Harmonisation and standardisation of sediment assays and monitoring strategies

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WFD from the view of:

Sediment assays

Standardisation and Harmonisation

Terminology ISO TC147/SC5

Monitoring and Quality Standards

Convenor: ISO TC147/SC5/WG9

Sediment – Biota - Watermatrix

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 Direktive
  - –Share existing standards operating procedures for bioassays
  - –Discuss which approach for sediment bioassays will be most successfull

- **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, hydromorpho-logical, ecological, or a combination of these factors.

M.J. van den Heuvel-Greve, H. Maas & C.A. Schipper - SedNet WG Sediment quality and impact assessment, 4th workshop San Sebastian, 10-11 June 2004

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 (moleculair, 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

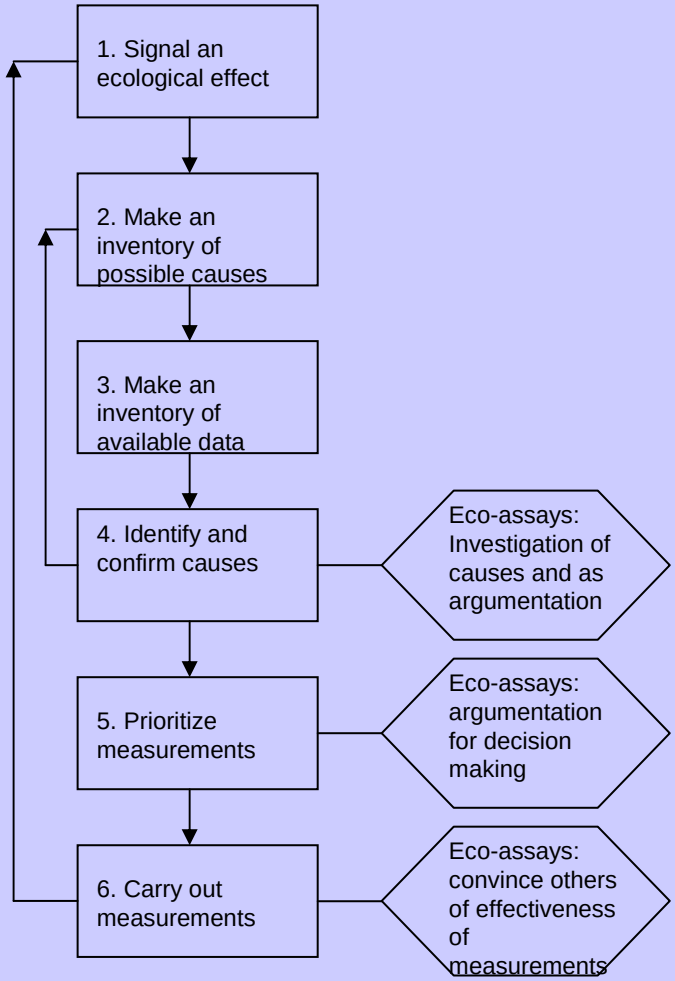
Possible use of bioassays within the WFD

Eco-assays as diagnostic tool to:

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

M.J. van den Heuvel-Greve, H. Maas & C.A. Schipper – SedNet WG Sediment quality and impact assessment, 4th workshop San Sebastian, 10-11 June 2004

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

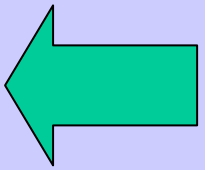
M.J. van den Heuvel-Greve, H. Maas & C.A. Schipper
SedNet 4th workshop San Sebastian, 10-11 June 2004

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



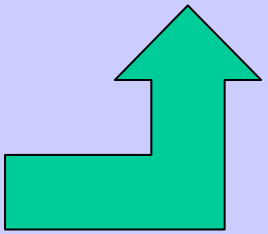
National Standards:

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, Potugal, Slovakia, Spain, Sweden, Switzerland, United Kingdom

ISO Members outside Europe

Japan, Russia, South Africa, Turkey, USA, Canada, Chile, Korea etc.

Implemented and validated Standardprotocols:

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)

