Harmonisation and standardisation of sediment assays and monitoring strategies

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www.tu-berlin.de/~oekotox

www.ecosystemhealth.net
WFD from the view of:
Sediment assays
Standardisation and Harmonisation
Terminology ISO TC147/SC5
Monitoring and Quality Standards
Convenor: ISO TC147/SC5/WG9
1. Biological components: Flora, Benthic Organism, Fishfauna and Population

2. Hydromorphological components

3. Physico-chemical components

4. Pollution by priority Substances: pressure (Annex II, 1.4) – AMPS/PHS and impact (Annex II 1.5) – effects (biota)

AMPS = Analysis and Monitoring of Priority Substances

PHS = Priority Hazardous Substances (33) – cf.2455/2001/EC
• **Goal:**
  – Present ideas how organismic, suborganismic tests / sediment bioassays can be used within a European Framework Directive
  – Share existing standards operating procedures for bioassays
  – Discuss which approach for sediment bioassays will be most successful

• **Contents:**
  1. The inventory on the possible use of bioassays within the WFD
  2. ISO/DIN standards operating procedures for sediment bioassays
  3. Use of sediment bioassays in Germany
  4. Biological components: Fish Population
  5. Sediments and good ecological status
  6. Chemical Monitoring and Bioassays .... Bioanalysis
  7. Conclusions
Possible use of sediment-bioassays within the WFD

The WFD describes:

• **Surveillance monitoring**
  The assessment and description of long-term ecological trends and an overall description of the waters to determine whether a good status has been or will be achieved.

• **Operational monitoring**
  The assessment of the status of the water mass of which it has become evident that it may not meet the environmental objectives and/or to assess the changes in the status of this water mass resulting from the programme of measures.

• **Investigative monitoring**
  The identification of the cause of the failure to achieve a “good ecological-status”.
Possible use of bioassays within the WFD

Bioassays are NOT mentioned in the WFD!

“Eco-assay”: a test that helps to determine what the cause is of a failing ecological status in a waterbody. The cause can be chemical, physical, hydromorphological, ecological, or a combination of these factors.

Possible use of bioassays within the WFD

Types of eco-assays:

- *In vivo* bioassays (whole-organism, lab)
- *In vitro* bioassays (cellular, lab)
- *In situ* bioassays (whole organism, field)
- Biomarkers (molecular, morphological, histopathological responses within organism)
- Toxic Identification Evaluation (TIE) / Effect Directed Analysis (EDA)
- Micro-mesocosm studies (semi-field experiments)
- Biomanipulation studies (manipulation of ecosystem)
- Models
Possible use of bioassays within the WFD

Eco-assays as diagnostic tool to:

1. Identify causes
2. Confirm causes
3. Prioritize measurements
4. Convince others of the effectiveness of measurements

Possible use of bioassays within the WFD

Diagnostic decision tree

Steps:
1. Ecological effect
2. Inventory of causes (theoretical)
3. Inventory of available data
4. Identification/confirmation of causes
5. Prioritization of measurements
6. Realization of measurements
Are Standards for Water-and Sediment-Quality Testing available?
Standards under ISO EN OECD ASTM

ISO = International Organisation for Standardisation
EN = European Organisation for Standardisation

National Standards:
AFNOR = Association Francaise de Normalisation
BSI = British Standard Institute
DIN = German Organisation for Standardisation
........... etc

OECD = Organisation for Economic Cooperation and Development
ASTM = American Society for Testing Materials
ISO and CEN Members:

Austria, Belgium, Czech Rep., Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxemburg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland, United Kingdom

ISO Members outside Europe

Japan, Russia, South Africa, Turkey, USA, Canada, Chile, Korea etc.
Implemented and validated Standard protocols:

**ISO / CEN** - Bioassays, **Sediments**

National Standards - DIN, AFNOR, BSI etc.

