Development of ecotoxicological test systems to assess contaminated sediments

Joint report no. 1: Acute and (sub)chronic tests with the model compound chlorpyrifos *

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</table>
SUMMARY

The studies described were carried out in the scope of the Netherlands Integrated Soil Program (PCBB) and cover the results on acute and chronic toxicity studies with the water flea *Daphnia magna* and larvae of the midge *Chironomus riparius*.

The studies were part of a joint project of the TNO Institute of Environmental Sciences (IMW-TNO), the Institute for Inland Water Management and Waste Water Treatment (RIZA) and the Netherlands Institute of Public Health and Environmental Protection (RIVM). The aim of the project is to develop and evaluate a set of test systems for polluted sediments. Preferably the test methods must be suitable to assess the toxic effects of both specific chemicals and polluted field sediments.

Choices in the methodology had to be made at the start of the project and the rationales for it are discussed in this report. Items to be mentioned are:

- Test organisms
- Substrate
- Treatment of sediment
- Exposure media
- Dilution water
- Test duration
- Test conditions
- Natural environmental parameters
- Model compounds

Tests carried out were acute and (sub)chronic tests with *D. magna* and *C. riparius*. The acute tests and the chronic tests with daphnias (reproduction tests) were carried out by each of the three institutes. Subchronic tests with larvae of the midge were carried out by two of the institutes involved. Ethyl-chlorpyrifos was used as a model compound.
The treatment of the sediment was a so called worst case approach to mimic processes in
the field as realistic as possible for the situation in the Netherlands. This meant that sedi-
ments were treated in such a way that oxidation may occur and the bioavailability of heavy
metals is enhanced.

In the acute tests various types of exposure media were investigated, viz:

- sediment/water system
- overlying water
- centrifuged overlying water
- pore water, prepared by centrifuging a range of contaminated sediments
- pore water, prepared by dilution of a pore water from a contaminated sediment.

The most important results of the various tests to be mentioned are:

- In the acute tests the preparation method of the exposure media appeared not to
  influence the results. Therefore it was decided to carry out the (sub)chronic tests with
  sediment/water systems and with elutriates.

- Both test organisms appeared to be suitable for sediment toxicity testing. In an acute
test they can be exposed simultaneously in the same test vessel. In all tests D. magna
was less sensitive to chlorpyrifos than the larvae of C. riparius. This can be attributed
to the specific working mechanism of the compound.

- The reference sediment chosen was useful from the point of view of a low control
  mortality. Storage conditions of sediments still need further attention.

- It can be recommended to follow existing guidelines for aquatic toxicity testing as far
  as possible, however, special attention must be given to environmental parameters,
such as ammonia, nitrite, nitrate, oxygen depletion and salinity, which can be limiting
  for the possibilities to carry out the test.

- In the (sub)chronic tests with D. magna and C. riparius low oxygen concentrations
  were measured. The lowest values measured were respectively 0.8 and 1.8 mg.l⁻¹. In
  the reproduction test with D. magna they may have influenced the results.
• The toxic effects observed in the tests with chlorpyrifos can be attributed to the aqueous phase. In the reproduction tests with *D. magna* a somewhat higher toxicity was found by two institutes in the sediment/water system than in the elutriate. However it is most likely that these results are influenced by low oxygen concentrations in the sediment/water system. It is therefore concluded that it is unlikely that contaminated sediment particles contributed to the toxic effects of chlorpyrifos for these organisms.

• The inter laboratory variation was low in all tests.

• The lowest NOEC values in the sediment/water systems, overlying water and pore water were (in mg.kg$^{-1}$ dry weight of sediment):

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Acute Test</th>
<th>(Sub)chronic test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. magna</em></td>
<td>0.18</td>
<td>0.056</td>
</tr>
<tr>
<td><em>C. riparius</em></td>
<td>0.032</td>
<td>0.032</td>
</tr>
</tbody>
</table>

• From the chemical analysis available a sorption coefficient for chlorpyrifos of ca. 17,000 litres per kg could be calculated.
1. **INTRODUCTION**

Sediments in the Netherlands are often contaminated with chemicals, which may lead to harmfull effects on aquatic ecosystems. It is the policy of the government to minimize the risk of this contamination. The government therefore conducts a policy, which on the one side aims at site specific problems, on purpose to diminish effects of local contaminants by remediation. On the other side the policy aims at the prevention of pollution, for which purpose concentrations of specific chemicals are determined that would have no adverse effects in the field situation. These data are used to develop sediment quality criteria for the presence of the chemical in sediments. For both approaches it is necessary to estimate (possible) adverse effects of a sediment or a specific chemical. Ecotoxicological testing is one of the methods to be used for this assessment. As ecotoxicological test methods are not available for sediments a project is carried out in the scope of the Netherlands Integrated Soil Research Program (PCBB) in order to develop a set of test systems to evaluate (polluted) sediments. The purpose of this project is the development and evaluation of biological test systems; the project is carried out jointly by RIZA, RIVM and IMW-TNO.

The main objective of the investigations is to obtain a set of acute and chronic test systems with both sediment/water and pore water as matrices. Due to the nature of sediments specific attention is given to the influences of limiting conditions, which may act as an extra stress during the test, such as ammonia, nitrate/nitrite, phosphate, chloride toxicity and oxygen depletion.

Some characteristics for "ideal" sediment assays are:

- The test organisms should be easy to culture in the laboratory or be available from the field and maintainable in the laboratory.
- The choice of test organisms must be primarily based on the aquatic ecosystems in the Netherlands.
- The tests must be as rapid and inexpensive as possible.
- The tests must be standardizable.
Based on the experience of the three institutes involved acute tests with *Daphnia magna* and *Chironomus riparius* as test animals were chosen to start the studies. Much attention was given to the treatment of the sediments tested and the preparation of the exposure media applied.

This report is the first of a series of joint reports from the three participating institutes and it covers the results on the acute and chronic toxicity studies carried out with the waterflea *D. magna* and the larva of the midge *C. riparius*. Ethyl-chlorpyrifos was used as a model compound. Detailed information of the work carried out is given in the separate reports of each institute (ref. 1, 2, 3, 4, 5, 6 and 7).

