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INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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No. 37, *Detailed Review Document on Classification Systems for Substances Which Pose an Aspiration Hazard (2002)*

No. 38, *Detailed Background Review of the Uterotrophic Assay Summary of the Available Literature in Support of the Project of the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to Standardise and Validate the Uterotrophic Assay (2003)*

No. 39, *Guidance Document on Acute Inhalation Toxicity Testing (in preparation)*

No. 40, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures Which Cause Respiratory Tract Irritation and Corrosion (2003)*

No. 41, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which in Contact with Water Release Toxic Gases (2003)*

No. 42, *Guidance Document on Reporting Summary Information on Environmental, Occupational and Consumer Exposure (2003)*

No. 43, *Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (2008)*

No. 44, *Description of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment (2003)*

No. 45, *Guidance Document on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long-range Transport (2004)*

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No. 59, *Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats (2006)*

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No. 63, *Guidance Document on the Definition of Residue (2006)*

No. 64, *Guidance Document on Overview of Residue Chemistry Studies (2006)*

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No. 74, *Detailed Review Paper for Avian Two-generation Toxicity Testing (2007)*

No. 75, *Guidance Document on the Honey Bee (Apis Mellifera L.) Brood test Under Semi-field Conditions (2007)*

No. 76, *Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol (2007)*

No. 77, *Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study (2007)*

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No. 84, *Report on the Workshop on the Application of the GHS Classification Criteria to HPV Chemicals, 5-6 July Bern Switzerland (2007)*

No. 85, *Report of the Validation Peer Review for the Hershberger Bioassay, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*

No. 86, *Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α-Reductase Inhibitor in Dose Response Studies by Multiple Laboratories (2008)*

No. 87, *Report of the Ring Test and Statistical Analysis of Performance of the Guidance on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (Transformation/ Dissolution Protocol) (2008)*

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No.89 *Retrospective Performance Assessment of the Test Guideline 426 on Developmental Neurotoxicity (2008)*

No.90 *Background Review Document on the Rodent Hershberger Bioassay (2008)*

No.91 *Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3) (2008)*

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No.93 *Report of the Validation of an Enhancement of OECD TG 211: Daphnia Magna Reproduction Test (2008)*

No.94 *Report of the Validation Peer Review for the 21-Day Fish Endocrine Screening Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2008)*

No.95 *Detailed Review Paper on Fish Life-Cycle Tests (2008)*

No.96 *Guidance Document on Magnitude of Pesticide Residues in Processed Commodities (2008)*

No.97 *Detailed Review Paper on the use of Metabolising Systems for In Vitro Testing of Endocrine Disruptors (2008)*

No. 98 *Considerations Regarding Applicability of the Guidance on Transformation/Dissolution of Metals Compounds in Aqueous Media (Transformation/Dissolution Protocol) (2008)*

No. 99 *Comparison between OECD Test Guidelines and ISO Standards in the Areas of Ecotoxicology and Health Effects*

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

OECD Environment Directorate,
Environment, Health and Safety Division

2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

FOREWORD

This document presents commonalities and differences between *OECD Guidelines for the Testing of Chemicals* (OECD Test Guidelines) and corresponding standards of the *International Organisation for Standardization* (ISO) in the areas of environmental effects (toxicity to aquatic and terrestrial organisms), fate (e.g., degradation and bioaccumulation) and health effects (skin irritation; skin sensitization; toxicokinetics) for the purpose of sharing background information across developers, users of the OECD Test Guidelines and experts in charge of the registration of chemicals.

This document is a snapshot of test methods as of 2007. Given that new or updated test methods will be developed, this document should be regarded as a living document which could be updated in the future, taking into account the progress of the work of both organisations and available resources.

Some OECD Test Guidelines and ISO standards were compared in 1998-1999 and available to the Working Group of National Coordinators of the Test Guidelines Programme (WNT) [ENV/MC/CHEM/TG/RD(98)2, September 1998; ENV/JM/TG/RD(99)3, April 1999]. France took the lead to update the comparison in the light of the developments of the OECD Test Guidelines Programme, as well as developments of ISO standards. The first draft was circulated to the WNT and to the Secretary and Chair of relevant ISO groups (TC 147/SC 5: Water quality/Biological methods; TC 190/SC 4: Soil quality/Biological methods) in September 2007 asking comments. Comments were received from Denmark and the United States. The second draft was approved at the WNT meeting in April 2008.

This document is published on the responsibility of the Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals of the OECD.

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CHAPTER 1: INTRODUCTION

OECD Test Guidelines

1. The *OECD Guidelines for the Testing of Chemicals* (*OECD Test Guidelines*) represent a basic set of tools that are primarily for use in regulatory safety testing and subsequent chemical product notification and chemical registration. They can also be used for a variety of other purposes including the selection/ranking of candidate chemicals during the development of new chemicals and products and in toxicology research. Since the OECD Test Guidelines have been adopted by the OECD Member countries, their use in the generation of data provides a common basis for the acceptance of data, together with the opportunity to reduce direct and indirect cost to governments and industry associated with testing and assessment of chemicals. According to the OECD Council Decision on Mutual Acceptance of Data (12 May 1981, C(81)30/Final; amended on 26 November 1997, C(97)186/Final: downloadable from: http://webdomino1.oecd.org/horizontal/oecdacts.nsf/linkto/C(81)30), data generated in one country in accordance with the OECD Test Guidelines – and in accordance with the *OECD Principles of Good Laboratory Practice* – should be accepted in OECD Member countries and non-member economies which signed up to Mutual Acceptance of Data for purposes of assessment of chemicals and other uses relating to protection of man and the environment.

Test Guidelines development process

2. The OECD Test Guidelines are periodically updated in order to keep pace with progress in science. In addition, new Test Guidelines are developed and agreed upon, based on specific needs identified by OECD Member countries. OECD-wide networks of National Coordinators and of national experts provide the opportunity for input from scientists in government, academia and industry. The structure of the OECD Test Guidelines Programme, the various responsibilities of those involved in the process and, in detail, the procedures that are followed during the development of new, or the updating of existing, Test Guidelines are provided by the *Guidance Document for the Development of OECD Guidelines for the Testing of Chemicals* (OECD, 1993; revised 2006: downloadable from: http://appli1.oecd.org/olis/2006doc.nsf/linkto/env-jm-mono(2006)20).

Areas

3. The OECD Test Guidelines are available free of charge on the OECD website (http://www.oecd.org/env/testguidelines), categorised in the following sections: Section 1: Physical Chemical Properties; Section 2: Effects on Biotic Systems; Section 3: Degradation and Accumulation; Section 4: Health Effects; Section 5: Other Test Guidelines. Section 5 encompasses all Test Guidelines which do not fall within other four sections. At this time, Section 5 consists of Test Guidelines on pesticides residue chemistry only.

ISO Standards[1]

4. *International Organisation for Standardization* (ISO) is a network of the national standards institutes of 157 countries, on the basis of one member per country, with a Central Secretariat in Geneva, Switzerland, that coordinates the system. ISO is a non-governmental organization and its members are not delegations of national governments. Many of its member institutes are part of the governmental structure of their countries, or are mandated by their government. On the other hand, other members have their roots uniquely in the private sector, having been set up by national partnerships of industry associations. ISO is able to act as a bridging organization in which a consensus can be reached on solutions that meet both the requirements of business and the broader needs of society, such as the needs of stakeholder groups like consumers and users.

Standards development process[2]

5. The need for an ISO standard is usually expressed by an industry sector, which communicates this need to a national member body. The latter proposes the new work item to ISO as a whole. Once the need for an International Standard has been recognized and formally agreed, the first phase involves definition of the technical scope of the future standard. This phase is usually carried out in working groups which comprise technical experts from countries interested in the subject matter. Once agreement has been reached on which technical aspects are to be covered in the standard, a second phase is entered during which countries negotiate the detailed specifications within the standard. This is the consensus-building phase. The final phase comprises the formal approval of the resulting draft International Standard (the acceptance criteria stipulate approval by two-thirds of the ISO members that have participated actively in the standards development process, and approval by 75% of all members that vote), following which the agreed text is published as an ISO International Standard.

Areas[3]

6. ISO has developed over 17000 International Standards on a variety of subjects and 1100 new ISO standards are published every year. The full range of technical fields can be seen from the listing International Standards (http://www.iso.org/iso/iso_catalogue) and purchasable on the ISO website: http://www.iso.org/iso/store.htm.

Scope and contents of this document

7. This document compares OECD Test Guidelines and ISO Standards in the areas of environmental effects (toxicity to aquatic and terrestrial organisms), fate (e.g., degradability and bioaccumulation) and health effects (Skin Irritation Test, Skin Sensitisation Test and Toxicokinetic Test). This document includes only comparable methods that have been developed by both organisations in the area of testing of chemical substances by using laboratory test. Historically many of the ISO ecotoxicology standards were developed mainly for chemicals testing, but now their major scope is testing environmental samples e.g. wastewater, sediments, soils and soil materials.

[1] http://www.iso.org/iso/about.htm (last accessed 12 February 2008)

[2] http://www.iso.org/iso/standards_development/processes_and_procedures/how_are_standards_developed.htm (last accessed 12 February 2008)

[3] http://www.iso.org/iso/iso_catalogue (last accessed 12 February 2008)

8. Tables 1-1, 1-2 and 1-3 present correspondences between OECD Test Guidelines Sections 2-4 and ISO standards. The lists include all Test Guidelines in the relevant sections, including Test Guidelines without comparable ISO standards.

9. Chapters 2-4 provide detailed comparisons between an OECD Test Guideline and its equivalent ISO standard. Annex to this document outlines other ISO standards which are related to OECD Test Guidelines in the area of health effects but which has no need to be compared.

Table 1-1: OECD Test Guidelines Section 2: Effects on biotic systems and corresponding ISO Standards

OECD TEST GUIDELINES			CORRESPONDING ISO STANDARDS		Table in this document
Ref	Title	Adopted or Most recently updated	Ref	Adopted or Most recently updated	
201	Alga, Growth Inhibition Test	2006	8692	2005	Table 2-1
202	*Daphnia* sp. Acute Immobilisation Test	2004	6341	1996 / 1998	Table 2-3
203	Fish, Acute Toxicity Test	1992	7346	1996	Table 2-5
204	Fish, Prolonged Toxicity Test: 14-Day Study	1984	10229	1994	Table 2-6
205	Avian Dietary Toxicity Test	1984	No equivalent ISO standard		NA
206	Avian Reproduction Test	1984	No equivalent ISO standard		NA
207	Earthworm, Acute Toxicity Tests	1984	11268-1	1993	Table 2-10
208	Terrestrial Plants, Growth Test	2006	11269-2	2005	Table 2-9
209	Activated Sludge, Respiration Inhibition Test	1984	8192	2007	Table 2-8
210	Fish, Early-Life Stage Toxicity Test	1992	No equivalent ISO standard		NA
211	*Daphnia magna* Reproduction Test	1998	10706	2000	Table 2-4
212	Fish, Short- term Toxicity Test on Embryo and Sac-fry Stages	1998	12890	1999	Table 2-7
213	Honeybees, Acute Oral Toxicity Test	1998	No equivalent ISO standard		NA
214	Honeybees, Acute Contact Toxicity Test	1998	No equivalent ISO standard		NA
215	Fish, Juvenile Growth Test	2000	No equivalent ISO standard		NA
216	Soil Microorganisms: Nitrogen Transformation Test	2000	14238	1997	Table 2-13
217	Soil Microorganisms: Carbon Transformation Test	2000	No equivalent ISO standard		NA
218	Sediment-Water Chironomid Toxicity Using Spiked Sediment	2004	No equivalent ISO standard		NA
219	Sediment-Water Chironomid Toxicity Using Spiked Water	2004	No equivalent ISO standard		NA
220	Enchytraeid Reproduction Test	2004	16387	2004	Table 2-12

221	*Lemna* sp. Growth Inhibition Test	2006	20079	2005	Table 2-2
222	Earthworm Reproduction Test (*Eisenia fetida / Eisenia andrei*)	2004	11268-2	1998	Table 2-11
224	Determination of the activity of anaerobic bacteria - reduction of gas production from anaerobically sewage sludge	2007	No equivalent ISO standard		NA
227	Terrestrial Plant Test: Vegetative Vigour Test	2006	No equivalent ISO standard		NA

[NB: Ref/Table numbers to be checked again before declassification.]

Table 1-2: OECD Test Guidelines Section 3: Degradation and Accumulation and corresponding ISO Standards

OECD TEST GUIDELINES			CORRESPONDING ISO STANDARDS		Table in this document
Ref	Title	Adopted or Most recently updated	Ref	Adopted or Most recently updated	
301A	Ready Biodegradability: DOC Die-Away Test	1992	7827	1994	Table 3-1
301B	Ready Biodegradability: CO_2 Evolution Test	1992	9439	1999	Table 3-2
301C	Ready Biodegradability: Modified MITI Test (I)	1992	No equivalent ISO standard		NA
301D	Ready Biodegradability: Closed Bottle Test	1992	10707	1994	Table 3-3
301E	Ready Biodegradability: Modified OECD Screening Test	1992	No equivalent ISO standard		NA
301F	Ready Biodegradability: Manometric Respirometry Test	1992	9408	1999	Table 3-4
302A	Inherent Biodegradability: Modified SCAS Test	1981	9887	1992	Table 3-6
302B	Inherent Biodegradability: Zahn-Wellens/EMPA Test	1992	9888	1998	Table 3-7
302C	Inherent Biodegradability: Modified MITI Test (II)	1981	No equivalent ISO standard		NA
303A	Simulation Test – Aerobic Sewage Treatment: Activated Sludge Units	2001	11733	2004	Table 3-8
303B	Simulation Test – Aerobic Sewage Treatment: Biofilms	2001	No equivalent ISO standard		NA
304A	Inherent Biodegradability in Soil	1981	11266	1994	Table 3-12
305	Bioconcentration: Flow-Through Fish Test	1996	No equivalent ISO standard		NA
306	Biodegradability in Seawater	1992	16221	2001	Table 3-10
307	Aerobic and Anaerobic Transformation in Soil	2002	No equivalent ISO standard		NA
308	Aerobic and Anaerobic Transformation in Aquatic Sediment Systems	2002	No equivalent ISO standard		NA
309	Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test	2004	14592-1	2002	Table 3-9

310	Ready Biodegradability - CO_2 in sealed vessels (Headspace Test)	2006	14593	1999	Table 3-5
311	Anaerobic Biodegradability of Organic Compounds in Digested Sludge: by Measurement of Gas Production	2006	11734	1995	Table 3-11
312	Leaching in Soil Columns	2004	No equivalent ISO standard		NA

[NB: Ref/Table numbers to be checked again before declassification.]

Table 1-3: OECD Test Guidelines Section 4: Health effects and corresponding ISO standards

OECD TEST GUIDELINES			CORRESPONDING ISO STANDARDS		Table in this document
Ref	Title	Adopted or Most recently updated	Ref	Adopted or Most recently updated	
401	Acute Oral Toxicity	Deleted 2002	No equivalent ISO standard		NA
402	Acute Dermal Toxicity	1987	No equivalent ISO standard		NA
403	Acute Inhalation Toxicity	1981	No equivalent ISO standard		NA
404	Acute Dermal Irritation/Corrosion	2002	10993-3	1995	Table 4-1
405	Acute Eye Irritation/Corrosion	2002	No equivalent ISO standard		NA
406	Skin Sensitisation (GMPT)	1992	10993-3	2002-2006	Table 4-2
406	Skin Sensitisation (Buehler Test)	1992	10993-3	1995	Table 4-3
407	Repeated Dose 28-Day Oral Toxicity Study in Rodents	1995	No equivalent ISO standard		NA
408	Repeated Dose 90-Day Oral Toxicity Study in Rodents	1998	No equivalent ISO standard		NA
409	Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	1998	No equivalent ISO standard		NA
410	Repeated Dose Dermal Toxicity:90-Day	1981	No equivalent ISO standard		NA
411	Subchronic Inhalation Toxicity: 90-Day	1981	No equivalent ISO standard		NA
412	Repeated Dose Inhalation Toxicity: 28/14-Day	1981	No equivalent ISO standard		NA
413	Subchronic Inhalation Toxicity: 90-Day	1981	No equivalent ISO standard		NA
414	Prenatal Developmental Toxicity Study	1981	No equivalent ISO standard		NA
415	One-Generation Reproduction Toxicity	1983	No equivalent ISO standard		NA
416	Two-generation Reproduction Toxicity Study	1983	No equivalent ISO standard		NA
417	Toxicokinetics	1992	10993-3	1997	Table 4-4
418	Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure	1995	No equivalent ISO standard		NA

419	Delayed Neurotoxicity of Organophosphorus Substances: 29-Day Repeated Dose Study	1995	No equivalent ISO standard	NA
420	Acute Oral toxicity – Acute Toxic Class Method	2001	No equivalent ISO standard	NA
421	Reproduction/Developmental Toxicity Screening Test	1995	No equivalent ISO standard	NA
422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	1996	No equivalent ISO standard	NA
423	Acute Oral Toxicity – Acute Toxic Class Method	2001	No equivalent ISO standard	NA
424	Neurotoxicity Study in Rodents	1997	No equivalent ISO standard	NA
425	Acute Oral Toxicity: Up-and-Down Procedure	2006	No equivalent ISO standard	NA
427	Skin Absorption: *In Vivo* Method	2004	No equivalent ISO standard	NA
428	Skin Absorption: *In Vitro* Method	2004	No equivalent ISO standard	NA
429	Skin Sensitisation: Local Lymph Node Assay	2002	No equivalent ISO standard	NA
430	*In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)	2004	No equivalent ISO standard	NA
431	*In Vitro* Skin Corrosion: Human Skin Model Test	2004	No equivalent ISO standard	NA
432	*In Vitro* 3T3 NRU Phototoxicity Test	2004	No equivalent ISO standard	NA
435	*In Vitro* Membrane Barrier Test Method for Skin Corrosion	2006	No equivalent ISO standard	NA
451	Carcinogenicity Studies	1981	No equivalent ISO standard	NA
452	Chronic Toxicity Studies	1981	No equivalent ISO standard	NA
453	Combined Chronic toxicity/Carcinogenicity Studies	1981	No equivalent ISO standard	NA
471	Bacterial Reverse Mutation Test	1997	No equivalent ISO standard	NA
472	Genetic Toxicology: *Escherichia coli*, Reverse Assay	Deleted:1997 (Merged w/ TG 471)	No equivalent ISO standard	NA
473	*In Vitro* Mammalian Chromosome Aberration Test	1997	No equivalent ISO standard	NA
474	Mammalian Erythrocyte Micronucleus Test	1997	No equivalent ISO standard	NA
475	Mammalian Bone Marrow Chromosome Aberration Test	1997	No equivalent ISO standard	NA

476	*In Vitro* Mammalian Cell Gene Mutation Test	1997	No equivalent ISO standard	NA
477	Genetic Toxicology: Sex-Linked Recessive Lethal Test in *Drosophilia melanogaster*	1984	No equivalent ISO standard	NA
478	Genetic Toxicology: Rodent dominant Lethal Test	1984	No equivalent ISO standard	NA
479	Genetic Toxicology: *In Vitro* Sister Chromatid Exchange assay in Mammalian Cells	1986	No equivalent ISO standard	NA
480	Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay	1986	No equivalent ISO standard	NA
481	Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay	1986	No equivalent ISO standard	NA
482	Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *In Vitro*	1986	No equivalent ISO standard	NA
483	Mammalian Spermatagonial Chromosome Aberration Test	1997	No equivalent ISO standard	NA
484	Genetic Toxicology: Mouse Spot Test	1986	No equivalent ISO standard	NA
485	Genetic Toxicology: Mouse Heritable Translocation Assay	1986	No equivalent ISO standard	NA
486	Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*	1997	No equivalent ISO standard	NA

[NB: Ref/Table numbers to be checked again before declassification.]

CHAPTER 2: OECD TEST GUIDELINES SECTION 2 - EFFECTS ON BIOTIC SYSTEMS

Aquatic Toxicity Tests

Algae Test

- Alga, Growth Inhibition Test (OECD TG n°201/ISO 8692): Table 2-1

Freshwater aquatic plant Test

- *Lemna* sp. Growth Inhibition Test (OECD TG n°221/ISO 20079): Table 2-2

Daphnia Tests

- *Daphnia* sp., Acute Immobilisation Test (OECD TG n°202/ISO 6341): Table 2-3
- *Daphnia magna* Reproduction Test (OECD TG n°211/ISO 10706): Table 2-4

Fish Tests

- Fish, Acute Toxicity Test (OECD TG n°203/ISO 7346-1,2,3): Table 2-5
- Fish, Prolonged Toxicity Test (OECD TG n°204/ISO 10229): Table 2-6
- Fish, Early-life Stage Toxicity Test (OECD TG n°212/ISO 12890): Table 2-7

Bacterial Test

- Activated Sludge, Respiration Inhibition Test (OECD TG n°209/ISO 8192): Table 2-8

Terrestrial Toxicity Tests

Plant

- Terrestrial Plants, Growth Test (OECD TG n°208/ISO 11269-2): Table 2-9

Soil Fauna

- Earthworm, Acute Toxicity Test (OECD TG n°207/ISO 11268-1): Table 2-10
- Earthworm, Reproduction Test (OECD TG n°222/ISO 11268-2): Table 2-11
- Enchytraeid Reproduction Test (OECD TG n°220/ISO 16387): Table 2-12

Micro-organisms

- Soil micro-organisms: Nitrogen transformation test (OECD TG n°216/ISO 14238): Table 2-13

Table 2-1: Alga, Growth Inhibition Test (OECD TG 201/ISO 8692)

	OECD	ISO	Comments
Title	Freshwater Alga and Cyanobacteria, Growth Inhibition Test	Water quality - Fresh water algal growth inhibition test with unicellular green algae	
Reference	201	8692	
Year	2006	2004	
Test species			
Species	- *Pseudokirchneriella subcapitata,* formely known as *Selenastrum capricornutum* (ATCC 22662, CCAP 278/4, 61.81 SAG) - *Desmodesmus subspicatus*, formely known as *Scenedesmus subspicatus* (86.81 SAG) - *Navicula pelliculosa* (UTEX 664) - *Anabaena flos-aquae* (UTEX 1444, ATCC 29413, CCAP 1403/13A) - *Synechococcus leopoliensis* (UTEX 625, CCAP 1405/1) Other species can be used	- *Pseudokirchneriella subcapitata,* formely known as *Selenastrum capricornutum* (ATCC 22662 or CCAP 278/4) - *Desmodesmus subspicatus*, formely known as *Scenedesmus subspicatus* Chodat (86.81 SAG)	
Test conditions			
Temperature	Temperature in the range of 21-24ºC controlled at ± 2ºC	23 ± 2ºC	
Lighting	Continuous uniform fluorescent light (e.g. cool-white or daylight type)	Under continuous white light	
Light intensity	Green algae: 60-120 µE/m^2/s (equivalent to 4440 – 8880 lux for cool-white light). The light intensity shall be maintained within ± 15% from the average light intensity over the incubation area. Some species, in particular *Anabaena flos aquae*, need a lower light intensity (e.g. 40-60 µE/m^2/s)	60-120 µE/m^2/s. An equivalent range of 6000 to 10000 lux is acceptable for light-measuring instruments calibrated in lux. The light intensity at the average level of the test media shall be homogenous within ± 10 %	
Vessels	Glass vessels (size and volume not specified)	Not specified	

Table 2-1: Alga, Growth Inhibition Test (OECD TG 201/ISO 8692)

	OECD	ISO	Comments
Medium	Two alternative media are recommended: OECD and AAP medium. Modification of the growth media may be necessary for specific purposes, e.g. when testing metals, chelating agents or testing different pH values. For growth inhibition tests performed with *Navicula pelliculosa*, both media must be supplemented with Na_2SiO_3, $9H_2O$.	The medium is equal to the one described in the OECD Guideline (OECD medium). After equilibration, adjust pH if necessary to 8.1 ± 0.2.	
Performance of the test			
Test duration	Normally 72 hours However, shorter or longer test durations (e.g. 48 – 96 hours) are acceptable if validity criteria are met.	72 hours ± 2 hours	
Initial cell concentration	*Pseudokirchneriella subcapitata*: 0.5 - 1.0 x 10^4cells/ml *Desmodesmus subspicatus:* 2 - 5 x 10^3 cells/ml *Navicula pelliculosa*, *Anabaena flos-aquae:* 1.0 x 10^4cells/ml *Synechococcus leopoliensis*: 0.5 – 1.0 x 10^5 cells/ml	≤ 10^4 cells/ml	
Range finding test	Optional. There is no clear statement about the preliminary test	It is advisable to perform a preliminary range-finding test covering several orders of magnitude. Replication of test concentrations is not necessary	

Table 2-1: Alga, Growth Inhibition Test (OECD TG 201/ISO 8692)

	OECD	ISO	Comments
Test concentrations	At least five concentrations in a geometric series with a factor not exceeding 3.2 The concentration series should cover the range causing 5 - 75% inhibition of algal growth rate. A limit test may be performed (e.g. 100 mg/L or limit of the solubility of the chemical in the test medium). For test substances showing a flat response curve, a higher factor is acceptable.	Algae should be exposed to concentrations of the tests sample in a geometric series with a factor not exceeding 3.2. The concentrations should be chosen to obtain one inhibition level below and one inhibition level above the intended ErCx. Additionally, at least two inhibition levels between 10 % and 90 % should be included in the concentration range. A limit test may be performed to demonstrate the absence of toxicity (six replicates for the treatment and the control).	
Number of replicates	Three replicates at each test concentration, and ideally twice that number of controls. If determination of the NOEC in not required, the test design may be altered to increase the number of concentrations and reduce the number of replicates.	Three replicates of each test substance concentration, and six controls.	
Solubilising agent	Acetone, t-butyl alcohol, DMF; maximum concentration ≤ 100µl/l in the final test medium.	Not specified. The standard refers to two others documents ISO 14442 and ISO 5667-16 for poorly soluble chemicals.	
Reference substance	It is recommended to test a reference substance such as 3,5 dichlorophenol at least twice a year. Potassium dichromate can also be used for green algae.	It is recommended to test a reference substance in order to prove the validity of the test system (3,5 dichlorophenol or potassium dichromate).	

