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Detailed Review Paper on Aquatic Testing Methods for Pesticides and Industrial Chemicals

Part 1: Report

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No. 11

DETAILED REVIEW PAPER ON AQUATIC TESTING METHODS FOR PESTICIDES AND INDUSTRIAL CHEMICALS

PART 1: REPORT

(Part 2 (Annexes) is available on request from the Secretariat)

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Also published in the Series on Testing and Assessment:

No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (1993; reformatted 1995)

No. 2, *Detailed Review Paper on Biodegradability Testing* (1995)

No. 3, *Guidance Document for Aquatic Effects Assessment* (1995)

No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment* (1995)

No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing* (1996)

No. 6, *Report of the Final Ring-test of the* Daphnia magna *Reproduction Test* (1997)

No. 7, *Guidance Document on Direct Phototransformation of Chemicals in Water* (1997)

No. 8, *Report of the OECD Workshop on Sharing Information about New Industrial Chemicals Assessment* (1997)

No. 9, *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application* (1997)

No. 10, *Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data* (1998)

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonize 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 subsidiary 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 subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

The work of the OECD related to chemical safety is carried out in the **Environmental Health and Safety Programme**. As part of its work on chemical testing, the OECD has issued several Council Decisions and Recommendations (the former legally binding on Member countries), as well as numerous Guidance Documents and technical reports. The best known of these publications, the **OECD Test Guidelines**, is a collection of methods used to assess the hazards of chemicals and of chemical preparations such as pesticides and pharmaceuticals. These methods cover tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. The OECD Test Guidelines are recognised worldwide as the standard reference tool for chemical testing.

More information about the Environmental Health and Safety Programme and its publications (including the Test Guidelines) is available on the OECD's World Wide Web site (see page 6).

The Environmental Health and Safety Programme co-operates closely with other international organisations. This document was produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), 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. UNITAR joined the IOMC in 1997 to become the seventh Participating Organization. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

The OECD is one of the international organisations with a leading role in the promotion of internationally acceptable methods for the testing of chemicals for regulatory purposes. These chemicals include, among others, industrial chemicals, pesticides, food additives and pharmaceuticals.

The development of OECD Test Guidelines in a specific area starts with a Detailed Review Paper (DRP) when it is considered essential that the "state-of-the-art" in the area under review first be assessed. Criteria that apply to DRPs, as well as further details of OECD Test Guideline development procedures, are given in OECD Environment Monograph No. 76, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (1995).

The *Detailed Review Paper on Aquatic Testing Methods for Pesticides and Industrial Chemicals* is the eleventh in the OECD Series on Testing and Assessment. (Before 1995, a number of other OECD publications concerning chemical testing and assessment appeared in the OECD Environment Monograph series.) The objective of this DRP was to review the area of aquatic toxicity testing, including the pelagic (water) and benthic (sediment) environment, in order to identify whether, in the light of scientific developments, there was a need to revise existing OECD Test Guidelines or to develop new Guidelines.

The *Detailed Review Paper on Aquatic Testing Methods for Pesticides and Industrial Chemicals* is divided into two parts. *Part 1* is the main report, and *Part 2 (Annexes)* contains detailed background information relating to a number of topics in the Report.

This Detailed Review Paper was prepared and collated by Denmark, with the contribution of the Netherlands for sediment test methods. The DRP was circulated to, and greatly appreciated by, OECD Member countries. They considered it a comprehensive and exhaustive review and a good basis for prioritising the development of OECD Test Guidelines in aquatic toxicity testing. As a joint activity of the OECD Test Guidelines and Risk Assessment Programmes, a Working Group on Aquatic Toxicity Testing was then established to (i) discuss the DRP and comments received from Member countries, and (ii) propose priorities for revision and development of Test Guidelines in the aquatic toxicity testing area. This Working Group met in Copenhagen, Denmark, on 29-30 June 1995. The report of the Working Group Meeting is included in this document.

The recommendations of the Meeting regarding the proposed priorities for development and revision of OECD Test Guidelines in the aquatic toxicity area were subsequently endorsed by the National Co-ordinators of the Test Guidelines Programme at their 6th Meeting in December 1995. New work initiated within the Test Guidelines Programme as a result of this DRP and the subsequent Working Group Meeting is also indicated in this document.

The OECD's Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals recommended that this document be derestricted. It is published on the responsibility of the Secretary-General of the OECD.

Detailed Review Paper on Aquatic Testing Methods for Pesticides and Industrial Chemicals

Prepared for the Organisation for Economic Co-operation and Development

Authors:

The pelagic part of this review was prepared by P. Kristensen, J. Tørsløv, E. Bjørnestad, G. Petersen and L. Samsøe-Petersen (VKI Water Quality Institute, Denmark). The work was supervised by a steering group chaired by H. Tyle (DK-EPA). The members of the steering group were K.O. Kusk and F. Bro-Rasmussen, Technical University of Denmark, T. Källqvist, NIVA, Norway, M. Tarkpea, SNV, Sweden, and J. Mossin, DK-EPA, Denmark.

The benthic part of the review was prepared by C.J. Roghair, M.A.H. Wolters-Balk and J. van der Wal (RIVM, the Netherlands). Sediment methods were collected and compiled by R.N. Hooftman and S. Gimeno (TNO, the Netherlands). The sediment part of the report was supervised by J.H.M. de Bruijn (DGM, the Netherlands), C. van de Guchte (RIZA, the Netherlands) and R.N. Hooftman (TNO, the Netherlands).

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Executive Summary

Literature searches were made to investigate developments and advances in methods for assessing aquatic toxicity of chemicals, including testing methods for the pelagic (water) and benthic (sediment) environments. The searches were restricted to laboratory testing methods, excluding field studies, and tests with single species (or mixed cultures for microorganisms), excluding multispecies tests and microcosm and mesocosm studies.

The Detailed Review Paper (DRP) covered national and international standardised methods required in testing schemes for hazard and risk assessment of chemicals (industrial chemicals and pesticides) as well as published or unpublished methods describing testing protocols and endpoints relevant for future OECD Test Guideline development. Nearly 450 pelagic and 260 benthic testing methods developed over the last decade were reviewed.

The methods collected, covering various trophic levels and different types of endpoints, have been grouped according to eight scenarios based on (i) pelagic and benthic environmental compartments, (ii) marine and freshwater environments, and (iii) "warm" and "cold" temperature regimes.

In order to identify testing methods for future OECD Test Guideline development, the collected methods were evaluated and scored in terms of (a) scientific validity, sensitivity, specificity, and reproducibility, and (b) input with respect to significant endpoints not yet sufficiently covered by OECD Test Guidelines. The evaluation procedure was based on a set of criteria as follows:

- **practical feasibility of the test method**: technical performance, test duration, availability and maintenance of test organism, exposure system, equipment and labour costs;

- **validity of the test method**: reproducibility, sources of potential error, range of tolerance to environmental conditions;

- **usefulness in prognoses***:* geographical representativeness, ecological representativeness, extrapolation of endpoints, general sensitivity, relevance of exposure route and test conditions;

- **level of standardisation**.

Based on (1) data requirements in current hazard/risk assessment schemes, (2) the evaluation and scoring of testing methods, and (3) considerations regarding the potential need of aquatic toxicity tests for regulatory purposes, the DRP made a number of recommendations for OECD Test Guideline development in pelagic and benthic testing areas. These recommendations have been ranked in the following three categories:

- **Primary recommendation (Group 1):** methods recommended for inclusion in the OECD Test Guidelines Programme, as they are needed in existing hazard/risk assessment schemes;

- **Secondary recommendation (Group 2):** methods presumably needed in the future for guideline development, as they are recommended in some national assessment schemes and/or considered to represent key ecological groups;

- **Tertiary recommendations (Group 3):** methods not needed in the immediate or near future.

The recommendations arising from this review (see Chapter 7 of the DRP, "Recommendations") can be summarised as follows:

***Pelagic tests*:**

Group 1:
(a) Freshwater higher plant test (*Lemna*) ;
(b) Marine algae test (*Skeletonema, Phaeodactylum*, etc.) ;
(c) Marine crustacean test (*Acartia, Tisbe, Mysisdopsis*, etc.) ;
(d) Life cycle freshwater (*Danio, Pimephales*) and marine (*Cyprinodon*) fish test.

Group 2:
(a) Marine higher plant test (*Zostera*) ;
(b) Marine macroalgae test (*Champia, Gracilera*) ;
(c) Freshwater crustacean acute test (*Daphnia, Ceriodaphnia, Neomysis*) ;
(d) Freshwater insect test (*Aedes, Acroneuria*, etc.) ;
(e) Amphibian test (*Xenopus, Rana*) ;
(f) Freshwater and marine rotifer test (*Brachionus*) ;
(g) Sea urchin test (*Echinodermata, Lytechinus*, etc.) ;
(h) Marine mollusc test (*Crassostrea, Mytilus*) ;
(i) Freshwater and marine protozoa test (*Tetrahymena*) ;
(j) Bacteria test (*Pseudomonas*).

***Benthic tests*:**

Group 1:
(a) Marine annelid acute test (*Arenicola*) ;
(b) Marine crustacean acute test (*Corophium*) ;
(c) Sea urchin acute test (*Lytechinus, Echinocardium*).

Group 2:
(a) Freshwater annelid test (*Tubifex, Lumbriculus*, etc.) ;
(b) Freshwater (*Hyalella*, etc.) and marine (*Corophium*, etc.) crustacean test;
(c) Freshwater insect acute and subchronic test (*Chironomus*) ;
(d) Freshwater aschelminthes/nematod test (*Panagrellus*) ;
(e) Marine mollusc test (*Macoma*) ;
(f) Bacteria test (methanogenic bacteria).

An OECD Working Group on Aquatic Toxicity Testing discussed the DRP and proposed priorities for revision and development of Test Guidelines in the aquatic toxicity testing area.

With respect to the recommendations from the OECD Working Group on Aquatic Toxicity Testing (see "Final Report of the OECD Working Group Meeting on Aquatic Toxicity Testing" in this document), the following Test Guidelines were given high priority by the National Co-ordinators of the OECD Test Guidelines Programme (see "Recommendations for Guideline Revision and Development") and should be developed according to the indicated order (i.e. from the most to the least urgent):

Pelagic tests:

1. Crustacean, saltwater sp., acute and reproduction tests:
2. Higher plant, Kormophyta (*Lemna*), growth test;
3. Fish, full and/or partial life cycle test;
4. Microalgae, freshwater and saltwater sp., growth test – revision of TG 201;

5a. Mollusc, saltwater sp., acute on early life stages and shell deposition tests;

5b. Bacteria, sludge bacteria, nitrification test;

and revision of TG 202, Part I: *Daphnia* sp., Acute Immobilisation Test (test duration extended to 48 hours).

Benthic tests:

1. Insect (*Chironomus*), acute and chronic (growth and emergence) test;
2. Crustacean (amphipod), saltwater sp., acute and growth tests;

3a. Annelid, freshwater sp., acute and reproduction tests;

3b. Annelid, saltwater sp., acute test;

4. Crustacean, freshwater sp. (*Hyalella*), acute and growth tests.

Résumé

Une étude bibliographique a été menée afin de faire le point sur les méthodes d'évaluation de la toxicité aquatique des produits chimiques, qui comprennent des méthodes d'essai pour les milieux pélagique (eau) et benthique (sédiment). La recherche s'est limitée aux méthodes de laboratoire, excluant les études de terrain, et aux essais avec une seule espèce (ou des cultures mixtes dans le cas des microorganismes) excluant les essais avec plusieurs espèces et les études en microcosme et en mésocosme.

Le Document d'examen détaillé (DED) a pris en compte les méthodes normalisées nationales et internationales requises dans les systèmes d'essais pour l'évaluation des dangers et des risques des produits chimiques (produits chimiques industriels et pesticides), ainsi que les méthodes publiées et non publiées, décrivant des protocoles d'essais et indiquant des critères d'effet, appropriées au développement futur de Lignes directrices de l'OCDE. Près de 450 méthodes pélagiques et 260 méthodes benthiques developpées au cours de ces dix dernières années ont été examinées.

Les méthodes recueillies, qui couvrent divers niveaux trophiques et différents types d'effets, ont été regroupées selon huit scenarios basés sur (i) les compartiments environnementaux pélagique et benthique, (ii) les milieux marin et d'eau douce, et (iii) les régimes de température "chaud" et "froid".

Afin d'identifier les méthodes d'essai pouvant faire l'objet de futures Lignes directrices de l'OCDE pour les essais, les méthodes ainsi rassemblées ont été évaluées et notées en termes de (a) validité scientifique, sensibilité, spécificité et reproductibilité, et (b) contribution concernant des effets significatifs encore insuffisamment pris en compte dans les Lignes directrices de l'OCDE pour les essais. La procédure d'évaluation reposait sur l'ensemble de critères suivants :

- **faisabilité d'un point de vue pratique de la méthode** : réalisation technique, durée de l'essai, disponibilité et entretien de l'organisme d'essai, système d'exposition, coût de l'équipement et coût du travail ;

- **validité de la méthode** : reproductibilité, sources d'erreur potentielle, zone de tolérance aux conditions environnementales ;

- **utilité dans les pronostics** : représentativité géographique, représentativité écologique, extrapolation des effets, sensibilité générale, bien-fondé des voies d'exposition et des conditions d'essai ;

- **niveau de normalisation**.

Sur la base (1) des données requises dans les systèmes d'évaluation des dangers/risques, (2) de l'évaluation et la notation des méthodes d'essai, et (3) de considérations concernant le besoin potentiel d'essais de toxicité aquatique à des fins réglementaires, le DED fait un certain nombre de recommandations pour le développement de Lignes directrices de l'OCDE pour les essais dans les domaines pélagique et benthique. Ces recommandations sont groupées en trois catégories :

- **Première recommandation (Groupe 1) :** méthodes qu'il est recommandé d'inclure dans le Programme de l'OCDE sur les Lignes directrices pour les essais : elles sont nécessaires dans les systèmes existants d'évaluation des dangers/risques;

- **Seconde recommandation (Groupe 2) :** méthodes dont on présume qu'on le besoin futur pour le developpement de Lignes directrices : elles sont recommandées dans quelques systèmes d'évaluation nationaux et/ou sont considérées représenter des groupes écologiques clés;

- **Troisième recommandation (Groupe 3) :** méthodes qui ne sont pas nécessaires dans un avenir proche ou lointain.

Les recommandations issues de cette étude (cf. la section 7 du DED, "Recommendations") peuvent être résumées ainsi :

Essais pélagiques :

Groupe 1 :
(a) Essai sur plante supérieure d'eau douce (*Lemna*) ;
(b) Essai sur algue marine (*Skeletonema*, *Phaeodactylum*, etc.) ;
(c) Essai sur crustacé marin (*Acartia*, *Tisbe*, *Mysisdopsis*, etc.) ;
(d) Essai de cycle de vie sur poissons d'eau douce (*Danio*, *Pimephales*) et marin (*Cyprinodon*).

Groupe 2 :
(a) Essai sur plante supérieure marine (*Zostera*) ;
(b) Essai sur macroalgue marine (*Champia*, *Gracilera*) ;
(c) Essai aigu sur crustacé d'eau douce (*Daphnia*, *Ceriodaphnia*, *Neomysis*) ;
(d) Essai sur insecte d'eau douce (*Aedes*, *Acroneuria*, etc.) ;
(e) Essai sur amphibien (*Xenopus*, *Rana*) ;
(f) Essai sur rotifères d'eau douce et marin (*Brachionus*) ;
(g) Essai sur oursin de mer (*Echinodermata*, *Lytechinus*, etc.) ;
(h) Essai sur mollusque marin (*Crassostrea*, *Mytilus*) ;
(i) Essai sur protozoaires d'eau douce et marin (*Tetrahymena*) ;
(j) Essai sur bactéries (*Pseudomonas*).

Essais benthiques :

Groupe 1 :
(a) Essai aigu sur annélide marin (*Arenicola*) ;
(b) Essai aigu sur crustacé marin (*Corophium*) ;
(c) Essai aigu sur oursin de mer (*Lytechinus*, *Echinocardium*).

Groupe 2 :
(a) Essai aigu sur annélide d'eau douce (*Tubifex*, *Lumbriculus*, etc.) ;
(b) Essai sur crustacé d'eau douce (*Hyalella*, etc.) et marin (*Corophium*, etc.) ;
(c) Essais aigu et subchronique sur insecte d'eau douce (*Chironomus*) ;
(d) Essai sur aschelminthes/nématode d'eau douce (*Panagrellus*) ;
(e) Essai sur mollusque marin (*Macoma*) ;
(f) Essai sur bactéries (bactéries méthanogènes).

Un Groupe de travail de l'OCDE sur les essais de toxicité aquatique a discuté le DED et il a proposé des priorités pour la révision et le développement de Lignes directrices pour les essais dans le domaine de la toxicité aquatique.

En ce qui concerne les recommandations issues du groupe de travail de l'OCDE sur les essais de toxicité aquatique (cf. la section "Report of the OECD Working Group Meeting on Aquatic Toxicity Testing"), les Co-ordinateurs nationaux du programme de l'OCDE sur les Lignes directrices pour les essais ont accordé une forte priorité aux Lignes directrices suivantes (cf. la section "Recommendations for Guideline Revision and Development" ci-après) qui devraient être développées dans l'ordre indiqué (c'est-à-dire de la plus urgente à la moins urgente) :

Essais pélagiques :

1. Essais aigu et de reproduction sur crustacé d'eau de mer ;
2. Essai sur plante supérieure kormophyte (*Lemna*) ;
3. Essai de cycle de vie partiel et/ou complet sur poisson ;
4. Essai de croissance sur microalgues d'eau douce et d'eau de mer - revision de la Ligne directrice 201 ;

5a. Essais aigu sur les stades précoces de développement et de croissance de la coquille sur mollusque d'eau de mer ;

5b. Essai de nitrification sur bactéries des boues.

et révision de la Ligne directrice 202, Partie I: *Daphnia* sp., essai d'immobilisation immédiate (durée de l'essai étendue à 48 heures).

Essais benthiques :

1. Essais aigu et chronique (croissance et émergence) sur insecte (*Chironomus*) ;
2. Essais aigu et de croissance sur crustacé d'eau de mer (amphipode) ;

3a. Essais aigu et de reproduction sur annélide d'eau douce ;

3b. Essai aigu sur annélide d'eau de mer ;

4. Essais aigu et de croissance sur crustacé d'eau douce (*Hyalella*).

Recommendations for Guideline Revision and Development

There was a high degree of consensus from the National Co-ordinators of the OECD Test Guidelines Programme when they were asked to consider the recommendations made in the Detailed Review Paper and the subsequent proposals from the OECD Working Group on Aquatic Toxicity Testing. In general, Member countries agreed with both the proposed priority given to the various work items and the proposed actions.

The recommendations of the OECD Working Group Meeting in Copenhagen in June 1995, approved by the 6th Meeting of the National Co-ordinators of the Test Guidelines Programme in December 1995, with respect to the revision and development of OECD Test Guidelines on aquatic toxicity testing are summarised in **Tables R.1 and R.2** for pelagic and benthic tests, respectively.

In summary, the following tests were given a high priority for OECD Test Guideline development and are listed according to their priority (from the most urgent to the least urgent), as agreed upon by the National Co-ordinators of the Test Guidelines Programme:

Pelagic tests:

1. ➢ Crustacea, saltwater sp., acute and reproduction tests;
2. ➢ Higher plant, Kormophyta (*Lemna*), growth test;
3. ➢ Fish, full and/or partial life cycle test;
4. ➢ Microalgae, freshwater and saltwater sp., growth test – revision of TG 201;
5. ➢ Mollusca, saltwater sp., acute on ELS and shell deposition tests;
➢ Bacteria, sludge bacteria, nitrification test.

and revision of TG 202, Part I: *Daphnia* sp., 24h-EC_{50} Acute Immobilisation Test (48h study).

Benthic tests:

1. ➢ Insecta, *Chironomus*, acute and chronic (growth and emergence) tests;
2. ➢ Crustacea (amphipod), saltwater sp., acute and growth tests;
3. ➢ Annelida, freshwater sp., acute and reproduction tests;
➢ Annelida, saltwater sp., acute test;
4. ➢ Crustacea, freshwater sp. (*Hyalella*), acute and growth tests.

Table R1: Priorities for development/revision of OECD Test Guidelines for pelagic toxicity testing

Pelagic tests			Methods available	Action	Workload	Ring-test	Priority score (*)
Taxonomic group	**Species**	**Endpoints**					
Kormophyta	Freshwater species (*Lemna*)	Growth	ISO, AFNOR, US EPA, ASTM	New TG	Moderate	Possibly required	2
Microalgae	Freshwater species (add blue-greens, diatoms)	Growth	OECD 201	Revise 201	Moderate	Required	4
	Saltwater sp. (*Skeletonema, Gymnodium,* etc.)	Growth	OECD 201, ISO, ASTM, PARCOM	Revise 201	Moderate	Required	4
Crustacea	Freshwater species (*Daphnia*)	Acute (immobilisation) EC50-48h	OECD 202	Revise 202	Very small (editorial work)	Not required	–
	Saltwater sp. (*Tisbe, Nitocra, Mysidopsis, Acartia*, etc.)	Acute (survival) and reproduction	PARCOM, US EPA	New TG or Revise 202?	Large	Required	1
Fish	Freshwater and saltwater species	Life cycle (full and/or partial life cycle test)	US EPA	New TG	Very large	Required	3
Bacteria	Sludge bacteria	Nitrification	ISO	New TG	Moderate	Possibly required	5
Mollusca	Saltwater sp. (*Crassostrea, Mytilus, Mercanaria*)	Acute: ELS-48h and shell deposition-96h	TSCA, FIFRA, ASTM, US EPA	New TG (one TG)	Large	Required	5

(*): Priority score for development of OECD Test Guidelines from 1 (most urgent) to 5 (least urgent)

Table R2: Priorities for development/revision of OECD Test Guidelines for benthic toxicity testing

Benthic tests			Methods available	Action	Workload	Ring-test	Priority score (*)
Taxonomic group	**Species**	**Endpoints**					
Insecta	Freshwater species (*Chironomus*)	Acute (survival) and growth/emergence	ASTM, US EPA, BBA	New TG (one TG – separate tests for acute and chronic endpoints)	Moderate	Existing ring-tests (EU, BBA, US EPA)	1
Crustacea (Amphipod)	Freshwater species (*Hyalella*)	Acute (survival) and growth	ASTM, US EPA, Environment Canada	New TG (one TG – separate tests for acute and chronic endpoints)	Moderate	–	4
Crustacea (Amphipod)	Saltwater sp. (*Corophium, Ampelisca, Leptocheirus*)	Acute (survival)	Literature	New TG	Large	Existing ring-tests (EU, PARCOM)	2
Annelida	Freshwater sp. (e.g. *Tubifex, Lubriculus*)	Acute (survival) and reproduction	Literature	New TG (one TG – separate tests for acute and chronic endpoints)	Large	–	3
	saltwater sp. (e.g. *Arenicola, Neanthes*)	Acute (survival)	PARCOM	New TG	Large	–	3

(*): Priority score for development of OECD Test Guidelines from 1 (most urgent) to 4 (least urgent)

Work in Progress in the OECD Test Guidelines Programme

The development and revision of Test Guidelines for environmental aquatic toxicity is only one of the many areas covered by the OECD Test Guidelines Programme. For this reason, it is not possible to tackle immediately all those areas of environmental toxicity testing identified by Member countries as having high priority. At their 6th Meeting in December 1995, the National Co-ordinators of the Test Guidelines Programme agreed that work should focus initially on the development of a Guideline for aquatic higher plant (*Lemna* test) and Guideline(s) for sediment-dwelling organisms (Chironomidae test). Work on the revision of the *Daphnia*, Acute Immobilisation Test (OECD 202, Part I) should also be initiated.

Further, the 8th National Co-ordinators Meeting in April 1997 recommended that revision of the Alga, Growh Inhibition Test (OECD 201) be included in the 1997 workplan.

Other areas identified as being of high priority by the DRP, the Working Group and Member countries will be addressed as the work progresses.

PREFACE

At the Second Meeting of the National Co-ordinators of the OECD Test Guidelines Programme in September 1991, it was decided that a Detailed Review Paper (DRP) concerning aquatic ecotoxicity testing methods should be prepared. The purpose of the document was to assist the OECD National Co-ordinators in their discussions regarding the identification of aquatic toxicity test methods to be included in the OECD Test Guidelines Programme.

The Test Guidelines Programme deals with testing methods to be used for the effects, hazard and risk assessment of chemicals, to facilitate harmonized testing and assessment systems in OECD countries. Therefore, the recommendations to be made should take into consideration:

- the need for new testing guidelines as identified in the (draft) schemes for environmental effects, hazard and risk assessment of chemicals (industrial chemicals and pesticides);

- ecological considerations regarding the need for additional aquatic toxicity data for strengthening the predictive ability of the existing assessment schemes, including the refinement stage of the assessment;

- the state-of-the-art regarding existing standardised and non-standardised aquatic ecotoxicity testing methods which presently are not included in the OECD Test Guidelines Programme but which might be considered for future guideline development due to the testing methodology used and the organisms and toxicity endpoints studied.

An important objective of the Detailed Review Paper was to cover testing methods for the pelagic and the benthic (sediment) environment. The identified methods should be applicable to the testing of both industrial chemicals and pesticides.

The review paper has been limited to the investigation of testing methods at the organism/species level. Only for microorganisms have testing methods at community or higher organisational level been included. Methods which aim exclusively at investigating the effects measured on physiological, histological or biochemical endpoints have not been included.

The number of aquatic toxicity testing methods published within the last decade is enormous, and evaluating and compiling all of them would be an almost endless task. Thus, the methods included in the Detailed Review Paper have been restricted to:

1) nationally and internationally standardised methods;

2) methods (published or unpublished) in which the species tested or endpoint studied are not already represented in existing OECD Test Guidelines.

Papers reporting the use of already published standard test methods have not been included unless these papers introduced a major modification of the original method.

At an early stage, National Co-ordinators of the Test Guidelines Programme, experts from OECD countries, and the various national standardisation organisations were requested to forward papers that were of relevance to the Detailed Review Paper. Most OECD countries responded, and a

comprehensive number of papers were received (see Annex M for the list of contributors). These papers were supplemented with relevant aquatic toxicity testing methods which were collected from the scientific literature.

Two interim draft reports were prepared in October 1993 and August 1994, respectively. These drafts were circulated to selected experts in Germany, Norway, Sweden and the United States for comments. A final draft version of the DRP was completed in March 1995 and was circulated by the Secretariat to all OECD Member countries for comments in April 1995.

As a joint activity of the OECD Test Guidelines and Risk Assessment Programmes, a Working Group on Aquatic Toxicity Testing was established to (i) discuss the DRP and comments received from Member countries, and (ii) propose priorities for revision and development of Test Guidelines in the aquatic toxicity testing area. This OECD Working Group met in Copenhagen, Denmark, in June 1995. The report of the Working Group Meeting is included in this document.

In reviewing the DRP with respect to the evaluation of test methods, the Working Group Meeting discussed the definitions used in the DRP for (i) pelagic and benthic tests, (ii) warm and cold water temperatures, (iii) freshwater and marine environments, (iv) acute, subchronic and chronic effects, and (v) short-term and long-term duration. Although it was recognised that the definitions used were acceptable for the objective of the DRP, i.e. the identification of potential candidates for OECD Test Guideline development, a number of amendments to some definitions (pelagic and benthic tests, acute-subchronic-chronic effects) were proposed. In particular, it was recommended that (i) the term "subchronic" should not be used and should be replaced with "chronic", and (ii) the term "life-cycle/multigeneration" should be introduced.

The Working Group proposal for amendments to the definitions used in the DRP was submitted to the National Co-ordinators for consideration, with the request that they give their opinion on the changes necessary to the DRP. Responses received again gave evidence of discrepancy between the different approaches regarding the definitions and terminology used for characterising ecotoxicity tests in terms of acute-subchronic-chronic tests. It was deemed that, in the absence of scientific (and regulatory) consensus on this issue, the definitions used in the DRP (i) were useful and acceptable for the specific purpose of the DRP, and (ii) must not be regarded as "official" OECD terminology for use in the Test Guidelines.

This DRP is based on the compilation of more than 600 pelagic and benthic testing methods. It should be noted that the primary search for methods in the scientific literature was ended in 1993, and that methods published since have not been systematically compiled. In 1994 (and early 1995) a number of reports were issued from scientific workshops and from some national authorities. These reports have, as far as possible, been consulted and taken into account in the review.

The reader may notice that several test methods are discussed twice in the benthic chapters of the report. The benthic toxicity tests listed in Table 6.1 and discussed in Section 6.2 are standardised methods which are comparable to the pelagic standard methods discussed in Chapter 5. The benthic standard methods are also discussed in Sections 6.3-6.6. These sections contain all references for the methods, including non-standardised test methods and other scientific studies, which may be of interest for OECD Test Guideline development.

1. INTRODUCTION

1.1 Current use of OECD Test Guidelines and other standardised testing methods

Within the last decade, several aquatic ecotoxicological testing methods have been internationally standardised (OECD, ISO, EU) in order to comply with the need for internationally acceptable data for predicting the environmental effects, hazard and risk of chemical substances. Internationally standardised testing methods that are relevant to the aquatic environment are restricted to short-term chronic test methods on planktonic freshwater unicellular algae, short-term acute and long-term (sub)chronic[1] test methods on planktonic freshwater crustaceans (daphnids), and short-term acute and short-term and long-term subchronic test methods on freshwater fish. In addition to the internationally standardised methods, a number of testing methods on species representing other taxonomic groups than those above have been adopted as national standards for various purposes.

Aquatic effects, hazard and risk assessment strategies including all or some of the above types of methods are presently being discussed within the OECD (OECD 1994, 1995), in the United States (Landis et al. 1993, Cairns and Niederlehner 1995, SETAC 1994a), and in the EU (1993a, 1994) as well as in a number of other national and international fora.

The evaluation schemes adopted or discussed for the aquatic environment may be grouped into those focusing on:

- hazard identification, referring only to relevant intrinsic properties of chemicals. An example is the EU classification criteria for labelling of substances "dangerous for the environment"; (EC 1993b)

- effects assessment, e.g. for the establishment of surface water or sediment quality criteria (CSTE 1993, VROM 1994, US EPA 1986);

- generic risk assessment of chemicals and pesticides (e.g. OECD 1989a, EC 1993a, US EPA 1986, EC 1994, RIVM/VROM/WVC 1994);

- assessment of specific types of chemicals such as detergents and petroleum products (e.g. AIS 1992, CONCAWE 1991);

- assessments focusing on the specific use pattern of chemicals in specific types of aquatic environments (e.g. CONCAWE 1991, PARCOM 1993);

- assessments focusing on the discharge of complex effluents to specific types of environments (e.g. US EPA 1991, S-EPA 1990, DK-EPA 1994).

Data derived from short-term exposure studies on representatives of aquatic primary producers (planktonic algae), primary consumers (daphnids), and secondary (and tertiary) consumers (fish) are usually required at an initial screening level of the various assessment schemes. Testing methods applied

[1] Refer to Chapter 4 for definition of terms (*short-term, long-term, acute, subchronic* and *chronic*).

to substances (or mixtures) that, at the initial screening, have been identified as possessing a potential effect/hazard/risk for the aquatic environment are usually (sub)chronic short-term or long-term studies with the same three taxonomic groups as mentioned above, and often also the same species (all three species or the most sensitive species after short-term exposure).

In the first mentioned type of assessment schemes (hazard identification), only intrinsic properties already covered by existing OECD Test Guidelines are used.

Generic aquatic assessment schemes are also based on relatively few screening methods (and standard emission and generic exposure scenarios), which lead to an assessment of the potential "risk" to the aquatic environment in general or to the concerned aquatic environment. Although a risk quotient is elaborated in most of the generic assessment schemes, the final evaluation in terms of assessment of "risk" to the aquatic environment is very limited with regard to the actual prediction of the probability that an adverse ecological effect will occur. In practice, the generic risk assessment schemes may thus be regarded as "comprehensive chemical ranking or priority-setting systems" based on the principles of environmental risk assessment.

1.2 Generic effects and risk assessment schemes for the aquatic environment

Effect assessment may be defined as the identification and quantification of the (potential) adverse effects of chemical substances (OECD 1995) on individuals, populations, and, ideally, also on biological communities and ecosystems. The principles for assessing effects on the structure and dynamic function of populations, biological communities, and ecosystems or environmental compartments are therefore important but outside the scope of this document. The different approaches for deriving PEC (Predicted Environmental Concentration) (OECD 1995), estimating PNEC (Predicted Non Effect Concentration), and conducting generic hazard and risk assessment for the aquatic environment by use of assessment (application) factors or statistically based extrapolation methods have recently been comprehensively discussed elsewhere (OECD 1989a, 1989b, 1992a, 1993b, 1994a, 1995, EC 1993a, 1994, Linders et al. 1992, Lynch 1993, Zeeman 1995).

At the initial screening level, where testing of short-term toxicity to three species (algae, crustaceans and fish) is normally requested, PNEC is estimated by the application of a relatively high assessment factor to the most sensitive of the three tested species (OECD 1995, EC 1993a, EC 1994). The initial generic aquatic effect assessment, performed using this limited amount of data, is intended to identify a conservative and protective PNEC value. Should the estimated environmental concentration (PEC) be above the predicted No Effect Concentration (PNEC) of the ecosystem, additional data may be requested for refinement of the PNEC and/or the PEC.

Today, the testing methods normally applied for the refinement of the PNEC are restricted to relatively long-term aquatic toxicity studies on the same species as those applied at the initial screening level, as no other standard long-term test methods are available.

Refinement of the initial PNEC estimate may also be directed to special (aquatic) environmental compartments, dependent on the physico-chemical properties of the concerned chemical substance and the (standard) release scenario. Most assessment strategies point out especially the need for testing methods to assess the effect of sorptive chemicals on the benthic compartment (OECD 1995), but other aquatic ecosystems may be of concern in relation to the use and environmental release pattern of certain types of chemicals, such as those used for offshore oil activities or certain types of chemicals released into highly exposed aquatic environments (e.g. the Rhine, the Great Lakes, the Mediterranean, the North Sea, the Baltic Sea).

In an administrative context, generic aquatic effect assessments are normally not aimed at predicting the type of effect that might be observed in the environment. If identification of the type of effect were the object, a comprehensive testing programme would be necessary, involving the study of effect mechanisms and types of effects together with thresholds for both structural and functional aspects of concerned types of aquatic ecosystems, by employing single species testing (including physiological, morphological and biochemical studies) and mesocosm and probably also field studies.

1.3 Other types of assessment schemes for the aquatic environment

The assessment schemes, which go beyond the generic level, are based on, for example, the need for assessment of special groups of chemicals (e.g. detergents), special use pattern (e.g. use of chemicals for off-shore drilling and exploration), and/or special release scenarios (e.g. emission of waste water or drilling chemicals to defined types of aquatic environments).

For the assessment of specific groups of chemicals, the knowledge of the characteristic inherent properties of the group of chemicals may lead to a request for testing methods for special sensitive taxonomic groups or sensitive toxic endpoints, or for tests with a longer duration than the initial screening tests. Also, the testing of more than one representative from a special sensitive taxonomic group may be requested. Special knowledge of the use pattern may lead to a more precise estimate of the release volume, and thus lead to a request for a refined assessment based on more data compared to the initial assessment step. Knowledge of specific release scenarios, and thus of specific types of environments of concern, may lead to a request for the testing of organisms of specific ecological or commercial significance for the type of environment or environmental compartment in question (e.g. pelagic or benthic environment, freshwater or marine water, low or high temperature regime).

Certain aquatic risk assessment strategies allowing for assessments related to more specific aquatic environments or compartments of concern, e.g. sediments, cold waters and the marine environments, have recently been proposed (Norton et al. 1992, US EPA 1992a, UK 1993, OECD 1994a, 1995). The possibility of developing more relevant approaches is, however, limited in practice, as presently no international standardised test guidelines exist for meeting the data requirements of such schemes.

Recently, assessment schemes for the marine environment have been developed recommending the testing of organisms (marine algae, marine crustaceans and marine fish) that are not currently included in the OECD Test Guidelines Programme. The schemes will be applicable for environmental risk assessment of offshore chemicals and mud for the marine cold water environment (the North Sea) (PARCOM 1993). Likewise, assessment schemes for the evaluation of chemical substances adsorbing significantly onto sediments have recently been proposed by referring to data derived by employing benthic organisms (EC 1993a, EC 1994, CCME 1994, OECD 1992b).

The initial generic assessments may lead to an identification of environmental compartments of special concern, to taxonomic groups of special sensitivity, or, in general, to a request for a refined assessment. The above mentioned assessment schemes, which currently go beyond the generic step, may be used for identification of future needs and also for assessment steps beyond the existing generic assessment schemes. The present aquatic OECD Test Guidelines include primarily warm water species representative of the pelagic grazing food chain in freshwater systems. Therefore, test results obtained by employing these methods may primarily be applicable for generic aquatic effects/hazard/risk assessments and for assessment of risk to pelagic fresh and warm water grazing food chains of aquatic ecosystems.

Future test method requirements will depend on the aquatic effects, hazard and risk assessment strategies to be applied at the initial assessment level, as well as at successive levels. Thus, the identification of needs for future Test Guideline development should be guided by the already adopted assessment methods, as well as by the trends in the discussion on future hazard and risk assessment strategies. On the other hand, the recommendations made for the development of new Test Guidelines may also be considered when the data requirements and assessment strategies are to be updated in the future. It is a general observation that most of the existing (generic) assessment schemes have been strongly influenced by the practical possibilities provided by the existing OECD Test Guidelines and less influenced by the identified needs for data to fulfil the objectives of the schemes.

The data requirements, assessment and testing strategies applied today for industrial chemicals and pesticides are not very well co-ordinated in most countries; however, many attempts to improve the co-ordination of test guideline development and basic risk assessment principles have recently been made both nationally and internationally (OECD 1995). It is therefore assumed that in the future industrial chemicals and pesticides will be assessed using identical risk assessment principles, taking into consideration the differences in effect mechanisms, use-release-exposure patterns, and data availability and requirements.

1.4 Future requirements for aquatic ecotoxicity testing methods

Future needs for new aquatic test guidelines for effects and hazard or risk assessment of chemicals and pesticides will probably be directed to the following primary objectives:

- Aquatic effects, hazard and risk assessment schemes for certain types of aquatic environments may require toxicity data on organisms from the type of aquatic environment in question.

- Estimation of the aquatic effects of certain groups of chemicals may require additional data on a number of species from specially sensitive taxonomic groups.

- Refinement stages of generic aquatic effects, hazard and risk assessment schemes may require additional data (more species and/or longer duration of tests) for, for example, estimation of PNEC by statistical distribution methods (cf. OECD 1994a, EC 1994) or improvement of the significance of a statistical extrapolation.

Proposals for future test guidelines should be based on ecologically important taxonomic groups from a structural and functional point of view. It is likewise important that testing methods applied in the context of generic aquatic effects, hazard and risk assessments are applicable for routine laboratory testing and that the methods have a high degree of repeatability and reproducibility.

1.5 About this DRP

This Detailed Review Paper only includes methods that have already been standardised to some extent by national or international standardisation bodies, protocols not currently standardised but supported by sufficient data documenting their validity and performance (published in pre-reviewed international journals), and protocols recommended nationally for specific uses.

The paper is restricted to methods involving the exposure of organisms of similar age, and methods handling populations of single species/mixed cultures (microorganisms). Only laboratory methods are considered. Multispecies, microcosm and mesocosm methods (field and laboratory) are not

included in this review. Such methods were the object of recently published reports (WRC 1990, EC 1994, Emans et al. 1993, Okkerman et al. 1992, SETAC-Europe 1991, 1992, SETAC 1992, Tourt 1988, Van Leeuwen et al. 1994).

Physiological, morphological and biochemical methods are not dealt with in this paper. These types of methods have, besides detailed studies on mechanisms of toxic action (e.g. for establishing QSARs), primarily been applied in biological *in situ* monitoring as exposure biomarkers (US EPA 1990a, Peakall 1994, Goksøyr and Förlin 1992). Although the ability of some biomarkers to identify certain morphological, anatomical, physiological and/or biochemical stress to or impact on organisms when exposed to low concentrations of certain types of chemical substances may be important, a causality between such end-points and effect end-points at higher biological organisation levels needs further documentation. Most biomarkers are only sensitive to certain classes of chemicals and therefore in general are not applicable for the generic assessment of chemicals. Therefore, biomarkers have until now neither been included in the context of chemical legislation nor in the OECD Test Guidelines Programme. Some biomarkers may, however, be valuable as "early warning systems" when assessing chemicals with certain kinds of specific mode of toxic action (Bradbury et al. 1990, Pritchard 1993), even though they are left out of consideration in this paper.

An important objective of the DRP is to identify a number of methods for potential future Test Guideline development among the very high number of available testing methods. In order to meet a presently unknown future development in environmental effects, hazard and risk assessment schemes, methods have been identified within different environmental compartments (pelagic and benthic), environments (marine and freshwater) and temperature regimes ("warm" and "cold"). Within these eight environmental scenarios, testing methods have been identified covering various trophic levels as well as different types of endpoints. It has not been the intention to actually propose to the OECD Test Guidelines Programme that the development of methods within all eight scenarios should be initiated. Instead, this framework has been chosen to facilitate a selection of methods which may be applicable for the objectives of assessment schemes.

Due to the limited availability of standardised methods and protocols, the benthic part of this paper also includes research carried out in order to assess the toxicity of sediments. Appropriate recommendations from the recent Workshop on Sediment Toxicity Assessment (WOSTA workshop), assembled in a guidance document (SETAC, 1994b), are taken into account in the evaluation of the benthic test methods. Methods, protocols, and other references which were evaluated in order to recommend benthic toxicity tests included whole sediment, elutriate, porewater and sediment suspension methods. As benthic tests are to be asked for at higher levels of hazard or risk assessment schemes, with the objective of assessing the effect threshold to benthic species in whole sediment, the primary recommended methods only comprise whole sediment tests. Elutriate and porewater tests are to be considered useful in this context only as initial screening tests.

1.6 Outline of the procedure used for elaboration of the DRP

Recommendation of aquatic toxicity testing methods to be considered for the OECD Test Guidelines Programme has been based on two parallel activities (**Figure 1.1**):

A: Testing strategies included in aquatic effects, hazard and risk assessment procedures currently applied or under discussion for future application for industrial chemicals and pesticides/biocides, as well as chemical mixtures, were collected. Special emphasis has been given to data requirements of the strategies/procedures for the assessment of ecotoxicity effects. The schemes that recommend/request ecotoxicity data other than, or in

addition to, the acute and chronic toxicity to species recommended in the existing OECD Test Guidelines have primarily been highlighted.

B: Available aquatic testing methods were collected from National Co-ordinators of the OECD Test Guidelines Programme and other experts, from international and national standardisation organisations, and from international scientific journals. The methods were briefly evaluated with respect to their relevance in relation to the objectives of the Detailed Review Paper, the level of scientific documentation, and the novelty of the methods in relation to the existing OECD Test Guidelines. An important objective of the initial sorting process was to select methods involving organisms representing different taxonomic groups, trophic levels, feeding strategies, climatic zones and habitats in the pelagic and benthic compartments. The methods collected were further evaluated using a set of formalised criteria (as described in Chapter 4).

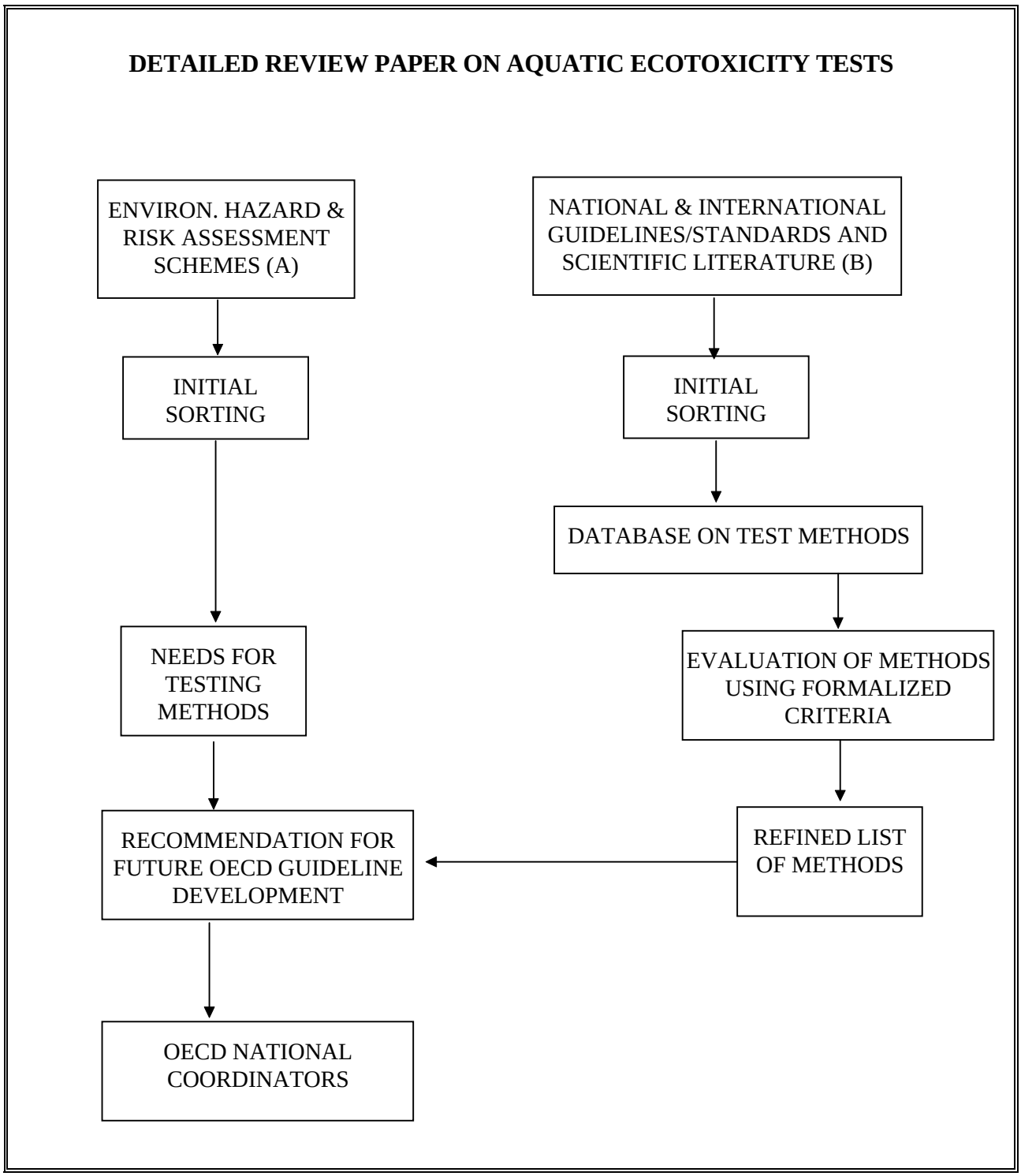
Based on the data requirements of currently available assessment schemes (Task A), on the list of testing methods (Task B), and on considerations regarding the potential future need for aquatic testing methods in effects, hazard and risk assessment schemes, methods have been recommended according to the following three categories:

- **Primary recommendation (Group 1):** Methods needed for existing effects, hazard and risk assessment (draft) schemes for chemicals and/or pesticides as adopted by international organisations (e.g. OECD, UN, PARCOM), communities (EU) and industrial organisations (e.g. ECETOC, AIS, CONCAWE). The methods identified are expected to require only a small amount of work in order to be ready for standardisation (Group 1a), or are expected to be ready for standardisation after sufficient scientific documentation has been provided (Group 1b). These methods should be considered for OECD ring-testing unless sufficient ring-testing has already been performed. An OECD Test Guidelines proposal should be drafted as soon as possible for methods in Group 1a, and in the near future for those in Group 1b.

- **Secondary recommendation (Group 2):** Methods presumably needed in the future, as they are recommended for the assessment of chemicals, pesticides and/or complex mixtures in national adopted or draft schemes for effects, hazard and risk assessment, or in schemes proposed in recent scientific literature. Methods that are considered to meet ecologically defined needs are also included. The methods identified are expected to require only a small amount of work in order to be ready for standardisation (Group 2a) or expected to be ready for standardisation after sufficient documentation has been provided (Group 2b). The drafting of an OECD Test Guideline should be considered in the future (Group 2a), or establishment of necessary scientific documentation should be prompted as the methods might be considered for Test Guideline development in the future (Group 2b).

- **Tertiary recommendation (Group 3):** No immediate or near future needs for the methods have been identified. The methods may be requested in special cases. They may be more or less ready for standardisation, but initiation of a standardisation process within the OECD Test Guidelines Programme is not recommended in the near future.