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**

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

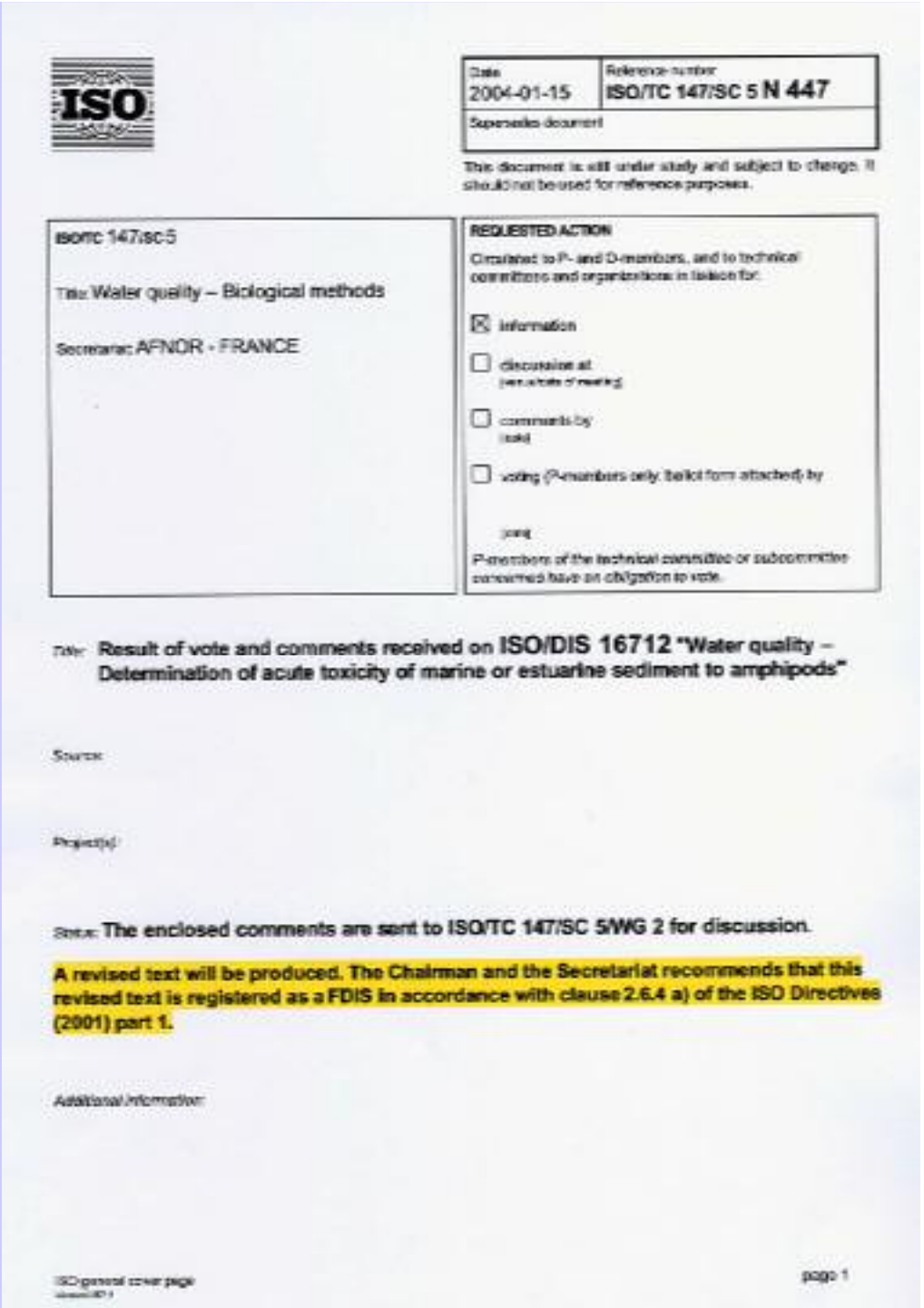
The toxicity is characterised (quantified) by how many times a sample must be diluted in a ratio 1:2 until there no longer toxicity = **highest dilution factor without an effect**

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 memberstates and to include their technical comments into the standard



ISO

Date	Reference number
2004-01-15	ISO/TC 147/SC 5 N 447
Supersedes document	

This document is still under study and subject to change. It should not be used for reference purposes.

ISO/TC 147/SC 5

Title: Water quality – Biological methods

Secretariat: AFNOR - FRANCE

REQUESTED ACTION

Circulated to P- and O-members, and to technical committees and organizations in liaison for:

☒ information

☐ discussion at [venue/date of meeting]

☐ comments by [date]

☐ voting (P-members only: ballot form attached) by [date]

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

Title: Result of vote and comments received on ISO/DIS 16712 "Water quality – Determination of acute toxicity of marine or estuarine sediment to amphipods"

Source:

Project(s):