Table 2-1: Alga, Growth Inhibition Test (OECD TG 201/ISO 8692)

	OECD	ISO	Comments
Data and Reporting			
Response variables	- Average specific growth rate - Yield	- Average specific growth rate	
Frequency of measurements	- The algal biomass is determined at least daily. - The pH is measured at the beginning of the test and at the end of the test. The pH of the control medium should not increase by more than 1.5 units during the test.- The concentration of the test substance is analysed at the beginning of the test and at the end of the test (lowest, highest concentrations and a concentration around EC_{50})	- The cell density is determined at least every 24 hours. - The pH is measured at the beginning and at the end of the test (each concentration and control). The appearance of the cells and the identity of the test organism should be confirmed by microscopy	
Expression of results	ErCx (e.g. 50 %) - NOEC	ErCx (e.g. 10 %, 50 %) LID: lowest effective dilution (for waste water)	
Validity	- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour period. This corresponds to a specific growth rate of 0.92 day^{-1}. For the most frequently used species, the specific growth rate is substantially higher (*P. subcapitata*: 1.5-1.7; *D. subspicatus*: 1.2-1.5; N. *pelliculosa*: 1.4; A. *flos-aquae*: 1.2-1.4; S. *leopoliensis*: 2.0-2.4) - The mean coefficient of variation of average specific growth rate (days 0-1; 1-2; 2-3) must not exceed 35% in control cultures. - The coefficient of variation of average specific growth rates in control cultures must not exceed 7% for *P. subcapitata* and *D. subspicatus* and 10 % for other species	- The average control growth rate shall be at least 1.4 day^{-1}. This growth rate corresponds to an increase in cell density by a factor 67 in 72 hours. - The variation coefficient of the control growth rates shall not exceed 5%. - The control pH shall not have increased during the test by more than 1.5 relative to the pH of the growth medium.	

Table 2-2: *Lemna* sp.Growth Inhibition Test (OECD TG 221/ISO 20079)

	OECD	ISO	Comments
Title	*Lemna* sp., Growth Inhibition Test	Water quality – Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) – Duckweed growth inhibition test	
Reference	221	20079	
Year	2006	2005	
Test species			
Species	*Lemna minor* or *Lemna gibba*	*Lemna minor*	
Test conditions			
Temperature	24 ± 2ºC	24 ± 2ºC in the test vessels The temperature should be maintained throughout the test within ±1ºC in all vessels.	
Lighting	Continuous warm or cool-white fluorescent lighting	Continuous warm or cool-white fluorescent lighting	
Light intensity	85-135 µE/m^2/s (equivalent to 6500 – 10000 lux). Any differences from the selected light intensity should not exceed the range of ± 15% over the test area.	85-135 µE/m^2/s. The light intensity shall not exceed ± 15% of the selected value in the test area.	
Vessels	Beakers, crystallising dishes or Petri dishes made of glass or other chemically inert material. The test vessels should have a minimum depth of 20 mm and a volume of 100 ml of test medium.	Glass beakers, crystallising dishes or Petri dishes with a minimum volume of 150 ml. A minimum volume of test solution of 100 ml is recommended. The diameter of the test vessel should be chosen in order to avoid the overlapping of the fronds at the end of the test.	
Medium	- *Lemna minor*: modified Swedish Standard medium (SIS) - *Lemna gibba*: 20X AAP medium	The modified Steinberg medium is recommended. The modified APHA medium (for testing metal substances and metal contaminated effluents) and the modified Swedish Standard (SIS) medium are	

Table 2-2: *Lemna* sp.Growth Inhibition Test (OECD TG 221/ISO 20079)

	OECD	ISO	Comments
		also suitable.	
Performance of the test			
Test duration	7 days	7 days	
Initial frond number	9 to 12 fronds per vessel. Colonies consisting of 2 to 4 fronds are transferred from the inoculum culture to the test vessels. The number of fronds and colonies should be the same in each test vessel.	10 to 16 fronds per vessel. Colonies consisting of 2 to 3 fronds are transferred from the inoculum culture to the test vessels.	
Range finding test	Optional. There is no clear statement about the preliminary test	Optional. There is no clear statement about the preliminary test	
Test concentrations	At least five concentrations in a geometric series with a factor not exceeding 3.2. Justification should be provided if fewer than five concentrations are used. Two test designs are described: determination of ECx and estimation of LOEC/NOEC. If the estimation of NOEC is not required, the test design may be modified to increase the number of concentrations and reduce the number of replicates per concentration. However, the number of controls should be at least three. A limit test may be performed (e.g. 100 mg/l or limit of the solubility of the chemical in the test medium). The number of treatment replicates should be doubled.	A geometric series of at least five concentrations. At least one measured inhibition value for the intended ECx should be below and one above to the ECx to be estimated and three or more values should be other than 0 % and 100 % inhibition. A limit test may be performed (six replicates for the treatment and the control).	
Number of replicates	Three replicates at each test concentration and for the control.	Three replicates at each test concentration and six controls. The number of replicates may be increased for the assessment of $EC_{<20}$.	

Table 2-2: *Lemna* sp.Growth Inhibition Test (OECD TG 221/ISO 20079)

	OECD	ISO	Comments
Solubilising agent	As far as possible the use of solvents, emulsifiers and dispersants should be avoided. When such compounds are used (e.g. acetone, DMF), the concentration shall be ≤ 0.1 ml/l in the final test medium.	As far as possible the use of solvents and dispersants should be avoided. When such compounds are used the concentration shall be ≤ 0.1 ml/l in the final test medium.	
Reference substance	It is advisable to test a reference substance such as 3,5 dichlorophenol at least twice a year or in parallel to the determination of the toxicity of a test substance if testing is carried out at a lower frequency.	It is advisable to test a reference chemical (3,5 dichlorophenol and/or potassium chloride) regularly in order to check the test procedure and the sensitivity of the strain. Nickel sulphate may be an additional reference substance.	
Data and Reporting			
Response variables	- Average specific growth rate, - Yield. These response variables are calculated on the basis of frond number and at least one of the following parameter: total frond area, dry weight, fresh weight.	- Average specific growth rate The average specific growth rate is calculated on the basis of frond number and one of the following parameter: total frond area, dry weight, chlorophyll-a concentration.	
Frequency of measurements	*Toxicity parameters*: - Frond number: at least 2 measurements within the test period - Total frond area: at the beginning, during and at the end of the test. - Dry or fresh weight: at the beginning and at the end of the test. *Test concentrations*: - The concentrations of the test substance are analysed at appropriate intervals. • Static test - The test concentrations should be determined at least at the beginning and at the end of the test. •Semi-static test	*Toxicity parameters*: - Frond number: every 48h to 72 hours for the controls; at the beginning and at the end of the test for the test concentrations - Total frond area: at the beginning and at the end of the test. - Dry weight: at the beginning and at the end of the test. - Chlorophyll-a concentration: at the beginning and at the end of the test. Observations of any visual sign of phytotoxicity are recorded at the end of the test. *pH*:	

Table 2-2: *Lemna* sp.Growth Inhibition Test (OECD TG 221/ISO 20079)

	OECD	ISO	Comments
	1) When the concentration is not expected to remain within ± 20 % of the nominal: it is necessary to analyse all freshly prepared test solutions and the same solutions prior to each renewal in all test concentrations. 2) When the concentration is not expected to remain within ± 20 % of the nominal but the initial concentrations are repeatable and stable: chemical determinations may be carried out on only the highest and lowest test concentrations. • Flow-through test A similar sampling regime as for semi-static tests is appropriate including analysis at the beginning, mid-way through and at the end of the test. In this type of test, the flow rate of diluent and test substance or test substance stock solution should be checked daily. *pH*: 1) If a static test design is used, the pH should be determined at the beginning and at the end of the test in each treatment. 2) If a semi-static test design is used, the pH should be determined in all freshly prepared test solutions and in the same solutions prior to each renewal in all test concentrations.	The pH should be determined at the end of the test in each treatment.	
Expression of results	- ECx (e.g.50 %) - NOEC/LOEC	ECx (e.g. 10,20,50 %)	

Table 2-2: *Lemna* sp.Growth Inhibition Test (OECD TG 221/ISO 20079)

	OECD	ISO	Comments
Data and Reporting (continued)			
Validity	The doubling time of frond number in the control must be less than 2.5 days (60h) corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of $0.275d^{-1}$.	The mean number of fronds in the control shall have at least a average specific growth rate of $0.275d^{-1}$. This corresponds to a doubling time of about 2.5 days and a 7-fold increase of the mean number of fronds at the end of the test. The EC_{50} (frond number) for 3,5 dichlorophenol should be in the range 2.2 – 3.8 mg/l using the modified Steinberg medium. For potassium chloride, the EC_{50} should be in the range 5.5 – 10 g/l using APHA medium.	

Table 2-3: *Daphnia* sp., Acute Immobilisation Test (OECD TG 202/ISO 6341)

	OECD	ISO	Comments
Title	*Daphnia* sp., Acute Immobilisation Test	Water quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test	
Reference	202	6341	
Year	2004	1996 + technical corrigendum 1998	
Test species			
Species	*Daphnia magna* Straus or any other suitable Daphnia species (e.g. *Daphnia pulex*)	*Daphnia magna* Straus (Cladocera, Crustacea)	
Age	≤ 24 hours old. Not the first brood progeny. If the test medium is different from the culture medium, brood daphnids should be maintained in the test medium for at least 48 hours prior to the start of the test	≤ 24 hours old. At least third generation. Gravid females shall be transferred in the test medium. The newly released neonates are collected within 24 hours.	
Test conditions			
Temperature	The temperature should be within the range 18-22ºC. For each test, the temperature should be constant within ± 1ºC.	20 ± 2 ºC	
Lighting	Controlled light-dark cycle: 16 hours light / 8 hours dark. Complete darkness is acceptable especially for chemicals unstable under light.	Controlled light-dark cycle: 16 light / 8 dark or complete darkness (Photo-degradable chemicals shall be tested in the dark)	
Light intensity	Not specified	Not specified	
Vessels	Tubes or beakers made of glass or other chemically inert material	Vessels made of chemically inert material (e.g. glass test tubes or beakers)	

Table 2-3: *Daphnia* sp., Acute Immobilisation Test (OECD TG 202/ISO 6341)

	OECD	ISO	Comments
Medium	Natural water (surface or ground water), reconstituted water (e.g. ISO, M4 or M7 media) or dechlorinated tap water are acceptable as holding and dilution water. The pH should be in the range 6-9 and hardness in the range 140 – 250 mg/l (as $CaCO_3$) for *Daphnia magna*.	- Reconstituted water (ISO medium) pH: 7.8 ± 0.2 hardness: 250 ± 25 mg/l (as $CaCO_3$) molar Ca/Mg ratio: close to 4:1 dissolved oxygen concentration: above 7 mg/l - Natural water with pH and hardness characteristics similar to reconstituted water	
Performance of the test			
Test duration	48 hours	24 hours 48 hours (if desired)	
Preliminary test	Optional. Single series of widely spaced concentrations; 5 daphnids per concentration; duration 48 hours or less.	- Single series of concentrations - Generally chosen in geometric progression	
Test concentrations	At least five concentrations in a geometric series with a factor not exceeding 3.2. The highest concentration should preferably result in 100 % immobilisation, and the lowest concentration should preferably give no observable effect. A limit test may be performed (e.g. 100 mg/l or limit of the solubility of the chemical in the test medium).	The range of concentration is at least three percentages of immobilisation between 10 % and 90 %. Limit test can be performed at 100 mg/l.	
Solubilising agent	As far as possible the use of solvents, emulsifiers and dispersants should be avoided.	The concentration of the solvent in the final test solution shall not exceed 0.1 ml/l	
Number of animal and Loading	At least 20 animals, preferably divided into four groups of five animals each should be used at each test concentration and for the control. At least 2 ml of test solution should be provided for each animal.	At least 20 animals. The total number of *Daphnia magna* shall not exceed 20 per vessel. The density of animals per vessel shall not exceed 1 *Daphnia* per 2 ml.	

Table 2-3: *Daphnia* sp., Acute Immobilisation Test (OECD TG 202/ISO 6341)

	OECD	ISO	Comments
Reference substance	It is recommended to test a reference substance every month and at least twice a year. Potassium dichromate is one of the recommended reference substance.	Periodically. Potassium dichromate is the recommended reference substance.	
Data and Reporting			
Frequency of observations	Mobility of daphnids shall be determined at 24 and 48 hours.	Mobility of daphnids shall be determined at 24 and 48 hours (if appropriate).	
Frequency of measurements	The concentration of the test substance is analysed at the beginning of the test and at the end of the test, as a minimum at the lowest and the highest concentrations. The dissolved oxygen and the pH are measured at the beginning and the end of the test in the control and in the highest test substance concentration.	The analyses of test substances should be conducted, and if analyses show that standard deviation of the concentrations measured during the test is not greater than 20 % of the mean, the median inhibitory concentrations may be calculated from the means of the measurements rather than from the initial concentrations. - Oxygen concentration: immediately after counting the immobilised *Daphnia magna* in the test container with the solution of lowest concentration at which all the *Daphnia magna* have been immobilised.	
Expression of results	- EC_{50} 48 hours with 95% confidence limits (24 hours optional). - Slope of the concentration response curve. - Where the standard methods of calculating EC_{50} are not applicable, the highest concentration causing no immobility and the lowest concentration causing 100 % immobility should be used as an approximation of the EC_{50}.	- EC_{50} 24 h and 48h (if appropriate). - The highest concentration tested producing no immobilisation of Daphnia. - The lowest concentration tested producing 100 % immobilisation of Daphnia.	

Table 2-3: *Daphnia* sp., Acute Immobilisation Test (OECD TG 202/ISO 6341)

	OECD	ISO	Comments
Validity	- In the control, no more than 10 % of the daphnids should have been immobilised. - The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.	- The percentage immobilisation of the controls is less than or equal to 10 %. - The dissolved oxygen concentration at the end of the test is ≥ 2 mg/l. - The EC_{50} 24 hours of the potassium dichromate is within the range of 0.6 – 2.1 mg/l.	

Table 2-4: *Daphnia magna* Reproduction Test (OECD TG 211/ISO 10706)

	OECD	ISO	Comments
Title	*Daphnia magna* Reproduction Test	Water quality - Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea)	
Reference	211	10706	
Year	1998	2000	
Test species			
Species	*Daphnia magna* Straus Other *Daphnia* species may be used provided they meet the validity criteria. Clone A is recommended. Other clones are acceptable, provided that the Daphnia culture is shown to meet the validity criteria.	*Daphnia magna* Straus There is no statement about clone in the ISO Standard. However, the clone used should be mentioned in the test report.	
Other information about species	- Less than 24 hours old, - Must not be first brood progeny.	- Less than 24 hours old, - Should be from the second to the fifth brood, - From a healthy stock with < 20 % mortality, where males or ephippia are absent, and there is no delay in the production of the first brood or discoloured animals.	
Test conditions			
Temperature	The temperature should be within the range 18-22ºC. For each test, the temperature should be constant within ± 1ºC	The temperature should be within the range 18-22 ºC. For each test, the temperature should be constant within ± 1ºC (e.g. 18-20ºC, 19-21ºC or 20-22ºC)	
Lighting	Controlled light-dark cycle: 16 light / 8 dark	Controlled light-dark cycle: 16 light / 8 dark	
Light intensity	≤ 15 - 20 $\mu E/m^2/s$	Preferably 600 – 800 lux. The light intensity shall not exceed 1200 lux.	
Test system	- Semi-static system, - Flow-through system.	- Semi-static system, - Flow-through system.	

Table 2-4: *Daphnia magna* Reproduction Test (OECD TG 211/ISO 10706)

	OECD	ISO	Comments
Vessels	Beakers made of glass or other chemically inert material	Vessels made of chemically inert material (e.g. test tubes or glass beakers, 50 ml or 100 ml capacity)	
Medium	- M7 and M4 media - ASTM reconstituted hard freshwater with seaweed extract. If the test substance contains metals, the M4 and M7 medium are not recommended. Other media are acceptable provided that the validity criteria are met. The test media should satisfy the following characteristics: • Dissolved oxygen concentration: > 3 mg/l, • pH: 6 - 9 (within 1.5 pH unit during the test), • Hardness: > 140 mg/l (as $CaCO_3$), • TOC level in the medium before addition of the algae < 2 mg/l.	- M7 and M4 media - ASTM reconstituted hard freshwater - Natural water If the test substance contains metals, the M4 and M7 medium are not recommended. The test media should satisfy the following characteristics • Dissolved oxygen concentration: 95% of air saturation value, • pH: 8.0 ± 0.5, • Hardness: > 140 mg/l (as $CaCO_3$), • TOC level in the medium before addition of the algae < 5 mg/l.	
Performance of the test			
Test duration	21 days	21 days	
Acclimation	About 3 weeks (one generation) if the culture conditions are different from test conditions.	About 3 weeks (one generation) if the culture conditions are different from test conditions.	
Preliminary test	Daphnia acute immobilisation test or range finding study.	Daphnia acute immobilisation test or range finding study.	
Test concentrations	At least five exposure concentrations arranged in a geometric series with a separation factor not exceeding 3.2. Justification should be provided if fewer than five concentrations are used.	At least five exposure concentrations arranged in a geometric series with a separation factor not exceeding 3.2.	
Solubilising agent	As far as possible the use of solvents, emulsifiers and dispersants should be avoided. When such compounds are used, the concentration shall be ≤ 0.1 ml/l in the final test medium.	The concentration of solvent or dispersant shall be ≤ 0.1 ml/l. The concentration of solubilising agent should be same in all recipients.	

Table 2-4: *Daphnia magna* Reproduction Test (OECD TG 211/ISO 10706)

	OECD	ISO	Comments
Loading	- *Semi-static tests*: at least 10 animals individually held at each test concentration and control (50-100 ml of medium in each vessel). - *Flow-through tests*: 40 animals divided into four groups of 10 animals at each test concentration (a smaller number of test organisms may be used and a minimum of 20 animals per concentration divided into two or more replicates with an equal number of animals is recommended).	- *Semi-static tests*: at least 10 animals individually held at each test concentration and control. - *Flow-through tests*: there is no statement about the number of animals.	
Reference substance	There is no statement about the reference substance.	There is no statement about the reference substance.	
Data and Reporting			
Frequency of observations	- Number of offspring (including aborted eggs, dead offspring): preferably daily, - Mortality among the parent animals: preferably daily, at least at the same time as offspring are counted.	- Number of offspring (including males, ephippia eggs): at least three times a week, - Mortality among the parent animals: at least three times a week, - Length, dry weight of parent animals (option): at the end of the test.	
Frequency of measurements	- *Test concentrations*: • Semi-static test 1) When the concentration is expected to remain within ± 20 % of the nominal: as a minimum, in the highest and lowest concentrations, when freshly prepared and at the time of renewal on one occasion during the first week of the test and each week thereafter. 2) When the concentration is not expected to remain within ± 20 % of the nominal: when freshly prepared and at renewal in all test concentrations. 3) When the concentration is not expected to remain within ± 20 % of the nominal but the initial	- *Test concentrations*: • Semi-static test 1) When the concentration is expected to remain within ± 20 % of the nominal: in the highest and lowest concentrations, when freshly prepared and at the time of renewal on one occasion during the first week of the test and each week thereafter. 2) When the concentration is not expected to remain within ± 20 % of the nominal: at lest weekly. If concentrations are within 20 % of initial measured concentrations, analyses shall be conducted on only the highest and lowest exposure concentrations.	

Table 2-4: *Daphnia magna* Reproduction Test (OECD TG 211/ISO 10706)

	OECD	ISO	Comments
	concentrations are repeatable and stable: chemical determinations could be reduced in weeks 2 and 3 of the test to the highest and lowest test concentrations. • Flow-through test A similar sampling regime as for semi-static tests is appropriate. - *Oxygen concentration, temperature, hardness, pH*: at least once a week, in fresh and old media, in the control(s) and in the highest test substance concentration.	• Flow-through test A similar sampling regime for semi-static tests is appropriate. - *Oxygen concentration, temperature, hardness, pH*: weekly and at the beginning and the end of one of the renewal periods, in the control(s) and in the highest test substance concentration. - Light intensity shall be recorded (no statement about frequency of measurement).	
Expression of results	- LOEC, NOEC - ECx (50, 10 ,20) The results are expressed according to the total number of living offspring produced per parent animal alive at the end of the test.	- LOEC, NOEC - ECx (50, 10 ,20) The results are expressed according to the total number of living offspring produced per parent animal alive at the end of the test.	
Validity	- The mortality of the parent animals (female *Daphnia*) in controls does not exceed 20 % at the end of the test. - The mean number of offspring produced per parent animal surviving at the end of the test is ≥ 60 in the controls.	- The mortality of adults in controls is < 20 % at the end of the test. - The mean number of offspring per parent in controls is ≥ 60 %. - The presence of living males in controls is < 20 % at the end of the test. - The coefficient of variation of the reproductive output, based on the number of juveniles per parent per day, should not exceed 20 % in controls.	

Table 2-5: Fish, Acute Toxicity Test (OECD TG 203/ISO 7346-1, 2, 3)

	OECD	ISO	Comments
Title	Fish, Acute Toxicity Test	Water quality - Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static method - Part 2: Semi-static method - Part 3: Flow-through method	The ISO 7346 includes three separate test methods.
Reference	203	7346 -1, -2, -3	
Year	1992	1996	
Test species			
Species	- Zebra fish (*Danio rerio*) - Fathead Minnow (*Pimephales promelas*) - Common carp (*Cyprinus carpio*) - Ricefish (*Orizias latipes*) - Guppy (*Poecilia reticulata*) - Bluegill (*Lepomis macrochirus*) - Rainbow trout (*Oncorhynchus mykiss*) Other species can be used.	Zebra fish (*Brachydanio rerio*) The following species can also be used: - Fathead Minnow (*Pimephales promelas*) - Ricefish (*Orizias latipes*) - Guppy (*Poecilia reticulata*) - Bluegill (*Lepomis macrochirus*) The test method can be adapted to other freshwater species, to brackish and marine species.	
Size of fish	- Zebra fish: 2.0 ± 1.0 cm - Fathead Minnow: 2.0 ± 1.0 cm - Common carp: 3.0 ± 1.0 cm - Ricefish: 2.0 ± 1.0 cm - Guppy: 2.0 ± 1.0 cm - Bluegill: 2.0 ± 1.0 cm - Rainbow trout: 5.0 ± 1.0 cm	Zebra fish: 3 ± 0.5 cm (which corresponds to a mass of 0.3 ± 0.1 g)	
Acclimation	The fish must be held: - in the laboratory for at least 12 days before the test, - and at least 7 days in the water quality to be used in the test.	The fish must be held: - in the laboratory for at least two weeks before the test, - and at least 7 days in the water quality and test conditions to be used in the test.	

