{ow}).

For the assessment of secondary poisoning, the foodchain

soil $\Rightarrow$ earthworm $\Rightarrow$ wormeating bird or mammal

is proposed as a representative example in the Guidance Document. The PEC in food organisms (earthworms) is calculated from the PEC_{soil} and a bioconcentration factor (BCF). The concentration in grass and crops is used to assess effects on cattle and humans. The PNEC for secondary poisoning (consumption of earthworms by birds or mammals) should be calculated from dietary test data with relevant species. Acute lethal doses tests cannot be used.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing a compartment specific assessment, by allowing a chemical-specific assessment, or by increasing the field relevance of the assessment.

Compartment specific assessment

Toxicity tests for soil-dwelling organisms are required, as extrapolation from aquatic tests is erroneous. Extrapolation does not take the specific conditions of exposure through soil into account (exposure through pore-water and consumption of soil; partitioning between soil and pore water), and as the extrapolation is based on the P_{ow} , it is only valid for non-polar, organic substances.

The bioaccumulation of chemical compounds in earthworms is different from bioaccumulation in fish (Annex II bioaccumulation test). Fish are mainly exposed through the gills, and accumulation through dermal uptake or via food are minor pathways. Earthworms are mainly exposed through oral uptake (soil consumption). Furthermore, elimination rates may differ, as they belong to different taxonomic groups. In fish bioaccumulation tests, the chemical is distributed over the water (test medium) and the fish, whereas bioaccumulation in earthworms involves three compartments (soil, pore water, earthworm). Therefore, a specific bioaccumulation test with earthworms should be considered.

When secondary poisoning is expected to be important, specific tests for worm-eating mammals or birds can be required. Available (dietary) toxicity data should be used as much as possible, to reduce the number of test animals required.

Chemical specific assessment

As stated before, soil toxicity data is especially required for polar organic or non-organic chemicals, as the extrapolation from aquatic toxicity data is not possible for these compounds.

Increase of field relevance

No guidelines are available for semi-field scale, multispecies toxicity tests for the soil compartment (like mesocosm studies in the aquatic compartment).

Combination with lysimeter studies is possible, but not common practise. In field studies, the effects of chemicals on arthropod or nematodes communities have been studied. Semi-field soil toxicity studies need further research (on feasibility *and* benefits) before demanding for authorisation.

Table 7 Potential data requirements for effects assessment (PNEC calculation) of substances in the compartment soil.

Potential data requirements, additional to annex III	Benefits/ rationale	Compatibility	Feasibility
Acute toxicity tests for soil-dwelling species	Required for a compartment specific PNEC calculation (instead of calculation from aquatic data)	Test result (EC_{50} , NOEC) can be used directly in calculation rules for PNEC calculation	Test guidelines available, e.g. Earthworm (OECD 207 and ISO test) and collembola (BBA guideline, draft ISO 11267). Furthermore, arthropod tests are available.
Chronic toxicity test for soil dwelling species	Required for a compartment specific PNEC calculation, under chronic exposure conditions	Data suitable for risk analysis	Test guidelines available, e.g. earthworm reproduction test or enchytraeidae reproduction test (BBA guideline, EU ring test project)
Soil micro-organism community test	- Enables compartment specific assessment, when data on side-effects to soil microbes is required (nitrogen mineralisation, nitrification, carbon mineralisation). - Ecologically relevant process	Test result (EC_{50} , NOEC) can be used directly in calculation rules for PNEC calculation	OECD draft This proposal attempts to consolidate the following guidelines - BBA (1990), EPA (1987), ISO 14240 and ISO 14238 (1997)
Toxicity test for terrestrial plants	- Useful, because effects on plants cannot be extrapolated from animal tests - Possibilities for combination with bioaccumulation test	Data suitable for risk assessment	Test guidelines available, e.g. OECD 208 (Terrestrial plants) and ISO guidelines
Bioconcentration test in soil-dwelling animals and terrestrial plants	Enables compartment specific BCF (instead of extrapolation from P_{ow} or fish test BCF)	Data suitable for risk analysis	No standard guidelines, but combination with earthworm or terrestrial plant toxicity test is feasible.
Toxicity test for worm-eating predators	- Required for assessment of secondary poisoning - Increase of field relevance compared to using rat test data	Data suitable for risk assessment	Dietary tests for bird species available (required for Annex II?)