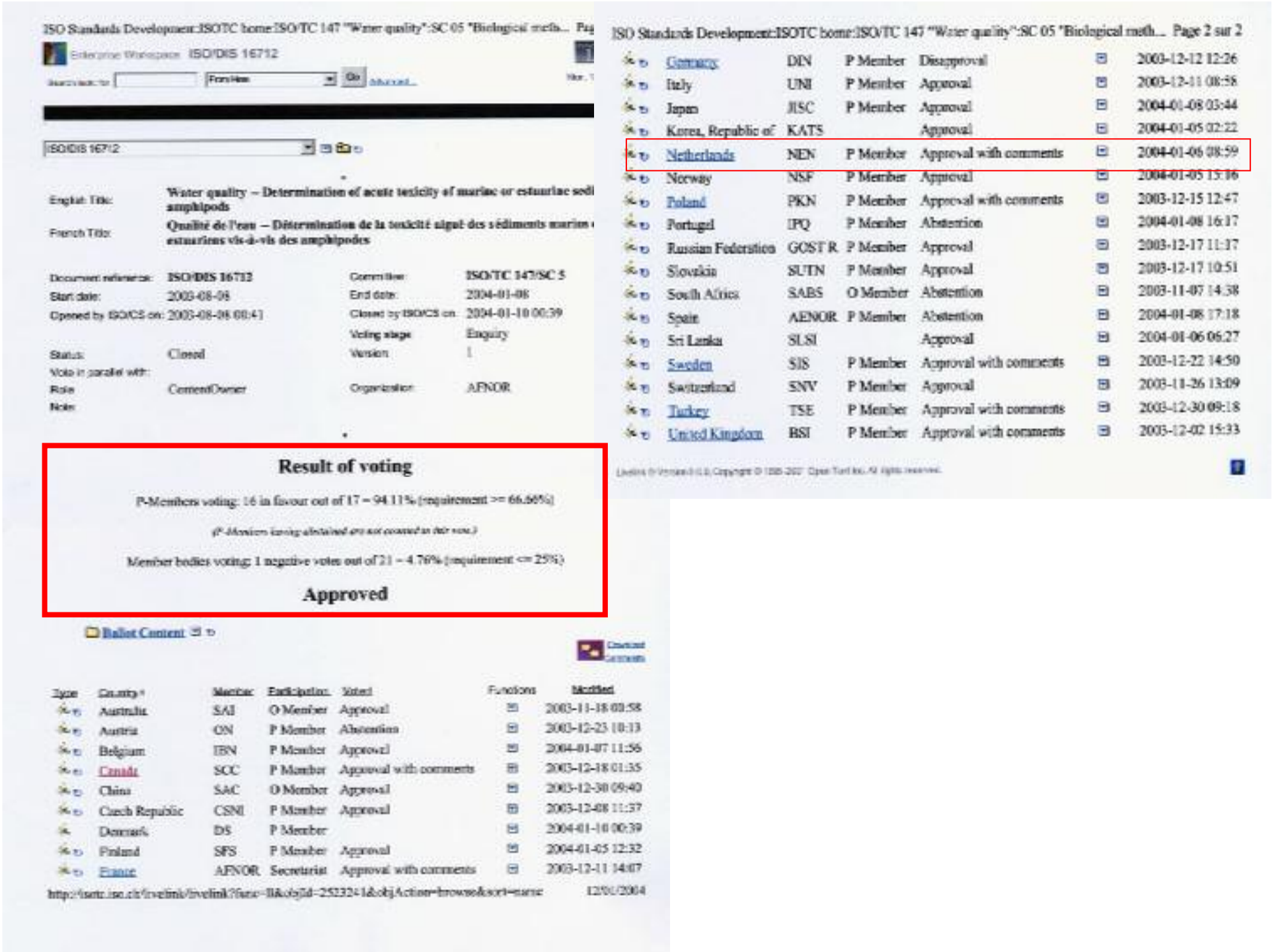
Status: The enclosed comments are sent to ISO/TC 147/SC 5/WG 2 for discussion.

A revised text will be produced. The Chairman and the Secretariat recommends that this revised text is registered as a FDIS in accordance with clause 2.6.4 a) of the ISO Directives (2001) part 1.

Additional information:

ISO general cover page

page 1



ISO Standards Development:ISOTC home:ISO/TC 147 "Water quality":SC 05 "Biological meth... Page 2 sur 2

Germany	DIN	P Member	Disapproval		2003-12-12 12:26
Italy	UNI	P Member	Approval		2003-12-11 08:58
Japan	JISC	P Member	Approval		2004-01-08 03:44
Korea, Republic of	KATS		Approval		2004-01-05 02:22
Netherlands	NEN	P Member	Approval with comments		2004-01-06 08:59
Norway	NSF	P Member	Approval		2004-01-05 15:16
Poland	PKN	P Member	Approval with comments		2003-12-15 12:47
Portugal	IPQ	P Member	Abstention		2004-01-08 16:17
Russian Federation	GOST R	P Member	Approval		2003-12-17 11:17
Slovakia	SUTN	P Member	Approval		2003-12-17 10:51
South Africa	SABS	O Member	Abstention		2003-11-07 14:38
Spain	AENOR	P Member	Abstention		2004-01-08 17:18
Sri Lanka	SLSI		Approval		2004-01-06 06:27
Sweden	SIS	P Member	Approval with comments		2003-12-22 14:50
Switzerland	SNV	P Member	Approval		2003-11-26 13:09
Turkey	TSE	P Member	Approval with comments		2003-12-30 09:18
United Kingdom	BSI	P Member	Approval with comments		2003-12-02 15:33

ISO Standards Development:ISOTC home:ISO/TC 147 "Water quality":SC 05 "Biological meth...