Table 2-5: Fish, Acute Toxicity Test (OECD TG 203/ISO 7346-1, 2, 3)

	OECD	ISO	Comments
Test conditions			
Temperature	- Zebra fish: 21-25°C - Fathead Minnow: 21-25°C - Common carp: 20-24°C - Ricefish: 21-25°C - Guppy: 21-25°C - Bluegill: 21-25°C - Rainbow trout: 13-17°C The temperature should be constant within a range of 2°C.	23 ± 1ºC in the test vessels	
Lighting	12 to 16h photoperiod daily	12 to 16h photoperiod daily	
Vessels	Tanks made of chemically inert material and of a suitable capacity	Tanks of a suitable capacity (e.g. capacity > 10 l) with a large surface allowing exchanges between air and test solutions	
Medium	- Good quality natural water - Reconstituted water - Drinking water (dechlorinated if necessary) Waters with following characteristics are preferable: - Total hardness: 10 - 250 mg/l expressed as calcium carbonate, - pH: 6.0 - 8.5.	Reconstituted water ISO 7346: - 294.0 mg/l $CaCl_2$ $7H_2O$, - 123.3 mg/l $MgSO_4$ $7H_2O$, - 63.0 mg/l $NaHCO_3$, - 5.5 mg/l KCl, - pH: 7.8 ± 0.2, - Dissolved oxygen concentration: at least 90 % of air saturation value, - Total hardness: approx. 250 mg/l expressed as calcium carbonate.	
Performance of the test			
Test duration	Preferably 96 hours	96 hours	
Preliminary test	There are no details on the preliminary test.	- Five concentrations in an adequate geometric series, - Three fish in each vessel, - Twice a day, note the number of dead fish and the dissolved oxygen concentration.	
Test concentrations	At least five concentrations in a geometric series with a factor preferably not exceeding 2.2.	At least five concentrations in an approximately geometric series including the lowest	

Table 2-5: Fish, Acute Toxicity Test (OECD TG 203/ISO 7346-1, 2, 3)

	OECD	ISO	Comments
	A limit test may be performed (100 mg/l) in order to demonstrate that the LC50 is greater than this concentration.	concentration killing all the fish in the preliminary test, and the highest non-lethal concentration in 96 hours. A limit test may be performed (100 mg/l or the limit of aqueous solubility, whichever is the lower) in order to demonstrate that the LC50 is greater than this concentration.	
Number of replicates	One replicate.	One replicate.	
Solubilising agent	Organic solvents, emulsifiers or dispersants may be used. The concentration should not exceed 100 mg/l.	The concentration of organic solvent shall not exceed 100 ml/l or 100 mg/l, whichever is the greater.	
Number of fish and loading	At least 7 fish - Static test: ≤ 1.0 g fish/litre, - Semi static test: ≤ 1.0 g fish/litre, - Flow-through test: higher loading than 1.0 g fish/litre can be accepted.	At least 7 fish (10 fish for Limit Test) - Static test: ≤ 1.0 g fish/litre, - Semi static test: ≤ 1.5 g fish/litre, - Flow-through test: ≤ 1.5 g fish/litre.	
Reference substance	There is no statement on reference substances in the OECD Guideline.	When there is a change of stock population, carry out a test using the reference substance (e.g. $K_2Cr_2O_7$)	
Data and Reporting			
Frequency of observations	At least after 24, 48, 72 and 96h. 3 and 6h after the start of the test are desirable.	At least daily, but can be made more frequently, for example to enable median periods of survival to be calculated for each concentration.	
Frequency of measurements	- *Concentration of test substance*: there is no recommendation on the frequency of measurements of test concentrations. - *pH*: at least daily (in semi-static systems the pH should be measured prior to and after water renewal). - *Dissolved oxygen*: at least daily.	- *Concentration of test substance*: • Static test: at least at the beginning and at the end of the test (if the substance is shown to be stable). • Semi static test: at least at the beginning and end of the first and final renewal periods (if the substance is shown to be stable), or at least at the beginning and end of each renewal period throughout the duration (if the substance is shown	

Table 2-5: Fish, Acute Toxicity Test (OECD TG 203/ISO 7346-1, 2, 3)

	OECD	ISO	Comments
	- *Temperature*: at least daily.	to be unstable). • Flow-through test: at least at the beginning and end of the test (both stock solutions and out going solutions from the test vessels). - *pH*: at least daily (in semi-static systems the pH should be measured prior to and after water renewal). - *Dissolved oxygen*: at least daily (in semi-static systems the DOC should be measured prior to and after water renewal). - *Temperature*: at least daily (in semi-static systems the temperature should be measured prior to and after water renewal).	
Expression of results	- LC50 (24, 48, 72 and 96 hours if possible), - Maximum concentration causing no mortality during the test, - Minimum concentration causing 100 % mortality during the test.	- LC_{50} (24, 48, 72 and 96 hours if possible) If insufficient data are available to estimate the LC_{50}: - Maximum concentration causing no mortality during the test, - Minimum concentration causing 100 % mortality during the test.	
Validity	- Mortality in the control(s) should not exceed 10 % (or one fish if less than ten are used) at the end of the test. - Constant condition should be maintained as far as possible throughout the test and, in necessary, semi-static or flow-through systems should be used. - The dissolved oxygen concentration must have been at least 60 % of the air saturation value (ASV) throughout the test. - There must be evidence that the concentration of the test substance has been correctly maintained, and	- Mortality in the control should not exceed 10 % or one fish per tank. - The proportion of control fish showing abnormal behaviour did not exceed 10 % or one per tank. - The dissolved oxygen concentration in the test solutions during the test was at least 60 % ASV. - The concentrations of the test substance were not known (or suspected) to have declined significantly throughout the test. - The 24h-LC_{50} of the reference chemical, if available, for the stock of fish was in reasonable	

Table 2-5: Fish, Acute Toxicity Test (OECD TG 203/ISO 7346-1, 2, 3)

	OECD	ISO	Comments
	preferably it should be at least 80 % of the nominal concentration throughout the test. The results should be based on measured concentrations if the deviation from the nominal concentration is greater than 20 %.	agreement with results obtained previously in the same laboratory.	

Table 2-6: Fish, Prolonged Toxicity Test (OECD TG 204/ISO 10229)

	OECD	ISO	Comments
Title	Fish, Prolonged Toxicity Test: 14-day Study	Water quality - Determination of prolonged toxicity of substances to freshwater fish - Method for evaluating the effects of substances on the growth rate of rainbow trout [*Oncorhynchus mykiss Walbaum* (Teleostei, Salmonidae)]	
Reference	204	10229	
Year	1984	1994	
Test species			
Species	- Zebra-fish (*Danio rerio*) - Fathead Minnow (*Pimephales promelas*) - Common carp (*Cyprinus carpio*) - Ricefish (*Orizias latipes*) - Guppy (*Poecilia reticulata*) - Bluegill (*Lepomis macrochirus*) - Rainbow trout (*Oncorhynchus mykiss*) Other species can be used.	- Rainbow trout (*Oncorhynchus mykiss*) The test method can be adapted to other freshwater species, to brackish and marine species.	
Size of fish	Recommended total length - Zebra-fish: 2.0 ± 1.0 cm - Fathead Minnow: 2.0 ± 1.0 cm - Common carp: 3.0 ± 1.0 cm - Ricefish: 2.0 ± 1.0 cm - Guppy: 2.0 ± 1.0 cm - Bluegill: 2.0 ± 1.0 cm - Rainbow trout: 5.0 ± 1.0 cm	3 - 5g at the start of the test (the range for individual masses shall be within ±10 % of the arithmetic mean of the individual masses)	
Acclimation	The fish must be held: - in the laboratory for at least 12 to 15 days before the test, - and at least 7 days in the water quality to be used in the test.	At least two weeks in the water and conditions (feeding, lighting) to be used in the test.	

Table 2-6: Fish, Prolonged Toxicity Test (OECD TG 204/ISO 10229)

	OECD	ISO	Comments
Other information about fish	There is no explanation about marking.	Fish can be individually recognised if the freeze-branding technique or an equivalent method, but this is not mandatory.	
Test conditions			
Temperature	- Zebra fish: 21-25°C - Fathead Minnow: 21-25°C - Common carp: 20-24°C - Ricefish: 21-25°C - Guppy: 21-25°C - Bluegill: 21-25°C - Rainbow trout: 13-17°C	At a constant temperature of 12.5 to 17.5 ºC within ± 1 ºC.	
Lighting	12 to 16h photoperiod daily	12 to 16h photoperiod daily.	
Vessels	Containers of suitable capacity in relation to the recommended loading.	Chemically inert vessels of 45 litres capacity. The lateral surfaces should be opaque.	
Medium	- Reconstituted water, - Good quality natural water, - Drinking water supply (dechlorinated if necessary). Waters with following characteristics are preferable: - Total hardness: 50 to 250 mg of $CaCO_3$ per litre - pH: 6.0 to 8.5	Suitable water for the long-term survival and growth of the test fish. The water should have the following characteristics: - The pH of dilution water should be within the range 6.7 to 8.5 (during a given test the pH of the dilution water shall not vary by more than ± 0.2 pH units from the mean value), - Total hardness: 20 to 300 mg of $CaCO_3$ per litre. The reconstituted water ISO 7346 is suitable.	
Feeding	Several times daily (2% dry weight related to the initial fish weight).	4 % of the fish wet weight per day. The food is provided two times per day (at least 5 hours between the two feeding periods). After 14 days, the fish are re-weighted; the amount of food is adjusted accordingly.	
Performance of the test			
Test duration	Normally 14 days but the test duration can be	28 days	

Table 2-6: Fish, Prolonged Toxicity Test (OECD TG 204/ISO 10229)

	OECD	ISO	Comments
	extended by one or two weeks.		
Preliminary test		Acute toxicity test according to ISO 7346-2 or ISO 7346-3.	
Test concentrations	The test concentrations chosen must permit the determination of both the threshold levels for lethal and other observable effects, and the NOEC value. Concentrations of the substance in excess of 100 mg/l need not be tested if a threshold level has not been reached up to this concentration.	Based on the results from the acute toxicity test. At least five concentrations, forming an geometric progression (the factor shall not exceed 3.162). The highest concentration should not usually be less than 10 % or greater than 32 % of the 96h LC_{50}. If fewer than five concentrations are used, this shall be justified.	
Number of replicates	One replicate.	One replicate.	
Solubilising agent	Organic solvents, emulsifiers or dispersants may be used. The concentration should not exceed 100 mg/l	When a solubilising agent is used, the final concentration should not exceed 0.1 ml/l (preferably be below 0.01 ml/l) in the test medium and should be the same in all test vessels.	
Loading	At least 10 fish / concentration. - Semi-static tests: ≤ 1.0 g fish/litre - Flow-through systems: higher loading than 1.0 g fish/litre can be acceptable.	16 fish / concentration. Maximum loading is about 1.4 g fish/litre. (16 fish × 4 g/fish / 45 l ≈ 1.4 g fish/litre)	
Reference substance	There is no statement about reference substance.	There is no statement about reference substance.	
Data and Reporting			
Frequency of observations	- *Mortality*: at least once a day - *Observed effects*: it is recommend that daily records be kept of all observed effects, but a minimum of three observation sessions per week must be conducted. - *Weight and length of fish*: at the termination of the test (Representative samples of the test population should be weighted and measured before the test	- *Mortality and abnormal behaviour*: preferably daily. - *Weight and length*: 0, 14th day and 28th day. (If each fish is identified, record individually. If fish is not individually identified, mean increases in length or mass are calculated.).	

Table 2-6: Fish, Prolonged Toxicity Test (OECD TG 204/ISO 10229)

	OECD	ISO	Comments
	starts)		
Frequency of measurements	- *Test concentration*: • Flow-through test: at the beginning of the test • Semi-static test: at the beginning of the test, immediately prior to the first renewal of the test solution and at the termination of the test. - *pH*: at least twice a week. - *Dissolved oxygen*: at least twice a week. - *Temperature*: at least twice a week.	- *Test concentration*: at least at the beginning, middle and end of the test. (Preferably, 3 days before the start of the test, 2 days before the start of the test, then once a week). - *pH*: at least once daily - *Dissolved oxygen*: at least once daily. - *Temperature*: at least once daily.	
Expression of results	- NOEC, - Threshold levels of lethal and other observed effects.	- LOEC, NOEC, - IC_{10} (if individual growth rates have not been measured, IC_{10} can be estimated using the mean specific growth rate data).	
Validity	- The mortality in the controls should not exceed 10 % at the end of the test. - The dissolved oxygen concentration should be at least 60 % of the air saturation value throughout the test. - There should be evidence that the concentrations of the test substance had been correctly maintained (it should be at least 80 % of the nominal concentration) over the test period. The results should be based on measured concentrations if the deviation from the nominal concentration is greater than 20 %. - In semi-static procedures, aeration can be used, provided it does not lead to a significant loss of test substance.	- The mortality of the control fish shall not exceed 10 %. - The dissolved oxygen concentration in the test solutions during the test is at least 70 % ASV. - The concentrations of the test substance remain within ± 20 % of the median value throughout the test. - The temperature is in the range of 12.5 - 17.5ºC and does not vary by more than 2ºC.	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
Title	Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages	Water quality - Determination of embryo-larval toxicity to freshwater fish - Semi-static procedure	
Reference	212	12890	
Year	1998	1999	
Test species			
Species	- Zebra fish (*Danio rerio*) - Rainbow trout (*Oncorhynchus mykiss*) - Common carp (*Cyprinus carpio*) - Fathead minnow (*Pimephales promelas*) - Ricefish / Medaka (*Orizias latipes*) - Other species can be used (There is a list of well-documented species).	- Zebra fish (*Danio rerio*) Other freshwater species can be used.	
Test conditions			
Temperature	- Zebra fish: 25 ± 1°C - Rainbow trout: 10±1°C for embryos, 12±1°C for larvae - Common carp: 21-25°C Fathead minnow: 25 ± 2°C - Ricefish: 24 ± 1°C for embryos, 23 ± 2°C for larvae	The testing atmosphere is maintained at 26 ± 2ºC.	
Lighting	- Zebra fish: 12h to 16h light photoperiod - Rainbow trout: 12h to 16h light photoperiod (darkness for larvae until one week after hatching) - Common carp: 12h to 16h light photoperiod - Fathead minnow: 16h light photoperiod - Ricefish: 12h to 16h light photoperiod	12h light/12h dark, 14h light/10h dark or 16h light/8h dark	
Feeding	No food is provided.	No food is provided.	
Vessels	Glass or other chemically inert vessels. The dimension of the vessels should be large enough to allow compliance with the loading rate.	Shallow vessels (ca 100 ml capacity), Petri dish type with an inner diameter of ca 100 mm and equipped with a cover.	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
Test conditions (continued)			
Medium	Any water which conforms to the following characteristics and allows survival of test species in control in compliance with validity criteria. Chemical characteristics of acceptable dilution water: - Particular matter: < 20 mg/l - Total organic carbon: < 2 mg/l - Unionised ammonia: < 1 µg/l - Residual chlorine: < 10 µg/l - Total organophosphorus pesticides: < 50 ng/l - Total organochlorine pesticides + PCBs: < 50 ng/l - Total organic chlorine: < 25 ng/l The pH should remain within a range of ± 0.5 unit. For *Danio rerio*, optimal values for pH and hardness are 7.8 and 250 mg/l expressed as $CaCO_3$ respectively.	Reconstituted water: - 117.6 mg/l $CaCl_2\,2H_2O$, - 49.3 mg/l $MgSO_4\,7H_2O$, - 25.9 mg/l $NaHCO_3$, - 2.3 mg/l KCl. The reconstituted water shall have the following characteristics: - pH: 7.5 ± 0.2 (if necessary it should be adjusted) Dissolved oxygen concentration: 90 to 100 % of air saturation value at 26°C. - Total Hardness: corresponding to (100 ± 10) mg/l expressed as $CaCO_3$.	
Performance of the test			
Test duration	Depends on the species tested: - Zebra fish: 8-10 days - Rainbow trout: 50-55 days - Common carp: 8-9 days - Fathead minnow: 8-9 days - Ricefish / Medaka: 13-16 days	10 days The test may be prolonged up to 14 days.	
Start of the test	The test should start preferably 30 minutes after the eggs have been fertilised. For eggs obtained from a commercial supplier, the test should be initiated within 8 hours after fertilisation.	2 - 4 hours after spawning.	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
Test concentrations	Based on the results of acute toxicity test. Normally five concentrations (spaced by a constant factor not exceeding 3.2). Concentrations of the substance higher than the 96 h LC_{50} or 10 mg/l, whichever is lower, need not be tested.	Based on the results from the acute toxicity test. At least six concentrations forming a geometric progression: the two highest give a significant effect on hatching or on survival and at least the lowest produces no significant effect (e.g., 2, 1, 1/2, 1/4, 1/8 and 1/16 × LC_{50} of the acute lethal toxicity test).	
Number of replicates	At least three replicates for each test concentration and for the control.	Two replicates for each test concentration and four controls.	
Solubilising agent	When a solubilising agent is used, the final concentration should not exceed 0.1 ml/l in the test medium and should be the same in all test vessels.	The concentration of solvent in the final test solution shall not exceed 0.1 ml/l	
Number of eggs and loading	Sufficient number to meet statistical requirements. At least 30 eggs, divided equally between at least three replicate test chambers, should be used per concentration. The loading rate should be low enough in order that a dissolved oxygen concentration of at least 60 % of the air saturation value can be maintained without aeration. For flow-through test, a loading rate not exceeding 0.5 g/l per 24 hours and not exceeding 5 g/l of solution at any time has been recommended.	15 eggs per dish After 24 hours a variable number of the 15 embryos will have died and these eggs become white. Record the number of dead eggs in each dish and reduce the number of viable eggs per dish to maximum of 10 when transferring to new solutions. Determination of median times for hatching and survival shall only be based on these remaining 10 individuals.	
Reference substance	There is no statement about the reference substance.	There is no statement about the reference substance.	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
Data and Reporting			
Frequency of observations	- *Hatching and survival*: at least once daily (It may be desirable to make more frequent observations at the beginning of the test). - *Abnormal appearance and behaviour*: at adequate intervals depending on the duration of the test and the nature of the abnormality. - *Individual weight (dry weight preferably) and individual length*: at the end of the test.	- Hatching and survival: daily (every morning and afternoon on the third and fourth days).	
Frequency of measurements	- *Test concentrations*: • Semi-static test 1) When the concentration is expected to remain within ± 20 % of the nominal: as a minimum, in the highest and lowest concentrations, when freshly prepared and at the time of renewal on at least three occasions spaced evenly during the test. 2) When the concentration is not expected to remain within ± 20 % of the nominal: when freshly prepared and at renewal in all test concentrations. 3) When the concentration is not expected to remain within ± 20 % of the nominal, it is necessary to analyse all test concentrations when freshly prepared and at the time of renewal but following the same regime. • Flow-through test A similar sampling regime as for semi-static tests is appropriate. - *pH*: in all vessels at the beginning and end of each water renewal and at least weekly for flow-trough tests. - *Dissolved oxygen*: in all vessels, three times during	- Dissolved oxygen content, - pH, - Temperature, Measurement should be done with the highest and lowest concentrations first in the new and in the old solutions. If the differences in the values are large between these solutions then all the solutions shall be measured.	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
	the test ; in semi-static tests, preferably at the beginning and end of each water renewal and at least weekly for flow-trough tests. - Total hardness should be measured once in the controls and one vessel at the highest concentration. - Temperature should be measured daily and preferably be monitored continuously in at least one test vessel		
Expression of results	LOEC, NOEC, Data for statistical analysis: - cumulative mortality, - number of healthy larvae at the end of test, - time to start of hatching and end of hatching (i.e. 90 % hatching in each replicate), - number of larvae hatching each day, - length and weight of surviving animals, - number of deformed larvae or of abnormal appearance, - number of larvae exhibiting abnormal behaviour.	NEC, ECx, LOEC, NOEC Data for statistical analysis: - median time for hatching, - survival (eggs, larvae).	

Table 2-7: Fish, Early-life Stage Toxicity Test (OECD TG 212/ISO 12890)

	OECD	ISO	Comments
Data and Reporting (continued)			
Validity	- The dissolved oxygen concentration must be between 60 and 100 % of the air saturation value throughout the test. - The water temperature must not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species. -Overall survival of fertilised eggs in the controls and, where relevant, in the solvent-only controls must be greater than or equal to the limits defined for the selected species (Annexes 3 and 4).	-The concentration of dissolved oxygen in the controls has been maintained between 70 and 110 % of the air-saturation value for dilution water at 26 ºC. - The temperature in the test solutions has been maintained at (26 ± 2)ºC. - The pH in all fresh solutions has been 7.5 ± 0.2. - If the test is prolonged the median time for survival in the controls shall be 12 - 15 days. - More than 70 % of the embryos (eggs) in the controls were alive after 24 hours. - The median times for hatching were 2 days to 4 days in the controls. - The survival of larvae in the controls after ten days was > 90 %. - If the test is prolonged the median time for survival in the controls is 12 days to 16 days.	

Table 2-8: Activated Sludge, Respiration Inhibition Test (OECD TG 209/ISO 8192)

	OECD	ISO	Comments
Name	Activated Sludge, Respiration Inhibition Test	Water quality - Test for inhibition of oxygen consumption by activated sludge (carbon and ammonium oxidation)	The oxygen consumption of activated sludge organisms commonly comprises two main components: hetero-trophic carbonaceaous respiration and auto-trophic nitrification. In the ISO method, the inhibition of the oxygen uptake by all sludge
Reference	209	8192	
Year	1984	2007	
Test species			
Inoculum	Normally activated sludge from a sewage treatment plant, where possible, treating predominantly domestic sewage. If this is not possible, the inoculum may be obtained from a sewage work treating predominantly industrial waste water, but used only after de-adaptation.	Activated sludge should be taken from the exit of the aeration tank of a waste water plant treating predominantly domestic sewage. Depending on the purpose of the test, any type of activated sludge at a suitable suspended solids concentration (e.g. 2 to 4 g/l) can also be used.	
Test conditions			
Temperature	20 ± 2ºC	22 ± 2ºC	
Lighting	No information.	No information.	
Vessels	e.g. 1000 ml beakers.	1000 ml beakers filled with 500 ml of mixture. 250 to 300 ml bottles BOD bottles or Erlenmeyers flasks with stoppers are also possible.	

Table 2-8: Activated Sludge, Respiration Inhibition Test (OECD TG 209/ISO 8192)

	OECD	ISO	Comments
Medium	Synthetic sewage medium (100-fold OECD medium).	Synthetic sewage medium (100-fold OECD medium).	organisms, herotrophic micro-organisms and the oxidation of ammonium salts by nitrifying micro-organisms may be separately expressed from measurement of the rate in the absence and presence of ATU, a specific inhibitor of the oxidation of ammonium to nitrite by first stage nitrifiers.
Performance of the test			
Test duration	30 min and/or 180 min.	Usually 30 min up to 180 min even more (e.g. up to 27 h)	
Concentration of inoculum	1.6 g/l of suspended solids in the test medium.	1.5 g/l of suspended solids in the test medium.	
Range finding test	Optional. There is no special statement about this preliminary test.	At least three concentrations for example 1, 10, 100 mg/l. Ideally, the lowest concentration should have no effect on the oxygen consumption.	

Table 2-8: Activated Sludge, Respiration Inhibition Test (OECD TG 209/ISO 8192)