Figure 1.1: Flow chart outlining steps followed in the elaboration of this Detailed Review Paper



2. TESTING METHODS REQUESTED IN ENVIRONMENTAL EFFECTS, HAZARD AND RISK ASSESSMENT SCHEMES

Formalised and draft schemes for the assessment of environmental effects, hazard and/or risk of industrial chemicals and pesticides have been reviewed. The primary aim has been to identify the aquatic ecotoxicological testing methods currently recommended/requested to be used for deriving the data applied in these schemes. Relatively few schemes have currently been formalised in a regulatory context. Therefore, also draft schemes, which currently are under debate nationally as well as in international communities and organisations, and recent contributions from the scientific literature have been included. The reviewed schemes are listed in **Table 2.1**.

For the schemes focusing on the assessment of new chemicals within the EU (EC 1993a, ECETOC 1993 a.o.) only the OECD testing methods equivalent to present OECD Test Guidelines have been requested, although a need for additional testing of those substances having specific target environments (e.g. sediments) has been indicated. Most of the assessment schemes handling existing chemicals are primarily based on data from the currently adopted guidelines, although all existing data of sufficient quality are included in the assessment (e.g. OECD 1992a, UK 1993, van der Zandt and van Leeuwen 1992, RIVM, VROM, WVC 1994, OECD 1994a, 1994b).

Based on the gross list of effects, hazard and risk assessment schemes (Table 2.1), the following types of assessment schemes have been further outlined:

- schemes currently adopted in international organisations (e.g. OECD) and communities (e.g. the European Union) for initial generic assessment of new industrial chemicals. Although these schemes only recommend the current OECD Test Guidelines or standardised methods which are in accordance with the principles of the respective OECD Test Guidelines, the schemes have been outlined to serve as a background for comparison;

- schemes adopted in a national legislative framework or proposed for future updates of existing national or international schemes for assessment of chemicals or pesticides;

- adopted or proposed schemes for assessment of specific groups of chemicals (e.g. detergents, chemicals used for oil drilling and exploration) by international industrial organisations (e.g. AIS, CONCAWE);

- adopted or proposed schemes focusing on specific use patterns of chemicals in specific types of aquatic environments (e.g. CONCAWE, PARCOM);

- adopted or proposed schemes for the assessment of chemical mixtures (leachate, waste water) discharged to specific types of environments (e.g. US EPA, S-EPA, DK-EPA).

Of the four last types of schemes, only those that include recommendations for methods other than (or in addition to) the current OECD Test Guidelines (or equivalent methods) have been outlined.

Although testing methods and assessment schemes for complex mixtures are not currently included in the OECD work programme, these schemes have been outlined as well. The most interesting aspects of schemes for the assessment of mixtures are not the actual recommendations for the testing of

the mixtures, but the requirements and assessment framework for schemes focusing on specific types of environments and the recommendations made for the testing of organisms considered to be valid representatives of the specific types of environments or compartments in question. Thus the objectives for testing of mixtures and testing of specific groups of chemicals being emitted to specific types of environments (e.g. PARCOM) may be similar.

Testing methods applied for the assessment of chemical mixtures may also be applied for testing of single chemicals and pesticides as long as they comply with the requirements of an OECD Test Guideline.

The assessment schemes regarding the recommendations for testing methods, species, endpoints and guideline or protocol are outlined in Annex A (see the companion volume, *Part II: Annexes*). A short description of the tier or "trigger" system applied is also given.

In Tables **2.2-2.5**, the testing methods or testing requirements identified in the reviewed assessment schemes are compiled for fish, crustaceans, plants (algae, vascular plants) and "other" taxonomic groups. Specifications of the testing method recommended for each taxonomic group in the various schemes have been listed (Part a of the tables), as have the species recommended for testing (Part b of the tables). The duration of the test (long-term: >7 days; short-term: ≤ 7 days), endpoints applied (acute/subchronic/chronic), and the references of the methods have been included (refer to Chapter 4 for definitions).

As some of the schemes have identified test species from freshwater, brackish water and marine environments, this separation has been maintained in the tables, although only a distinction between marine (all saline environments) and freshwater species is made in the recommendations for development of new test guidelines. The distinction between the three types of aquatic environments, as well as between cold and warm temperature regimes, is not distinct for many species. Some of the species are normal residents in more than one type of environment (e.g. brackish and freshwater), and some are cosmopolites (e.g. found in both cold and warm marine environments all over the world). For andromous species (e.g. *Gasterosteus aculeatus, Salmo trutta*), the early life stages are found in freshwater and the juveniles/mature fish in a brackish (*Gasterosteus*) or the marine (*Salmo*) environment. In this review, the test temperature actually used in the test is applied for rubricating the organism if the preferable residential environment for the species is not distinct (e.g. *Daphnia magna* and *D. pulex*). For andromous species, the actual residence of the tested stage is used for rubrication of the species. Therefore, *Gasterosteus* is registered in brackish water for short-term acute toxicity studies and in freshwater for early life stage studies (Table 2.2b).

As would be expected, the endpoints currently applied for fish, crustaceans and algae in the OECD Test Guidelines (i.e. lethality, immobilisation and inhibition of growth, respectively, for the three taxonomic groups) are also applied in other standard methods with these taxonomic groups. However, the list of species recommended for testing in some of these methods is considerably different from in the equivalent OECD Test Guideline. Also, recommendations for taxonomic groups other than algae, crustaceans and fish have been identified (vascular plants, protozoans, rotifera, planaria, annelida, bivalvia, echinoidea, insecta and amphibia).

Table 2.1: Environmental effects, hazard and risk assessment schemes for the aquatic environment

Schemes in which recommendations/requests have been made for ecotoxicity methods other than those already adopted as OECD Test Guidelines are outlined in Annex A.

References to Annex A are given in brackets (C: Chemicals, P: Pesticides, W: Waste water).

TESTING SUBJECT	ASSESSMENT SCHEME
Industrial chemicals	**International communities/organisations/industrial organisations:** · OECD (1994a): Guidance Document for Aquatic Effects Assessment. OECD Environment Monograph No. 92. Paris **(C3)** · OECD (1994b): SIDS Manual. Screening Information Data Set Manual of the OECD Programme on the Co-operative Investigation of High Production Volume Chemicals. Revised draft, July 1994. · European Commission (EC) (1993): Risk Assessment of Notified New Substances, Technical Guidance Document. Technical Guidance Documents in support of the Risk Assessment Directive (93/67/EEC) for New Substances Notified in accordance with the Requirements of Council Directive 67/548/EEC, Brussels **(C2)** · European Commission (EC) (1994): Risk Assessment of Existing Substances, Technical Guidance Document (XI/919/94-EN). Technical Guidance Documents in support of the Commission Regulation (EC) No. 1488/94 on Risk Assessment of Existing Substances in Accordance with Council Regulation (EEC) No. 793/93 · PARCOM (1993): Harmonized system for the testing, evaluation and control of the use and discharge of chemicals offshore under the remit given to the Paris Commission in the Final Declaration of the Third North Sea Conference **(C8)** · AIS (1992): Practical aspects of environmental hazard assessment of detergent chemicals in Europe. Consensus draft from AIS 2nd workshop, Limelette, June 1992 **(C1)** · CONCAWE (1991): Ecotoxicological testing of petroleum products: A tier testing approach. Report No. 91/56, July 1991 **(C4)**

Table 2.1, continued

	National assessment schemes: · US Environmental Protection Agency (1985): Guidelines for deriving numerical national water quality criteria **(C6)** · US Environmental Protection Agency (1988): Toxicity Substances Control Act (TSCA), Code of Federal Regulations 40, July 1988 **(C9)** · RIVM, WROM, WRC (1994): Uniform System for the Evaluation of Substances (USES), version 1.0. National Institute of Public Health and Environmental Protection (RIVM), Ministry of Housing, Spatial Planning and the Environment (VROM), Ministry of Welfare, Health and Cultural Affairs (WVC). The Hague, Ministry of Housing, Spatial Planning and the Environment. Distribution No. 11144/150
	Scientific contributions: · Landner, L. (1989): Systems for Testing and Hazard Evaluation of Chemicals in the Aquatic Environment (ESTER). A manual for an initial assessment. KEMI Report No. 4/89 **(C5)** · Walker, J. (1990): Chemical fate, bioconcentration and environmental effects testing: Proposed testing and decision criteria. Toxicity Assessment: An International Journal, Vol. 5, pp. 103-134 **(C7)**

Table 2.1, continued

Pesticides	**International communities/organisations/industrial organisations:** · EPPO (1991): Proposal for aquatic organism risk assessment scheme for pesticides. Report of the subgroup on aquatic organisms, July 1991 **(P1)** · Lynch, M. (1993): Study concerning the inclusion of active substances in Annex 1 to Council Dir. 91/414/EEC (January 1993) **(P6)** **National assessment schemes:** · Environment Canada (1993): C. Boutin, K.E. Freemark and C.J. Keddy, Proposed Guideline for the registration of chemical pesticides: Nontarget plant testing and evaluation. Technical Report Series No. 145. Headquarters 1993, Canadian Wildlife Service **(P3)** · Germany (1993): Criteria for assessment of plant protection products in the registration procedure. *Mitteilungen aus der BBA*, Heft 285, Berlin **(P2)** · USA (1990): Pesticide Assessment Guidelines, Hazard Evaluation: Wildlife and Aquatic Organisms. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Code of Federal Regulations, 40 CFR 162, Subdivision E **(P7)** **Scientific contributions:** · Klein, A.W. and J. Goedicke (1993): Environmental assessment of pesticides under Directive 91/414/EEC **(P4)** · Linders, J., H. Clausen, C. Hansen, A. Klein and W. Klein (1992): Environmental criteria for pesticides. Recommendations from the Northern European Workshop on Environmental Hazard and Risk Assessment of Pesticides, Bilthoven, the Netherlands, 23-25 March 1992 **(P5)** · USA (1991): Technical support document for water quality-based toxic control (EPA/505/2-90-001), March 1991 **(W1)** · Sweden (1990): Biological-chemical characterisation of industrial waste water. Swedish Environmental Protection Agency **(W3)**

Table 2.1, continued

	· Denmark (1994): Technical guideline for hazard and risk assessment of industrial effluents. Environmental project report No. 256 Danish Environmental Protection Agency **(W2)** · United States Environmental Protection Agency (US EPA) and US Army Corps of Engineers: Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual. Inland Testing Manual (Draft), EPA-823-B-94-002, 1994 **(W4)** · Keddy, C., J.C. Greene and M.A. Bonnell (1994): A review of whole organism bioassays for assessing the quality of soil, freshwater sediment and freshwater in Canada. The National Contaminated Sites Remediation Programme. Environment Canada, Ecosystem Conservation Directorate, Evaluation and Interpretation Branch, Ottawa, Ontario, Scientific Series No. 198 **(W5)** · US EPA (1994): EPA's Contaminated Sediment Management Strategy. US Environmental Protection Agency, Office of Water. EPA 823-R-94-001, August 1994 **(W6)**

Table 2.2a: Ecotoxicity testing methods for fish, as recommended for environmental hazard and risk assessments

ST: short-term exposure (≤ 7 days); LT: long-term exposure (> 7 days); AC: acute effects (lethal or sublethal effects registered after a short exposure period related to the life span of the organism); C: chronic effects (effects observed after an exposure period covering the entire life span of the organism); SC: sub-chronic effects (effects observed after an exposure period covering a significant part of the life cycle or covering life stages or life processes believed to be especially sensitive). References to Annex A are given in / /. (C: Industrial chemicals; P: Pesticides; W: Waste water.)

TAX. GROUP	METHOD/ ENDPOINT	TEST DURATION & TYPE OF TEST		APPLICATION	REF. OF METHOD	ASSESSMENT SCHEME
BONE FISH	reproduction	ST	SC	chemicals, level 2 or 3	n.d.	EC 1993 **/C2/**
				waste w., confirmatory test	Landner (1985)	SW-EPA 1990 **/W3/**
	egg & sacfry	ST	SC	existing chemicals, scoring	ISO-dr.	ESTHER 1990 **/C5/**
				Detergents, confirmatory, tier 2	OECD draft	AIS 1992 **/C1/**
				chemicals, level 1	OECD-dr	EC 1993 **/C2/**
				waste w., refinement of PNEC	OECD-dr	DK-EPA 1994 **/W4/**
				waste w., refinement of PNEC	ISO-dr	S-EPA 1990 **/W3/**
	repro., egg & sacfry	LT	SC	existing chemicals, scoring	n.d.	ESTHER 1990 **/C5/**
	embryo-larvae	ST (7d)	SC	waste water, dilution < 100:1	US EPA	US EPA 1991 **/W1/**
				waste w., refinement of PNEC	US EPA	S-EPA 1990 **/W3/**
	growth test	LT (28d)	SC	chemicals, level 1	OECD-dr	EC 1993 **/C2/**
				pesticides, refinement level	OECD-dr	EPPO 1991 **/P1/**
				pesticides, continued exp.	OECD-dr	Lynch 1993 **/P6/**
	partial life cycle	LT	SC	new and existing chemicals, tier 2	ASTM, EPA	Walker 1990 **/C7/**
	life cycle	LT	C	chemicals, level 2 or 3	n.d.	EC 1993 **/C2/**
				oil chemicals, tier 3	n.d.	CONCAWE 1991 **/C4/**
				existing chemicals, scoring	n.d.	ESTHER 1990 **/C5/**
				pesticides, tier 3	n.d.	US-FIFRA 1990 **/P7/**

Table 2.2b: Compilation of fish species recommended for testing in relation to different testing methods

Bolding indicates species recommended in current OECD Test Guidelines or draft guidelines (w: warm water species; c: cold water species; n.d.: not defined).

METHOD/ END POINT	FRESHWATER	BRACKISH WATER	MARINE WATER
acute lethal	***Brachydanio rerio* (w), *Pimephales promelas* (w), *Oryzias latipes* (w), *Poecilia reticulata* (w), *Lepomis macrochirus* (w), *Oncorhynchus mykiss* (c), *Cyprinus carpio* (c)**, *Rasbora heteromorpha* (harlequin, w), *Salmo salar* (brown trout, c), *Ictalurus punctatus* (channel catfish, w), *Salvelinus fontinalis* (brook trout, c), *Perca fluviatilis* (perch, c), *Gasterosteus aculeatus* (stickleback, c)	*Alburnus alburnus* (bleak, c), *Clupea harengus* (herring, c), *Platichthys flesus* (flounder, c), *Gasterosteus aculeatus* (stickleback, c)	*Cyprinodon variegatus* (sheepshead minnow, w), *Menidia beryllina*, *M. medinia*, *M. peninsulae* (silversides, w), *Platichthys flesus* (flounder, c), *Clupea harengus* (herring, c), *Scophthalmus maximus* (turbot, c), *Limanda limanda* (dab, c), *Citharicthys stigmaeus*, *Leuresthes tenuis*
reproduction	n.d.	n.d.	n.d.
egg & sacfry (9-11 d)	***Brachydanio rerio* (w), *Pimephales promelas* (w), *Carassius auratus* (w), *Lepomis macrochirus* (w), *O. mykiss* (c), *Cyprinus carpio* (c),**	*Platichthys flesus* (flounder, c), *Clupea harengus* (herring, c)	***Menidia peninsulae* (w), *Clupea harengus* (c), *Gadus morhua* (c)**, *P. flesus* (c), *Scophthalmus maximus* (turbot, c)
repro., egg & sacfry	*Brachydanio rerio* (w)	n.d.	n.d.
embryo-larvae (7d)	*Pimephales promelas* (w)	n.d.	*Cyprinodon variegatus* (sheepshead minnow, w), *Menidia beryllina* (inland silverside, w)
Early life stage test	***O. mykiss* (c)**, ***Pimephales promelas* (w)**, ***Brachydanio rerio* (w)**, ***Oryzias latipes* (w)**, ***Oncorhynchus kisutch* (coho salmon, w)**, ***O. tschawytscha* (chinook salmon, w), *Salmo trutta* (brown trout, c), *Salvelinus fontinalis* (brook trout, c)**, ***S. namaycush* (lake trout, c)**, ***Esox lucius* (northern pike, c)**, ***Catostomus commersoni* (White sucker, ? w)**, ***Lepomis macrochirus* (bluegill, w)**, ***Ictalurus punctatus* (channel catfish, w)**, ***Jordanella floridae* (flagfish, w), *Gasterosteus aculeatus* (three-spined stickleback, c), *Cyprinus carpio* (common carp, c)**	n.d.	***Menidia menidia* (Atlantic silverside, w), *M.. peninsulae* (tidewater silverside, w)**
growth test	***O. mykiss* (c), *B. rerio* (w)**	n.d.	n.d.
partial life cycle	n.d.	n.d.	n.d.
life cycle	n.d.	n.d.	n.d.

Table 2.3a: Ecotoxicological testing methods for crustaceans as recommended for environmental hazard or risk assessment schemes

ST: short-term exposure (≤ 7 days); LT: long-term exposure (> 7 days); AC: acute effects (lethal or sublethal effects registered after a short exposure period related to the life span of the organism); C: chronic effects (effects observed after an exposure period covering the entire life span of the organism). SC: sub-chronic effects (effects observed after an exposure period covering a considerable part of the life cycle or covering life stages or life processes believed to be specially sensitive). References to Annex A are given in / /. (C: Industrial chemicals; P: Pesticides; W: Waste water.)

TAX. GROUP	METHOD/ ENDPOINT	TEST DURATION & TYPE OF TEST		APPLICATION	REF. OF METHOD	ASSESSMENT SCHEME
CRUSTA-CEANS	acute toxicity	ST (24-96h)	LC-50, inhibition	most assessment schemes at screening level (*Daphnia* a.o.)	OECD a.o.	–
		ST (benthic) (10d)	LC-50	whole sediment test, screening test	Env-Can	Env-Can 1994 **/W5/** US EPA 1994 **/W6/**
		LT (benthic) (28d)	AC	whole sediment test, definitive test	Env-Can	Env-Can 1994 **/W5/**
	reproduc. & early devel.	ST (7d)	SC	waste water, dilution < 1:100 (*Ceriodaphnia*)	US EPA	US EPA 1991 **/W1/**
				waste water, refinement stage (*Ceriodaphnia*)	US EPA	S-EPA 1990 **/W3/**
	reproduc. & devel.	LT (14d)	SC	new and exist. chemicals, tier 3 (*Daphnia* sp.)	US EPA	US EPA 1988 **/C9/**
				pesticides, continued exposure (*Daphnia magna*)	n.d.	Lynch 1993 **/P6/**
				waste water, Stage 2, marine env. (*Nitocra spinipes*)	National Stand.	S-EPA 1990 **/W3/**
				waste water, tier 2, brackish env. (*Nitocra spinipes*)	DS 2209	DK-EPA 1994 **/W2/**
	life cycle	LT (21-28d)	C	most assessment schemes at refinement stage (*Daphnia* a.o.)	OECD a.o.	–

Table 2.3b: Compilation of crustacean species recommended for testing in relation to different testing methods

Bolding represents species recommended in current OECD Test Guidelines
(w: warm water species; c: cold water species; n.d.: not defined).

METHOD/ ENDPOINT	FRESHWATER	BRACKISH WATER	MARINE WATER
PELAGIC ENVIRONMENT			
Acute toxicity	***Daphnia magna* (w)**, *D. pulex* (w), *Ceriodaphnia dubia* (w), *Gammarus fasciatus* (c), *G. Pseudolimnaeus* (c), *G. lacusstris* (c)	*Nitocra spinipes* (c), *Crangon crangon* (c), *Hyalella azteca, Penaeus aztecus* (brown shrimp, c), *P. duorarum* (pink shrimp, c), *P. setiferus* (c)	*Mysidopsis bahia* (w), *Acartia tonsa* (c), *Tisbe battagliai* (c), *Neomysis americana, Holmesimysis costata, Palaemonetes* sp.
Reproduction & early development	*Ceriodaphnia dubia* (w)	n.d.	*Mysidopsis bahia* (w)
Reproduction & development	***Daphnia magna***, *D. pulex*	*Nitocra spinipes* (c)	n.d.
Life cycle	***Daphnia magna***, *D. pulex*	n.d.	*Mysidopsis bahia* (w), *Acartia tonsa* (c)
BENTHIC ENVIRONMENT			
Acute toxicity	(*Daphnia magna*), *Hyalella azteca*	*Nitocra* spinipes (c), *Corophium insidiosum* (c), *Eohaustorius plumulosus*	*Corophium volutator* (c), *Ampelisca abdita, Rhepoxynius abronius, Leptocheirus plumulosus*

Table 2.4a: Ecotoxicological testing methods for algae and vascular plants as recommended for environmental hazard or risk assessment schemes

ST: short-term exposure (≤ 7 days); LT: long-term exposure (> 7 days); AC: acute effects (lethal or sublethal effects registered after a short exposure period related to the life span of the organism); C: chronic effects (effects observed after an exposure period covering the entire life span of the organism); SC: subchronic effects (effects observed after an exposure period covering a considerable part of the life cycle or covering life stages or life processes believed to be specially sensitive). References to Annex A are given in / /. (C: Industrial chemicals; P: Pesticides; W: Waste water.)

TAX. GROUP	METHOD/ ENDPOINT	TEST DURATION & TYPE OF TEST		APPLICATION	REF. OF METHOD	ASSESSMENT SCHEME
Microalgae	growth inhibition	ST	C	most schemes	OECD a.o.	OECD a.o.
	test battery	ST	(C)	waste water, stage 2	Claesson (1986)	S-EPA 1990 **/W3/**
Macroalgae	fertilization	ST (7-9d)	SC	waste water, refinement of PNEC	US EPA	US EPA 1991 **/W1/**
	reproduction	ST (24h)	SC	waste water, refinement of PNEC	US EPA	US EPA 1991 **/W1/**
Vascular plants	growth (*Lemna* sp.)	ST	SC	chemical scoring	n.d.	ESTHER 1989 **/C5/**
				water quality criteria	ASTM, EPA	Stephan 1985 **/C6/**
				chemicals, tier 2	US EPA	US EPA 1988 **/C9/**
				pesticides, refinement of PNEC	US EPA	EPPO 1991 **/P1/**
				pesticides, tier 2	n.d.	Canada 1993 **/P3/**
				waste water, tier 2	US EPA	DK-EPA 1994 **/W2/**
				waste water, stage 1	SIS-dr	S-EPA 1990 **/W3/**
				leachate, contaminated site, definitive test	ASTM, US EPA	Env-Can **/W5/**
	growth, 30 species	n.d.	n.d.	pesticides, tier 1	US EPA	Canada 1993 **/P3/**
	growth, rooted submerged	n.d.	n.d.	pesticides, tier 3	n.d.	Canada 1993 **/P3/**
		LT (28d)	SC	waste water, tier 2 (*Zostera marina*)	protocol	DK-EPA 1994 **/W2/**
	growth, emergent plants	n.d.	n.d.	pesticides, tier 3	n.d.	Canada 1993 **/P3/**

Table 2.4b: Compilation of algae and vascular plant species recommended for testing in relation to different testing methods

Bolding indicates species recommended in current OECD Test Guidelines
(w: warm water species; c: cold water species).

METHOD/ ENDPOINT	FRESHWATER	BRACKISH WATER	MARINE WATER
microalgae, growth	***Selenastrum capricornutum*** (green algae), *Scenedesmus quadricauda*, ***S. subspicatus***, ***Chlorella vulgaris***, *Anabaena, Navicula, Nitzschia palea, Monoraphidium griphitti, Microcystis* sp.	–	*Skeletonema costatum* (diatom), *Phaeodactylum tricornutum, Thallassiosira pseudonana, Isochrysis balbana*
macroalgae, fertilization	n.d.	n.d.	*Champia parvula* (red algae)
macroalgae, reproduction	n.d.	n.d.	*Laminaria saccharina* (kelp)
vascular plants, growth	*Lemna minor, Lemna gibba*	*Zostera marina*	*Zostera marina*

Table 2.5a: Ecotoxicity testing requirements/recommendations for taxonomic groups other than fish, crustaceans, algae and plants

ST: short-term exposure (≤ 7 days); LT: long-term exposure (> 7 days); AC: acute effects (lethal or sublethal effects registered after a short exposure period related to the life span of the organism); C: chronic effects (effects observed after an exposure period covering the entire life span of the organism); SC: sub-chronic effects (effects observed after an exposure period covering a considerable part of the life cycle or covering life stages or life processes believed to be specially sensitive). References to Annex A are given in / /. (C: Industrial chemicals, P: Pesticides, W: Waste water.)

TAX. GROUP	METHOD/ ENDPOINT	TEST DURATION & TYPE OF TEST		APPLICATION	REF. OF METHOD	ASSESSMENT SCHEME
Bacteria	nitrification	ST	AC	waste water, stage 1	ISO	S-EPA 1990 **/W3/**
	sludge resp.	ST	AC	waste water, stage 1	OECD	S-EPA 1990 **/W3/**
	P. putida, growth	ST (72h)	C	contaminated water	ISO (1991)	Env-Can 1994 **/W5/**
				waste water, tier 2	ISO-dr	DK-EPA 1994 **/W2/**
	Microtox^{R}	ST	AC	chemical scoring	ISO-dr	ESTHER 1989 **/C5/**
				contaminated water	Env-Can	Env-Can 1994 **/W5/**
				waste water, tier 2	ISO-dr	DK-EPA 1994 **/W2/**
	n.d.	n.d.	n.d.	contaminated water/ sediment	n.d.	Env-Can 1994 **/W5/**
				oil chemicals, tier 2	n.d.	CONCAWE 1991 **/C4/**
Protozoa	growth inhib	ST	C	waste water, tier 2	–	DK-EPA 1994 **/W2/**
Rotifera	IC-50	ST (24h)	AC	screening, contaminated water	Snell & Persoone (1989)	Env-Can 1994 **/W5/**
	reproduction	ST (48h)	SC	definitive test, cont. water	ASTM draft	Env-Can 1994 **/W5/**
	n.d.	n.d.	n.d.	water quality criteria	ASTM	Stephan 1985 **/C6/**
Planaria	n.d.	n.d.	n.d.	waste water, tier 2	n.d.	DK-EPA 1994 **/W2/**
Annelida	n.d.	n.d.	n.d.	water quality criteria	n.d.	Stephan 1985 **/C6/**
	reproduction	n.d.	n.d.	definitive, cont. water	ASTM draft	Env-Can, 1994 **/W5/**
	survival	ST (10d)	AC	waste water, tier 2 (sediment)	Thain (1990, 1991)	DK-EPA 1994 **/W2/**

Table 2.5a, continued

Annelida	n.d.	n.d.	n.d.	waste water, tier 3 (sediment)	Wiederholm (1989)	S-EPA 1990 **/W3/**
Bivalvia	n.d.	n.d.	n.d.	water quality cr.	n.d.	Stephan 1985 **/C6/**
	shell growth	ST	SC	chemicals, tier 2	US EPA	US EPA 1988 **/C9/**
				pesticides, tier 2	ASTM	US EPA 1990 **/P7/**
				emission, dredged materials	ASTM	US EPA 1994 **/W5/**
				waste water, tier 2	Strømgren (1993)	DK-EPA 1994 **/W2/**
	larvae survival	ST	SC	pesticides, tier 2	ASTM	US EPA 1990 **/P7/**
				waste water, tier 2	SNV (83)	S-EPA 1990 **/W3/**
				waste water tier 2	n.d.	DK-EPA 1994 **/W2/**
Echinoidea	survival	LT (21d)	SC	oil chemicals, sediments	PARCOM-dr	PARCOM 1993 **/C8/**
				waste water, tier 2, sed.	PARCOM-dr	DK-EPA 1994 **/W2/**
	survival, embryo develop.	ST (48h)	SC	emission, dredged matr.	US EPA	US EPA 1994 **/W4/**
		ST (80min)	SC	emission, dredged matr.	ASTM	US EPA 1994 **/W4/**
	fertilization	ST (<2h)	SC	waste water, refinement of PNEC	US EPA-dr	US EPA 1991 **/W1/**
Insecta	larvae survival	ST	AC	water quality criteria	ASTM	Stephan 1985 **/C6/**
				chemicals, tier 2, sediments	Swartz (1985) a.o.	Walker 1990 **/C7/**
				chemicals, tier 1,2, sed.	US EPA	US EPA 1988 **/C9/**
				pesticides, sediments	n.d.	Germany 1993 **/P2/**
				pesticides, tier 3, sed.	n.d.	US EPA 1990 **/P7/**
				waste water, tier 3, sed.	ASTM a.o.	DK-EPA 1994 **/W2/**
		ST (benthic, 10 days)	AC	contam. water, screening	ASTM a.o.	Env-Can, 1994 **/W5/**
				dredged material	EPA	US EPA, 1994 **/W6/**
Amphibia	survival, develop.	LT	SC	water quality criteria	ASTM (96h)	Stephan 1985 **/C6/**
				chemicals, tier 2, sed.	US EPA-dr (30 d)	Walker 1990 **/C7/**
				chemicals, tier 2, sed.	US EPA-dr	US EPA 1988 **/C9/**

Table 2.5b: Recommended species other than fish, crustacean, algae and vascular plants for the various assessment schemes

Bolding indicates that an OECD Test Guideline or draft guideline already exists, including the species/community in question.

TAX. GROUP	METHOD/ ENDPOINT	FRESHWATER	BRACKISH WATER	MARINE WATER
PELAGIC ENVIRONMENT				
Bacteria	nitrificat.	activated sludge	n.d.	n.d.
	respiration	activated sludge	n.d.	n.d.
	growth	*Pseudomonas putidae*	n.d.	
	luminescence			*Photobacterium* sp. ("Microtox")
Protozoa	growth	*Tetrahymena* sp.	n.d.	*Uronema marina*
Rotifera	growth	*Brachionus* sp., *B. calyciflorus*	n.d.	n.d.
Bivalvia	larvae survival	n.d.	n.d.	*Crassostrea* sp., *Mytilus edulis*
Echinoidea	fertilization, acute tox./ embryo dev.	–	n.d.	*Strongylocentrotus* sp., *Lytechinus pictus*, *Dendraster* sp.
Insecta	survival	*Baetis rhodani*, *Cloeon bipunctata*	–	–
Amphibia	survival, developm.	tadpole short-term	–	–
BENTHIC ENVIRONMENT				
Bacteria	nitrificat.	natural community	n.d.	n.d.
Bacteria	respiration	natural community	n.d.	n.d.
Planaria	growth	n.d.	n.d.	n.d.
Annelida	survival	*Tubifex tubifex*, *Pristina leidyi*	n.d.	*Arenicola marina*, *Nereis virens*, *Neanthes arenaceodentata*
Bivalvia	shell growth	*Unio* sp., *Anodonta imbecillis*	n.d.	*Crassostrea virginica* (eastern oyster), *C. gigas* (Pacific oyster), *Mercenaria mercenaria* (hard clam), *Mytilus edulis* (blue mussel), *Abra alba*
Echinoidea	survival	–	n.d.	*Echinocardium cordatum*
Insecta	survival	*Chironomus tentans*, *C. riparus*, *Hexagenia limbata*	–	–
Amphibia	long-term, survival, develop. (21d)	tadpole	–	–

In most of the schemes, a possibility of requesting additional and specific ecotoxicity data is indicated according to case-by-case procedures. The trigger for requesting additional data is either based on a need for refining the PNEC (lowering the assessment factor when toxicity data are available for more species representing other taxa than fish, algae and crustaceans) or for deriving a PNEC for an environmental compartment other than the freshwater pelagic environment (i.e. the benthic compartment or marine environments). The identified "non-specific" requirements/recommen dations are given below.

Refinement of the PNEC for the pelagic environment [references to Annex A, where the assessment schemes are outlined, are given in brackets (C: Industrial chemicals, P: Pesticides, W: Waste water)]:

- AIS (detergents): chronic toxicity to two species from taxa other than the fish, crustaceans and algae applied at the initial step (tier 2) (**C1**)

- EC (technical guidance to Dir 93/67/EEC): additional testing to species other than those applied at the base set level (level 1, 2) and testing another species of algae, should algae be the most sensitive of the three species tested at base set level (**C2**)

- OECD (guidance for aquatic effects assessment): chronic toxicity to two species representing taxa other than those applied at the initial level of testing (**C3**)

- CONCAWE (petroleum products): toxicity to species other than the fish, crustaceans and algae applied at tier 1 (tier 2 data) (**C4**)

- Walker (1990), US EPA (1988) (industrial chemicals, TSCA): for most sensitive species at tier 1, another species from this trophic level/taxa should be tested for acute toxicity (level 1, 2) (**C7**)

- EPPO (pesticide registration): acute and/or chronic toxicity to other invertebrates than those applied at base set level (tier 2) (**P1**)

- Lynch (uniform principles discussion paper for pesticides): toxicity to non-target organisms at risk other than those tested at the initial level (acute and chronic toxicity to algae, crustaceans and fish) (**P6**)

- Klein (1993), Linders (1992) (discussion papers related to EC Uniform Principles for pesticides): acute and chronic toxicity to species other than algae, crustaceans and fish. Should represent the environment of concern (**P4, P5**)

Elaborating a PNEC for a specific environment of concern:

- AIS: chronic toxicity to "relevant" biota (tier 3) (**C1**)

- CONCAWE: toxicity to sediment-living organisms (tier 2) (**C4**)

- Walker (1990), US EPA (1988): toxicity to benthic organisms (tier 2) (**C7, C9**)

- PARCOM (oil, chemicals discharged offshore): toxicity to marine sediment reworker species (**C8**)

- Lynch: toxicity to other non-target organisms at risk (also environments at risk?) (**P6**)
- BBA (registration of pesticides, Germany): toxicity to benthic organisms (**P2**)
- Klein (1993), Linders (1992): acute and chronic toxicity to two (other) species representing the environment of concern (**P4, P5**)
- FIFRA (registration of pesticides, USA): organisms applied for testing are selected based on the environment of concern (tier 2) (**P7**)
- US EPA (waste water permits): organisms representing the marine or freshwater environment (pelagic/sediment), depending on the environment of concern (**W1**)
- S-EPA (Sweden, industrial effluents): testing requirement for specific environments decided according to case-by-case procedures (Stage 3) (**W3**)
- DK-EPA (Denmark, industrial effluents): organisms representing the freshwater, brackish or marine environment (pelagic/sediment), depending on the environment of concern (**W2**)

The non-specific requirements/recommendations end up in the following general requests when a refinement of the PNEC is needed (higher testing levels than the initial screening level):

- testing one to two species from taxonomic groups other than fish, crustaceans and algae (pelagic environment) (seven schemes)
- testing species from an environment/compartment of concern (twelve schemes)
  - benthic organisms when the sediment is of concern
  - marine organisms when the marine environment is of concern

At present, the OECD Test Guidelines include testing regimes and organisms of relevance primarily for the pelagic freshwater environment.

Comparing the existing OECD Test Guidelines (and the current drafts) with the requirements identified above in the various assessment schemes, the potential needs for developing new guidelines or amending the existing guidelines to cover more species may be identified for the pelagic and benthic compartment (**Tables 2.6 and 2.7**).

Although the testing requirements are primarily focused on the assessment of the threshold of toxicity to the "natural" aquatic environment, the testing of toxicity to bacteria (and possibly also the request in some schemes for testing protozoa) is generally aimed at the protection of waste water treatment plants. The international standardised methods for bacteria, the sludge respiration test (OECD, ISO, EU), and the nitrification inhibition test (ISO) use activated sludge as biomass for the tests. As there may be a future need for further testing methods for the assessment of risk to this biological compartment (e.g. anaerobic inhibition tests, biological phosphorous removal), the biological waste water treatment step should also be defined as an important compartment in line with the above eight "natural" compartments. On the other hand, there is also a need for the development of testing methods for communities of bacteria in "natural" compartments, as suggested in some of the schemes.

Table 2.6: General trend in aquatic effects, hazard and risk assessment schemes regarding the need for standardised methods for aquatic, pelagic toxicity testing

The "frequency of need" is indicated by the reference numbers of the schemes requesting the method (refer to Annex A). w: warm water environment, c: cold water environment, ?: not suggested in any schemes, but should be considered as a potential area for test guideline development to cover the request for species representing other compartments/environments than in the existing guidelines. References to Annex A are given in / /. (C: Industrial chemicals, P: Pesticides, W: Waste water.)

TAX. GROUP	METHOD/END POINT	FRESHWATER PELAGIC ENV.	MARINE/BRACKISH PELAGIC ENV.	SCHEMES REF. (ANNEX A)
Fish	acute toxicity	covered by exist. TG	covered by exist. TG	–
	egg and sacfry	covered by exist. draft TG	covered by exist. draft TG	–
	FELS	covered by exist. TG	covered by exist. TG	–
	growth test	c: C2, P1, P6	c/w: ?	EC, 1993 **/C2/** EPPO, 1991 **/P1/** Lynch, 1993 **/P6/**
	life cycle/partial life cycle	w: C2, C4, C5, P7	c/w: ?	EC, 1993 **/C2/** CONCAWE, 1991 **/C4/** ESTHER, 1989 **/C5/** FIFRA 1990 **/P7/**
Crustacea	acute toxicity	covered by exist. TG (*Daphnia* sp.) *Ceriodaphnia*	w: C9, W4 (*Mysidopsis*) c: C8, W2 (*Acartia, Tisbe*)	US EPA, 1988 **/C9/** PARCOM, 1993 **/C8/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W5/**
	chronic, life cycle	covered by exist. TG (*Daphnia*, subchronic)	w: C9, W3 (*Mysidopsis*) c: C8, W2 (*Acartia*)	US EPA, 1988 **/C9/** S-EPA, 1990 **/W3/** PARCOM, 1993 **/C8/** DK-EPA, 1994 **/W2/**
Microalgae	growth inhibition	covered by exist. TG	C7, C8, C9, W2, W3 (*Skeletonema*) C8, W2 (*Phaeodactylum*)	Walker, 1990 **/C7/** PARCOM, 1993 **/C8/** US EPA, 1988 **/C9/** Env-Can, 1993 **/P3/** DK-EPA, 1994 **/W2/** S-EPA, 1990 **/W3/**
Macroalgae	fertilization, reproduction	–	c: C9 (Laminaria, *Champia*)	US EPA, 1993 **/C9/**
Vascular plants	growth inhibition	c: C9, P1, W3, W2, W5 (*Lemna*)	c: W3 (*Zostera*)	US EPA, 1993 **/C9/** EPPO, 1991 **/P1/** Env-Can, 1993 **/P3/** DK-EPA, 1994 **/W3/** S-EPA, 1990 **/W2/** Env-Can, 1994 **/W5/**
Bacteria	respiration a.o.	c/w: C4, W5, W3, W2	c/w: ?	Env-Can, 1994 **/W5/** DK-EPA, 1994 **/W3/** S-EPA, 1990 **/W2/** CONCAWE, 1991 **/C4/**
Bivalvia	larvae survival	c/w: ?	c: C9, W3, W2, W4 (*Crassostrea, Mytilus*)	US EPA, 1988 **/C9/** S-EPA, 1990 **/W3/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W4/**

Table 2.6, continued

Echinoidea	fertilization	–	c: C8, W1, W2, W4 (sea urchin)	US EPA, 1991 **/W1/** PARCOM, 1993 **/C8/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W4/**
Insecta	larvae survival	c: C9, C7, P2, W2 (*Baetis* sp., *Cloeon* sp. a.o.)	–	US EPA, 1988 **/C9/** Walker, 1990 **/C7/** Germany, 1993 **/P2/** DK-EPA, 1994 **/W2/**
Amphibia	Survival, develop.	w: C9, C7	–	US EPA, 1988 **/C9/** Walker, 1990 **/C7/**

Table 2.7: General trend in environmental effects, hazard and risk assessment schemes regarding the need for standardised methods for aquatic, benthic toxicity testing

The "frequency of need" is indicated by the reference numbers of the schemes requesting the method (refer to Annex A). w: warm water environment; c: cold water environment; ?: not suggested in any schemes, but should be considered a potential area for Test Guideline development in order to cover the request for species representing other compartments/ environments than the existing guidelines.

TAX. GROUP	METHOD/END POINT	FRESHWATER SEDIMENT	MARINE & BRACKISH SEDIMENT	SCHEMES REF. (ANNEX A)
Crustaceans	acute toxicity	c: C9, W2, W4, W6 (*Gammarus* sp., *Hyalella* a.o.)	c: C8, W2, W4, W5, W6 (*Nitocra* sp., *Corophium* sp., *Hyalella azteca*)	US EPA, 1988 **/C9/** PARCOM, 1993 **/C8/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W4/** Env-Can, 1994 **/W5/** US EPA, 1994 **/W6/**
Bacteria	respiration a.o.	C4, W2, W3, W5	?	CONCAWE, 1991 **/C4/** DK-EPA, 1994 **/W2/** S-EPA, 1990 **/W3/** Env-Can, 1994 **/W5/**
Protozoa	growth	W2	W2	DK-EPA, 1994 **/W2/**
Annelida	survival	c: C6, W2, W3, W4, W5 (*Tubifex* sp.)	c: W2, W3 (Arenicola sp., *Nereis* sp.)	Stephan, 1985 **/C6/** DK-EPA, 1994 **/W2/** S-EPA, 1990 **/W3/** US EPA, 1994 **/W4/** Env-Can, 1994 **/W5/**
Bivalvia	growth	c: C9, W2 (*Unio* sp., *Anodonta* sp.)	c: W2 (*Crassostrea* sp., *Mytilus* sp., *Albra abra*)	US EPA, 1988 **/C9/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W4/**
Echinoidae	survival	–	c: C8, W2 (*Echinocardium* sp.)	PARCOM, 1993 **/C8/** DK-EPA, 1994 **/W2/**
Insecta	larvae survival	c: C7, C9, P2, W2, W4, W5, W6 (C*hironomus* sp. a.o.)	–	Walker, 1990 **/C7/** US EPA, 1988 **/C9/** Germany, 1993 **/P2/** DK-EPA, 1994 **/W2/** US EPA, 1994 **/W4/** Env-Can, 1994 **/W5/** US EPA, 1994 **/W6/**
Amphibia	survival (21d)	c: C9 (tadpole)	–	US EPA, 1988 **/C9/**

3. METHODS COLLECTED FROM OECD MEMBER COUNTRIES, SCIENTIFIC LITERATURE, AND STANDARDISATION ORGANISATIONS

The primary sources for collection of ecotoxicological testing methods were:

- international standards/guidelines;
- national standards of the OECD countries, made available through the National Co-ordinators, environmental protection agencies, or national standardisation organisations;
- testing methods published in international journals, conference proceedings, handbooks, etc., restricted to those differing from national and international standards (with respect to e.g. test species, endpoints, exposure duration, and/or life stages tested);
- unpublished test protocols supplied with sufficient test data documenting the reproducibility and validity of the method, made available through the National Co-ordinators.

A total of 449 pelagic and 258 benthic methods have been compiled. Testing methods/references are listed in Annex L.

Testing methods promoted via the National Co-ordinators of the OECD Test Guidelines Programme, and methods collected from national and international standardisation organisations and from various ring-test and research reports, formed the initial basis of the collection of pelagic test methods. In the case of those taxonomic groups for which the eight environmental scenarios (Chapter 4) were not sufficiently covered with respect to endpoints, trophic levels, etc. (all taxonomic groups other than microalgae, freshwater crustaceans and fish), a literature search was carried out in the BIOSIS computerized literature database. Furthermore, recent volumes of the most frequently used ecotoxicological journals were searched manually.

Three review papers were initially used to select articles covering benthic test methods (Burton 1991, Burton and Scott 1992, Giesy and Hoke 1989). A literature search carried out in BIOSIS in July 1993 was expected to cover most of the relevant literature. The collection of methods was then ended. An exception was made for some ring-tested or (draft) standardised methods which became available after July 1993. Due to the large amount of articles available, it was necessary to restrict the methods to be evaluated to approximately 200. Priority was given to standardised methods and to recent articles. Papers were not included in the evaluation procedure in case a comparable test method had already been described in the database and the paper did not contain information on another species, method, preparation of exposure media, exposure time or effect parameter.

The methods were reviewed with respect to the following main categories of information:

1) Identification

The reference of the test method, including a reference identification number and identification of the submitter of the method (contact person).

2) Test organism

The species used in the test method was identified and grouped taxonomically. The ecological habitats, aquatic compartments and climatic zones for the species were specified. The trophic level of the test organism, the feeding mechanism, and exposure routes under environmental conditions were also included. All information referred to the life stages used in the test, as organisms may have different ecological functions and habitats at different life stages.

3) Specification of test method

Detailed information on the test method was registered, including the endpoints applied, details regarding test exposure (regime, route and duration), and other relevant specification of test conditions, feeding, use of equipment, and method of statistical evaluation of data.

4) Benthic test methods

Information of specific relevance for sediment tests was registered separately: exposure conditions, physico-chemical characterisation of the sediment, etc.

5) Test of reference substances

The results from testing of chemical substances were used in evaluating the sensitivity and reproducibility of the test methods. The test results for selected chemicals were registered if available, and the approximate number of chemicals tested was indicated.

The test methods compiled are outlined in **Tables 3.1 and 3.2**. Testing methods for the assessment of biological processes in waste water treatment plants are included in the bacteria group.

Table 3.1: Reference numbers of pelagic test methods compiled

The figures refer to the reference numbers, listed in Annex L.
Refer to Chapter 4 for definitions of endpoints and compartments.

Algae, micro	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	22, 181	22, 181	21, 194	21, 194
Subchronic				
Chronic	14, 77, 186, 187, 188, 226, 370, 374, 375, 376, 377, 378, 379, 380, 437	14, 77, 186, 187, 188, 226, 370, 374, 375, 376, 377, 378, 379, 380, 437	65, 184, 185, 371, 372, 437	65, 184, 185, 371, 372, 437
Algae, macro	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic			8, 44, 57	8, 38, 43, 57
Chronic				
Kormophyta	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic	66, 88	191		202
Chronic				
Arthropoda, crustaceans	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	28, 402	11, 10, 17, 49, 58, 59, 67, 207, 215, 217, 224, 231, 238, 277, 323, 400, 401	12, 60, 73, 75, 87, 348, 349, 414, 417, 418, 419, 420	47, 210, 211, 212, 213, 214, 216, 219, 220, 222, 230, 412
Subchronic		18	80, 406, 407	208, 221, 408, 410
Chronic		6, 50, 68, 81, 89, 218, 223, 324	55, 74	409

Table 3.1, continued

Arthropoda, insects	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	91, 138, 144, 161, 174	23, 85, 125, 126, 127, 128, 129, 131, 132, 133, 134, 135, 136, 137, 162, 163, 146, 147, 148, 161, 168, 169, 170, 171, 172, 175, 180		
Subchronic	33	139, 145, 152, 160, 164, 165, 439		
Chronic				
Aschelminthes	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	9		381	
Subchronic				
Chronic	31, 190, 205			
Bacteria	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	206, 382, 383, 384	382, 383, 384		193
Subchronic				
Chronic	192, 408	192, 408		
Chordata, amphibians	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	83, 98, 117, 118, 122, 124	92, 93, 94, 95, 96, 97, 102, 103, 104, 106, 107, 108, 109, 110, 111, 112, 113, 114, 119, 120, 121		
Subchronic	99	90, 105, 115		
Chronic				

Table 3.1, continued

Chordata, fish, all groups	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	3, 4, 19, 62, 227, 239, 240, 252, 299, 300, 301, 304, 305, 385, 386, 387, 388, 389, 390, 392, 393, 394, 395, 396, 397	16, 63, 76, 78, 247, 279, 297, 298, 391, 398	64, 241, 250, 251, 306, 307, 308, 309, 311, 312, 314, 321, 322	15, 310, 313, 315, 316, 317, 318, 319, 320, 438
Subchronic	1, 2, 32, 36, 71, 155, 228, 242, 244, 245, 248, 249, 253, 254, 255, 256, 257, 259, 262, 263, 264, 265, 266, 267, 273, 274, 283, 289, 291, 292, 326, 327, 328, 337, 338, 339, 341, 347, 351, 355, 356	79, 229, 245, 258, 260, 261, 271, 275, 284, 285, 286, 287, 288, 290, 325, 329, 330, 331, 332, 333, 334, 335, 336, 352, 354, 357, 358, 359, 360, 361, 362, 364, 365	52, 53, 54, 243, 268, 280, 281, 282, 293, 294, 295, 342, 343, 344, 350	246, 269, 270, 272, 278, 296, 340, 346
Chronic	26, 276, 428			
Cnidaria	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute			368	
Subchronic	373		203, 442, 443	448
Chronic	7			
Echinodermata	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic			56, 235, 236, 413	233, 234, 237
Chronic				

Table 3.1, continued

Mollusca	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute		209, 416		
Subchronic	5	415	421	41, 46, 72, 86, 403, 404, 405, 411
Chronic				
Plathyhelminthes	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	195			
Subchronic	197, 198, 367			
Chronic				
Protozoa	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	196, 204	204, 440		
Subchronic				
Chronic	13, 199, 369, 436	13, 199, 369, 436, 440	189	189

Table 3.2: Reference numbers of benthic test methods compiled

The figures refer to the reference numbers, listed in Annex L.
Refer to Chapter 4 for definitions of endpoints and compartments.