8. Generic data requirements for a Sewage Treatment Plant

8.1 Exposure assessment

Current situation

A Sewage Treatment Plant is an important environmental compartment from the viewpoint of risk assessment, because it is situated between industrial processes or communal discharges, and the environment (surface water). Degradation of chemicals in a STP can prevent environmental exposure. No specific test data for STPs are demanded in Annex II of the directive.

In the EC Technical Guidance Document, tabulated results of the SimpleTreat model (Struijs, 1996) are presented to estimate the removal of a chemical in a STP as function of its P_{ow} , Henry-coefficient and (ready or inherent) biodegradability. This information is provided by the core data of Annex II.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing a compartment specific assessment, by allowing a chemical-specific assessment, or by increasing the field relevance of the assessment.

Compartment specific assessment

As stated, the data from Annex II is sufficient for an estimation (model calculations) of the removal in a STP. However, tests are available to simulate the specific conditions in a STP, thus enabling the measurement of the removal rate. When these tests are performed in combination with ^{14}C labelled substances, a mass balance can be constructed to determine the degradation and removal by adsorption to sludge.

Chemical specific assessment

No additional data for enabling a chemical specific assessment is required.

Increase of field relevance

Simulation tests have a high level of field relevance.

Table 8 Potential data requirements for exposure assessment (PEC calculation) of substances in a STP.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Continuous activated sludge test	- Provides compartment specific data, simulating some of the conditions found in Sewage treatment plants. ¹⁴C analysis enables construction of mass balances (degradation, removal)	Provides the most reliable predictive information suitable for risk analysis of the Sewage treatment plant route.	The OECD 303A guideline is the accepted international standard.

8.2 Effects assessment

Present situation

In Annex IIA of the Biocidal Products Directive, the following data regarding the calculation of a PNEC for a substance is included:

- VII 7.4 Inhibition of microbiological activity

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing a compartment specific assessment, by allowing a chemical-specific assessment, or by increasing the field relevance of the assessment.

Compartment specific assessment

An activated sludge respiration inhibition is appropriate to assess toxic effects of substances on micro-organisms in STPs.

Chemical specific assessment

No additional data for enabling a chemical specific assessment is required.

Increase of field relevance

No additional data for increasing the field relevance of the assessment is required.

Table 9 Potential data requirements for effects assessment (PNEC calculation) of substances in a STP.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Inhibition of activity of micro-organisms in STP	Required for assessing the effects on micro-organisms in sewage sludge	Test result can be used in risk assessments.	OECD 209 is the standard test guideline

9. Generic data requirements for air

9.1 Exposure assessment

Current situation

In the EC Technical Guidance Document, direct ecological effects due to exposure of organisms to chemicals in the air are not taken into account. However, a concentration in the air is calculated ($PEC_{local,air}$ and $PEC_{regional,air}$) to estimate atmospheric deposition to soil and surface water, and to assess the amount inhaled by humans.

In the Technical Guidance Document, simple calculation rules and default values are proposed to calculate the concentration in the atmosphere. These defaults are produced by performing calculations with the OPS air transport model. As a result of this approach, only Henry's law constant is needed, which can be calculated from the water solubility, the vapour pressure and the molecular weight, which are present in Annex II.

Potential data for Annex III

Data to be considered for inclusion in Annex III should ensure and improve the risk assessment by allowing a compartment specific assessment, by allowing a chemical-specific assessment, or by increasing the field relevance of the assessment.