	OECD	ISO	Comments
Test concentrations	At least five concentrations spaced by a constant factor preferably not exceeding 3.2 (at least three concentrations when reference substance is tested).	At least five concentrations in a logarithmic series.	
Number of replicates	Two controls, no statement for test concentrations.	Two replicates (one with ATU).	
Reference substance	3,5-dichlorophenol.	3,5-dichlorophenol. Alternatively, N-methylaniline can be used as reference chemical, especially for inhibition of nitrification processes.	
Data and Reporting			
Measurements	Oxygen consumption after aeration time of 30 minutes and/or 3 hours.	Oxygen consumption after aeration time of 30 minutes and, if required 3 hours.	
Expression of results	EC_{50}	EC_{50}	
Validity	- The two control respiration rates are within 15 % of each other. - The EC_{50} (3 hours) of 3,5-dichlorophenol is in the accepted range 5 to 30 mg/l.	- *Reference substance*: if the EC_{50} of the reference substance does not lie in the expected range, repeat the test with activated sludge from another source: • 3,5 DCP: 2 to 25 mg/l for total respiration; 5 to 40 mg/l for heterotrophic respiration; 0.1 to 10 for nitrification respiration, • N-methylaniline; 0.1 to 5 mg/l. - The total blank control oxygen uptake rate is higher than 20 mg/g.	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
Name	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	Soil quality - Determination of the effects of pollutants on soil flora Part 2: Effects of chemicals on the emergence and growth of higher plants	ISO11269 contains two parts. Part 1 is a method for the measurement of inhibition of root growth.
Reference	208	11269-2	
Year	2006	2005	
Test plants			
Number of species	The number of species to be tested is not specified in the guideline.	A minimum of two species should be selected at least one from each of the categories (there are two categories).	
Categories of species	Historically most used species and potential non crop species are given in annex (annexes 2 and 3 respectively). The species listed in annex 2 are presented below: *Monocotyledonous:* - Onion (*Allium cepa*), - Oat (*Avena sativa* L.), - Barley (*Hordeum vulgare* L.), - Perennial ryegrass, (*Lolium perenne* L.), - Rice (*Oryza sativa* L.), - Rye (*Secale cereale* L.), - Grain sorghum, Shattercane (*Sorghum bicolor* L.), - Wheat (*Triticum aestivum* L.), - Corn (*Zea mays* L.). *Dicotyledonous*:	*Category 1 (monocotyledonous)*: - Rye (*Secale cereale* L.), - Ryegrass, perennial (*Lolium perenne* L.), - Rice (*Oryza sativa* L.), - Oat (common or winter) (*Avena sativa* L.), - Wheat, soft (*Triticum aestivum* L.), - Barley (spring or winter) (*Hordeum vulgare* L.), - Sorghum, common (or shattercane or durra, white or millet, great) (*Sorghum bicolor* (L.) Moench), - Sweetcorn (*Zea mays* L.). *Category 2 (dicotyledonous)*: - Mustard, white (*Sinapis alba*), - Rape [or rape (summer) or rape (winter)] (*Brassica napus* (L.) ssp. *napus*),	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
	- Carrot (*Daucus carotta*), - Sunflower (*Helianthus annuus*), - Lettuce (*Lactuca sativa*), - White mustard (*Sinapis alba*), - Chinese cabbage (*Brassica campestris var. chinensis*), - Oilseed rape (*Brassica napus*), - Cabbage (*Brassica oleracea var capitata*), - Turnip (*Brassica rapa*), - Garden cress (*Lepidium sativum*), - Radish (*Raphanus sativus*), - Sugar Beet (*Beta vulgaris*), - Cucumber (*Cucumis sativus*), - Soybean (*Glycine max,G. soja*), - Mung bean (*Phaseolus aureus*), - Dwarf bean, French bean, Garden bean (*Phaseolus vulgaris*), - Pea (*Pisum sativum*), - Fenugreek (*Trigonella foenum-graecum*), - Birdsfoot trefoil (*Lotus corniculatus*), - Red clover (*Trifolium pratense*), - Vetch (*Vicia sativa*), - Flax (*Linum usitatissimum*), - Buckwheat (*Fagopyrum esculentum*), - Tomato (*Solanum lycopersicon*).	- Radish, wild (*Raphanus sativus* L), - Turnip, wild (*Brassica rapa* ssp. *rapa* (DC.) Metzg.), - Birdsfoot fenugreek (*Trifolium ornithopodioides),* - Lettuce (*Lactuca sativa* L.), - Cress, garden (*Lepidium sativum* L.), - Tomato (*Lycopersicon esculentum* Miller), - Bean (*Phaseolus aureus* Roxb.).	
Test conditions			
Temperature	22 ± 10°C.	Suitable for maintaining normal growth of each species.	
Lighting	Photoperiod: minimum 16 hours of light. Light intensity: 350 ± 50 µE/m^2/s, wavelength 400 – 700 nm.	16 hours/day.	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
	Additional lighting may be necessary if intensity decreases below 200 μE/m^2/s.		
Soil moisture content	There is no special statement about soil moisture content. Bottom watering of test containers (e.g. by using glass fiber wicks) is recommended.	Daily adjustment of the moisture content of the soil is necessary to maintain a predetermined percentage water holding capacity (e.g. 80 % for *Avena sativa*, 60 % for *Brassica rapa*)	
Humidity	70 ± 25 %.	Suitable for maintaining normal growth of each species.	
Vessels	Containers shall be non-porous plastics or glazed pots. The pots must be large enough to allow normal growth.	Containers shall be non-porous plastics or glazed pots with a top internal diameter between 85 and 95 mm. The pots should be adjusted to the size of the specific test species.	
Soils			
Characteristics	- Sandy loam, loamy sand or sandy clay loam soil that contains up to 1.5 % organic carbon. - Field soils should be sieved to a 2 mm mesh. - Artificial substrates may be used for the testing of chemicals (e.g. acid washed quartz sand, mineral wool and glass beads).	Field soils shall be passed through a sieve of square mesh 4 – 5 mm. The soil shall have the following characteristics: - Carbon content: < 1.5 % (3 % organic matter) - Fine particles content: < 20 % dry mass - pH: 5.0 - 7.5 Sand should be added to bring the organic or fine particle content of natural soil to within the approved limits.	
Performance of the test			
Duration	Usually 14 to 21 days after 50 % of the control seedlings have emerged.	No sooner than 14 days and no later than 21 days after 50 % of the control seedlings have emerged.	
Soil treatment (incorporation of chemicals into the soil)	The test substance can be incorporated into the soil or applied to the soil surface. Substances which are water soluble or suspended in water can be added to water, and then the solution is mixed with soil with an appropriate mixing device. The volume of water added should be same for each test concentration.	Any method ensuring an even distribution of the chemical throughout the soil may be used, excluding the use of surfactants. There are detailed explanations of recommended methods for incorporation in annex as follows: - recommended method for mixing chemicals that are soluble in water with the soil,	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
	Substances with a low water solubility should be dissolved in a suitable volatile solvent (e.g. acetone, ethanol) and mixed with sand. The solvent is removed from sand using a stream of air. For crop plant protection products (CPPP), the substance is sprayed onto the soil surface simulating typical spray tank applications.	- recommended method for mixing chemicals with low solubility in water with the soil. - recommended for mixing chemicals that are soluble in a solvent with the soil, - recommended methods of mixing solid chemicals with the soil - problems due to evaporation.	
Test design	Randomised block design.	Randomised block design.	
Number of replicates	At least four replicates at each test concentration and for the control.	Four replicates at each test concentration and for the control.	
Number of seeds	The number of seeds planted per pot depends upon the species, pot size and test duration. The maximum plant density would be around 3 - 10 seeds per 100 cm^2 depending of the size of the seeds. The total number of seeds should be at least 20 for each test concentration and control.	10 seeds / pot. After the emergence assessment within each pot, the number of seedlings is reduced to give a total of five evenly spaced representative specimens of the plants in the pots.	
Preliminary test	When necessary, a range finding test can be performed. The concentrations/rates should be widely spaced (e.g. 0.1, 1.0, 10, 100, 1000 mg/kg dry soil). For CPPP, the concentrations/rates could be based on the recommended/maximum concentration or application rate (e.g. 1/100, 1/10, 1/1 recommended/maximum concentration or application rate).	A preliminary test is used to find the range of concentrations affecting soil quality: 0.0 (control), 1, 10, 100 and 1000 mg/kg of oven dried soil.	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
Test concentrations	The selected concentrations/rates should encompass the ECx or ERx values that are to be determined. For example, if a EC_{50} value is required, it would be desirable to test at concentrations/rates that produce a 20 to 80 % effect. At least five concentrations/rates in a geometric series (with a factor not exceeding 3) are recommended. Substances need not be tested at concentrations higher than 1000 mg/kg dry soil. A limit test (single concentration/rate) may be performed.	Geometric series (preferably with a factor not exceeding two) to give an ECx estimate or an estimate of the lowest concentration that induces reduced emergence and growth (LOEC). The ECx approach requires at least three test concentrations resulting in partial kill or growth reductions. Substances need not be tested at concentrations higher than 1000 mg/kg of oven-dried soil. A limit test may be performed in order to demonstrate that the LOEC is beyond the limit concentration.	
Reference substance	A reference substance may be tested at regular intervals. No reference substances are recommended for this test. Alternatively, historical biomass or growth measurement of controls could be used to evaluate the performance of the test system.	Sodium trichloroacetate and boric acid are recommended. A reference test should be carried out if any major changes in operating procedures are introduced.	
Data and Reporting			
Measurements	- The number of plants that emerge per replicate and percentage as compared to the controls, - The biomass of surviving plant per replicate (wet weight immediately after harvest or dry weight after oven drying at 60ºC) and percentage as compared to the controls, - The shoot height (if appropriate), - The observation of any visual damage (chlorosis, necrosis, stem deformation...) qualitatively and quantitatively.	- The number of plants that emerge per replicate and mean number, - The total mass of shoots of seedlings per replicate and mean number at harvest (either fresh mass weighted immediately after cutting the shoots or dry mass after oven drying at 70ºC to 80ºC for 16 hours), - The observation of any visual damage (chlorosis, necrosis, stem deformation...).	

Table 2-9: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD TG 208/ISO 11269-2)

	OECD	ISO	Comments
Expression of results	- NOEC/LOEC for seedling emergence, shootweight, visual injury. - ECx or ERx (e.g. 25, 50 %) for seedling emergence, shoot weight, visual injury	- NOEC for growth reduction/emergence, - LOEC for growth reduction/emergence, - ECx (e.g. 10, 50 %) for growth reduction.	
Validity	- The seedling emergence shall be at least 70 % in the controls. - The seedling, in the controls, shall not exhibit visible phytotoxic effects and the plants shall exhibit only normal variation in growth and morphology for that particular species. - The mean survival of emerged control seedlings shall be at least 90 % for the duration of the study. - Environmental conditions for a particular species are identical and growing media shall contain the same amount of soil matrix, support media, or substrate from the same source.	Emergence shall be sufficient to provide seven healthy seedlings per pot in the control.	

Table 2-10: Earthworm, Acute Toxicity Tests (OECD TG 207/ISO 11268-1)

	OECD	ISO	Comments
Name	Earthworm, Acute Toxicity Tests	Soil quality - Effects of pollutants on earthworms (*Eisenia foetida*) Part 1: Determination of acute toxicity using artificial soil substrate	
Reference	207	11268 - 1	
Year	1984	1993	
Test Species			
Species	- *Eisenia foetida* foetida. - *Eisenia foetida* andrei. Where possible *E. foetida* foetida should be used. Other species may be used.	- *Eisenia foetida* foetida. - *Eisenia foetida* andrei.	
Age	Adult (at least two months old with clitellum).	Adult (at least two months old with clitellum).	
Weight	300 - 600 mg (individual wet weight).	300 - 600 mg (individual wet weight).	
Acclimation	24h in an artificial soil.	There is no statement about acclimation.	
Test conditions			
Temperature	20 ± 2ºC.	20 ± 2ºC.	
Lighting	Under continuous light (to ensure that worms remain in the test medium throughout duration of test).	Controlled light-dark cycle: 12h light/12h dark - 16h light/8h dark.	
Light intensity	400 - 800 lux.	400 - 800 lux.	
Feeding	No food is provided.	No food is provided.	
Vessels	Glass containers of approximately one litre (i.e. crystallising dishes or spoutless beakers) covered with glass lids or perforated plastic film.	Glass containers (capacity 1 to 2 litres), non tightly closed, to allow exchanges between the medium and the atmosphere.	
Artificial soil			
Soil quantity per container	750 g (wet soil).	500 g (dry weight).	

Table 2-10: Earthworm, Acute Toxicity Tests (OECD TG 207/ISO 11268-1)

	OECD	ISO	Comments
Artificial soil constitution	- Sphagnum peat : 10 % (as close to pH 5.5 - 6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content), - Kaolin clay: 20 % (kaolinite content preferably above 30 %), - Industrial sand: 70 % (fine sand should be dominant with more than 50 % of the particles between 50 and 200 microns). The pH is adjusted to 6.0 ± 0.5 (by addition of $CaCO_3$).	- Sphagnum peat: 10 % (finely ground and with no visible plant), - Kaolin clay: 20 % (containing not less than 30 % kaolinite), - Industrial sand: 70 % (fine sand should be dominant with more than 50 % of the particles between 50 and 200 microns), The pH is adjusted to 6.0 ± 0.5 (by addition of $CaCO_3$, usually 0.5% of the dry mass of the components).	
Moisture content	About 35 %. The complete mixture should be moist but not so wet that water appears when the artificial soil is compressed. With some peat, a moisture content of over 35 % may be suitable.	40 % - 60 % of the maximum water holding capacity of the artificial soil.	
Performance of the test			
Test duration	14 days.	14 days.	
Number of animals	10 per container.	10 per container.	
Number of replicates	Four replicates at each test concentration and for the control.	Four replicates at each test concentration and for the control.	
Soil treatment (incorporation of chemicals into the soil)	The following methods are recommended to incorporate chemicals into the artificial soil: - An emulsion or dispersion of the test substance in deionised water is mixed with the artificial soil or sprayed over it. - The test substance can be dissolved in a small volume of a suitable organic solvent (e.g.: hexane, acetone, chloroform). The solvent should be allowed to evaporate. - The test substance can be mixed with 10 g of fine ground sand. This mixture is then mixed with artificial soil.	The following methods are recommended to incorporate chemicals into the artificial soil: - *Water soluble chemicals*: the quantity of test substance required to obtain the desired concentration is dissolved in the deionised water used to wet the test substrate or in a part of it. This solution is mixed thoroughly with the test substrate. - *Substances insoluble in water but soluble in organic solvents*: the quantity of test substance required to obtain the desired concentration is dissolved in a volatile solvent (such as acetone or hexane) and mixed it thoroughly with the artificial	

Table 2-10: Earthworm, Acute Toxicity Tests (OECD TG 207/ISO 11268-1)

	OECD	ISO	Comments
		soil. After evaporation of the solvent, the artificial soil sand is mixed with the deionised water. - *Substances insoluble in water and organic solvents*: the quantity of test substance required to obtain the desired concentration is mixed thoroughly with 10 g of fine quartz sand. The sand, the artificial soil and the water are mixed thoroughly.	
Preliminary test	A simple paper contact test is described as an optional screening test in the OECD Guideline. In artificial soil: 0.01, 0.1, 1.0, 10, 100, 1000 mg/kg.	Five concentrations: for example 0.1, 1.0, 10, 100, 1000 mg/kg. Substances are not tested at concentrations higher than 1000 mg/kg.	
Test concentrations	Five concentrations in a geometric series. One concentration resulting in no mortality and one resulting in total mortality should be used.	Five concentrations providing a geometric progression between the highest concentration causing no mortality and the lowest concentration causing total mortality in the preliminary test.	
Reference substance	Chloroacetamide, Occasionally.	Chloroacetamide (expected EC_{50} is 20 - 80 mg/kg).	

Table 2-10: Earthworm, Acute Toxicity Tests (OECD TG 207/ISO 11268-1)

	OECD	ISO	Comments
Data and Reporting			
Frequency of observations	The mortality is assessed after 7 and 14 days.	The mortality is assessed after 7 and 14 days.	
Frequency of measurement	- *Moisture content*: at the beginning and at the end of the test, - *pH*: at the beginning of the test.	- *Moisture content*: at the beginning and at the end of the test, - *pH*: at the beginning of the test, - *Wet weight of the worms*: at the beginning and at the end of the test.	
Expression of results	LC_{50}	- LC_{50} (mortality) - NOEC (mortality or loss in biomass).	
Validity	The mortality in the controls should not exceed 10 % at the end of the test.	- The mortality in the control is < 10 % at the end of the test. - The average loss of biomass of the worms in the control does not exceed 20 % at the end of the test.	

Table 2-11: Earthworm, Reproduction Test (OECD TG 222/ISO 11268-2)

	OECD	ISO	Comments
Name	Earthworm, Reproduction Test (*Eisenia fetida* / *Eisenia andrei*)	Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) Part 2: Determination of effects on reproduction	
Reference	222	11268 - 2	
Year	2004	1998	
Test Species			
Species	- *Eisenia fetida.* - *Eisenia andrei.*	- *Eisenia fetida fetida.* - *Eisenia fetida andrei.*	
Age	Adult worms (between two months and one year old with clitellum). Individuals in a test group should not differ in age by more than four weeks.	Adult worms (between two months and one year old with clitellum). Individuals in a test group should not differ in age by more than four weeks.	
Weight	250 - 600 mg (individual wet weight).	300 - 600 mg (individual wet weight).	
Acclimation	24h in an artificial soil substrate	1 to 7 days in an artificial soil substrate. The same food as the one used during the test is given in a sufficient amount.	
Test conditions			
Temperature	20 ± 2ºC.	20 ± 2ºC.	
Lighting	Controlled light-dark cycle: preferably 16h light / 8h dark.	Controlled light-dark cycle: between 12 h light / 12 h dark and 16 h light / 8 h dark.	
Light intensity	400 - 800 lux in the area of test containers.	400 - 800 lux in the area of test containers.	
Feeding	5 g of food (oatmeal, cow or horse manure) are spread on the soil surface one day after adding the worms and moisten with potable water (about 5 to 6 ml per container). Thereafter, food is provided once a week during the first four weeks. After removal of adults, a further 5 g of food is added to each test container. No further feeding takes place during the remaining 4 weeks of the test.	5 g of food (e.g. cow manure) are spread on the soil surface one day after adding the worms and moisten with potable water (about 5 to 6 ml per container). The same amount of food is added once a week during the first four weeks. After removal of adults, a further 5 g of food is added to each test container. No further feeding takes place during the remaining 4 weeks of the test.	

Table 2-11: Earthworm, Reproduction Test (OECD TG 222/ISO 11268-2)

	OECD	ISO	Comments
Vessels	Containers made of glass or other chemically inert material of about one to two litres capacity. The containers should have a cross-sectional area of approximately 200 cm^2. The design of the container cover should permit gaseous exchange between the substrate and atmosphere and access of light (e.g. perforated transparent cover) whilst preventing the worms from escaping.	Containers of one to two litres capacity with a cross-sectional area of approximately 200 cm^2. The containers shall permit gaseous exchange between the substrate and atmosphere and access of light (e.g. by means of perforated transparent cover). The containers shall have provision to prevent the worms from escaping.	
Artificial soil			
Soil quantity per container	500 to 600 g (dry weight) in order to obtain a moist substrate depth of 5-6 cm	500 to 600 g (dry weight) in order to obtain a moist substrate depth of 5-6 cm	
Artificial soil constitution	- Sphagnum peat : 10 % (as close to pH 5.5 - 6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content), - Kaolin clay: 20 % (kaolinite content preferably above 30 %), - quartz sand: 70 % (fine sand should be dominant with more than 50 % of the particles between 50 and 200 microns), - Calcium carbonate (pulverized, analysis grade): 0.3 to 1.0 % to obtain an initial pH of 6.0 ± 0.5.	- Sphagnum peat : 10 % (air-dried, finely ground, and with no visible plant remains), - Kaolin clay: 20 % (air dried, containing not less than 30 % kaolinite), - industrial quartz sand: 70 % (air-dried, predominantly fine sand with more than 50 % by mass of particle size 50 and 200 microns, dependent on $CaCO_3$ needed), - Calcium carbonate (pulverised, analysis grade): about 0.5 % to bring the pH of wetted substrate to 6.0 ± 0.5 at the start of the test.	
Moisture content	40 to 60 % of the maximum water holding capacity (WHC) of the artificial soil substrate. The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically. Losses are replenished as necessary with deionised water. The water content should not vary by more than 10 % from that at the start of the test.	40 to 60 % of the maximum water holding capacity (WHC) of the artificial soil substrate. The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically and, if necessary, by replenishing lost water. At the end of the test, the water content shall not differ by more than 10 % from that at the beginning of the test.	

Table 2-11: Earthworm, Reproduction Test (OECD TG 222/ISO 11268-2)

	OECD	ISO	Comments
Performance of the test			
Test duration	56 days.	56 days.	
Number of animals	10 worms per container.	10 worms per container.	
Number of replicates	According to the experimental design: - *determination of NOEC*: four replicates for each test concentration and eight controls, - *determination of ECx*: two replicates for each test concentration and six controls, - *combined determination of NOEC and ECx*: four replicates for each test concentration and eight controls.	At least four replicates for each test concentration and control.	
Soil treatment (incorporation of chemicals into the soil)	The following methods are recommended to incorporate chemicals into the artificial soil: - *Water soluble substances*: a solution of the test substance is prepared in deionised water immediately before starting the test, in a quantity sufficient for all replicates of one concentration. A co-solvent may be required to facilitate for the preparation of the test solution. The solution is then mixed thoroughly with the soil substrate. - *Substances insoluble in water but soluble in organic solvents*: the test substance is dissolved in a small volume of suitable organic solvent and then sprayed onto, or mixed into, a small quantity of fine quartz sand. The solvent is then removed by evaporation in a fume hood. The treated sand is then mixed it thoroughly with the pre-moistened artificial soil. Deionised water is then added to reach a final moisture content of 40 to 60 % of maximum WHC. - *Substances insoluble in water and organic solvents*: the quantity of test substance required to obtain the desired concentration is mixed thoroughly with 10 g	The following methods are recommended to incorporate chemicals into the artificial soil: - *Water soluble substances*: the quantity of the test substance is dissolved in the water required for the replicates of one concentration (or that portion of it necessary to wet the soil substrate). The solution is mixed thoroughly with the soil substrate before introducing it into a test container. - *Substances insoluble in water but soluble in organic solvents*: the quantity of test substance required to obtain the desired concentration is dissolved in a volatile solvent (such as acetone or hexane) and mixed with a portion of quartz sand. The solvent is evaporated by placing the container under a fume hood. The treated sand, the remainder of the basic substrate and the water are mixed thoroughly before introducing into the test containers. Ultrasonic dispersion, organic solvents, emulsifiers or dispersants may be used to disperse	

Table 2-11: Earthworm, Reproduction Test (OECD TG 222/ISO 11268-2)

	OECD	ISO	Comments
	of fine quartz sand. The mixture is then mixed it thoroughly with the pre-moistened artificial soil. Deionised water is then added to reach a final moisture content of 40 to 60 % of maximum WHC.	substances with low aqueous solubility. When such auxiliary substances are used, all test concentrations and one additional control should contain the same minimum amount of auxiliary substance.	
Preliminary test	Acute toxicity test (14 days) conducted with, for example, five concentrations: 0.1, 1.0, 10, 100, 1000 mg/kg (dry weight). One replicate for each test concentration and control.	Acute toxicity test (14 days) conducted with four concentrations: 1.0, 10, 100, 1000 mg/kg (dry weight). One replicate (10 worms) for each test concentration and control.	
Test concentrations	According to the experimental design: - *determination of NOEC*: five to twelve concentrations in a geometric series (spaced by a constant factor not exceeding 2.0), - *determination of ECx*: an adequate number of concentrations to cause four statistically different mean responses (the spacing factor may vary: less than or equal to 1.8 in the expected effect range and above 1.8 at the higher and lower concentrations), - *combined determination of NOEC and ECx*: eight concentrations in a geometric series (spaced by a constant factor not exceeding 1.8). A limit test may be performed to demonstrate that the NOEC is greater than the limit concentration (i.e. 1000 mg/kg).	There is no statement about the number of test concentrations. The concentrations should be spaced by a constant factor not exceeding 2.0. Substances do not need to be tested at concentrations higher than 1000 mg/kg of soil substrate (dry weight).	
Reference substance	Carbendazim or benomyl (significant effects in the range 1 to 5 mg active ingredient /kg (dry weight)). At least once a year, or when testing is carried out at a lower frequency, in parallel to the test substance.	Carbendazim (effects on reproduction should be observed in the range 1 to 5 mg active ingredient /kg (dry weight)).	
Data and Reporting			

Table 2-11: Earthworm, Reproduction Test (OECD TG 222/ISO 11268-2)

	OECD	ISO	Comments
Frequency of observations	The living worms are observed, counted and weighted on day 28. At the end of the second 4-week period, the number of juveniles hatched from cocoons and cocoon number are determined. Any unusual behaviour is also recorded.	The living worms are observed, counted and weighted on day 28. At the end of the second 4-week period, the number of juveniles hatched from cocoons are determined. Any unusual behaviour is also recorded.	
Frequency of measurement	*pH and moisture content of the artificial soil substrate*: at the beginning and at the end of the test The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically.	*pH and moisture content of the artificial soil substrate*: at the beginning and at the end of the test The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically.	
Expression of results	- NOEC, - ECx (e.g. 10, 50 %).	- NOEC, - LOEC, - EC 50.	
Validity	- The mortality of adults in the controls over the initial 4 weeks of the test shall be lower than 10 %. - Each control replicate shall have produced at least 30 juveniles at the end of the test. - The coefficient of variation for the reproduction in the control shall not exceed 30 %	- The mortality of adults in the controls is lower than 10 %. - The rate of production of juveniles is at least 30 per control container. - The coefficient of variation for reproduction does not exceed 30 %.	

Table 2-12: Enchytraeid, Reproduction Test (OECD TG 220/ISO 16387)

	OECD	ISO	Comments
Name	Enchytraeid, Reproduction Test	Soil quality - Effects of pollutants on *Enchytraeidae* (*Enchytraeus* sp.) Determination of effects on reproduction and survival	
Reference	220	16387	
Year	2004	2004	
Test Species			
Species	*Enchytraeus albidus* Other species may be used (*E. crypticus*, *E. buchholzi*, *E. luxuriosus*, *E. bulbosus*).	*Enchytraeus albidus* Other species may be used (*E. crypticus*, *E. buchholzi*, *E. luxuriosus*, *E. bulbosus*).	
Age	Adult worms (with eggs in the clitellum region).	Adult worms (with eggs in the clitellum region).	
Weight / Size	Approximately 10 mm.	Approximately 15 mm.	
Acclimation	At least 24h and up to 72 h in untreated artificial soil.	At least 24h in untreated artificial soil.	
Test conditions			
Temperature	20 ± 2ºC.	20 ± 2ºC.	
Lighting	Controlled light-dark cycle: preferably 16h light / 8h dark.	Controlled light-dark cycle: preferably 16h light / 8h dark.	
Light intensity	400 - 800 lux in the area of test containers.	400 - 800 lux in the area of test containers.	
Feeding	50 mg of rolled oats is mixed into the soil surface before adding the worms. Additional feeding (25 mg) is placed on the soil surface weekly during the first three weeks. No further feeding takes place during the remaining 3 weeks of the test.	50 mg of rolled oats is mixed into the soil surface before adding the worms. Additional feeding (25 mg) is placed on the soil surface weekly during the first four weeks. No further feeding takes place during the remaining 2 weeks of the test.	
Vessels	Test vessels made of glass or other chemically inert material. Glass jars (e.g. volume: 0.20 – 0.25 l; diameter ≈ 6 cm) are suitable. The vessels should have transparent lids (e.g. glass or polyethylene) allowing gas exchange between the soil and atmosphere.	Test containers made of glass of 0.20 to 0.25 l capacity with lids. The containers (diameter of 6 cm approximately) should contain an amount of artificial soil corresponding to 20 g (dry mass). Th lids shall permit gaseous exchange between the substrate and atmosphere (glass or perforated plastic film).	

Table 2-12: Enchytraeid, Reproduction Test (OECD TG 220/ISO 16387)

	OECD	ISO	Comments
Artificial soil			
Soil quantity per container	20 g (dry weight).	20 g (dry weight).	
Artificial soil constitution	- Sphagnum peat : 10 % (air-dried and finely ground, 2 ± 1 mm), - Kaolin clay: 20 % (kaolinite content preferably above 30 %), - quartz sand: 70 % approximately (fine sand should be dominant with more than 50 % of the particles between 50 and 200 microns), - Calcium carbonate (pulverised, analysis grade): 0.3 to 1.0 % to obtain an initial pH of 6.0 ± 0.5. Maximum Water Holding Capacity (WHC) and pH are determined on pre-moistened artificial soil (half of the required final water content).	- Sphagnum peat : 10 % (air-dried and finely ground, 2 ± 1 mm), - Kaolin clay: 20 % (kaolinite content preferably above 30 %), - quartz sand: 69 % approximately (fine sand should be dominant with more than 50 % of the particles between 50 and 200 microns), - Calcium carbonate (pulverised, analysis grade): 0.3 to 1.0 % to obtain an initial of pH 6.0 ± 0.5. Maximum Water Holding Capacity (WHC) and pH are determined on pre-moistened artificial soil (half of the required final water content).	
Moisture content	40 to 60 % of the maximum water holding capacity (WHC) of the artificial soil substrate. The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically. The water content should not vary by more than 10 % from that at the start of the test.	40 to 60 % of the maximum water holding capacity (WHC) of the artificial soil. The water content of the soil substrate is maintained throughout the test by re-weighting the containers once a week.	
Performance of the test			
Test duration	42 days.	42 days.	
Number of animals	10 per container.	10 per container.	