Short-term test methods

Bacteria	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4073	4113, 4088, 4100	4091	4005, 4019, 4027, 4078, 4166, 4192, 4207
Subchronic				
Chronic	4029, 4197, 4048, 4054, 4099	4100, 4016	4079	
Algae	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic		4126		
Chronic	4052, 4101, 4177	4071, 4127, 4128, 4129, 4130, 4131, 4132, 4072		
Chordata, fish, all groups	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				4087
Subchronic	4070			4086
Chronic				

Table 3.2, continued

Long-term test methods

Bacteria	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic				
Chronic	4197			
Algae	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic				
Chronic			4193, 4194, 4195, 4196	
Aschelminthes	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic				
Chronic				

Table 3.2, continued

Short-term test methods

Annelida	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4179, 4157	4093, 4094, 4095, 4202	4154	4035
Subchronic				
Chronic				
Mollusca	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4155, 4199		4036, 4156, 4206	4024, 4183, 4184, 4226
Subchronic				
Chronic				
Arthropoda, crustaceans	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4056, 4106, 4135, 4136, 4138, 4165, 4170, 4171, 4180, 4181, 4080, 4105		4103, 4065, 4066, 4114, 4115, 4172	4001
Subchronic			4032, 4033, 4034	
Chronic				
Arthropoda, insects	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4096, 4159, 4018, 4214, 4081, 4152, 4205	4076, 4116		
Subchronic	4204			
Chronic				

Table 3.2, continued

Long-term test methods

Annelida	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4163, 4157, 4239, 4240, 4253	4122	4002, 4004, 4187, 4090, 4249	4146, 4188, 4228, 4026
Subchronic	4044, 4179	4092	4046	
Chronic	4124, 4157, 4161, 4203			
Mollusca	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4250			4025
Subchronic			4164	4049
Chronic				
Arthropoda, crustaceans	Freshwater		Marine	
	Warm	Cold	Warm	Cold
Acute	4012, 4104, 4137, 4105, 4231, 4248	4141	4102, 4117, 4167, 4168, 4118, 4007, 4008, 4232, 4233, 4234, 4235, 4237, 4238, 4244, 4245, 4246, 4255, 4257, 4258	4020, 4037, 4038, 4039, 4040, 4085, 4185, 4006, 4007, 4008, 4009, 4186, 4198, 4227, 4144, 4236, 4242, 4243, 4246, 4256, 4257
Subchronic	4012, 4021, 4022, 4139, 4143		4119	4208, 4209
Chronic	4056, 4109, 4082, 4112		4145, 4174, 4182	

Table 3.2, continued

Long-term test methods (continued)

Arthropoda, insects	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4013, 4140			
Subchronic	4013, 4097, 4098, 4142, 4011, 4015, 4075, 4083, 4084, 4111, 4134, 4158, 4254	4045		
Chronic	4108, 4191, 4011			

Short-term test methods

Enchinodermata	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute			4149, 4150	4050, 4051, 4151
Subchronic				
Chronic				
Chordata, amphibians	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4043, 4057			
Subchronic				
Chronic				
Chordata, fish, all groups	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4041, 4042, 4058, 4060, 4062, 4178, 4059	4047, 4120	4014	4153, 4200, 4201
Subchronic				
Chronic				

Table 3.2, continued

Long-term test methods

Echinodermata	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				4003, 4023, 4074, 4123, 4225
Subchronic				4031, 4190
Chronic				4189
Chordata, amphibians	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute				
Subchronic				
Chronic				
Chordata, fish, all groups	**Freshwater**		**Marine**	
	Warm	**Cold**	**Warm**	**Cold**
Acute	4107		4162	
Subchronic	4061, 4063	4055, 4121		
Chronic				

4. EVALUATION OF TESTING METHODS

4.1 Introduction

In order to identify testing methods applicable for future OECD Test Guideline development, the following procedure has been applied:

1) Laboratory testing methods have been collected and taxonomically grouped as shown in Tables 3.1 and 3.2

2) All collected methods have been thoroughly evaluated regarding issues of importance for the requirements of an OECD Test Guideline. A formalised set of evaluation criteria has been applied (Section 4.3).

3) The methods have been grouped according to the natural habitat of the tested organism for the stage of development tested, leading to the following eight potential assessment scenarios:

   Pelagic compartment:

   - Cold (cold-temperate) marine environment
   - Cold freshwater environment
   - Warm (warm-temperate – tropical) marine environment
   - Warm freshwater environment

   Benthic compartment:

   - Cold (cold-temperate) marine environment
   - Cold freshwater environment
   - Warm (warm-temperate – tropical) marine environment
   - Warm freshwater environment

4) For each of the eight scenarios above, applicable methods have been identified, representing as far as possible different trophic levels within the grazing and the degrading food chain.

5) For each of the trophic levels, acute, subchronic and chronic test methods have as far as possible been identified.

As many environmental effects, hazard and risk assessment schemes also request data on evaluation of the protection of biological processes in waste water treatment plants, a ninth assessment scenario primarily focusing on the testing of microbial communities has been included:

- Biological waste water treatment "compartment"

The overall framework is outlined in **Figure 4.1** and the definition of terms used throughout this report is given in **Table 4.1**.

The selection of test methods for Test Guideline development has been based on the established set of formalised evaluation criteria. In the evaluation process, it was nevertheless necessary to use some degree of expert judgement concerning how each method should be regarded according to these evaluation criteria. It is noted that a detailed descriptive justification for the evaluation of each individual method is not provided here, mainly because this would have expanded the report too considerably.

4.2 Definition of "pelagic" and "benthic" test methods

The review covers test methods with pelagic and with benthic organisms. For the purpose of environmental effects, hazard and risk assessments, test methods should as far as possible reflect the conditions in the relevant environmental compartment as regards exposure route, choice of test organism, physical and chemical test conditions, etc. The choice of test scenario (benthic or pelagic) will primarily depend on the properties of the chemical to be tested, i.e. testing of benthic species is expected to be requested if the chemical is likely to sorb to particulate matter or if the chemicals tend to sink because of a combination of high density and low water solubility.

The discrimination between pelagic and benthic testing methods is, however, not straightforward. For example, *Daphnia* is often used to rank the hazard of polluted sediments by testing the toxicity of elutriates. As the objective is to assess the toxicity of the sediment, the test is generally regarded as a benthic test even though the test organism used is pelagic. From an effects or risk assessment point of view, the use of pelagic organisms for effects assessment in sediments would be justifiable if the statistical distribution of inherent threshold toxicity levels for comparable toxic endpoints were not significantly different and if sediment particles as a possible exposure route for benthic (detritivorous) organisms were of minor importance compared to porewater. As there is currently insufficient scientific documentation of these hypotheses, there is a need for testing benthic organisms.

In the context of this review, and for effects, hazard and risk assessments in general, there is a need for a pragmatic definition in order to discriminate between methods related to the pelagic and to the benthic environment (pelagic and benthic tests, respectively).

Definition of pelagic tests: Methods are based on life cycle stages of organisms that live, feed and respire in the pelagic environment. Exposure is predominantly via the process of feeding and respiration. In the pelagic test, the organisms are exposed to materials added to water, without the presence of sediments.

Definition of benthic tests: Methods are based on life cycle stages of organisms that live, feed and respire in the benthic environment. Exposure is predominantly via the process of feeding and/or respiration. In a benthic test, the organisms are exposed in a whole sediment system (i.e. a non-disturbed sediment layer with overlying water).

The suggested definitions are based on the assumption that the route of exposure in relation to the habitat of the organism is the primary determinant of potential toxicity differences to pelagic and benthic organisms. In other words, an organism or life stage predominantly exposed to chemicals from the pelagic environment via food and water is identified as a pelagic organism, irrespective of the habitat of the organism or life stage

Figure 4.1 Evaluation strategy for aquatic toxicity testing methods

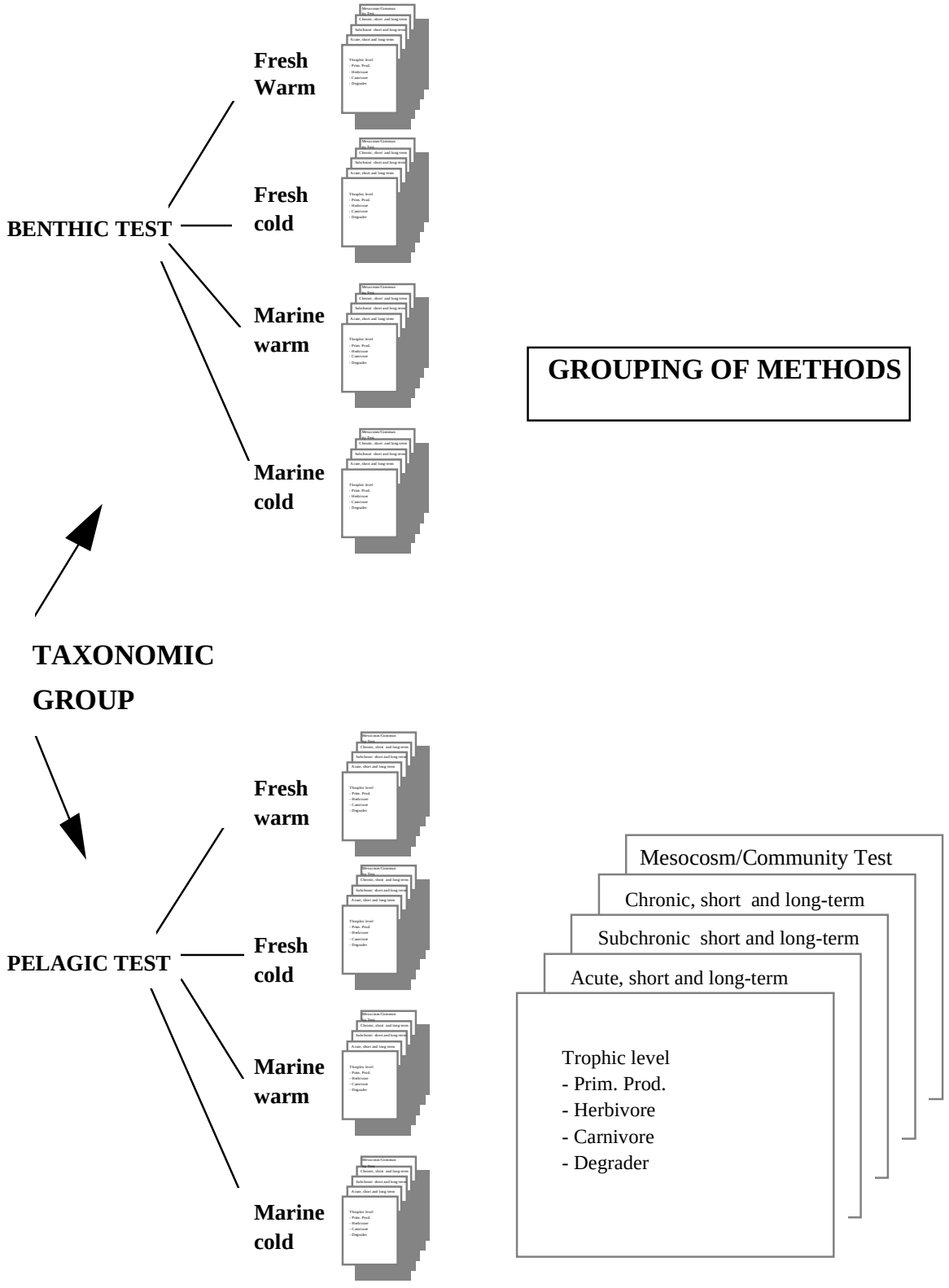


Table 4.1 Definitions of terms used in the evaluation of test methods

Term	Definition	Remarks
Acute	Short exposure in relation to the life span of the organisms.	
Subchronic	The exposure period covers a significant part of the life cycle or covers life stages (e.g. early life stages) or life processes (e.g. reproduction) considered to be especially sensitive	
Chronic	Effects observed during exposure of the entire life cycle of the organism	
Cold	Mean annual temperature = 5-18 °C	Cold temperate zone, Tait (1972)
Warm	Mean annual temperature ≥ 18 °C	Warm-temperate and tropical zones, Tait (1972)
Short-term		Benthic tests in general have longer duration than pelagic tests.
Pelagic	*≤ 7 days*	
Benthic	*≤ 10 days*	
Long-term		
Pelagic	*> 7 days*	
Benthic	*> 10 days*	

The primary argument for this definition is based on the rationale behind the effects, hazard and risk assessment methodology and not on prevailing ecological definitions of the benthic and pelagic community

Pelagic test methods thus include test methods based on benthic organisms which in terms of food uptake and respiration are related to the pelagic environment: e.g. organisms living in streams or the littoral zone attached to rocks or stones (epibenthos) and benthic organisms that feed by filtering the overlying pelagic water phase.

In practice, benthic test methods will predominantly be tests with detritivorous organisms, as herbivorous organisms living in or on the sediment are mainly nourished by and respirate in water from the pelagic environment. The presumably best choice for benthic test species would be those representatives of the infauna (living in the sediment) that both respirate and nourish themselves in the sediment (e.g. annelids, some of the chironomids).

Use of pelagic organisms for testing contaminated sediments should generally not be recommended for environmental effects, hazard and risk assessment. For initial screening purposes, however, the use of this type of testing may be useful.

Infauna organisms living in the porewater of the benthic environment (e.g. bacteria, protozoans) should, although they are benthic organisms, be tested in a water-only system (single-species testing) or in a whole sediment-water system (community testing).

4.3 Test method evaluation procedures

Test methods within each of the following taxonomic groups have been evaluated with the purpose of identifying methods that meet the requirements for routine testing. The methods are grouped according to the taxonomy used by Barnes (1974), but for pragmatic reasons some groups (e.g. Kormophyta, bacteria) represent a higher level in the taxonomical hierarchy than others (e.g. crustaceans or insects), as the numbers of available test methods in some groups are sparse. The list is not complete, as taxonomic groups that do not include aquatic species have been omitted.

Algae, micro	(green algae, cyanobacteria)
Algae, macro	
Annelida oligochaeta	Polychaeta
Arthropoda	Arachnida (spiders, etc.)
Arthropoda	Crustaceans, all groups
Arthropoda	Insects
Aschelminthes	Nematodes, rotifers, etc.
Bacteria	Bryozoa
Chordata	Amphibians
Chordata	Ascidians
Chordata	Fish
Cnidaria	Jellyfish, sea anemones, corals, hydrozoans
Echinodermata	Sea lilies, sea urchins, sea cucumbers, sea stars, brittle stars
Kormophyta	Plants
Mollusca	Clams, mussels, squids, snails
Plathyhelminthes	Planarians
Protozoa	Ciliates, flagellates

For Nermertinea and Porifera, no aquatic test methods have been identified in the collected material.

The evaluation of the collected methods has been performed with respect to three main subjects:

- practical feasibility of the test method
- validity of the test method
- usefulness in prognostic testing

The main evaluation criteria are further divided into a number of more specific items for the evaluation. The test methods have been scored (A, B or C) according to each of the criteria specified in **Table 4.2**. In general, a score of A is given to aspects which comply with requirements for a standardised method.

The scores obtained are not summarised, but rather used for assessing the overall profile of the method.

Some aspects of a method may be more crucial for its applicability as a standard method than others. A CC score is given instead of a C for issues that immediately disqualify the method from standardisation. For example, if the test procedure is judged to be extremely difficult to perform, or if the test organism is impossible to keep in culture and collection in the field is limited to a few months per year, a score of CC is obtained.

The same evaluation criteria have as far as possible been used for both pelagic and sediment test methods. Some inherent differences between the testing environments have, however, necessitated the use of different criteria for some of the evaluation items.

Practical feasibility of the test method

Technical performance of the method

The technical performance, i.e. the complexity and degree of difficulty of the method, is judged by comparison with existing OECD Test Guidelines. As standardised test methods are intended for routine use by various laboratories, they should preferably be easy to perform. Methods demanding highly specialised laboratory equipment or technical experience are given a low score for technical perfor mance.

Compared to the pelagic "water-only" tests, benthic tests need preparation of the testing system such as spiking of the sediment. Therefore, the technical performance of benthic tests is different from that of pelagic tests but not necessarily more complex.

Duration of tests

In general, the duration of the test should be as short as possible for cost reasons, and sufficiently long for valid observations of the toxicity endpoints included in the test. Preferably, the test duration should be less than 60 days, which is the maximum duration of the OECD Test Guideline for bioaccumulation in fish (TG 305) and fish early life stage test (TG 210). The expressions “short-term” and “long-term” are applied for characterising the duration of the test. In this Detailed Review Paper, the following definitions have been applied:

Pelagic tests:

Short-term: ≤ 7 days
Long-term: > 7 days

Benthic tests:

Short-term: ≤10 days
Long-term: > 10 days

The seven-day cut-off value for pelagic tests has been chosen as this period will not conflict with any of the titles of methods included in the database [US EPA: seven-day short-term chronic toxicity to fish and crustaceans (EPA 600/4-89/001)].

Due to partition kinetics of chemicals in sediments and uptake kinetics of benthic organisms, the duration of a benthic test should, for comparable endpoints, be longer than a pelagic test (i.e. weeks rather than days). It may be added that the relatively longer duration of a benthic test does not necessarily lead to

a more costly test, as the complexity of the test determines the costs to a greater extent than duration alone. A longer test duration is a negative aspect, however, when results are needed at a short notice.

Availability and maintenance of test organism

Sufficient biological knowledge should preferably be available regarding the maintenance of cultures of the test organism in the laboratory. As culturing is expected to be possible only for relatively few species, a minimum requirement is that the species should be easily collected in the field most of the year and should be easily and successfully maintained in the laboratory for a period of at least twice the testing period (acclimatisation period + test duration).

Exposure system

It is important that the concentrations of the test substance remain constant during the test period. In order to be able to handle easily degradable and other "difficult" test substances, the exposure system of the test method should include a methodology for semistatic and flow-through procedures and/or use of dried test vessels.

For most benthic test methods, only static exposure systems have been used. For degradable substances (which may continuously be emitted into the environment), or when development of an effect in time has to be studied, renewal of the sediment (or the overlying water) is important. A flow-through system, in which the overlying water is (semi)continuously renewed, is currently not developed for routine testing but is primarily used for research purposes.

Costs of equipment and labour

The costs of equipment and labour should not considerably exceed the costs of the present short-term/long-term OECD Test Guidelines. Very laborious test methods should not be recommended for routine testing unless the endpoint(s) is judged to be highly important. OECD recommends that new test guidelines not require unique equipment or unique technical experience (OECD 1993a).

For benthic tests, a longer duration of the test is to be anticipated, which may or may not influence the costs compared to comparable pelagic tests.

Validity of test methods

Evaluation of the validity of a test method should include an assessment of its reproducibility, the possible sources of error, and the range of tolerance of the test species to environmental conditions.

Reproducibility

Low reproducibility and high variation in response parameters will lead to a reduced sensitivity of the method. Possible sources of error due to the test system, resulting in high variation of response parameters, should be as few as possible and in general should not exceed the performance of corresponding OECD Test Guidelines.

For a pelagic test method with good reproducibility, there should be no more than a factor of 5 between the highest and lowest test result obtained for a reference substance at different laboratories. A factor of 10 represents a method with medium to low reproducibility. Greater differences should not be accepted for a standard test method unless there are reasons to believe that research work may lead to an improvement of the method as regards its reproducibility.

Less reproducibility is to be accepted for benthic tests, as they are generally more complex and more difficult to standardise and thus show a higher degree of variability in the results. In general, if results from repeated studies do not differ by more than one order of magnitude, the procedures of benthic tests are considered adequate (SETAC, 1994b).

The composition of the sediment influences the availability of the chemical to the organisms, and therefore the toxicological effect. When natural sediments are used in benthic toxicity tests, variation in responses will exceed those in water-only tests.

Another source of variation is the test organism. Benthic tests more frequently use test animals from the wild. This might lead to higher background mortality and variation in susceptibility compared to pelagic tests. Because the route of exposure in benthic tests is generally more complex than in pelagic tests, the variation in susceptibility between species with different life history strategies may be considerable (SETAC, 1994b).

As it has not been the purpose of this review to collect additional data on the reproducibility of the test methods, in order to supplement the very often limited information provided in the test protocol, limited or missing documentation of reproducibility has not been used to exclude any methods.

Sources of potential error

The complexity of the test procedure, in terms of handling of test organisms (inducing stress to the organisms), maintenance of test conditions, and the complexity of the exposure system, may affect the possibility of introducing errors. These include random errors leading to a high variability in the observations as well as systematic errors.

Methods with relatively few critical steps are preferred. If, however, the test method includes critical steps, documentation of the uncertainty, or recommendations on how to minimise the possible error, should be included.

If the test procedure involves many critical and very complicated procedures compared to the existing OECD Test Guidelines, the test method is not recommended for standardisation (score CC).

Compared to water-only tests, there are generally more critical phases in benthic tests. Most relate to the preparation of the benthic test system. The methodology and the time needed to reach chemical equilibrium between the water and sediment particles are of critical importance (SETAC 1994b, ISPRA 1995). They can affect the extent of equilibration, the concentration distribution within the final individual test samples, and the variability in distribution of test material in samples taken from a bulk treatment.

For hydrophobic chemicals, the time needed to reach chemical equilibrium between the particle and pore water phases may range from days to months. If this is not taken into account, additional variation in toxic response may result. Especially for screening of elutriate or porewater, this is a very critical aspect.

As the critical steps are more or less the same for all benthic tests, these steps have been handled in the evaluation of the exposure system.

Range of tolerance to environmental conditions

The robustness of the test organism is of importance to the applicability of the test method for standardisation. The conditions required by the test organism with respect to chemicophysical (e.g. oxygen tension, pH, temperature, light) and biological (e.g. feeding) factors should preferably be documented, and the prescribed test conditions should be well defined within these requirements in order to avoid stressing of the organism.

It should be noted that robustness to various environmental factors should not automatically lead to the conclusion that the method is less sensitive to a chemical stressor. Sensitivity is primarily determined by inherent biological factors and the reproducibility and precision of the method in question.

If the test organism has a very narrow tolerance to one or more environmental factors, which may be very difficult to sufficiently control under laboratory conditions, it may not be suitable for routine testing and will thus obtain a score of CC.

Usefulness in prognoses

Geographical representativeness

The applicability of a standard test method depends, among other factors, on the range of different environments, in terms of geographical zones and ecological compartments, which the test organism inhabits and thus may represent. Organisms with a relatively narrow geographical distribution are usually not preferred as test organisms, but may be of relevance for effect assessment of specific aquatic environments.

Endemic species, which can represent only very specific types of environment (e.g. rock pools), are not recommended for aquatic hazard and risk assessments and thus are not recommended for OECD Test Guideline development (e.g. *Artemia salina*) (score of CC).

Ecological representativeness

Test organisms should preferably represent a life form that is abundant in the environment and thus important for the structure and/or function of the aquatic ecosystem. Moreover, as the selection procedure applied in this review aims at identifying applicable test species within different taxonomic groups, species that represent life forms that are typical of the taxonomic group are preferred.

Specialised life forms that are neither important to the ecological compartment that they represent, nor representative of life forms within the taxonomic group, are not recommended for standardisation (score of CC).

Extrapolation of endpoints

The present review primarily includes endpoints related to the population and the organism level of organisation. Endpoints at the community level are limited to microorganisms. Physiological endpoints are included when they are measured as an overall response of exposed organisms or communities, but are otherwise excluded.

The endpoint should as far as possible indicate an effect of "ecological relevance". Effects on survival, growth and reproductive success of organisms are normally considered as being of primary relevance in this respect (OECD 1992a, Zeeman and Gilford 1993). Other endpoints considered to be of ecological relevance are avoidance, gross deformities or visible tumours (van Leeuwen 1990).

Physiological and biochemical responses of individual organisms may be of ecological significance as well, but in general they are difficult to directly interpret in an ecological context. Based on the above considerations, toxicity endpoints may be compiled in the following five groups:

1) endpoints at population or community level:

   - population survival and growth
   - age structure
   - fecundity
   - species composition and community tolerance

2) endpoints related to individuals or groups of organisms of similar age:

   - survival/lethality (or immobilisation)
   - growth and survival of specific life stages
   - reproduction and survival or early life stages
   - avoidance/behavioural effects
   - gross deformities and morphological effects

3) endpoints related to specific toxicity mechanisms of substances (e.g. genotoxicity)

4) physiological endpoints (e.g. effects on metabolic processes, inhibition of respiration)

5) biochemical endpoints (induction or inhibition of enzymatic activity, etc.)

Testing methods may include several endpoints. Life cycle tests include some or all of the endpoints listed in 2), while short-term acute toxicity studies only focus on survival.

In the literature, the terms *acute, subacute, subchronic* and *chronic* are used in an inconsistent manner. In particular, there are different approaches for characterising tests extending beyond the acute toxicity testing period. In this Detailed Review Paper the following terms and definitions are applied:

Acute effects:

Lethal or sublethal effects observed after a short exposure period in relation to the life span of the organisms.

Subchronic effects:

Lethal and sublethal effects observed after an exposure period covering a significant part of the life cycle that gives an indication of long-term (chronic) effects, often by focusing on critical or sensitive stages.

Chronic effects:

Effects observed during exposure of the entire life cycle of the organism. The often seen definition of "chronic" as tests covering at least 90 per cent of the life span of the organism is not considered applicable, as crucial life stages may be omitted in the 10 per cent life span not exposed (e.g. fertilization or yolksac stage of salmon fish). Chronic studies on microorganisms (e.g. algae and protozoans) often cover several generations of the organisms (multigeneration tests). In this review, these studies have also been termed chronic tests.

Results from subchronic tests are often applied as an estimate for chronic effects. In literature, subchronic toxicity studies, according to the above definition, are often referred to as chronic tests. In the present review, the above definitions have been used for all the methods irrespective of the terms used by the authors of the testing methods.

General sensitivity

Although "no species is the most sensitive to all chemicals" is a well known phrase, it is important to deal with the relative sensitivity of species within a taxonomic group for a range of chemicals. Closely related species may vary in their "general" sensitivity due to differences in physiological processes, different levels of activity of detoxification systems, uptake and depuration processes, etc. Also, different positions of taxonomically related species in the food web may lead to different sensitivities. The general sensitivity of the test methods may, however, be difficult to assess due to missing or insufficient data. No systematic search for toxicity data has been made for the collected testing methods. For the benthic tests a search in the AQUIRE database has, however, been performed. These data may give an initial impression of the sensitivity of benthic species, but are not sufficient for a valid assessment of the relative sensitivity of the species for identification of preferences.

Relevance of exposure route and test conditions

As far as possible, the route of exposure applied for the test should not be in conflict with the exposure route of the organism in the field (i.e. pelagic organisms should preferably not be applied for benthic tests and vice versa). Furthermore, the test conditions in general (i.e. temperature, salinity, hardness of the water) should not be in conflict with the conditions found in the habitat of the organism, although the organism may.

If both the exposure route and the test conditions differ significantly from the conditions found in the natural habitat of the organism, the method is not recommended (score of CC).

Level of standardisation

The level of standardisation of a method is evaluated from its status in a standardisation process, e.g. a national standard method is often at a higher level of standardisation than a method published in an international journal. Other relevant information concerns whether international or national ring-tests have been conducted. The level of standardisation indicates how much effort is needed to develop the method into a guideline. Test methods adopted as national or international standards may be adopted as OECD Test Guidelines with only limited effort. Thus, the level of standardisation is an important issue in the assessment of methods with similar qualities, but is not crucial for a method that may cover an identified need in assessment schemes.

Summarising the scores

The scores obtained in the evaluation, according to the criteria in Table 4.2, are summarised in order to obtain a single overall score for the method. The overall score is obtained by expert judgement applied to the method’s scoring profile. Some of the evaluation criteria are considered more important than others. Methods with CC scores are not applicable for routine testing. A CC score is obtained only for aspects that are regarded as important for their applicability as a standard test method, i.e. technical performance, availability of test organisms, sources of potential error, range of tolerance to environmental conditions, environmental relevance, extrapolation of endpoints, and relevance of exposure route and test conditions.

It is emphasised that the overall score is a relative score indicating the applicability of the method compared to other methods within the same taxonomic group:

1) The test method is highly relevant and possesses the quality needed for standardised aquatic toxicity testing. The organism is a valid representative of the taxonomic group and type of aquatic environment in question. The method is expected to require relatively limited efforts to be ready for standardisation.

2) The test method is relevant and possesses the qualities needed for standardised aquatic toxicity testing, but further scientific documentation is needed before it is recommended for standardisation.

3) The test method is not applicable for routine testing.

The methods in Group B have been further assessed by the reviewer regarding the expected outcome of "further scientific documentation" (see the annexed method evaluation). If the documentation needed is expected to be relatively easy to provide and non-problematic for the quality of the method as a future test guideline, an A is indicated; if the opposite is the case, a C is indicated.

Methods scored A have been further assessed in Chapters 5 and 6. To be able to select a sufficient number of methods within each taxonomic group to cover the eight (nine) scenarios, some of the methods scored B, which most likely will be upgraded to a score of A when sufficient documentation has been made available, are also further assessed in the following chapters.

4.4 Procedure for identification of "key" test methods

Seen in an ecological, recreational and commercial framework, the key taxonomic groups whose inclusion in testing programmes should be considered are those involved in the primary routes for mineralisation, energy flux and nutrient/carbon cycling within the aquatic compartment. The set of test species should preferably include representatives from each of the following groups (Committee of the Council of the Netherlands, 1989):

- primary producers (vascular plants, algae)
- microbial saprophages (e.g. bacteria)
- saprophages/detrivores (e.g. insect larvae, annelids, crustaceans)
- herbivores/primary consumers (e.g. protozoans, crustaceans, insect larvae, bivalves, fish)
- carnivores (e.g. crustaceans, insect larvae, fish)

Many of the taxonomic groups have representatives at more than one trophic level, and many species cover more than one trophic position and/or cover different positions at different stages of the life cycle. A number of model scenarios may therefore be elaborated, depending on the feeding strategy of the organism in relation to route of exposure (habitat considerations).

In addition to selecting representatives from the various trophic levels, the selection of representatives of species threatened by extinction (e.g. amphibia) might also be considered (Committee of the Council of the Netherlands, 1989). Recreationally and/or commercially important species might be included as well (Smrchek et al. 1993).

Table 4.2: Criteria for evaluation of pelagic and benthic toxicity testing methods

ITEM	RATING	PELAGIC TESTS	BENTHIC TESTS
1. PRACTICAL FEASIBILITY OF THE TEST METHOD			
Technical performance	A	The performance of the method is comparable to internationally adopted routine tests with algae, crustaceans and fish (acute, subchronic and chronic, respectively)	
	B	More difficult to perform than the existing routine tests, but within the ability of routine test laboratories.	
	CC	Extremely difficult to perform, requires special training of staff and/or equipment not expected to be available in laboratories performing routine testing.	
Duration of long-term tests	A	Duration 8-28 days	Duration 11-28 days
	B	Duration 29-60 days	Duration 29-60 days
	C	Duration over 60 days	Duration over 60 days
Availability and maintenance of test organisms	A	Sufficient documentation for relatively easy maintenance in the laboratory for several generations.	
	B	Cannot be held in culture under laboratory conditions, but can easily be maintained for at least twice the test duration (acclimatisation period and test duration) and can easily be purchased from supplier or sampled during most seasons (≥ six months) in sufficient quantities.	
	CC	Insufficient documentation for minimum maintenance in the laboratory, cannot be easily purchased from supplier, or can be sampled only during a limited period of the year (< 6 months).	
Exposure system	A	Static, semi-static and flow-through exposure systems are all described and sufficiently documented for the method.	Static and semistatic and/or flow-through procedures have been described

ITEM	RATING	PELAGIC TESTS	BENTHIC TESTS
1. PRACTICAL FEASIBILITY OF THE TEST METHOD (continued)			
	B	Only static and semi-static procedures have been described and documented.	Only static procedures have been described
	C	Only static procedures have been described and documented.	
Costs, equipment	A	Normal laboratory equipment for routine testing (e.g. OECD Test Guidelines) is sufficient.	
	B	Low level of investments may be needed (less than 10,000 ECU/US$)	
	C	High level of investments may be needed (over 10,000 ECU/US$)	
Costs, labour	A	Corresponding to short-term routine tests (e.g. OECD Test Guidelines)	Corresponding to long-term static or flow-through test methods (e.g. OECD Test Guidelines)
	B	Corresponding to long-term routine tests (e.g. OECD Test Guidelines)	Flow-through tests with contaminated overlying water
	C	More laborious than long-term routine tests.	More laborious than under A and B
2. VALIDITY OF TEST METHOD			
Reproducibility	A	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 5.	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 10.
	B	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 10.	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 20.
	C	The LC/EC50 values for reference chemicals tested at different laboratories lie above a factor of 10.	The LC/EC50 values for reference chemicals tested at different laboratories lie above a factor of 20.

ITEM	RATING	PELAGIC TESTS	BENTHIC TESTS
Sources of potential error	A	Potential critical phases are few, sufficiently documented, and should not be critical for routine laboratories.	
	B	Potential critical phases are few, but not all of them are sufficiently documented. The critical steps may be of significance for performance of the test.	
	CC	A relatively large number of critical steps are involved, which are not sufficiently documented.	
Range of tolerance to environmental conditions	A	The test organism can tolerate the test conditions used in terms of temperature, oxygen, pH, light regime, feeding, salinity a.o., as well as the range and variations of these during test and maintenance.	As pelagic tests, and tolerates a wide range in sediment particle size and content of organic carbon.
	B	Range of tolerance is documented, but tolerance to some of the environmental parameters may cause problems for routine laboratories.	
	CC	Some of the environmental conditions are likely to give problems for routine testing and maintenance.	
3. USEFULNESS IN PROGNOSES			
Geographical representativeness	A	Test organism is represented in many geographical areas (cosmopolite).	
	B	Test organism is represented in one geographical area only.	
	CC	Endemic species, relicts and other organisms with a very narrow geographic distribution.	

ITEM	RATING	PELAGIC TESTS	BENTHIC TESTS
Ecological representativeness	A	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group and may be a dominating or ecologically important species and thus be of importance for the structure of the ecosystem.	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group and is an infauna organism.
	B	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group but does not dominate its natural environment.	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group and is an epibenthic organism.
	CC	The test organism represents a specialised type of life form within the taxonomic group and does not dominate its natural environment.	The test organism represents a specialised type of life form within the taxonomic group and is not a member of the benthic community.
Extrapolation of endpoints: Usefulness and significance in risk assessments	A	Ecologically highly relevant endpoints: at community or population level, e.g. population growth, age structure, fecundity. For microorganisms, functional endpoints are used.	
	B	Ecologically relevant endpoints: survival/growth of individual or groups of organisms, behavioural responses, etc.	
	CC	Less ecologically relevant endpoints related exclusively to specific toxicity mechanisms, physiological or biochemical endpoints at the organism level.	
General sensitivity	A	The species/system is documented to be highly sensitive to a wide range of chemicals.	
	B	In general, as sensitive as the organisms presently applied for routine testing (in OECD Test Guidelines).	
	C	In general, less sensitive than the organisms presently applied (in OECD Test Guidelines).	
Relevance of exposure route and test conditions	A	The abiotic and biotic conditions in the test and route of exposure during the test simulate well the conditions in the natural habitat of the species. The organism is tested in a water-only system.	The abiotic and biotic conditions in the test and route of exposure during the test simulate well the conditions in the natural habitat of the species. The organism is tested in a whole sediment-water system, i.e. possible exposure routes are particles, porewater and overlying water.

ITEM	RATING	PELAGIC TESTS	BENTHIC TESTS
	B	The route of exposure or the abiotic/biotic conditions in the test differs significantly from the natural habitat of the organism.	
	CC	Both the route of exposure and the abiotic/biotic conditions in the test differs significantly from the natural habitat and exposure route of the organism.	
4. LEVEL OF STANDARDISATION			
	AA	International standard/guideline	
	A	National standard method/guideline or the method has been subject to national (or international) ring-testing (at least five laboratories), or international draft standard is in progress.	National standard method/guideline or the method has been subject to national (or international) ring-testing (at least five laboratories), or international draft standard is in progress, or the method is extensively commented in an international expert group (e.g. PARCOM).
	B	National standard method/guideline, but not yet ring-tested or national draft guideline in progress.	
	C	Method is published in an international peer-reviewed journal or protocol with sufficient documentation for publication.	

A surrogate species may be considered in some cases to replace an ecologically relevant test species, provided that the surrogate species is at least as sensitive as the key species and easier to handle under laboratory test conditions (Smrchek et al. 1993).

In addition to the trophic level and habitat of the organisms, the representativeness of environmental compartments should be considered for both the pelagic and the sediment compartments: freshwater and marine environments combined with cold-temperate or warm-temperate/tropical environments.

If all the above combinations are included, the number of potential testing scenarios will end up with the same problems as the selection of relevant test species: the limits due to the costs of the testing programme. Therefore, a limited number of scenarios should be selected which can define the range of responses to be expected in similar scenarios (Emans et al. 1992). This logic is in parallel to the "cluster hypothesis" for single species (Smrchek et al. 1993). The elaboration of a number of testing scenarios may also be needed, to make it possible for the regulatory authorities to select specific testing scenarios for predicting threshold effect concentrations in specific environments/compartments of concern.

Norton et al. (1992) have elaborated ten critical issues in the design of effects assessment procedures. Those related to ecological aspects are listed below:

- flexibility in the choice of test protocols
- predictions based on key tests or exposure-related studies
- uncertainty analysis for evaluation of extreme cases
- the application of expert judgement

The problem of applying single species in the prediction of threshold levels for environmental compartments (function and structure) has been addressed in a number of publications during the past decade (e.g. Cairns and Niederlehner 1995). The inherent assumptions involved have been defined as follows (UK 1993):

- *although ecosystem sensitivity is a complex attribute it may be approximated in terms of sensitivity of the most sensitive species, although for localised discharges, some consideration needs to be given to site specific sensitive species*

- *protection of community structure (e.g. species list, diversity, size- and age-class) ensures protection of ecosystem function (e.g. fixation and transfer of energy, productivity, resistance to perturbation, recycling of nutrients)*

Also, the selection of ecologically "relevant" endpoints may be related to different environments. For example, the growth and development of the early life stages of fish, bivalvia and echinodea are especially ecologically relevant for the pelagic environment, whereas the adult stages of e.g. bivalvia and echinodea are primarily of relevance for the benthic environment.

A number of key scenarios may be elaborated based on the overall descriptors, i.e. pelagic/ sediment compartment, grazing/detritus food web, marine/freshwater environments, and cold/warm water environments (**Table 4.3**).

Table 4.3: Key taxonomic groups considering the selection of ecotoxicity test species for specific environmental compartments

	FOOD WEB	MARINE ENV. COLD/WARM	FRESHWATER ENV. COLD/WARM
PELAGIC COMPARTMENT	grazing	microalgae, macroalgae, protozoans, fish, crustaceans, bivalvia, molluscs, echinoids, a.o.	microalgae, vascular plants, protozoans, insects, fish, crustaceans, bivalvia, molluscs, a.o.
	detritus	bacteria, protozoans, crustaceans, a.o.	bacteria, protozoans, crustaceans, insects, a.o.
BENTHIC COMPARTMENT	grazing	–	–
	detritus	bacteria, protozoans, annelids, molluscs insects, crustaceans, echinoderms, Cnidaria, (fish), a.o.	bacteria, protozoans, annelids, molluscs, crustaceans, plathyhelminthes, (fish), a.o.

Based on the definition of benthic and pelagic test methods suggested above (Section 4.2), the grazing food web has primarily been included in the pelagic environment and the detritus food web in the benthic environment.

The exposure of detritivorous organisms located in the pelagic environment is not considered significantly different from the exposure of the primary members of the grazing food web (seen in a hazard/risk assessment context). For hydrophobic substances, the pelagic organisms are generally exposed to a minor degree compared to the benthic or infauna organisms nourished by detritus. In other words, an effect assessment based on organisms from the benthic fauna may be expected to lead to a protective estimate of PNEC, and also for the members of the detritus food web living in the pelagic region.

Assessment of effects, hazard/risk in regard to benthic organisms is expected to be performed in the refinement phase of the assessment schemes and primarily for chemicals with sorptive properties. For readily soluble and non-sorptive chemicals, only the assessment of effects, hazard/risk in regard to the pelagic environment seems relevant. This type of chemicals will expose members of both the grazing and detritus food web in the pelagic environment. Therefore, it seems reasonable to argue that organisms from the detritus food web are to some extent also represented in pelagic testing scenarios.

The detritus food web related to the benthic compartment is represented by organisms scraping surfaces of particles (many crustaceans and insect larvae/nymphs), organisms filtering the water (many insect larvae and nymphs), and the sediment reworkers eating their way though the sediment (e.g. annelids). In practice, the above discrimination is not straightforward, as the grazing and detritus food web in the benthic compartment may to some extent be occupied by the same organisms (e.g. filterfeeders, organisms scraping surfaces for bacteria, algae and other types of "*aufwuchs*").

In an effects, hazard and risk assessment framework, however, the food web may be of minor importance compared to the physical habitat of the organisms. The infauna may be expected to be exposed to relatively higher concentrations of sorptive chemicals than the pelagic and epibenthic organisms. Therefore, representatives of the sediment "reworkers" (e.g. annelids, cnidaria, echinodenus and insect larvae) may be a favourable device for benthic test organisms (as tested in a whole sediment system).

Most of the currently adopted aquatic effects, hazard and risk assessment schemes include testing methods for the assessment of toxicity to the microorganisms for the protection of the biological treatment step in waste water treatment plants. Only one method is currently included in the OECD Test Guidelines, i.e. the aerobic respiration inhibition test with activated sludge bacteria community (TG 209). Other methods have, however, been included in the work programme of the OECD Test Guidelines Programme: a nitrification inhibition test and inhibition of anaerobic respiration. In line with the above focus on the need to cover testing of representatives from different trophic levels in the "natural" environment, the same procedure may be used for treatment plants.

It is a general observation that the nitrification test is considered considerably more sensitive than the aerobic respiration test, and this may be the case for other "specialised" biological processes, e.g. the biological phosphorous removal process. Therefore, proposals for new methods should include processes that are the most sensitive processes in the treatment plant and not the overall respiration of the microbial community. It may be added that the biologically activated sludge and the biofilter are highly specialised communities of not only bacteria but also e.g. fungi, protozoans and nematodes, which take an active part in the process of the highly efficient degrader community.

Thus, the conceptual framework for environmental model scenarios applied for the present Detailed Review Paper includes eight scenarios (Figure 4.1) and a scenario focusing on the protection of biological treatment of waste water:

- **four scenarios primarily focusing on the pelagic grazing (and detritus) food web**: fresh and marine water environments in combination with warm-temperate/tropical and cold-temperate environments;

- **four scenarios focusing on the benthic detritus food web**: fresh and marine water environments in combination with warm-temperate/tropical and cold-temperate environments.

- **one scenario focusing on biological waste water treatment** (aerobic and anaerobic treatment).

Key ecological organisms, organisms of recreational/commercial interest, and taxonomic groups threatened by extinction have as far as possible been identified for each of these scenarios, in order to fulfil the objective of obtaining a flexible system of testing methods to be available to environmental effects, hazard and risk assessment of chemicals and pesticides.

5. PELAGIC TEST METHODS

5.1 Evaluation process

According to the characteristics considered to be important for a standardised pelagic test method, as outlined in Table 4.2, the collected methods have been thoroughly evaluated (Annexes C-F). As discussed in Section 4.3, the overall result of the evaluation has been indicated by ranking the methods A, B and C, respectively.

Out of the more than 400 pelagic test methods, a considerable number are currently nationally adopted standard methods or are in the process of standardisation. Or detailed protocols for the methods have been elaborated and are in use for various purposes. For the standardised methods and for methods where a standardised use has been intended, most of the aspects in Table 4.3 regarding test method characteristics were available and sufficiently documented as well. In general, these methods have received the highest overall scores and are thus the primary background for the further evaluations made below. The level of documentation of testing methods collected from the scientific literature is much more variable, as a considerable number of these methods are not intended for standardisation but only for research. Most of these studies have been given a B as the overall score. Only in the case of taxonomic groups for which a sufficient number of methods with A scores were not identified have methods with a score of B been included in the evaluation process below.

The ranking of the methods according to the eight aquatic scenarios has not been straightforward for a number of methods, as the test organism often belongs to more than one of the scenarios in regard to preferences for temperature regimes, trophic level and/or habitat in the environment. As far as possible, the methods have been assigned to the scenario where the tested life stage has its highest preference. Where sufficient information was not available in the reviewed papers/protocols, relevant textbooks and/or specialists have been consulted. For the methods where the necessary information could not be made available, the method was ranked on the basis of the actual test conditions for the organism. Epibenthic species, which are predominantly nourished as herbivores, have been regarded as pelagic and not benthic organisms. Some of the species having a preference for detritus have also been included in the chapter on benthic methods (e.g. *Gammarus*).

Organisms that may live in porewater, e.g. protozoans, have been included in this chapter because they are most frequently tested in a water-only system and may be members of the pelagic environment as well.

Rooted vascular plants may be exposed to organic chemicals via the part in the sediment (roots) and the part in the pelagic environment (leaves). The available methods have been addressed in the pelagic part of the report, as it is expected that the relatively most important exposure route for organic chemicals to rooted plants is via the leaves. However, very little scientific documentation of exposure of, and effects on, rooted aquatic plants is generally available.

For similar methods, i.e. test organisms from the same taxonomic group, trophic level and endpoints, the reference to the method (including the most detailed information) has been indicated as a key reference. Other supplementary references may be recommended also.

The general sensitivity of the method and (perhaps more crucial) the relative variability of the data obtained by its use are attributes that are important for selection of a method for guideline development. For the pelagic methods, no search for toxicity data has been made and only information included in the reviewed papers/documents regarding reproducibility and sensitivity have been transferred to the database. However, this information has generally been very limited and for most methods not sufficient for a relative ranking of the methods.

5.2 Warm freshwater environment

The methods assessed to be candidates for standardisation are outlined in **Table 5.1**. In addition to the taxonomic groups already represented in the OECD Test Guidelines Programme, methods involving representatives from the following taxonomic groups have been identified as suitable for standardisation:

Higher plants
Insect larvae
Aschelminthes (rotifers a.o.)
Bacteria
Amphibia larvae
Planarians
Cnidaria (*Hydra*)
Protozoans

Group 1:

Methods needed for existing (draft) international aquatic effects, hazard and risk assessment schemes

Group 1a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term subchronic growth inhibition test with *Lemna gibba*

The need for a test method with vascular plants has been identified by the European Plant Protection Organisation (1991) and by five other assessment schemes, two of these focusing on the assessment of industrial chemicals (US EPA) and pesticides (Environment Canada). Vascular plants are taxonomically a broad group of organisms, and, although the genus *Lemna* is restricted to shallow lakes and ponds, the species may be an acceptable representative of the group in the aquatic environment. *Lemna gibba* (and *L. minor*), are relatively easy to culture in the laboratory, and the test method is uncomplicated compared to the existing guidelines. The testing method has been adopted by US EPA /66/ and ASTM /88/ (refer to **Table 5.2**).

Long-term chronic life cycle toxicity test with fathead minnow (*Pimephales promelas*) and zebra fish (*Brachydanio rerio*)

The confirmatory steps of many assessment schemes suggest life cycle tests with fish, among others EU and CONCAWE. The method with zebra fish has been applied for research work and is not in

the process of being standardised. A US EPA guideline has been adopted for fathead minnow. This guideline may, however, be extended to include zebra fish as well.

Group 1b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

No methods have been identified.

Group 2:

Methods presumably needed in the near future as they are recommended in national assessment schemes and/or considered to represent key ecological groups

Group 2a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term acute test with the crustacean *Neomysis mercedis*

Acute tests with crustaceans are required by a broad range of assessment schemes, but no warm water species are currently included in the OECD Test Guidelines (TG 202). The method with *Neomysis mercedis* is available as an ASTM standard /402/.

Short-term acute and chronic toxicity test with the rotifer *Brachionus*

Two assessment schemes recommend testing of Rotifera. A procedure for testing of acute toxicity is described by the ASTM, but a procedure for chronic toxicity is also available /190/. The rotifers represent a group of small pelagic filtrators (microzooplankton) which are of importance for carbon cycling in the aquatic ecosystem.

Group 2b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term acute toxicity study with Amphibia (*Xenopus laevis*)

Tests with amphibian larvae have been identified in two schemes for assessment of chemicals (USA). *Xenopus* may represent a threatened animal group, the amphibians. The test organism may be cultured in the laboratory. The method is considered ready for standardisation after minor improvements (development of flow-through systems). The method has been adopted by ASTM.

Short-term subchronic study with Amphibia (*Xenopus laevis*)

This method may be combined with the acute toxicity method above.

Short-term chronic toxicity test with the protozoan *Tetrahymena pyriformis*

The protozoans are an important group of organisms in the aquatic environment, being an important link in the detritus food chain. Testing with protozoans is recommended in one draft assessment scheme for waste water (DK). Several test methods are available, and recently a (limited) international ring-test with *Tetrahymena pyriformis* has been performed. The test is based on inhibition of growth, is of short duration, and is easy to perform. It will thus require only little effort to be standardised. Testing with protozoans is recommended in an assessment scheme for waste water.