Enterprise Workspace ISO/DIS 16712

ISO/DIS 16712

English Title: **Water quality -- Determination of acute toxicity of marine or estuarine sedi... amphipods**

French Title: **Qualité de l'eau -- Détermination de la toxicité aiguë des sédiments marins ... estuariens vis-à-vis des amphipodes**

Document reference:	ISO/DIS 16712	Committee:	ISO/TC 147/SC 5
Start date:	2003-08-08	End date:	2004-01-08
Opened by ISO/CS on:	2003-08-08 08:41	Closed by ISO/CS on:	2004-01-10 00:39
		Voting stage	Enquiry
Status:	Closed	Version:	1
Vote in parallel with:			
Role	CentralOwner	Organization:	AFNOR
Note:			

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 votes out of 21 = 4.76% [requirement <= 25%]

Approved

Ballot Content

Type	Country	Member	Participation	Voted	Functions	Modified
	Australia	SAI	O Member	Approval		2003-11-18 03:58
	Austria	ON	P Member	Abstention		2003-12-23 10:13
	Belgium	IBN	P Member	Approval		2004-01-07 11:56
	Canada	SCC	P Member	Approval with comments		2003-12-18 01:35
	China	SAC	O Member	Approval		2003-12-30 09:40
	Czech Republic	CSNI	P Member	Approval		2003-12-08 11:37
	Denmark	DS	P Member			2004-01-10 00:39
	Finland	SFS	P Member	Approval		2004-01-05 12:32
	France	AFNOR	Secretariat	Approval with comments		2003-12-11 14:07

http://isotc.iso.ch/livelink/livelink?func=ll&objId=2523241&objAction=browse&sort=name 12/01/2004

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

Date	Reference number
2004-04-05	ISO/TC 147/SC 5 N 459
Supersedes document	

This document is still under study and subject to change. It should not be used for reference purposes.

ISO/TC 147/SC 5

Title: Water quality - Biological methods

Secretariat: AFNOR

REQUESTED ACTION

Circulated to P- and O-members, and to technical committees and organizations in liaison for:

☒ information

☐ discussion at (place/date of meeting)

☐ comments by (date)

☐ voting (P-members only: ballot form attached) by (date)

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

Title: **Vote and comments received on ISO/CD 21427-2 "Water quality – Evaluation of genotoxicity by measurement of the induction of micronuclei – Part 2 : Mixed population method using the cell line V79"**

Source: ISO/TC 147/SC 5 secretariat

Project(s):

Status: **The enclosed comments are sent to ISO/TC 147/SC 5/WG 9 for discussion. A revised text will be produced by WG 9.**

The Chairman and the Secretariat recommends that this revised text is submitted for registration as a DIS in accordance with clause 2.5.3 c) of ISO Directives (2001) part 1.

Additional information:

ISC general cover page
Version 01.1

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

P-MEMBER	MEMBER	YES	NO	ABST.	No reply	Comments (enclosed)
Austria	P	X				
Belgium	P	X				
Canada	P	X				
Czech Republic	P	X				
Denmark	P	X				
Finland	P	X				
France	P	X				X
Germany	P	X				
Italy	P	X				
Japan	P				X	
Netherlands	P	X				
Norway	P				X	
Poland	P			X		
Portugal	P				X	
Russian Federation	P				X	
Slovakia	P				X	
Spain	P	X				
Sweden	P	X				
Switzerland	P	X				
Turkey	P	X				
United Kingdom	P				X	
TOTAL	21	14	0	1	6	1

TWO-LETTER COUNTRY CODE FOR MEMBER BODIES *
(First column of the report of voting)

FR	FRANCE

* See ISO 3166-1:1997.

Template for comments and secretariat observations

Date: 2004-04-05	Document: **ISO/TC 147/SC 5 N 459**

1	2	(3)	4	5	(6)	(7)
MB[1]	**Clause No./ Subclause No./ Annex** (e.g. 3.1)	**Paragraph/ Figure/Table /Note** (e.g. Table 1)	**Type of com-ment**[2]	**Comment (justification for change) by the MB**	**Proposed change by the MB**	**Secretariat observations** on each comment submitted
FR	Scope		te	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	Add the following terms to the scope : "The described 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."	
FR	4		te	Out of the scope of the standard	Delete the last paragraph of clause 4 ("The test facility ..... have been tested")	
FR	5		te	The sentence in the middle of clause 5 ("therefore, test items, ...under test conditions.") needs to be clarified		
FR	6.2.2		ed	Explanation and consistency	Give the meaning of NADP	
FR	6.4.2		ed	Explanation	Explain the terms "Passage the cells" or use another verb.	
FR	6.5.1		ed	To avid ambiguity	Change "it should also be prepared" to "it shall have been prepared"	
FR	7 and 8.1		te	For completeness	Add a sub-clause for " 0,22 µm sterile filter" in 7 and add reference to this sub-clause in 8.1 (into the last but one sentence, after "sterile filters")	
FR	8.1		te	The harmonisation of the storage conditions defined in both the parts of the standard shall be considered.	Put the sentence "If this not possible ...respectively." in a nota.	
FR	9.3		te	For clarity	Delete the terms "Take into account the following criteria" and change the 4 alineas to	