Table 2-12: Enchytraeid, Reproduction Test (OECD TG 220/ISO 16387)

	OECD	ISO	Comments
Number of replicates	According to the experimental design: - *determination of NOEC*: four replicates for each test concentration and eight controls, - *determination of ECx*: four replicates and four controls, - *combined determination of NOEC and ECx*: four replicates for each test concentration and eight controls.	According to the experimental design: - *determination of NOEC*: four replicates for each test concentration and eight controls, - *determination of ECx*: two replicates for each test concentration and six controls, - *combined determination of NOEC and ECx*: four replicates for each test concentration and eight controls.	
Soil treatment (incorporation of chemicals into the soil)	The following methods are recommended to incorporate chemicals into the artificial soil: - *Water soluble substances*: a solution of the test substance is prepared in deionised water in a quantity sufficient for all replicates of one concentration. (it is recommended to use to use an appropriate quantity of water to reach the required moisture content). The solution is then mixed thoroughly with the pre-moistened soil substrate. - *Substances insoluble in water but soluble in organic solvents*: the test substance is dissolved in a small volume of suitable organic solvent (e.g. acetone) and then sprayed onto, or mixed into, a small quantity of fine quartz sand (e.g. 2.5 g). The solvent is then removed by evaporation in a fume hood. The treated sand is then added to the pre-moistened artificial soil and thoroughly mixed with the appropriate amount of water to obtain the moisture content required. - *Substances insoluble in water and organic solvents*: the quantity of test substance required to obtain the desired concentration is mixed thoroughly with 2.5 g of fine quartz sand. The mixture is then mixed it thoroughly with the pre-moistened artificial soil after	The following methods are recommended to incorporate chemicals into the artificial soil: - *Water soluble substances*: a solution of the test substance is prepared in deionised water immediately before starting the test, in a quantity sufficient for all replicates of one concentration (it is convenient to use the amount of water necessary to reach the final moisture content). The solution is then mixed thoroughly with the soil substrate. - *Substances insoluble in water but soluble in organic solvents*: the quantity of test substance, required to obtain the desired concentration, is dissolved in a small volume of organic solvent (acetone or hexane) and mixed with a portion of the fine quartz sand required. The solvent is then removed by evaporation in a fume hood. The treated sand is then mixed it thoroughly with the water and the artificial soil. - *Substances insoluble in water and organic solvents*: the quantity of test substance required to obtain the desired concentration is mixed thoroughly with 10 g of fine quartz sand. The mixture is then mixed it thoroughly with the pre-	

Table 2-12: Enchytraeid, Reproduction Test (OECD TG 220/ISO 16387)

	OECD	ISO	Comments
	adding the appropriate amount of water to obtain the moisture content required.	moistened artificial soil and with the amount of deionised water necessary to obtain the final moisture content.	
Preliminary test	Range-finding test conducted at about five concentrations: 0.1, 1.0, 10, 100, 1000 mg/kg (dry weight). Mortality, changes in behaviour and additionally the presence of juveniles are assessed after 14 days. One replicate is performed for each test concentration and control. Substances are not tested at concentrations higher than 1000 mg/kg.	Range-finding test conducted at about five concentrations: 0.1, 1.0, 10, 100, 1000 mg/kg (dry weight). Mortality and additionally the presence of juveniles are assessed after 14 days. One replicate is performed for each test concentration and control Substances are not tested at concentrations higher than 1000 mg/kg.	
Test concentrations	According to the experimental design: - *determination of NOEC*: at least five concentrations in a geometric series (spaced by a constant factor not exceeding 1.8), - *determination of ECx*: at least five concentrations ; the concentrations should bracket the ECx to enable interpolation and not extrapolation (the spacing factor may vary: less than or equal to 1.8 in the expected effect range and above 1.8 at the higher and lower concentrations), - *combined determination of NOEC and ECx*: eight concentrations in a geometric series (spaced by a constant factor not exceeding 1.8). If no effects are observed at the highest concentration in the range-finding test, a limit test may be performed to demonstrate that the NOEC is greater than the limit concentration (i.e. 1000 mg/kg).	According to the experimental design : - *determination of NOEC*: at least five concentrations in a geometric series (spaced by a constant factor not exceeding 2.0), - *determination of ECx*: twelve concentrations (the spacing factor may vary: being smaller at low concentrations; larger at high concentrations. The concentrations shall be spaced by a factor not exceeding 2.0). - *combined determination of NOEC and ECx*: six to eight concentrations in a geometric series (spaced by a constant factor not exceeding 2.0).	
Reference	Carbendazim (DEROSAL^{TM}) (significant effects in	Carbendazim (EC 50 reproduction in the range 1.2	

Table 2-12: Enchytraeid, Reproduction Test (OECD TG 220/ISO 16387)

	OECD	ISO	Comments
substance	the range 1.2 ± 0.8 mg active ingredient /kg (dry weight)). The reference substance should be tested at regular interval and possibly included in each test.	± 0.8 mg/kg (dry weight)). At least once a year, or in parallel to the test substance.	
Data and Reporting			
Frequency of observations	After, 21 days, the adults are removed and counted. Morphological and behaviour changes of the adults worms are noted. At the end of the second 3-week period, the offspring hatched from cocoons are recorded	After, 21 days, the adults are removed and counted. Morphological and behaviour changes of the adults worms are noted. At the end of the second 3-week period, the offspring hatched from cocoons are recorded.	
Frequency of measurement	*pH and moisture content of the artificial soil substrate*: at the beginning and at the end of the test. The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically.	*pH and moisture content of the artificial soil substrate*: at the beginning and at the end of the test. The water content of the soil substrate is maintained throughout the test by re-weighting the containers periodically.	
Expression of results	- NOEC, - ECx.	- NOEC, - ECx.	
Validity	- The mortality of adults in the controls after the first 3 weeks of the test should not exceed 20 %. - Each control replicate should have produced at least 25 juveniles at the end of the test. - The coefficient of variation for reproduction in the control should not exceed 50 %.	- The mortality of adults in the controls after the first 3 weeks of the test should not exceed 20 %. - Each control replicate should have produced at least 25 juveniles at the end of the test. - The coefficient of variation for reproduction in the control should not exceed 50 %.	

Table 2-13: Soil micro-organisms: Nitrogen Transformation Test (OECD TG 216/ISO 14238)

	OECD	ISO	Comments
Name	Soil Microorganisms: Nitrogen Transformation Test	Soil quality – Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	
Reference	216	14238	
Year	2000	1997	
Test conditions			
Temperature	20 ± 2°C	20 ± 2°C.	
Lighting	Dark.	Dark.	
Moisture content	40 - 60 % of the maximum WHC. The moisture content of the soil should be maintained during the test within ± 5°C.	Pore water pressure: about 0.02 Mpa, or 40 - 60 % max. WHC. The moisture content of the soil should be maintained during the test within ± 5°C.	
Vessels	Containers made of inert material. Incubation of soil samples can be performed in two ways: - as bulk samples of each treated soil and control, - or as a series of individual and equally sized sub-samples of each treated soil and control. When volatile substances are tested, sealable and gas tight containers should be used (one quarter of the volume of the container is filled with the soil sample).	Not specified. Incubation of soil samples can be performed in two ways: - as bulk samples of each treated soil and control, - or as a series of individual and equally sized sub-samples of each treated soil and control.	

Table 2-13: Soil micro-organisms: Nitrogen Transformation Test (OECD TG 216/ISO 14238)

	OECD	ISO	Comments
Soil			
Soil characteristics	One single soil is used. The recommended soil should have the following characteristics: - Sand content: 50 - 75 % - pH: 5.5 - 7.5, - Organic carbon content: 0.5 - 1.5 %, - The carbon content of the microbial biomass should be at least 1 % of the total soil organic carbon.	The recommended soil should have the following characteristics: - pH: < 5.0, - Organic carbon content: 0.5 - 1.5 %, - Low clay content.	
Storage	The use of soil freshly collected from the field is preferred. If storage can not be avoided, soil may be stored in the dark at 4 ± 2°C for a maximum of 3 months. During storage, aerobic conditions should be ensured. If soils are collected from areas where they are frozen for at least 3 months per year, storage for 6 months at minus 18 – 22°C can be considered.	The soil may be stored in the dark at 4 ± 2°C for a maximum of 3 months. During storage, aerobic conditions should be maintained.	
Pre-incubation	If the soil is stored (4°C or – 18°C), pre-incubation is recommended for a period between 2 and 28 days	Not specified	
Amendment with organic substrate	The soil should be amended with a suitable organic substrate, e.g. powered lucerne-grass-green meal (main component *Medicago sativa*) with a C/N ratio between 12/1 and 16/1. The recommended ratio is 5g of lucerne per kg of soil (dry weight).	The soil should be amended with a suitable organic substrate: - powered lucerne-grass-green meal with a C/N ratio ≤ 16/1, - pulverised horn, - any other relevant organic nitrogen substrate.	
Soil quantity per container	Not specified.	e.g. 10 - 100 g.	
Performance of the test			
Duration	*For non agrochemicals*: 28 days. *For agrochemicals*: If the rate of nitrate formation in treated and control	28 days	

Table 2-13: Soil micro-organisms: Nitrogen Transformation Test (OECD TG 216/ISO 14238)

	OECD	ISO	Comments
	samples differ by more than 25 % on day 28, the test is continued until a difference equal or less than 25 % is obtained, or for a maximum of 100 days.		
Soil treatment (incorporation of chemicals into the soil)	The test substance is usually applied using a carrier: water or inert solid such as fine quartz sand (0.1 – 0.5 mm). Liquid carriers other than water (e.g. organic solvents such as acetone, chloroform) should be avoided. If sand is used as carrier it can be coated with the test substance dissolved or suspended in an appropriate solvent. The solvent is removed by evaporation before mixing with the soil. A ratio of 10 g of soil per kg of soil (dry weight) is recommended.	The test substance is usually applied using a carrier: - water, - inert solid such as fine quartz sand or test soil. If sand or soil is used as carrier it can be coated with the test substance dissolved or suspended in an appropriate solvent. The solvent is removed by evaporation before mixing with the soil.	
Test concentrations	*For non agrochemicals*: A geometric series of at least five concentrations *For agrochemicals*: At least two concentrations (the lower should reflect at least the maximum amount expected to reach the soil under practical conditions), e.g. the Predicted Environmental Concentration and five time that concentration. If substances, that contain high quantities of nitrogen, are tested at high concentration, appropriate controls (i.e. soil plus test substance without plant meal) must be included in the test.	A geometric series of at least five concentrations.	
Number of replicates	At least 3 replicates for each test concentration and for the control.	At least 3 replicates for each test concentration and for the control.	
Reference substance	Not specified.	Not specified.	

Table 2-13: Soil micro-organisms: Nitrogen Transformation Test (OECD TG 216/ISO 14238)

	OECD	ISO	Comments
Data and Reporting			
Data reporting	Nitrate formation rate.	Nitrate formation rate.	
Frequency of measurement	*For non agrochemicals:* - 0 and day 28, - An intermediate measurement may be performed if necessary, e.g. at day 7, *For agrochemicals*: - 0, 7, 14, 28 days - If the test should be prolonged, further measurements should be made at 14 days interval after day 28.	0 and day 28.	
Expression of results	- *For non-agrochemicals*: ECx (i.e. 10, 25, 50), - *For agrochemicals:* Percentage deviation from control.	ID_{25}, ID_{50}.	
Validity	The variation between control replicates should be less than ± 15 %.	There is no statement about the validity criteria.	

CHAPTER 3: OECD TEST GUIDELINES SECTION 3 - DEGRADATION AND ACCUMULATION

Aerobic biodegradability

Ready Biodegradability Tests in aqueous medium

- DOC Die-Away Test (OECD TG n°301A/ISO 7827): Table 3-1
- CO_2 Evolution Test (OECD TG n°301B/ISO 9349): Table 3-2
- Closed Bottle Test (OECD TG n°301D/ISO 10707): Table 3-3
- Manometric Respirometry Test (OECD TG n°301F/ISO 9408): Table 3-4
- CO_2 in sealed vessels (Headspace Test) (OECD TG n°310/ISO 14539): Table 3-5

Inherent Biodegradability Tests

- Modified SCAS Test (OECD TG n°302A/ISO 9887): Table 3-6
- Zahn-Wellens/EMPA Test (OECD TG n°302B/ISO 9888): Table 3-7

Simulation Tests in aqueous medium

- Simulation Test - Aerobic Sewage Treatment (Activated Sludge units) (OECD TG n°303A/ISO 11733): Table 3-8
- Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD TG n°309/ ISO 14592-1): Table 3-9

Biodegradation in seawater

- Biodegradability in Seawater (OECD TG n°306/ISO 16221): Table 3-10

Anaerobic biodegradability

- Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production (OECD TG n°311/ISO 11734): Table 3-11

Soil Biodegradation Test

- Biodegradability in Soil (OECD TG n°304A/ISO 11266): Table 3-12

Table 3-1: DOC Die-Away Test (OECD TG 301A/ISO 7827)

	OECD	ISO	Comments
Name	DOC Die-Away Test	Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of dissolved organic carbon (DOC)	
Reference	301A	7827	
Year	1992	1994	
Inoculum			
Sources of Inoculum	- Activated sludge collected from the aeration tank of a sewage treatment plant or laboratory-scale unit treating predominantly domestic sewage, - Secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage, - Surface waters (e.g. river, lake), - Soils, - Mixture of these different sources.	- Activated sludge collected from the aeration tank of a treatment plant or laboratory plant dealing with predominantly domestic sewage - Secondary effluent collected from a treatment plant or a laboratory plant dealing with predominantly domestic sewage - Surface water - Mixture of these different sources	
Pre-adaptation	Not applicable	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, Zahn-Wellens), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	Aeration of activated sludge (in mineral medium) or secondary effluent for 5 - 7 days at the test temperature. Pre-conditioning may improve the precision of the methods by reducing blank values.	There is no statement.	
Test system			
Reagents	Analytical grade	Analytical grade	
Medium	Synthetic mineral medium	Synthetic mineral medium	Same mineral

Table 3-1: DOC Die-Away Test (OECD TG 301A/ISO 7827)

	OECD	ISO	Comments
	The concentration of the main elements in the mineral medium are the following (mg/l): - P: 116, - N: 1.3, - Na: 86, - K: 122, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be within the range 7.4 ± 0.2		medium is required in the OECD Test Guideline and the ISO standard.
Vessels	Conical flasks of 0.25 to 2 litres capacity.	Conical flasks of suitable capacity (e.g. 2 litres).	
Reference substance	- Aniline (freshly distilled), - Sodium acetate, - Sodium benzoate.	- Aniline, - Sodium acetate, - Sodium benzoate.	
Test conditions			
Temperature	22 ± 2ºC.	At a temperature of 20 – 25ºC	
Lighting	Dark or diffused light.	Dark or diffused light.	
Performance of the test			
Duration	The test lasts normally for 28 days. Tests may be ended before 28 days: i.e. as soon as the biodegradation curve has reached a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not been reached by day 28, but in such cases, the chemical would not be classified as readily biodegradable.	The test lasts normally for 28 days. If a sufficient degree (> 80 %) and a constant level of degradation is attained, the test may be ended before the end of the test period. The test also can be extended by 1 to 2 weeks, if degradation has obviously started but has not reached a plateau.	
Concentration of test substance	10 - 40 mg DOC/l.	10 - 40 mg DOC/l.	
Concentration of inoculum	The concentration of inoculum in the test flasks should satisfied the following conditions:	The concentration of inoculum in the test flasks should satisfied the following conditions:	

Table 3-1: DOC Die-Away Test (OECD TG 301A/ISO 7827)

	OECD	ISO	Comments
	- Suspended solids: ≤ 30 mg/l. - Secondary effluent from treatment plant: ≤ 100 ml/l. - Number of cells/l: approximately $10^7 – 10^8$.	- sufficient to give a population which offers enough biodegradation activity. - degrades the reference substance by the stipulated percentage. - gives between 10^3 to 10^6 active cells/ml. - gives not more than the equivalent of 30 mg/l suspended solids of activated sludge in the final mixture. - the content of DOC in the inoculum should be less than 10 % of the initial content of DOC introduced by the test substance.	
Number of vessels	In a typical test, the following flasks are used: - 2 flasks containing the test substance and inoculum, - 2 flasks containing only the inoculum (blank), - 1 flask containing the reference substance and the inoculum (procedure control). And preferably and when necessary: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control), - 1 flask containing the test substance, the inoculum and the sterilising agent (adsorption control), - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control).	The test should include: - At least 2 flasks containing the test substance and inoculum, - At least 2 flasks containing only the inoculum, - At least 1 flask containing the reference substance and the inoculum. And when necessary: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control), - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control).	
Data and Reporting			
Data reporting	- DOC, - Specific analysis of the test substance and intermediates (optionally).	- DOC, - Specific analysis of the test substance (optionally).	
Frequency of measurement	DOC: sufficient number of samples are taken to allow the percentage removal in the 10-day window to be assessed, but no precise sampling pattern can be described.	- *DOC*: At the beginning of the test and end of the test and at least at three regular intermediate time intervals. - *Volume of medium*: before each sampling (in	

Table 3-1: DOC Die-Away Test (OECD TG 301A/ISO 7827)

	OECD	ISO	Comments
	However, if samples are preserved, it is recommended to take samples every day or every two days.	order to compensate the possible loss of water).	
Expression of results	- Percentage degradation from DOC removals, - Percentage degradation from specific analysis of the test substance (when appropriate), - Lag phase, - Degradation time, - 10-day window, - Maximum level of degradation.	- Percentage degradation from DOC removals, - Percentage degradation from specific analysis of the test substance (when appropriate), - Lag phase, - Degradation time, - Maximum level of degradation.	
Validity	The test is considered as valid if: - The difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, is less than 20 %, - The percentage degradation of the reference substance has reached the pass level (70 %) by day 14. In addition, when a toxicity control is performed in parallel, the percentage degradation should be higher than 35 % at day 14 (If not , the test substance can be assumed to be inhibitory).	The test is considered as valid if : - in the test flasks with the same test concentration and inoculum, the difference between the extreme degradation values found is less than 20 % DOC removal at the end of the test. - the percentage degradation of the reference substance is more than 70 % at day 14.	

Table 3-2: CO_2 Evolution Test (OECD 301B/ISO 9439)

	OECD	ISO	Comments
Name	CO_2 Evolution Test	Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Carbon dioxide evolution test	
Reference	301B	9439	
Year	1992	1999	
Inoculum			
Sources of Inoculum	- Activated sludge collected from the aeration tank of a sewage treatment plant or laboratory-scale unit treating predominantly domestic sewage, - Secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage, - Surface waters (e.g. river, lake), - Soils, - Mixture of these different sources.	- Activated sludge collected from the aeration tank of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Influent or effluent of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Surface water, - Mixture of these different sources.	
Pre-adaptation	Not applicable.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, Zahn-Wellens), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	Aeration of activated sludge (in mineral medium) or secondary effluent for 5 - 7 days at the test temperature. Pre-conditioning may improve the precision of the methods by reducing blank values.	To reduce the influence of the blank, it may be helpful to precondition the inoculum, e.g. by aerating it, from 1 to 7 days, before use.	
Test systems			
Reagents	Analytical grade.	Analytical grade.	
Medium	Synthetic mineral medium. The concentration of the main elements in the mineral medium are the following (mg/l):	Synthetic mineral medium.	Same mineral medium is required in the OECD Test

Table 3-2: CO_2 Evolution Test (OECD 301B/ISO 9439)

	OECD	ISO	Comments
	- P: 116, - N: 1.3, - Na: 86, - K: 122, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be in the range 7.4 ± 0.2.		Guideline and the ISO standard.
Vessels	Flasks of 2.0 to 5.0 litres capacity.	Glass vessels (e.g. Erlenmeyer flasks or bottles) allowing gas purging and shaking or stirring.	
Reference substance	- Aniline (freshly distilled), - Sodium acetate, - Sodium benzoate.	- Aniline, - Sodium benzoate.	
Test conditions			
Temperature	22 ±2ºC.	20 - 25ºC. Within one test, the variation of temperature shall not exceed ± 2°C.	
Lighting	Dark or diffused light.	Dark or diffused light.	
Performance of the test			
Duration	The test lasts normally for 28 days. Tests may be ended before 28 days: i.e. as soon as the biodegradation curve has reached a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not been reached by day 28, but in such cases, the chemical would not be classified as readily biodegradable.	Usually, the maximum test period 28 days should not exceed 28 days. If a nearly constant level of CO_2 formation is attained (plateau phase) and no further biodegradation is expected, the test is considered to be completed. The test also can be extended by 1 to 2 weeks, if degradation has obviously started but has not reached a plateau.	
Concentration of test substance	10 - 20 mg DOC or TOC/l.	10 - 40 mg/l of organic carbon.	
Concentration of inoculum	The concentration of inoculum in the test flasks should satisfied:	The concentration of inoculum in the test flasks should satisfied the following conditions:	

Table 3-2: CO_2 Evolution Test (OECD 301B/ISO 9439)

	OECD	ISO	Comments
	- Suspended solids: ≤ 30 mg/l. - Secondary effluent from treatment plant: ≤ 100 ml/l. - Number of cells/l: approximately $10^7 – 10^8$.	- sufficient to give a population which offers enough biodegradation activity. - degrades the reference substance by the stipulated percentage. - gives between 10^3 to 10^6 CFU per ml in the final mixture. - gives not more than the equivalent of 30 mg/l suspended solids of activated sludge in the final mixture. - the content of DOC provided by the inoculum should be less than 10 % of the initial content of DOC introduced by the test substance.	
Number of vessels	In a typical test, the following flasks are used: - 2 flasks containing the test substance and inoculum, - 2 flasks containing only the inoculum (blank), - 1 flask containing the reference substance and the inoculum (procedure control). And preferably and when necessary: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control), - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control).	The test should include: - At least 2 flasks containing the test substance and inoculum, - At least 2 flasks containing only the inoculum (blank), - At least 1 flask containing the reference substance and the inoculum (procedure control). And if needed: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control), - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control).	
Data and Reporting			
Data reporting	- CO_2 released, - DOC (optionally), - Specific analysis of test substance (optionally).	- CO_2 released, - DOC (optionally), - Specific analysis of test substance (optionally), - pH.	
Frequency of	*CO_2*:	*CO_2:*	

Table 3-2: CO_2 Evolution Test (OECD 301B/ISO 9439)

	OECD	ISO	Comments
measurement	- During the first ten days, every two or three days, - Until 28th day, at least every five days, so that the 10-day window period can be identified. *DOC and/or specific analysis (optionally):* - 0 and 28th day.	- At regular intervals, depending on the carbon dioxide evolution rate. *DOC*: - At the beginning and the end of the test or at regular intervals during the test period. *pH*: - At the end of the test.	
Expression of results	- Percentage degradation from released CO_2, - Percentage of DOC removal (when appropriate), - Percentage degradation from specific analysis of the test substance (when appropriate), - Lag phase, - Degradation time, - 10-day window, - Maximum level of degradation.	- Percentage degradation from released CO_2 - Percentage of DOC removal (when appropriate) - Percentage degradation from specific analysis of the test substance (when appropriate).	
Validity	The test is considered as valid if: - The IC (Inorganic Carbon) content of the test substance suspension in the mineral medium at the beginning of the test is less than 5 % of the TC (Total carbon). - The percentage degradation of the reference substance has reached the pass levels (60 %) by day 14. - The difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, is less than 20 %. In addition: - When a toxicity control is performed in parallel, the percentage degradation should be higher than 25 % at day 14 (If not , the test substance can be assumed to	The test is considered as valid if: - The amount of DIC (Dissolved Inorganic Carbon) at the beginning of the test is less than 5 % of the organic carbon of the test substance. - The amount of carbon dioxide which has evolved from the inoculum blank at the end of the test is about 40 mg/l and does not exceed 70 mg/l. - The percentage degradation of the reference substance is greater than 60 % on the 14th day. In addition: - If a toxicity control is included, the percentage degradation of the reference substance should be higher than 40 % at the end of the test (If not, the test substance is assumed to be inhibitory), - If the pH value is outside the range 6.0 - 8.5 and	

Table 3-2: CO_2 Evolution Test (OECD 301B/ISO 9439)

	OECD	ISO	Comments
	be inhibitory), - The total CO_2 evolution in the inoculum blank at the end of the test should not normally exceed 40 mg/l medium. If greater values than 70 mg CO_2 /l are obtained, the data and experimental technique should be examined critically.	the percentage degradation of the test substance is less than 60 %, the test should be repeated with a lower concentration of test substance.	