Short-term acute toxicity test with *Aedes aegyptii*

Four assessment schemes recommend testing with insect larvae, one of them for assessment of waste water. This taxonomic group is ecologically important in the pelagic environment, although most of the species (larvae, nymphs) are members of the benthic fauna/infauna. *Aedes aegyptii* is an epibenthic filterfeeding species. The method is considered feasible as a low-cost screening method in line with the existing Test Guidelines for short-term acute toxicity. The test method needs further development, especially regarding the exposure system.

Group 3:

No immediate or near future needs for the methods have been identified. The methods may be more or less ready for standardisation.

Long-term subchronic toxicity test method with Planaria (*Dugesia dorotocephala*)

Most planarians are epibenthic carnivorous/omnivorous species. The primary endpoints of the method are survival and regeneration.

The species belonging to this taxonomic group are seldom represented in high numbers in aquatic systems.

Short-term acute toxicity to Planaria (*Dugesia dorotocephala*)

The principles and endpoints of the method may be included in the method listed above.

Long-term subchronic toxicity to insect nymph (*Epeorus latifolium*)

The method is considered to be relatively costly to perform. The method with *Aedes aegyptii* /26/ is preferred.

Short-term subchronic toxicity to fish (*Pimephales promelas*)

The endpoints studied are already covered in the Fish Early Life Stage Test (TG 210) and partly in the OECD draft TG regarding the egg and sacfry test.

Table 5.1: Warm freshwater environment, acute toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Crustacea	*Neomysis mercedis*	O	ST	survival	A	A	2a	402	28
Insecta	*Aedes aegyptii*	H/D	ST	larvae survival	B	A	2b	144	91,138
Amphibia	*Xenopus laevis*	H	ST	larvae survival	B	B	2b	98	83,117, 122
Fish	Various species	C	ST	survival	OECD TG 203	–	–	–	–
	Various species	C	LT	survival	OECD TG 204	–	–	–	–
Aschelminthes	*Brachionus calyciflorus*	H	ST	survival	A	B	2a	9	31,190, 205
Plathyhelminthes	*Dugesia dorotocephala*	C	ST	behaviour	B	C	3	195	–

Table 5.1, continued: subchronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Kormophyta (plants)	*Lemna gibba*	P	ST	growth	A (US EPA, ASTM, AFNOR)	A	1a	88	66
Insecta	*Epeorus latifolium*	H	LT	survival, development	B	A	3	33	–
Amphibia	*Xenopus laevis*	H	LT	survival, development	B	B	2b	99	–
Fish	*Brachydanio rerio* and others	C	ST	egg & yolksac-larvae develop., survival, growth	OECD draft	–	–	265	–
	Pimephales promelas and others	C	ST	larvae develop., survival, growth	A (US EPA)	C	3	242	244
	Various species	C	LT	early life stage develop., survival, growth	OECD TG 210	–	–	–	–
Cnidaria	*Hydra attenuata*	O	ST	teratogenesis	B	C	3	373	–
Plathyhelminthes	*Dugesia dorotocephala* and *D. japonica*	C	ST	regeneration, survival	B	C	3	197,198	367

Table 5.1, continued: chronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, micro	Various species	P	ST	growth	OECD TG 201	–	–	–	–
Bacteria	*Pseudomonas putida*	D	ST	growth	A	A	2a	192	–
Aschelminthes	*Brachionus urceolaris*	H	ST	survival, reproduction	B	B	2a	31	9,190, 209
Fish	*Pimephales promelas*	C	LT	reproduction, growth, survival, development	US EPA 670/4-73-001	A	1a	428	–
	Brachydanio rerio	C	LT	reproduction, growth, survival, development	B	A	(1a)	276	26
Cnidaria	*Hydra* sp.	O	ST	reproduction, survival, development	B	B	3	7	–
Protozoans	*Tetrahymena pyriformis*	D	ST	growth	A	A	2b	436	

Short-term subchronic toxicity to Cnidaria (*Hydra altenuata*)

The method has been developed for studying teratogenesis. The testing principles presumably may be applied for other endpoints as well. The taxonomic group is presumably only an important group in certain types of aquatic environments.

Short-term chronic toxicity to Cnidaria (*Hydra* sp.)

Life cycle testing methods with *Hydra*. The taxonomic group may dominate, in some periods, in special environments.

5.3 Cold freshwater environment

The methods assessed to be candidates for Test Guideline development are outlined in **Table 5.2.** Methods based on organisms within the following groups, which are not already represented in the OECD Test Guidelines Programme, are identified as suitable for standardisation:

Higher plants
Crustaceans (*Gammarus* sp.)
Insect larvae
Bacteria
Amphibians
Protozoans

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

Group 1a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Test methods with vascular plants are requested in six assessment schemes.

A test with *Lemna* is recommended for the warm freshwater environment (see Section 5.2). *Lemna* is easy to maintain in culture and the test method is relatively uncomplicated. The testing method suggested has been adopted as a Swedish standard.

Group 1b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

No methods to be recommended.

Group 2:

Methods presumably needed in the near future as they are recommended in national assessment schemes and/or considered to represent key ecological groups

Group 2a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Group 2b
Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term acute and long-term subchronic toxicity tests with the amphibians *Rana pipiens*, *Ambystoma mexicanum* and *A. texanum*

The recommendation of tests with amphibian larvae has been identified in two assessment schemes (USA). Among the available methods for acute toxicity, two tests with the herbivore *Rana pipiens* and the carnivore *Ambystoma mexicanum* are recommended, but other procedures are also available. Two subchronic tests with *Rana pipiens* and *Ambystoma texanum* are recommended.

Short-term acute toxicity test with the crustaceans *Gammarus pulex* and *G. italicus*

The test method is recommended in two assessment schemes (testing of chemicals and waste water). *Gammarus*, being a detritivore, represents an important link in the food chain in many freshwater environments. Several *Gammarid* species can be maintained in culture and the tests are relatively easy to perform.

Short-term acute toxicity tests with insect larvae: *Aedes atropalpus*, *Aeronueria lycorias*, *Hydropsyche pellucidula* and *H. contubernalis*

Acute toxicity tests with insect larvae are required by several assessment schemes. The test with the herbivorous larvae of the mosquito *Aedes atropalpus* is inexpensive and relatively well documented, even though development is still needed.

The test with the predacious larvae of the stone fly *Acroneuria lycorias* is relatively labour intensive and not well documented, but it is currently the best available test for carnivorous insect larvae.

The two species of *Hydropsyche* (*H. pellucidula* and *H. contubernalis*) are both widely distributed, and both tests require further development. Investigations of the general sensitivity of these species are needed before standardisation can be initiated.

The species above are all epibenthic filter feeding species.

Short-term chronic toxicity test with the protozoan *Tetrahymena pyriformis*

The growth test with *Tetrahymena* is recommended for the warm as well as the cold freshwater environment (see Section 5.2).

Table 5.2: Cold freshwater environment, acute toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Crustacea	*Ceriodaphnia dubia*	H	ST	survival	A (US EPA)	A	3	58	49
	Daphnia magna and *D. pulex*	H	ST	survival	OECD TG 202	A	–		
	Gammarus pulex and *G. italicus*	D	ST	survival	B	A	2b	224	231,215
Insecta	*Aedes atropalpus*	H	ST	larvae survival	B	A	2b	175	
	Aeroneuria lycorias	C	ST	nymph survival	B	A	2b	148	
	Hydropsyche pellucidula and *H. contubernalis*	O/D	ST	juvenile survival	B	A	2b	172,180	23,162, 135
Amphibia	*Rana pipiens*	H	ST	survival	B	B	2b	95	92,107, 119,102
	Ambystoma mexicanum	C	ST	survival	B	B	2b	121	97,108, 109
Fish	*Oncorhynchus mykiss* a.o. species	C	ST	survival	OECD TG 203	A	–	–	–
	Oncorhynchus mykiss	C	LT	survival	OECD TG 204	B	–	–	–

Table 5.2, continued: subchronic toxicity testing methods

Taxonomic Group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Kormophyta	*Lemna minor*	P	ST	growth	A	A	1a	191	66,88
Crustacea	*Ceriodaphnia dubia*	H	ST	reproduction, survival	A	A	3	18	–
	Daphnia sp.	H	LT	reproduction, survival	OECD TG 202	–	–	–	–
Insecta	*Cloeon triangulifer*	H	ST/LT	survival moulting	B	A	2b	165	164
Amphibia	*Rana pipiens*	H	LT	ELS, embryotox	B	B	2b	105	–
	Ambystoma texanum	C	LT	embryotox-respiration	B	B	2b	90	–
Fish	*Oncorhynchus mykiss*	C	LT	ELS, survival, growth, embryotox a.o.	OECD TG 210, OECD draft	–	–	–	–

Table 5.2, continued: chronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, micro	Various species	P	ST	growth	OECD TG 201	–	–	–	–
Bacteria	*Pseudomonas putida*	D	ST	growth	A (ISO draft)	A	3	192	–
Protozoans	*Tetrahymena pyriformis*	D	ST	growth	A	A	2b	436	13,199, 20

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

Short-term acute toxicity to crustaceans (*Ceriodaphnia dubia*)

The endpoints of this method are included in OECD TG 202 with *Daphnia magna* and *D. pulex*. In future updates of the Test Guideline, the *Ceriodaphnia* species should be included.

Short-term subchronic toxicity to Crustaceans (*Ceriodaphnia dubia*)

The method is adopted as a US EPA as well as an Environment Canada test guideline. The method may be applied as a short-term screening method for chronic toxicity for some chemicals. The endpoints studied are already considered to be included in OECD TG 202.

Short-term chronic toxicity (growth) test with the bacteria *Pseudomonas putida*

The use of this test is included in two assessment schemes (complex mixtures). The growth test with *P. putida* is available as a German standard and an ISO draft guideline, and the test is relatively easy and inexpensive to perform. The ecological relevance of this single species bacterial test may be debatable.

5.4 Warm marine environment

The methods expected to be candidates for Test Guideline development are outlined in **Table 5.3**. Methods based on organisms within the following groups, which are not already represented in the OECD Test Guidelines Programme, are identified as suitable for standardisation:

Microalgae
Macroalgae
Crustaceans
Aschelminthes
Amphibians
Fish
Echinoderms
Cnidarians
Protozoans

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

Group 1a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term chronic toxicity test with the microalgae *Skeletonema costatum* and *Phaeodactylum triconutum*

Algae growth tests are requested in most assessment schemes but no tests for marine algae are currently included in the OECD Test Guidelines. A draft ISO standard is available and will probably be adopted in the near future.

Group 1b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

No methods to be recommended.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

Group 2a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term acute and subchronic toxicity test with the rotifer *Brachionus plicatilis*

Data on toxicity to rotifers are requested in two assessment schemes (Stephan, 1985 and Environment Canada, 1994). A procedure is described by the US-ASTM. As mentioned in Section 5.2, the rotifers are relevant as they represent microzooplankton, which is important for the carbon and nutrient cycling in the aquatic ecosystem.

Short-term subchronic toxicity test with the macroalgae *Champia parvula*

Tests with macroalgae are included in one assessment scheme (US EPA). The present test, which is a US EPA standard, is based on growth and reproduction. Similar test systems with other species are available (refs. 8 and 44).

Short-term acute toxicity tests with the crustaceans *Mysidopsis bahia* and *Penaeus aztecus* a.o.

Acute tests with crustaceans are required by a broad range of assessment schemes. Test methods for the species mentioned are available as US EPA standard tests and are uncomplicated to perform compared to the existing OECD Test Guidelines. *Mysidopsis bahia*, and probably also *Penaeus aztecus*, can be maintained in culture and the species are suitable as test organisms representing omnivorous crustaceans from the warm pelagic marine environment.

Long-term subchronic and chronic tests with the crustaceans *Mysidopsis bahia*

Four assessment schemes include data on reproduction of crustaceans. Standard methods with *Mysidopsis bahia* are available from US-ASTM (reproduction, subchronic test) and US EPA (growth, chronic).

Short-term subchronic test with the sea urchins *Arbacia punctulata* and *Lytechinus pictus*

Subchronic test methods with sea urchins are available as Canadian and US EPA standards. The methods are relatively easy to perform, but the test organisms need to be collected from the environment as sufficient knowledge of culturing is not available. Subchronic endpoints based on tests with sea urchins (fertilization, development) are included in four assessment schemes, of which three schemes are focusing on waste water assessment.

Group 2b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term chronic toxicity (growth) test with the protozoan *Uronema marinum*

Growth test with protozoans is recommended in one scheme for assessment of complex mixtures. The test is easy to perform and is possible to standardise with only little effort.

Short-term acute toxicity test with the macroalgae *Gracilaria tenuistipitata*

Tests with macroalgae are included in one assessment scheme (US EPA). The present test is based on short-term growth, but needs to be further developed before standardisation can be recommended.

Long-term subchronic toxicity test with the hydrozoans *Eirene viridula* and *Cordylophora caspia*

Tests with hydrozoans have not been requested by any of the assessment schemes reviewed. The taxonomic group may, however, be ecologically significant in the marine environment. *E. viridula* is a cosmopolite species in the marine environment and is frequently found in both warm and cold environments. *C. caspia* is primarily found in brackish cold water environments. Both species may be cultured in the laboratory. The endpoints studied are asexual reproduction and growth.

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

No methods recommended.

Table 5.3: Warm marine environment, acute toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, macro	*Gracilaria tenuistipitata*	P	ST	growth	B	A	2b	44	–
Crustaceans	*Mysidopsis bahia* a.o.	O	ST	survival	A	A	2a	73	60,420
	Penaeus aztecus a.o.	O	ST	survival	A	B	2a	75	348,349
Aschelminthes	*Brachionus plicatilis*	H	ST	survival	A	A	2a	381	–
Fish	*Cyprinodon variegatus* a.o.	C	ST	survival	OECD 203	–	–	–	–

Table 5.3, continued: subchronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, macro	*Champia parvula*	P	ST	growth, reproduction	A	A	2a	57	8
Crustaceans	*Mysidopsis bahia* a.o.	O	LT	survival, reproduction	A	A	2a	80	406,407
Fish	*Menidia peninsulae*	C	ST	ELS, survival, growth, hatchability	OECD draft	–	–	268	280,52, 54
	Menidia peninsulae a.o.	C	LT	ELS, survival, hatchability, growth, malformation	OECD 210	–	–	344	
Cnidaria (Coelenterata)	*Eirene viridula*	C	LT	asexual reproduction	B	B	2b	443	
	Cordylophora caspia							442	
Echinodermata	*Lytechinus pictus*	O	ST	reproduction, fertility	A	A	2a	236	56,413

Table 5.3, continued: chronic toxicity testing methods

Taxonomic Group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, micro	*Skeletonema costatum* a.o.	P	ST	growth	ISO draft	A	1a	–	–
Crustaceans	*Mysidopsis bahia*	O	LT	survival, growth	A	A	2a	55	–
Fish	*Cyprinodon variegatus*	C	LT	reproduction, growth, survival, development	A	B (US EPA)	2a	435	–
Protozoans	*Uronema marinum*	O	ST	growth	B	A	2b	189	–

5.5 Cold marine environment

The methods assessed to be candidates for standardisation are outlined in **Table 5.4**. Methods involving representation from the following taxonomic groups, which are not already represented in the OECD Test Guidelines Programme, are identified as suitable for standardisation:

Microalgae
Macroalgae
Higher plants
Crustaceans
Fish
Echinoderms
Molluscs
Protozoans

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

Group 1a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term chronic toxicity test with the microalgae *Skeletonema costatum* and *Phaeodactylum triconutum*

These algae species can be regarded as both cold and warm water species. An ISO draft method is available (see Section 5.4).

Short-term acute toxicity test with the crustaceans *Acartia tonsa*, *Tisbe battgliai* and *Nitocra spinipes*

An ISO draft method for these species is available. The tests are easy to perform compared to corresponding OECD Test Guidelines. The test species are representative for planktonic crustaceans in the cold marine (or brackish) environment. The test species may be maintained in laboratory culture.

Group 1b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term subchronic and long-term chronic test with the crustaceans *Acartia tonsa*, *Centrophages hamatus* and *Eurytemora affinis*

Four assessment schemes request subchronic/chronic toxicity data for marine crustacean species. Due to the ecological importance of this taxonomic group, the process of Test Guideline development should be initiated as soon as sufficient scientific documentation has been made available.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups.

Group 2a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

Short-term subchronic test with the macroalgae *Champia parvula*

Macroalgae are included in one assessment scheme for assessment of chemicals (US EPA). A US EPA standard is available with growth and ability to form reproductive elements as endpoints. Other methods are also available (*Ceramium strictum* and *Porphyra yezoensis*).

Short-term subchronic test with the sea urchins *Strongylocentrotus* sp. and *Dendraster exentricus*

Basically, the standard test from Environment Canada is recommended for the warm marine environment (see Section 5.4). The method is relatively easy to perform, but the test organisms have to be collected from the environment. Subchronic endpoints with sea urchins (reproduction and growth) are included in four assessment schemes (including three schemes for waste water assessment).

Short-term subchronic tests with bivalvia *Crassostrea* sp., *Mytilus edulis* and *Mercenaria mercenaria*

Four different assessment schemes include bivalvia as test organisms, which reflects their importance in the coastal environment. A US-ASTM standard method based on survival and development of embryos and larvae has been adopted.

Long-term subchronic test with the plant *Zostea marina*

Toxicity data from testing of vascular plants are requested in six assessment schemes, but only in one of these (a waste water scheme) has a marine vascular plant test been recommended. *Zostea* is widespread and may represent higher plants in the cold marine and brackish water environment. The test needs to be further developed, however.

Group 2b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term chronic toxicity (growth) test with the protozoan *Uronema marinum*

This test is recommended for the warm as well as the cold marine environment (see Section 5.4). Growth tests with protozoans are recommended in one draft scheme for waste water assessment. The test is easy to perform and is possible to standardise with little effort.

Table 5.4: Cold marine environment, acute toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Crustacea	*Acartia tonsa* a.o.	H/O	ST	survival	ISO draft	A	1a	212	213,214, 230,219, 220
Fish	*Cymatogaster* sp. a.o.	C	ST	survival	OECD 203	–	1a	–	–

Table 5.4, continued: subchronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Algae, macro	*Porphyra yezoensis*	P	LT	growth	B	A	2b	38	–
	Ceramium strictum	P	ST	reproduction	B	A	2b	8	57,38
	Champia parvula	P	ST	reproduction	A	A	2a	57	8,38
Kormophyta	*Zostea marina*	P	LT	growth	B	A	2a	202	–
Crustacea	*Acartia tonsa*	H	ST	survival, fertility	B	A	1b	410	208,221
	Centrophages hamatus	H	ST	survival, fertility	B	A	1b	208	410,221
	Eurytemora affinis	H	ST	fertility	B	A	1b	221	208,410

Table 5.4, continued: chronic toxicity testing methods

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Fish	*Clupea harengus*	C	LT	ELS, hatchability, survival, growth	OECD draft	–	–	269	–
	Gasterosteus aculeatus	C	LT	ELS, survival, hatchability	OECD 210	–	–	340	–
Echinodermata	*Strongylocentrotus* sp. a.o.	O	ST	fertility	A	A	2a	233	243,237
Mollusca	*Crassostrea* sp. a.o.	H	ST	reproduction	A	A	2a	86	72,41, 46,403, 411
Algae, micro	*Skeletonema costatum* a.o.	P	ST	Growth	ISO draft	A	1a	371, 372	–
Crustaceans	*Acartia tonsa*	H	LT	Reproduction, survival	B	A	1b	409	–
Protozoans	*Uronema marinum*	O	ST	Growth	B	A	2b	189	–

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

No methods recommended.

5.6 Biological waste water treatment

The methods that should be considered to be candidates for standardisation are outlined in **Table 5.5**. Only tests with consortia/communities of bacteria have been proposed in assessment schemes.

Group 1:

Methods needed for existing international (draft) effects, hazard and risk assessment schemes

Group 1a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

No methods recommended.

Group 1b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

No methods recommended.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

Group 2a

Methods recommended for inclusion in the OECD Test Guidelines Programme, as they are expected to require only little effort to be ready for standardisation:

<u>Short-term acute tests with nitrifying bacteria</u>

A test for inhibition of the nitrification in activated sludge (ISO guideline 9509) is recommended for OECD Test Guideline development. The method is highly relevant for assessment of substances emitted to waste water treatment plants. Tests with bacteria are requested in three different assessment schemes, two of them focusing on assessment of waste water.

Group 2b

Methods to be considered for Test Guideline development after sufficient scientific documentation has been provided:

Short-term chronic toxicity to fermenting bacteria (anaerobic sludge)

Effects of chemicals on fermentation processes should be considered for future assessment schemes. An ISO draft standard is currently elaborated and is expected to be adopted in the near future.

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

No methods recommended.

Table 5.5: Short-term acute and chronic toxicity test methods, treatment plants

Taxonomic group	Species	Trophic level	LT/ST	Endpoints	Readiness for standard. AA/A/B	Relevance for hazard/risk assessment	Recom.	Key ref.	Support ref.
Bacteria	nitrifying consortia	D	ST	rate of nitrification	AA (ISO)	A	2a	384	–
Bacteria	fermenting bacteria	D	ST	anaerobic respiration	B	A	2b	408	–
Bacteria	aerobic respirative consortia	D	ST	aerobic respiration	OECD TG 209	–	–	382	–

6. BENTHIC TEST METHODS

6.1 Evaluation process

As stated in the Introduction, only a few benthic methods concern a standardised method or protocol. Most references used in this review refer to research carried out to assess the toxicity of sediments, without aiming at developing a widely applicable toxicity test method. As sufficient information on reproducibility, sources of potential error, range of tolerance of the organisms to environmental conditions, geographical distribution and general sensitivity to chemicals was often not available in the papers, these criteria could not be used for discrimination between the methods.

Due to this situation, it was decided to slightly modify the evaluation process compared to the process followed for the pelagic test methods.

First, the standard and ring-tested methods were selected from the available literature and evaluated according to the scoring system outlined in Section 4.3. The detailed evaluation of these methods is given in Annex G.

From these standard and ring-tested methods, all those that met the five characteristics considered to be important for a benthic toxicity test were selected. Most of these characteristics were mentioned as being important ones by the participants in the WOSTA workshop:

1) The test is carried out with a benthic or epibenthic species (ecological representativeness).
2) Possible exposure routes are via sediment particles (ingestion and contact), porewater and overlying water, as in a whole sediment system (relevance of exposure route).
3) The duration of the exposure phase is long enough for chemicals to be taken up by organisms (arbitrarily fixed at ten days or longer) (duration of test).
4) Endpoints are ecologically relevant (extrapolation of endpoints).
5) Test organisms can easily be cultured in the laboratory or sampled from the field (availability of test organism).

The remaining references, which are non-standardised or ring-tested methods, were also evaluated according to the scoring system outlined in Section 4.3 (results are given in Annexes H-K). As most of these studies were not aimed at presenting a testing method, it was not attempted to give an overall score indicating the applicability of the study. Instead, only those methods that met the five characteristics mentioned above were selected.

All the selected methods and studies were grouped according to the taxonomic group they are addressing. They are presented in the **Tables 6.1-6.5**. Depending on individual evaluation scores, key references and supporting references were chosen. Where several methods fulfilled the five characteristics, the expected best choice is presented in the tables. For some methods, ASTM guidelines were available, as well as recent ASTM-based EPA guidelines. In those cases, the EPA guidelines were chosen as key references. Remarks are made on their need for hazard and risk assessment schemes.

Some of the methods and studies that were not allocated a high score in this tiered approach were selected and allocated to one of the tables (Tables 6.2-6.5), as they might fill a "taxonomic" or "endpoint" gap and therefore might be useful in the process of Test Guideline development.

Because of the combination of an (epi)benthic species tested in a whole sediment system, the procedure of selecting the available methods has been very difficult. In view of the aim of the OECD Test Guidelines, this selection is thought to be justifiable, but some explanation might be useful. Benthic test methods are asked for in order to assess the risks of sediment-bound chemicals for benthic and epibenthic species. As the bioavailability of this type of chemicals to (epi)benthic organisms is insufficiently understood, all possible exposure routes to these organisms have to be taken into account. When testing detritivorous (epi)benthic species in a whole sediment system, all potential exposure routes may be reflected. Therefore, elutriate and porewater tests with pelagic species are thought to be useful as screening methods but not to be optimal for fulfilling the aim of an OECD benthic test method (view shared by SETAC 1994). Test methods with pelagic species are recommended in Chapter 5. Depending on the purpose of testing, these pelagic species can be tested in a water-only system with a known PEC (e.g. estimated porewater concentration) or tested in an elutriate or porewater.

Sediment suspension methods can be viewed as something in between elutriate and whole sediment tests. It is doubtful whether the sediment particles contribute as a relevant exposure route in these tests.

WOSTA workshop participants considered "general sensitivity" to be an important factor for ranking purposes. Although it has not been the purpose of this review to collect additional information, some additional research was carried out. The database AQUIRE (update of June 1994) was consulted in order to assess the susceptibility of a species to metals, pesticides and industrial chemicals, as far as information was available. For this purpose, toxicity values for the species were compared with those for algae, daphnids and fish, using three toxicity classes: < 0.1 mg/l, 0.1-1.0 mg/l and > 1.0 mg/l.

For the items "availability" and "costs (equipment and labour)", another important item according to the WOSTA workshop, the TNO Institute of Environmental Sciences in Delft, the Netherlands, was consulted.

Some additional remarks on the evaluation process:

- The description of test methods was often too incomplete to describe the method fully according to the data registration form.

- The procedure for sediment preparation and spiking was considered not to be species-dependant, and therefore not to differentiate between the methods. Hence, this part of the method is not evaluated. Some recommendations are made in Chapter 7.

Table 6.1: Standardised and ring-tested methods that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints

Environment	Taxonomic group	Species	Endpoint	Level	Trophic level	Duration	Ref. No.
cold fresh	Insecta	*Hexagenia limbata*	survival	2a	degrader	acute	4247
warm fresh	Annelida	*Tubifex tubifex*	survival	2a	degrader	acute	4239
		Lumbriculus variegatus	survival	2a	degrader	acute	4240
		Pristina leidyi	survival	2a	degrader	acute	4253
	Mollusca	*Anodonta imbecillis*	survival	2a	herbivore	acute	4250
	Crustacea	*Hyalella azteca*	survival, growth, behaviour and reproduction	2a	degrader	acute and subchronic	4012
		Hyalella azteca	survival (and growth)	2a	degrader	acute	4231
		Hyalella azteca	survival	2a	degrader	acute	4248
	Insecta	*Chironomus riparius*	survival, growth and emergence	2a	degrader	acute and subchronic	4011
		Chironomus riparius	survival	2a	degrader	acute	4251
warm fresh	Insecta	*Chironomus riparius*	survival, growth and behaviour	2b	degrader	subchronic	4015
		Chironomus riparius	survival, emergence and behaviour	2b	degrader	subchronic	4075

Environment	Taxonomic group	Species	Endpoint	Level	Trophic level	Duration	Ref. No.
warm fresh	Insecta	*Chrironomus tentans*	survival, growth and emergence	2a	degrader	acute and subchronic	4013
		Chrironomus tentans	survival and growth	2a	degrader	acute	4241
		Chrironomus tentans	survival	2a	degrader	acute	4252
		Hexagenia limbata	survival	2a	degrader	acute	4247
cold marine	Annelida	*Arenicola marina*	survival	1a	degrader	acute	4188/ 4228
	Crustacea	*Rhepoxynius abronius*	survival, avoidance and reburying ability	2a	degrader herbivore carnivore	acute	4006
		Rhepoxynius abronius	survival	2a	degrader herbivore carnivore	acute	4256
		Rhepoxynius abronius	survival (and reburying ability)	2a	degrader herbivore carnivore	acute	4243
cold marine	Crustacea	*Eohaustorius estuarius*	survival, avoidance and reburying ability	2a	degrader?	acute	4007
		Eohaustorius estuarius	survival (and reburying ability)	2a	degrader?	acute	4242

Environment	Taxonomic group	Species	Endpoint	Level	Trophic level	Duration	Ref. No.
cold marine	Crustacea	*Ampelisca abdita*	survival, growth, avoidance and behaviour	2a	degrader herbivore	acute	4008
		Grandidierella japonica	survival, avoidance and reburying ability	2a	degrader herbivore	acute	4009
		Grandidierella japonica	survival	2a	degrader herbivore	acute	4257
		various species: *Amphiporeia virginiana, Corophium volutator, Eohaustorius estuarius, Eohaustorius washingtonianus, Foxiphalus xiximeus, Leptocheirus pinguis, Rhepoxynius abronius*	survival, avoidance and reburying ability	2a	primarily degraders	acute	4040
		Bathyporeia sarsi	survival and reburying ability	1a	degrader	acute	4085
		Corophium sp.	survival and avoidance	1a	degrader	acute	4198
		Corophium sp.	survival	2a	degrader	acute	4246
		Corophium volutator	survival	1a	degrader	acute	4227
		Holmesimysis costata	survival	2a	omnivore	acute	4236
	Echinodermata	*Echinocardium cordatum*	survival, condition, avoidance and reburying ability	2a	degrader	acute	4003

Environment	Taxonomic group	Species	Endpoint	Level	Trophic level	Duration	Ref. No.
cold marine	Crustacea	*Echinocardium cordatum*	survival, avoidance, reburying ability and behaviour	1a	degrader	acute	4023/ 4225
warm marine	Annelida	*Nereis virens*	survival, growth and behaviour	2a	degrader	acute	4002/ 4004
		Nereis virens	survival, growth and behaviour	2a	degrader	acute	4187
		Neanthes arenaceodentata	survival	2a	degrader	acute	4249
	Crustacea	*Eohaustorius estuarius*	survival, avoidance and reburying behaviour	2a	degrader?	acute	4007
		Ampelisca abdita	survival, growth and behaviour	2a	degrader	acute	4008
		Ampelisca abdita	survival	2a	degrader herbivore	acute	4244
		Ampelisca abdita	survival	2a	degrader herbivore	acute	4258
		Corophium sp.	survival	2a	degrader	acute	4246
		Leptocheirus plumulosus	survival (and reburying ability)	2a	degrader	acute	4245
		Leptocheirus plumulosus	survival	2a	degrader	acute	4255
		Grandidierella japonica	survival	2a	degrader herbivore	acute	4257

Environment	Taxonomic group	Species	Endpoint	Level	Trophic level	Duration	Ref. No.
warm marine	Crustacea	*Mysidopsis bahia*	survival	2a	degrader omnivore	acute	4232
		Mysidopsis bigelowi	survival	2a	degrader omnivore	acute	4233
		Mysidopsis almyra	survival	2a	degrader omnivore	acute	4234
		Neomysis americana	survival	2a	omnivore	acute	4235
		Penaeus sp.	survival	2a	degrader	acute	4237
		Palaemonetes sp.	survival	2a	degrader	acute	4238

6.2 Standard and ring-tested methods

Standard or ring-tested methods recommended in assessment schemes are important sources for the final recommendation. ASTM methods belong to this group, as well as PARCOM ring-test protocols. Environment Canada has also published several standardised methods. Recently, several EPA guidelines were published. Some of these guidelines are based on ASTM methods. They are evaluated as independent guidelines.

A selection of these methods, which fulfil the five characteristics mentioned above, are considered to be key methods which can be used for OECD Test Guideline development. Table 6.1 presents the methods that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints.

Detailed scoring of these methods is presented in Annex G.

6.2.1 Cold freshwater environment

There are no candidates for Group 1. The only candidate is for Group 2a and represents the Insecta.

Long-term acute test with the insect *Hexagenia limbata*

An acute test with the mayfly *Hexagenia limbata* is recommended by the US EPA (1994a).

6.2.2 Warm freshwater environment

There are no candidates for Group 1. Candidates for Group 2a are methods involving representatives of the Annelida, Mollusca, Crustacea and Insecta.

Long-term acute tests with the annelids *Tubifex tubifex*, *Lumbriculus variegatus* and *Pristina leidyi*

Acute tests with the three annelid species *Tubifex tubifex*, *Lumbriculus variegatus* and *Pristina leidyi* are recommended by the US EPA (1994a). Annelids are also recommended by Stephan et al. (1985) and the Swedish EPA (1990).

Long-term acute test with the mollusc *Anodonta imbecillis*

An acute test with the paper pond shell clam *Anodonta imbecillis* is recommended by US EPA (1994a).

Long-term acute and subchronic tests with the crustacean *Hyalella azteca*

Benthic toxicity tests with crustaceans are required in several schemes (US EPA 1994a,d, DK-EPA 1993, S-EPA 1990, Walker 1990, Stephan et al. 1985). Recommended species are, amongst others, *Hyalella azteca* and *Gammarus pulex*. For *H. azteca*, ASTM and US EPA standards are available. The ASTM method covers both acute and subchronic exposure. In 1995, a test method for growth and survival in sediment using *H. azteca* will be published by Environment Canada.

Long-term acute and subchronic tests with the insect *Chironomus riparius*

Tests with *Chironomus riparius* are recommended by the Danish EPA (1993) and US EPA (1994a). A test with *C. riparius* is also be requested by the BBA (1993). An ASTM method for this species covers both acute and subchronic exposure. The available US EPA method only covers acute exposure. Two subchronic methods with *C. riparius* were recently ring-tested (refs. 4015 and 4075). These descriptions, meant for pesticide testing, are not as thorough as the ASTM method. They might support the drafting of a possible Test Guideline on *C. riparius*. Currently, efforts being are made in the Netherlands to start an international ring-test on *C. riparius* (ref. 4254). This subchronic test starts with the exposure of eggs.

Long-term acute and subchronic tests with the insect *Chironomus tentans*

A test on this species is recommended by US EPA (1994a,d) and Walker (1990). The ASTM method for *C. tentans* covers both acute and subchronic exposure. The available US EPA method only covers acute exposure.

US EPA (1988) has recommended a test with *C. decorus*. This method might be replaced by the methods with other Chironomidae.

Long-term acute test with the insect *Hexagenia limbata*

An acute test with the mayfly *Hexagenia limbata* is recommended by the US EPA (1994a).

6.2.3 Cold marine environment

Candidates for Group 1a are methods involving representatives of the Annelida, Crustacea and Echinodermata.

Long-term acute test with the annelid *Arenicola marina*

This method has been recommended by PARCOM (1993) and the Danish EPA (1993). The method is available as a PARCOM ring-test protocol. *A. marina* is less sensitive than the currently applied OECD species.

Long-term acute tests with crustaceans

Tests with marine amphipods have been requested in two schemes (PARCOM 1993, Walker 1990) and are recommended by the US EPA (1994a, d). PARCOM recommends its own ring-test protocols on *Corophium* sp. or *Bathyporeia sarci*. Following their recent choice, *C. volutator* is preferred (ref. 4227). *C. volutator* can be cultured in the laboratory, but this might need some more study. Control mortality can be too high (pers. comm.).

Species used in ASTM, Environment Canada and/or US EPA methods are a.o. *Eohaustorius estuarius, Rhepoxynius abronius, Corophium* sp. and *Grandidierella japonica*. The only standard known for crustaceans other than amphipods is that for the mysid shrimp *Holmesimysis costata*. This method is recommended by the US EPA (1994a).

Long-term acute test with the echinoid *Echinocardium cordatum*

Two schemes request tests with echinodermata (PARCOM 1993, DK-EPA 1993). Both schemes recommend the PARCOM ring-test protocol on *E. cordatum*. This species might not be easily available all over the world.

6.2.4 Warm marine environment

There are no candidates for Group 1. Candidates for Group 2a are methods involving representatives of the Annelida and the Crustacea.

Long-term acute tests with the annelids *Nereis virens* and *Neanthes arenaceodentata*

The method for *Nereis virens* is available as a PARCOM ring-test protocol and is suggested by the Danish EPA (1993). The method for *Neanthes arenaceodentata* is recommended by US EPA (1994a).

Long-term acute tests with crustaceans

Amphipods are recommended by US EPA (1994a,d) and by Walker (1990). For the warm marine environment, several amphipods are available which are used in the ASTM standard method and in the US EPA methods.

Methods for other crustaceans are also recommended by US EPA (1994a). These species are *Mysidopsis* sp., *Neomysis americana, Penaeus* sp. and *Palaemonetes* sp.

6.2.5 Standard and ring-tested methods not selected

Methods which are standardised or ring-tested, but not inserted in Table 6.1 because they do not fulfil all five characteristics, are:

- a method with the freshwater algae *Selenastrum capricornutum* (ref. 4052, pelagic species, elutriate as test medium)
- a method with the marine mollusc *Abra alba* (refs. 4183 and 4226, a physiological endpoint, sediment suspension as test medium, the test chemical is micro-encapsulated)
- methods with the marine crustacean *Chaetogammarus marinus* (refs. 4001 and 4020, water as test medium)
- methods with various echinoids (refs. 4050/4051, not easily available, pelagic life stage, elutriate as test medium)
- a method with the marine fish *Scophthalmus maximus* (refs. 4200/4201, water accommodated fraction as test medium)

Several of these are also short-term methods.

Table 6.2: Methods and studies that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints

Environment: cold freshwater

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
Bacteria	sediment bacteria	mineralisation	ST	2b	degrader	chronic	4016	
Annelida	*Stylodrilus heringianus*	survival and avoidance	ST	2b	degrader	acute	4093	
	Limnodrilus hoffmeisteri	survival and avoidance	ST	2b	degrader	acute	4094	
	Stylodrilus heringianus	survival and growth		2b	degrader	subchron	4092	
Crustacea	*Gammarus lacustris*	survival		2b	degrader	acute	4141	
Insecta	*Hexagenia limbata*	survival		2a	degrader	acute	4247	
Pisces	*Noemacheilus barbatulus*	growth	ST	3	carnivore	acute	4047	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

6.3 Cold freshwater environment

Methods that fulfil the requirements of 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily sampled in the field and which has 5) ecologically relevant or highly relevant endpoints are presented in Table 6.2. A few methods do not fulfil all five characteristics, but might be useful in the process of Test Guideline development. These methods can be recognised in Table 6.2 by a short-term exposure period or, in other cases, by a remark made in the text.

Detailed scoring of the methods is presented in Annex H.

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

There are no candidates for Group 1.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

The following method is considered to belong to Group 2a.

Long-term acute test with the insect *Hexagenia limbata*

An acute test with the mayfly *Hexagenia limbata* is recommended by US EPA (1994a). Toxicity to insects is also required by the Danish EPA (1993). However, it recommends a *Chironomus* species, as specifically mentioned in some other schemes. For the cold water environment, a test method using *H. limbata* is available.

The following methods are considered to belong to Group 2b.

Short-term chronic test with bacteria

The use of bacteria for testing the sediment environment has not been specifically discussed in the context of the assessment schemes. However, the bacteria are considered to be an ecologically important group of organisms. Methods with anaerobic bacteria are described (ref. 4016; a detailed description was published in 1994). These methods are short-term methods, but exposure can be regarded as chronic due to the fast uptake of chemicals. Endpoints are mineralisation (e.g. acetate). The methods use radio-labelled substrates which makes them less attractive.

Acute and subchronic toxicity tests with the annelids *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*

One assessment scheme demands an acute toxicity test with e.g. annelids (Stephan et al., 1985). The US EPA recommends sediment tests with three warm freshwater species (1994a). The Swedish EPA also recommends a test with the warm freshwater species *Tubifex tubifex*.

For the cold water environment, methods might be developed with the species *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*. Two key references refer to short-term methods. The duration of the method should therefore be extended. These species are generally less sensitive than the current OECD species. Superficial breathing is reported in the case of *S. heringianus*.

A long-term subchronic study has been carried out with *S. heringianus*, in which survival and growth have been studied.

Long-term acute toxicity test with the crustacean *Gammarus lacustris*

Acute toxicity testing with benthic crustaceans has been requested by US EPA (1994a,d) and Stephan et al. (1985). The recommended species, *Hyalella azteca*, is a warm freshwater species. *Gammarus lacustris* could serve as a cold freshwater species. This species is more sensitive than the current OECD species. *Gammarus* sp. are also recommended for pelagic toxicity testing. The recommended benthic method with *G. lacustris* uses whole sediment as test system. However, the authors prefer testing with *Hyalella azteca* rather than *G. lacustris*.

Test guideline 40 CFR 795.120 is used by the US EPA as a gammarid acute toxicity test. A description of this method was not available at the time of evaluation. According to recent personal information, this guideline is still in use as a sediment toxicity test.

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

Acute toxicity test with the fish *Noemacheilus barbatulus*

Benthic tests with fish are not mentioned in the assessment schemes. A short-term study has been carried out with the stone loach *Noemacheilus barbatulus*. Although this species is mainly exposed through other routes than via sediment particles, it has dermal contact with sediment because it is tested in a whole sediment system. The species might be used in a case where a fish test needs to be developed.

Table 6.3: Methods and studies that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints

Environment: warm freshwater

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
Archaebacteria	methanogens	methanogenesis		2b	degrader	chronic	4197	
	various	enzymatic activity, bacterial respiration and cell counts			degrader	chronic		4029
Aschelminthes	*Panagrellus redivivus*	survival and growth	ST	2b	?	subchronic	4070	
Annelida	*Tubifex tubifex*	survival		2a	degrader	acute	4239	
	Tubifex tubifex	reproduction		2b	degrader	chronic	4161	
	Lumbriculus variegatus	survival, growth, reproduction and burrowing behaviour		2b	degrader	chronic	4044	
	Lumbriculus variegatus	survival		2a	degrader	acute	4240	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: warm freshwater

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
Annelida	*Lumbriculus variegatus*	survival (growth and reproduction)			degrader	acute (chronic)		
	Pristina leidyi	survival		2a	degrader	acute	4253	
	Branchiura sowerbyi	survival, growth, behaviour and appearance		2b	degrader	acute	4163	
Mollusca	*Anodonta imbecillis*	survival		2a	herbivore	acute	4250	
	Corbicula fluminea	survival	ST	2b	herbivore	acute	4155	
Crustacea	*Hyalella azteca*	survival, growth, behaviour and reproduction		2a	degrader	(acute and) subchronic	4012	
	Hyalella azteca	survival (and growth)		2a	degrader	acute	4231/ 4248	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: warm freshwater

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
Crustacea	*Diporeia* sp.	survival and behaviour		2b	degrader	acute	4104	
Insecta	*Chironomus riparius*	survival, growth and emergence		2a	degrader	acute and subchronic	4011	
	Chironomus riparius	survival		2a	degrader	acute	4251	
	Chironomus riparius	survival, growth and behaviour			degrader	subchronic		4015
	Chironomus riparius	survival, emergence and behaviour			degrader	subchronic		4075
	Chironomus riparius	survival, growth, behaviour, head width and mentum deformation			degrader	subchronic		4083
	Chironomus riparius	hatchability, ELS, survival	ST		degrader	subchronic		4204

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: warm freshwater

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
Insecta	*Chironomus riparius*	survival, growth and abnormal behaviour			degrader	subchronic		4254
	Chironomus tentans	survival, growth and emergence		2a	degrader	acute and subchronic	4013	
	Chironomus tentans	survival and growth		2a	degrader	acute	4241/ 4252	
	Paratanytarsus parthenogeneticus	hatchability		3	degrader	chronic	4108	
	Hexagenia limbata	survival		2a	degrader	acute	4247	
Pisces	*Ictalurus punctatus*	survival	ST	3	carnivore	acute	4062	
	Ictalurus punctatus	survival		3	carnivore	subchron	4063	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

6.4 Warm freshwater environment

Methods that fulfil the requirements of 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints are presented in Table 6.3. A few methods do not fulfil all five characteristics but might be useful in the process of Test Guideline development. These methods can be recognised in Table 6.3 by a short-term exposure period or, in other cases, by a remark made in the text.

Detailed scoring of the methods is presented in Annex I.

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

There are no methods available for Group 1.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

The following methods are considered to belong to Group 2a. In the process of Test Guideline development, some references belonging to Group 2b (see Table 6.3) might be used to give a Test Guideline a broader application.

Long-term acute and chronic toxicity tests with annelids

Testing of annelid species is recommended by US EPA (1994a), Stephan et al. (1985) and the Swedish EPA (1990). These species are *Tubifex tubifex, Lumbriculus variegatus* and *Pristina leidyi*. An acute toxicity method is also available for *Branchiura sowerbyi*.

Chronic methods have been developed with *Tubifex tubifex* and *Lumbriculus variegatus*.

B. sowerbyi seems to be as sensitive as the current OECD species, whereas the other two are less sensitive. A chronic test with *B. sowerbyi* has not yet been developed. Superficial breathing is reported in the case of *L. variegatus.*

Acute toxicity tests with the molluscs *Anodonta imbecilis* and *Corbicula fluminea*

The Danish EPA requires testing with molluscs. *Unio* sp. are recommended, but a method is not mentioned. *Anodonta imbecilis* is recommended, and a method has been described by US EPA (1994a). *Corbicula fluminea* is a species that has also been used, however, in short-term studies. A standard method with this species is not available. Although these molluscs are tested in a whole sediment system, exposure is mainly via overlying water. These species seem to be less sensitive than the current OECD species.

Long-term acute and subchronic toxicity tests with the crustaceans *Hyalella* azteca and *Diporeia* sp.

Acute tests on *Hyalella azteca* are recommended by US EPA (1994a,d) and by Stephan et al. (1985). Recent guidelines are those of US EPA. Stephan refers to the ASTM method. There are several other references on *H. azteca* available, but they do not add new endpoints or other essential items.

Diporeia sp. is the only other available epibenthic crustacean. This organism was used in a long-term study and should be sampled in the field.

Long-term acute and subchronic toxicity tests with the insect *Chironomus*

Methods with *Chironomus riparius* are required by the Danish EPA (1993) and US EPA (1994a). A test with *C. riparius* might also be requested by the BBA (1993). The tests are available as a US EPA method as well as an ASTM method. Methods with *C. tentans* are also available as US EPA methods and as an ASTM standard method. A test on this species is recommended by the US EPA (1994a,d) and by Walker (1990) and might be requested by the BBA (1993). US EPA (1988) has recommended a test with *C. decorus*. This method might be replaced by the above mentioned methods with other Chironomidae.

Several additional methods use *C. riparius*. Differences between these methods mainly concern the exposure duration and the endpoints. Two endpoints mentioned are head width and mentum deformations. The value of these laborious endpoints is still under discussion. The endpoints hatchability and ELS, survival have to be carried out in water. Several references are included in the database because of special characteristics: use of artificial sediment (ref. 4133, and also 4075), use of a flow-through system (ref. 4134), and food as possible exposure route (ref. 4158).

Two methods with *C. riparius* were recently ring-tested (refs. 4015 and 4075). These descriptions, meant for pesticide testing, are not as thorough as the ASTM method. They might support the drafting of a possible Test Guideline on *C. riparius*. Currently, efforts are being made in the Netherlands to start an international ring-test on *C. riparius* (ref. 4254). This semi-static test method starts with the exposure of egg masses. Larvae will be exposed until the fourth larval stage.

Long-term acute test with the insect *Hexagenia limbata*

An acute test with the mayfly *Hexagenia limbata* is recommended by the US EPA (1994a).

Toxicity tests with amphibians

The US EPA (1988) as well as Walker (1990) request a (subchronic) tadpole benthic test. The EPA-recommended guideline is a proposed guideline, which has not yet been officially published. At the time of evaluation, no description was available. However, the test method is being used.

In this review, acute short-term test methods with *Xenopus laevis* and *Rana pipiens* are recorded. The test with *R. pipiens* is carried out in a whole sediment system. The endpoint is survival at an early life stage. The life stage used for testing is considered to be pelagic. Therefore, this method should not be used as a benthic test. The species can be cultured in the laboratory, but the availability might give problems. Furthermore, amphibian species are not favourable test species in some countries for animal welfare reasons.

The following methods are considered to belong to Group 2b.

Chronic test with bacteria

The use of bacteria with reference to the sediment environment has not been required in the assessment schemes. However, the bacteria are considered to be an ecologically important group of organisms. A method with bacteria might be based on ref. 4197. The bacteria are tested in a sediment suspension, but may also be tested in an anaerobic whole sediment system. The endpoint is methanogenesis.

Short-term subchronic toxicity test with the nematode *Panagrellus redivivus*

A test with nematodes is not required in the assessment schemes, but might be of interest due to their ecological importance. The nematode *Panagrellus redivivus* is used in a short-term test with an unpreferable solvent extract as test system.

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation

Long-term subchronic toxicity test with the fish *Ictalurus punctatus*

Benthic tests with fish are not mentioned in the assessment schemes. The benthic fish *Ictalurus punctatus* is used in a whole sediment system for studying acute and subchronic effects. Although this species is mainly exposed through other routes than via sediment particles, it has dermal contact with sediment because it is tested in a whole sediment system.

Long-term chronic toxicity test with the insect *Paratanytarsus parthenogeneticus*

A chronic test method has been developed with the insect *Paratanytarsus parthenogeneticus*. Tests with insects, mainly chironomid sp., are required, but a chronic test was not mentioned. The test with *P. parthenogeneticus* is carried out in water.