IMW-TNO carried out the acute experiments in compliance with the OECD Rules of Good Laboratory Practice and the other studies in accordance with the current OECD Good Laboratory Practice Principles (ref. 8). The latter was also the case for all experiments carried out by RIVM.

A list of definitions for sediment toxicology as used in this report is given in Annex A.
2. RATIONALES FOR CHOICES IN METHODOLOGY

Rationales for species selection and test design have recently been given by Giesy et al. (ref. 9). Some characteristics for “ideal” sediment assays are:

2.1 Test organisms

Both benthic and pelagic organisms are chosen to evaluate the various exposure routes. In the present project the choice of the test organisms was further determined on the basis of the following requirements:

- Representatives for various trophic levels.
- Representing various species and variation in life style.
- Possibility to culture or, in the case of animals collected in the field, to keep in the laboratory.
- Availability of more or less standardized methods in aquatic toxicology.
- Availability of ecological and ecotoxicological know how.
- Suitability for the assessment of the degree of pollution of field sediments and to study sediments spiked with individual chemicals.
- Ecologically relevant to Dutch aquatic ecosystems.
- Organisms considered as suitable test organisms are:
  - Pelagic organisms – bacteria (microtox), algae, crustaceans, fishes.
  - Benthic organisms – insects, worms and protozoa.

In the present study *D. magna* and *C. riparius* were used as test organisms in combined acute tests and separately in (sub)chronic tests. These organisms fulfill the requirements stated above almost completely. Other organisms will be used in another part of the project.

The daphnias used were less than 24 hours old at the start of the tests (conform existing OECD and EC guidelines for acute and chronic toxicity tests). In the studies with chlorpyrifos second instar larvae of *C. riparius* were used. In future experiments it will be investigated whether the egg stage is more sensitive. Test methods then have to be adapted, because it is not possible to track eggs or first instar larvae in sediment.
2.2 Reference substrate

2.2.1 Choices of substrates

Possibilities for the choice of reference substrates are:
1. Sediments collected in the field from more or less clean locations.
2. Substrates artificially made from natural components.
3. Substrates made from substitute materials.

2.2.2 Sediments collected in the field

The idea is that sediments collected in the field with a normal density of certain species fulfill the demands of the habitat for the concerning species. In advance it is not possible to estimate the required properties (such as grain size and food availability) of the sediment and an equivocal characterization is difficult to made. However, an advantage of using a field sediment as a reference is the aimed resemblance with contaminated sediments, to be assessed, which enables a further interpretation of results. Because pollution is everywhere (e.g. PAHs) the sampled sediments will never be completely “clean” in this project. It is not part of the present project to investigate the possible influence on experiments.

Storage and treatment of sediments will influence their properties and the partitioning of (added) substances in the environmental compartments can be disturbed compared with the field situation. Guidance for the handling of sediments is given in the report of the OECD workshop on effects assessment of chemicals in sediment (ref. 10). Fresh reference sediments have to be collected from the field at regular times. At this moment there is not enough insight to give expiry periods for sediments.

In this project a relatively clean sediment from the Schoonrewoerdse Wiel is used. This is a former sedimentation area of the river Rhine.
2.2.3 Substrates artificially made from natural components

Natural components as present in sediments (such as clay minerals, organic carbon, sand) can be joined to an artificially substrate. As for field sediments there is only a limited knowledge what the habitat requirements for the organisms used are. Advantage of the use of artificially substrates is the possibility to store the components as dry material; although the drying itself may influence their binding properties. The influence of grain size and organic carbon content on the development and growth of chironomids was investigated by Hof (ref. 11). In another part of this project some materials as kaoline, sand and mixtures of it were tested for their applicability in chironomid tests. For chironomids the general conclusion from these experiments was that a natural sediment was the most optimal habitat.

2.2.4 Substrates made from substitute materials

For special purposes substitute materials are used incidentally. For example glass beads can be applied in aquatic tests to provide test animals an opportunity to hide themselves to prevent cannibalism; midge larvae can be cultured in unbleached cellulose or agar. In this project however this type of substrates will not be used.

2.3 Treatment of sediment

The method described in this report applies to the sediment of the Schoonrewoerdse Wiel and is based on preliminary experiences of IMW-TNO and RIZA. The following choices have been made:

2.3.1 Seaving

The sediment was seaved through a 500 μm sieve to remove pieces of organic material, stones, shells and naturally occurring organisms. The mesh wide may not be fine enough to remove all organisms present, such as oligochaetes and young midge larvae.
2.3.2 Irradiation

An additional treatment was necessary for the sediment of the Schoonrewoordse Wiel to remove or kill remaining organisms. Irradiation was applied because it is expected that it will not interfere as much with the sediment properties as other methods (freezing, drying). Irradiation is not carried out for sterilizing purposes.

2.3.3 Storage

To minimize alterations during storage the sediment has to be kept cool. Time between sampling and start of the tests was kept as short as possible. For the time being a temperature of 4°C and an expiry time of three months was chosen for the storage of sediments used for spiking.

2.3.4 Oxidation of sediment

A worst case approach in the treatment of sediment was chosen. This means that sediments were treated in such a way that oxidation of reduced material can occur and the bioavailability of heavy metals is enhanced. Oxidation processes in the field are considered as realistic, because they occur on places where animals get in touch with polluted sediments (by processes as bioturbation, bio-irrigation, shipping).

2.3.5 Aeration of the test systems

Aeration may sometimes be necessary, because due to the reducing properties of the sediment, low oxygen concentrations may occur affecting the test results. It is realized that some compounds may be stripped by aeration.
2.4 Dilution water

Dutch Standard water (DSW or DSWL) was used. This is the standard water mostly used in ecotoxicology laboratories in the Netherlands in aquatic toxicology studies for the preparation of test media and for the culture of test animals. It is prepared by addition of several salts to distilled water (DSW) or to groundwater from Linschoten (= DSWL).