**OECD** - Bioassays

**ASTM** - Bioassays, **Sediments**

**PARCOM / OSPARCOM** - **Sediments**
ISO Standards for Sediment testing:
- whole sediment - pore water - elutriate

Sediment contact biotests

Bacteria: *Athrobacter globiformis* (ISO NWI)

Nematoda: *Caenorhabdities elegans* (ISO NWI)

Fish egg test (ISO 15088)

Higher plant test: *Lemna minor* (ISO 20079)

Genotoxicity, umu assay *Salmonella typhemurium* (ISO 13829)

Sediment exposure with Flatfish: biochemical response – EROD (ISO 23893-2/AWI)
The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = highest dilution factor without an effect

Bacteria:

1. **Luminescent bacteria test ISO 11348** (Determination of the inhibitory effects of water samples on the light emission of *Vibrio fisheri*)
2. **Growth inhibition test ISO 10712** (cell multiplication inhibition test with *Pseudomonas putida*)
3. **Kinetic luminescent bacteria test – determination of the inhibitory effects of sediments and other solids – ISO NWI/AWI** with *Vibrio fisheri*
ISO-TC 147-SC5 / CEN-TC 230  Standards for sediment testing: pore water and elutriate

The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = highest dilution factor without an effect

Invertebrates:

Determination of the inhibition of the mobility of *Daphnia magna* (Cladocera, Crustacea) - ISO 10706

Determination of acute toxicity of marine or estuarine sediment to amphipods – acute immobilisation test - ISO/DIS 16712
Voting on an ISO Standard (marine or estuarine sediments to amphipoda...) between the delegates of the ISO member states and to include their technical comments into the standard.
Result of voting

P-Members voting: 16 in favour out of 17 = 94.11% (requirement >= 66.66%)
(P-Members having abstained are not counted in the vote.)

Member bodies voting: 1 negative vote out of 21 = 4.76% (requirement <= 25%)

Approved
ISO-TC 147-SC5 / CEN-TC 230 Standards for sediment testing: pore water and elutriate

Vertebrates:

Fish eggs - determination of the non-acute toxicity to fish eggs – ISO/AWI 15088

Algae and aquatic plants:

Fresh water algal growth inhibition test with *Desmodesmus subspicatus* and *Selenastrum capricornutum* – ISO 8692

Duckweed (*Lemna minor*) growth inhibition test – ISO/DIS 20079

Estuarine and marine sediments:

Algae *Phaeodactylum tricornutum* – ISO/CD 10253
ISO-TC 147-SC5 / CEN-TC 230 Standards for sediment testing: pore water and elutriate

The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = highest dilution factor without an effect

Monitoring of sediments with “subanimal testing parameters“

Genotoxicity

Determination of Genotoxicity with *Salmonella typhimurium* using the umu assay (ISO 13829) and/or the AMES assay (ISO 16240)

Determination of Genotoxicity by measurements of the induction of micronuclei with amphibia larvae and a cell line – ISO 21427-1 and ISO 21427-2
**REQUESTED ACTION**

- Information
- Discussion at meeting
- Comments by phone
- Voting (P-members only; ballot form attached by

P-members of the technical committee or subcommittee concerned have an obligation to vote.

**VOTES OF ISO/TC 147/SC 5 MEMBERS ON ISO/CD 21427-2**

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<th>P-MEMBER</th>
<th>MEMBER</th>
<th>YES</th>
<th>NO</th>
<th>ABST.</th>
<th>No reply</th>
<th>Comments (enclosed)</th>
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**TOTAL**

| 21 | 14 | 0 | 1 | 6 | 1 |

**TWO-LETTER COUNTRY CODE FOR MEMBER BODIES**

(First column of the report of voting)