Table 6.4: Methods and studies that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints

Environment: cold marine

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
Aschelminthes	*Monhystera disjuncta*	survival	ST	2b	degrader	acute	4087	
	Monhystera disjuncta	reproduction and growth	ST	2b	degrader	subchronic	4086	
Annelida	*Arenicola marina*	survival		1a	degrader	acute	4188/ 4228	
	Nereis virens *Glycera dibranchiata* *Nephtys caeca*	burrowing behaviour (food uptake)			degrader	subchronic		4146
Mollusca	*Abra alba*	survival and fecal pellet production	ST	2b	degrader	acute	4183/ 4226	
	Abra alba	survival and fecal pellet production	ST	2b	degrader	acute	4184	
	Macoma baltica	survival		2b	degrader	acute	4025	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: cold marine

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
Crustacea	*Rhepoxynius abronius*	survival (and reburying ability)		2a	degrader herbivore carnivore	acute	4243/ 4256	
	Rhepoxynius abronius	survival, avoidance and reburying ability			degrader herbivore carnivore	acute		4006
	Eohaustorius estuarius	survival (and reburying ability)		2a	degrader ?	acute	4242	
	Eohaustorius estuarius	survival, avoidance and reburying ability			degrader ?	acute		4007
	Ampelisca abdita	survival, avoidance, growth and behaviour		2a	degrader herbivore	acute	4008	
	Grandidierella japonica	survival		2a	degrader herbivore	acute	4257	
	Grandidierella japonica	survival, avoidance and reburying ability			degrader herbivore	acute		4009

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: cold marine

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
Crustacea	various species: *Amphiporeia virginiana* *Corophium volutator* *Eohaustorius estuarius* *Eohaustorius washingtonianus* *Foxiphalus xiximeus* *Leptocheirus pinguins* *Rhepoxynius abronius*	survival, avoidance and reburying ability		2a	primarily degraders	acute	4040	
	Bathyporeia sarsi	survival and reburying ability		1a	degrader	acute	4085	
	Corophium sp.	survival and avoidance		1a	degrader	acute	4198	
	Corophium sp.	survival			degrader	acute		4246
	Corophium volutator	survival		1a	degrader	acute	4227	
	Holmesimysis costata	survival		2a	omnivore	acute	4236	
	Corophium insidiosum	survival and reproduction		2b	degrader	subchronic	4208	
	Microdeutopus gryllotalpa	survival and reproduction		2b	degrader	subchronic	4209	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: cold marine

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
Echinodermata	*Echinocardium cordatum*	survival, avoidance, reburying ability and behaviour		1a	degrader	acute	4023/ 4225	
	Lytechinus pictus	survival, growth and reproduction		2b	degrader	chronic	4189	
	Lytechinus pictus	survival, growth, reproduction and behaviour		2b	degrader	subchronic	4190	
Pisces	*Scophthalmus maximus*	survival	ST	3	carnivore	acute	4200/ 4201	
	Pseudopleuronectes americanus	growth and biochemical responses	ST	3	carnivore	acute	4153	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

6.5 Cold marine environment

Methods that fulfil the requirements of 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints are presented in Table 6.4. A few methods do not fulfil all five characteristics, but might be useful in the process of Test Guideline development. These methods can be recognised in Table 6.4 by a short-term exposure period or, in other cases, by a remark made in the text.

Detailed scoring of the methods is presented in Annex J.

Group 1:

Methods needed for existing international (draft) effects, aquatic hazard and risk assessment schemes

The following methods are considered to belong to Group 1a. In the process of Test Guideline development some references, belonging to group b, might be used to give a Test Guideline a broader application.

Long-term acute toxicity test with the annelid *Arenicola marina*

Tests with marine annelids are required by PARCOM (1993) and the Danish EPA (1993). Both refer to the PARCOM protocol with *Arenicola marina*.

Burrowing behaviour as endpoint is studied with the annelids *Nereis virens*, *Glycera dibranchiata* and *Nepthtys caeca*. This endpoint might be of interest when developing a Test Guideline.

Long-term acute and subchronic toxicity tests with crustaceans

Several assessment schemes require toxicity tests with crustaceans (US EPA 1994a,d, PARCOM 1993, DK-EPA 1993, Walker 1990). PARCOM recommends the amphipods *Corophium volutator* and *Bathyporeia sarci*. Other recommended amphipods are *Rhepoxynius abronius*, *Eohaustorius estuarius*, *Grandidierella japonica*, *Corophium* sp. and *C. insidiosum*. As a non-amphipod crustacean, *Holmesimysis costata* is recommended (although it does not burrow in the sediment layer). Table 6.1 shows the standard methods, guidelines and ring-test protocols which are made available by ASTM, Environment Canada, PARCOM, and the US EPA. These methods are also listed in Table 6.4, together with some other methods that fulfil the five characteristics mentioned above. Not available as standard methods, but interesting for Test Guideline development, are the subchronic tests with endpoints on reproduction with *C. insidiosum* and *Microdeutopus gryllotalpa*. Several references on *C. volutator* (4037, 4038, 4039), *C. spinicorne* (4185), and *R. abronius* (4186) fulfil the five characteristics, but are not listed in Table 6.4 because it is felt that they do not add new points to the methods mentioned above.

Long-term acute toxicity test with the echinoid *Echinocardium cordatum*

Toxicity tests with the echinoid *Echinocardium cordatum* are mentioned by PARCOM (1993) and the Danish EPA (1993). Both recommend the PARCOM protocol.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

The following methods are considered to belong to Group 2b.

Toxicity tests with the molluscs *Abra alba* and *Macoma baltica*

The Danish EPA requires testing with molluscs. *Abra alba* and *Macoma baltica* are recommended. For *A. alba*, the PARCOM ring-test protocol is mentioned. One of the endpoints of this method is fecal pellet production, the ecological relevancy of which is debatable. The test is short in duration and carried out in a sediment suspension, which is not a preferred test system. Ref. 4184 describes a study with *A. alba* in a whole sediment system.

No guideline for a test with *Macoma baltica* is recommended. In the database, a reference is given to a long-term acute study, which is carried out in a water and sand system.

Long-term subchronic and chronic toxicity tests with the sea urchin *Lytechinus pictus*

A (sub)chronic test might be developed with the sea urchin *Lytechinus pictus*. The exposure time in these studies was 49 and 60 days. Reproduction is one of the endpoints. Tests with sea urchins are not asked for in the assessment schemes. However, this might be an interesting reference, as there are few marine species for which reproduction can be studied.

Short-term acute and subchronic toxicity tests with the nematode *Monhystera disjuncta*

A test with nematodes is not required in the assessment schemes, but might be of interest due to their function in ecosystems. An available species is *Monhystera disjuncta*, which lives on the surfaces of sediments. However, this species was studied in a water-only system. The exposure time was short in both studies.

The allocation of nematodes to the pelagic or the benthic part of this review paper might be an item for discussion. As *M. disjuncta* is most abundant on sediment surfaces, it is allocated to the benthic part.

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

Acute toxicity tests with the fish *Pseudopleuronectes americanus* and *Scophthalmus maximus*

Benthic tests with fish are not mentioned in the assessment schemes. A short-term test method has been developed with the turbot *Scophthalmus maximus*. This method is available as a PARCOM protocol. The test system is a water-accommodated fraction. A short-term study in whole sediment is carried out with the winter flounder *Pseudopleuronectes americanus*. Apart from growth, the endpoints concern biochemical responses, which are of minor importance to the scope of this document.

Table 6.5: Methods and studies that provide 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints.

Environment: warm marine

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
Annelida	*Nereis virens*	survival, growth and behaviour		2a	degrader	acute	4187	
	Nereis virens	survival, growth and behaviour			degrader	acute		4002/ 4004
	Neanthes arenaceodentata	survival		2a	degrader	acute	4249	
	Nereis arenaceodentata	survival and growth			degrader	acute		4090
	Nereis arenaceodentata	survival and growth		2b	degrader	subchronic	4046	
Crustacea	*Eohaustorius estuarius*	survival, avoidance and reburying behaviour		2a	degrader?	acute	4007	
	Ampelisca abdita	survival		2a	degrader herbivore	acute	4244/ 4258	
Crustacea	*Ampelisca abdita*	survival, growth and behaviour			degrader herbivore	acute		4008

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: warm marine

Taxonomic group	Species	Endpoints	Remark*	Level	Trophic level	Duration	Key ref.	Suppl. ref.
	Ampelisca abdita	survival, reproduction, growth and population growth		2b	degrader herbivore	chronic	4174	
	Corophium sp.	survival		2a	degrader	acute	4246	
	Grandidierella japonica	survival		2a	degrader herbivore	acute	4257	
	Amphiascus tenuiremis	survival and reproduction		2b	degrader	chronic	4182	
	Microarthridion littorale	survival and reproduction	ST	2b	herbivore?	subchronic	4032	
	Paronychocamptus wilsoni	survival and reproduction	ST	2b	herbivore?	subchronic	4033	
Crustacea	*Enhydrosoma propinquum*	survival and reproduction	ST	2b	herbivore?	subchronic	4034	
	Leptocheirus plumulosus	survival (and reburying ability)		2a	degrader	acute	4245/ 4255	
	Leptocheirus plumulosus	survival, growth and reproduction		2b	degrader	subchronic	4119	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

Environment: warm marine

Taxonomic group	**Species**	**Endpoints**	**Remark***	**Level**	**Trophic level**	**Duration**	**Key ref.**	**Suppl. ref.**
	Leptocheirus plumulosus	survival			degrader	acute		4117/ 4118
	Mysidopsis bahia	survival		2a	degrader omnivore	acute	4232	
	Mysidopsis bigelowi	survival		2a	degrader omnivore	acute	4233	
	Mysidopsis almyra	survival		2a	degrader omnivore	acute	4234	
Crustacea	*Neomysis americana*	survival		2a	omnivore	acute	4235	
	Penaeus sp.	survival		2a	degrader	acute	4237	
	Palaemonetes sp.	survival		2a	degrader	acute	4238	
Pisces	*Leistomus xanthurus*	survival		3	carnivore	acute	4162	

* *Where a method or study might be useful for OECD Test Guideline development but is short-term in duration, an indication (ST) is given. Other remarks concerning deviations from the five characteristics are given in the text.*

6.6 Warm marine environment

Methods that fulfil the requirements of 1) a long-term exposure in 2) a whole sediment system to 3) a benthic or epibenthic species which 4) can be cultured in the laboratory or easily be sampled in the field and which has 5) ecologically relevant or highly relevant endpoints are presented in Table 6.5. A few methods do not fulfil all five characteristics but might be useful in the process of Test Guideline development. These methods can be recognised in Table 6.5 by a short-term exposure period or, in other cases, by a remark made in the text.

Detailed scoring of the methods is presented in Annex K.

Group 1:

Methods needed for existing international (draft) aquatic effects, hazard and risk assessment schemes

There are no methods available for Group 1.

Group 2:

Methods presumably needed in the near future as they are recommended in some national assessment schemes and/or considered to represent key ecological groups

The following methods are considered to belong to Group 2a. In the process of Test Guideline development, some references, belonging to Group 2b, might be used to give a Test Guideline a broader application. Examples are references concerning subchronic or chronic studies.

Long-term acute and subchronic toxicity tests with the annelids *Nereis virens* and *Neanthes arenaceodentata*

The *Nereis virens* method is available as a PARCOM ring-test protocol and has been recommended by the DK-EPA (1993). *Nereis virens* is a sediment reworking worm, but it also swims in the water layer. A test method with *Neanthes (Nereis) arenaceodentata* is recommended by US EPA (1994a).

A subchronic test was developed with the annelid *N. arenaceodentata*. However, a subchronic test method is not specifically requested in the assessment schemes.

Long-term acute, subchronic and chronic toxicity tests with crustaceans

Several assessment schemes recommend toxicity tests with crustaceans (US EPA 1994a,d, PARCOM 1993, DK-EPA 1993, Walker 1990). The species recommended by PARCOM are cold marine species. The US EPA recommends several species for the warm marine environment. These species are *Ampelisca abdita*, *Leptocheirus plumulosus*, *Corophium* sp. and *Grandidierella japonica*. *Eohaustorius estuarius* might also be used for warm temperatures. Other recommended crustaceans are *Mysidopsis* sp., *Neomysis americana*, *Penaeus* sp. and *Palaemonetes* sp. (most of them do not burrow in the sediment layer; *Penaeus* sp. are an exception to this). The methods are available as ASTM standard methods and US EPA methods.

None of the assessment schemes mentions subchronic or chronic tests with crustaceans. A 58-day reproduction and population growth study might be used for developing a chronic test with *A. abdita*. However, the test system used was a sediment suspension. A 21-day reproduction study was carried out with *Amphiascus tenuiremis* and might serve to develop a subchronic test. Reproduction studies are carried out with three brackish copepods, *Microarthridion littorale*, *Paronychocamptus wilsoni* and *Enhydrosoma propinquum*. The tests are short-term, however. The brackish amphipod *Leptocheirus plumulosus* is used in several tests, among which a reproduction test. The acute tests differ from each other in test duration and the life stage of the organisms (adults/juveniles).

Group 3:

No immediate needs for the methods have been identified. The methods may be more or less ready for standardisation.

Long-term acute toxicity test with the fish *Leistomus xanthurus*

Benthic tests with fish are not mentioned in the assessment schemes. An acute study was carried out with the spot *Leistomus xanthurus*.

7. RECOMMENDATIONS

The identification of aquatic ecotoxicity test methods for the OECD Test Guidelines Programme is primarily based on the following assessment criteria:

- There should be a regulatory need for the methods, either as a direct request in adopted or drafted aquatic effects, hazard or risk assessment schemes, or a recommendation for the type of methods to be included in future updates.

- The test methods should as far as possible fulfil the requirements of an OECD Test Guideline in terms of uncomplicated test procedures, inexpensive equipment, easy culturing or sampling of test organism, low costs for testing, etc.

- The test organism should as far as possible be a valid representative of its taxonomic group, trophic level, and environmental compartment/habitat.

- The test methodology should as far as possible facilitate the study of acute as well as subchronic/chronic effects, and test procedures should be available for the handling of "difficult" substances.

- The recommended methods, together with the existing Test Guidelines, should preferably fulfil the need for a future improved flexibility in the choice of testing methods for specific purposes, either for effects/hazard/risk assessment in specific types of compartments or for assessment of chemicals/pesticides with specific properties.

Test methods fulfilling most of the above requirements have been identified in Chapters 5 and 6.

The methods are recommended for the OECD Test Guidelines Programme at three levels:

- **Primary recommendation (Group 1):**

  Methods needed for existing effects, hazard and risk assessment schemes for chemicals and/or pesticides, as adopted or drafted by international organisations (e.g. OECD, UN, PARC), communities (EU) and industrial organisations (e.g. ECETOC, AIS, CONCAWE). The methods identified are expected to require only a small amount of work to be ready for standardisation (Group 1a) or to be ready for standardisation after sufficient scientific documentation has been provided (Group 1b). These methods should be considered for OECD ring-testing unless sufficient ring-testing has already been performed. An OECD Test Guidelines proposal should be drafted as soon as possible for methods in Group 1a, and in the near future for Group 1b.

- **Secondary recommendation (Group 2):**

  Methods presumably needed in the future, as they are recommended for the assessment of chemicals, pesticides and/or complex mixtures in national adopted or draft schemes for effects, hazard and risk assessment or in schemes proposed in recent scientific literature. Methods that are considered to meet ecologically defined needs are also included. The methods identified are expected to require only a small amount of work in order to be ready

for standardisation (Group 2a), or to be ready for standardisation after sufficient documentation has been provided (Group 2b). The drafting of an OECD Test Guideline should be considered in future (Group 2a), or the establishment of necessary scientific documentation should be prompted as the methods might be considered for Test Guideline development in the future (Group 2b).

- **Tertiary recommendations (Group 3):**

  No immediate or near future needs for the methods have been identified. The methods may be requested in special cases. The methods may be more or less ready for standardisation, but the initiation of a standardisation process within the OECD Test Guidelines Programme in the near future is not recommended.

The test methods recommended at the first and second level (Groups 1 and 2 above) should be considered for Test Guideline development. For practical reasons, it may not be possible to initiate Test Guideline development for all the recommended methods at the same time. An identification of the test methods with highest priority is therefore needed.

Highest priority should be given to test methods that are:

- requested/recommended in assessment schemes adopted (or proposed) by national or international communities/organisations;
- requested/recommended for the assessment of industrial chemicals or pesticides;
- ready for standardisation;
- of high ecological relevance.

It is recommended that whenever possible the Test Guidelines to be developed take the form of "framework" guidelines for taxonomic groups rather than for single species, and that specific guidance for testing the single species within the taxonomic group be Annexed to the Test Guideline. This approach may make it easier to test representatives from various environmental compartments belonging to the same taxonomic group in a comparative way, and thus improve the possibility of obtaining comparative effect thresholds for different environmental compartments. This approach may furthermore limit the amount of work necessary for the development of Test Guidelines. The study of acute and subchronic/chronic toxicity should, whenever possible, be included in the same Test Guideline as well, for the same reasons as given above.

Specific recommendations for the OECD Test Guidelines Programme, regarding the development of new Test Guidelines, are given below for the pelagic and benthic environment.

7.1 Pelagic environment

An overview of the primary and secondary recommended test methods is shown in a food web frame in **Figures 7.1-7.2 and 7.3-7.4** for the freshwater and marine environments, respectively. The methods are further outlined in **Tables 7.1 and 7.2** for the primary and secondary recommended test methods.

Figure 7.1: Pelagic warm freshwater environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

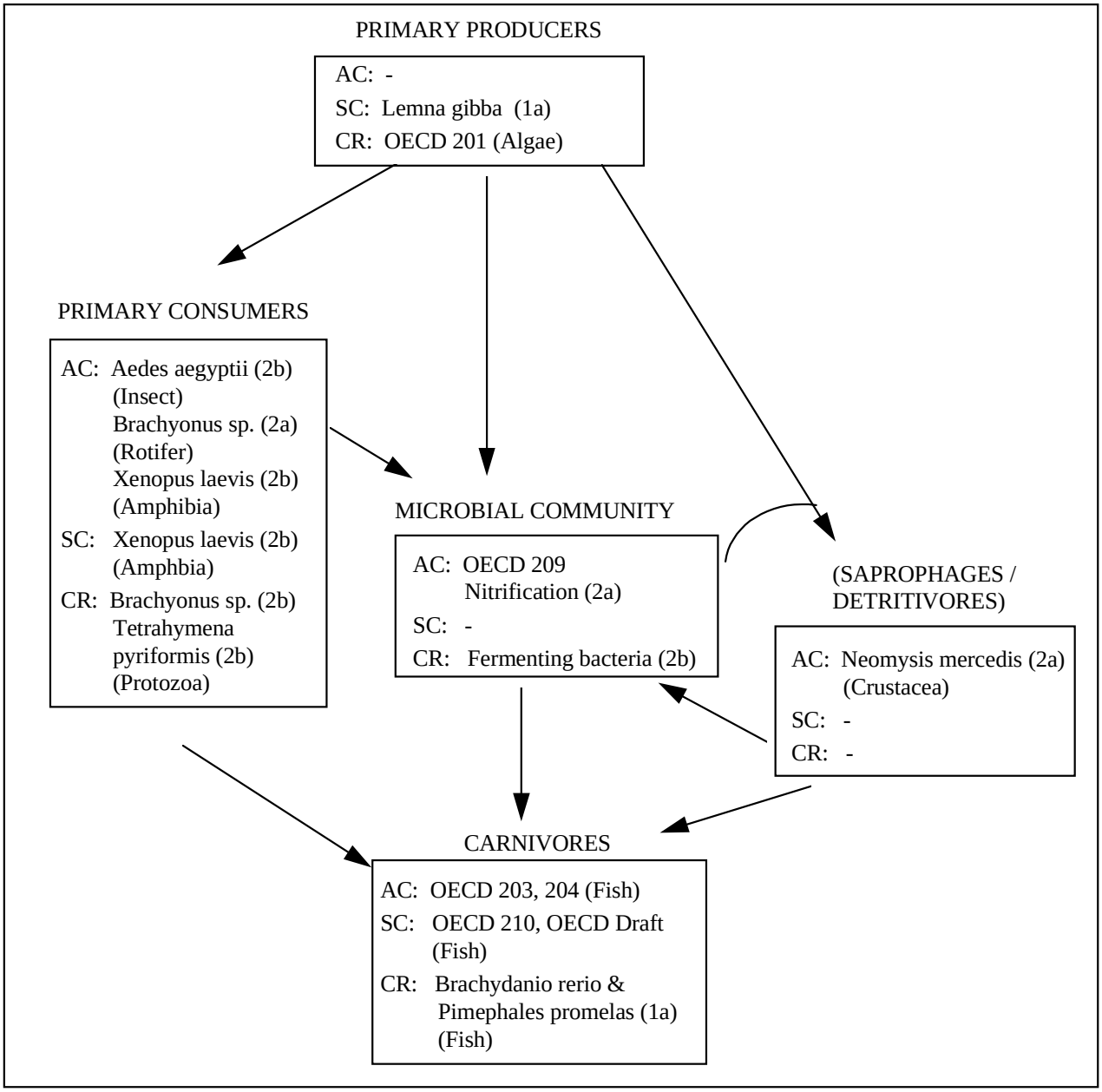


Figure 7.2: Pelagic cold freshwater environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

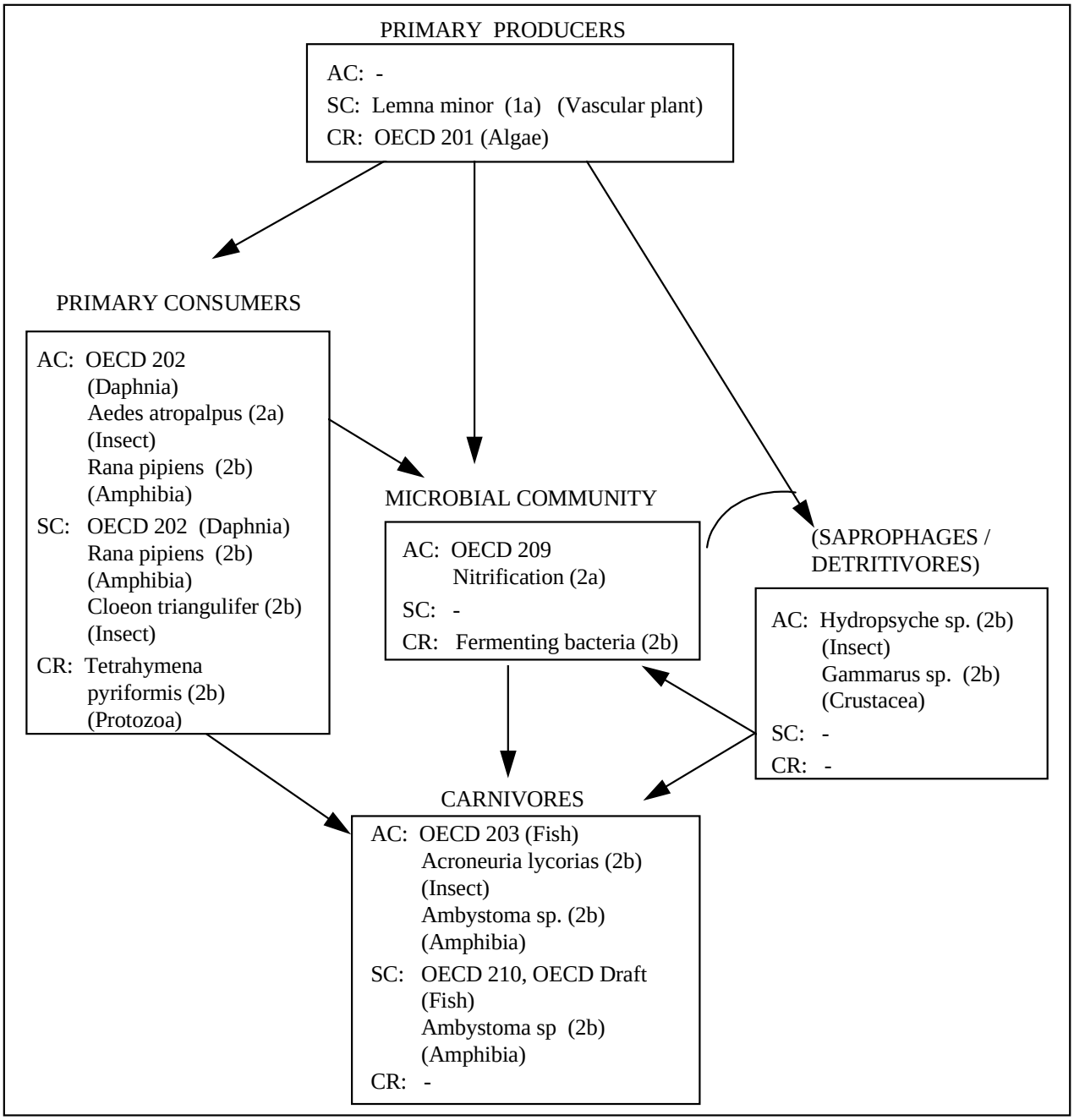


Figure 7.3: Pelagic warm marine environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

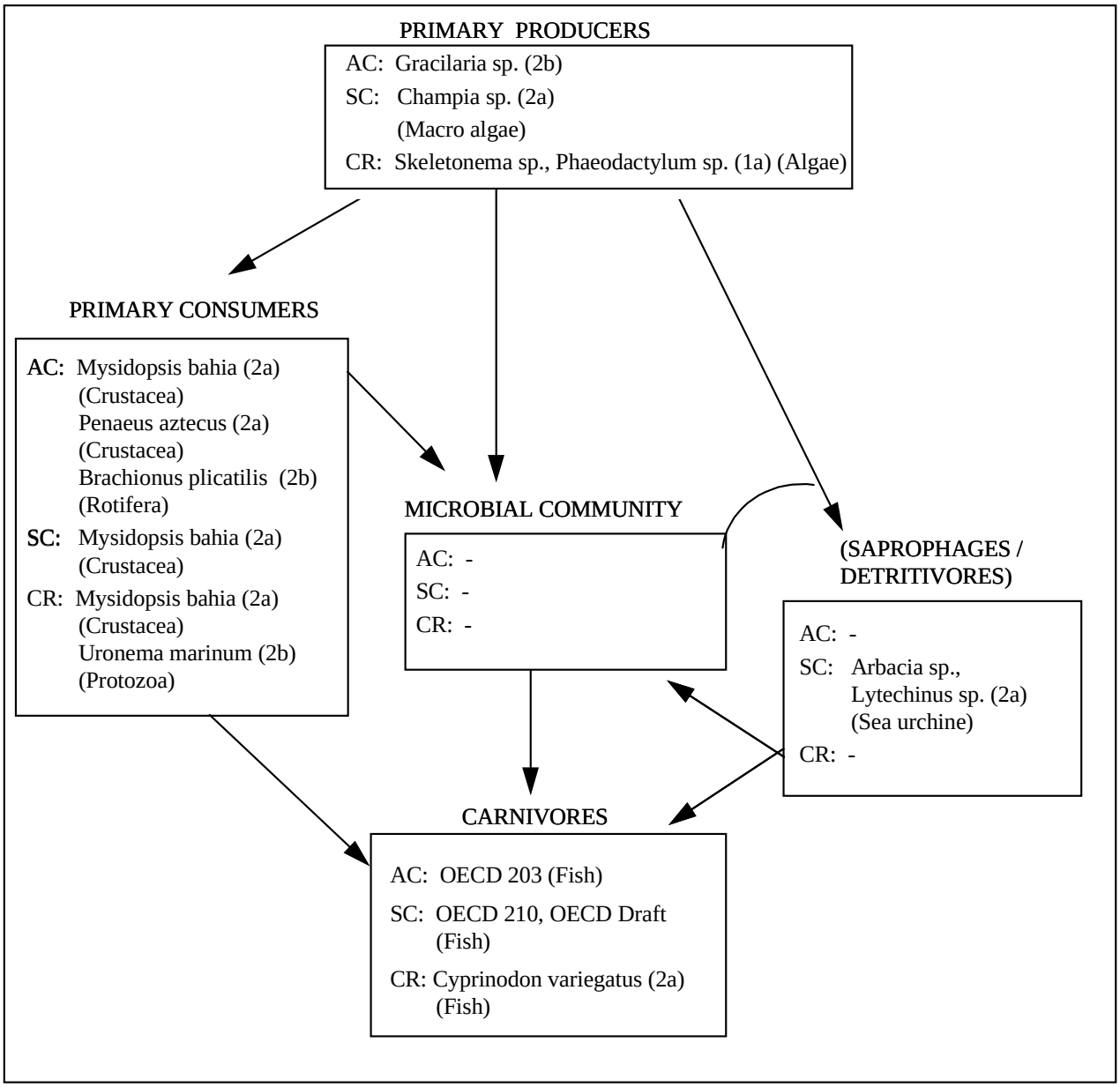


Figure 7.4: Pelagic cold marine environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, C: subchronic, CR: chronic)

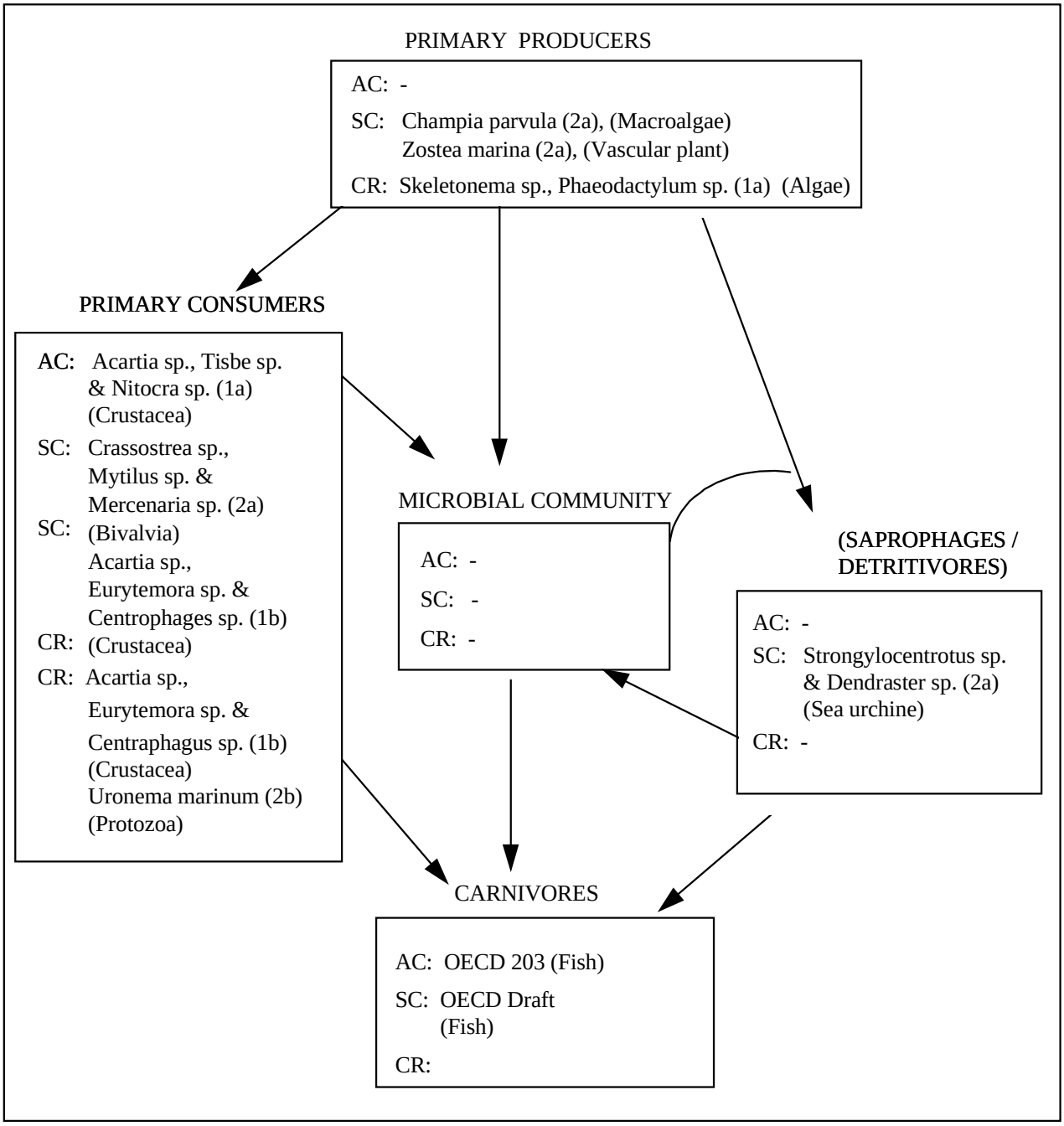


Table 7.1 Methods recommended for inclusion in the OECD Test Guidelines Programme (Groups 1a + 1b) as they are needed for existing international (draft) aquatic effects, hazard or risk assessment schemes

Taxonomic group	Test organism	Acute (AC) Subchronic (SC) Chronic (C)	Short-term (ST) Long-term (LT)	References (Ref. No. in brackets)
Warm freshwater environment				
Kormophyta (higher plants)	*Lemna gibba*	SC	ST	US EPA (66) ASTM (88)
Fish	*Brachydanio rerio* *Pimephales promelas*	C	LT	Bresh et al. 1990 (276) US EPA (428)
Cold freshwater environment				
Kormophyta (higher plants)	*Lemna minor*	SC	ST	US EPA (191)
Warm marine environment				
Algae, micro	*Skeletonema costatum* *Phaeodactylum tricornutum*	C	ST	ISO draft (371) US EPA (65)
Cold marine environment				
Algae, micro	*Skeletonema costatum* *Phaeodactylum tricornutum*	C	ST	ISO draft (371) US EPA (65)
Arthropoda, Crustacea	*Acartia tonsa* *Tisbe battagliai* *Nitocra spinipes*	AC	ST	ISO draft (212-214)
	Acartia tonsa	SC	ST	Johansen 1988 (410)
	Centropages hamatus	SC	ST	Cowles 1983 (208)
	Erytemora affinis	SC	ST	Berdugo 1977 (221)
	Acartia tonsa	C	LT	Møhlenberg 1994 (409)

Table 7.2 Methods recommended in some national assessment schemes and/or considered to represent key ecological groups (Groups 2a and 2b)

Taxonomic group	**Test organism**	**Acute (AC) Subchronic (SC) Chronic (C)**	**Short-term (ST) Long-term (LT)**	**References (Ref. No. in brackets)**
Warm freshwater environment				
Arthropoda, Insecta	*Aedes aegyptii*	AC	ST	Kiviranta et al. 1993 (144)
Arthropoda, Crustacea	*Neomysis mercedis*	AC	ST	US EPA (402)
Chordata, Amphibia	*Xenopus laevis*	AC SC	ST LT	de Zwart 1987 (98) Dumpert 1987 (98)
Aschelminthes	*Brachionus calyciflorus* *B. urceolaris*	AC C	ST ST	Holdway 1988 (3) Hatakeyama 1991 (31)
Protozoa	*Tetrahymena pyriformis*	C	ST	(436)
Bacteria	*Pseudomonas putida*	C	ST	ISO draft (192)
Cold freshwater environment				
Chordata, Amphibia	*Rana pipiens* *Ambystoma mexicanum*	AC SC	ST LT	Berriee 1993 (95) Sloff 1980 (121)
Arthropoda, Crustacea	*Gammarus pulex* *G. italicus*	AC	ST	McCahon 1988 (224)
Arthropoda, Insecta	*Aedes astropalpus* *Acroneuria lycorias* *Hydropsyche* sp.	AC	ST	Tousignant 1992 (175) Schere 1986 (148) Canmargo 1992 (172) Vouri 1993 (180)
Protozoa	*Tetrahymena pyriformis*	C	ST	(436)
Bacteria	*Pseudomonas putida*	C	ST	ISO draft (192)

Table 7.2, continued

Taxonomic group	Test organism	Acute (AC) Subchronic (SC) Chronic (C)	Short-term (ST) Long-term (LT)	References (Ref. No. in brackets)
Warm marine environment				
Algae, macro	*Champia parvula* *Gracilaria tenuistipitata*	SC AC	ST ST	US EPA (57) Haglund (44)
Arthropoda, Crustacea	*Mysidopsis bahia* *Penaeus aztecus* a.o.	AC SC C	ST LT LT	US EPA (73) ASTM (80) US EPA (55)
Echinodermata	*Arbacia punctulata* *Lytechinus variegatus*	SC	ST	Environment Canada (236)
Aschelminthes	*Brachionus plicatilis*	AC	ST	ASTM (381)
Protozoa	*Uronema marinum*	C	ST	Parker 1979 (189)
Fish	*Cyprinodon variegatus*	C	LT	US EPA (435)
Cold marine environment				
Algae, macro	*Champia parvula* *Ceramium strictum*	SC	ST	US EPA (57)
Kormophyta	*Zostera marina*	SC	LT	protocol (202)
Echinodermata	*Strongylocentrotus* sp. *Dendraster excentricus*	SC	ST	Environment Canada (233)
Mollusca	*Crassostrea* sp. *Mytilus edulis* *Mercenaria mercenaria*	SC	ST	ASTM 1986 (403-405)
Protozoa	*Uronema marinum*	C	ST	Parker 1979 (189)

The following methods are considered to be of highest priority for Test Guideline development in the nearest future:

Primary producers

Microalgae (marine environment)

The present OECD Test Guideline 201 includes only freshwater species representing warm and cold water environments. It is recommended that the marine water species *Skeletonema costatum* and *Phaeodactylum tricornutum* be included in the existing Test Guideline when it is next updated. An ISO draft and US EPA guideline for these species are available. It is recommended that the update of the OECD Test Guideline await the finalisation of the ISO work.

Vascular plants (freshwater environment)

It is recommended that one Test Guideline be developed covering both the warm freshwater species *Lemna gibba* and the cold freshwater species *Lemna minor*. An ASTM standard method and a US EPA guideline have been adopted for *Lemna gibba,* and a Swedish EPA standard for *Lemna minor*. A French AFNOR standard for *Lemna minor* is being developed.

Primary consumers

Crustaceans (marine environment)

Acute toxicity tests with crustaceans are requested in most assessment schemes but only the (cold) freshwater environment is represented in the existing OECD Test Guideline (TG 202, *Daphnia*). Several standardised test methods are available for marine and brackish water species. For the warm marine environment, ASTM and US EPA standardised methods are available for studying acute, subchronic and chronic effects (*Mysidopsis bahia, Penaeus aztecus* a.o.).

For the cold marine environment, an ISO proposal and a PARCOM guideline are available for testing acute toxicity to the marine species *Acartia tonsa*, *Tisbe battagliai* and *Nitocra spinipes* (brackish water). A number of test methods for subchronic and chronic tests to cold marine water species are available in the scientific literature.

It is recommended that two Test Guidelines be developed for warm water and cold water marine species, based on the above test methods. They should preferably cover subchronic/chronic toxicity in addition to acute toxicity.

Crustaceans (freshwater environment)

The primary habitat of the species recommended for testing in Test Guideline 202, *Daphnia magna* and *D. pulex,* is cold water ponds. In order to enable testing for a warm water species as well, it is recommended that the species *Neomysis mecedis* and/or *Ceriodaphnia dubia also be* included at the next update of Test Guideline 202. Both species are included in ASTM and US EPA guidelines.

Protozoans (freshwater and marine environment)

Although no assessment schemes for industrial chemicals and pesticides recommend testing of protozoans, it is recommended that a Test Guideline be developed for this taxonomic group. The primary reason is that protozoans are an important ecological link between the microbial community/primary producers and the secondary consumers in the detritus food chain. Low-cost methods have been developed for both the marine and the freshwater species (short-term chronic tests), and, for *Tetrahymena pyriformis*, a relatively limited international ring-test of the method has been performed. Both the species *Tetrahymena pyriformis* and *Uronema marinum* are distributed in both warm and cold regions of the freshwater and marine environment.

It is recommended that a Test Guideline be developed for the protozoans *Tetrahymena pyriformis* and *Uronema marinum*, preferably combined in one guideline with Annexed testing details for the two species.

Microbial community

Nitrifying bacteria (waste water treatment plant)

The protection of nitrification processes is of increasing concern in relation to waste water treatment. It is the general experience that these processes are more vulnerable to chemical toxification than the general aerobic degradation processes, as measured by the respiration inhibition of activated sludge (OECD TG 209).

An ISO standard method has been adopted, but the standard method needs updating.

Thus, although no schemes for the assessment of chemicals or pesticides request the method at present, it is recommended that a Test Guideline for inhibition of nitrification processes be developed.

Saprophages/detritivores

Sea urchins (marine water environment)

It is recommended that an OECD Test Guideline be developed covering both the warm marine species *Arbacia punctulata* and *Lytechinus variegatus* and the cold marine species *Strongylocentrotus* sp. and *Dendraster excentricus.* All four species are included in an Environment Canada guideline. Effects on fertilization and development of early life stages are studied (short-term subchronic test methods). Larvae are nourished by both plankton and detritus. Therefore they are considered to be primary consumers and detritivorous organisms.

Although no schemes for generic assessment of chemicals or pesticides currently recommend or request toxicity data on sea urchins, it is recommended that a Test Guideline be developed in order to cover this ecologically important taxonomic group of the marine environment.

Secondary consumers

Fish (freshwater and marine environment)

A number of assessment schemes recommend that a full life cycle fish test be performed at the refinement stages of the assessment. It is therefore recommended that a Test Guideline be developed in order to meet this demand.

The Test Guideline should preferably include the warm freshwater species *Pimephales promelas* (fathead minnow) and *Brachydanio rerio* (zebra fish), as well as the warm water marine species *Cyprinodon variegatus* (sheepshead minnow). Specific testing requirements may be Annexed in the guideline. For the minnows, US EPA guidelines are available.

Primary recommendations, summary

An overview of the test methods recommended in relation to the environments represented by the test organisms is given in **Table 7.3**.

The primary recommended test methods in combination with the existing OECD Test Guidelines will increase the number of taxonomic groups available for subchronic/chronic testing from three (algae, crustaceans, fish) to five (algae, vascular plants, crustaceans, protozoans, fish) for the freshwater environment, the microbial community not included.

For the marine environment, the number of taxonomic groups will be increased from the present one group (fish) to five groups at subchronic/chronic testing level (microalgae, crustaceans, protozoans, sea urchins, fish).

7.2 Benthic environment

An overview of the primary and secondary recommended test methods is shown in a food web frame in **Figures 7.5-7.6 and 7.7-7.8** for the freshwater and marine environments, respectively. The methods are further outlined in **Tables 7.4 and 7.5**. Most of these methods concern detritivores or mixed detritivores/herbivores/carnivores.

Based on the role of benthic methods in the risk assessment of sediment-bound chemicals, it is proposed to give preference to the long-term testing of infaunal species in whole sediment systems, thereby including all possibly relevant exposure routes via sediment particles, porewater and overlying water. Methods using other systems (elutriates, porewater or sediment suspensions) and organisms that are predominantly exposed via the pelagic environment in the process of feeding and respiration are of low preference. This holds also for testing with a short exposure time, as field exposure to sediment-bound chemicals will be long-term in character.