Table 3-3: Closed Bottle Test (OECD 301D/ISO 10707)

	OECD	ISO	Comments
Name	Closed Bottle Test	Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of biochemical oxygen demand (closed bottle test)	
Reference	301D	10707	
Year	1992	1994	
Inoculum			
Sources of Inoculum	- Secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage. - Surface waters (e.g. river, lake).	- Secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage. - Surface water. - Mixtures from these different sources.	
Pre-adaptation	Not applicable.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, Zahn-Wellens), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	If required the inoculum may be pre-conditioned by aerating the secondary effluent without other treatment or addition, for 5 - 7 days at the test temperature.	If the oxygen consumption in the blank bottles without test substance is too high (> 1.5 mg/l at the end of the test), a preconditioning by aeration of the inoculum between 1 and 7 days is recommended. This may help to reduce the oxygen consumption of the microorganisms in the blank.	
Test system			
Reagents	Analytical grade.	Analytical grade.	
Medium	Synthetic mineral medium. The concentration of the main elements in the mineral medium are the following (mg/l):	Synthetic mineral medium.	Same mineral medium is required in the

Table 3-3: Closed Bottle Test (OECD 301D/ISO 10707)

	OECD	ISO	Comments
	- P: 11.6, - N: 0.13, - Na: 8.6, - K: 12.2, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be in the range 7.4 ± 0.2.		OECD Test Guideline and the ISO standard.
Vessels	BOD bottles with glass stoppers (e.g. 100 - 125 ml or 250 - 300 ml capacity).	BOD bottles with glass stoppers of 250-300 ml capacity.	
Reference substance	- Aniline (freshly distilled), - Sodium acetate, - Sodium benzoate.	- Aniline, - Sodium acetate, - Sodium benzoate.	
Test conditions			
Temperature	22 ± 2ºC. Within one test, the temperature should be constant.	20 – 25°C. Within one test, the temperature shall be maintained within ± 1ºC.	
Lighting	Dark.	Dark.	
Performance of the test			
Duration	28 days.	28 days.	
Concentration of test substance	2 - 10 mg/l (usually 2 mg/l). For poorly degradable substances and those with a low ThOD, concentrations within the range 5 - 10 mg/l can be used. In some case, it would be advisable to run parallel series of test substance at two different concentrations (e.g. 2 and 5 mg/l).	Usually 2 mg/l. For poorly degradable substances and those with a low ThOD, concentrations up to 10 mg/l can be used. In some case, it would be advisable to run parallel series of test chemical at two different concentrations (e.g. 2 and 5 mg/l).	
Concentration of inoculum	From one drop (0.05 ml) to 5 ml per litre of medium. The volume should give between 10^4 to 10^6 cells/l in the test vessels.	From one drop (0.05 ml) to 5 ml per litre of medium The concentration of inoculum in the test flasks should satisfied the following conditions: - Sufficient to give a population which offers	

Table 3-3: Closed Bottle Test (OECD 301D/ISO 10707)

	OECD	ISO	Comments
		enough biodegradation activity, - degrades the reference substance by the stipulated percentage, - gives between 10^3 to 10^6 active cells/ml.	
Number of vessels	In a typical test, the following flasks are used: - At least 10 bottles containing the test substance and inoculum (test suspension), - At least 10 bottles containing only the inoculum (inoculum blank), At least 10 bottles containing the reference substance and inoculum (procedure control), And when necessary: - 6 bottles containing the test substance, the reference substance and the inoculum (toxicity control). However, to ensure being able to identify the 10-day window about twice as many bottles would be necessary.	The test should include: - At least 10 bottles containing the test substance and inoculum, - At least 10 bottles containing only the inoculum, At least 10 bottles containing the reference substance and inoculum. And when necessary: -At least 6 bottles containing the test substance, the reference substance and the inoculum.	
Data and Reporting			
Data reporting	- Concentration of dissolved oxygen. - Concentration of nitrite and nitrate (for N-containing test substances).	- Concentration of dissolved oxygen. - Concentration of nitrite and nitrate (for N-containing test substances).	
Frequency of measurements	*Concentration of dissolved oxygen*: - At least weekly ; weekly samples should allow the assessment of percentage removal in a 14-day window, whereas sampling every 3 - 4 days should allow the 10-day window to be identified. *Concentration of nitrite and nitrate (for N-containing test substances):* - Same time schedule as oxygen measurement.	*Concentration of dissolved oxygen*: - At least weekly. *Concentration of nitrite and nitrate (for N-containing test substances):* - At the beginning and at the end of the test.	
Expression of	- Percentage biodegradation (by dividing the specific	Percentage biodegradation by dividing the specific	

Table 3-3: Closed Bottle Test (OECD 301D/ISO 10707)

	OECD	ISO	Comments
results	BOD by the specific ThOD or COD (if the ThOD can not be calculated)), - Lag phase, - Degradation time, - 10-day window, - Maximum level of degradation. For N-containing test substances, calculate the correction for the oxygen consumed by nitrification.	BOD by the specific ThOD or COD (if the ThOD can not be calculated). For N-containing test substances, calculate the the correction for the oxygen consumed by nitrification.	
Validity	The test is considered as valid if: - The oxygen depletion in the inoculum blank does not exceed 1.5 mg dissolved oxygen/l after 28 days, - The residual concentration of oxygen in the test bottles does not fall below 0.5 mg/l at any time, - The difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, is less than 20 %, - The percentage degradation of the reference substance has reached the pass levels (60 %) by day 14. In addition, when a toxicity control is performed in parallel, the percentage degradation should be higher than 25 % at day 14 (If not , the test substance can be assumed to be inhibitory).	The test is considered as valid if: - The oxygen depletion in the inoculum blank control does not exceed 1.5 mg/l after 28 days, - The residual concentration of oxygen in the test bottle does not fall below 0.5 mg/l at any time,. - The difference of extremes of replicate values at the end of the test is less than 20 % (if one of three replicates is outside this range, consider it as an outlier and use the remaining values). - The percentage degradation of the reference substance has reached 60 % after 14 days. In addition, when a toxicity control is performed in parallel, the percentage degradation should be higher than 25 % at day 14 (If not , the test substance can be assumed to be inhibitory).	

Table 3-4: Manometric Respirometry Test (OECD TG 301F/ISO 9408)

	OECD	ISO	Comments
Name	Manometric Respirometry Test	Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer	
Reference	301F	9408	
Year	1992	1999	
Inoculum			
Sources of Inoculum	- Activated sludge from the aeration tank of a sewage treatment plant or laboratory-scale unit treating predominantly domestic sewage, - Sewage effluents from a treatment plant or laboratory-scale unit receiving predominantly domestic sewage, - Surface waters (e.g. river, lake), - Soils, - Mixture of these different sources.	- Activated sludge collected from the aeration tank of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Influent or effluent of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Surface water, - Mixture of these sources.	
Pre-adaptation	Not applicable.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, Zahn-Wellens), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	Aeration of activated sludge (in mineral medium) or secondary effluent for 5 - 7 days at the test temperature. Pre-conditioning may improve the precision of the methods by reducing blank values.	To reduce the influence of the blank, it may be helpful to pre-condition the inoculum, e.g. by aerating it, up to one week, before use.	
Test system			
Reagents	Analytical grade.	Analytical grade.	

Table 3-4: Manometric Respirometry Test (OECD TG 301F/ISO 9408)

	OECD	ISO	Comments
Medium	Synthetic mineral medium. The concentration of the main elements in the mineral medium are the following (mg/l): - P: 116, - N: 1.3, - Na: 86, - K: 122, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be in the range 7.4 ± 0.2.	Synthetic mineral medium.	Same mineral medium is required in the OECD Test Guideline and the ISO standard.
Vessels	There is no information regarding the test vessels.	There is no information regarding the test vessels.	
Reference substance	- Aniline (freshly distilled), - Sodium acetate, - Sodium benzoate.	- Aniline, - Sodium benzoate.	
Carbon dioxide absorber	- Potassium hydroxide solution, - Soda lime pellets, or another absorbent.	- Potassium hydroxide solution (about 10 mol/l) - Soda lime pellets or another absorbent	
Test conditions			
Temperature	22 ± 2ºC. The temperature shall be maintained within ±1ºC throughout the test.	20 – 25°C. The temperature shall be maintained within ±1ºC throughout the test..	
Lighting	There is no information regarding lighting.	Dark or diffuse light.	
Performance of the test			
Duration	The test lasts normally for 28 days. Tests may be ended before 28 days: i.e. as soon as the biodegradation curve has reached a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not	Usually, the maximum test period 28 days should not exceed 28 days. If a nearly constant level of O_2 consumption is attained (plateau phase) and no further biodegradation is expected, the test is considered to be completed.	

Table 3-4: Manometric Respirometry Test (OECD TG 301F/ISO 9408)

	OECD	ISO	Comments
	been reached by day 28; but in such cases, the chemical would not be classified as readily biodegradable.	The test may be extended by 1 to 2 weeks, if degradation has obviously started but has not reached a plateau.	
Concentration of test substance	100 mg test substance/l (giving 50 - 100 mg ThOD/l).	100 mg test substance/l equivalent to at least 100 mg ThOD/l.	
Concentration of inoculum	The concentration of inoculum in the test flasks should satisfied the following conditions: - Suspended solids: ≤ 30 mg/l, - Secondary effluent from treatment plant: ≤ 100 ml/l, - Number of cells/l: approximately $10^7 – 10^8$.	The concentration of inoculum in the test flasks should satisfied the following conditions: - sufficient to give a population which offers enough biodegradation activity, - degrades the reference substance by the stipulated percentage, - gives between 10^3 to 10^6 CFU per ml in the final mixture, - gives not more than the equivalent of 30 mg/l suspended solids of activated sludge in the final mixture, - the content of DOC provided by the inoculum should be less than 10 % of the initial content of DOC introduced by the test substance. Generally, 1 to 10 ml of inoculum are sufficient for 1000 ml of test solution.	
Number of vessels	In a typical test, the following flasks are used: - 2 flasks containing the test substance and inoculum, - 2 flasks containing only the inoculum (blank), - 1 flask containing the reference substance and the inoculum (procedure control), and preferably and when necessary: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control), - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control).	The test should include: - At least 2 flasks containing the test substance and inoculum, - At least 2 flasks containing only the inoculum (blank), - At least 1 flask containing the reference substance and the inoculum (procedure control). And if needed: - 1 flask containing the test substance, the	

Table 3-4: Manometric Respirometry Test (OECD TG 301F/ISO 9408)

	OECD	ISO	Comments
		reference substance and the inoculum (toxicity control), - 1 flask containing the test substance and the sterilising agent (abiotic sterile control).	
Data and Reporting			
Data reporting	- Concentration of dissolved oxygen, - DOC (optional), - Specific analysis of test substance (optional), - Concentration of nitrite and nitrate (for N-containing test substances), - pH.	- Concentration of dissolved oxygen, - DOC (optional), - Specific analysis of test substance (optional), - Concentration of nitrite and nitrate (for N-containing test substances), - pH.	
Frequency of measurement	*Concentration of dissolved oxygen*: - daily measurement for non automatic respirometer, *DOC, and/or specific analysis (optionally):* - 0 and 28th day, *Concentration of nitrite and nitrate (for N-containing test substances):* - 0 and 28th day, *pH*: -28th day.	*Concentration of dissolved oxygen*: - no specific requirement for non automatic respirometer, *DOC, and/or specific analysis (optionally):* - 0 and 28th day, *Concentration of nitrite and nitrate (for N-containing test substances):* - 0 and 28th day, *pH*: -28th day.	
Expression of results	- Calculate the percentage biodegradation by dividing the specific BOD by the specific ThOD or COD (if the ThOD can not be calculated). - When optional determinations of specific chemical and/or DOC are made, calculate the percentage degradation. - Lag phase, - Degradation time,	Calculate the percentage biodegradation by dividing the specific BOD by the specific ThOD or COD (if the ThOD can not be calculated). When optional determinations of specific chemical and/or DOC are made, calculate the percentage degradation. For N-containing test substances, calculate the correction for the oxygen consumed by	

Table 3-4: Manometric Respirometry Test (OECD TG 301F/ISO 9408)

	OECD	ISO	Comments
	- 10-day window, - Maximum level of degradation, - For N-containing test substances, calculate the correction for the oxygen consumed by nitrification.	nitrification.	
Validity	The test is considered as valid if: - The oxygen uptake of the inoculum blank is normally 20 - 30 mg O_2/l and not be greater than 60 mg/l in 28 days (values higher than 60 mg/l require critical examination of the data and experimental technique), - The difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, is less than 20 %, - The percentage degradation of the reference substance has reached the pass levels (60 %) by day 14. In addition: - when a toxicity control is performed in parallel, the percentage degradation should be higher than 25 % at day 14 (If not , the test substance can be assumed to be inhibitory), - If the pH value is outside the range 6.0 - 8.5 and the oxygen consumption by the test substance is less than 60 %, the test should be repeated with a lower concentration of test substance.	The test is considered as valid if: - The amount of BOD in the inoculum blank is normally 20 to 30 mg O_2/l and not be greater than 60 mg/l at the end of the test, - The percentage degradation of the reference substance is greater than 60 % on the 14^{th} day. In addition: - When a toxicity control is performed in parallel, the percentage degradation should be higher than 40 % at the end of the test (If not , the test substance can be assumed to be inhibitory), - If the pH value at the end of the test is outside the range 6.0 - 8.5 and if the percentage degradation of the test substance is less than 60 %, it is advisable to repeat the test with a lower concentration of the test substance.	

Table 3-5: CO_2 in Sealed Vessels – Headspace Test (OECD TG 310/ISO 14593)

	OECD	ISO	Comments
Name	Ready biodegradability - CO_2 in sealed vessels (Headspace Test)	Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)	
Reference	310	14593	
Year	2006	1999	
Inoculum			
Sources of Inoculum	- Activated sludge collected from the aeration tank of a sewage treatment plant or laboratory-scale unit treating predominantly domestic sewage, - Secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage, - Surface waters, - Soils.	- Activated sludge collected from the aeration tank of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Influent or effluent of a full-scale or a laboratory waste water treatment plant dealing with predominantly domestic sewage, - Surface water.	
Pre-adaptation	Not applicable.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, Zahn-Wellens), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	Aeration of activated sludge (in mineral medium, concentration 30 mg/l) for 5 - 7 days at the test temperature. Pre-conditioning may improve the precision of the methods by reducing blank values.	To reduce the influence of the blank, it may be helpful to precondition the inoculum from activated sludge plant, e.g. by aerating it up to one week, before use.	
Test systems			
Reagents	Analytical grade.	Analytical grade.	
Medium	Synthetic mineral medium. The concentration of the main elements in the	Synthetic mineral medium.	Same mineral medium is

Table 3-5: CO_2 in Sealed Vessels – Headspace Test (OECD TG 310/ISO 14593)

	OECD	ISO	Comments
	mineral medium are the following (mg/l): - P: 116, - N: 1.3, - Na: 86, - K: 122, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be in the range 7.4 ±0.2		required in the OECD Test Guideline and the ISO standard. The medium is identical to the one used in DOC Die away test, CO_2 evolution test, manometric respirometry.
Vessels	Glass serum bottles sealed with butyl rubber stoppers and crimp-on aluminium seals (recommended size "125 ml" corresponding to a total volume of around 160 ml).	Gas-tight glass vessels (e.g. serum bottles of 160 ml capacity, sealed with butyl rubber septa and aluminium scrimp seals or any other gas-tight system). A headspace to liquid ration of 1:2 is usual.	
Reference substance	- Aniline, - Sodium benzoate, - ethylene glycol, - 1-octanol (when testing poorly soluble substances).	- Aniline, - Sodium benzoate.	
Test conditions			
Temperature	20 ± 1ºC.	20 - 25ºC. The variation of temperature shall not exceed ± 2°C throughout the test.	
Lighting	Dark.	Dark or diffused light.	
Performance of the test			
Duration	The test lasts normally for 28 days. Tests may be ended before 28 days: i.e. as soon as the biodegradation curve has reached a plateau.	The test lasts normally for 28 days but can be prolonged if degradation has started. The test may be finished before 28 days, if biodegradation has reached a plateau.	
Concentration of	2 - 40 mg C/l (usually 20 mg C/l).	2 - 40 mg OC/l (usually 20 mg OC/l).	

Table 3-5: CO_2 in Sealed Vessels – Headspace Test (OECD TG 310/ISO 14593)

	OECD	ISO	Comments
test substance			
Concentration of inoculum	The concentration of inoculum in the test flasks should satisfied the following conditions: - sufficient to give adequate biodegradative activity, - degrades the reference substance by the stipulated percentage, - gives between 10^2 to 10^5 CFU per ml in the final mixture, - normally gives a concentration of 4 mg/l suspended solids in the final mixture when activated sludge is used (concentrations up to 30 mg/l may be used, but may significantly increase the CO_2 production of the blanks), - contributes less than 10 % of the initial content of DOC introduced by the test substance, - is generally 1 - 10 ml of inoculum for 1 litre of test solution.	The concentration of inoculum in the test flasks should satisfied the following conditions: - sufficient to give a population which offers enough biodegradation activity, - degrades the reference substance by the stipulated percentage, - gives between 10^2 to 10^5 CFU per ml in the final mixture, - normally gives a concentration of 4 mg/l suspended solids in the final mixture when activated sludge is used (concentrations up to 30 mg/l are generally possible, but may influence significantly the CO_2 production of the blanks and are therefore nor recommended), - the quantity of DOC provided by the inoculum should be less than 10 % of the initial content of DOC introduced by the test substance, - generally 1 to 10 ml of inoculum are sufficient for 1 litre of test solution.	
Number of vessels	In a typical test, the following set of bottles are used: - test vessels containing the test substance and inoculum, - blank controls containing only the inoculum and any chemicals, solvent, agents or glass fiber filters used to introduce the test substance into the test vessels, - procedure control vessels containing the reference substance and the inoculum. And if needed: - vessels containing the inoculum, the test substance, and the reference substance at the same	The test should include: - test vessels containing the test substance and inoculum, - blank controls containing only the inoculum, - procedure control vessels containing the reference substance and the inoculum. And if needed: - vessels containing the inoculum, the test substance, and the reference substance for checking the possible inhibitory effect of the test substance,	

Table 3-5: CO_2 in Sealed Vessels – Headspace Test (OECD TG 310/ISO 14593)

	OECD	ISO	Comments
	concentrations for checking the possible inhibitory effect of the test substance, - vessels for checking the possible abiotic degradation containing the test substance without inoculum and a sterilising agent ($HgCl_2$), or sterilised by other means (e.g. by autoclaving). It is recommended to analyse 3 replicate bottles at each time interval during the test and at least 5 replicates at the end of the test.	- vessels for checking the possible abiotic degradation containing the test substance without inoculum sterilised by autoclaving or addition of a suitable inorganic toxic substance (e.g. $HgCl_2$).	
Data and Reporting			
Data reporting	- TIC (total inorganic carbon) concentration, - DOC (optionally), - Specific analysis of the test substance (optionally), - pH (optionally, a value less than 6.5 could indicate that nitrification has occurred).	- TIC concentration, - DOC (optionally), - Specific analysis of the test substance (optionally).	
Frequency of measurement	*TIC*: - At least weekly or more frequently (e.g. twice per week) if a complete degradation curve is required. *DOC and/or specific analysis (optionally):* - Not specified. *pH*: - Not specified.	*TIC*: - At least weekly or more frequently if a complete degradation curve is required. *DOC and/or specific analysis (optionally):* - At the beginning and the end of the test.	
Expression of results	- Percentage degradation from IC increase, - Percentage of DOC removal (when appropriate), - Percentage degradation from specific analysis of the test substance (when appropriate). - Lag phase, - 10d-window, - Biodegradation phase, - Plateau phase.	- Percentage degradation from TIC increase - Percentage of DOC removal (when appropriate) - Percentage degradation from specific analysis of the test substance (when appropriate). - Lag phase, - Biodegradation phase, - maximum level of biodegradation, - Plateau phase.	

Table 3-5: CO_2 in Sealed Vessels – Headspace Test (OECD TG 310/ISO 14593)

	OECD	ISO	Comments
Validity	The test is considered as valid if: - The mean percentage degradation of the reference substance is higher than 60 % by the 14th day of incubation, - The mean amount of TIC in the control (blank control) is higher than 3 mg/l at the end of the test.	The test is considered as valid if: - The mean percentage degradation of the reference substance is higher than 60 % on the 14th day of incubation, - The mean amount of TIC in the control (blank control), at the end of the test, is lower or equal to 15 % of the organic carbon added initially as the test substance.	

Table 3-6: Modified SCAS Test (OECD TG 302A/ISO 9887)

	OECD	ISO	Comments
Name	Inherent Biodegradability: Modified SCAS Test	Water quality - Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Semi-continuous activated sludge method (SCAS)	
Reference	302A	9887	
Year	1981	1992	
Inoculum			
Sources of Inoculum	Activated sludge collected from a sewage treatment plant treating predominantly domestic sewage.	- Activated sludge collected from a waste water treatment plant, Depending on the purpose of the test, the waste water treatment plant should receive predominantly domestic waste water. - Activated sludge may also be taken from laboratory treatment plant. In order to get many different species and bacterial strains, it may be possible to make a mixture from different sources.	Since the conditions provided by the test are highly favourable to the selection and/or adaptation of micro-organisms capable of degrading the test substance, the procedure may also be used to produce acclimatised inocula for use in other tests.
Pre-adaptation	There is no statement about pre-adaptation.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (this test, Zahn-Wellens test), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	Normally up to two weeks (until DOC in the supernatant liquor at the end of each aeration cycle is less than 12 mg/l).	Normally up to two weeks (the degree of DOC elimination is usually about 80 %. When synthetic sewage is used, more than 90 % of the DOC is removed).	
Materials			
Reagents	No specific reagents.	Not specified.	

Table 3-6: Modified SCAS Test (OECD TG 302A/ISO 9887)

	OECD	ISO	Comments
Medium	Settled domestic sewage.	- Domestic sewage, - Synthetic sewage. After dilution, the concentration of the main elements in the synthetic sewage are the following (mg/l): C: 105, N: 46, P: 5. The pH is in the range: 7.0 - 7.5.	
Vessels	There is no statement about test vessels.	SCAS unit of 250 to 300 ml capacity filled with 150 ml.	
Reference substance	No specific substance is recommended, but results of OECD/EEC ring test are provided	No specific substance is recommended, but results of ring test are provided.	
Test conditions			
Temperature	Not specified.	20 - 25ºC.	
Lighting	Not specified.	Dark or diffused light.	
Performance of the test			
Duration	Not specified At least 12 weeks (when the substances showing little or no biodegradation)	Not specified But based on knowledge, between 12 and 26 weeks	
Concentration of test substance	20 mg/l DOC.	20 mg/l DOC. If the test substance is not toxic, higher concentrations (e.g. 50 mg/l) may used.	
Concentration of inoculum	There is no information regarding the concentration of suspended solids.	1 to 4 g/l of suspended solids.	
Effective detention period	36 h	36 h	
Data and Reporting			
Data reporting	DOC (in supernatant liquors).	- DOC, - Specific analysis of the test substance (optionally, if primary biodegradation is to be	

Table 3-6: Modified SCAS Test (OECD TG 302A/ISO 9887)

	OECD	ISO	Comments
		followed).	
Frequency of DOC measurements	Daily. Less frequent analysis is allowed.	Daily, if the value is changing significantly. Otherwise, less frequently (when no biodegradation is observed, two or three times weekly).	
Expression of results	Percentage biodegradation from DOC removal.	Percentage removal of DOC.	
Validity	There is no statement regarding the validity of the test.	There is no statement regarding the validity of the test.	

Table 3-7: Zahn-Wellens/EMPA Test (OECD TG 302B/ISO 9888)

	OECD	ISO	Comments
Name	Zahn-Wellens/EMPA Test	Water quality - Evaluation of the ultimate aerobic biodegradability of organic compounds in aqueous medium - Static test (Zahn-Wellens method)	
Reference	302B	9888	
Year	1992	1999	
Inoculum			
Sources of inoculum	Activated sludge from a sewage treatment works (BOD_5 of effluent should be < 25 mg/l). In special cases, it may be possible to make a mixture from different sources (e.g. other treatment plants, soil extracts, river water, etc.), to get as many different species and bacterial strains as possible.	Activated sludge from the aeration tank of a biological waste water treatment plant Depending on the purpose of the test, the waste water treatment plant should receive predominantly domestic waste water. Activated sludge may also be taken from laboratory treatment plant. In order to get many different species and bacterial strains, it may be possible to make a mixture from different sources.	
Pre-adaptation	Pre-adapted inoculum may be used (new exposition of the same activated sludge to the test substance).	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests (SCAS, this test), from treatment plants dealing with similar substances or from contaminated areas (This should be clearly stated in the test report).	
Pre-conditioning	-	-	
Materials			
Reagent	Analytical grade.	Analytical grade.	
Medium	Synthetic mineral medium The concentration of the main elements in the mineral medium are the following (mg/l): - P: 116, - N: 1.3,	Synthetic mineral medium	Same mineral medium is required in the OECD Test Guideline and

Table 3-7: Zahn-Wellens/EMPA Test (OECD TG 302B/ISO 9888)

	OECD	ISO	Comments
	- Na: 86, - K: 122, - Mg: 2.2, - Ca: 9.9, - Fe: 0.05 – 0.10. The pH should be in the range 7.4 ± 0.2.		the ISO standard. The medium is identical to the one used in DOC Die away test, CO_2 evolution test and manometric respirometry.
Vessels	Cylindrical glass vessels of 1 to 5 litres capacity equipped with a stirrer of inert material.	Glass vessels of 1 to 5 litres capacity equipped with agitators with glass or metal stirrers.	
Reference substance	- Ethylene glycol, - Diethylene glycol, - Aniline, - Lauryl sulfonate.	- Ethylene glycol, - Diethylene glycol, - Aniline, - Sodium benzoate.	
Test conditions			
Temperature	20 - 25ºC	20 - 25ºC. Within one test, the temperature shall be maintained within ±1ºC.	
Lighting	Dark or diffuse light.	Dark or diffuse light.	
Performance of the test			
Duration	The test lasts normally for 28 days. The test may be ended before 28 days: i.e. as soon as the biodegradation curve has reached a plateau. The test may be extended if adaptation occurs in the final days of the test period.	Usually, the maximum test period should not exceed 28 days. If a sufficient level of DOC removal is attained (plateau phase) and no further elimination is expected, the test is considered to be completed. The test may be extended by 1 to 2 weeks, if degradation has obviously started but has not reached a plateau.	
Concentration of test substance	DOC: 50 mg/l - 400 mg/l. COD: 100 mg/l - 1000 mg/l.	DOC: 50 - 400 mg/l. COD: 100 - 1000 mg/l.	