2.5 Sediment/water ratio

A sediment/water ratio of 1:4 was applied. Considerations as minimal dilution (worst case approach), minimal water volume for pelagic test animals, realistic exposure (compared to the field situation) and international recommendations are taken into account for this choice (ref. 12).

2.6 Length of the test

2.6.1 Acute tests

The length of the acute tests was 96 hours for both D. magna and C. riparius, conform many acute tests in aquatic toxicology.

2.6.2 (Sub)chronic tests

The reproduction tests with D. magna lasted 21 days conform existing OECD and EC guidelines. For the (sub)chronic tests with C. riparius exposure periods of 10 days and 21 days were chosen for the aqueous media and sediment/water systems respectively. It is known that the larvae can stand a period of 10 days in aqueous media without the presence of a substrate.
2.7 Test- and limiting conditions

Test conditions as temperature, pH, light rithm etc were in conformity with international guidelines for aquatic toxicology. Additionally special attention is given to limiting conditions as oxygen concentrations, nitrite/nitrate, chloride and ammonia.

2.8 Test substance

The present study was carried out with ethyl-chlorpyrifos, one of the model compounds in the Netherlands Integrated Soil Research Program. Other model compounds to be investigated are: 3,4-dichloroaniline, cadmium (as chloride) and pentachlorophenol.
3 MATERIAL AND METHODS

Details on the experiments carried out are given in the separate reports of the three participating institutes (ref. 1, 2, 3, 4, 5, 6 and 7).

3.1 Test organisms and test parameters

The test animals used were the waterflea *D. magna* and the larva of the midge *C. riparius*.

*D. magna* is cultured in the laboratory according to standardized methods. At the start of the test the animals were less than 24 h old. *C. riparius* is also cultured under standard conditions. The test animals used were in the second larval stage.

Test parameters used were immobility (acute test), reproduction and mortality (chronic test) for daphnia and mortality, growth, head width and mentum deformations (the latter two were only determined by TNO) for *C. riparius*. EC50 (mobility daphnia) and LC50 (mortality daphnia and midge larva) were determined. Additionally the condition of the test animals was assessed and NOEC values for mobility, mortality and condition were determined.

3.2 Test substance

3.2.1 Origin and dosing

The test substance used was ethyl-chlorpyrifos (Riedel de Haen, purity 99%). It was dosed from concentrated solutions in tertiairy butyl alcohol (TBA); the concentration of the solvent was 33 µl TBA/l⁻¹ (RIVM) or 100 µl/l⁻¹ (RIZA and IMW-TNO). RIVM used dimethyl sulfoxide (= DMSO) in the “water only” test (chlorpyrifos dissolved in standard water).
3.3 Preparation of exposure media

3.3.1 Reference sediment

The sediment was diluted with standard water (1:1 v/v) and seaved through a 500 μm seave and sterilized with 10 kGy. This pre-treatment of the sediments was carried out jointly by the three institutes involved.

For the preparation of all exposure media sediment suspensions of 40 g dry weight of sediment per litre were prepared (= 1:4 v/v).

3.3.2 Acute tests

In the acute (combined) tests with daphnias and larvae of the midge five (RIZA and IMW-TNO) or four (RIVM) types of exposure media were applied:

- sediment/water system
- overlying water
- centrifuged overlying water
- pore water, prepared by centrifuging a range of contaminated sediments
- pore water, prepared by dilution of a pore water from a contaminated sediment of 1.8 mg/kg (not by RIVM)

Sediment suspensions were prepared by adding chlorpyrifos in a stock solution to a sediment suspension of 40 g dw.sediment L⁻¹ in dilution water. The contaminated suspensions were stirred vigorously for about 20 hours at 17°C or 20°C.

The sediment/water system was prepared by pipetting the suspensions into the glass vials used in the test.

The overlying water was prepared by allowing the sediment suspension to stand for about 24 hours at 20°C and then the overlying water was poured off carefully. By centrifuging the overlying water the centrifuged overlying water was prepared.
Pore water was prepared by shaking the remaining sediment suspension and centrifuging. The supernatant was called pore water. Two types of pore water were prepared, one by centrifuging every sediment separately for the various concentrations and the other by dilution of pore water derived from 1.8 mg.kg\(^{-1}\) contaminated sediment. The concentration of the latter are given in volume percentages of the parent pore water.

A scheme of the preparation method is given in Figure 1. Additionally a “water only” test with chlorpyrifos dissolved in dilution water was carried.

*Figure 1* Preparation of test media for sediment toxicity tests with chlorpyrifos.
The dosed amounts of the test substance in the sediment suspensions were 0, 0 (solvent control), 0.032, 0.056, 0.10, 0.18, 0.32, 0.56 and 1.0 mg per kg dry weight of sediment. RIZA and IMW-TNO tested an extra dosed amount of 1.8 mg per kg dry weight of sediment.

The “water only” test was carried out by RIVM with solutions of 0, 0 (solvent control), and 0.0032-320 μg.l⁻¹ (spaced by a factor 3.2). IMW-TNO tested solutions of 0, 0 (solvent control), and 0.032-3.2 μg.l⁻¹ (factor 1.8). RIZA tested solutions of 0, 0 (solvent control), and 0.10-0.56 μg.l⁻¹ (factor 1.8) for C. riparius and 0, 0 (solvent control) and 0.032-0.32 μg.l⁻¹ (factor 1.8) for Daphnia magna.
3.3.3 Reproduction tests with *D. magna*

The exposure media for the chronic tests were:
- sediment/water system
- elutriate

The way of preparation of the aqueous media did not influence the results of the acute tests with chlorpyrifos it was therefore decided to prepare one type of aqueous medium (an elutriate) by mixing the test substance with a sediment suspension (40 g dry weight per litre of sediment) and to remove the elutriate by centrifugation of this slurry.

The dosed amounts of the test substance in the sediment/water systems and the elutriates were 0, 0 (solvent control), 0.032 (RIZA) or 0.056 (RIVM and IMW-TNO) – 0.56 mg per kg dry weight of sediment (spaced by a factor 1.8).