Table 7.3: Test organisms from existing OECD Test Guidelines and test methods recommended for Test Guideline development in relation to the environmental compartments they represent

(ac: acute, sc: subchronic, cr: chronic; capital letters: existing OECD Test Guideline)

	Freshwater		**Marine**	
	Warm	Cold	Warm	Cold
Primary producers				
Microalgae	CR	CR	cr	cr
Vascular plants	sc	sc	–	–
Primary consumers				
Crustaceans	ac,sc	AC,SC	ac,sc,cr	ac,sc,cr
Protozoa	cr	cr	cr	cr
Microbial community				
Inhibition of active sludge respiration	AC	AC		
Inhibition of nitrification	ac	ac		
Omnivores/detritivores				
Sea urchins	–	–	sc	sc
Carnivores				
Fish	AC,SC,cr	AC,SC,cr	AC,SC,cr	AC,SC

Figure 7.5: Benthic cold freshwater environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

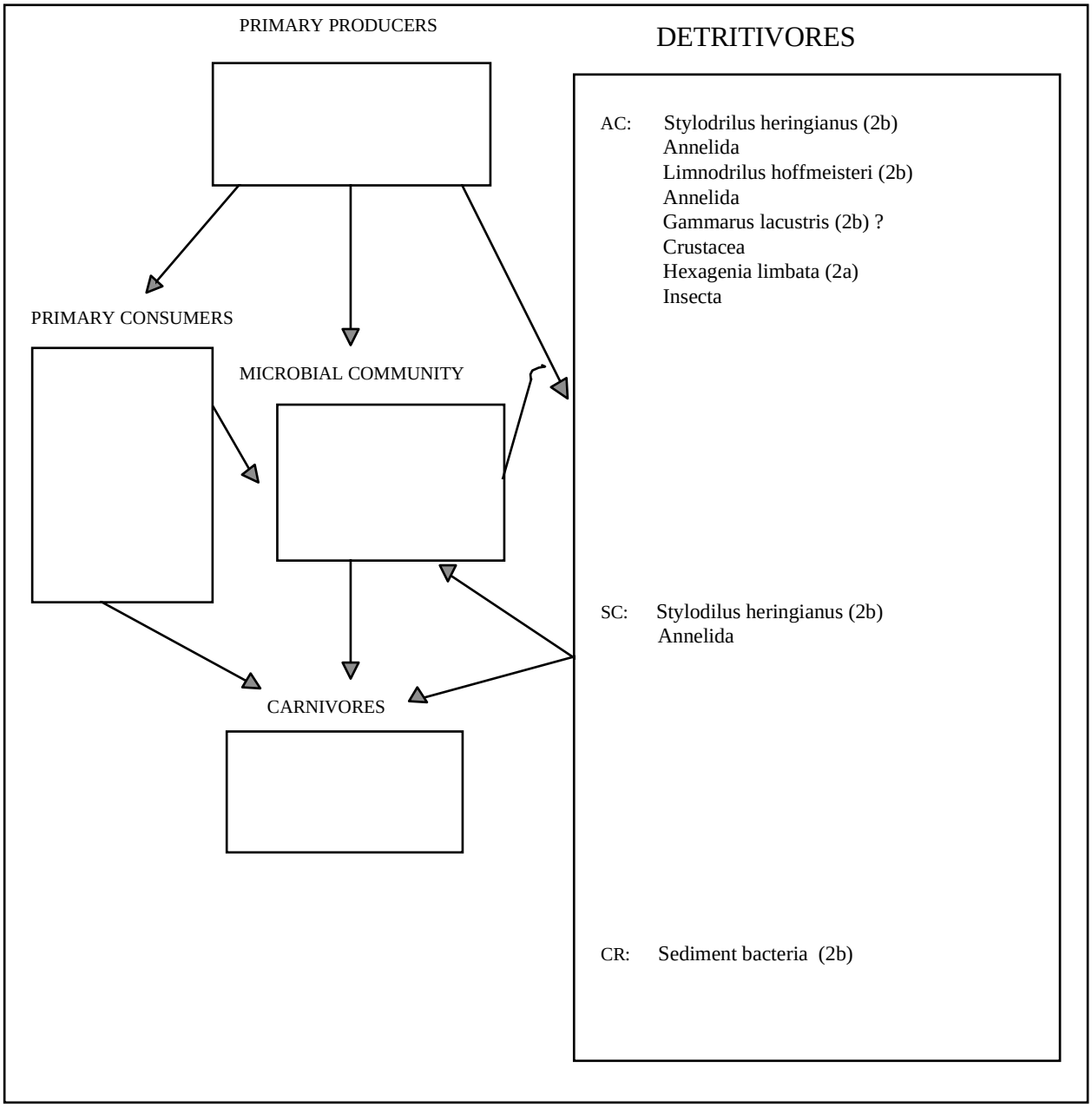


Figure 7.6: Benthic warm freshwater environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

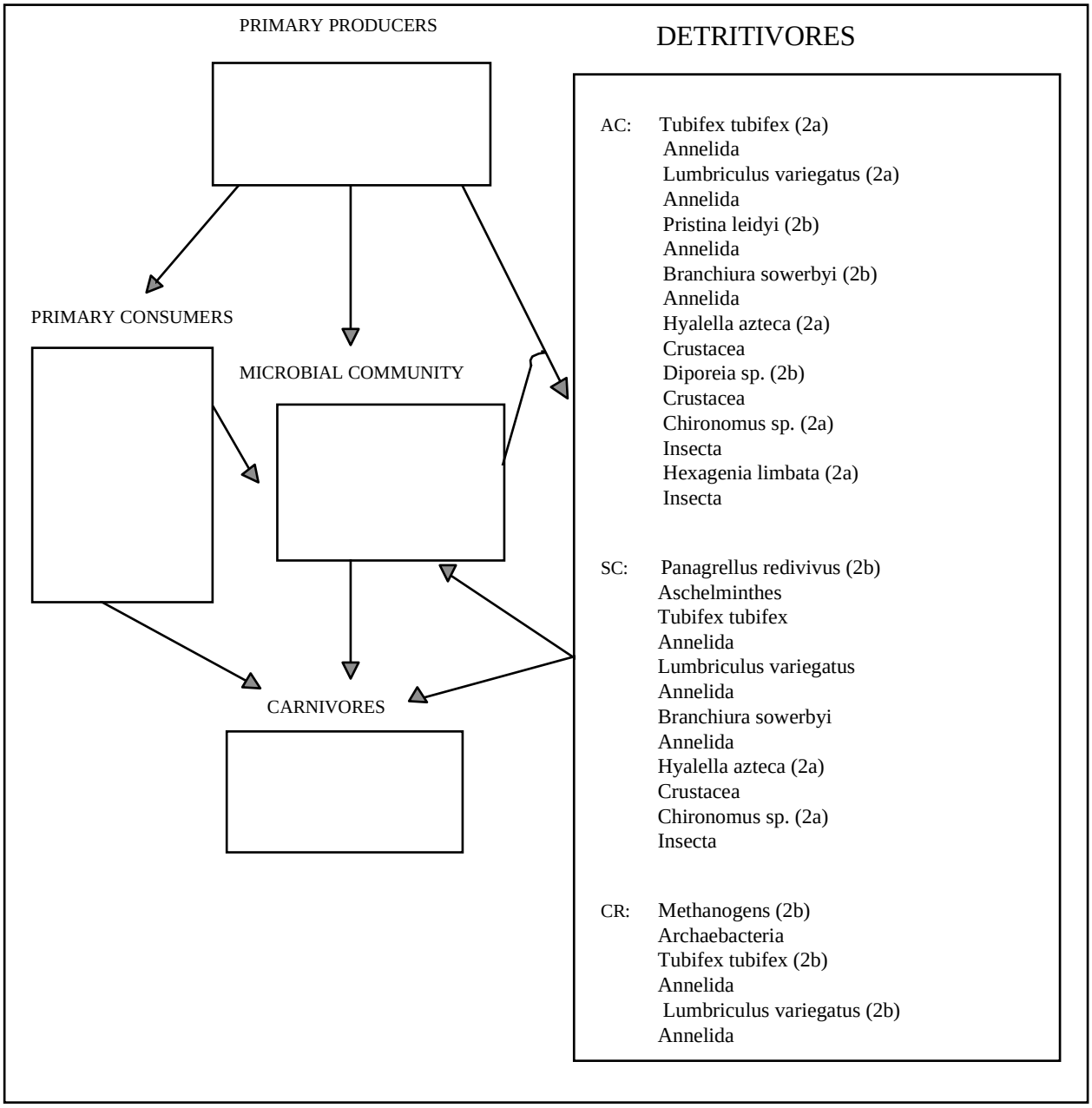


Figure 7.7: Benthic cold marine environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)

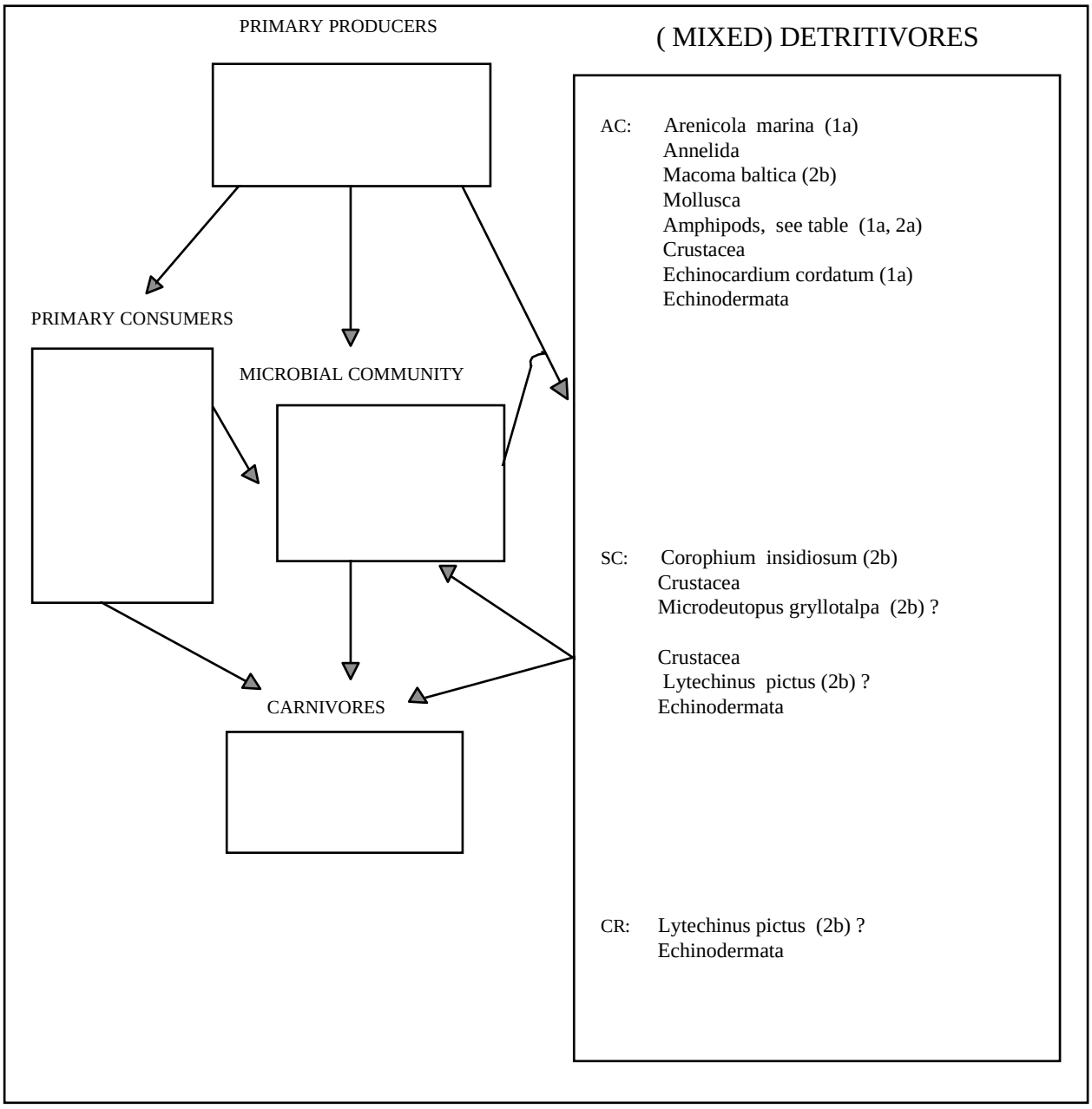
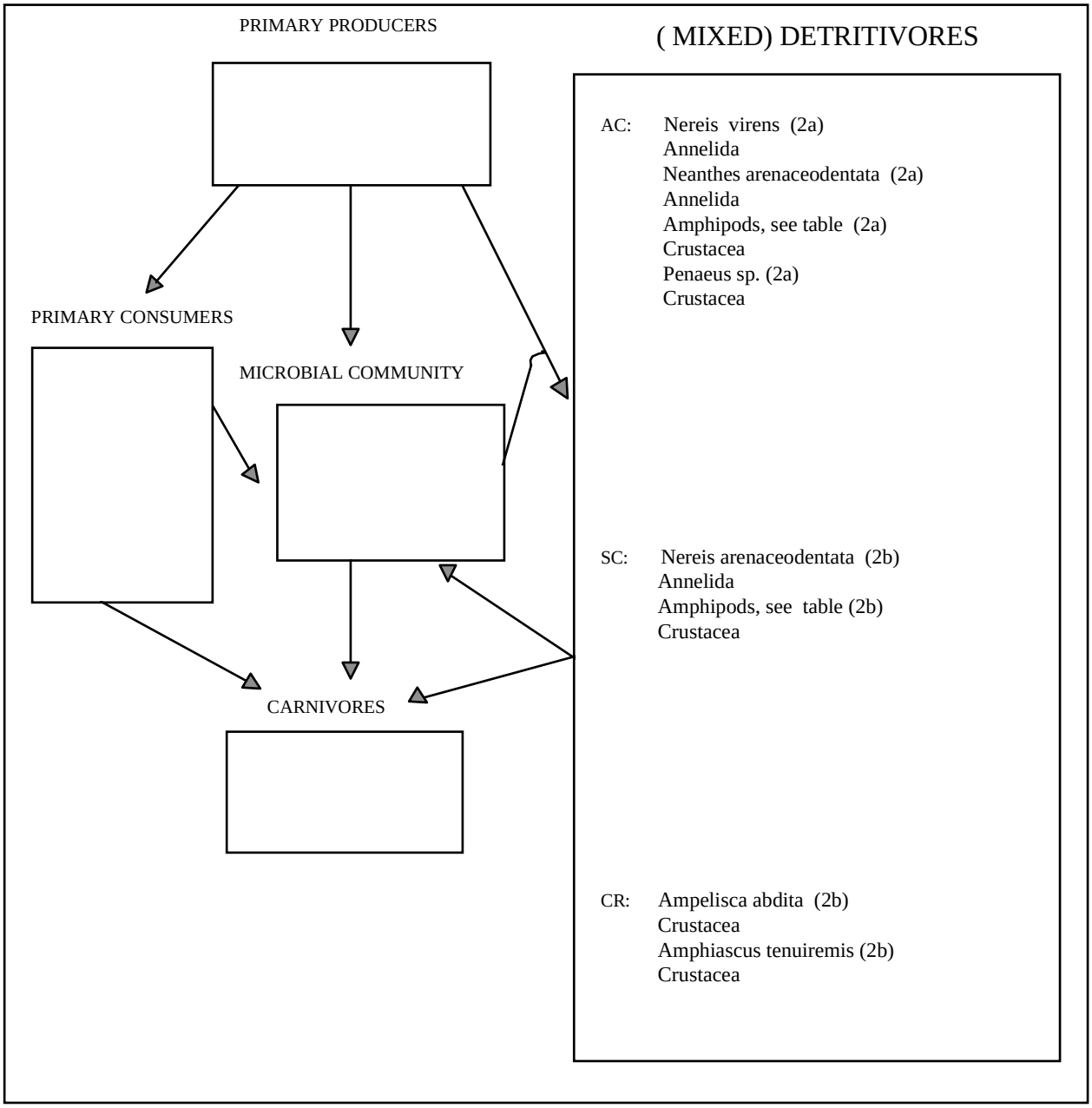


Figure 7.8: Benthic warm marine environment

Primary (1a and 1b) and secondary (2a and 2b) recommendations
(AC: acute, SC: subchronic, CR: chronic)



The following methods are considered to be of highest priority for Test Guideline development in the near future:

Chronic test with methanogenic bacteria

Although none of the assessment schemes require a test with bacteria, a test method with this functionally important group of organisms is thought to be useful. A relatively easy method is available. The endpoint of this method is methanogenesis.

Subchronic test with freshwater nematodes

A test with nematodes is not required in the assessment schemes, but might be of interest due to their ecological importance. The nematode *Panagrellus redivivus* is used in a short-term test.

Acute, subchronic and chronic toxicity tests with freshwater annelids

Annelids play an important role in the degradation of materials in aquatic ecosystems. They can be found in huge numbers and may represent a large amount of the biomass in sediments. Due to their feeding mechanism, the benthic species are exposed to pollutants via sediment particles as well as via porewater. Therefore, Test Guideline development with annelids in whole sediment systems is strongly recommended.

Toxicity tests with (freshwater) annelids are recommended in three schemes. Recommended species are *Tubifex tubifex*, *Lumbriculus variegatus* and *Pristina leidyi*. For these species, standard acute toxicity tests are available. Together with *Branchiura sowerbyi*, these species may serve as warm freshwater species. *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri* are useful as cold freshwater species. From the species recommended in this review, *Branchiura sowerbyi* is known to live mainly in the sediment layer. *Stylodrilus heringianus* and *Lumbriculus variegatus* are reported to live in the sediment as well as in the water layer.

The duration of the methods should preferably be long-term. The endpoints of the acute test method may be survival and burrowing behaviour. Subchronic and chronic tests might be developed with at least the warm water species and possibly with *S. heringianus*. These methods should include sublethal endpoints as reproduction (possible with all warm water species; *P. leidyi* might be an exception to this) and growth.

Acute toxicity tests with freshwater molluscs

This test is required in one assessment scheme. For *A. imbecillis*, a US EPA standard method is available. The endpoint is survival. *Corbicula fluminea* might be another useful species, although it has not been used for routine toxicity testing. No cold freshwater species can be recommended. As it is thought that the exposure of these molluscs via sediment particles might be of minor importance, the development of the relevant guidelines should have a lower priority.

Acute and subchronic toxicity tests with freshwater crustaceans

Three assessment schemes specifically ask for an acute toxicity test with *Hyalella azteca*. Standards for this method are available. This amphipod is an epibenthic detritivore which will burrow in the sediment surface. It is common throughout the entire American continent.

The US EPA guidelines could serve as key references for developing an OECD Test Guideline with this warm water species. It is recommended to develop an acute as well as a subchronic method, including sublethal endpoints as growth and reproduction. Another species for the warm environment might be *Diporeia* sp., for which no standard method exists.

For the cold water environment, *Gammarus lacustris* might be a suitable species. An acute test method is available with the endpoint survival.

Acute and subchronic toxicity tests with freshwater insect larvae

Toxicity tests with chironomid larvae are recommended in at least five assessment schemes. *Chironomus riparius* as well as *C. tentans* and *C. decorus* are mentioned. These larvae have a direct contact with the sediment by building tubes or cases in the sediment layer and eating sediment particles. As benthic annelids, midge larvae may represent a large amount of the sediment biomass and several species are known to ingest sediment particles. The testing of these species in whole sediment systems is therefore strongly recommended.

Several methods are available. An EU acute test method with *C. riparius* was ring-tested in 1994. A BBA long-term test method with *C. riparius* was also ring-tested in 1994. It is recommended to use the US EPA standards as key references for acute methods with the species *C. riparius* and *C. tentans*. Endpoints of these methods are survival (and possibly growth) for the acute test method. For the subchronic method, the ASTM standard may be used as key reference. Growth and emergence are endpoints for this method. Several supporting references should be used for including other life stages and endpoints. No method can be recommended for *C. decorus*.

For the cold as well as the warm water environment, *Hexagenia limbata* might be used. A US EPA standard method is available with the endpoint of survival. Another possibility might be to adjust the above mentioned chironomid test methods to cold temperatures.

Toxicity tests with amphibians

The US EPA requires a subchronic tadpole benthic test. However, according to the evaluation carried out, such a method is not recommended because the species is not considered to be benthic. Some information on benthic tests with amphibians is given in Section 6.4.

Acute and subchronic tests with marine nematodes

A test with nematodes is not required in the assessment schemes, but might be of interest due to their ecological importance. For the marine environment, *Monhystera disjuncta* might be useful. The species has not been used for routine toxicity testing. As *M. disjuncta* lives upon the sediment surface, Test Guideline development for this species has a lower priority compared to guideline development for infaunal species.

Acute toxicity tests with marine annelids

Three schemes require toxicity tests with marine annelids. The recommended species are *Arenicola marina*, *Neanthes (Nereis) arenaceodentata* and *Nereis virens*. The first is a cold water species, the latter two are warm water species. *N. virens* is reported to be benthic as well as pelagic. For all species, standard methods are available. The endpoints for *A. marina* and *N. arenaceodentata* are survival; the *N. virens* method also includes growth and behaviour.

Acute toxicity test with marine molluscs

This type of test is required in one assessment scheme. The recommended species are *Abra alba*, for which a PARCOM protocol exists, and *Macoma baltica*. The PARCOM protocol with *A. alba* is not recommended because of the physiological endpoint (apart from survival), the sediment suspension as test system, and because of the short exposure time. The reference concerning *M. baltica* does not have these disadvantages. However, this species was used in a study, not in a test method. Both species are cold water species.

Acute and subchronic toxicity tests with marine crustacea

At least four assessment schemes recommend acute toxicity tests with marine crustaceans. For this group of species, ASTM standard methods, Environment Canada guidelines, PARCOM ring-test protocols, an EU ring-test protocol and US EPA standard methods are available for cold water and warm water species. The US EPA standards use amphipods as well as other crustaceans. These acute test methods address in most cases the endpoints survival, avoidance and reburying ability.

It is recommended to include reproduction as an endpoint, which makes the test method a subchronic test. Reproduction is studied for several cold and warm water species, which are not included in the above mentioned protocols.

Most of the non-amphipod crustaceans mentioned above do not burrow in the sediment layer. Test Guideline development for these species is therefore thought to have a lower priority.

Acute, subchronic and chronic toxicity test with echinoids

Two schemes ask for the acute toxicity test with *Echinocardium cordatum*. A PARCOM protocol is available for this test. *E. cordatum* might not be easily available all over the world. Attention should be given to the possibility of including species of other regions.

Subchronic and chronic studies were carried out with the sea urchin *Lytechinus pictus*. Although this species is not used for routine toxicity testing, it might be useful for the development of a subchronic or chronic test guideline.

Preparation of test systems

An important part of a benthic toxicity test is the preparation of the test systems. The results of the test might be strongly influenced by sediment characteristics, i.e. organic carbon content, particle size distribution, acid volatile sulphide content, and redox potential. Especially for organic compounds, the equilibration time – the time period employed to let the test chemical equilibrate between water and sediment particles – is of critical importance. Together with the spiking method, it may affect (SETAC 1994):

- the extent of equilibration,
- the concentration distribution within the final individual test samples (whether from bulk or single-sample preparations), and
- the distribution (variability) of test material in samples taken from a bulk treatment.

It is recommended to develop guidance concerning the preparation of sediment test systems. This guidance should include the collection and storage of reference sediments, the preparation of artificial sediments, and the characterisation and spiking of the sediments. Species-specific requirements or other information may be given in the relevant test guideline.

Literature that should be consulted consists of the ASTM guideline for the preparation of test systems (ASTM, 1991), the proceedings of the WOSTA workshop (SETAC, 1994) and the guidance documents which will be published by Environment Canada in 1995 on a *Hyalella azteca* benthic toxicity test, a test using larvae of freshwater midges, and a document on the measurement of test precision using control sediment spiked with a reference toxicant. Recently (January 1995), a workshop was held in Ispra, Italy, which addressed the topic of soil and sediment preparation in toxicity tests.

Table 7.4 Methods recommended for inclusion in the OECD Test Guidelines Programme (Groups 1a, 1b) as they are needed for existing (draft) aquatic hazard/risk assessment schemes

Taxonomic group	**Test organism**	**AC/SC/C**	**ST/LT**	**References**
Cold freshwater environment				
No methods available				
Warm freshwater environment				
No methods available				
Cold marine environment				
Annelida	*Arenicola marina*	AC	LT	PARCOM 1993/1994 (4188/4228)
Crustacea	*Bathyporeia sarsi*	AC	LT	PARCOM/Van der Hurk 1990 (4085)
	Corophium sp.	AC	LT	PARCOM 1993 (4198)
	Corophium volutator	AC	LT	PARCOM 1994 (4227)
Echinodermata	*Echinocardium cordatum*	AC	LT	PARCOM 1993/1994 (4023/4225)
Warm marine environment				
No methods available				

Table 7.5 Methods presumably needed in the near future as they are recommended in some assessment schemes and/or considered to represent key ecological groups (Groups 2a, 2b). "?" refers to species about which it is uncertain that they are burrowing organisms.

Taxonomic group	Test organism	AC/SC/C	ST/LT	References
Cold freshwater environment				
Bacteria	sediment bacteria	C	ST	Van Beelen et al. 1990 (4016)
Annelida	*Stylodrilus heringianus*	AC		Keilty et al. 1988 (4093)
		SC	LT	Keilty et al. 1990 (4092)
	Limnodrilus hoffmeisteri	AC		Keilty et al. 1988 (4094)
Crustecea	*Gammarus lacustris ?*	AC	LT	Nebeker et al. 1984 (4141)
Insecta	*Hexagenia limbata*	AC	LT	US EPA 1994a (4247)
Warm freshwater environment				
Chordata, Amphibia	*Rana pipiens*	AC	ST	Berriee 1993 (95)
	Ambystoma mexicanum	SC	LT	Sloff 1980 (121)
Protozoa	*Tetrahymena pyriformis*	C	ST	Tyle 1993 (20)
Archaebacteria	methanogens	C	LT	Van Vlaardingen et al. 1992 (4197)
Aschelminthes	*Panagrellus redivivus*	SC		Gregor et al. 1989 (4070)
Annelida	*Tubifex tubifex*	AC	LT	US EPA 1994a (4239)
		C	LT	Reynolds et al. 1991 (4161)
	Lumbriculus variegatus	AC	LT	US EPA 1994a (4240)
		C	LT	Dermott et al. 1992 (4044)
	Pristina leidyi	AC	LT	US EPA 1994a (4253)
	Branchiura sowerbyi	AC	LT	Roghair et al. 1993 (4163)

Table 7.5, continued

Taxonomic group	Test organism	AC/SC/C	ST/LT	References
Warm freshwater environment (continued)				
Crustacea	*Hyalella azteca*	AC	LT	US EPA 1994a/b (4231/4248)
		(AC)SC	LT	ASTM 1991 (4012)
	Diporeia sp.	AC	LT	Landrum et al. 1991 (4104)
Insecta	*Chironomus riparius*	AC	LT	US EPA 1994a (4251)
		(AC)SC	LT	ASTM 1991 (4011)
	Chironomus tentans	AC	LT	US EPA 1994 a/b (4241/4252)
		(AC) SC	LT	ASTM 1991 (4013)
	Hexagenia limbata	AC	LT	US EPA 1994a (4247)
Cold marine environment				
Mollusca	*Macoma baltica*	AC	LT	Bryant et al. 1985 (4025)
Crustacea	*Rhepoxynius abronius*	AC	LT	US EPA 1994a/c (4243/4256)
	Eohaustorius estuarius	AC	LT	US EPA 1994c (4242)
	Ampelisca abdita	AC	LT	ASTM 1990 (4008)
	Grandidierella japonica	AC	LT	US EPA 1994a (4257)
	Amphipoeria virginiana	AC		
	Corophium volutator		LT	Env Can 1992 (4040)
	Eohaustorius estuarius			
	Eohaustorius washingtonianus			
	Foxiphalus xiximeus			
	Leptocheirus pinguins			
	Rhepoxynius abronius			
	Corophium insidiosum	SC	LT	Bjørnestad et al. (4208)
	Microdeutopus gryllotalpa	SC	LT	Bjørnestad et al. ? (4209)
Echinodermata	*Lytechinus pictus ?*	SC	LT	Thompson et al. 1991 (4190)
		C	LT	Thomson et al. 1989 (4189)

Table 7.5, continued

Taxonomic group	Test organism	AC/SC/C	ST/LT	References
Warm marine environment				
Annelida	*Nereis virens*	AC	LT	PARCOM 1993 (4187)
	Neanthes arenaceodentata	AC	LT	US EPA 1994 a/c (4249)
	Nereis arenaceodentata	AC	LT	Dillon et al. 1993 (4046)
Crustacea	*Eohaustorius estuarius*	AC	LT	ASTM 1990 (4007)
	Ampelisca abdita	AC	LT	US EPA 1994a/c (4244/4258)
		C	LT	Scott et al. 1989 (4174)
	Corophium sp.	AC	LT	US EPA 1994a (4246)
	Grandidirella japonica	AC	LT	US EPA 1994a (4257)
	Leptocheirus plumulosus	AC	LT	US EPA 1994a/c (4245/4255)
		SC	LT	McGee et al. 1993 (4032)
	Microrarthridion littorale ?	SC		Chandler 1990 (4032)
	Paronychocamptus wilsoni ?	SC		Chandler 1990 (4033)
	Enhydrosoma propinquum ?	SC		Chandler 1990 (4034)
	Amphiascus tenuiremis ?	C	LT	Strawbridge et al. 1992 (4182)
	Penaeus sp.	AC	LT	US EPA 1994a (4237)

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9. LIST OF ABBREVIATIONS AND ACRONYMS

AIS (D)	Association Internationale de la Savonnerie et de la Détergence
ASTM	American Society for Testing and Materials
AFNOR	Association Française de Normalisation
BBA	Biologishe Bundesanstalt für Land und Forstwirtschaft
CCME	Canadian Council of Ministers of the Environment
CONCAWE	Oil companies' European organization for environment, health and safety
DK-EPA	Danish Environmental Protection Agency
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EEC	European Economic Community
EU	European Union
EPPO	European and Mediterranean Plant Protection Organization
ESTHER	System for Testing and Hazard Evaluation of Chemicals
FELS	Fish Early Life Stage
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (US)
KEMI	National Chemical Inspectorate (Sweden)
PARCOM	Paris Commission
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
RIVM	National Institute of Public Health and Environmental Protection (the Netherlands)
SETAC	Society of Environmental Toxicology and Chemistry
TSCA	Toxic Substances Control Act (US)
UN	United Nations
USES	Uniform System for the Evaluation of Substances (the Netherlands)

VROM	Ministry of Housing, Spatial Planning and the Environment (the Netherlands)
WVC	Ministry of Welfare, Health and Cultural Affairs (the Netherlands)
WRC	Water Research Centre (UK)

10. ACKNOWLEDGEMENT

At an early stage, National Co-ordinators of the OECD Test Guidelines Programme, experts from OECD countries, and the various national standardisation organisations were requested to forward papers of relevance for the Detailed Review Paper. The following information was requested:

- aquatic national standard testing methods, as well as published or unpublished methods describing testing procedures and endpoints of relevance for future OECD Test Guideline development (pelagic as well as benthic methods);

- formalised and draft schemes for environmental effects, hazard and risk assessment of chemicals, pesticides and chemical mixtures (e.g. industrial effluents);

- papers or documents dealing with statistical treatment of ecotoxicity data and procedures for testing of “difficult” substances (low water soluble or adsorbable chemicals).

Most OECD countries responded, and a comprehensive number of papers were received. These papers were supplemented with relevant aquatic toxicity testing methods which were collected from the scientific literature. The countries and contact persons that responded to the request are listed in Annex M to this report.

The authors thank all those who responded, initially by providing the information and/or, after reading earlier drafts, with useful suggestions and criticisms.

The Danish part of the project was financially supported by the Danish Environmental Protection Agency (DK-EPA) and the Commission of the European Communities, Directorate-General Environment, Nuclear Safety and Civil Protection, Brussels.

Final Report of the OECD Working Group Meeting on Aquatic Toxicity Testing

Copenhagen, Denmark, 29-30 June 1995

Note: The recommendations of the OECD Working Group Meeting in Copenhagen in June 1995 were approved by the 6th Meeting of the National Co-ordinators of the Test Guidelines Programme in December 1995, with respect to the revision and development of OECD Test Guidelines on aquatic toxicity testing.

Table of Contents

Annexes:

Introduction and Background

At the 2nd Meeting of the National Co-ordinators of the OECD Test Guidelines Programme (TGP), in September 1991, it was decided that a Detailed Review Paper (DRP) concerning aquatic ecotoxicity testing methods should be prepared. The DRP should cover testing methods for the pelagic (water) and benthic (sediment) environment, and the identified methods should be applicable to both industrial chemicals and pesticides.

Therefore, a DRP on "Aquatic Testing Methods for Pesticides and Industrial Chemicals" has been prepared and collated by Denmark, with the contribution of the Netherlands for sediment test methods. The work was initiated in 1992. Two interim draft reports were prepared in October 1993 and August 1994, respectively, and the final draft of the DRP was completed in March 1995. This document consists of an extensive inventory and evaluation of existing methods for water/sediment toxicity testing. It also makes recommendations for OECD Test Guideline development and updating.

The DRP was circulated for comments to OECD Member countries by the OECD Secretariat in April 1995, with a deadline for response of 12 June 1995. In order to assist in reporting comments and facilitate the collation of responses, a questionnaire relating to the DRP recommendations was added to the DRP by the Secretariat. Member countries were requested to indicate: (i) priorities for the development of OECD Test Guidelines in water/sediment toxicity testing, and (ii) the amount of work that would be involved.

At their 5th Meeting in October 1994, the National Co-ordinators agreed that after circulation of the DRP for comments, a small Working Group should be established to discuss the comments received and to propose priorities for revision and development of Test Guidelines in the area concerned. The National Co-ordinators further suggested that the Working Group Meeting should be held back-to-back with the SETAC-Europe Annual Congress in June 1995, in Denmark, assuming that many of the experts to be involved in the Working Group would participate in the SETAC Congress.

At their meeting in January 1995, the OECD’s Hazard Assessment Advisory Body (HAAB) requested that the Working Group be a joint activity of the Test Guidelines and Hazard Assessment Programmes.

As agreed at the 5th National Co-ordinators' Meeting, the Secretariat developed proposals for membership of the Working Group. In consultation with the Danish organisers of the meeting of this Working Group, an effort was made to arrange for a balanced participation from Member countries including government, industry and testing facilities. The Working Group was established in May 1995, and the proposed composition was then submitted to the National Co-ordinators of the TGP and members of the HAAB for approval and possible additional nominations.

The Working Group members were provided with the DRP and the questionnaire relating to the DRP recommendations. They were also requested to complete this questionnaire, with a deadline of 14 June 1995, so that the compilation of the responses/comments would be available at the Working Group Meeting.

The Working Group Meeting was hosted by the Danish Environmental Protection Agency. It was held at Eigtveds Pakhus, Asiatisk Plads 2, Copenhagen, on 29-30 June 1995. The Meeting was chaired by Professor Finn Bro-Rasmussen of the Technical University of Denmark, and Robert Morcock of US EPA assisted as rapporteur. There were 29 participants from 13 OECD Member countries, the

European Commission and industry, and one observer from Argentina (see List of Participants in Annex 2).

Objectives, Focus and Structure of the Working Group Meeting

The objectives of the Working Group Meeting were to discuss the Detailed Review Paper on "Aquatic Testing Methods for Pesticides and Industrial Chemicals" and comments received from Member countries in order to:

(i) identify the needs for development and/or revision of OECD Test Guidelines for assessing effects on pelagic and benthic organisms;

(ii) propose priorities for development/revision of OECD Test Guidelines;

(iii) make proposals for drafting the selected Test Guidelines.

The Working Group Meeting focused on the Test Guidelines to be developed for assessing effects of chemicals in pelagic and benthic, freshwater and marine organisms covering:

- laboratory tests, excluding field studies;
- tests with single species (or mixed cultures for microorganisms), excluding tests with multispecies, microcosms and mesocosm studies;
- toxicity tests for testing of general chemicals and pesticides, excluding bioassays with effluent and field contaminated sediments.

The Meeting was organised around a series of Plenary Sessions and Working Group Sessions. The Working Group Sessions involved two Breakout Groups which considered the following topics: pelagic toxicity testing methods [Group 1] and benthic toxicity testing methods [Group 2]. The membership of each Breakout Group is shown in Annex 3.

Each Group reported during the Plenary Sessions on progress made. The Plenary Sessions provided the opportunity to provide feedback to each Breakout Group from all participants and to reach consensus on priorities for development/revision of OECD Test Guidelines for pelagic and benthic toxicity testing. There were three Working Group Sessions and three Plenary Sessions, including the final Plenary Session.

Inputs

Participants were provided with the following documents prior to the meeting:

- the Detailed Review Paper on "Aquatic Testing Methods for Pesticides and Industrial Chemicals", including annexed material;
- the questionnaire relating to the DRP recommendations, which was provided by the Secretariat to both Member countries and members of the Working Group;

- "Priority-setting for the Revision and Development of Test Guidelines Applicable to Pesticides. Recommendations of the Ecotoxicology Task Force for Aquatic Test Guidelines and Current Status in OECD Work" (see Annex 6).

The following documents were made available in the meeting room:

- a compilation of Member countries' comments on the DRP, including responses to the questionnaire (see Annex 7);

- a summary of Member countries' responses to the questionnaire relating to the DRP recommendations for Test Guideline development (see Annex 8);

- a compilation of all Working Group members' comments on the DRP, including responses to the questionnaire (see Annex 9).

At the Meeting, participants were also provided with the following information:

- an overview of the DRP and its recommendations, presented by Dr. Preben Kristensen, WQI, Denmark and Dr. Carla Roghair, RIVM, the Netherlands;

- a summary of (i) comments on the DRP and its recommendations and (ii) responses to the questionnaire received from Member countries and from Working Group members, presented by the OECD;

- a proposed schedule for issues to be addressed in Working Group discussions.

Discussion Issues

In order to focus the discussion of the Working Group Meeting, and to help achieve the objectives outlined above, each Breakout Group addressed a set of issues relating to the topics to be dealt with, i.e. (i) review and comment on the DRP, (ii) structure of the future Test Guidelines, (iii) identification of the needs for pelagic and benthic tests, (iv) proposed priorities for development of OECD Test Guidelines, (v) proposals for drafting the selected guidelines. The discussion issues were common to the two Breakout Groups. The topics addressed are listed below, with an indication of the issues/questions discussed.

<u>Discussion topics for the Breakout Groups</u>

- Review and comment on the DRP:

  - Principles used for evaluation of test methods (Chapter 4);
  - Definitions used: pelagic/benthic, cold/warm, freshwater/marine, acute/subchronic/chronic, short-term/long-term (Chapter 4);
  - Evaluation criteria for scoring of the pelagic/benthic test methods (Chapters 4, 5 and 6).

- Issues relating to the general structure of future Test Guidelines:

  - Can/should the guideline cover representatives from warm (tropical/subtropical) as well as cold (temperate, arctic) environments?
  - Can/should the guideline cover marine as well as freshwater species?
  - Can/should the guideline cover taxonomic groups (with representatives from some or all of the above compartments) or single species?
  - Can/should the guideline include acute, subchronic and chronic endpoints?
  - Is there a need to treat pesticides and general chemicals differently in terms of (1) taxonomic groups/species, (2) endpoints to be covered, and (3) test design?

- Identification of the needs for pelagic and benthic tests:

  - Which trophic levels should be covered? (Is there a need for methods for assessing effects in specific ecologically/recreationally/commercially important species and/or species threatened by extinction?)
  - Which taxonomic groups/species should be covered?
  - Which endpoints should be covered for each taxonomic group/species?

- Proposed priorities for the revision and development of OECD Test Guidelines, taking Member countries' comments into account as well as the priority-setting for development and revision of TGs applicable to pesticides:

  - Recommendations for the revision and development of OECD Test Guidelines:
    - Which taxa/species should be recommended?
    - Which endpoints should be covered?

  - Anticipated workload for each proposed Guideline:
    - Is there an international/national guideline/standard/protocol which could be used as a basis for OECD Test Guideline development?
    - Should the development/revision await the outcome of work initiated in other fora?
    - Is there a need for ring-testing of the test method?

  - Priority-setting of the proposed Test Guidelines.

- Proposals for drafting the selected Guidelines:

  - How to share the work?
  - Which country would take the lead in drafting guidelines?
  - Which country would take responsibility for initiating additional experimental work and/or ring-testing?
  - What is the expected time schedule for the development?

Summary of Working Group and Plenary Session Discussions

This section summarises and collates the outcome of the discussions of the two Breakout Groups. It focuses on the major issues raised and areas of agreement reached in the Working Group and Plenary Sessions. The reports of the Breakout Groups are presented in Annexes 4 and 5.

Review of and comment on the DRP

The DRP was well appreciated by the Member countries, being regarded as a comprehensive and exhaustive review which constituted a good basis for prioritising the development of OECD Test Guidelines in aquatic toxicity testing. Participants discussed the DRP with respect to the evaluation of test methods, addressing the following issues: (i) principles used for evaluation of test methods, (ii) definitions used, and (iii) evaluation criteria for scoring of pelagic and benthic test methods.

Principles used for evaluation of test methods

The evaluation of test methods was based on the identification of methods according to eight assessment scenarios involving pelagic and benthic compartments, cold and warm waters, and freshwater and marine environments. The Meeting, as well as the Member countries, agreed to the principles used for evaluation of test methods.

Definitions used

The Meeting discussed the definitions used in the DRP for (i) pelagic and benthic tests, (ii) warm and cold water temperatures, (iii) freshwater and marine environments, (iv) acute, subchronic and chronic effects, (v) short-term and long-term test durations. It was agreed that the definitions used were acceptable for the objective of the DRP, namely the identification of potential candidates for OECD Test Guideline development. However, a number of amendments to some definitions were proposed in regard to the use of these terms in future OECD Test Guidelines. Since the DRP will be published by the OECD, the definitions should be in accordance with those used in the OECD Test Guidelines. Member countries' comments did not indicate any concern with respect to the definitions in the DRP.

Pelagic and benthic tests

The Meeting suggested that definitions of "pelagic" and "benthic" test methods be amended. The two Breakout Groups were not of the same opinion as to changes to be made, in particular regarding the definition for benthic tests. The Benthic Group disagreed with the Pelagic Group's proposal in which benthic tests should include tests where organisms are exposed in a "water only" system for those small organisms (meiofauna) which live in the pore water of the sediment. No consensus was reached on this item. A proposal for amended definitions of pelagic tests and benthic tests, taking into account the suggestions of the Meeting, is presented in Annex 1 to this Workshop Report. This proposal would be submitted to the National Co-ordinators of the Test Guidelines Programme at their next meeting in December 1995.

Warm and cold water temperatures

Participants considered that distinction between "warm" and "cold" species was used for convenience. It was recommended that "cold" temperatures be defined as <18°C.

Freshwater and marine environments

Participants accepted the definitions of "freshwater" and "marine" environments. The term "saltwater" should be preferred to "marine".

Acute, subchronic and chronic effects

The definitions for "acute", "subchronic" and "chronic" were discussed and a number of amendments were proposed.[2] The main recommendations were as follows:

- the term "subchronic" should not be used and should be replaced with "chronic", i.e. chronic tests may be defined as test methods where lethal and sublethal effects are studied during an exposure period covering a considerable part of the life cycle;

- the term "life cycle/multigeneration" should be used for tests where the exposure period is sufficient for one or more generations to be exposed (previously indicated as "chronic").

It was agreed that a proposal taking into account the above recommendations would be made to the National Co-ordinators of the Test Guidelines Programme for consideration at their next meeting in December 1995. The proposal for amendments to the definitions is presented in Annex 1.

Short-term and long-term test durations

Participants stated that periods specified in the DRP were used for convenience. It was recommended that short-term exposure be defined as < 10 days and long-term exposure be defined as ≥ 10 days.

Evaluation criteria for scoring of the pelagic and benthic test methods

The two Breakout Groups discussed the scoring of pelagic and benthic test methods with respect to the evaluation criteria used, e.g. practical feasibility, validity, usefulness in prognoses (predictiveness) and level of standardisation. Participants recognised the relevance of using such criteria and considered that scoring of test methods in the DRP is a relative evaluation system facilitating the selection of methods. It appears that Member countries generally agreed with the criteria used for the scoring of pelagic and benthic test methods, since they had no specific comments on this item.

Issues relating to the general structure of future Test Guidelines

The Meeting discussed several issues raised in the DRP relating to the general structure of future Test Guidelines. Should the guideline (i) cover representatives from warm and cold environments? (ii) cover freshwater and saltwater species? (iii) cover taxonomic groups or single species? (iv) include acute, subchronic and chronic effects? (iv) cover the testing of pesticides and general chemicals?

Warm and cold environments

In accordance with the general opinion of Member countries, the Meeting considered that distinction between warm water and cold water species was justified for practical reasons and should not constitute a matter of principle. Participants agreed that the Test Guideline should recommend the use of either warm or cold water species, as appropriate, and indicate the thermal ranges appropriate to each species.

2 In this report, the terms *acute*, *subchronic* and *chronic* are used according to the definitions given in the DRP.

Freshwater and marine species

The Meeting considered that, where feasible, the Test Guideline may cover marine as well as freshwater species, but separate Guidelines might be regarded as more practical. Participants were of the opinion, as were most Member countries, that this issue should not constitute a matter of principle and should be considered on a case-by-case basis.

Taxonomic groups versus single species

The Meeting agreed that guidelines can be based on taxonomic groups, but it was stressed that the degree of specificity in the Test Guideline will depend on the particular species and may be more important for chronic exposure. Member countries appeared to have different opinions on a "framework" Test Guideline for taxonomic groups, arguing for the usefulness of such a principle, but also indicating the difficulty in applying it.

Acute, subchronic and chronic effects

Participants were of the opinion that, where feasible, the Test Guideline may cover acute as well as subchronic/chronic endpoints. It was stressed that it may be technically difficult to include acute and chronic endpoints in a single study design. With respect to this issue, Working Group discussions reflected Member countries' opinions.

Pesticides versus general chemicals

The Meeting agreed that there was no need for separate guidelines for effects assessment of pesticides and general chemicals in pelagic/benthic organisms. However, because of different exposure scenarios of pesticides *versus* general chemicals, it was recommended that the guidelines be flexible to provide recommendations on the specific variations of the test design. Member countries did not make specific comments on this item.

Identification of the needs for pelagic and benthic tests

The Breakout Groups discussed the need for development/revision of Test Guidelines for assessment of effects in pelagic and benthic organisms respectively. Each Group identified the need for test methods in the area concerned in terms of (i) which taxonomic group/species should be represented, and (ii) which endpoints should be covered. The proposals of each Group were then discussed in Plenary Session. The recommendations of the Meeting are summarised below.

Taxonomic groups/species

It was emphasised that the use of representative taxonomic groups for selection of test species is a first requirement and should be preferred to an approach based on trophic levels. Taxonomic groups and species to be represented were discussed for both pelagic and benthic tests.

Pelagic test methods

The Pelagic Group identified the taxonomic groups/species to be recommended for pelagic tests and proposed two lists of recommendations.

List 1: Recommended taxa/species based on the test methods which were given initial high priority according to (i) the DRP recommendations (methods ranked in Group 1 and/or Group 2), (ii) Member countries' comments on the DRP (methods given High to Medium priorities), and (iii) the Pesticide Task Force on Ecotoxicology (methods given High or Medium priorities).

Test methods on List 1 should be developed/revised as soon as possible.

List 2: Taxa/species which were given relative high priority in the DRP (methods ranked in Group 2), but were not given high priority by Member countries nor by the Pesticide Task Force on Ecotoxicology.

Test methods on List 2 should be considered for Test Guideline development in the future.

Lists 1 and 2 of the recommended taxa/species for pelagic tests agreed upon at the Working Group Meeting are summarised in **Tables 1 and 2.**

Benthic test methods

The recommendations regarding the taxonomic groups to be considered for development of benthic test methods are summarised in **Table 3**.

Endpoints

Endpoints to be covered were discussed for each selected taxonomic group/species, for both pelagic and benthic test methods.

Pelagic test methods

With respect to the pelagic organisms on List 1, the recommendations for endpoints to be covered are summarised in **Table 4**.

With respect to the methods on List 2, it was recommended that higher priority should be given to the development of life cycle studies and/or methods covering chronic endpoints than to acute endpoints.

Benthic test methods

Regarding the endpoints to be covered for benthic tests, the recommendations are summarised in **Table 5**.

Table 1: List 1: taxanomic groups/species recommended for pelagic tests (to be considered as soon as possible)

Taxonomic group	Environment: freshwater/saltwater	Species
Kormophyta	freshwater	*Lemna gibba, Lemna minor*
Microalgae	freshwater	add other species, e.g. blue-green, diatoms [TG 201]
	saltwater	e.g. *Skeletonema, Gymnodium, Phaeodactylum*
Crustacea	freshwater	*Daphnia* sp. (no other species) [TG 202]
	saltwater	e.g. *Tisbe, Nitocra, Mysidopsis, Acartia*
Fish	freshwater	species to be selected for life cycle test
	saltwater	species to be selected for life cycle test
Bacteria	–	nitrifying bacteria (aerobic activated sludge bacteria)
Mollusca	saltwater	e.g. *Crassostrea, Mytilus, Mercenaria*

Table 2: List 2: taxanomic groups/species recommended for pelagic tests (to be considered in the future)

Taxonomic group	Environment: freshwater/saltwater	Species
Protozoa	freshwater	e.g. *Tetrahymena pyriformis*
	saltwater	e.g. *Uronema marinum*
Echinodermata	saltwater	species to be selected
Aschelminthes (Rotifera)	freshwater	species to be selected (e.g. *Brachionus*)
	saltwater	species to be selected (e.g. *Brachionus*)
amphibia	freshwater	species to be selected
Macroalgae	saltwater	species to be selected

Table 3: Taxonomic groups/species to be considered for benthic tests

Taxonomic group	Environment: freshwater/saltwater	Species
Insecta	freshwater	*Chironomus* sp., *Polypedilum*
Crustacea (Amphipod)	freshwater	*Hyalella azteca*
Crustacea (Amphipod)	saltwater	species to be selected (e.g. *Corophium*, *Ampelisca*, *Leptocheirus*)
Annelida	freshwater	species to be selected (e.g. *Tubifex*, *Lumbriculus*)
	saltwater	species to be selected (e.g. *Arenicola*, *Neanthes*)

Table 4: Recommended endpoints for pelagic tests on List 1

Pelagic tests		Endpoints
Taxonomic group	**Species**	
Kormophyta	freshwater (*Lemna*)	growth
Microalgae	freshwater and saltwater sp.	growth
Crustacea	freshwater species (*Daphnia*)	acute (immobilisation, EC50-48h)
	saltwater sp. (*Tisbe, Nitocra, Acartia, Mysidopsis, Penaeus*)	acute (survival) and reproduction
Fish	freshwater and saltwater sp.	full and/or partial life cycle test
Bacteria	sludge bacteria	inhibition of nitrification
Mollusca	saltwater species (*Mytilus, Crassostrea, Mercenaria*)	acute on early life stages, 48h and shell deposition, 96h

Table 5: Recommended endpoints to be covered by benthic tests

Benthic tests		Endpoints
Taxonomic group	**Species**	
Insecta	freshwater species (*Chironomus, Polypedilum*)	acute (survival) and growth/emergence
Crustacea (Amphipod)	freshwater species (*Hyalella azteca*)	acute (survival) and growth
Crustacea (Amphipod)	saltwater sp. (e.g. *Corophium, Ampelisca, Leptocheirus*)	acute (survival) and growth
Annelida	freshwater sp. (e.g. *Tubifex, Lumbriculus*)	acute (survival) and reproduction
	saltwater sp. (e.g. *Arenicola, Neanthes*)	acute (survival)

Proposed priorities for development/revision of OECD Test Guidelines

The Meeting proposed priorities for development/revision of OECD Test Guidelines. Member countries' comments were also taken into account, as well as the "Priority-setting for Revision and Development of Test Guidelines Applicable to Pesticides" established in 1993. The selected Test Guidelines were discussed with respect to the anticipated workload that their development would involve. The workload was estimated taking into account (i) availability of existing guidelines/methods/protocols, (ii) need for ring-test, and (iii) specific issues to be solved (e.g. standardisation of sediments and dosing for benthic tests).

The Breakout Groups discussed the order of priority for development of the selected Guidelines, from the most urgent to the least urgent. Group 1 assigned priority scores from 1 to 5 for the development of pelagic Test Guidelines. Group 2 assigned priority scores from 1 to 4 for the development of benthic Test Guidelines. The proposals of each Breakout Group were discussed in Plenary session. It was agreed that the priorities for pelagic and benthic test development should be considered entirely separately.

The recommendations of the Meeting with respect to revision and development of OECD Test Guidelines in aquatic toxicity testing are summarised in **Tables 6 and 7** for pelagic tests and benthic tests respectively.

Proposals for drafting the selected guidelines

Proposals for drafting the selected guidelines in terms of (i) how to share the work and (ii) which country could take the lead should be discussed at the next National Co-ordinators' Meeting of the Test Guidelines Programme in December 1995.

Summary and further work

The Meeting agreed on: (i) recommendations for amendments to the DRP, and (ii) proposed priorities for development and/or revision of OECD Test Guidelines in aquatic toxicity testing.

Amendments to the DRP

A number of corrections to the text, including editorial changes, were proposed. These corrections, as well as those derived from Member countries' comments, would be included in the final version of the DRP. It was proposed that the deadline for comments from Working Group participants be 2 October 1995.

With respect to the amendments to the definitions used in the DRP (i.e. pelagic and benthic tests, acute/subchronic/chronic effects), a proposal in line with the recommendations of the Meeting is presented in Annex 1. This proposal will be submitted for consideration to the National Co-ordinators of the Test Guidelines Programme at their next Meeting in December 1995. The definitions agreed upon at the NCs' Meeting should be included in the revised version of the DRP.

Proposed priorities for OECD Test Guideline development

The Meeting agreed on priorities for development and/or revision of OECD Test Guidelines in pelagic and benthic toxicity testing. The outcome of this Meeting regarding the proposed priorities for development and/or revision of OECD Test Guidelines for pelagic/benthic toxicity testing will be submitted for consideration to the National Co-ordinators of the Test Guidelines Programme at the next Meeting in December 1995.

Table 8 summarises the proposed priorities for OECD Test Guideline development in aquatic toxicity testing according to recommendations: (1) as proposed in the DRP, (2) as agreed by the Working Group Meeting, (3) as indicated by OECD Member countries in response to the questionnaire relating to the DRP recommendations, (4) as proposed by the Pesticide Task Force on Ecotoxicology in 1993. It reveals that opinions are generally in agreement as to the need for development and/or revision of several OECD Test Guidelines.