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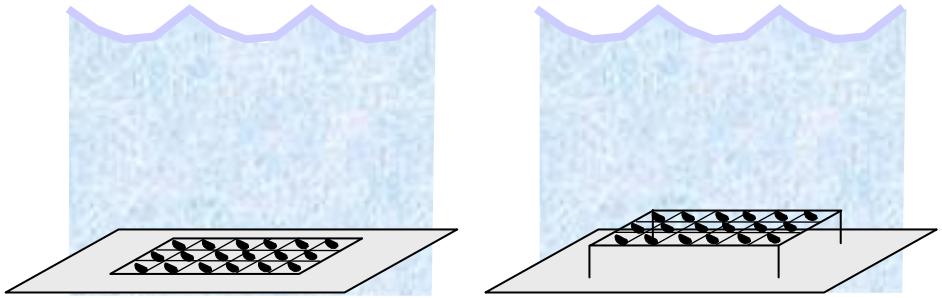
Determination of Genotoxicity by measurements of the induction of micronuclei with amphibia larvae and a cell line – ISO 21427-1 and ISO 21427-2

Monitoring Strategies

Salazar, M.H., Salazar, S.M. 2001. Standard guide for conducting in situ Field bioassys with marine, estuarine and freshwater bivalves. In: Annual book of ASTM standards. West Conshohocken, PA: American Society for Testing and Materials



Directly on Sediment Above Sediment using Legs

Fixed Bottom Placement

Ecotoxicological Sediment Classification **(HABAB-WSV 2000, Directive for Dredged Inland Material Management in Federal Inland Waterways HABAK-WSV 1999, Directive for Dredged Inland Material Management in Federal Coastal Waterways)**

Highest dilution factor without effect	Toxicity class	Designation of toxicity class
Original sample	0	Toxicity not detectable
1:2	I	Very low toxicity
1:4	II	Slightly toxic
1:8	III	Moderately toxic
1:16	IV	Elevated toxicity
1:32	V	High toxicity
≤ (1:64)	VI	Very high toxicity

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

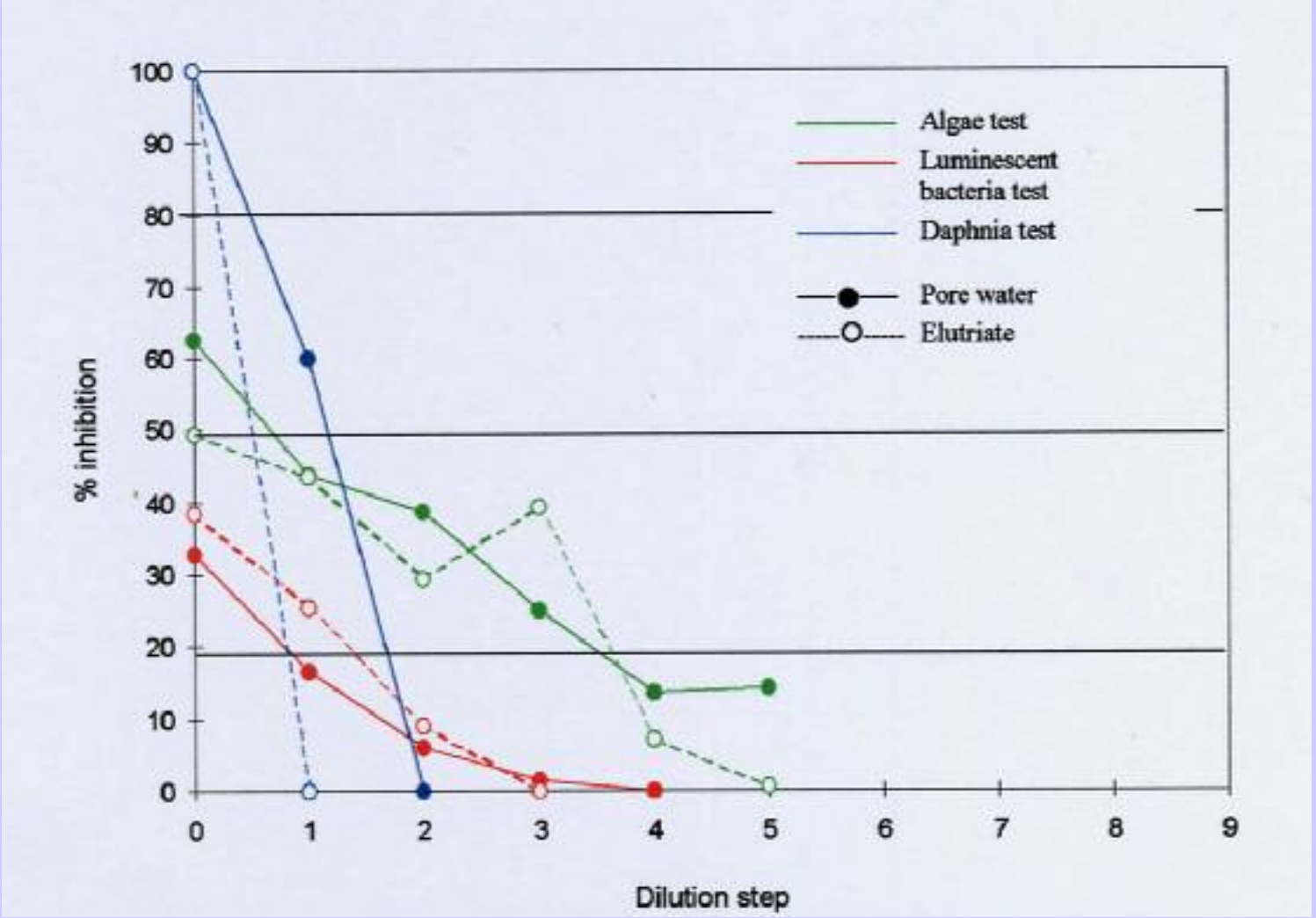
Luminescent bacteria test

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)