Additionally a “water only” test with chlorpyrifos dissolved in dilution water was carried out by RIVM with solutions of 0, 0 (solvent control) and 0.0032-1.0 μg.l⁻¹ (spaced by a factor 3.2).

3.3.4 (Sub)chronic tests with *C. riparius*

The exposure media for the (sub)chronic tests chosen on basis of the results of the acute tests were:
- sediment/water system
- elutriate

These media were prepared as described for the reproduction tests with *Daphnia magna* (see 3.3.3).

The dosed amounts of the test substance were 0, 0 (solvent control), 0.018, 0.032, 0.056, 0.10, 0.18 mg per kg dry weight of sediment.
3.4 Test conditions

3.4.1 Acute tests

A summary of the experimental conditions in the (combined) acute tests (excluded the “water only tests) with D. magna and C. riparius is given here below.

Type of test : static
Aeration during the test : no
Dilution water : DSW (RIVM and RIZA) and DSWL (IMW-TNO)
Sediment/water ratio : 1:4 (v/v); corresponding with ca. 40 g dry
weight sediment per litre
Temperature : 20 ± 1°C
Length of the test : 96 hours
Volume test medium : 10 ml
Number of test animals per test vessel : one daphnia and one larva of the midge (combined exposure)
Food : Chlorella pyrenoidosa (10 µl 1.10^9 cells.l^-1)
and/or Tetramin (50 µl of a 2% suspension); no
food was added in the sediment/water test sys-
tems
Observation times : 24, 48, 72 and 96 h for both daphnias and
larvae of the midge, with exception of a single
observation at t = 96 h for the larvae in the
sediment/water system.
Measurements : pH, oxygen concentration (all institutes),
NH_3-NH_4^2, NO_2/NO_3 (RIVM), chlorpyrifos
concentrations (RIZA and IMW-TNO).
### 3.4.2 Chronic tests with *D. magna*

A summary of the experimental conditions in the reproduction tests (excluded the “water only” tests) with *D. magna* is given here below.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>semi-static; replacement weekly by RIZA and IMW-TNO and twice weekly by RIVM (sediment/water systems) and twice weekly (elutriates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeration during the test</td>
<td>no</td>
</tr>
<tr>
<td>Dilution water</td>
<td>DSW (RIVM and RIZA) and DSWL (IMW-TNO)</td>
</tr>
<tr>
<td>Sediment/water ratio</td>
<td>1:4 (v/v); is ca. 40 g dry weight sediment per litre</td>
</tr>
<tr>
<td>Temperature</td>
<td>20 ± 1°C</td>
</tr>
<tr>
<td>Length of the test</td>
<td>21 days</td>
</tr>
<tr>
<td>Volume test medium</td>
<td>50 ml</td>
</tr>
<tr>
<td>Number of test animals per test vessel</td>
<td>one (parent) daphnia</td>
</tr>
<tr>
<td>Food: RIVM</td>
<td><em>C. pyrenoidosa</em>, fed to satiation</td>
</tr>
<tr>
<td>RIZA</td>
<td><em>C. pyrenoidosa</em>, daily</td>
</tr>
<tr>
<td>IMW-TNO</td>
<td><em>C. pyrenoidosa</em> and yeast daily</td>
</tr>
<tr>
<td>Observation times</td>
<td>each replacement time and end of the test (RIVM and IMW-TNO), daily (RIZA)</td>
</tr>
<tr>
<td>Measurements</td>
<td>pH, oxygen concentration (all institutes), NH₃, NO₂/NO₃ (RIVM), chlorpyrifos concentrations (RIZA and IMW-TNO).</td>
</tr>
</tbody>
</table>
3.4.3  (Sub)chronic tests with *C. riparius*

A summary of the experimental conditions in the (sub)chronic tests with *C. riparius* is given here below.

Type of test : semi-static; replacement sediment/water system weekly and elutriate twice per week

Aeration during the test : no

Dilution water : DSW (RIZA) and DSWL (IMW-TNO)

Sediment/water ratio : 1:4 (v/v); corresponds with ca. 40 g dry weight sediment per litre

Temperature : 20 ± 1°C

Length of the test : 10 days (elutriates) or 21 days (sediment/water system)

Volume test medium : 400 ml (IMW-TNO) or 50 ml (RIZA) elutriate; 50 ml sediment/water system

Number of test animals per test vessel : 25; test with triplicate test vessels

Food : twice per week with a Trouvit (RIZA) or a Tetramin (IMW-TNO) suspension; 50 μl in the first week; 100 and 200 μl in the second and third week respectively.

Observation times : each replacement time and at the end of the test

Measurements : pH, oxygen concentration (all institutes), NH₃, NO₂/NO₃ (RIVM), phosphate (RIZA), chlorpyrifos concentrations (RIZA and IMW-TNO).
3.5 Differences in the methods used by the institutes

There were only some minor differences in the methods used by the three institutes.

Culture *D. magna*

RIVM : in 70% DSW (via a carbon filter), prepared from demi water and 30% pond water; at a temperature of 19 ± 1°C.
RIZA : in water from the lake IJssel (sterilized with UV); at a temperature of 20 ± 1°C.
IMW-TNO : in DSWL, prepared from ground water from Linschoten; at a temperature of 20 ± 1°C

Culture *C. riparius*

RIVM : on a substrate of sediment/sand with DSW; at 23 ± 2°C
RIZA : Water of the lake IJssel, diluted with demi water (hardness 90 mg CaCO₃ per litre) and sediment as substrate; at 23 ± 2°C
IMW-TNO : on a substrate of sediment with DSWL; at 20 ± 1°C

Centrifuge speed

RIVM : 3500 rpm = 3000 g for 20 minutes
RIZA : 3000 g for 20 minutes
IMW-TNO : 2000 rpm = 1000 gₘₐₓ for 30 minutes