Compartment specific assessment

As stated in the introduction, the data required in Annex II of the directive are sufficient to estimate the PEC_{air} with the calculation rules provided in the Technical Guidance Document. When a more sophisticated assessment is required, information about degradation rates is necessary (degradation by OH radicals, photodegradation). This information is only relevant for calculation of concentrations on a regional scale; timescales for exposure to local (point) sources are too small for degradation processes to be of importance.

For some biocidal products or applications, a test to determine the evaporation rate from treated material to the air may be required.

Chemical specific assessment

There are no additional data requirements based on specific chemical characteristics. Establishing the vapour pressure and molecular weight can be performed for all chemicals without specific problems.

Increase of field relevance

Due to the large simplifications in the assessment procedure, semi-field or field

measurements may will strongly improve the assessment. However, no test guidelines or practises are available.

Table 10 Potential data requirements for exposure assessment (PEC calculation) of substances in the compartment air.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Photodegradation rate and degradation rate by OH radicals in air	– Required for a realistic assessment of regional air concentrations – Only relevant for a more sophisticated assessment	Not required for local scale calculations; only relevant in combination with more complex transport models	No test guidelines; OECD Monograph available (1992)
Evaporation rate (from treated material)	– Required to estimate environmental emissions – Only relevant for some application types	Needs to be known prior to risk assessment	Depends on treated material and product (formulation). Will be elaborated in relevant release scenario's

9.2 Effects assessment

Present situation

As stated before, the direct exposure of organisms to contaminated air is not considered in the EC Technical Guidance Document. No toxicity data for organisms exposed to atmospheric contamination is required in Annex II of the Directive. Additional data can however be considered, to perform a qualitative assessment.

Compartment specific assessment

Some toxicity tests for species exposed through the air are available, from the field of beneficial species testing. It is useful to require one or more of these tests for substances that are likely to be emitted to the air. The inhalation toxicity to mammals (Annex II) can also be used for a qualitative effects assessment.

Chemical specific assessment

Effects other than those based on toxicity (e.g., effects of radicals, or acids) can be important to consider, but tests or guidelines are not available.

Increase of field relevance

No additional data for improving the field relevance is required.

Table 11 Potential data requirements for effects assessment (PNEC calculation) of substances in the compartment air.

Potential data requirements, additional to annex II	Benefits/ rationale	Compatibility	Feasibility
Acute and chronic toxicity for exposure by air	Enable a qualitative compartment specific risk assessment	Not compatible with quantitative risk assessment methods; qualitative assessment (expert judgement) required	Some tests available, e.g. Honeybees (EPPO Guideline), terrestrial plants (gassing), lichens. Toxicity tests for terrestrial plants or arthropod species could possibly be modified for exposure through air.

10. Use of generic data requirements

The data requirements, introduced in the previous chapters, are summarised in Table 12.

Table 12 Potential test data for Annex III

Compartment	Nr.	Potential tests for Annex III
Fresh surface water (incl. sediments)	I.1	Die-away biodegradation test with specific chemical analyses or using a radiolabel at environmentally realistic biocide concentrations (fresh water)
	I.2	Aerobic/anaerobic biodegradation in freshwater sediment/water systems, with specific chemical analyses or using a ¹⁴ C radiolabel.
	I.3	Biodegradation in fresh water microcosms or mesocosms, i.e. field simulation tests.
	I.4	Leaching rate (from treated material to fresh surface water)
	I.5	Chronic fresh water toxicity tests
	I.6	Fresh water mesocosm (semi-field scale) toxicity tests
	I.7	Toxicity test (fresh water) sediment-dwelling species
Fresh or marine surface water	I/II.1	Sediment-water partitioning coefficient (fresh or marine)
	I/II.2	Bioconcentration factor for other aquatic organisms than fish (fresh or marine)
	I/II.3	Specific toxic action tests (biomarkers) (fresh or marine)
	I/II.4	Toxicity test for hazardous degradation products (fresh or marine)
	I/II.5	Toxicity test for fish-eating predators (fresh or marine)
Marine water (incl. sediments)	II.1	Seawater die-away biodegradation test with specific chemical analyses or using a radiolabel at environmentally realistic biocide concentrations.
	II.2	Aerobic/anaerobic biodegradation in marine sediment/water systems, with specific chemical analyses or using a ¹⁴ C radiolabel.
	II.3	Biodegradation in marine microcosms, i.e. field simulation tests.
	II.4	Leaching rate (from treated material to marine surface water)
	II.5	Toxicity test for marine fish, algae, crustacean
	II.6	Toxicity test sediment-dwelling species (marine)
	II.7	Chronic marine toxicity tests
	II.8	Marine mesocosm (semi-field scale) toxicity tests
Soil (incl. groundwater)	III.1	Aerobic biodegradation in soil, using specific chemical analysis or a ¹⁴ C label
	III.2	Leaching rate (to ground water) in disturbed or undisturbed soil columns.
	III.3	Biodegradation and leaching rate (to ground water) in lysimeter studies
	III.4	Leaching rate (from treated material to soil)
	III.5	Acute toxicity tests for soil-dwelling species