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)

Highest dilution level without effect	Dilution factor	pT-value	Toxicity classes		Management categories	
			7-level system	Designation	4-level assessment	Colour coding
Original sample	2^{0}	0	0	toxicity non-detectable	unpolluted	0
1:2	2^{-1}	1	I	very slightly toxic	uncritically polluted	I
1:4	2^{-2}	2	II	slightly toxic		II
1:8	2^{-3}	3	III	moderately toxic	critically polluted	III
1:16	2^{-4}	4	IV	distinctly toxic		IV
1:32	2^{-5}	5	V	highly toxic	hazardous	V
≤ (1:64)	$\leq 2^{-6}$	≥ 6	VI	extremely toxic		VI

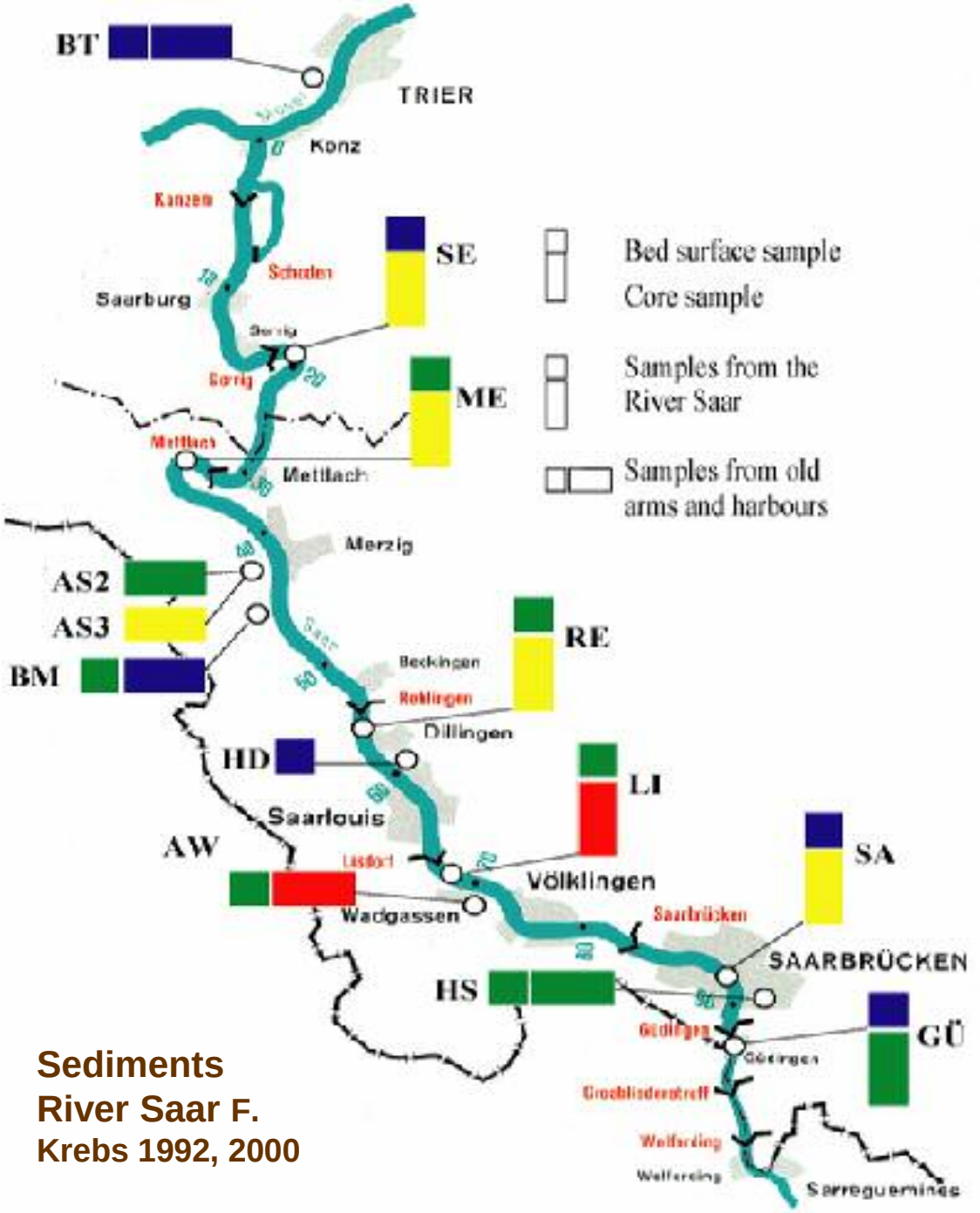
Colour coding and Managementcategories:

blue = unpolluted

Green = uncritically polluted

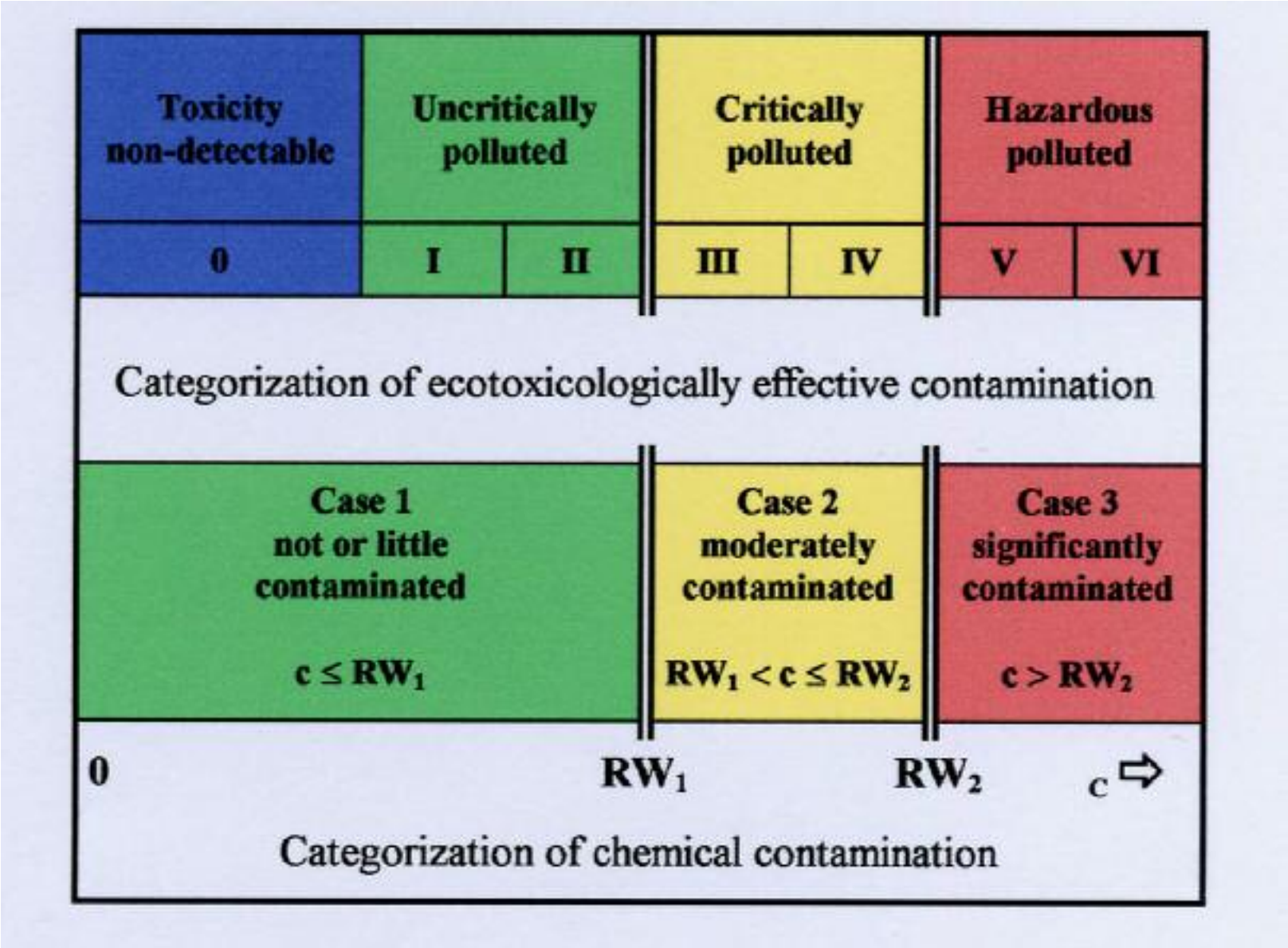
Yellow = critically polluted

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 concequences:

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
- biomarkes, 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**