Light regime and temperature range

RIVM : 12 h light, 12 h dark, temperature 20 ± 1°C
RIZA : 16 h light, 8 h dark, temperature 20 ± 2°C
IMW-TNO : 16 h light, 8 h dark with transition periods of 30 minutes, temperature 20 ± 1°C

Dosing of test substance from the organic solvent (tertiary butyl alcohol = TBA)

RIVM : 33 µl TBA.L⁻¹
RIZA : 100 µl TBA.L⁻¹
IMW-TNO : 100 µl TBA.L⁻¹
Specific for acute (combined) tests

Feeding during the test:

RIVM : did not feed the test animals in the "water only" test; in the other tests Tetramin and *C. pyrenoidosa* were given

RIZA : Tetramin and *C. pyrenoidosa*

IMW-TNO : *C. pyrenoidosa*

Calculations used

RIVM : Spearman-Karber (ref. 13)

RIZA : Kooymann method (ref. 14) and Spearman-Karber (13)

IMW-TNO : Methods of Kooymann (ref. 14) and Van der Hoeven (ref. 15)

Measurement of pH values and oxygen concentrations

RIVM : in additional vessels

RIZA : in additional vessels

IMW-TNO : in the test vessels used in the experiments

Specific for reproduction tests with *D. magna*

Feeding during the test:

RIVM : *C. pyrenoidosa*

RIZA : *C. pyrenoidosa*

IMW-TNO : *C. pyrenoidosa* and yeast

Calculations and statistics

RIVM : Gulley et al (ref. 16) and two-tailed Dunnett-test

RIZA : Kooymann (ref. 14), Spearman-Karber (ref. 13) and two-tailed Dunnett-test

IMW-TNO : Kooymann (ref. 14), Van der Hoeven (15) and two-tailed Dunnett test
Specific for (sub)chronic tests with *C. riparius*

Feeding during the test
RIZA : Trouvit, three times per week
IMW-TNO : Tetramin, three times per week

Calculations and statistics
RIZA : Kooyman (ref. 14) and Spearman-Karber (ref. 13)
IMW-TNO : Kooyman (ref. 14) and two-tailed Dunnett test
4. RESULTS AND DISCUSSION

4.1 Acute (combined) tests

4.1.1 Experimental results

A summary of the acute toxicity tests as carried out by the three participating institutes is given in Table 1. Detailed results of the experiments carried out are given in the separate reports of the three institutes (ref. 1, 3 and 6).
Table 1  Summary of the results of the acute toxicity tests with D. magna and C. riparius and chlorpyrifos.

<table>
<thead>
<tr>
<th>Sediment/water system</th>
<th>96 h EC50/LC50 1)</th>
<th>96 h NOEC 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dosed amount (mg.kg⁻¹.d.w.)</td>
<td>dosed amount (mg.kg⁻¹.d.w.)</td>
</tr>
<tr>
<td><strong>D. magna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.58 (0.39-0.87)</td>
<td>0.32</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.32-0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>TNO</td>
<td>0.42 (0.32-0.56)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>C. riparius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.34 (0.24-0.48)</td>
<td>0.10</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.09 (0.08-0.11)</td>
<td>0.056</td>
</tr>
<tr>
<td>TNO</td>
<td>0.13 (0.11-0.16)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Centrifuged overlying water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D. magna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.75 (0.62-0.91)</td>
<td>0.56</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.45 (0.40-0.50)</td>
<td>0.32</td>
</tr>
<tr>
<td>TNO</td>
<td>0.42 (0.32-0.56)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>C. riparius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.15 (0.12-0.18)</td>
<td>0.10</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.18-0.32</td>
<td>0.18</td>
</tr>
<tr>
<td>TNO</td>
<td>0.20 (0.16-0.24)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Overlying water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D. magna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.43 (0.36-0.52)</td>
<td>0.32</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.32-0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>TNO</td>
<td>0.42 (0.32-0.56)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>C. riparius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.10-0.18</td>
<td>0.032</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.14 (0.13-0.16)</td>
<td>0.10</td>
</tr>
<tr>
<td>TNO</td>
<td>0.22 (0.18-0.27)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Pore water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D. magna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.55 (0.44-0.69)</td>
<td>0.32</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.32-0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>TNO</td>
<td>0.39 (0.29-0.51)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>C. riparius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.12 (0.10-0.15)</td>
<td>0.10</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.18-0.32</td>
<td>0.18</td>
</tr>
<tr>
<td>TNO</td>
<td>0.22 (0.17-0.27)</td>
<td>0.10</td>
</tr>
<tr>
<td>**&quot;Water only&quot; 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D. magna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.58 (0.39-0.87)</td>
<td>0.32</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.21 (0.18-0.23)</td>
<td></td>
</tr>
<tr>
<td>TNO</td>
<td>0.11</td>
<td>&lt; 0.032</td>
</tr>
<tr>
<td></td>
<td>0.22 (0.17-0.29)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>C. riparius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIZA</td>
<td>0.34 (0.24-0.48)</td>
<td>0.10</td>
</tr>
<tr>
<td>TNO</td>
<td>0.09 (0.05-0.16)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

- **Pore water; concentrations prepared by dilution of a pore water derived from a sediment of 1.8 mg.kg⁻¹ 4)**
  - **D. magna**
    - RIZA: 35 (29-42) 18
    - TNO: 16 (12-22) 10
  - **C. riparius**
    - RIZA: 17 (12-23) 10
    - TNO: 13 (6.8-18) 3

1) EC50 mobility for D. magna; confidence limits in brackets
2) LC50 mortality for C. riparius; confidence limits in brackets
3) NOEC mobility for D. magna, mortality for C. riparius
4) Concentrations in µg.l⁻¹

Concentrations in volume percentages of the dosed amount of the "parent" pore water
A survey of the highest and lowest values obtained for both test organisms in the various test systems is given for each laboratory in Table 2. The results of the test with pore water, obtained by dilution of a “parent pore water” is not taken into account, because this test was carried out by two of the three institutes.