	III.6	Chronic toxicity test for soil dwelling species
	III.7	Soil micro-organism community test
	III.8	Toxicity test for terrestrial plants
	III.9	Bioconcentration test in soil-dwelling animals and terrestrial plants
	III.10	Toxicity test for worm-eating predators
STP	IV.1	Continuous activated sludge test
	IV.2	Inhibition of activity of micro-organisms in STP
Air	V.1	Photodegradation rate and degradation rate by OH radicals in air
	V.2	Evaporation rate (from treated material to air)
	V.3	Acute and chronic toxicity for exposure by air

The data is presented per environmental compartment. When, in a certain stage of the life cycle of a biocidal product type, emissions to a specific environmental compartment occur, Table 12 can be used as basis for constructing data requirements for this product type.

As stated before, the data listed in this report is a ‘long list’ of data. Data that will ensure and improve the result of the risk assessment is included, and pro’s and con’s are discussed. The choice however which data should actually be included in Annex III, is not made in this report. This decision should (and will) be taken in a process of discussion between regulatory authorities, EC and chemical industry (and other parties concerned), and is more a political than a scientific discussion. However, by making the benefits, the compatibility and feasibility explicit, the discussions can take place on a rational and scientific basis.

In the release estimation part (Report “BIOEXPO - Release estimation of 23 biocidal product types”), guidance is given on the use of the ‘long lists’, with reference to the characteristics of the product types.

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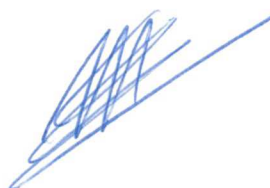
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Appendix I Biodegradability tests

Aquatic biodegradability tests

No	Name	Principle	Scope	Recommended usage
ISO 7827 OECD 301A	DOC die-away method Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability by analysis of dissolved organic carbon (DOC)	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - Measurement of the removal of dissolved organic carbon (DOC) to determine the ultimate biodegradability in 28 days. - The inoculum may be obtained from a variety of sources, e.g. activated sludge, sewage effluents, surface waters, soils or a mixture of these. - Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble at the test concentration (10-40 mg/l DOC); - non-volatile, or having a negligible vapour pressure; - not significantly adsorbable on glass and activated sludge; - not inhibitory to the test micro-organisms at the test concentration.	Test method for primary and ultimate biodegradation of water soluble test compounds.
ISO 9439 OECD 301B	CO ₂ evolution / modified Sturm method Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of released carbon dioxide	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - Measurement of the biogenically produced carbon dioxide (CO₂) to determine the ultimate biodegradability in 28 days. - Evaluation of the test results by comparing the CO₂ with the theoretical amount. - For water soluble test compounds the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water.	The test can be used for organic compounds which are: - water-soluble at the test concentration (10-40 mg/l DOC); - water-insoluble in the test conditions; - non-volatile, or having a negligible vapour pressure; - not inhibitory to the test micro-organisms at the test concentration.	Test method for water soluble and poorly soluble test compounds using an analytical parameter of strictly biological origin.
OECD 301C	MITI (I) method Evaluation in an aqueous medium of the "primary and ultimate" aerobic biodegradability of organic compounds - Method by analysis of O ₂ consumption.	Stirred, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - Measurement of the oxygen consumption to determine the ultimate biodegradability in 28 days. - Uses an inoculum specially cultured in a sludge unit from at least 10 sites "where a variety of chemicals are discharged", the micro-organisms are considered as un-adapted following continuous incubation. - The primary biodegradation may be calculated from chemical analysis of the parent compound and ultimate biodegradation can be confirmed by DOC analysis.	The test can be used for organic compounds which are: - water soluble or to some extent poorly soluble - adsorptive - volatile	Test method uses a fixed high dosage of compound (100 mg.l ⁻¹). Some suitability claimed for poorly soluble substances. Relatively conservative regulatory test