Table 3-7: Zahn-Wellens/EMPA Test (OECD TG 302B/ISO 9888)

	OECD	ISO	Comments
Concentration of inoculum	Sludge concentration: - between 0.2 and 1.0 g/l in the final volume. The ratio between inoculum and test substance (as DOC) should be between 2.5:1 and 4:1.	Sludge concentration: - For 50 mg/l DOC: 0.2 g/l of suspended solids in final mixture, - For 400 mg/l DOC, 1.0 g/l of suspended solids in the final mixture, - For concentrations > 50 and < 400 mg/l : within the range 0.2 – 1.0 g/l.	
Number of vessels	In a typical test, the following flasks are used: - 1 or 2 vessel(s) containing the test substance and the inoculum, - 1 or 2 vessel(s) containing only the inoculum (blank), - 1 vessel containing the reference substance and the inoculum (procedure control), And if needed: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control).	The test should include: - At least 1 flask containing the test substance and inoculum, - At least 1 flask containing only the inoculum (blank), - At least 1 flask containing the reference substance and the inoculum (procedure control), And if needed: - 1 flask containing the test substance and the sterilising agent (abiotic sterile control).	
Data and Reporting			
Data reporting	- DOC or COD (in duplicate), - Specific analysis of test substance (optionally, if primary biodegradation is to be followed), - pH.	- DOC or COD (at least in duplicate), - Specific analysis of test substance (optionally, if primary biodegradation is to be followed), - pH.	
Frequency of measurements	*DOC or COD*: - (3 ± 0.5) h after addition of the test substance (adsorption of test substance on activated sludge), - on at least 4 occasions in the interval between the 1st and 27th day, - on the 27th and 28th days, (or if the plateau is attained in less than 28 days, on the last two days of the test). Additional sampling may be necessary in order to	*DOC or COD:* - (3 ± 0.5) h after starting the test (adsorption of test substance on activated sludge), - on at least three intermediate time intervals (e.g. 7th, 14th and 21st day), - on two consecutive days at the end of the test (normally 27th and 28th day). *Specific analysis of test substance*: - No specific statement,	

Table 3-7: Zahn-Wellens/EMPA Test (OECD TG 302B/ISO 9888)

	OECD	ISO	Comments
	describe the reaching of the plateau or if adaptation is to be followed. *pH*: - At regular intervals (e.g. on each day of sampling; the pH is adjusted to pH 6.5 – 8.0 (if necessary)).	*pH:* - At regular intervals (e.g. on each day of sampling; the pH is adjusted to pH 7.0 ± 0.5 if necessary).	
Expression of results	- Percentage degradation from DOC or COD removal, - Percentage primary degradation of the test substance (optional), - Adaptation phase, - Biodegradation phase.	- Percentage degradation or elimination (if case of high adsorption) from DOC removals, - Percentage degradation from specific analyses of the test substance (when appropriate), - Lag phase, - Degradation time, - Maximum level of degradation.	
Validity	The test is valid if: - the removal of the reference substance (procedure control) is at least 70 % within 14 days, - the removal of DOC (or COD) in the test suspension takes place relatively gradually over days or weeks.	The test is valid if the percentage degradation of the reference substance (procedure control) is greater than 70 % on the 14^{th} day.	

Table 3-8: Simulation Test - Aerobic Sewage Treatment (Activated Sludge units) (OECD TG 303A/ISO 11733)

	OECD	ISO	Comments
Name	Simulation Test - Aerobic Sewage Treatment - Activated Sludge Units	Water quality - Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium - Activated sludge simulation test	
Reference	303A	11733	
Year	2001	2004	
Inoculum			
Sources of inoculum	- Activated sludge from aeration tank of a well operated waste water treatment plant or laboratory-scale activated sludge unit receiving predominantly domestic sewage, - Effluent from a domestic biological waste water treatment plant. In order to get as many different species and bacterial strains as possible, it may be helpful to add inocula from different sources (e.g. surface water).	- Activated sludge from the aeration tank of an efficiently operated biological waste water treatment plant or from laboratory treatment plant receiving predominantly domestic sewage, - Effluent from a domestic biological waste water treatment plant. To get as many different species and bacterial strains as possible, it may be helpful to add inocula from different sources.	
Test system			
Reagents	Grade not specified.	Analytical grade.	
Medium	- Domestic sewage (fresh settled sewage collected daily from treatment plant receiving domestic sewage), - Synthetic sewage (e.g. peptone, meat extract, urea, K_2HPO_4, NaCl, $CaCl_2$, $MgSO_4$). This synthetic sewage gives a mean DOC concentration in the influent of 100 mg/l, - Mixture of domestic and synthetic sewage.	- Synthetic sewage, 3 synthetic sewage are given in the standard (from a mean DOC of 100 mg/l in the influent to 180 mg/l). - Domestic sewage, - Mixture of synthetic and domestic sewage.	The composition of 1st synthetic sewage is similar to the one given in the OECD test guideline.
Vessels	- Activated sludge plant model (Hussman unit), The aeration vessel should have a capacity of about 3	- Activated sludge plant model (Hussman unit), The aeration vessel should have a capacity of	

Table 3-8: Simulation Test - Aerobic Sewage Treatment (Activated Sludge units) (OECD TG 303A/ISO 11733)

	OECD	ISO	Comments
	litres, the secondary clarifier a capacity of about 1.5 litres, - Porous pot unit.	about 3 litres, the secondary clarifier a capacity of about 1.5 litres, - Porous pot unit.	
Reference substance	e.g.: - Adipic acid, - 2-phenyl phenol, - 1-naphthol, - diphenic acid, - 1-naphthoic acid.	Not specified.	
Test conditions			
Temperature	20 - 25ºC.	20 - 25ºC.	
Lighting	Not specified.	Dark or diffused light.	
Performance of the test			
Duration	Normally, less than 12 weeks after addition of test substance. Once the units are operating efficiently, allow from 1 week to a maximum of 6 weeks after introduction of the test substance for adaptation to reach a steady state. Then, obtain at least 15 values in the plateau phase, normally lasting 3 weeks (one measure each weekday over 3 weeks), for the evaluation of the test results.	Normally, less than 12 weeks after addition of test substance. Once the units are operating efficiently, allow from 1 week to a maximum of 6 weeks after introduction of the test substance for adaptation to reach a steady state. Then, obtain at least 15 values in the plateau phase, normally lasting 3 weeks (one measure each weekday over 3 weeks), for the evaluation of the test results.	
Concentration of test substance	DOC: normally between 10 and 20 mg/l in the influent (upper limit 50 mg/l). The concentration may be reduced to 5 mg/l DOC or less if toxic effects are likely to occur (only if suitable specific analytical method is available).	DOC: normally between 10 and 20 mg/l in the influent (upper limit 50 mg/l). If toxic effects are likely to occur, the concentration may be reduced but not less than 5 mg/l DOC for analytical reasons. Lower tests concentrations may be used if the primary degradation is determined using specific analysis.	
Concentration of activated sludge	Concentration of dry matter is about 2.5 g/l.	Concentration of dry matter is about 2.5 g/l.	

Table 3-8: Simulation Test - Aerobic Sewage Treatment (Activated Sludge units) (OECD TG 303A/ISO 11733)

	OECD	ISO	Comments
Number of vessels	In a typical test, the following units are used: - At least 1 unit containing the test substance and the inoculum, - At least 1 unit containing only the inoculum (control), and, if needed: - 1 unit containing the reference substance and the inoculum (procedure control).	The following units are used: - 1 unit containing the test substance and the inoculum, - 1 unit containing only the inoculum (control).	
Data and Reporting			
Data reporting	- DOC or COD (influent and effluent), - Specific analysis of test substance (optionally, if primary biodegradation is to be followed), - Suspended solids, - Dissolved oxygen concentration, - Temperature, - pH.	- DOC or COD (influent and effluent) - Specific analysis of test substance (optionally, if primary biodegradation is to be followed), - Suspended solids, - Dissolved oxygen concentration, - Temperature, - pH	
Frequency of measurements	*DOC or COD:* - Daily (24 hours composite samples), *Specific analysis of the test substance:* - - Daily (24 hours composite samples), *Suspended solids:* - At least weekly (discard surplus sludge to maintain the concentration in the range 1 – 3 g/l), *pH, temperature, dissolved oxygen concentration:* . - at regular intervals (between daily and weekly).	*DOC or COD:* - Daily (24 hours composite samples), *Specific analysis of the test substance:* - - Daily (24 hours composite samples), *Suspended solids:* - At least weekly (discard surplus sludge to maintain the concentration in the range 1 – 3 g/l), *pH, temperature, dissolved oxygen concentration:* - at regular intervals (between daily and weekly).	
Expression of results	- Percentage elimination of test substance from DOC or COD removal. - Percentage primary degradation of the test substance (optional) - Lag phase, - Plateau phase.	- Percentage elimination of test substance from DOC or COD removal. - Percentage primary degradation of the test substance (optional) - Lag phase, - Plateau phase.	

Table 3-8: Simulation Test - Aerobic Sewage Treatment (Activated Sludge units) (OECD TG 303A/ISO 11733)

	OECD	ISO	Comments
Validity	The test is valid if: - the degree of DOC or COD elimination in the control unit is higher than 80 % after 2 weeks and no unusual observation have been made. In addition, - if a toxicity control is performed in parallel, the percentage degradation should be higher than 90 %. - if the test is performed under nitrifying conditions, the mean concentration in the effluents should be lower than 1 mg/l ammonia-N and 2 mg/l nitrite-N.	The degree of DOC or COD degradation in the control units should be higher than 80 % after 2 weeks and no unusual observations have been made. If the test is performed under nitrifying conditions, the mean concentration in the effluent should be lower than 1 mg/l ammonia and 2 mg/l nitrite.	

Table 3-9: Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD 309/ISO 14592-1)

	OECD	ISO	Comments
Name	Aerobic Mineralisation in Surface Water – Simulation biodegradation test	Water quality – Evaluation of the aerobic biodegradability of organic compounds at low concentrations – Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions	
Reference	309	14592-1	
Year	2004	2002	
Presentation of the document	This simulation test is a laboratory shake flask test to determine rates of aerobic biodegradation of organic substances in samples of natural surface water (fresh, brackish or marine). The concentrations of the test substance should represent the expected range of concentrations in the environment. Both radio-labelled or non-labelled test substances can be used in the test. The test is performed in batch by incubating the test substance with either surface water only ("pelagic test") or surface water amended with suspended solids/sediments ("suspended sediment test"). For recalcitrant substances, a prolonged incubation for up to several months may be required in order to achieve sufficient degradation. The test should be initiated by use of semi-continuous procedure (one third of the volume is replaced every 2 weeks with freshly collected water with the test substance added to the initial concentration).	This standard specifies a test method for evaluating the biodegradability of organic substance by aerobic micro-organisms by means of shake-flask batch test. It is applicable to natural surface water, free from coarse particles to simulate a pelagic environment ("pelagic test") or to surface water with suspended sediments to simulate a water body with suspended sediments ("suspended sediment test"). The standard is applicable to organic substances present in lower concentrations (below 100 µg/l) than those of natural carbon substrates also present in the system. Under these conditions, the test substances serve as a secondary substrate and the kinetics for biodegradation would be expected to be first-order ("non-growth kinetics").	

Table 3-9: Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD 309/ISO 14592-1)

	OECD	ISO	Comments
Inoculum			
Sources of inoculum	- Surface water ("pelagic test"), - Surface water amended with suspended solids/sediments of 0.01 to 1 g/l dry weight ("suspended sediment test"). There is no addition of inoculum to the one already present in water or suspended solids.	- Surface water ("pelagic test"), - Surface water with suspended solids/sediments to obtain a level of 0.1 to 1 g/l dry weight ("suspended sediment test"). Usually, there is no addition of inoculum to the one already present in water or suspended solids. In some cases, an optional test may be carried out with a nutrient-rich or nutrient-enriched surface water or with membrane-filtered secondary effluent of a waste water treatment plant as the substrate amended with 3 mg/l (dry weight) of activated sludge as inoculum.	
Test system			
Medium	Natural surface water (fresh, brackish or marine): - Surface water ("pelagic test"), - Surface water amended with suspended solids/sediments of 0.01 to 1 g/l dry weight ("suspended sediment test").	- Surface water ("pelagic test"), - Surface water with suspended solids/sediments to obtain a level of of 0.1 to 1 g/l dry weight ("suspended sediment test").	
Vessels	Conical or cylindrical flasks of appropriate capacity (e.g. 0.5 to 1.0 litre) closed with silicone or rubber stoppers, or in serum flasks with CO_2-tight lids (e.g. with butyl rubber septa). For non volatile test substance that are not radio-labelled, gas tight stoppers or lids are not required ; loose cotton plugs are suitable.	Conical or cylindrical flasks of appropriate capacity (e.g. 0.5 to 1.0 litre) closed with silicone or rubber stoppers, or in serum flasks with CO_2-tight lids (e.g. with butyl rubber septa). For non volatile test substance that are not radio-labelled, gas tight stoppers or lids are not required ; loose cotton plugs are suitable. The flask may be sterilised by heating or autoclaving in order to be sure that no bacterial contamination occurs.	

Table 3-9: Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD 309/ISO 14592-1)

	OECD	ISO	Comments
Reference substance	- Aniline, - Sodium benzoate.	Preferably aniline (optional).	
Test conditions			
Temperature	20 - 25°C or field temperature. The temperature shall be maintained within ± 2°C throughout the test.	20 - 25°C or field temperature.	
Lighting	Dark or diffused light.	Dark or diffused light.	
Performance of the test			
Duration	The duration of the test should normally not exceed 60 days (unless the semi-continuous procedure with periodical renewal of the test suspension is applied). However, the test period for the batch test may be extended to a maximum of 90 days.	There is no statement about the test duration.	
Concentration of test substance	Less than 1 to 100 µg/l (but maximum concentration below 10 µg/l are preferred) At least, two different concentrations should be used. The concentrations should differ from each other by a factor of 5 to 10. For radio-labelled substances, a specific activity of approx. 50 µCi/mg (1.85 MBq) or more is preferred in order to facilitate ^{14}C measurements. For substance with high water solubility and low volatility, a stock solution can be prepared in deionised water. The volume of stock solution should be held to the practical minimum (< 10 % of the final volume). When the use of solvent is necessary, an additional solvent control is also prepared. The solvent should not affect the stability of the test substance in water. The solvent should be stripped off to an extremely small quantity so that it does not significantly	1 to 100 µg/l. The test substance is added at two concentration levels. Radio-labelled substances may be used directly or mixed with unlabelled test substances. A suitable activity for counting is often in the range 80 to 170 Bq per sample for analysis (an activity of 15 to 30 Bq/ml in the test flask results initially 75 to 150 Bq with a sample size of 5 ml). For water-soluble substances, a stock solution can be prepared in deionised water. For poorly water-soluble substances, the test substance is dissolved in a minimum amount of volatile organic solvent. The solvent should be stripped off so that it does not significantly increase the DOC concentration of the surface water. Other techniques to introduce the test substance in	

Table 3-9: Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD 309/ISO 14592-1)

	OECD	ISO	Comments
	increase the DOC concentration of the test water or suspension. Other techniques to introduce the test substance in the test flasks may be used as described in ISO 10634 and OECD guidance document on aquatic toxicity testing of difficult substances (n°23).	the test flasks may be used as described in ISO 10634.	
Concentration of inoculum	There is no statement about the concentration of inoculum. However, in the ISO ringtest, the water examined were reported to have a bacterial biomass corresponding to 10^3 to 10^4 colony forming units per ml.	There is no statement about the concentration of inoculum.	
Number of vessels	The test should include: - At least 2 flasks for each concentration of the test substance (preferably a minimum of 3) or multiple test flasks for each concentration, if whole flasks are harvested at each sampling time, - At least 2 flasks for each test concentration for mass balance calculation, - At least 1 flask containing only the test water (blank), - 2 flasks containing the reference substance at 10 µg/l (reference control), - 1 or 2 flasks containing sterilised test water and test substance for examining possible abiotic degradation or other non-biological removal of the test substance (sterile control), - 2 flasks containing test water and reference substance to examine the possible adverse effects of the solvent by degradation of the reference substance (solvent control).	The test should include: - At least 2 flasks for each concentration (at least 2) of the test substance, - At least one flask containing the surface water only (blank), And optionally: - At least 2 flasks containing the reference substance (test performance), - At least 1 flask containing sterilised surface water and test substance for examining possible abiotic degradation or other non-biological removal of the test substance (sterile control).	
Data and Reporting			

Table 3-9: Aerobic Mineralisation in Surface Water – Simulation biodegradation test (OECD 309/ISO 14592-1)

	OECD	ISO	Comments
Data reporting	- Residual ^{14}C concentration, - Residual concentration of test substance (if specific chemical analysis is used), - pH, - Dissolved oxygen concentration.	- Residual ^{14}C concentration, - Residual concentration of test substance (if specific chemical analysis is used).	
Frequency of measurements	Periodically.	At suitable intervals in the course of the test.	
Expression of results	- Fraction of ^{14}C mineralised, - Mass balance, - Level of primary degradation (if specific analysis is used), - Lag phase, - Degradation rate constant (First-order or pseudo first-order rate constant), - Degradation half-life, - Degradation curve.	- Fraction of ^{14}C mineralised, - Mass balance, - Level of primary degradation (if specific analysis is used), - Lag phase, - Degradation rate constant (First-order or pseudo first-order rate constant), - Degradation half-life.	
Validity	- The reference substance should be degraded in less than 2 weeks (for aniline in a the ISO ringtest, adapted degradation rate constants ranged from 0.3 to 1.7 day^{-1}). - The total recovery (mass balance) at the end of the test should be between 90 and 110 % for radio-labelled substances, whereas the initial recovery at the beginning of the experiment should be between 70 and 10 % for non-labelled substances.	If the optionally tested, reference substance is not sufficiently degraded within the expected time interval, the test is suspect and its validity shall be further verified, or alternatively the test shall be repeated using a new sample of water (for aniline in a interlaboratory test, rate constants ranged from 0.3 to 1.7d^{-1}).	

Table 3-10: Biodegradability in Seawater (OECD TG 306/ISO 16221)

	OECD	ISO	Comments
Name	Biodegradability in Seawater	Water quality – Guidance for determination of biodegradability in the marine environment	
Reference	306	16221	
Year	1992	2001	
Presentation of the document	This guideline presents two methods: - a seawater variant of the modified OECD Screening Test (Shake Flask Method), - a seawater variant of the Closed Bottle Test.	This standard specifies five methods for assessing the biodegradability of organic substances in the marine environment. These methods are the followings: DOC removal (ISO 7827), closed bottle test (ISO 10707), two-phase closed bottle test (ISO 10708), CO_2 evolution test (ISO 9439) and CO_2 in Sealed Vessels – Headspace Test (ISO 14593). The tests can be performed in natural seawater or artificial seawater.	
Inoculum			
Source of inoculum	No addition of inoculum to the one already present in seawater.	- *Seawater*: usually no addition of inoculum to the one already present in seawater, - *Artificial seawater*: filtrated seawater, suspension of marine sediments, bacteria from sea water aquarium filter.	
Pre-adaptation	No information.	In certain circumstances, pre-exposed inocula may be used. Pre-exposed inocula can be obtained from laboratory biodegradation tests or from contaminated areas (This should be clearly stated in the test report).	

Table 3-10: Biodegradability in Seawater (OECD TG 306/ISO 16221)

	OECD	ISO	Comments
Pre-conditioning	If the DOC content of the sample is found to be high, it is recommended that the seawater be aged for about a week prior to use.	To reduce the DOC concentration or BOD in the blank, it may be helpful to precondition the inoculum e.g. by aerating it in the test conditions up to one week, before use.	
Test system			
Medium	Natural seawater supplemented with mineral nutrients (same stock solutions and same amount of stock solutions used for the preparation of synthetic medium for TG 301A, 301B, 301F, 310).	- Natural seawater supplemented with mineral nutrients (same stock solutions and same amount of stock solutions used for the preparation of synthetic medium for TG 301A, 301B, 301F, 310 except solutions containing $CaCl_2$ and $MgSO_4$), - Artificial seawater.	
Reference substance	- Aniline, - Sodium acetate, - Sodium benzoate.	- Aniline, - Sodium benzoate.	
Test conditions			
Temperature	15 – 20°C. Within one test, the temperature should be maintained within ± 2°C (Shake Flask Method), within ± 1°C (Closed bottle test).	15 - 25°C. Within one test, the temperature should be maintained within ± 1°C.	
Lighting	No information.	Dark or diffused light.	
Performance of the test			
Duration	*Shake Flask method*: The recommended maximum duration is about 60 days. The test may be ended before as soon as the biodegradation curve has reached a plateau. The test may be extended if degradation has obviously started by day 60. *Closed bottle test*: The recommended duration is 28 days.	The maximum duration is 60 days. The test may be ended before as soon as the biodegradation curve has reached a plateau.	
Concentration of the test substance	- 5 – 40 mg DOC/l (Shake Flask Method), - 2 – 10 mg test substance /l (Closed bottle test).	- 5 - 40 mg DOC/l (DOC removal, ISO 7827), - 2 - 10 mg test substance /l (closed bottle test, ISO 10707),	

Table 3-10: Biodegradability in Seawater (OECD TG 306/ISO 16221)

	OECD	ISO	Comments
		- 100 mg ThOD /l (two-phase closed bottle test, ISO 10708), - 20 mg TOC/l (CO_2 evolution test, ISO 9439), - 20 - 40 mg TOC/l (CO_2 in Sealed Vessels – Headspace Test, ISO 14593).	
Concentration of inoculum	There is no statement.	About 10^5 cells/ml in the test vessels.	
Number of vessels	*For the Shake Flask Method, the following flasks are used*: - 2 flasks containing the test substance, - 2 flasks containing supplemented seawater only (blank), - 1 flask containing the reference substance (procedure control), And: - 1 flask containing the test and the reference substance (toxicity control, optional), - 1 flask containing the test substance and the sterilising agent (abiotic sterile control, optional). *For the closed bottle test, the following flasks are used*: - At least 8 flasks containing the test substance, - At least 8 flasks containing supplemented seawater only (blank), - At least 8 flasks containing the reference substance (procedure control), And when necessary : - At least 6 flasks containing the test and the reference substance (toxicity control, optional).	The test should include: - At least 2 flasks containing the test substance and inoculum, - At least 2 flasks containing only the inoculum (blank), - At least 1 flask containing the reference substance and the inoculum (procedure control), And if needed: - 1 flask containing the test substance, the reference substance and the inoculum (toxicity control) - 1 flask containing the test substance and the sterilising agent (abiotic sterile control).	
Data and Reporting			
Data reporting	*Shake Flask Method*: - DOC (in duplicate),	According to the method used as a basis: - DOC or COD,	

Table 3-10: Biodegradability in Seawater (OECD TG 306/ISO 16221)

	OECD	ISO	Comments
	Closed bottle test: - concentration of dissolved oxygen.	- TIC concentration, - concentration of dissolved oxygen, - CO_2 released, - Specific analysis of test substance (optionally, if primary biodegradation is to be followed), - pH.	
Frequency of measurement	*Shake Flask Method*: - No fixed time schedule. *Closed Bottle Test*: - At least on days 0 ,5, 15 an 28.	According to the method used as a basis	
Expression of results	- Percentage degradation, - Lag phase, - Degradation time.	- Percentage degradation - Percentage degradation from specific analyses of the test substance (when appropriate) - Lag phase, - Degradation time, - Maximum level of degradation.	
Validity	*Shake Flask Method*: - The percentage degradation of the reference substance should be comparable to the results obtained in the ringtest (the lag phase and time to achieve 50 % degradation excluding lag phase were 1 to 4 days and 1 to 7 days respectively for sodium benzoate and 1 to 10 days and 1 to 10 days respectively for aniline). *Closed Bottle Test*: - The percentage degradation of the reference substance is comparable to the results obtained in the ringtest (the lag phase and time to achieve 50 % degradation excluding lag phase were 0 to 2 days and 1 to 4 days respectively for sodium benzoate and 0 to 7 days and 2 to 12 days respectively for aniline).	The test is valid if the percentage degradation of the reference substance (procedure control) is greater than 60 % (70 % for DOC removal measurement) on the 14^{th} day.	

Table 3-10: Biodegradability in Seawater (OECD TG 306/ISO 16221)

	OECD	ISO	Comments
	- The blank respiration should not exceed 30 % of the oxygen in the bottle.		