**Table 2** Highest and lowest 96 h EC50 (mobility)/LC50 values and NOEC values for mobility and mortality in the acute tests with chlorpyrifos

<table>
<thead>
<tr>
<th>Dosed amounts (mg kg(^{-1}) d.w.)</th>
<th><strong>D. magna</strong></th>
<th><strong>C. riparius</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>96 h EC50</td>
<td>96 h NOEC</td>
<td>96 h LC50</td>
</tr>
<tr>
<td>RIVM</td>
<td>0.32-0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>RIZA</td>
<td>0.43-0.75</td>
<td>0.32-0.56</td>
</tr>
<tr>
<td>IMW-TNO</td>
<td>0.39-0.42</td>
<td>0.18-0.32</td>
</tr>
</tbody>
</table>

The variation in the results of each laboratory for the various test systems used was within a factor 2-3. As this is quite acceptable as an intra laboratory variation it is not likely that the sediment particles contributed to the acute toxic effects of chlorpyrifos. Furthermore the inter laboratory variation appeared to be very low.

*C. riparius* was more sensitive to chlorpyrifos than *D. magna*, which very likely can be attributed to the working mechanism of chlorpyrifos.
Low oxygen concentrations were measured by all institutes in some exposure media. A review of the lowest concentrations measured is given in Table 3.

**Table 3** Lowest oxygen concentrations during the acute toxicity tests as measured by the three institutes.

<table>
<thead>
<tr>
<th></th>
<th>Concentration mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIVM</td>
</tr>
<tr>
<td>sediment/water system</td>
<td>2.0</td>
</tr>
<tr>
<td>overlying water</td>
<td>2.6</td>
</tr>
<tr>
<td>centrifuged overlying water</td>
<td>4.9</td>
</tr>
<tr>
<td>pore water</td>
<td>8.1</td>
</tr>
<tr>
<td>pore water (from a parent pore water)</td>
<td>*</td>
</tr>
<tr>
<td>&quot;water only&quot; test</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* test not carried out

It appears that the majority of the test sytems had oxygen concentrations below 60% of the saturation value (5.5 mg.l⁻¹ at 20°C), which is required in aquatic toxicology according to most guidelines. There are however no indications that these low levels affected the results and it is likely that due to the nature of sediments rather low oxygen levels must be accepted in acute tests or aeration must be considered.

**Table 4** Lowest and highest pH values during the acute toxicity tests as measured by the three institutes.

|                                | Highest and lowest pH value |
|                                | RIVM   | RIZA   | IMW-TNO |
| sediment/water system          | 7.1-8.0 | 6.9-7.3 | 7.3-7.9 |
| overlying water                | 7.1-8.4 | 7.0-7.8 | 7.3-8.3 |
| centrifuged overlying water    | 7.3-8.3 | 8.0    | 7.4-8.3 |
| pore water                     | 7.4-8.0 | 7.5-7.8 | 7.1-8.3 |
| pore water (from a parent pore water) | *     | 7.1-7.8 | 8.1-8.3 |
| "water only" test              | 7.9-8.3 | 7.4-8.0 | 7.8-8.6 |

* test not carried out
4.2 Reproduction tests with *Daphnia magna*

A summary of the reproduction tests with *D. magna* as carried out by the three participating institutes is given in Table 5. Detailed results of the experiments carried out are given in the separate reports of the three institutes (ref. 2, 4 and 7).

*Table 5*  Summary of the results of the reproduction tests with *D. magna* and chlorpyrifos.

<table>
<thead>
<tr>
<th></th>
<th>Dosed amounts (mg.kg$^{-1}$ d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mortality</td>
</tr>
<tr>
<td></td>
<td>21 d LC50</td>
</tr>
<tr>
<td>RIVM</td>
<td></td>
</tr>
<tr>
<td>sed/water</td>
<td>0.11</td>
</tr>
<tr>
<td>elutriate</td>
<td>0.32-0.56</td>
</tr>
<tr>
<td>RIZA</td>
<td></td>
</tr>
<tr>
<td>sed/water</td>
<td>0.078</td>
</tr>
<tr>
<td>elutriate</td>
<td>0.076</td>
</tr>
<tr>
<td>IMW-TNO</td>
<td></td>
</tr>
<tr>
<td>sed/water</td>
<td>0.14</td>
</tr>
<tr>
<td>elutriate</td>
<td>0.22</td>
</tr>
<tr>
<td>RIVM</td>
<td></td>
</tr>
<tr>
<td>“water only” **</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.032-0.10</td>
</tr>
</tbody>
</table>

* The reproduction of the surviving daphnias was not significantly inhibited compared with those of the control animals at any of the dosed amounts tested. Therefore the NOEC for reproduction is equal to those for mortality.

** Two tests were carried out.

Low oxygen concentrations were measured by all institutes in the tests with elutriates and sediment/water systems. A review of the lowest values measured is given in Table 6.
Table 6  Lowest oxygen concentrations during the reproduction tests with D. magna as measured by the three institutes.

<table>
<thead>
<tr>
<th></th>
<th>Concentration mg.l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIVM</td>
</tr>
<tr>
<td>sediment/water</td>
<td>0.8</td>
</tr>
<tr>
<td>elutriate</td>
<td>2.1</td>
</tr>
<tr>
<td>&quot;water only&quot;</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* test not carried out

The lowest low oxygen concentrations, below 60% of the saturation value (5.5 mg.l⁻¹ at 20°C) were measured, particularly in the sediment/water systems.

At RIVM and IMW-TNO the toxicity of chlorpyrifos in the sediment/water system was higher than at exposure to the elutriates. Chemical analysis at IMW-TNO showed that the chlorpyrifos concentrations in the elutriates and the overlying water in the sediment/water systems were at the same level. It can therefore not completely be excluded that sediment particles had some contribution to the observed toxic effects, however it is expected that these results are influenced by the stress of low oxygen concentrations.

The minor differences between the institutes are most likely also the result of this oxygen stress.

A summary of the lowest and highest pH values measured is given in Table 7 and it is not likely that the pH values affected the results.

Table 7  Lowest and highest pH-values during the reproduction tests with D. magna as measured by the three institutes.