It should be borne in mind that the Working Group Meeting selected the tests for OECD Test Guideline development taking into account the recommendations of the DRP, OECD Member countries and the Pesticide Task Force. The selected guidelines were all given high priority for development, and the Working Group Meeting assigned priority scores from the most urgent to the less urgent.

In summary, the following tests were given a high priority for OECD Test Guideline development and are listed according to their priority (from the most urgent to the least urgent) as agreed upon at the Meeting:

Pelagic tests:

1. ➢ Crustacea, saltwater sp., acute and reproduction tests;
2. ➢ Higher plant, Kormophyta (*Lemna*), growth test;
3. ➢ Fish, full and/or partial life cycle test;

4. ➢ Microalgae, freshwater and saltwater sp., growth test - Revision of TG 201;
5. ➢ Mollusca, saltwater sp., acute on ELS and shell deposition tests;
   ➢ Bacteria, sludge bacteria, nitrification test.

and revision of TG 202, Part I: *Daphnia* sp., 24h-EC_{50} Acute Immobilisation test (48h study).

Benthic tests:

1. ➢ Insecta, *Chironomus*, acute and chronic (growth and emergence) tests;
2. ➢ Crustacea (Amphipod), saltwater sp., acute and growth tests;
3. ➢ Annelida, freshwater sp., acute and reproduction tests;
   ➢ Annelida, saltwater sp., acute test;
4. ➢ Crustacea, freshwater sp. (*Hyalella*), acute and growth tests.

Table 6: Priorities for development/revision of OECD Test Guidelines for pelagic toxicity testing

Pelagic tests			Methods available	Action	Workload	Ring-test	Priority score(*)
Taxonomic group	**Species**	**Endpoints**					
Kormophyta	Freshwater species (*Lemna*)	Growth	ISO, AFNOR, US EPA, ASTM	New TG	Moderate	Possibly required	2
Microalgae	Freshwater species (add blue-greens, diatoms)	Growth	OECD 201	Revise 201	Moderate	Required	4
	Saltwater sp. (*Skeletonema, Gymnodium* etc.)	Growth	OECD 201, ISO, ASTM, PARCOM	Revise 201	Moderate	Required	4
Crustacea	Freshwater species (*Daphnia*)	Acute (immobilisation) EC_{50}-48h	OECD 202	Revise 202	Very small (editorial work)	Not required	–
	Saltwater sp. (*Tisbe, Nitocra, Mysidopsis, Acartia*, etc.)	Acute (survival) and reproduction	PARCOM, US EPA	New TG or Revise 202?	Large	Required	1
Fish	Freshwater and saltwater species	Life cycle (full and/or partial life cycle test)	US EPA	New TG	Very large	Required	3
Bacteria	Sludge bacteria	Nitrification	ISO	New TG	Moderate	possibly Required	5
Mollusca	Saltwater sp. (*Crassostrea, Mytilus, Mercanaria*)	Acute: ELS-48h and shell deposition-96h	TSCA, FIFRA, ASTM, US EPA	New TG (one TG)	Large	Required	5

(*): Priority score for development of OECD Test Guidelines from 1 (most urgent) to 5 (least urgent)

Table 7: Priorities for development/revision of OECD Test Guidelines for benthic toxicity testing

Benthic tests			Methods available	Action	Workload	Ring-test	Priority score (*)
Taxonomic group	**Species**	**Endpoints**					
Insecta	Freshwater species (*Chironomus*)	Acute (survival) and growth/emergence	ASTM, US EPA, BBA	New TG (one TG - separate tests for acute and chronic endpoints)	Moderate	Existing ring-tests (EU, BBA, US EPA)	1
Crustacea (Amphipod)	Freshwater species (*Hyalella*)	Acute (survival) and growth	ASTM, US EPA, Environment Canada	New TG (one TG - separate tests for acute and chronic endpoints)	Moderate	–	4
Crustacea (Amphipod)	Saltwater sp. (*Corophium, Ampelisca, Leptocheirus*)	Acute (survival)	Literature	New TG	Large	Existing ring-tests (EU, PARCOM)	2
Annelida	Freshwater sp. (e.g. *Tubifex, Lubriculus*)	Acute (survival) and reproduction	Literature	New TG (one TG - separate tests for acute and chronic endpoints)	Large	–	3
	saltwater sp. (e.g. *Arenicola, Neanthes*)	Acute (survival)	PARCOM	New TG	Large	–	3

(*): Priority score for development of OECD Test Guideline from 1 (most urgent) to 4 (least urgent)

Table 8: Priorities for OECD Test Guideline development in aquatic toxicity testing according to recommendations from (1) DRP, (2) Working Group Meeting, (3) OECD Member countries, (4) Pesticide Task Force on Ecotoxicology

Test Guidelines				Proposed priorities from			
Taxonomic group	**FW (freshwater), SW (saltwater) species**	**Endpoints**	**Action**	**DRP (1)**	**Wkg. Group Meeting (2)**	**OECD Member countries (3)**	**Pesticide Task Force (4)**
Pelagic tests							
Kormophyta	FW (*Lemna*)	Growth	New TG	Group 1	2	High	High
Microalgae	FW (add blue-greens, diatoms) and SW (e.g. *Skeletonema, Gymnodium*)	Growth	Rev 201	Group 1	4	High	High
Crustacea	FW (*Daphnia*)	Acute (EC50-48h)	Rev 202	Group 2	–	High to Medium	High
	SW (e.g. *Tisbe, Nitocra, Mysidopsis, Acartia*)	Acute and reproduction	New TG?	Group 1	1	High to Medium	Low
Fish	FW and SW	Life cycle test	New TG	Group 1	3	High	Medium
Bacteria	FW (sludge bacteria)	Nitrification	New TG	Group 2	5	Mixed opinions (High, Medium, Low)	0
Mollusca	SW (e.g. *Crassostrea, Mytilus, Mercenaria*)	Acute on ELS and shell deposition	New TG	Group 2	5	Mixed opinions (High, Medium, Low)	not considered
Benthic tests							
Insecta	FW (*Chironomus*)	Acute and growth/emergence	New TG	Group 2	1	High	High
Crustacea (Amphipod)	FW (*Hyalella*)	Acute and growth	New TG	Group 1	4	High to Medium	not considered
Crustacea (Amphipod)	SW (e.g. *Corophium, Ampelisca*)	Acute	New TG	Groups 1 & 2	2	High	not considered
Annelida	FW (e.g. *Tubifex, Lumbriculus*)	Acute and reproduction	New TG	Group 2	3	Mixed opinions (High, Medium, Low)	not considered
	SW (e.g. *Arenicola, Neanthes*)	Acute	New TG	Group 1	3	Mixed opinions	not considered

(1), (2), (3), (4): See notes on next page

Notes:

(1) Priorities for development of OECD Test Guidelines according to DRP recommendations:

- Group 1: Methods needed for existing effects, hazard and risk assessment schemes for chemicals and/or pesticides as adopted or drafted by international organisations, communities and industrial organisations. These methods should be considered for OECD ring-testing unless sufficient ring-testing has already been performed. An OECD Test Guidelines Proposal should be drafted as soon as possible or in the near future.

- Group 2: Methods presumably needed in the future as they are recommended for assessment of chemicals, pesticides and/or complex mixtures in national adopted or draft schemes for effects, hazard and risk assessment, or schemes proposed in recent scientific literature. Methods that are considered to meet ecologically defined needs are also included. The drafting of an OECD Test Guideline should be considered in the future, or establishment of necessary scientific documentation should be prompted as the methods might be considered for Test Guideline development in future.

(2) Priority scores for development of OECD Test Guidelines, assigned by the Working Group Meeting:

- 1 (most urgent) to 5 (least urgent) for pelagic tests;
- 1 (most urgent) to 4 (least urgent) for benthic tests.

(3) Priorities for development of OECD Test Guidelines assigned by OECD Member countries in response to the questionnaire relating to the DRP recommendations:

- High: The method covers endpoints which are included in existing data requirements of a considerable number of countries and at an early level of testing/assessment, and/or covers relevant taxonomic groups which are not yet represented. The method is scientifically justified.
- Medium: The method covers endpoints which are included in existing data requirements of a limited number of countries, or only required at a higher level of testing assessment, and/or covers relevant taxonomic groups which are not sufficiently represented. The method is scientifically justified.
- Low: The method is scientifically justified; however there are no requirements for data on endpoints covered by the test.

(4) Priorities for development of OECD Test Guidelines applicable to pesticides assigned by the Pesticide Task Force on Ecotoxicology in 1993. Priorities (i.e. High, Medium, Low) were assigned taking into consideration:

- The frequency with which any particular data requirement is requested for pesticide registration;
- The revision/developments proposed by Member countries in the Test Guidelines survey, the number of countries supporting work on particular studies, and whether they satisfy the data requirements listed in the survey;
- The recommendations of the Pesticide Working Group;
- The availability of existing methods from other fora.

ANNEX 1

PROPOSAL FOR AMENDMENTS TO THE DEFINITIONS USED IN THE DRP, ACCORDING TO THE RECOMMENDATIONS OF THE WORKING GROUP MEETING

I. Definition of "pelagic" and "benthic" test methods

Existing definitions in the DRP:

Definition of pelagic tests: Methods based on (life stages of) organisms inhabiting the pelagic environment, or (life stages of) organisms being exposed predominantly via the pelagic environment in the process of feeding and respiration. In the pelagic test, the organisms are exposed in a "water only system".

Definition of benthic tests: Methods based on (life stages of) organisms inhabiting the benthic environment and being exposed predominantly via the benthic environment in the process of feeding and/or respiration. In a benthic test, the organisms are exposed in a whole sediment system (i.e. a non-disturbed sediment layer with overlying water).

Proposal for amended definitions:

Definition of pelagic tests: Methods based on life cycle stages of organisms that live, feed and respire in the pelagic environment. Exposure is predominantly via the process of feeding and respiration. In a pelagic test, the organisms are exposed to materials added to water, without the presence of sediments.

Definition of benthic tests: Methods based on life cycle stages of organisms that live, feed and respire in the benthic environment. Exposure is predominantly via the process of feeding and/or respiration. In a benthic test, the organisms are exposed in a whole-sediment system (i.e. a non-disturbed sediment layer with overlying water).

II. Definition of acute, subchronic and chronic effects

Existing definitions in the DRP:

Acute effects: Lethal or sublethal effects observed after a short exposure period related to the life span of the organisms.

Subchronic effects: Lethal and sublethal effects observed after an exposure period covering a considerable part of the life cycle or covering life stages or life processes (e.g. reproduction) believed to be especially sensitive compared to other life stages.

Chronic effects: Effects observed during exposure of the entire life cycle of the organism. The often seen definition of "chronic" as tests covering at least 90 per cent of the life span of the organism is not considered applicable, as crucial life stages may be omitted in the 10 per cent life span not exposed (e.g. fertilization or yolksac stage of salmon fish). Chronic studies on microorganisms (e.g. algae and protozoans) often cover several generations of the organisms (multigeneration tests). In this review, these studies have also been termed chronic tests.

Proposal for amended definitions:

Acute effects: Lethal or sublethal effects observed after a short exposure period in relation to the life span of the organism.

Chronic effects: Lethal and sublethal effects observed after an exposure period covering a considerable part of the life span of the organism (e.g. 15 per cent or more). The exposure period is sufficient to cover some important life processes, e.g. reproduction, growth.

Life cycle/multigeneration effects: Effects observed during exposure of the entire life cycle of the organism, i.e. tests where the exposure period is sufficient for one or more generations to be exposed (e.g. from egg (F_0) to egg (F_1), juvenile to juvenile).

ANNEX 2

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ANNEX 3

COMPOSITION OF THE BREAKOUT GROUPS

Breakout Group 1: Pelagic Toxicity Testing Methods

Chair: Jack De Bruijn, VROM, the Netherlands
Rapporteurs: Preben Kristensen, WQI, Denmark and John Solbé, Unilever, UK

Finn Bro-Rasmussen, Denmark University, Denmark
Robert Graney, ACPA, Bayer Miles, USA
Mark Hinman, Exxon, USA
Peter Hodson, Environment Canada, Canada
Torsten Källqvist, NIVA, Norway
Ursula Klaschka, UBA, Germany
Robert Morcock, US EPA, USA
Guido Persoone, Gent University, Belgium
Keith Solomon, Guelph University, Canada
Maria Tarpkea, SNV, Sweden
Henrik Tyle, Danish EPA, Denmark
Paule Vasseur, CSE Metz, France

Jorge Herkovits, ICAS, Argentina (Observer)

Breakout Group 2: Benthic Toxicity Testing Methods

Chair: Peter Matthiessen, MAFF, UK
Rapporteurs: Herbert Köpp, BBA, Germany, Peter McCahon, GIFAP, Rhône-Poulenc, France, and Carla Roghair, RIVM, Netherlands

Rudiger Bias, BASF, Germany
Ebba Tiberg, KemI, Sweden
Juan Gonzalez-Valero, Ciba-Geigy, Switzerland
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Shigehisa Hatakeyama, Inst. Environment, Japan
Ross Hyne, NSW EPA, Australia
Steve Maund, ACPA, Zeneca, UK
Ossi Meyn, US EPA, USA
Richard Stephenson, Shell, Netherlands
Elvira Tsani-Bazaca, EC/JRC, Italy

ANNEX 4

REPORT OF BREAKOUT GROUP 1 ON PELAGIC TOXICITY TESTING METHODS

Chairman: Jack de Bruijn, VROM, the Netherlands
Rapporteurs: Preben Kristensen, VKI, Denmark and John Solbé, Unilever, UK

I. Description of the Group's activities

The Group on Pelagic Toxicity Testing Methods started with a discussion on the definitions and selection criteria that were used in the DRP. Subsequently, the Group elaborated two priority lists, one with high priority methods to be recommended for OECD Test Guideline development as soon as possible, and one with methods, or rather taxonomic groups, for which test guidelines should be developed at a somewhat longer term. When agreement on these lists was achieved, some more detailed discussions were held on the expected type of activities necessary for Test Guideline development and more specific priorities within the lists were achieved.

II. Review and comments on the DRP

The Group reviewed the DRP and the following comments were made:

1) A number of corrections to the text were proposed. These corrections will be included in the final version of the DRP. Deadline for comments from the Working Group experts was set at October 1, 1995.

2) In general, it was agreed that the definitions used in the DRP (i.e. "acute", "subchronic", "chronic", "warm water species", "cold water species", "pelagic tests", "benthic tests", "short-term" and "long-term") were feasible for the objective of the DRP: to set up a transparent methodology for identification of potential future candidates for new OECD TGs.

   A number of amendments to these definitions were, however, suggested for the future use of these terms in OECD Test Guideline work:

   - The term "subchronic" should not be used in OECD Test Guidelines.
   - "Chronic" should replace "subchronic": i.e. chronic tests may be defined as "test methods in which lethal and sublethal effects are studied during an exposure period covering a considerable part of the life cycle or an exposure period (which needs not be longer than an acute test) where effects may be caused in especially sensitive life stages (e.g. larvae; seedling) or life processes (e.g. reproduction).
   - "Life cycle/multigeneration test" should be used to cover the definition in the DRP for chronic tests, i.e. test methods where the exposure period is sufficient for one or more generations to be exposed (e.g. from egg (F_0) to egg (F_1)).

- The definitions used for warm and cold water species should not be used for OECD TGs. Where possible, Guidelines should be developed that are suitable for testing of either cold or warm water species. These Guidelines should indicate the thermal ranges appropriate to species in order to help make choices within a taxonomic group.
- The definition for benthic tests does not cover meiofauna or mesofauna living in the pore water which, according to the DRP, may be tested in a "water-only" exposure system. The following amended definition was suggested: "Methods based on (life stages of) organisms inhabiting the benthic environment and being exposed predominantly via the benthic environment in the process of feeding and/or respiration. In a benthic test, the organisms are exposed in a whole-sediment system (i.e. during the test, an undisturbed sediment layer with overlying water) or in a water-only system for those organisms which live in the pore water of the sediment".
- "Short-term" and "long-term" are not useful in the OECD TG context. The duration of the test should appear in the title of the test.

It was agreed that a proposal in line with the above amendments for "acute tests", "chronic tests" and "life cycle tests" be made for the consideration of the National Co-ordinators at their meeting later this year (December 1995). The definitions agreed upon at the NCs meeting (provided they do not involve major changes of the DRP) should be included in the final version of the report.

3) The overall procedure used for rating (scoring) the testing methods in the DRP was generally accepted as a relative evaluation system facilitating the transparency of the selection of methods. According to some experts, endemic species, relicts and other organisms with a very narrow geographic distribution should not *per se* be excluded from potential test guidelines (i.e. given a CC in the scoring system: the method is not applicable for standardisation). In this respect, the question was raised whether OECD is looking both for methods that are complementary to existing Test Guidelines and for cheaper alternatives.

4) Where feasible, Test Guidelines may be based on taxonomic groups, and may cover acute as well as chronic endpoints and/or cover marine as well as freshwater species. In general, the possibility of elaboration of "framework" guidelines is believed to be better for acute than for chronic tests and will depend on the particular species/taxonomic groups.

5) Generally, there is no need for specific pesticide Test Guidelines.

III. Elaboration of a list of pelagic testing methods to be given high priority for test guideline development

Based on the recommendations made in the DRP (primary and secondary recommendations), the comments received from the National Co-ordinators (as compiled and distributed by the OECD Secretariat) and the recommendations made by the OECD Task Force on Ecotoxicology, Pesticide Activity (OECD Document TG\TGP 93-2.111), a list of high priority testing methods was elaborated. This list (**List 1**) is outlined below. At this stage of the selection process no differentiation in priority was made among the methods.

LIST 1:

High priority list of testing methods to be considered for OECD Test Guideline development

- Kormophyta (vascular plants) :

  to elaborate a new TG with *Lemna* sp. (acute test)

- Freshwater microalgae:

  to include more species in TG 201 than the present green algae (e.g. blue-green algae, diatoms) and extend the test duration from 72h to 96h

- Marine microalgae:

  to extend TG 201 or to elaborate a new TG for a multigeneration test with marine microalgae

- Crustaceans:

  1) revision of TG 202, Part I to extend the test duration from 24 to 48 hrs

  2) elaboration of a new TG for marine species, e.g. *Acartia, Tisbe, Nitocra* (endpoints to be decided)

- Fish:

  elaboration of new TG on life cycle tests with marine and freshwater species (species to be decided)

- Bacteria:

  elaboration of new TG with nitrifying bacterial consortia

- Mollusca:

  elaboration of a new TG with marine mollusca (species and end-points to be decided)

During the elaboration of List 1, the following tentative recommendations were excluded:

– Updating TG 202, Part 1 to include also *Ceriodaphnia*: This update was not considered to be important as *Ceriodaphnia* is not presently recommended for testing of chemicals or pesticides in any of the OECD countries. Information on toxicity to *Ceriodaphnia* is not likely to improve the data background for effects assessment as long as data for *Daphnia* sp. are available.

- Testing method with *Pseudomonas putida*. The method is not recommended for testing of chemicals or pesticides in any of the OECD countries. The NCs were wrongly asked to consider this method due to an error in the DRP (included in the list of recommended methods, although given low priority in other parts of the DRP). Instead, a testing method with nitrifying bacterial consortia should have been included in the list of methods forwarded to NCs. The Group agreed to include this method in the list of high priority methods for OECD TG development (List 1).

In addition to List 1, the Group discussed methods such as those that are recommended in the DRP (primary recommendations) but not given high priority by the NCs nor by the OECD Task Force (e.g. testing methods with protozoans and echinoderms). Also, proposals were brought forward from the Group which were included in the DRP as secondary recommendations (e.g. testing methods with amphibians, aschelminthes and macroalgae).

After the methods had been reviewed, an additional list of methods which should also be considered by the NCs for Test Guideline development in the future was elaborated. This list (**List 2**) is outlined below.

LIST 2:

Methods considered to be of relatively high priority for Test Guideline development by the Working Group, although not included in the recommendations given by the National Co-ordinators

- Protozoans:

  New TG for freshwater and marine species

- Echinoderms:

  New TG for marine species

- Amphibians:

  New TG for freshwater species: species to be decided

- Aschelminthes:

  New TG on acute and/or chronic toxicity test with freshwater and/or marine Rotifera

- Macroalgae:

  New TG on growth inhibition and/or inhibition of reproduction with marine species.

In addition to these methods, the Pelagic Group identified a future need for tests with fungi and rooted emergent macrophytes and, in general, the need for considering endpoints of relevance for specifically acting chemicals, e.g. genotoxicity endpoints and endpoints for endocrine disrupters.

IV. Outline and prioritising of methods given high priority for OECD Test Guideline development

1. High priority methods: OECD Test Guideline work should be initiated as soon as possible (List 1)

The methods included in List 1 were reviewed by the Group regarding available guidelines/ standards, expected work load, and need for ring-testing. Although these methods were all given a high priority, indicating that Test Guideline development should start as soon as possible, the experts were finally asked to prioritise three of the methods listed with regard to which methods to handle first. It was agreed that the updating of TG 202, Part I (increasing the duration of the test from 24 to 48 hrs) could be excluded from the prioritising process, as this activity was considered to be editorial work by the OECD Secretariat.

The methods are listed below according to the outcome of this voting procedure:

a) Editorial work:

Updating TG 202, Part I regarding increased duration from 24 to 48 hrs

b) Prioritised list:

1. <u>Marine crustaceans</u>:

- *Acartia*, *Tisbe*, *Nitocra*, *Mysidopsis*, *Penaeus*, a.o.
- acute and reproduction test method
- standardised methods available: PARCOM (cold water species), US EPA (warm water species)
- consider whether TG 202 could be used as a starting point/framework
- **workload: high**
- **ring-test needed**

2. <u>Freshwater vascular plants</u>:

- *Lemna minor, Lemna gibba*
- growth test method
- national standards available (US EPA, ASTM, Swedish standard, AFNOR draft); Swedish method has been put forward to ISO
- work in OECD should be co-ordinated with ISO
- **workload: moderate**
- **ring-test possibly needed**

3. <u>Fish</u>:

- species to be decided
- life cycle test and/or partial life cycle test
- existing US EPA method available for warm marine and warm freshwater species but needs considerable work; however, a life cycle test is preferred

- **workload: very high**
- **ring-test needed**

4. <u>Freshwater microalgae</u>:

- to include more freshwater species in OECD TG 201: blue-green algae, diatoms, a.o. and extend the test duration to 96h
- standards available, including existing TG 201
- workload: moderate
- ring-test possibly needed

5. <u>Marine microalgae</u>:

- *Skeletonema* sp., Gymnodium, *Phaeodactylum*
- standards available, including ISO, ASTM, PARCOM guideline.
- **workload: moderate**
- **ring-test possibly needed**

6. <u>Bacteria</u>:

- nitrification test with microbial consortia from aerobic waste water treatment plants
- method requested in EU scheme for chemicals because of concerns related to waste water treatment plants
- ISO standard available but needs updating
- the work should be co-ordinated with ISO
- **workload: medium**
- **ring-test needed**

7. <u>Marine molluscs</u>:

- *Crassostrea*, *Mytilus*, a.o.
- two test methods proposed: acute (48h, early life stages) and 96h, shell deposition. The shell deposition test is often recommended in USA.
- ASTM, TSCA and FIFRA methods available
- **workload: high for both methods**
- **ring-testing needed**

2. Other methods given relatively high priority for OECD Test Guideline development (List 2)

Taxonomic groups included in List 2 were discussed in general without going into details regarding the potential test species and endpoints. The main evaluation criteria as used in the DRP were considered: practicability (excl. cost), validity, usefulness, level of standardisation and cost.

Although cost is difficult to assess for a taxonomic group as such, it was anticipated that testing methods with organisms having relatively short life cycles are expected to be less costly to perform than tests with organisms having longer life cycles. The cost is, however, very much dependent on the endpoint studied.

The priorities agreed (high, medium, low) are outlined in the following table:

Taxonomic group	**Practicability (excl. cost)**	**Validity**	**Usefulness**	**Level of standardisation**	**Cost**
Protozoa	H	H	H	M	$
Echinodermata	M	L	H	M	$$
Amphibia	H	?	H	L[1]	$$
Aschelminthes (Rotifera)	H	H	H	M[2]	$
Macroalgae	H	M	H	M	$($)

[1] *ASTM method being developed presently (guidance document available).*

[2] *ASTM method available. The method is considered to have significant potential for international standardisation.*

H: High, M: Medium, L: Low, $: relatively low costs, $$: reasonable costs and within the costs for performance of existing OECD TGs (including costs for culturing/collection and maintenance).

Amphibia tests may also include endpoints for genotoxicity. As in the real world this taxonomic group is in danger (and laboratory culturing is possible), toxicity data may be needed to set environmental quality criteria that specifically take into account the protection of amphibians. Some experts noted, however, that animal welfare considerations may prevent acceptance of further testing methods with vertebrates.

It was agreed that higher priority should be given to the development of life cycle studies and/or to methods covering chronic endpoints rather than acute endpoints.

Other aspects than the five main evaluation issues above, such as 1) needs for regulatory purposes and 2) ecological aspects, were not included in the above prioritising. However, a regulatory need might be higher for testing representatives from classes of species not presently represented in the existing OECD Test Guidelines (and not in the high priority list (List 1) above). This aspect might indicate a higher priority for the development of test guidelines for protozoans, echinoderms and aschelminthes than for macroalgae (the class being represented by microalgae) and Amphibians (the class (vertebrate) being represented by fish).

Thus the general outcome of the discussion on priority for the List 2 methods was that highest priority for OECD Test Guideline development should be given to: protozoans, echinoderms, and aschelminthes (Rotifera).

ANNEX 5

REPORT OF BREAKOUT GROUP 2 ON BENTHIC TOXICITY TESTING METHODS

Chairman: Peter Matthiessen, MAFF, UK
Rapporteurs: Herbert Köpp, BBA, Germany, Peter McCahon, GIFAP, Rhône-Poulenc, France, and Carla Roghair, RIVM, the Netherlands

Starting points for the discussions

To provide information to participants in the Working Group Meeting and to facilitate the discussion, several documents had been prepared and served as starting points:

- the Detailed Review Paper (DRP) on "Aquatic Testing Methods for Pesticides and Industrial Chemicals",
- a compilation of OECD Member countries' comments on the DRP,
- a compilation of Working Group members' comments on the DRP,
- the recommendations of the OECD Pesticide Task Force on Ecotoxicity Test Guidelines Priority-setting.

Objectives

Based on the information made available through the various documents, as well as on the experience of the participants, the Group should discuss and identify :

- sediment toxicity tests for guideline development through OECD;
- priorities for the development;
- starting points (i.e. available protocols/methods) or approaches for drafting proposals, including an estimation of the workload.

Certain issues were specifically excluded from evaluation by the Working Group Meeting. Since the OECD Test Guidelines Programme focuses on test methods for industrial chemicals and pesticides, neither bioassays with contaminated sediments nor testing methods for effluent were discussed.

Further, following the definition used in the DRP, only whole-sediment toxicity tests with infaunal species were considered by the Benthic Group since their exposure to sediment-associated chemicals is more realistic in whole-sediment designs and are of more concern than the exposure of epibenthic species which are predominantly exposed via the overlying pelagic water phase.

Both Breakout Groups were also to limit their discussions to tests on the single species level, because these are far more frequently required than multispecies or mesocosm tests and thus more in need of international harmonization. Finally, it was not the task of the meeting to discuss or recommend hazard or risk assessment strategies.

Principles for the choice of benthic test methods

1. Need

Several activities, both international and national, concerning the development of sediment toxicity tests are already under way or have been ongoing for some time (e.g. by the Paris Commission, the Commission of the European Union, the ASTM, the German BBA, among others). Recently adopted risk assessment schemes for regulatory purposes (e.g. Directive 91/414/EEC and its Annexes for pesticides registration in the EU, or the EPA FIFRA regulations 40 CFR part 158) require sediment toxicity tests in certain cases. Thus, the Benthic Group concluded that there is an urgent need to combine the scattered activities and to start working towards internationally harmonized guidelines. The OECD, its Member countries having signed the Agreement on Mutual Acceptance of Data, should take the lead.

2. Criteria

The authors of the DRP used a set of scoring criteria to develop recommendations on the need and the priority of tests for guideline development. These scoring criteria can be grouped into four categories: practical feasibility, scientific validity, usefulness of prognosis, level of standardisation.

These criteria were found to be valid and useful for reviewing the available test methods and protocols. The Group focused its discussion on two criteria:

(1) Usefulness of prognosis/predictiveness in the scoring system comprises geographical representativeness, ecological representativeness, extrapolation of endpoints, general sensitivity, and relevance of exposure route and test conditions. It is therefore the most complex of the four categories used.

Referring to this broad criterion, the finally recommended tests were selected based on ecological representativeness (benthic species have a high score), endpoints (survival, growth and reproduction have a high score), and relevance of exposure route (testing in whole sediment system has a high score).

However, the use of benthic test results in a quantitative risk assessment was considered more difficult as compared to pelagic tests (e.g. preciseness of PECs: consequently PEC/PNEC ratios).

Additionally, existing risk assessment schemes need to be developed further with regard to benthic tests and their use (e.g. more clearly defined trigger values/properties, use of the results).

Thus, the Group proposed to rename this criterion *as representativeness with regard to ecology and exposure.*

(2) The level of standardisation was not considered an important criterion for driving benthic test recommendations, due to the lack of widely accepted or even standardised methods.

3. Taxonomic groups

Benthic communities can be characterised as predominantly detritivorous, while pelagic systems are based more on primary consumers. The taxonomic groups which dominate pelagic testing (planktonic algae, crustacea and fish) are of less importance in benthic environments as compared to other groups like annelids, echinoderms, insect larvae and crustacea with different feeding mechanisms (shredders, detritivores, grazers). Further, some of these groups are not represented in pelagic communities. Some priority should therefore ideally be given to taxonomic groups which are not represented in pelagic testing, although this is not of overriding importance.

For implementation in risk assessment schemes, sediment testing should be kept at a minimum by using application factors, data from pelagic species, and careful consideration of data on fate and bioavailability of the compound concerned in sediments.

Like the participants in the WOSTA meeting, the Group emphasised that lack of knowledge of bioavailability is the driving factor for toxicity to sediment organisms, not a theoretically higher overall sensitivity of benthic as compared to pelagic organisms. Toxicity endpoints for several taxonomic groups are already measured in pelagic studies. There is consequently little need for sediment toxicity tests covering more than a few taxa.

Finally, when selecting certain species or taxa for guideline development, care should be taken that these organisms are available in as many OECD countries as possible (preferably by culture).

4. Temperature

The climatic conditions and the range of water temperature vary widely among and even within OECD Member countries. Hence, test guidelines which cover both cold water and warm water species could be more easily adopted into regulatory practice on a broader scale.

Due to slower test chemical degradation, cold water tests can be considered as the worst case (as compared to warm water tests) and should therefore be preferred. However, this may not be possible for all relevant species due to their ecological requirements (e.g. reproduction might only occur in warm water; limited geographical distribution, etc.). In conclusion, this was considered a practical issue (which can be dealt with on the individual species/test guideline level) rather than a matter of principle.

5. Salinity

In contrast with the issue of temperature range, the Group concluded that guidelines applicable to both freshwater and marine species will probably be more difficult to develop than specific guidelines. Two main practical problems were identified:

- Exposure scenarios for marine and freshwater species may differ widely, thus possibly requiring different test designs in some cases;

- Marine taxa often have more or different life stages.

Thus, separate marine and freshwater guidelines were regarded as more feasible. However, this was also considered more a practical issue than a matter of principle.

6. Endpoints/duration of tests

Benthic organisms are very likely to be exposed to chemicals which are persistent, have a tendency to adsorb to particulate matter, and partition into the sediment (WOSTA report). Due to this exposure scenario, the Group regarded it as appropriate that subchronic/chronic endpoints are preferred in Test Guideline development.

There might be practical difficulties in including both acute and chronic endpoints in a single study design, but at least subchronic and chronic endpoints should be combined. In this context, it should be noted that long-term exposures are not necessarily disproportionately more time-consuming for experimenters than short tests.

Further, the Group took note that the definitions used in the DRP for short/long term exposure should read as follows: short-term < 10 days; long-term ≥ 10 days.

7. Pesticides versus industrial chemicals

Existing OECD aquatic toxicity Test Guidelines were primarily developed for industrial chemicals, but have been widely applied to pesticides. The Group agreed that in benthic testing there is no need for strictly separate guidelines for both groups of chemicals. However, because of different exposure scenarios of pesticides versus industrial chemicals, there may be specific requirements as to how the test chemical should be applied to the system. Such differences in the test design could be readily addressed and incorporated into a single guideline by providing recommendations on the specific variations of the design.

Selection of taxonomic groups

Note: It should be recognised that the choice of tests for further development was based on information available at the time of the Meeting. Given the developmental status of sediment toxicity tests in general, further tests may become available at a later stage. The same applies to the exclusion of epibenthic species from consideration at this stage.

For a detailed discussion, the Group referred to section 7.2 of the DRP. Due to a general lack of methods ready for standardisation [Group 1 (1a and 1b), primary recommendation; for the definitions, see DRP], Groups 2a and 2b (secondary recommendation) were also considered. The following table provides an overview of the main methods discussed and the Benthic Group' s conclusions on the methods listed under Groups 1-2b in the DRP.

Table I: Freshwater environment

Taxonomic group	Test organism	Acute (ac) subchr. (sc) chronic (c)	Items of discussion	Conclusion of the Benthic Group
DRP recommendation: Group 1				
No Test Guideline reference available				
DRP recommendation: Group 2a				
Insecta	*Hexagenia limbata*	ac	- no culture method available - long life cycle (univoltine) - geographical limitations - availability is a problem	not selected
	Chironomidae	ac/sc	- broad international experience with response of all larval stages to many chemicals - three ring-tests completed (EU, BBA, US EPA) - ease of culture - acute and subchronic test methods available - species should be infaunal and feeding mainly on particles - check: practical differences (e.g. temperature range, time to emergence) between species and resistance of certain strains	selected
	Polypedilum sp.		- see Chironomidae - add as possible species to guideline	
Annelids	*Tubifex tubifex*	ac/c	- not yet present in guidelines as a taxonomic group	
	Lumbriculus variegatus	ac/c	- more widely used for bioaccumulation studies - seem to be tolerant - sc/c endpoints for future consideration	selected
Crustacea (amphipods)	*Hyatella azteca*	ac/sc	- shorter life cycle than *Gammarus* - culture easier, but international availability needs confirmation - temp. = 23°C; cooler temperature possible? - work is ongoing but probably large workload	selected
DRP recommendation: Group 2b				
Annelids	*Stylodrilus heringianus*	ac/c	- not yet present in guidelines as a taxonomic group	
	Limnodrilus hoffmeisteri	ac/c	- more widely used for bioaccumulation studies - seem to be tolerant - sc/c endpoints for future consideration	selected
Crustacea	*Gammarus* sp.	ac	- epibenthic species - cannibalistic - long life cycle	not selected
Bacteria		c	- no requirements for bacterial studies - information already available from degradation studies water/sediment biodegradation studies)	not selected

Table II: Marine environment

Taxonomic group	Test organism	Acute (ac) Subchr. (sc) Chronic (c)	Items of discussion	Conclusion of the Benthic Group
DRP recommendation: Group 1				
Annelida	*Arenicola marina*	ac	- culture not possible - PARCOM successfully ring-tested - geographical distribution? - acute endpoints simple - sublethal endpoint (casting rate) has potential - breeding cycle makes chronic endpoint non-viable	selected
Crustacea	*Bathyporeia sarsi* *Corophium volutator*	ac ac	- can be held in laboratory, but not yet be cultured - subchronic endpoint under development - two ring-tests completed (EU, PARCOM) - can be held in lab, culture method under development - control mortality unacceptable at certain periods of year - cannibalistic under some circumstances?	selected
Echinodermata	*Echinocardium cordatum*	ac	- sensitive, reproducible - culturing not possible - difficult to collect - large size leads to practical testing problems	not selected
DRP recommendation: Group 2a/2b				
Crustacea	*Penaeid* sp.	ac	- if a true infaunal species is available and capable of culture	possibly selected
Crustacea (amphipods)	*Rhepoxynius abronius* *Eohaustorius estuarius* *Grandidierella* *Corophium* sp.	ac ac ac ac	- culture problems - promising for acute effects	
	Ampelisca abdita *Leptocheirus pinguins*	ac/sc ac/sc	- *Ampelisca/Leptocheirus* may be promising for subchronic test	selected (all amphipods)
Annelida	*Nereis virens*	ac	- cannibalism in culture - not typically benthic (adults carnivorous and pelagic) - not sensitive	not selected
	Neanthes arenaceodentata	ac/sc	- sc growth endpoint may not be sensitive - potential for future?	possibly selected

Recommended priorities for the OECD Test Guidelines Programme

I. Freshwater

Among the methods/taxa discussed, the **Chironomidae** were considered to be most appropriate in terms of Test Guideline development. There is broad international experience with several species and all larval stages, exposed to a broad range of chemicals. Suitable acute and subchronic endpoints have been identified. Several test designs are available, at least two of them having been successfully ring-tested. So far, insects are not represented in aquatic toxicity Test Guidelines of OECD. Hence, the Chironomidae were given **highest priority**.

The **freshwater annelids** also represent a new taxonomic group. Considerable experience for some species with culturing and with bioaccumulation studies (long-term study design) as well as toxicity tests (mainly bioassays) is available. However, further research with regard to culturing, species selection, subchronic endpoints, etc. is still required. The Group assigned the annelids the **second-highest priority** among the freshwater organisms.

As to the crustacean ***Hyalella azteca***, more research and development work (e.g. availability in culture, possibility to test at lower temperatures) is required before a guideline can be drafted. Although the workload may be less than for the annelids, the priority for *Hyalella* was decided to be lower because there are already standard test guidelines for pelagic crustacea.

II. Marine

The marine **amphipod** crustacea were considered as the most promising of the saltwater taxa for test guideline development. There is widespread experience with several species, mainly with acute endpoints, but work on subchronic endpoints is progressing. At least for *Corophium*, a method for culturing in the laboratory is in an advanced stage of development. Thus the marine amphipods were given **priority over the marine annelids** where the Group identified a similar need for further research and development work, as with the freshwater annelids.

III. Overall priorities for guideline development

In conclusion, the Benthic Group assigned overall priorities as follows:

1. Chironomidae – ac/sc
2. marine amphipods – ac/sc
3. freshwater and marine annelids – ac/sc
4. *Hyalella* – ac/sc

Estimated workload

For each of the four taxa recommended for guideline development, there is a high estimated work load for the standardisation of sediments, dosing, and possibly analysis (see below). As to the selected priorities, the specific workload was estimated by the Group as follows:

1. Chironomidae:

Less workload than with any other taxonomic groups on culturing, endpoints, protocols. At least three international ring-tests (EU, BBA, US EPA) successfully performed.

2. Marine amphipods:

High estimated work load for culture method, subchronic endpoints, sediment selection, etc. Some guidelines available as starting points. At least two international ring-tests (EU, PARCOM) successfully performed.

3. Annelids:

High estimated work load including some research and development, e.g. culturing, protocol development, endpoints.

4. *Hyalella azteca*:

Less work than for annelids, but crustacea are already represented as a taxonomic group in testing, hence the lower priority. Some research and development required.

Specific issues concerned with the drafting of recommended Test Guidelines

1. Chironomidae:

Acute and subchronic endpoints can be addressed in the same TG, but these should be measured in separate tests. When recommending certain species and strains, consideration should be given to their possible resistance to some chemicals.

2. Amphipods:

Acute and subchronic endpoints should be combined in the same TG, but these should be measured in separate tests.

3. Annelids:

Freshwater and marine species should not be combined in a single Test Guideline.

General issues for the drafting of benthic Test Guidelines

The Benthic Group identified several issues as applicable to benthic toxicity tests in general. Hence, they should be addressed for all Test Guidelines in a similar manner. Research devoted to such general issues should therefore be co-ordinated as much as possible and its results be made available to all specialist drafting groups.

- Guidance on when to perform benthic toxicity tests

There is agreement that persistence of a substance and its partitioning into the sediment indicate the possible need for a benthic toxicity test. However, some participants in the Group stated that rapid degradability does not necessarily preclude presence in sediment (e.g. continuous discharge of a labile substance may also result in long-term exposure of benthic organisms). Further, the role of a PEC/PNEC ratio, that is of toxicity data from pelagic species as a criterion for sediment testing, is still under discussion. Given this uncertain picture, the Test Guidelines to be developed should refer to guidance on the types of substances and/or exposure scenarios which may trigger benthic toxicity tests. Such guidance may also be drafted and published separately under the OECD Hazard Assessment Programme. When drafting this guidance, consideration should be given to existing hazard/risk assessment schemes as well as to the results of the previous workshops listed below:

- WOSTA "Workshop on Sediment Toxicity Assessment", Renesse, the Netherlands, 8-10 November 1993 (guidance document available from SETAC; eds. Hill, I.R., Matthiessen, P. and Heimbach, F.);

- SETAC workshop on "Sediments in Risk Assessment", Monterey, USA, April 1995 (report in draft).

- Sediment selection/standardisation

One of the main objectives of a benthic toxicity test is the assessment of bioavailability of the substance concerned. Hence, those factors which are known to influence the bioavailability of a chemical need to be standardised as much as possible during the Test Guideline development. The type of sediment, particularly properties such as organic carbon content and particle size distribution, has a crucial influence on the results of a sediment toxicity test. Both natural and artificial sediments could be used. However, any type chosen must be suitable for the test organisms and be able to sustain them with as little additional feeding as possible (see below).

As to **natural sediments**, it was noted that two broad types of sediment have been selected for fate studies (results of the OECD Workshop on "Selection of Soils/Sediments", Belgirate, Italy, 18-20 January 1995; Final Report, June 1995). Conducting both fate and toxicity studies with the same sediment could prove very valuable in interpreting the results of both tests (e.g. bioavailable portion in the toxicity test; biological activity in the fate study). If these tests were conducted in parallel or in a co-ordinated manner, analytical sampling in the toxicity test might be reduced. However, for any natural sediments chosen, clear specification of the range of important parameters (e.g. OC content, particle size distribution) is needed. Each range should then be as narrow as possible to reduce variability. Specifications close to the standard sediments chosen for fate testing would be valuable.

Artificial sediments have been used successfully (e.g. in the BBA ring-test) and are recommended by the Group for further standardisation and use in routine testing, where possible. However, they are still the subject of several research programmes.

- Dosing of the test substance

Most methods available use spiked sediment. Spiked sediment (spiking of sediment/water slurries) was considered to be advantageous in many cases (e.g. by ensuring homogenous concentrations). However, it may be necessary in higher-tier tests or for specific risk assessment procedures to use other means of application in order to simulate certain exposure scenarios (e.g. overspray or run-off of agricultural pesticides). Thus, the Group concluded that the drafting groups should consider a robust design that allows combination of several exposure scenarios into the same TG.

- Equilibration time

When spiking a sediment, the time to equilibration for different substances can vary from hours to weeks. Existing guidance on equilibration time ranges from 24 hours to 14 days (ASTM, WOSTA report), with the additional requirement that >80 per cent equilibrium should be reached (WOSTA report); thus, to establish this, chemical analysis has to be performed.

Clearly, a short equilibration time before the start of a test will produce worst-case results due to high bioavailability. A long equilibration time, however, might introduce additional variables in terms of biodegradability of the substance, the microbial activity of the sediment, and the food supply available to the test organisms. Hence, the Group concluded that a **short standard equilibration period** is to be preferred. Some focused research on this issue might be needed.

- Static vs. semi-static design

Considering that substances studied in sediment toxicity testing are likely to be persistent, the Group agreed that static test designs should primarily be developed. However, for labile chemicals which might persist in sediments due to continuous discharges, a semi-static test might be considered (degradability does not exclude presence in sediment). If possible, draft guidelines should offer both options.

- Chemical analysis

The analytical verification of test substance concentrations in sediments is often difficult and expensive to perform. For example, it may only be possible with radio-labelled material. Further, it needs to be decided whether particular phases should be analysed or whether the concentration in whole sediment is the most relevant measurement. From fate studies in sediment systems, unextractable residues are known to cause difficulties with obtaining a chemical mass balance and hence with the subsequent interpretation. If extraction methods for measuring the adsorbed phase are used, they may need standardisation to allow for comparability of results. Depending on available methodology (detection limit) and on the toxicity of the substance concerned, the size of test vessels and the amount of sediment used may need to be enlarged to allow for sufficient samples for analyses. However, other requirements like GLP also need to be considered.

Existing guidance on this issue is not very detailed (WOSTA report). For routine testing, clarification is needed and should be developed. Careful consideration should then be given to existing risk assessment procedures (e.g. which route of exposure in the sediment is addressed; which PECs are used, etc.). This evaluation should also consider the guideline for terrestrial soil toxicity tests (e.g. with earthworms) which uses a similar matrix to sediment and does not require analytical verification of test concentrations.

- Feeding

Additional feeding may influence the bioavailability of the test substance. It should and can be avoided in acute tests. For long-term tests, feeding is usually required to sustain the test organisms. The extent to which feeding is necessary depends on the type, microbial activity, and organic carbon content of the test sediment and should therefore be evaluated at the time of the sediment selection. Clear guidance as to the type and amount of additional food needs to be given for each species in a test guideline.

ANNEX 6

PRIORITY-SETTING FOR THE REVISION AND DEVELOPMENT OF TEST GUIDELINES APPLICABLE TO PESTICIDES

Recommendations of the Ecotoxicology Task Force for Aquatic Test Guidelines and Current Status in OECD Work

Introduction

At the 20th Joint Meeting of the OECD's Chemicals Group and Management Committee, which met in combined session with the Pesticide Forum on 26 May 1993, Member countries agreed that a three-year Pesticide Activity be established from January 1994. This Activity would follow the more limited pesticide project initiated in May 1992. The Pesticide Activity and a Proposed Workplan were approved by the Environment Policy Committee in June 1993.

It was agreed that work on Test Guidelines within the Pesticide Activity should be fully integrated into the Test Guidelines Programme. However, bearing in mind the large amount of Guideline work that had been identified by an earlier survey (see document PEST/WG/DOC2), it was recognised that priorities needed to be set before work could begin. It was therefore agreed that the first step in this process would be to establish three Task Forces, one for environmental fate and physical chemical properties, one for ecotoxicology, and one for human health and occupational exposure. The Task Forces would comprise pesticide experts (from government and industry) who would propose priorities for consideration by the National Co-ordinators at their October meeting, with a view to incorporation of the pesticide work into the Test Guidelines Programme. The recommendations of the Task Forces would also be reported to the next meeting of the Pesticide Forum in February 1994.

In July 1993, the Secretariat informed the Test Guideline National Co-ordinators of this process and of the composition of the Task Forces proposed (see letter ENV/EHS/HK/mc/93.198).

Priority-setting by the pesticide Task Forces

Individual Task Force members

From information provided by the Secretariat (see letter ENV/EHS/HK/mc/93.198 and enclosures), individual Task Force members were requested to:

(a) indicate priorities (i.e. High, Medium, Low) for the revision/development of the Guidelines recommended in the Test Guideline Survey or needed to satisfy the data requirements listed in the data requirements survey. In setting priorities, Task Force members were asked to take into consideration:

- the frequency with which any particular data requirement is requested for pesticide registration;

- the revision/developments proposed by Member countries in the Test Guidelines survey, the number of countries supporting work on particular studies, and whether they satisfy the data requirements listed in the survey

- the recommendations of the Pesticide Working Group;

- the availability of existing methods from other fora.

(b) indicate the amount of work that would be involved (i.e. small, moderate, large);

(c) indicate where single Guidelines were needed to cover certain data requirements and where endpoints could be combined into a single Guideline. For example, could the assessment of volatility of pesticides from soil, leaf surfaces and water be addressed within a single Guideline, or are separate Guidelines needed?

Task Force meetings

The Environmental Fate and Physical Chemical Properties, Ecotoxicology and Human Health and Exposure Task Forces met in Paris on 8, 10 and 16 September, respectively. The objective of these meetings was to review the individual responses of the members and to reach consensus on Guidelines considered to be of the highest priority. For these Guidelines, the Task Forces were also asked to reach consensus on how much work was required and on the way to proceed, i.e. any of the approaches as indicated in Monograph No. 76 on Test Guideline development (lead country, workshop, etc.), or a specific approach, more or less unique to the pesticides work (working group).

Recommendations of the Task Force on Ecotoxicology

The following table summarises the recommendations of the Ecotoxicity Task Force for work on aquatic Test Guidelines (1993) and indicates the current status of the work (May 1995).