Aquatic biodegradability tests, continued

No	Name	Principle	Scope	Recommended usage
ISO 10707 OECD 301 D Related to OECD 306	Closed bottle test. Evaluation, in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by analysis of biochemical oxygen demand	Static aquatic test system using organic test compounds as the sole source of carbon and energy of aerobic mixed micro-organisms. - Measurement of the biochemical oxygen demand (BOD) in completely filled closed bottles to determine the ultimate biodegradability within 28 days. - Evaluation of the test results by comparing the BOD with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD).	The test can be used for organic compounds which : - are water-soluble at the test concentration (2-10 mg/l); - are water-insoluble at the test conditions, provided a suitable dosing technique is used; - are volatile, provided a suitable dosing technique is used; - are inhibitory to the test micro-organisms down to the lowest test concentrations used.	Simple test method - used extensively has the advantage of relatively low test concentrations, conservative, used for volatile and toxic test compounds.
OECD 301 E	Modified OECD Screening Test.	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - DOC die-away is measured to follow biodegradation; the inoculum may be pre-adapted to measure inherent biodegradation in addition to ready biodegradability. - Primary biodegradation may be calculated from supplemental chemical analysis of the parent compound. - The inoculum is derived from the secondary effluent of a sewage treatment plant or laboratory scale unit receiving predominately domestic sewage.	The test can be used for organic compounds which are: - have a water solubility of at least 100 mg.l-1 - non-volatile, or having a negligible vapour pressure	Similar to DOC die-away method ISO 7827, OECD 301 A, but employs a relatively low concentration of organisms. Limited by requirement for high water solubility.
ISO 9408 OECD 301F	Manometric/ respirometric method. Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by determining the oxygen demand in a closed respirometer.	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - Measurement of the biochemical oxygen demand which (BOD) in a closed respirometer to determine the ultimate biodegradability within 28 days. - Evaluation of the test results by comparing the BOD with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD). - For water soluble test compounds the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water or use of specific analysis to determine the primary biodegradability.	The method test can be used for organic compounds which are: - water-soluble at the test concentration (10-40 mg.l-1 DOC); - non-volatile, or having a negligible vapour pressure; - not significantly adsorbable on glass and activated sludge; - not inhibitory to the test micro-organism at the test concentration.	Test method for water soluble and poorly soluble test compounds using a respirometer producing detailed biodegradation curves, using an analytical parameter of strictly biological origin.