| FR | FRANCE |

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<th>MB</th>
<th>Clause No./ Subclause No./ Annex (e.g. 3.1)</th>
<th>Paragraph/ Figure/Table /Note (e.g. Table 1)</th>
<th>Type of comment</th>
<th>Comment (justification for change) by the MB</th>
<th>Proposed change by the MB</th>
<th>Secretariat observations on each comment submitted</th>
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<tr>
<td>FR</td>
<td>Scope</td>
<td>te</td>
<td>As per resolution 21 taken by ISO/TC 147/SC 5 at Cardiff on 2003-10-09 (see document ISO/TC 147/SC 5 N 413) and for consistency with Part 1 of the standard</td>
<td>Add the following terms to the scope: &quot;The adsorbed method is applicable to: - aqueous effluents, aqueous leachates and eluates of soils, industrial waste or sewage treatment sludge; - surface and ground water; - water-soluble and water-miscible substances.&quot;</td>
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<tr>
<td>FR</td>
<td>4</td>
<td>te</td>
<td>Out of the scope of the standard</td>
<td>Delete the last paragraph of clause 4 (&quot;The test facility ..... have been tested&quot;)</td>
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<tr>
<td>FR</td>
<td>5</td>
<td>te</td>
<td>The sentence in the middle of clause 5 (&quot;therefore, toxic items, ..... under test conditions.&quot;) needs to be clarified.</td>
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<td></td>
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<td>FR</td>
<td>6.2.2</td>
<td>ed</td>
<td>Explanation and consistency</td>
<td>Give the meaning of NADP</td>
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<td>6.4.2</td>
<td>ed</td>
<td>Explanation</td>
<td>Explain the terms &quot;Passage the cells&quot; or use another verb.</td>
<td></td>
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<tr>
<td>FR</td>
<td>6.5.1</td>
<td>ed</td>
<td>To avoid ambiguity</td>
<td>Change &quot;It should also be prepared&quot; to &quot;it shall have been prepared&quot;</td>
<td></td>
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<tr>
<td>FR</td>
<td>7 and 8.1</td>
<td>te</td>
<td>For completeness</td>
<td>Add a sub-clause for &quot;0.22 µm sterile filter&quot; in 7 and add reference to this sub-clause in 8.1 (into the last but one sentence, after &quot;sterile filters&quot;)</td>
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<tr>
<td>FR</td>
<td>8.1</td>
<td>te</td>
<td>The harmonisation of the storage conditions defined in both the parts of the standard shall be considered.</td>
<td>Put the sentence &quot;If this not possible ..... respectively.&quot; In a note.</td>
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<tr>
<td>FR</td>
<td>9.3</td>
<td>te</td>
<td>For clarity</td>
<td>Delete the terms &quot;Take into account the following criteria&quot; and change the 4 alines to</td>
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1. MB = Member body (enter the ISO 3166 two-letter country code, e.g. CN for China; comments from the ISO/CS editing unit are identified by **)  
2. Type of comment: ge = general  te = technical  ed = editorial  

NOTE: Columns 1, 2, 4, 5 are compulsory.
ISO-TC 147-SC5 / CEN-TC 230  Standards for sediment testing: pore water and elutriate

The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = highest dilution factor without an effect

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Monitoring Strategies

Directly on Sediment

Above Sediment using Legs

Fixed Bottom Placement

The toxicity derived from the bioassay is characterized by how many times a sample has to be diluted in a ratio of 1:2 to render it no longer toxic (dilution factor).

By using more than one bioassay, the toxicity class of sediments is determined by the most sensitive organism within the test series.
As a standard, the following procedures are used:

**Algae test**

**Luminescent bacteria test**

**Daphnia test**

This characterizes a toxicity, assumed by an environment sampling of a model organism, by how many times a sampling must be diluted in a ratio of 1:2 in order that it is no longer toxic. For a numerical designation of the determined toxicity the pT value is used.

Biochemical tests (oxygen and nutrients)

**HABAB-WSV Directive for the Handling of Dreged Material**
Ecotoxicological examination of sediment samples from the River Saar, Krebs 2000 (Geometric dilution series with the factor 2)
Toxicity classes used by the Federal Institute of Hydrology in sediment assessment and ecotoxicological management categories - The ecotoxicological analyses are made with pore water and elutriate (Krebs 1988, 2000, 2004, HABAB-WSV 2000)

<table>
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<tr>
<th>Highest dilution level without effect</th>
<th>Dilution factor</th>
<th>pT-value</th>
<th>7-level system</th>
<th>Designation</th>
<th>Management categories</th>
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<td>Original sample</td>
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<td>0</td>
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<td>1:2</td>
<td>$2^{-1}$</td>
<td>1</td>
<td>I</td>
<td>very slightly toxic</td>
<td>uncritically polluted</td>
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<tr>
<td>1:4</td>
<td>$2^{-2}$</td>
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<td>II</td>
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<tr>
<td>1:8</td>
<td>$2^{-3}$</td>
<td>3</td>
<td>III</td>
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<td>III</td>
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<td>1:16</td>
<td>$2^{-4}$</td>
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<td>IV</td>
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<tr>
<td>1:32</td>
<td>$2^{-5}$</td>
<td>5</td>
<td>V</td>
<td>highly toxic</td>
<td>hazardous</td>
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<tr>
<td>≤ (1:64)</td>
<td>≤ $2^{-6}$</td>
<td>≥ 6</td>
<td>VI</td>
<td>extremely toxic</td>
<td>VI</td>
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</table>
Colour coding and Management categories:

blue = unpolluted
Green = uncritically polluted
Yellow = critically polluted
Red = hazardous