Table 3-11: Anaerobic Biodegradability of Organic Compounds in Digested Sludge (OECD TG 311/ISO 11734)

	OECD	ISO	Comments
Name	Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production	Water quality – Evaluation of the ultimate anaerobic biodegradation of organic compounds in digested sludge – Method by measurement of the biogas production	
Reference	311	11734	
Year	2006	1995	
Inoculum			
Source of inoculum	Digested sludge from a digester from a wastewater treatment plant which treats predominantly domestic sewage.	- Digested sludge from a digester from a wastewater treatment plant which treats predominantly domestic sewage. - Digested sludge collected from a laboratory treatment plant.	
Pre-adaptation	*For test substances which are or are expected to be poorly biodegradable*: The test substance is added to the digested sludge at an organic carbon concentration of 5 to 20 mg/l. The digested sludge is then incubated for up to 2 weeks (The conditions of the pre-exposure should be indicated in the test report).	*For test substances which are or are expected to be poorly biodegradable*: The test substance is added to the digested sludge at an organic carbon concentration of 5 to 20 mg/l. (The conditions of the pre-exposure should be indicated in the test report).	
Pre-conditioning	In order to reduce the background gas production and to decrease the influence of the blank controls, pre-digestion of the sludge may be considered. The sludge should be then allowed to digest without addition of any nutrients or substrates at 35 ± 2°C for up to 7 days.	In order to reduce the background gas production and to decrease the influence of the blank controls, pre-digestion of the sludge may be considered. The sludge should be then allowed to digest without addition of any nutrients or substrates at 35 ± 2°C for up to 7 days.	
Test system			
Reagents	Analytical grade reagents.	Analytical grade reagents.	
Medium	Synthetic mineral medium. The medium is sparged with nitrogen gas for about	Synthetic mineral medium. The medium is sparged with nitrogen gas for about	

Table 3-11: Anaerobic Biodegradability of Organic Compounds in Digested Sludge (OECD TG 311/ISO 11734)

	OECD	ISO	Comments
	20 minutes before adding the solution of reducing agent. The pH is adjusted to 7.0 ± 0.2 (if necessary).	20 minutes. The pH is adjusted to 7.0 ± 0.2 (if necessary).	
Vessels	Pressure-resistant glass test vessels of 0.1 to 1.0 litre capacity, fitted with a gas-tight septum capable of withstanding about 2 bar. The headspace volume should be about 10 to 30 % of the total volume. Glass serum bottles (recommended size "125 ml" corresponding to a total volume of around 160 ml), sealed with serum septa and crimped with aluminium rings, are recommended when the pressure is released at each sampling time.	Pressure-resistant glass test vessels of 0.1 to 1.0 litre capacity, fitted with a gas-tight septum capable of withstanding about 2 bar. The headspace volume should be about 10 to 30 % of the total volume. If the pressure is released regularly, a headspace volume of 10 % is sufficient.	
Reference substance	e.g.: - Phenol, - Sodium benzoate, - Polyethylene glycol 400.	e.g.: - Phenol, - Sodium benzoate, - Polyethylene glycol 400.	
Test conditions			
Temperature	35 ± 2°C.	35 ± 2°C.	
Lighting	No information.	No information.	
Performance of the test			
Duration	The recommended duration is 60 days unless the biodegradation curve obtained from the pressure measurement has reached a plateau phase. The test may be extended if the plateau phase is obviously not reached at the end of the normal incubation period.	The recommended duration is 60 days unless the biodegradation curve obtained from the pressure measurement has reached a plateau phase and the percentage degradation is higher than 50 %. The test may be extended if the plateau phase is obviously not reached at the end of the normal incubation period.	
Concentration of test substance	20 – 100 mg organic carbon /l. The test substance is added as stock solution, suspension, emulsion or directly as solid or liquid or as absorbed on to glass-fibre filter.	20 – 100 mg organic carbon /l. The test substance is added as stock solution, suspension, emulsion or directly as solid or liquid. If stock solutions are used, the volume added	

Table 3-11: Anaerobic Biodegradability of Organic Compounds in Digested Sludge (OECD TG 311/ISO 11734)

	OECD	ISO	Comments
	If stock solutions are used, the volume added should be less than 5% of the total volume of the reaction mixture. When the use of solvent is necessary, an additional solvent control is also prepared. Organic solvents which are known to inhibit methane production, such as chloroform and carbon tetrachloride should be avoided.	should be less than 5% of the total volume of the reaction mixture. When the use of solvent is necessary, organic solvents which are known to inhibit methane production, such as chloroform and carbon tetrachloride should be avoided.	
Concentration of digested sludge	- Concentration of total solids between 1 and 3 g/l in the test vessels. (or about 10 % of that in undiluted digested sludge). - IC concentration ≤ 10 mg/l in the final test suspension.	- Concentration of total solids between 1 and 3 g/l in the test vessels. (or about 10 % of that in undiluted digested sludge). - IC concentration ≤ 10 mg/l in the final test suspension.	
Number of vessels	The following vessels are used: - At least 3 vessels containing the test substance and the sludge inoculum, - At least 3 vessels containing the sludge inoculum (blank), - At least 3 vessels containing the reference substance and the sludge inoculum (procedure control). And: - At least 3 vessels containing the test and the reference substance and the sludge inoculum (toxicity control, conditional), - At least 3 vessels for pressure control chambers.	The following vessels are used: - At least 3 vessels containing the test substance and the sludge inoculum, - At least 3 vessels containing the sludge inoculum (blank), - At least 1 vessel for each reference substance containing the reference substance and the sludge inoculum (procedure control). And: - At least 1 vessel containing the test and the reference substance and the sludge inoculum (toxicity control, optionally).	
Data and Reporting			
Data reporting	- Gas production, - Inorganic Carbon in the supernatant liquor, - Specific analysis of test substance (optionally, if primary biodegradation is to be determined),	- Gas production, - Inorganic Carbon in the supernatant liquor, - Specific analysis of test substance (optionally, if primary biodegradation is to be determined),	

Table 3-11: Anaerobic Biodegradability of Organic Compounds in Digested Sludge (OECD TG 311/ISO 11734)

	OECD	ISO	Comments
	- pH.	- pH.	
Frequency of measurements	*Gas production*: - Periodically e.g. weekly, *Inorganic Carbon and pH*: - At the end of the test, *Specific analysis of the test substance*: - At the beginning and at the end of the test.	*Gas production*: - weekly, *Inorganic Carbon and pH*: - At the end of the test, *Specific analysis of the test substance*: - At the beginning and at the end of the test.	
Expression of results	Percentage biodegradation of the test substance.	Percentage biodegradation of the test substance.	
Validity	- Pressure readings should be used only from vessels that not show pink coloration (indicating absence of oxygen). - The percentage degradation of the reference substance should be higher than 60 % theoretical gas production within 60 days. - If the pH value at the end of the test has exceeded the range 6 - 8 and insufficient biodegradation has taken place, the test should be repeated with increased buffer capacity of the test medium.	- Pressure readings should be used only from vessels that not show pink coloration (indicating absence of oxygen). - The percentage degradation of the reference substance should be higher than 60 % theoretical gas production within 60 days. - If the pH value at the end of the test has exceeded the range 6 - 8 and insufficient biodegradation has taken place, the test should be repeated with increased buffer capacity of the test medium. And: - The gas production in the vessels containing the test and the reference substances should be at least the same as the one obtained in the vessel containing the reference substance only.	

Table 3-12: Inherent Biodegradability Test in Soil (OECD TG 304A/ISO 11266)

	OECD	ISO	Comments
Name	Inherent Biodegradability in Soil	Soil quality – Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions	In addition, ISO 14239 (1997) gives information on three different incubation systems: - Flow through system, - Soda lime column system, - Biometer system.
Reference	304A	11266	
Year	1981	1994	
Test system			
Reagent	Analytical grade.	Not specified.	
Vessels	Erlenmeyer flasks of 250 ml capacity fused to 50 ml round bottom tubes.	Not specified.	
Test substance	^{14}C-labeled substances (dissolved in acetone to give radioactivity of 37 – 185 KBq for 100 ml).	High purity substances (> 98 %), - ^{14}C-labeled substances, - Unlabelled substances.	
Carbon dioxide absorber	- Potassium hydroxide solution (0.1 M).	Not specified.	
Reference substance	No specific reference substance is recommended.	It is recommended to measure the microbial activity of the soil using a reference chemical. However, there is not statement in this guidance document about a specific reference substance.	

Table 3-12: Inherent Biodegradability Test in Soil (OECD TG 304A/ISO 11266)

	OECD	ISO	Comments
Soil			
Soil	- Alfisol (pH: 5.5 - 6.5; O.C: 1 - 1.5 %; clay content: 10 - 20 %; CEC: 10 - 15 mval), - Spodosol (pH: 4.0 - 5.0; O.C: 1.5 - 3.5 %; clay content: ≤ 10 %; CEC: < 10 mval), - Entisol (pH 6.6 - 8.0; O.C 1 – 4.0 %; clay content 11 - 25 %; CEC : >10 mval). In special cases, it is recommended to add two additional soils: - soil with high silt fraction content, - soil with a high clay content (30 %).	Not specified. Soil collection should be conducted according to ISO 10381-6. The following parameters should be determined: granulometry, water content, WHC, pH, organic matter content, CEC, nitrogen content.	
Soil moisture	40 % max. WHC.	Pore water pressure: -0.01 MPa to -0.031 Mpa (highest microbial activity), or 40 % - 60 % max. WHC.	
Amount of soil	50 g of soil (dry weight) per flask.	At least 50 g of soil (dry weight) per flask.	
Test conditions			
Temperature	22 ± 2ºC.	According to the purpose of the study The highest microbial activity is obtained for temperatures between 25 and 35°C. For soil from temperate area, a temperature within the range 10 - 25°C is relevant. Within one test, the temperature should be maintained within ± 2°C.	
Lighting	Dark.	Dark (except if photodegradation should be taken into consideration).	
Performance of the test			
Duration	≤ 64 days. Incubation time is sufficient when a total of 50 % CO_2 is obtained.	≤ 120 days.	
Concentration of test substance	100 µl of radioactive solution added on the soil surface (already placed in the Flask). The soil is then	According to the purpose of the study. The test substance can be incorporated in water, in solvent	

Table 3-12: Inherent Biodegradability Test in Soil (OECD TG 304A/ISO 11266)

	OECD	ISO	Comments
	mixed.	or after mixing with sand.	
Number of test vessels	4 replicates for test and control units.	At least 2 replicates.	
Data and Reporting			
Data reporting	- $^{14}CO_2$ TG304A includes two optional tests (respectively for volatile and persistent substances): - Estimation of evaporation, - Soil-extractable and non-extractable residues.	- $^{14}CO_2$, - CO_2, - O_2, - Specific analysis of test substance (if primary biodegradation is to be followed), - Soil-extractable and non-extractable residues, - Loss of parent compound.	
Frequency of CO_2 measurements	1, 2, 4, 8, 16, 32 and if necessary 64 days.	0, 2, 4, 8, 16, 32, 64, 120 days. At least 5 points are necessary to establish a degradation curve.	
Expression of results	Percentage of degradation based on CO_2 release.	- Percentage of degradation based on CO_2 release. - Percentage of degradation based on oxygen consumption. - Percentage degradation from specific analyses of the test substance. - DT 50, DT 90.	
Validity	There is no statement regarding validity criteria.	There is no statement regarding validity criteria.	

CHAPTER 4: OECD TEST GUIDELINES SECTION 4 - HEALTH EFFECTS

- Skin Irritation Test (OECD TG 404/ISO 10993-3): Table 4-1
- Skin Sensitisation Test (Maximization Sensitisation Test) (OECD TG 406/ISO 10993-3): Table 4-2
- Skin Sensitisation Test (Buehler Test) (OECD TG 406/ISO 10993-3): Table 4-3
- Toxicokinetic Test (OECD TG 417/ISO 10993-3): Table 4-4

Table 4-1: Skin Irritation Test (OECD TG 404/ISO 10993-3)

	OECD	ISO	Comments
Title	Acute Dermal Irritation /Corrosion	Biological evaluation of medical devices - Part 10: Test for irritation and sensitization	
Reference	404	10993-3	
Year	2002	1995	
Test animals			
Species	Albino rabbits Other species may be used if justified.	Albino rabbits	
Age of animals	Young adult	Young adult	
Weight of animals	Not specified.	Weight should be less than 2 kg.	
Sex of animals	Male and/or female animals	Male and/or female animals	
Number of animals	If it is suspected that the test substance might produce severe irritancy, a single animal test should be employed. Then if neither a corrosive effect nor a severe irritant effect is observed after a four hour exposure, the test should be completed using two additional animals. If it is expected that the test substance will not produce severe irritancy or corrosion, the test may be stared using three animals.	One animal shall initially be used to evaluate the test material. Unless a well-defined response is observed for solid or liquid materials, a minimum of two further animals shall be sued.	
Test conditions			
Temperature	20°C ± 3°C for rabbits	Not specified	
Humidity	30 - 70 %	Not specified	
Photoperiod	12 hours light / 12 hours dark	Not specified	
Performance of the test			
Exposure period	Four hours	≥ Four hours	
Dose level	Liquid: 0.5 ml Solid or semi-solid: 0.5 g	Liquid: 0.5 ml Powder : 0.5 g	

Table 4-1: Skin Irritation Test (OECD TG 404/ISO 10993-3)

	OECD	ISO	Comments
		Extracts and extractants: appropriate amount Solid: Not specified	
Observation periods	The duration of the observation period should not be fixed rigidly, but should be sufficient to evaluate fully the reversibility of the effects observed.	Observation periods need not exceed 14 days.	
Frequency of observations	Animals should be observed at 1 hour, 24 hours, 48 hours and 72 hours after patch removal.	Animals should be observed at 1 hour, 24 hours, 48 hours and 72 hours after patch removal.	
Animal-welfare consideration	Following substances do not need to be tested; - substances that have a pH of 2 or less or 11.5 or greater; - substances which have been shown to be highly toxic by the dermal route; - substances which, in an acute dermal toxicity test, have been shown not to produce irritation of the skin a t the limit test dose level of 2000 mg/kg body weight. In addition, substances for which corrosive properties are predicted on the basis of results from existing human and animal data, QSARs and in vitro tests may not be necessary to test.	Following substances do not need to be tested; - substances that have a pH of 2 or less or 11.5 or greater.	
Data and Reporting			
Interpretation of results	Dermal irritation is scored and recorded according to the numerical grading system.	Dermal irritation is scored and recorded according to the numerical grading system.	
Numerical grading system	**Erythema and eschar formation** 0 = no erythema 1 = very slight erythema (barely perceptible) 2 = well –defined erythema 3 = moderate erythema 4 = severe erythema (beet-redness) to eschar	**Erythema and eschar formation** 0 = no erythema 1 = very slight erythema (barely perceptible) 2 = well –defined erythema 3 = moderate erythema 4 = severe erythema (beet-redness) to eschar	

Table 4-1: Skin Irritation Test (OECD TG 404/ISO 10993-3)

	OECD	ISO	Comments
	formation preventing grading of erythema Max possible: 4 **Oedema formation** 0 = no oedema 1 = very slight oedema (barely perceptible) 2 = well –defined oedema (edges of area well-defined by definite raising) 3 = moderate oedema (raised approx. Mately 1 mm) 4 = severe oedema (raised more than 1 mm and extending beyond exposure area) Max possible: 4	formation preventing grading of erythema **Oedema formation** 0 = no oedema 1 = very slight oedema (barely perceptible) 2 = well –defined oedema (edges of area well-defined by definite raising) 3 = moderate odema (raised approx. Mately 1 mm) 4 = severe odema (raised more than 1 mm and extending beyond exposure area)	

Table 4-2: Skin Sensitisation (Maximization Sensitisation Test) (OECD TG 406/ISO 10993-3)

	OECD	ISO	Comments
Title	Skin Sensitisation	Biological evaluation of medical devices - Part 10: Test for irritation and sensitization	
Reference	406	10993-3	
Year	1992	2002 + 2006	
Test animals			
Species	Guinea pigs	Albino guinea pigs	
Age of animals	Young adult	Young adult	
Weight of animals	Not specified.	300 - 500g at the start of the test	
Sex of animals	Male and/or female animals If females are used, they shall be nulliparous and not pregnant.	Male and/or female animals If females are used, they shall be nulliparous and not pregnant.	
Number of animals	At least 10 animals for the treatment group At least 5 animals for the control group It may be necessary to double the number of animals in order to confirm weak sensitises.	At least 10 animals for the treatment group At least 5 animals for the control group It may be necessary to double the number of animals in order to confirm weak sensitises.	
Test conditions			
Temperature	20 °C ± 3 °C	Not specified	
Humidity	30 - 70 %	Not specified	
Photoperiod	12 hours light / 12 hours dark	Not specified	
Performance of the test			
Test procedure	Day 0: Intradermal Injections Day 6-8: Topical Application (remove the patches after 48 ± 2 hours) Day 20-22: Challenge (remove the patches after 24 hours)	Day 0: Intradermal Injections Day 7: Topical Application (remove the patches after 48 ± 2 hours) At least 14 days after the topical induction phase: Challenge (remove the patches after 24 ± 2 hours)	
Dose level	According to the results of preliminary test. The concentration of the test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-	According to the results of preliminary test. For the topical induction phase: the highest concentration causes slight erythema, but does not otherwise adversely affect the animals	

Table 4-2: Skin Sensitisation (Maximization Sensitisation Test) (OECD TG 406/ISO 10993-3)

	OECD	ISO	Comments
	to-moderate skin irritation. The concentration for the challenge exposure should be the highest non-irritant dose.	For the challenge phase: the highest concentration produces no erythema.	
Observation periods	Skin reaction should be observed at 24 hours and 48 hours after the removal of the patches.	Skin reaction should be observed at 24 hours, 48 hours and 72 hours after the removal of the patches.	
Rechallenge	One week after the first challenge, if needed.	One week after the first challenge, if needed.	
References substances	Hexyl cinnamic aldehyde, Mercaptobenzothiazole, Benzocaine	Not specified.	
Data and Reporting			
Interpretation of results	Skin reaction is scored and recorded according to the numerical grading system at each time interval.	Skin reaction is scored and recorded according to the numerical grading system at each time interval.	
Numerical grading system	0 = no visible change 1 = discrete or patchy erythema 2 = moderate and confluent erythema 3 = intense erythema and swelling	**Erythema and eschar formation** 0 = No erythema 1 = Slight erythema 2 = Well-defined erythema 3= Moderate erythema 4 = Severe erythema to slight eschar formation **Oedema formation** 0 = No erythema 1 = Slight erythema 2 = Well-defined erythema 3= Moderate erythema 4 = Severe oedema	

Table 4-3: Skin Sensitisation (Buehler Test) (OECD TG 406/ISO 10993-3)

	OECD	ISO	Comments
Title	Skin Sensitisation	Biological evaluation of medical devices - Part 10: Test for irritation and sensitization	
Reference	406	10993-3	
Year	1992	1995	
Test animals			
Species	Guinea pigs	Albino guinea pigs	
Age of animals	Young adult	Young adult	
Weight of animals	Not specified.	300 - 500g at the start of the test	
Sex of animals	Male and/or female animals If females are used, they shall be nulliparous and not pregnant.	Male and/or female animals If females are used, they shall be nulliparous and not pregnant.	
Number of animals	At least 20 animals for the treatment group At least 10 animals for the control group	At least 10 animals for the treatment group At least 5 animals for the control group It may be necessary to double the number of animals in order to confirm weak sensitises.	
Test conditions			
Temperature	20°C ± 3°C	Not specified	
Humidity	30 – 70 %	Not specified	
Photoperiod	12 hours light / 12 hours dark	Not specified	
Performance of the test			
Test procedure	Day 0: Induction (remove the patches after 6 hours) Day 6-8: Induction (remove the patches after 6 hours) Day 13-15: Induction (remove the patches after 6 hours) Day 27-29: Challenge (remove the patches after 6 hours)	Day 0: Induction (remove the patches after 6 hours) Day 7: Induction (remove the patches after 6 hours) Day 14: Induction (remove the patches after 6 hours) Day 28: Challenge (remove the patches after 6 hours)	
Dose level	According to the results of preliminary test.	According to the results of preliminary test.	

Table 4-3: Skin Sensitisation (Buehler Test) (OECD TG 406/ISO 10993-3)

	OECD	ISO	Comments
	The concentration of the test substance used for each induction exposure should be the highest non-irritating dose.	For the topical induction phase: the highest concentration causes no more than slight erythema, but does not otherwise adversely affect the animals. For the topical challenge phase: the highest concentration produces no erythema.	
Observation periods	Skin reaction should be observed at 24 hours and 48 hours after the removal of the patches.	Skin reaction should be observed at 24 hours and 48 hours after the removal of the patches.	
Rechallenge	One week after the first challenge, if needed.	One week after the first challenge, if needed.	
References substances	Hexyl cinnamic aldehyde, Mercaptobenzothiazole, Benzocaine	Not specified.	
Data and Reporting			
Interpretation of results	Skin reaction is scored and recorded according to the numerical grading system at each time interval.	Skin reaction is scored and recorded according to the numerical grading system at each time interval.	
Numerical grading system	0 = no visible change 1 = discrete or patchy erythema 2 = moderate and confluent erythema 3 = intense erythema and swelling	**Erythema and eschar formation** 0 = No erythema 1 = Slight erythema 2 = Well-defined erythema 3= Moderate erythema 4 = Severe erythema to slight eschar formation **Oedema formation** 0 = No erythema 1 = Slight erythema 2 = Well-defined erythema 3= Moderate erythema 4 = Severe oedema	

Table 4-4: Toxicokinetic Test (OECD TG 417/ISO 10993-3)

	OECD	ISO	Comments
Title	Toxicokinetics	Biological evaluation of medical devices - Part 16: Toxicokinetic study design for degradation products and leachables	
Reference	417	10993-3	
Year	1992	1997	
Test animals			
Species	One or more appropriate species	Appropriate species	
Age of animals	Young adult animals In special situations, very young or pregnant animals may be used.	Young adult animals	
Sex of animals	Sex preference is not mandatory, but under some circumstances both sexes may need to be studies.	Appropriate sex	
Number of animals	For absorption and excretion studies, there should be four animals in each dose group. If sexual dimorphism exists, four animals of each sex should be studies. In the case of studies with non-rodents, fewer animals may be used. When tissue distribution is being studied, the initial group size should take into account the number of animals of sacrifice. When metabolism is being studies, the group size is related to the needs of the study. For multiple-dose and multiple-time point studies, the group size should take into account the number of time points and planned sacrifice(s), but may not be smaller than two animals.	At least three for small animas / group At least two animals for larger species/ group	
Test conditions			
Temperature	22 ± 3°C	Not specified.	
Humidity	30-70 %	Not specified.	
Performance of the test			

Table 4-4: Toxicokinetic Test (OECD TG 417/ISO 10993-3)

	OECD	ISO	Comments
Test substances	- unlabelled - labelled	- unlabelled - labelled	
Dose level	In the case of single dose administration, at least two dose levels should be used. There should be a low dose at which not toxic effects are observed and a high dose at which there might be changes in toxicokinetic parameters or at which toxic effects occur. In the case of repeated-dose administration, the low dose is usually sufficient, but under certain circumstances a high dose may also be necessary.	Not specified.	
Route of administration	The test substance is usually administered orally by gavage or in the diet, applied to the skin, or administered by inhalation. Intravenous administration may be useful when determining the amount of absorption and the pattern of distribution soon after the administration of a substance.	The test substance should be administered by an appropriate route. This route should be relevant to the use of the medical device.	
Observation (Absorption)	The rate and extent of absorption of the administered substance can be determined by various methods, with and without reference groups, such as: - determination of the amount of test substance and/or metabolites in excreta, such as urine, bile, faeces, exhaled air and that remaining in the carcass; - comparison of a biological response (e.g. acute toxicity studies) between test and control and/or reference groups; - comparison of the amount of dose excreted renally in test and reference groups; - determination of the area under the plasma level/time curve of the test substance and/or metabolites and comparison with data from a	Following parameters should be measured; - blood; - serum; - excreta; - tissue concentrations.	

Table 4-4: Toxicokinetic Test (OECD TG 417/ISO 10993-3)

	OECD	ISO	Comments
	reference group.		
Observation (Distribution)	Two approaches are available; - useful quantitative information obtained using whole-body autoradiographic techniques; - quantitative information is obtained by sacrificing animals at different times after exposure and determining the concentration and amount of the test substance and/or metabolites in tissues and organs.	Studies may be - quantitative, determining levels in dissected tissues, - qualitative, using whole-body autoradiography (WBA); - semi-quantitative, using graded WBA standards.	
Observation (Excretion and Metabolism)	Excretion: urine, faeces and expired air and, in certain circumstances, bile are collected. The amount of test substance and/or metabolites in these excreta should be measured at several times after exposure, until about 95 per cent of the administered dose has been excreted or for seven days, whichever comes first. In special cases, the excretion of the test substance in the milk of lactating test animals may need to be determined. Metabolism: For determining the extent and pattern of metabolism, biological samples should be analysed by suitable techniques. Structures of metabolites should be elucidated and the metabolic pathways proposed in relation to the need to answer questions arising from previous	For studies of up to 14 days, the urine and faeces should be individually collected at 24 hours and then every 24 hours until the end of the experiment. In some study designs, animals may be sacrifices at intermediate times. Samples may be collected prior to 2 hours when it is probable that the test substance or its metabolites will be rapidly excreted. For studies of longer duration, sampling over the initial period should occur as for the short-term studies. Thereafter samples should be obtained for a continuous 24 hours period per assessment periods. The carcasses and/or target organs of the individual animals should be retained for analysis, and blood collected for analysis of plasma and whole-blood concentrations.	

ANNEX: OTHER ISO STANDARDS WHICH ARE RELATED TO OECD TEST GUIDELINES IN THE FIELD OF HEALTH EFFECTS

ISO 10993-3: Biological evaluation of medical devices
Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity

This standard provides a general idea of genotoxicity, carcinogenicity and reproductive toxicity, and does not provide any detail test methods. Practically, this standard suggests to performing tests according to current OECD Test Guidelines. Therefore there is no need to compare this standard with OECD Test Guidelines

ISO 10993-3: Biological evaluation of medical devices
Part 11: Tests for systemic toxicity

This standard also provides a general conception of systemic toxicity. Several OECD Test Guidelines and other organisations' guidelines are listed as references. Therefore there is no need to compare this standard with OECD Test Guidelines

ISO 13338: Determination of tissue corrosiveness of a gas or gas mixture

This standard provides a list indicating the corrosiveness of gasses/gas mixture, and a calculation method to the corrosivity of each of their components. Therefore there is no need to compare this standard with OECD Test Guidelines.

ISO 13344: Determination of the lethal toxic potency of fire effluents

This test method has been designed to provide data for use in the assessment of toxic fire hazard as a means for the evaluation of materials and products. This method subjects a test specimen to the combustion conditions of a specific laboratory fire model. Concentrations of the major gaseous toxicants in the fire effluent atmosphere are monitored over a 30 minutes period, and calculated as LC_{50}. Therefore there is no need to compare this standard with OECD Test Guidelines.

ISO TR 9122-2: Toxicity testing of fire effluents
Part 2: Guidelines for biological assays to determine the acute inhalation toxicity of fire effluents (basic principles, criteria and methodology)

The main objective of the ISO Technical Report is to provide researchers with basic background information on methods suitable to define the acute inhalation toxicity of fire effluents as generated by fire models. This report provides basic principles, criteria and methodology. Therefore there is no need to compare this standard with OECD Test Guidelines.