Aquatic biodegradability tests, continued

No	Name	Principle	Scope	Recommended usage
ISO 9887, OECD 302A, ASTM E-1625	Modified semi-continuous activated sludge method (SCAS) Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium.	Semi-static, aquatic test system using organic test compounds and easily biodegradable organic medium (sewage) as the source of carbon and energy for an inoculum of aerobic mixed micro-organisms (activated sludge) - Daily fill-and-draw of the test vessels with sewage and test compound and measurement of the removal of dissolved organic carbon (DOC) to determine the ultimate biodegradability within the test time of up to 26 weeks. - Evaluation of the test results by comparing the DOC concentration before and after the fill-and-draw procedure. - Additionally specific analysis can be used to determine the primary biodegradability of a test compound	The test can be used for organic compounds which are: - water-soluble at the test concentration (20- about 50 mg/l DOC); - non-volatile, or having a negligible vapour pressure; - not lost by foaming from the test solution; - not significantly adsorbable on glass and activated sludge; - not inhibitory to the test micro-organisms at the test concentration	Test with high biodegradation potential especially for compounds which are not easily degradable as well as waste water including co-metabolic degradation. Useful method for the pre-adaptation of an inoculum. Useful in risk analysis and has some predictive value for waste water treatment plants.
ISO 9888 OECD 302B	Zahn Wellens/ EMPA test Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Static test	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms (activated sludge). - Measurement of the removal of organic carbon (DOC) or chemical oxygen demand (COD) to determine the ultimate biodegradability and the elimination form water within 28 days; - Additionally specific analysis can be used to determine the primary biodegradability in an aqueous medium using standard methods;	The test can be used for organic compounds which are: - water-soluble at the test concentration (50-400 mg/l DOC); - non-volatile, or having a negligible vapour pressure; - not lost by foaming from the test solution; - not inhibitory to the test micro-organisms at the test concentration.	Static method for the evaluation of the degree of elimination (biodegradation and biotic removal) of test compounds and waste water - Useful in risk analysis has a good predictive value for waste water treatment plants.
ISO 11733 OECD 303A	Continuous activated sludge, coupled units simulation test (CAS). Evaluation of the elimination and the biodegradability of organic compounds in an aqueous medium.	Continuously operated, aquatic test system using organic test compounds and easily biodegradable organic medium (sewage) as the source of carbon and energy for an inoculum of aerobic mixed micro-organisms (activated sludge) - Daily addition of sewage and test to the test vessels and measurement of the dissolved organic carbon (DOC) or chemical oxygen demand (COD) in the influent and effluent to determine the ultimate biodegradability within the test time of up to 12 weeks. - Evaluation of the test results by calculating the DOC removal. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are : - water-soluble or satisfactory dispersible at the test concentration (10-20 mg/l DOC); - non-volatile, or having a negligible vapour pressure; - not inhibitory to the test micro-organisms at the test concentration.	Test used if detailed information in waste water treatment plants is required. Very effective predictions of sewage treatment effectiveness can be made: may need to be adapted to suit biocides due to toxicity.
ISO 11734	Anaerobic biogas production test Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production.	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of anaerobic mixed micro-organisms (digested sludge). - Measurement of the biogenic produced biogas (carbon dioxide and methane) by measurement of pressure and inorganic carbon (IC) to determine the ultimate biodegradability within 60 days. - Evaluation of the test results by comparing the biogas with the theoretical amount. - Additionally specific analysis can be used to determine the primary biodegradability of a test compound	The test can be used for organic compounds which are: - water soluble at the test concentration (20-100 mg.l⁻¹ organic carbon); - are water-insoluble at the test conditions, provided a suitable dosing technique is used; - are volatile, provided a suitable dosing techniques is used (case by case decision); - not inhibitory to the test micro-organisms at the test concentration.	Test used to evaluate anaerobic biodegradability