Sediments
River Saar F.
Krebs 1992, 2000
Management categories for sediments used by the Federal Institute of Hydrology. Chemical and ecotoxicological criteria according to HBAB-WSV (2000) and HABAK-WSV (1999) – c=contaminant concentration
“water bodies at risk“
(inland and transitional waters)

Implies that there is are significant consequences:
Operational monitoring (cf. Annex V)

Follow-up steps –

Report to the EC  March 2005

Water QS, EQS, AA-EQS, MAC-EQS

AA-EQS = anual average environmental quality standard
MAC-EQS = maximum allowable concentration
environmental quality standard
Monitoring strategies

Testbattery fresh water sediments (HABAB):

**Algae test**
Luminescent bacteria test
Daphnia test

Testbattery estuarine and marine sediments (HABAK, OSPARCOM):

- chronic sediment contact test
- biomarkers, and
- microcosm and mesocosm test

- the structure of benthos
- the contamination of marine organisms (bioaccumulation)
- the ecotoxicological properties of the sediment
- the eutrophication
ISO-TC 147-SC5 / CEN-TC 230 Standards and Harmonisation for sediment testing: pore water and elutriate

The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = highest dilution factor without an effect

New emerging ISO Standards

Biomarkers

Sediment exposure with bottom living fish: biochemical response – EROD (ISO 23893-2/AWI)

Sediment exposure and endocrine effects: hER and hAR receptor assays – New Work Item under ISO
Experimental Protocol:

simple competition assay

IgG = 150 kDa, ovalbumin = 46 kDa, E3G (estrone-3-glucuronide) = 468 Da

monoclonal anti-E3G IgG supplied by Unilever PLC

Physically adsorbed ovalbumin-E3G conjugate

Initially free/unknown E3G in solution

Initially anti-E3G IgG in solution

Gold SPR surface *UNREPLACEABLE*

Cranfield University
For sampling and evaluating sediments:

Use the advantages of the existing international Monitoring stations (IKSR, IKSE, IKSO, IKSD).

Use validated tools and historical data.
Summary

There is a broad variety of water and sediment biotests standardized after ISO, CEN, available of the different trophic levels

(1) The protocols of the bioassays includes the sampling and preparation steps of water and sediments prior to the test procedures

(2) Harmonisation of ISO Standards are progressed in the working groups by the participants from the ISO member states

(3) A new emerging protocol on endocrine effects will come up in the near future as an New Work Item for an ISO Standard

(4) Management tools under the HABAB (Freshwater sediments) and the HABAK (estuarine and marine sediments) directive demonstrates that only a certain set of Standards is needed and these are harmonized and contributed by ISO and CEN

(5) Bioassays will be implemented with the chemical monitoring: quality norm (QN)

Thank you very much for your attention!

http://www.afnor.fr
http://www.din.de/livelink
Water Quality Objectives (QO) for the protection of inland waters against dangerous substances [n.r. = not relevant; r = relevant, but no data available; [dw = dry weight]

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<tr>
<th>Substance</th>
<th>Aquatic communities</th>
<th>Fishery</th>
<th>Drinking Water supply</th>
<th>Sediments [µg/kg dw]</th>
<th>Water Quality Objectives [µg/l]</th>
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AMPS = Analysis and Monitoring of Priority Substances
PHS = Priority Hazardous Substances (33)
Derivation of Water Quality Objectives for the Protection of Inland Surface Waters against Heavy Metals. Use and Aquatic communities; use and Commercial and Sport Fishing; use and drinking watersupply; use and sediments (n.r. = not relevant).

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Endocrine effects Standards, OECD – ISO

National Standard DIN UA 7 / ISO

Sediment, pore water, elutriate:

Receptor Assays

ELRA-, Yes-, Callux-Assay, Sumpter
hER, hAR, Mc Donnell hER, hAR

Cell-proliferation: MCF7

Organismic Assays:

Vitellogenin-Synthesis-Assay: Fish

Imposex Molluscs, Crustaceans
pT-value -Sumpter/McDonnell/ELRA: 5α Dihydrotestosterone (hAR); 17β Estradiol (hER)

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