<table>
<thead>
<tr>
<th></th>
<th>Highest and lowest pH value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIVM</td>
</tr>
<tr>
<td>sediment/water</td>
<td>7.6-7.9</td>
</tr>
<tr>
<td>elutriate</td>
<td>7.9-8.5</td>
</tr>
<tr>
<td>&quot;water only&quot;</td>
<td>7.8-8.6</td>
</tr>
</tbody>
</table>

* test not carried out
4.3 (Sub)chronic tests with *Chironomus riparius*

A summary of the results of the (sub)chronic tests with larvae of the midge *C. riparius* as carried out by RIZA and IMW-TNO is given in Table 8. Detailed results are given in the separate reports of the three institutes (ref. 2 and 5).

**Table 8** Summary of the results for survival in the (sub)chronic tests with the larvae of the midge *C. riparius* and chlorpyrifos.

<table>
<thead>
<tr>
<th>Sediment/water</th>
<th>Elutriate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dosed amount (mg.kg(^{-1}) d.w.)</td>
</tr>
<tr>
<td></td>
<td>RIZA</td>
</tr>
<tr>
<td>10 d LC 50 ¹)</td>
<td>0.043</td>
</tr>
<tr>
<td>21 d LC 50 ²)</td>
<td></td>
</tr>
<tr>
<td>10 d NOEC ¹)</td>
<td>0.032</td>
</tr>
<tr>
<td>21 d NOEC ²)</td>
<td></td>
</tr>
</tbody>
</table>

¹) elutriate test  
²) sediment/water test

There were only minor variations (between the types of tests and the two laboratories) in the results of the tests with *C. riparius*. The small difference in sensitivity between both type of tests can be attributed to the longer exposure duration of the test with the sediment/water systems and it is not likely that the sediment particles contributed to the toxic effects of chlorpyrifos. The variation between both laboratories was also negligible.

Low oxygen concentrations were measured in the tests with elutriates and sediment/water systems. A review of the lowest values measured is given in Table 9.
Table 9  Lowest oxygen concentrations during the (sub)chronic tests with C. riparius as measured by RIZA and IMW-TNO.

<table>
<thead>
<tr>
<th></th>
<th>Concentration (mg.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIZA</td>
</tr>
<tr>
<td>sediment/water</td>
<td>1.8</td>
</tr>
<tr>
<td>elutriate</td>
<td>7.0</td>
</tr>
</tbody>
</table>

In particular low oxygen levels were measured in the sediment/water systems, however, there are no indications that they affected the results. It is known that chironomids can stand low oxygen levels.

A summary of the lowest and highest pH values measured is given in Table 10 and it is not likely that the pH values affected the results.

Table 10  Lowest and highest pH-values during the (sub)chronic tests with C. riparius as measured by RIZA and IMW-TNO.

<table>
<thead>
<tr>
<th></th>
<th>Highest and lowest pH value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIZA</td>
</tr>
<tr>
<td>sediment/water</td>
<td>7.1-7.7</td>
</tr>
<tr>
<td>elutriate</td>
<td>7.3-8.2</td>
</tr>
</tbody>
</table>

4.4 Chemical analysis and sorption coefficients

From the analysis data available from the acute and (sub)chronic tests at RIZA and IMWTNO the sorption coefficient (= Kp) of chlorpyrifos can be calculated. The sorption coefficient is the relation between the concentration of an organic substance in sediment (Cs in µg.kg⁻¹) and in water (Cw in µg.l⁻¹) and reflects the slope of the sorption-isotherm (Cs = Kp x Cw).

This sorption coefficient is normalized for the organic content (foc) of the sediment used (Koc = Kp/foc). For the Schoonrewoerdse Wiel the organic carbon content is ca. 10%.
The organic carbon sorption coefficient $K_{oc}$ for chlorpyrifos in the sediment of the Schoonrewoerdse Wiel was calculated to be 16.667 l.kg$^{-1}$ (IMW-TNO (sub)chronic tests); 17.250 l.kg$^{-1}$ (RIZA, (sub)chronic tests. From studies at the Winand Staring Centre for Integrated Land, Soil and Water Research in Wageningen a $K_{oc}$ of 13.600 l.kg$^{-1}$ is known.

4.5 Inter laboratory variation

There were only minor differences in the methods used by the three laboratories, which allows to establish the inter laboratory variation. From Tables 1 and 2 it is shown that in the acute tests the variation between the three laboratories was a factor 2-3. This result proves that the inter laboratory variation was very low. The results of the reproduction tests with *Daphnia magna*, showed also minor differences, which can be attributed to irregularities in the tests caused by low oxygen concentrations.

4.6 Routes of exposure

Comparison of the results of the acute “water only” tests (chlorpyrifos dissolved in dilution water) with the sediment-and pore water tests shows that the toxicity of chlorpyrifos in acute tests with *D. magna* and *C. riparius* can be attributed to the aqueous fraction. This is confirmed by the comparable toxicity of various test media (see section 4.1).

For the (sub)chronic tests with *C. riparius* it can also be excluded that there is any contribution of the sediment particles to the toxic effects observed.

In the reproduction tests with *D. magna* the results are less equivocal, however it is most likely that the somewhat higher toxicity found in the sediment/water system found by RIVM and IMW-TNO is caused by the stress of low oxygen concentrations.
5. CONCLUSIONS

With respect to the choices made as described in chapter 3, the following conclusions can be drawn:

- *D. magna* and *C. riparius* are suitable test organisms to use in sediment testing.
- The used reference sediment from the Schoonrewoerdse Wiel is useful from the point of view of low control mortality. There is only limited experience with other sediments, storage and artificial sediments. Attention to this will be given in another part of this project.
- The treatment of the sediment was a so-called “worst case” approach to mimic processes in the field. This is considered as the most realistic approach for the situation in the Netherlands. It must be noted that the methods used are quite different from the way of handling sediments in the United States, where it is tried to keep sediments as undisturbed as possible.
- It can be recommended to follow existing guidelines from aquatic toxicology as far as possible. It is however not possible to follow all the requirements due to limiting conditions, such as oxygen concentrations.
- The inter laboratory variation for the test systems used was low.
- *C. riparius* is more sensitive to chlorpyrifos than *D. magna*.
- The preparation method of the test media did not influence the test results in the acute tests.
- The toxic effects observed in the acute tests and in the (sub)chronic tests with *C. riparius* are caused by the aqueous fraction. It is unlikely that sediment particles contributed to the effects observed. In the reproduction test with *D. magna* two institutes found a higher toxicity in the sediment/water systems than in the elutriate. It is not likely that this is caused by the contribution of sediment particles to the toxic effects observed. Low oxygen concentrations in the sediment/water systems may have enhanced the toxicity of chlorpyrifos in this test medium. Therefore the latter environmental parameter needs more attention in this type of test.
6. REFERENCES

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_Daphnia magna_ and the larva of the midge _Chironomus riparius_ in a combined test.
Report IMW-TNO, R 91/090; study number MTB-89-0119-01.

Chronic toxicity tests with sediment/water systems and elutriates contaminated with
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Report IMW-TNO R 92/420.

Stofgerichte sediment-toetsen met de watervlo ( _Daphnia magna_ ) en de muggelarf
( _Chironomus riparius_ ) - acute testen met ethyl-chloorpyrifos.
Report RIZA, Lelystad; AOCE 92-03; Project PCBB-B6.

Stofgerichte sediment-toetsen met de watervlo ( _Daphnia magna_ ) – chronische testen
met ethyl-chloorpyrifos.

Stofgerichte sediment-toetsen met de muggelarf ( _Chironomus riparius_ ) – chronische
testen met ethyl-chloorpyrifos.

Ontwikkeling van sedimenttoxiciteits-toetsen;
I. De acute toxiciteit van sediment gecontamineerd met chloorpyrifos voor _Daphnia magna_ en _Chironomus riparius_.
Report RIVM 719102004.
   Ontwikkeling van sedimenttoxiciteits-toetsen;
   II. De chronische toxiciteit van sediment gecontamineerd met chloorpyrifos voor
   *Daphnia magna*.
   Report RIVM 719102012.

8. Good Laboratory Practice in the testing of chemicals (1982).

   Freshwater sediment toxicity bioassessment: Rationals for species selection and test
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   J. Great Lakes Res. 15, 539-569.

    Report of the OECD workshop on effects assessment of chemicals in sediment,
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ANNEX A  DEFINITIONS USED IN SEDIMENT TOXICOLOGY

BIOASSAY (= SEDIMENT TEST):
Ecotoxicological test to assess the toxicity of field sediments or spiked sediments on a certain organism.

CONTROL SEDIMENT:
Sediment from a relatively uncontaminated place, used for the testing of spiked sediments and field sediments to check the normal development of the test organisms used.

DILUTION WATER:
Standard water used for preparing sediment suspensions and the dilution of pore waters or elutriates.

ELUTRIATE:
Water extract of sediment suspension prepared by the mixing of a sediment with dilution water in a fixed volume ratio, subsequently followed by centrifuging the sediment suspension. The supernatant is the elutriate.

ENVIRONMENTAL TEST PARAMETERS:
The various characteristics of sediment, pore water, elutriate or overlying water, supposed to be critical for the results in ecotoxicological testing.

EQUILIBRIUM PARTITIONING:
Mathematical model to describe or predict the partitioning of a chemical between sediment particles and the water phase.

FIELD SEDIMENT:
Sediment obtained from a specific field location.

INTERSTITIAL WATER (= PORE WATER):
Water occupying space between the sediment particles and relatively simple removed, for instance by centrifuging or pressing.
IRRADIATED SEDIMENT:
Sediment treated with gamma irradiation to kill contaminating organisms.

OVERLAYING WATER:
Water phase above the sediment after sedimentation by standing.

MODEL COMPOUND:
Chemical used in the development of ecotoxicological test systems.

PORE WATER (see INTERSTITIAL WATER)

PRETREATMENT OF SEDIMENT:
Treatment of sediment as preparation for the use in sediment tests, such as:
• homogenisation by stirring, shaking or rolling
• dilution with water
• irradiation
• sedimentation of sediment and pouring off the overlying water

REFERENCE SEDIMENT:
Sediment, usually from a relatively clean site, used in the testing of sediments, to check the quality of the test animals. In this sediment growth and condition are normally good. The reference sediment would have the same composition and structure as the field sediment investigated.

SEDIMENT:
Particulate material lying below the water surface.
SEDIMENT CHARACTERISTICS:
Group of parameters which may be relevant for the interpretation of a sediment test; some of them are not determined on the sediment but in the water phase. Examples are:
- particle size ratio
- oxygen content
- NH4+
- nitrat/nitriet
- AVS
- pH
- BOD
- COD
- chloride
- sulphate
- redox potential
- conductivity
- salinity
- H2S
- presence of chemical pollutants (including oil)

SEDIMENT QUALITY CRITERIA;
Criteria used for the evaluation of possible adverse effects of polluted sediments on aquatic organisms. They include both test parameters and various established standards.

SEDIMENT SUSPENSION:
Suspension prepared by the mixing of sediment and dilution water in a fixed volume ratio (usually 1 : 4).

SEDIMENT TOXICITY TEST:
Ecotoxicological test (= bioassay) for the assessment of the toxicity of field sediments and spiked sediments.
SEDIMENT/WATER SYSTEM:
Exposure system prepared by the mixing of sediment and water in a fixed volume ratio (usually 1 : 4).

SOLVENT CONTROL:
Control sediment contaminated with a solvent, in an equal amount as the carrier used to dose the test substance for the spiking of sediments.

SPIKED SEDIMENT:
Sediment with added chemicals used for experimental purposes.

SUSPENDED SOLIDS:
Suspended particles present in overlaying water, pore water or elutriate.

TRIAD APPROACH:
Assessment of risks for ecosystems/organisms on the basis of chemistry, ecotoxicity and in situ biological studies on specific organisms.