RECOMMENDATIONS OF THE TASK FORCE ON ECOTOXICOLOGY

TEST AREA	SURVEY OF DATA REQUIREMENTS	TEST GUIDELINE SURVEY	Proposed priority	Anticipated workload	Task Force Comments (1993)	Current status in OECD work (May 1995)
	Requirements listed in survey	**Recommendations for revision/ development of Guidelines**				
AQUATIC TESTS						
Bacteria, algae and plants	Bacteria (aquatic) comment only	1. Develop test for bacteria (e.g. Microtox)	0	–	Not required for pesticides.	No activity
	Activated sludge, respiration inhibition test	2. Revise Guideline 209	0	–	Not applicable for pesticide registration, although may be useful for production purposes.	No activity
	Algae, growth inhibition	3. (a) Revise Guideline 201	HIGH *	Small	Deal with (a) and (b) in one Guideline by including more species. Refer to ASTM, US EPA methods.[1]	No activity
		3. (b) Develop algal test battery				
	Aquatic plant growth	4. Develop Guideline for aquatic macrophytes (e.g. *Lemna*)	HIGH	Small	US requirement for herbicides.[1]	No activity
Sediment invertebrates	Chronic toxicity and reproduction with benthic organisms like *Chironomus*	5. Develop tests for sediment invertebrates (acute and chronic)	HIGH	Large	Await outcome of DRP and other work. (NB. DRP available early 1994).	No activity
Freshwater planktonic invertebrates	Daphnia acute immobilisation test	6. No recommendation – Guideline 202, Part I OK?	HIGH *	Small	Revise in tandem with reproduction test. Increase test duration to 48 hours.[1]	No activity
	Daphnia life cycle	7. Revise Guideline 202, Part II	HIGH *	Large	Continue ongoing work. Final ring-test due to start January 1994.[1]	TG being revised in light of results of Final Ring-test (1994)
		8. Develop test for Ceriodaphnia reproduction	0	–	No requirement.	No activity
Marine/estuarine invertebrates (e.g. crustacean, oyster)	no requirement	9. Develop tests for marine/estuarine invertebrates	LOW	–	US requirement only.	No activity

TEST AREA	SURVEY OF DATA REQUIREMENTS	TEST GUIDELINE SURVEY	Proposed priority	Anticipated workload	Task Force Comments (1993)	Current status in OECD work (May 1995)
	Requirements listed in survey	**Recommendations for revision/ development of Guidelines**				
AQUATIC TESTS						
Fish	Fish acute toxicity LC50, freshwater: warm-water species	10. Revise Guideline 203	LOW	–	Newly revised (July 1992).[1]	No activity
	Fish acute toxicity LC50, freshwater: cold-water species					
	Marine or estuarine fish acute toxicity LC50/EC50					
	Chronic toxicity to fish or fish early life stage	11. Revise Guideline 204	LOW	–	Low priority given as new Guideline for fish growth test under development.[1]	No activity
		12. Develop chronic test (e.g. growth test)	HIGH *	Small/ Moderate	Development in progress.[1]	Fish Growth test: Nov. 1994 draft TG under revision in light of the last commenting round
		13. Develop fish embryo test – 7 days	0	–	No requirement.	No activity
		14. No recommendation for ELS test – TG 210 OK?	LOW	–	Guideline 210 considered OK.[1]	Egg and Sac-fry test: Nov. 1994 draft TG under revision in light of the last commenting round
	Fish reproduction and growth rate	15. Develop test for reproduction/life cycle	MEDIUM	–	Some discussion of whether a full life cycle test was needed, or whether this could be covered by a number of tests (test battery) looking at different, critical, stages. Await recommendations from DRP and research elsewhere (e.g. Germany).[1]	No activity
	Fish life cycle					
Amphibians	No requirement	16. Develop test(s) for amphibians	MEDIUM	–		No activity

TEST AREA	SURVEY OF DATA REQUIREMENTS	TEST GUIDELINE SURVEY	Proposed priority	Anticipated workload	Task Force Comments (1993)	Current status in OECD work (May 1995)
	Requirements listed in survey	**Recommendations for revision/ development of Guidelines**				
AQUATIC TESTS						
Field/mesocosm studies	Aquatic field testing (including non-target plants)	17. Develop test/guidance for field/mesocosm	HIGH/ MEDIUM	Moderate	SETAC (N. America and Europe) group working on guidance document for pond studies. Have SETAC document submitted to Secretariat via National Co-ordinator(s). Circulate to Member countries for comment in late '93. Decision not to actively pursue. Discussed possibility of extending document to include laboratory studies done under field conditions.	Work to be initiated
Other	Particular studies with fish and other aquatic organisms	18. Develop salinity challenge test	0	–		No activity

Other recommendations

1. **The Task Force identified the need for the development of guidance on how to test pesticide formulations, especially those with multiple active ingredients, in aquatic tests. A status report of current practice would be useful. It was felt that the issue of testing formulations could be included in the scope of the existing work, by the UK, on poorly soluble substances. The document should address, *inter alia*, analytical requirements, the need for testing above water solubility, and how data derived from such tests should be used/interpreted.**

ANNEX 7

COMPILATION OF MEMBER COUNTRIES' COMMENTS ON THE DETAILED REVIEW PAPER, INCLUDING RESPONSES TO THE QUESTIONNAIRE

The Detailed Review Paper (DRP) on "Aquatic Testing Methods for Pesticides and Industrial Chemicals" and a questionnaire relating to the DRP recommendations were circulated for comments to OECD Member countries in April 1995, with a deadline for response of June 1995.

Comments on the DRP, including responses to the Questionnaire, were received from twelve countries: Australia (Aus), Austria (Aut), Canada (Can), Finland (Fin), France (Fra), Germany (Ger), Italy (Ita), Japan (Jap), Norway (Nor), Sweden (Swe), United Kingdom (UK), United States (USA) and the European Commission (EC).

I: Pelagic test methods recommended by Member countries for OECD Guidelines Development

Water: w = warm water; c = cold water AC = acute test; SC = subchronic test; C = chronic test; ST = short-term test; LT = long-term test Footnotes [(a)(b)(c)] are explained at the end of the set of tables.

DRP recommendations for OECD TG development and proposed action						**Member countries' responses and comments**			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [(a)]**	**Proposed action:**		**Indicate priority [(b)] (i.e. High, Medium, Low)**		**Anticipated workload [(c)] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Freshwater environment									
Kormophyta, higher plant (*Lemna*)	w,c	SC/ST	1	One TG	US EPA, ASTM, S-EPA	High	Aus, Aut, Can, Fin, Fra, Ger, Ita, Jap, Nor, Swe, UK, USA, EC	Lar	USA
						Med		Mod	Aut, Fin, Fra (AFNOR ring-test)
						Low		Sm	Aul, Can, Ger, Ita, Nor, Swe, UK, EC
Arthropoda, Insecta (*Aedes*, *Acroneuria*, etc.)	w,c	AC/ST	2	One TG	Literature	High	UK	Lar	Can, Fra, Nor, UK, USA
						Med	Ita, Nor	Mod	Aus, Ita
						Low	Aus, Can, Fra, Jap, UK, USA, EC	Sm	Ger
Arthropoda, Crustacea (*Neomysis*, *Ceriodaphnia*)	w	AC/ST	2	Revise 202	US EPA, ASTM	High	Aus, Can, Fra (include *Ceriodaphnia*), Swe, UK, USA (only for *D. magna*, 48h-test)	Lar	USA
						Med	Ger, Ita, Jap (for *Cerio*), Nor	Mod	Can, Fra, Ita, Nor
						Low	EC, Jap (for *Neomycis*)	Sm	Aul, Ger, Swe, UK
(*Gammarus*)	c	AC/ST	2	One TG	Literature	High	Swe, UK	Lar	Can, Fin, Fra, UK, USA
						Med	Can, Fin, Ita, Jap, Nor, USA, EC	Mod	Aul, Ger, Ita, Nor, Swe, EC
						Low	Aul, Ger, Fra	Sm	

DRP recommendations for OECD TG development and proposed action						Member countries' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Chordata, Amphibia (*Xenopus, Rana*, etc.)	w,c w,c	AC/ST SC/LT	2 2	One TG	Literature	High	Fra (for SC/LT, genotox. as endpoint), Swe	Lar	Aul, Can, Fra, Nor, UK, USA
						Med	Aus, Ger, Ita, Jap, UK, USA	Mod	Ger, Ita, Swe
						Low	Can, Nor, EC	Sm	
Aschelminthes, Rotifera (*Brac Highonus*)	w w	AC/ST C/ST	2 2	One TG	Literature	High	Can, Swe	Lar	UK (for C/ST), Swe (for C/ST), USA
						Med	Aus, Ger, Fra, Ita, Jap, Nor, UK (for AC/ST)	Mod	Can, Fra, Ita, UK (for AC/ST)
						Low	UK (for C/ST), USA, EC(low sensitivity)	Sm	Aus, Ger, Swe (for AC/ST)
Protozoa (*Tetrahymena*)	w,c	C/ST	2	One TG	Literature	High	Fra, Jap	Lar	Can, Nor, UK, USA
						Med	Ger, Ita, Nor	Mod	Aus, Fra, Ger, Ita
						Low	Aus, Can, UK, USA, EC	Sm	
Fish (*Brachydanio, Pimephales*)	w	C/LT	1	One TG	US EPA, Literature	High	Aus, Aut, Can, Fin (include cold species), Fra, Ita, Jap, USA (+ *Salmonid*, cold species), EC	Lar	Aut, Fin, Nor, USA
						Med	Ger, Nor, Swe	Mod	Aus, Can, Fra, Ger, Ita, EC
						Low	UK	Sm	UK
Bacteria (*Pseudomonas*)	w,c	C/ST	2	One TG	ISO draft	High	Aut, Fra, Ger, Ita, Jap, EC	Lar	USA
						Med	Can, Swe	Mod	Aus, Can, UK
						Low	Aus, Nor, UK, USA	Sm	Aut, Fra, Ger, Ita, Nor, EC

DRP recommendations for OECD TG development and proposed action						Member countries' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Marine environment									
Algae, micro (*Skeletonema*, *Phaeodactylum*)	w,c	C/ST	1	Revise 201	ISO draft	High	Aus, Can, Fra, Ger, Ita, Jap (for *Skelet.*) Swe, UK, USA	Lar	USA
						Med	EC	Mod	Can, Ger
						Low		Sm	Aus, Fra, Ita, Nor, Swe, UK
Algae, macro (*Champia*, *Gracilaria*)	w,c w	SC/ST AC/ST	2 2	One TG	US EPA, Literature	High	Ger, Swe	Lar	Aus, UK, USA
						Med	Aus, Can, USA	Mod	Can, Ita, Nor, Swe
						Low	Fra, Jap, UK, EC	Sm	Ger
Higher plant, Kormophyta (*Zostea*)	c	SC/LT	2	One TG	Literature	High	Swe	Lar	Aus, Can, Ger, Nor, UK, USA
						Med	Aus, USA	Mod	Ita, Swe
						Low	Can, Fra, Ger, Jap, UK, EC (one TG for freshw. and marine)	Sm	
Arthropoda, Crustacea (various species)	c c c	AC/ST SC/ST C/LT	1 1 1	One TG	Literature	High	Fra, Ger, Nor, Swe, UK(for AC/ST and C/LT)	Lar	Can, Swe (for SC/ST, C/LT), USA
						Med	Aus, Can, Ita, Jap, USA, EC	Mod	Aus, Fra, Ger, Ita, Nor
						Low		Sm	Swe (for AC/ST), UK (for AC/ST), EC
	w w w	AC/ST SC/LT C/LT	2 2 2	One TG	US EPA, ASTM	High	Ita, Jap (*Mysidopsis*), Swe, UK (for SC/ST and C/LT), USA (*Mysids*)	Lar	USA
						Med	Aus, Can, Nor, BIAC (one TG for c and w species)	Mod	Aus, Can, Ita, Nor, UK (for SC/LT and C/LT)
						Low	Fra, Nor, UK	Sm	Swe, UK (for AC/ST)

DRP recommendations for OECD TG development and proposed action						Member countries' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Echinodermata, sea urchins (various species)	w,c	SC/ST	2	One TG	Can EPA	High	Aus, Can	Lar	UK, USA
						Med	Ita, Jap	Mod	Can, Ita, Nor
						Low	Nor, UK, USA, EC	Sm	Aus
Mollusca (*Crassostrea*, *Mytilus*, etc.)	c	SC/ST	2	One TG	ASTM	High	Aus, Fin, Fra, Ita, Swe, UK, USA	Lar	Can, Fin, USA
						Med	Nor	Mod	Nor
						Low	Can, EC	Sm	Swe
Aschelminthes, rotifera (*Brachionus*)	w	AC/ST	2	One TG: same as freshwater?	ASTM	High	Can, Swe (one TG for freshw. and marine)	Lar	USA
						Med	Aus, Fra (one TG for freshw. and marine), Ita, Jap	Mod	Aus, Can, Ita, Nor, UK
						Low	Nor, UK, USA, EC	Sm	Swe
Protozoa (*Tetrahymena*)	w,c	C/ST	2	Same TG as freshwater	Literature	High	Fra (one TG for freshw. and marine), Jap	Lar	Can, UK, USA
						Med	Ger, Ita, UK	Mod	Aus, Ger, Ita
						Low	Aus, Can, UK, USA, EC	Sm	Nor
Fish (*Cyprinodon*)	w	C/LT	2	Same TG as freshwater	US EPA	High	Fra (one TG for freshw. and marine), Ita, Swe, UK, USA, EC (one TG for freshw. and marine)	Lar	Aus, Can, No, USA
						Med	Aus, nor	Mod	Swe
						Low	Can, Ger,	Sm	Ger, UK

II: Benthic test methods recommended by Member countries for OECD Guidelines Development

Water: w = warm water; c = cold water AC = acute test; SC = subchronic test; C = chronic test; ST = short-term test; LT = long-term test Footnotes [(a)(b)(c)] are explained at the end of the set of tables.

DRP recommendations for OECD TG development and proposed action						**Member countries' responses and comments**			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [(a)]**	**Proposed action:**		**Indicate priority [(b)] (i.e. High, Medium, Low)**		**Anticipated workload [(c)] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Freshwater environment									
Annelida (various species)	w,c	AC/LT SC/LT C/LT	2 2 2	One TG?	US EPA, Literature	High	Aus, Aut, Ger, Ita (for SC/LT), Jap, EC	Lar	Aut, Nor, USA, EC
						Med	UK (SC/LT), USA	Mod	Aus, Can, Fra, Ger, UK (for AC/LT, SC/LT and C/LT)
						Low	Can, Fra (low sensitivity), Nor	Sm	Ita
Crustacea (various species)	w,c	AC/LT SC/LT	2 2	One TG	ASTM, US EPA, Literature	High	Aus, Aut, Ger, Ita (SC/LT), USA	Lar	Aut, Can, Nor, USA
						Med	Can, Fra, Jap, Nor, UK	Mod	Aus, Fra, Ger
						Low	EC	Sm	Ita, UK
Insecta (*Hexagenia*, *Chironomus*)	w,c	AC/LT SC/LT	2 2	One TG	ASTM, US EPA, Literature	High	Can, Fra, Ger, Ita (for *Chiro.*, SC/LT), Jap (for *Chiro.*) (for SC/LT), USA, EC	Lar	Aus, Nor, USA
						Med	Aus, Nor	Mod	Can, Fra, UK (for SC/LT)
						Low		Sm	Ger (EU ring-test, BBA method), Ita, UK (for AC/LT), EC
Aschelminthes, Nematode (*Panagrellus*)	w	SC	2	One TG	Literature	High	Ger	Lar	Aus, Can, Nor, USA
						Med	Jap	Mod	
						Low	Aus, Can, Fra, Ita, Nor, UK, USA	Sm	

DRP recommendations for OECD TG development and proposed action						Member countries' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Bacteria (Sediment bacteria)	c	C/ST	2	One TG?	Literature	High	Fin, Nor	Lar	Can, Fin, Fra, Ger, UK, USA
						Med	Aus, Fra, Ger, Jap	Mod	Aus, Nor
						Low	Can, Ita, UK, USA, EC	Sm	
Methanogenic bacteria (*Archaebacteria*)	w	C/LT	2	One TG	Literature	High	Fin, Nor	Lar	Aus, Can, Fra, Ger, UK, USA
						Med	Fra, Ger, Jap, UK	Mod	Fin, Nor
						Low	Aus, Can, Ita, USA, EC	Sm	
Marine environment									
Annelida (*Arenicola*)	c	AC/LT	1	One TG	PARCOM	High	Ger, Jap	Lar	USA
						Med	Ita, UK, USA, EC	Mod	Aus, Ita
						Low	Aus, Fra (low sensitivity)	Sm	Ger, UK, EC
(*Nereis, Neanthes*)	w	AC/LT SC/LT	2	One TG: same as cold water?	US EPA, Literature	High	Aus, Ger, Jap, UK, EC (same TG as freshwater)	Lar	UK (SC/LT), USA
						Med	Ita, USA	Mod	Aus, UK(AC/LT), Ita
						Low	Aus, Fra, Ita, Nor	Sm	Ger
Mollusca (*Macoma*)	c	AC/LT	2	One TG	Literature	High	Ger, UK, USA	Lar	Aus, Nor, USA
						Med	Jap	Mod	Ita, UK
						Low	Aus, Fra, Ita, Nor, EC	Sm	Ger

DRP recommendations for OECD TG development and proposed action						Member countries' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Crustacea (various species)	c	AC/LT	1	One TG	PARCOM	High	Fra (*Rhepoxyhius, Mysidopsis*) Ger, Ita (*Corophium*), Nor	Lar	USA
						Med	USA	Mod	Aus, Fra, Nor, UK
						Low	Aus	Sm	Ger
	w,c	AC/LT SC/LT C/LT	2	One TG?	US EPA, ASTM, Literature	High	Aus, Ger, Ita (*Corophium*), USA (species issue)	Lar	USA
						Med		Mod	Aus, UK
						Low		Sm	Ger
Echinoid (*Echinocardium*) (*Lytechinus*)	c	AC/LT	1	One TG	PARCOM	High	Fra	Lar	USA
						Med	Ita, Jap, UK	Mod	Aus, Fra, Nor, UK
						Low	Aus, Nor, USA	Sm	
	c	SC/LT C/LT	2	One TG?	Literature	High		Lar	UK, USA
						Med	Fra	Mod	Aus, Fra
						Low	Aus, Ita, UK, USA	Sm	

Footnotes:

(a) **Groups:** Groups 1 (primary recommendation) and 2 (secondary recommendation): see the DRP for definitions.

(b) **Priority:**

High The method covers endpoints which are included in existing data requirements of a considerable number of countries and at an early level of testing/assessment, and/or covers relevant taxonomic groups which are not yet represented. The method is scientifically justified.

Medium The method covers endpoints which are included in existing data requirements of a limited number of countries, or only required at a higher level of testing assessment, and/or covers relevant taxonomic groups which are not sufficiently represented. The method is scientifically justified.

Low The method is scientifically justified. However, there are no requirements for data on endpoints, covered by the test.

(c) **Anticipated workload:**

Large The test is or is not available as a defined protocol (from a Member country or international organisation); the test needs to be validated in a ring study with respect to its sensitivity, reproducibility and reliability.

Moderate The test is or is not available as a defined protocol and is well-described. Some validation is still considered necessary.

Small The test is available as a defined protocol and further validation is not considered necessary.

COMMENTS ON THE DRP BY MEMBER COUNTRIES:

(Page numbers refer to the circulated version of the document)

Aus
- Generally agrees with the recommendations (Chapter 7).
- Specifically, strongly supports the development of TGs for algae, vascular plants, invertebrates and vertebrates in benthic, marine and warm/tropical environments.
- There is a considerable need for developing a toxicity guideline for benthic organisms, and some merit in considering chronic or subchronic endpoints.
- In terms of setting the priorities (page 132 of the DRP), consideration should be given not only to whether there is a regulatory requirement, but also to the frequency of its potential or actual use. Other criteria used in setting priorities also require discussion. For instance, developing guidelines "ready for standardisation" should not be at the expense of tests urgently needed in other environmental compartments. Also, "high ecological relevance" may be quite hard to define, or variable from one region to another.
- Strongly supports the development of generic test guidelines (page 132 of the DRP) in which various species appropriate for various compartments or regions may be recommended (including, for example, tropical species).

Aut
- Comments only on the cold pelagic and benthic freshwater environments.
- Applauds the clear definitions of terms used in the evaluation of test methods (acute, subchronic, chronic, cold, warm, short-term pelagic/sediment, long-term pelagic/sediment).
- page 16: Table 2.2a. Include the new test with bone fish (SC/LT), ref.: n.d., Assessment scheme: Blübaum-Gronau and Hoffmann and Spieser and Krebs.

Can
- The DRP is a very thorough and comprehensive compilation of international aquatic testing methods and a very useful document for prioritising activity for the OECD Test Guidelines Program.
- Importance of validation and ring-testing of the test methods.
- Needs to review a model TG outline before commenting on the concept of "framework guidelines" for taxonomic groups.
- Supports the inclusion of acute and subchronic endpoints in the same guideline.

Fin
- DRP has a very pragmatic approach in regard to test evaluation procedures.
- Doesn't agree that none of the microorganism tests is recommended in category 1a or 1b. Microbial test should be ranked in high priority.
- Supports the recommendation that the test guidelines should be developed on the basis of taxonomic groups.
- Acute and subchronic/chronic endpoints could be included in the same guideline, but probably difficult.

Fra
- Emphasises the necessity to select sensitive (pelagic as well as benthic) species and not only cover relevant taxonomic groups. Sensitivity cannot be dissociated from the criterion of ecological relevance.
- Stresses the need of subchronic and chronic tests. Testing more species under the same conditions in acute tests will not provide valuable additional information to refine the assessment of chemicals.

- Acute test should include other endpoints than lethality (e.g. survival of early life stage, morphological effects...).
- Genotoxicity in aquatic species also needs to be addressed (e.g. SC test with amphibia/chordata).
- page 76: *Lemna* test is being standardised in France (AFNOR ring-test).

Ger
- The DRP is a very good comprehensive document of the numerous pelagic and benthic aquatic toxicity tests published all over the world.
- Test with chironomids should not have greater priority than test with nematodes.
- Rotifers and protozoa should have the same (medium) priority.
- Test with Hydra (attenuata) should be given high priority in freshwater (indicator of teratogenic effects).
- Test with fungi should also be considered for aquatic compartment.
- Marine microbial test should also be considered.
- There is a lack of benthic tests for acute and chronic toxicity, as compared with pelagic tests.
- For benthic tests it is proposed to focus on freshwater sediment rather than marine sediments (marine sediments are difficult to obtain and higher contamination levels are found in freshwater sediments).
- The differentiation between pelagic and benthic is welcome, but it is not necessary to differentiate between warm and cold water species.
- Useful to include acute and subchronic/chronic endpoints in the same guideline (as for *Daphnia*).

Ita
- The DRP was put together in a masterful and exhaustive way.
- In some cases, priorities (H, M, L) given according to the definitions may lead to "scores" contrasting with the primary recommendations given in the DRP (e.g. the chronic fish test should be ranked as Medium priority, as it is required only at a refinement level of assessment).
- Due to the importance of covering the benthic environment, acute test methods would be considered as high priority, but subchronic endpoints should be considered as well.
- In pelagic/freshwater test methods, insects need to be represented as well as rotifers (*Brachionus*).
- Agrees with recommendation of "framework guidelines", whenever possible.
- Agrees that acute and subchronic endpoints be included in the same guideline.

Jap
- Need for TGs for benthic compartment and much work may be necessary.
- Need for OECD TGs for marine organisms.
- The proposed species in the DRP seem rather arbitrarily selected. The selection criteria which should be applied are not followed (sensitivity, easily colonised, available at any time, representative of a trophic level, etc.).
- Need to select additional candidates for carnivorous species.
- Proposes a list of the organisms (cultured in laboratory) used in ecotoxicity tests.
- It would be "ideal" to develop TG for taxonomic groups, but very difficult to apply (different test conditions for the various species).
- Concern about the workload for developing new TGs (including ring-test).
- Stresses the need for development of TGs for agricultural pesticides.

Nor
- The selection of methods is based on the requirements in existing schemes. The fact that these schemes are based on existing guidelines may make this approach too conservative. It should be considered whether test guidelines for single species should cover all possible combinations of taxa.
- The criteria for selecting the methods to be included are sound, but it appears that these have not always been followed (e.g. the tests with microalgae include several methods which are almost identical and use one species, *Selenastrum*, while other tests including alternative species were not reviewed)
- Implementation of marine toxicity tests should be given high priority. Ring-tests are already carried out; therefore the workload is small.
- For freshwater algae tests, the list of green algae species is too limited. Several test methods, other than the OECD, ISO and EC tests, use alternative species. In algae test, include representatives of several taxonomic groups of microalgae, e.g. a cyanobacteria.
- Recommendations of the DRP should be considered as recommendations on categories of tests to be developed.

Swe
- Mainly agrees with the DRP recommendations on priorities for Test Guideline development.
- Supports the concept of filling taxonomic gaps in the food web instead of concentrating on certain species.
- Supports the concept that acute and subchronic/chronic endpoints should be included in the same guideline.
- Gives higher priority to amphibian test methods (very sensitive organism).
- Add *Ceramium* to the list of macroalgae in cold water.

UK
- Reluctant to support the approach outlined in the DRP, to develop guidelines based on taxonomic groups rather than single species, because of the difficulties in interpreting and comparing the results of tests from less well known species, and in validating such tests.

USA
- The document seems to be acceptable.
- Don't support the approach that guidelines be developed on the basis of taxonomic groups rather than single species. This is possible, but not particularly desirable because guidelines that involve appreciably different methods are sometimes challenging to incorporate into a single guideline. Concern for incorporating large taxonomic groups into a single guideline. This would still increase complexity of guidelines.
- Don't support the approach that acute, subchronic and chronic toxicity tests be included in the same guideline. Increases complexity of guidelines.
- The issue of whether or not to combine closely related warm and cold water species into a single guideline presents much less of a problem.
- The document is an ambitious effort to categorise what is now available and what is needed for the future in aquatic testing. There is some concern that guidelines developed for each of the environments and compartments listed become requirements for testing of an organism in every possible environment. Prefers to test the typical suite of organisms and, if needed, test additional specific organisms when the toxicity profile and release scenario dictate their need (comments from the American Crop Protection Association).
- page 21: In algae, *Anabaena* and *Navicula* should be added. Appropriate guidelines are available from the US EPA.
  page 25: Bacteria. Microtox should not be classified as a growth study (biochemical endpoint: luminescence).

page 46: Table 4.1. The cold water range overlaps with the warm temperature range. Also, most warm water studies are conducted at 20-25°C.
page 105: paras 5 and 7. The German BBA has a protocol for a 30-day *Chironomus riparius* study. *C. tentans* is not considered as sensitive as *C. riparius* in Europe.
page 132: The Guidelines should also refer to locations of the particular environmental compartments from a global perspective.
page 134: *Daphnia magna* (OECD TG 202) should be listed here. 20°C is the preferred temperature (see page 141, para 2, as well).
page 138:
* A guideline for the microalgae *Skeletonema* is available from the US EPA and PARCOM
* A PARCOM guideline exists for the crustacean *Acartia tonsa* (also page 141, para 3).
page 141: The sensitivity of protozoa is questioned. The indicator species approach should always be used with reference to sensitive species.

EC
- Test with rotifers not recommended due to low sensitivity.
- Chronic test with fish required for new and existing chemicals.
- Bacteria test requested in base-set for notification of new substances.
- In benthic test methods, test with nematode could be recommended only as alternative to annelida.
- In benthic test methods, test with annelid (*Arenicola*) could be recommended as an alternative to crustacea (*Corophium*).

ANNEX 8

SUMMARY OF MEMBER COUNTRIES' RESPONSES TO THE QUESTIONNAIRE RELATING TO THE DRP RECOMMENDATIONS FOR TEST GUIDELINE DEVELOPMENT

Pelagic test methods – freshwater environment

Test method	DRP recomm.: Group	Member countries' responses: Priority
Kormophyta - SC *Lemna*	1	OK. **High**
Insecta - AC *Aedes, Acroneuria*	2	Low
Crustacea *Ceriodaphnia* - AC [revise 202] *Gammarus* - AC	 2 2	 High to Medium Medium to Low
Amphibia *Xenopus, Rana*	2	Medium to Low
Rotifera Aschelminthes, *Brachionus* - AC, C	2	Medium to Low
Protozoa - C *Tetrahymena*	2	Medium to Low
Fish - C *Brachydanio*	1	OK. **High** Include marine sp. in same TG
Bacteria - C *Pseudomonas*	2	Mixed opinions: High, Med, Low

Pelagic test methods – marine environment

Test method	DRP recomm.: Group	Member countries' responses: Priority
Algae - C [rev. 201] *Skeletonema, Phaeodactylum*	1	OK. **High**
Algae, macro - SC and AC *Champia, Gracilaria*	2	Medium to Low
Kormophyta - SC *Zostea*	2	Medium to Low
Crustacea - AC, SC, C *cold species* *warm species*	 1 2	 High to Medium One TG for c and w species
Echinodermata - SC *various species*	2	Medium to Low
Mollusca - SC *Crassostrea, Mytilus*	2	High to Medium
Rotifera - AC *Brachionus*	2	Medium to Low
Protozoa - C *Tetrahyma*	2	Medium to Low
Fish - C *Cyprinodon*	2	High to Medium One TG freshw. and marine sp.

Benthic test methods – freshwater environment

Test method	**DRP recomm.: Group**	**Member countries' responses: Priority**
Annelida - AC, SC, C *various species*	2	Mixed opinions: High, Med, Low
Crustacea - AC, SC *various species*	2	High to Medium
Insecta - AC, SC *Chironomus*	2	**High**
Aschelminthes, Nematode - SC *Panagrellus*	2	Low
Bacteria - C *Sediment bacteria* *Methanogenic bacteria*	2 2	Mixed opinions: High, Med, Low

Benthic test methods – marine environment

Test method	**DRP recomm.: Group**	**Member countries' responses: Priority**
Annelida *Arenicola* - AC *Nereis*, *Neanthes* -AC, SC	1 2	Mixed opinions: High, Med, Low Mixed opinions: High, Med, Low
Mollusca - AC *Macoma*	2	Divided opinions between High and Low
Crustacea - AC, SC, C *Various species*	1 and 2	**High** Different species proposed
Echinoid *Echinocardium* - AC *Lytechinus* - SC, C	1 2	Medium to Low

Note: AC = acute; SC =subchronic; C = chronic

ANNEX 9

COMPILATION OF WORKING GROUP MEMBERS' COMMENTS ON THE DETAILED REVIEW PAPER, INCLUDING RESPONSES TO THE QUESTIONNAIRE

The Detailed Review Paper (DRP) on Aquatic Testing Methods for Pesticides and Industrial Chemicals, and a questionnaire relating to the DRP recommendations, were circulated for comments to members of the Working Group on Aquatic Toxicity Testing in May 1995, with a deadline for response of 14 June 1995.

Individual comments and/or responses to the Questionnaire were received from: Shigehisa Hatakeyama (SH), Herbert Köpp (HK), Rachel Fleming (RF), Guido Persoone (GP), Paule Vasseur (PV), Ursula Klaschka (UK), Peter McCahon (PMC), Richard Stephenson (RS), Torsten Källqvist (TK), Juan Gonzales-Valero (JGV), Maria Tarpkea (MT), Carla Roghair (CR), Keith Solomon (KS).

I: Pelagic test methods recommended by Member countries for OECD Guidelines Development

Water: w = warm water; c = cold water Type of test: AC = acute; SC = subchronic; C = chronic; ST = short-term; LT = long-term Footnotes [(a)(b)(c)] are explained at the end of the set of tables.

DRP recommendations for OECD TG development and proposed action						**WG members' responses and comments**			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [(a)]**	**Proposed action:**		**Indicate priority [(b)] (i.e. High, Medium, Low)**		**Anticipated workload [(c)] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Freshwater environment									
Kormophyta, higher plant (*Lemna*)	w,c	SC/ST	1	One TG	US EPA, ASTM, S EPA	High	TK, JGV, RF, HK, PV, UK, SH, MT, CR	Lar	
						Med	RS, KS (for *Lemna*, need other sp.)	Mod	JGV, HK, PV
						Low		Sm	TK, RF, MT, CR
Arthropoda, Insecta (*Aedes*, *Acroneuria*, etc.)	w,c	AC/ST	2	One TG	Literature	High		Lar	TK, JGV, PV, SH (for *Acroneuria*)
						Med	TK, HK, SH, CR, KS	Mod	HK,SH (for *Aedes*), CR
						Low	JGV, PV, RS	Sm	
Arthropoda, Crustacea (*Neomysis*, *Ceriodaphnia*)	w	AC/ST	2	Revise 202	US EPA, ASTM	High	PV(for *Ceriodaphnia*, one TG), SH, MT	Lar	
						Med	TK, KS	Mod	TK, JGV, PV
						Low	JGV, HK, UK, RS, CR	Sm	HK, SH (for *Ceriodaphnia*), MT
(*Gammarus*)	c	AC/ST	2	One TG	Literature	High	SH, MT	Lar	JGV, PV
						Med	TK, JGV, RF, HK, PV, KS	Mod	TK, RF, HK, MT, CR
						Low	UK, RS	Sm	

DRP recommendations for OECD TG development and proposed action						WG members' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Chordata, Amphibia (*Xenopus, Rana*, etc.)	w,c w,c	AC/ST SC/LT	2 2	One TG	Literature	High	RF, HK, PV(for SC/LT), MT	Lar	TK, JGV, RF, HK, CR
						Med	JGV, UK, SH, CR	Mod	PV, MT
						Low	TK, RS, KS	Sm	
Aschelminthes, rotifer (*Brachionus*)	w w	AC/ST C/ST	2 2	One TG	Literature	High	MT	Lar	HK, PV, MT (for C)
						Med	TK, RF, SH, CR, KS	Mod	RF, SH, KS
						Low	HK, PV, RS	Sm	MT(for AC)
Protozoa (*Tetrahymena*)	w,c	C/ST	2	One TG	Literature	High	PV, UK, CR	Lar	HK
						Med	TK, SH, KS	Mod	PV, SH, KS
						Low	HK, RS	Sm	TK
Fish (*Brachydanio, Pimephales*)	w	C/LT	1	One TG	US EPA, Literature	High	HK, PV, SH	Lar	TK, RS
						Med	TK, RF, RS, MT, KS (triggers to be assessed)	Mod	HK, PV, SH, KS
						Low	CR	Sm	RF
Bacteria (*Pseudomonas*)	w,c	C/ST	2	One TG	ISO draft	High	UK, MT, CR	Lar	PV
						Med	SH, MT	Mod	HK, SH, KS
						Low	TK, HK, PV, RS, KS (triggers to be assessed)	Sm	TK, MT

DRP recommendations for OECD TG development and proposed action						WG members' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Marine environment									
Algae, micro (*Skeletonema*, *Phaeodactylum*)	w,c	C/ST	1	Revise 201	ISO draft	High	TK, RF, PV, MT, CR	Lar	
						Med	RS, SH, KS	Mod	RS, SH, MT
						Low		Sm	TK, RF, PV, KS
Algae, macro (*Champia*, *Gracilaria*)	w,c w	SC/ST AC/ST	2 2	One TG	US EPA, Literature	High	TK, MT	Lar	PV
						Med	SH, CR	Mod	TK, SH, MT
						Low	PV, RS, KS	Sm	KS
Higher plant, Kormophyta (*Zostea*)	c	SC/LT	2	One TG	Literature	High	MT	Lar	TK, RF, PV
						Med	RF, SH, CR, KS	Mod	SH, KS
						Low	TK, PV, RS	Sm	
Arthropoda, Crustacea (various species)	c c c	AC/ST SC/ST C/LT	1 1 1	One TG	Literature	High	TK, RF (for *Tisbe*, *Acartia*), PV, SH, MT, CR	Lar	JGV, RS, MT(for SC and C)
						Med	JGV, RS, KS	Mod	TK, RF, PV, SH
						Low	UK	Sm	MT (for AC), KS
	w w w	AC/ST SC/LT C/LT	2 2 2	One TG	US EPA, ASTM	High	SH	Lar	PV, SH
						Med	TK, RF, PV, CR, KS	Mod	TK, JGV, RF, KS
						Low	JGV (one crustacea test with sensitive specie), RS	Sm	

DRP recommendations for OECD TG development and proposed action						WG members' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Echinodermata, sea urchins (various species)	w,c	SC/ST	2	One TG	Can EPA	High	RF, SH, CR	Lar	PV
						Med		Mod	TK, JGV, KS
						Low	TK, JGV, PV, RS	Sm	RF, SH, MT
Mollusca (*Crassostrea*, *Mytilus*, etc.)	c	SC/ST	2	One TG	ASTM	High	MT	Lar	PV
						Med	TK, JGV (only one TG for pelagic or benthic mollusca), RF, SH, KS	Mod	TK, SH, KS
						Low	PV, RS	Sm	JGV, RF, MT
Aschelminthes, rotifera (*Brachionus*)	w	AC/ST	2	One TG: same as freshwater?	ASTM	High	MT(one TG for freshw. and marine)	Lar	PV
						Med	SH	Mod	TK, JGV, SH, KS
						Low	TK, JGV, PV, RS, KS	Sm	
Protozoa (*Tetrahymena*)	w,c	C/ST	2	Same TG as freshwater	Literature	High	PV, UK	Lar	JGV
						Med	TK, SH	Mod	PV
						Low	JGV, RS, KS	Sm	TK, SH, KS
Fish (*Cyprinodon*)	w	C/LT	2	Same TG as freshwater	US EPA	High	PV, MT	Lar	TK
						Med	TK, JGV, SH, KS	Mod	JGV, PV, SH, MT, KS
						Low	RS	Sm	

II: Benthic test methods recommended for OECD Guidelines Development

Water: w = warm water; c = cold water Type of test: AC=acute; SC= subchronic; C= chonic; ST = short-term; LT = long-term Footnotes [(a)(b)(c)] are explained at the end of the set of tables.

DRP recommendations for OECD TG development and proposed action						**Working Group members' responses and comments**			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [(a)]**	**Proposed action:**		**Indicate priority [(b)] (i.e. High, Medium, Low)**		**Anticipated workload [(c)] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Freshwater environment									
Annelida (various species)	w,c	AC/LT SC/LT C/LT	2 2 2	One TG?	US EPA, Literature	High	RF, RS (one TG for benthic freshw. invertebrates), CR	Large	TK, HK, PV, RS, SH
						Med	HK (for C/LT), SH, KS	Mod	JGV, RF (for C/LT), KS
						Low	TK, JGV, HK (for AC/ST), PV (low sensitivity)	Small	
Crustacea (various species)	w,c	AC/LT SC/LT	2 2	One TG	ASTM, US EPA, Literature	High	SH	Large	TK, PV
						Med	TK, RF, HK (for SC/LT), PV (for *Gammarus*), CR, KS	Mod	JGV, RF, HK, KS
						Low	JGV, HK (for AC/LT), UK	Small	SH
Insecta (*Hexagenia*, *Chironomus*)	w,c	AC/LT SC/LT	2 2	One TG	ASTM, US EPA, Literature	High	JGV, RF, HK (for SC/LT), UK, SH, CR, KS	Large	TK, PV
						Med	TK	Mod	JGV, RF (for SC/LT), SH (for *Chironomus*), KS
						Low	HF (for AC/LT), PV	Small	RF (for AC/LT), HK
Aschelminthes, Nematode (*Panagrellus*)	w (*Panagrellus*)	SC	2 (*Panagrellus*)	One TG	Literature	High	UK	Large	TK, JGV, HK, PV, SH
						Med	RF, HK, CR	Mod	RF, KS
						Low	TK, JGV, PV, KS	Small	

DRP recommendations for OECD TG development and proposed action						Working Group Members' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Bacteria (Sediment bacteria)	c	C/ST	2	One TG?	Literature	High	TK, RF, UK	Large	RF,HK,PV
						Med	PV, SH, CR	Mod	TK, SH
						Low	JGV, HK, RS, KS	Small	
Methanogenic Bacteria (*Archaebacteria*)	w	C/LT	2	One TG	Literature	High	TK	Large	HK, PV, SH
						Med	RF, PV, CR	Mod	TK, RF
						Low	JGV, HK, RS, SH, KS	Small	
Marine environment									
Annelida (*Arenicola*)	c	AC/LT	1	One TG	PARCOM	High	JGV, RF, RS (one TG for benthic marine invertebrates), CR	Large	
						Med	UK, SH, KS	Mod	PV, SH
						Low	PV (low sensitivity)	Small	RF
(*Nereis, Neanthes*)	w	AC/LT SC/LT	2	One TG: same as cold water?	US EPA, Literature	High	RF, SH, CR	Large	PV
						Med	KS	Mod	SH
						Low	JGV (one annelid test with sensitive specie), PV (low sensitivity)	Small	RF
Mollusca (*Macoma*)	c	AC/LT	2	One TG	Literature	High	CR	Large	TK, PV
						Med	JGV (only one TG for pelagic or benthic mollusca), RF, UK, SH, KS	Mod	RF, SH
						Low	TK, PV (low sensitivity as AC/LT)	Small	

DRP recommendations for OECD TG development and proposed action						Working Group members' responses and comments			
Test organism: Taxonomic group (species)	**Water**	**Type of test**	**Group [a]**	**Proposed action:**		**Indicate priority [b] (i.e. High, Medium, Low)**		**Anticipated workload [c] (i.e. Large, Moderate, Small)**	
				number of TGs	**documents to be used as basis**				
Crustacea (various species)	c	AC/LT	1	One TG	PARCOM	High	TK, JGV (one TG), RF, PV (for *Rhepoxynius*, *Mysidopsis*), SH, CR, KS	Large	
						Med		Mod	TK, JGV, PV, SH
						Low		Small	RF
	w,c	AC/LT SC/LT C/LT	2	One TG?	US EPA, ASTM, Literature	High	JGV(same TG for representative species of crustacea), RF, SH, CR, KS	Large	SH
						Med		Mod	JGV, RF (for SC and C)
						Low		Small	RF (for AC/LT)
Echinoid (*Echinocardium*)	c	AC/LT	1	One TG	PARCOM	High	RF, PV, CR	Large	RF
						Med	JGV, SH	Mod	TK, JGV, PV, SH
						Low	TK, KS	Small	
(*Lytechinus*)	c	SC/LT C/LT	2	One TG?	Literature	High		Large	JGV
						Med	PV, SH, CR	Mod	PV
						Low	JGV, KS	Small	

Footnotes:

(a) **Groups:** Groups 1 (primary recommendation) and 2 (secondary recommendation): see the DRP for definitions.

(b) **Priority:**

High The method covers endpoints which are included in existing data requirements of a considerable number of countries and at an early level of testing/assessment, and/or covers relevant taxonomic groups which are not yet represented. The method is scientifically justified.

Medium The method covers endpoints which are included in existing data requirements of a limited number of countries, or only required at a higher level of testing assessment, and/or covers relevant taxonomic groups which are not sufficiently represented. The method is scientifically justified.

Low The method is scientifically justified. However, there are no requirements for data on endpoints, covered by the test.

(c) **Anticipated workload:**

Large The test is or is not available as a defined protocol (from a Member country or international organisation); the test needs to be validated in a ring study with respect to its sensitivity, reproducibility and reliability.

Moderate The test is or is not available as a defined protocol and is well-described. Some validation is still considered necessary.

Small The test is available as a defined protocol and further validation is not considered necessary.

COMMENTS ON THE DRP BY MEMBERS OF THE WORKING GROUP:

(Page numbers refer to the circulated version of the document)

PMC
- Major concern about such a large number of study types being proposed for review in the Detailed Review Paper.
- The DRP recommendation that test guidelines be developed on the basis of the need to include taxonomic groups rather than single species should be regarded as a higher tier requirement and not included as a standardised test guideline where the aim is to reduce variability.
- Recommendation that acute and subchronic/chronic endpoints should be included in the same guideline (use of chronic endpoints in short-term studies?) is worth consideration.
- Well validated test methods should be recommended as TG.

RS
- Need of clarification on definitions of acute, subchronic and chronic effects, and short-term and long-term durations of the tests.
- Agrees with DRP recommendations for priority-setting. The TG Programme should only address tests that are in Group 1, i.e. those required in existing or draft international schemes.
- Area requiring most discussion is the need for marine tests.

RF
- Two benthic freshwater test methods (ring-tested for the EU in 1994) were not included or referred to in the DRP. These are two ten-day mortality tests in the amphipod *Corophium volutator* and the midge larvae *Chironomus riparius*. Relating to the priority-setting for TG development, these tests may move from Group 2 to Group 1a or 1b. At present, there is an EC programme to standardise protocols for chronic tests with the same species.
- The approach of taxonomic guidelines may be a good idea for certain applications.
- Supports the approach of including chronic and acute endpoints in the same test guideline.

TK
- Framework guidelines for taxonomic groups rather than for single species, and inclusion of chronic and acute studies in the same guideline, may be useful in some cases.

HK
- Agrees on the principle of taxonomic test guidelines. However, a "framework guideline" needs to be confined to those members of a taxonomic group with similar ecological/habitat requirements.
- Combination of acute and (sub)chronic endpoints in the same guideline makes sense for test designs which include bioavailability (i.e. *Chironomus* tests with sediment). Such tests are typical for higher tiers. For base set testing, the combination of acute and chronic endpoints complicates the test design.

MT
- Agrees with recommendation to include taxonomic groups in TG.
- Preferable to include acute and subchronic/chronic endpoints in the same guideline.

KS
- Need to have a framework within which to assess the value of tests to make recommendation for aquatic toxicity testing. This framework for risk or hazard assessment would indicate how the test was to be used and would ask the question "what do you do with the data?".
- The choice of a chronic or acute test depends on the nature of the compound. Need to address the nature of the stressor in the choice of type of test.

- Doubts there is much inherent difference in the sensitivity of cold and warm water organisms. Same remark concerning sensitivity of saltwater and freshwater organisms.

UK
- DRP gives a very good overview and basis of decision to identify the needs for future test methods.
- Supports nematode testing due to their ecological relevance and easy maintenance in the laboratory.
- No need for a standardised test with *Artemia*.
- Importance of using organisms which can be cultured in the laboratory.
- Differentiation into warm/cold and marine/freshwater is too early at this time.
- Various amendments to the DRP were proposed:
  * page 44 (of the circulated version of the DRP): not clear about choice of the assessment scenarios, and what are the objectives of this grouping. Distinction between lentic and lotic is reasonable for pesticides. Distinction warm/cold seems justified only for fish. Differentiation in pelagic and benthic is reasonable for invertebrates.
  * page 105 (circulated version): for a long-term test with *Chironomus riparius*, a BBA method was validated in an international ring-test in 1994,
  * also page 105: BBA does not request a test with *Chironomus tentans*.
  * page 132 (circulated version): the approach by taxonomic group might be too general and not as precise as in guidelines for species.
  * page 133 (circulated version): the inclusion of acute and chronic tests in one guideline should be avoided. The guideline would be too large and therefore uncomfortable.

SH
- Most test organisms listed in tables in Chapter 7 are not described in Japan. However, it is considered that most toxicity tests cited in the tables may be conducted using similar organisms in Japan, although scientific names are different.
- Freshwater shrimp *Paratya compressa* improvisa is an excellent test organism. Recommended for testing of pesticides.

GP
- Congratulates the authors for the tremendous amount of work in collection and analysis of documents related to the subject and for the attempts to "distillate" recommendations for selection of test methods and species.
- Many of the proposed methods are highly complicated and costly. Relating to the current preoccupation with looking for simple and low-cost test methods, particular attention should be given to "alternative" microbiotests.
- Many comments on the DRP (page numbers refer to the circulated version):
  * page 5: section "Refinements of the Report": The statement about the selection of protocols appears questionable.
  * page 6: regrettable that tests based on "physiological, morphological and biochemical methods" are not dealt with in the Report.
  * page 6: distinction between warm and cold is difficult and not applicable.
  * page 21: *Mycrocystis* is microalgae.
  * page 28: Tables 2.6 and 2.7 do not address the real needs. Take criteria into consideration such as difficulties of performance of the tests, their cost (comparing the usefulness of new microbiotests versus the "conventional" existing tests).
  * page 31: Methods collected. The basis for selection is not clear.

* page 49: Practical feasibility of the test method. Stresses the utmost importance of this criterion. Reference to existing OECD Test Guidelines as point of comparison for the selection of tests can be questioned, since several of the OECD toxicity TGs are complex and costly...
* page 50: Importance of "availability and maintenance of test organisms" in the selection of test methods.
* page 53: "geographical representativeness" should be reconsidered in view of validation exercises. Some species with a relatively narrow geographical distribution can be as predictive as conventional species with broader distribution.
* page 54: Endpoints. The selection of avoidance/behavioural effects as ecologically relevant endpoint is questionable.
* page 56: Scores. The A, B, C scores are highly questionable in many cases.
* Chapters 5 and 6: Tables 5.1 to 5.5 and 6.1 to 6.5. Despite the efforts made by the authors, quite a number of pertinent literature references on particular tests are not included in this review.
* page 131: Recommendations. The selection made by the authors in many cases does not reflect the prerequisites, which are regulatory need, uncomplicated test procedures, inexpensive equipment, easy culturing of organisms, low cost for testing, etc. Several standardised and validated (currently used) and microbiotests are not taken into consideration or are not given first priority.