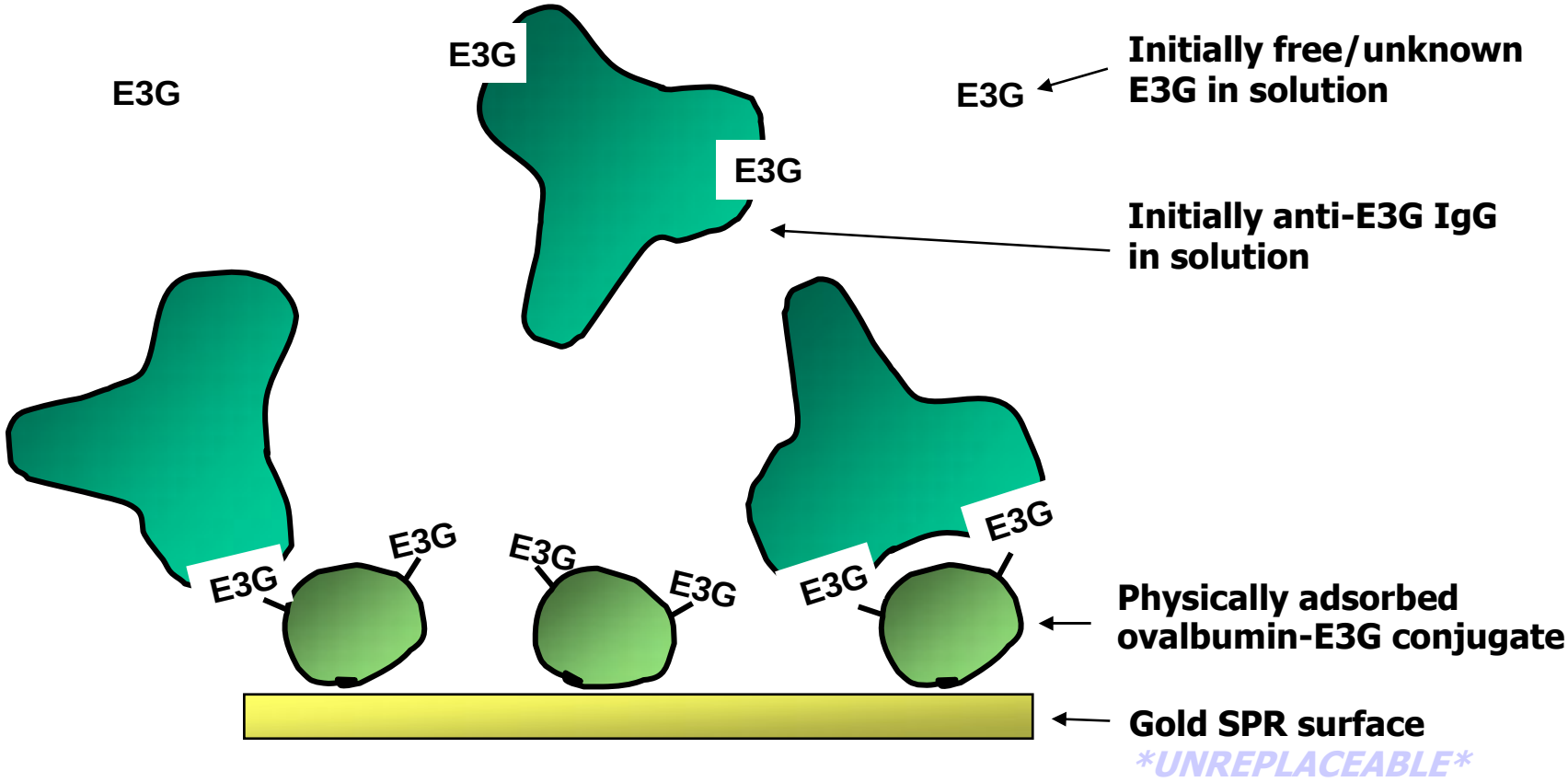
New emerging ISO Standards

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

For sampling and evaluating sediments:

Use the advantages of the existing international Monitoringstations (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)

(6) Monitoring in 2004 - impact assessment in 2006


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]

	Aquatic communities	Fishery	Drinking Water supply	Sediments	Water Quality Objectives
	[µg/l]	[µg/l]	[µg/l]	[µg/kg dw]	[µg/l]
2-Chloroaniline	0.1	n.r.	1.0	n.r.	0.1
4-Chloroaniline	0.01	n.r.	0.1	n.r.	0.1
1,4-Dichlorobenzene	10	r.	1	n.r.	1
Hexachlorobenzene	0.001	0.001	0.1	40	0.001
Hexachlorobutadien	0.01	r.	1	n.r.	0.01

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).

	Aquatic communities		Fisheries	Drinking Water supply	Sediments (susp. solids)
	[mg/kg dry weight]	[µg/l]	[µg/l]	[µg/l]	[mg/kg dry weight]
Pb	100	3.4	5	40	100
Cd	1.2	0.07	1	5	1.5
Cr	320	10	n.r.	50	100
Cu	80	4	n.r.	50	60
Ni	120	4.4	n.r.	50	50
Hg	0.8	0.04	0.1	1	1
Zn	400	14	n.r	3,000	200

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: 5a Dihydrotestosteron (hAR); 17ß Estradiol (hER)

Sediment-Sample	Sumpter		MC Donnell		ELRA
	hER	hAR	hER	hAR	
36	1	3	0	1	4
37	0	0	3	0	4
38	2	3	4	2	4
39	3	2	3	2	5
40	4	2	3	1	2
41	3	3	2	2	3
42	1	0	4	1	3
43	2	2	4	1	2