Aquatic biodegradability tests, continued

No	Name	Principle	Scope	Recommended usage
ISO 10708	Two-phase closed bottle test Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds. Method by determining the biochemical oxygen demand in a two-phase closed bottle test	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed micro-organisms. - Measurement of the biochemical oxygen demand (BOD) in closed bottles with head-space to determine the ultimate biodegradability within 28 days. - Evaluation of the test results by comparing the BOD including the use of oxygen from the headspace with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD). - For water soluble test compounds the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water or use of specific analysis to determine the primary biodegradability.	The test can be used for organic compounds which: - are water-soluble at the test concentration (100 mg.l⁻¹ ThOD); - are water-insoluble at the test conditions, provided a suitable dosing technique is used; - do not significantly adsorb or react with the oxygen electrode; - are inhibitory to the test micro organisms down to the lowest test concentrations used.	Test method especially for poorly water-soluble test compounds using simple equipment.
ISO 14592 Draft, ASTM E-1279	Die-away tests. 1. Shake-flask batch test 2. Continuous flow river model with attached biomass (not in ASTM guideline). Water quality evaluation of the aerobic biodegradability of organic compounds at low concentrations in water. Method follows the die-away of the parent compound.	Method for evaluating the biodegradability of organic compounds at low concentrations by aerobic micro-organisms in water. - Part 1. Is designed to simulate surface water or sediment-water suspensions, while Part 2. is a continuous-flow simulation of a river, including biomass attached to surfaces. - Evaluation of the test result is carried out by specific chemical analysis of the parent compound. - Part 1. uses stoppered flasks with an air headspace, while Part 2. uses an open cascade type system.	The test can be used for organic compounds which: are water soluble at the test concentration (preferably < 100µg.l⁻¹), this includes many 'poorly' soluble compounds;	Test method suitable for measuring the primary biodegradation of compounds at environmentally realistic concentrations.
OECD 306	Marine biodegradation test. 1. Shake flask method (seawater variant of the Modified OECD 301 E screening test). 2. Closed bottle method (seawater variant of the closed bottle test OECD 301D).	Method using natural seawater as the aqueous phase and the sole source of micro-organisms to evaluate the biodegradability. - For higher test substance concentrations, DOC removal or ThOD are measured in a shake-flask test lasting for up to 60d. - For lower test substance concentrations, in a more conservative test, DOC removal or ThOD are measured in a 28d closed bottle test (can also be extended to 56d).	The test can be used for organic compounds which: - are soluble at the test concentration (Shake flask method, 5-40 mg.l⁻¹ DOC; Closed bottle, 2-10 mg.l⁻¹ DOC) - are volatile providing suitable precautions are taken.	Relatively simple test methods suitable for measuring the ultimate biodegradation of organic chemicals in seawater. They are not simulation tests owing to the use of added nutrients, despite the use of natural seawater bacteria.
ISO 10634	Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.	Guidance describing several techniques for preparing poorly water-soluble organic compounds and introducing them into test vessels for a subsequent test on biodegradability in an aqueous medium using standard methods.	Guidance for poorly water-soluble compounds	

Soil biodegradation test.

No	Name	Principle	Scope	Recommended usage
OECD 304A There are closely related German BBA and Dutch CTB methods.	Inherent biodegradability in soil The method is designed to evaluate the mineralisation rate of ^{14}C labelled test compound in soil.	Aerobic or anaerobic method (56d) designed to evaluate the mineralisation rate of ^{14}C labelled test compound in soil by trapping $^{14}\text{CO}_2$ or $^{14}\text{CH}_4$ respectively, followed by scintillation counting. The method can also be used to analyse for the quantity and nature of bound residues.	The test can be used for organic compounds which are: • volatile or non-volatile; • soluble or insoluble in water • not inhibitory to micro-organisms.	The stipulation that compounds may not be inhibitory to microbes would seem to limit the application of the method. In practise, the method may be more flexible.
ISO 11266	Soil quality - Guidance on laboratory test for biodegradation of organic chemicals in soil under aerobic conditions	Aerobic incubation method (120 days) designed to evaluate the rate of disappearance of the test compound. The use of ^{14}C -labelled test compound allows for additional determinations.	The test can be used for organic compounds which are: • volatile or non-volatile; • soluble or insoluble in water - not inhibitory to micro-organisms.	The stipulation that compounds may not be inhibitory to microbes would seem to limit the application of the method. In practise, the method may be more flexible.
ISO 14239	Soil quality - Methods for measuring the mineralisation of organic chemicals in soil under aerobic conditions using laboratory incubation systems.	Method for trapping and measuring $^{14}\text{CO}_2$ in aerobic degradation tests.	The test can be used for organic compounds which are: • volatile or non-volatile; • soluble or insoluble in water - not inhibitory to micro-organisms.	The stipulation that compounds may not be inhibitory to microbes would seem to limit the application of the method. In practise, the method may be more flexible.