# FIELD MONITORING AND EFFECT ASSESSMENT OF EMERGING SUBSTANCES IN THE MARINE ENVIRONMENT: INTEGRATED APPROACHES AND FUTURE CHALLENGES

Dick Vethaak

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## Content

- Review status of integrated methods for chemical-biological effects monitoring of major emerging substances in the marine environment under OSPAR

- Present an integrated method that can be used for preliminary hazard assessment of emerging substances in WFD

- Discuss future challenges and opportunities

# Integrated chemical-biological effects monitoring under OSPAR- why?

- A much more effective assessment of ecosystem health
- Important  for assessment under the Marine Strategy Directive
- Improved scope for interpretation and understanding of monitoring results
- Cost-saving aspects

# Integrated chemical-biological effects monitoring - how?

Preconditions

- Simultaneous measurement of biological effects and chemical parameters, and primary or support parameters
- AQC and assessment tools
- Selection of appropriate packages of chemical and biological methods for monitoring that **explicitly link** each of the chemical determinands with the effects which they may cause

# OSPAR chemicals for priority action for which monitoring strategies have been adopted by OSPAR

- cadmium
- certain brominated flame retardant
- certain phthalates
- clotrimazole
- dicofol (Finland)
- 4-(dimethylbutylamino)diphenylamine (6PPD)
- dioxins and furans
- endosulphan
- lead and organic lead compounds
- HCH-isomers, including lindane
- mercury and organic mercury compounds
- methoxychlor
- musk xylenes and other musks
- nonylphenol/nonylphenol-ethoxylates
- octylphenol
- organic tin compounds
- pentachlorophenol (PCP)
- perfluorooctane sulphonate (PFOS)
- polychlorinated biphenyls (PCBs)
- polycyclic aromatic hydrocarbons (PAHs)
- short-chained chlorinated paraffins
- tetrabromobisphenol-A (TBBPA)
- trichlorobenzenes
- trifluralin
- 2,4,6 tri-*tert*-butylphenol

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# Packages of methods for chemical-biological effects monitoring

Emerging substances reviewed

Organotins, estrogenic substances, PAHs and alkylated PAHs, PCBs,

PC dibenzodioxins and furans, Brominated flame retardants, PFOS, Nanoparticles

Guidelines/literature used

- JAMP Guidelines for Monitoring Contaminants in sediment and biota
- JAMP guidelines for General Biological Effects Monitoring
- JAMP guidelines for Contaminant-specific Biological Effects Monitoring
  - -TBT-specific (gastropods)
  - - metal-specific
  - - PAH-specifc
- Reports of the ICES/OSPAR Workshops on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON)

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# OSPAR JAMP biological effects techniques – review and status

| Technique | JAMP | CEMP<br>cat/status | AQC |
|---|---|---|---|
| **TBT-Specifc Biological Effects** | | | |
| Imposex/intersex in gastropods | Yes | I - Mandatory | Q |
| **PAH-Specifc Biological Effects** | | | |
| CYP1A | Yes | II | Yes |
| PAH metabolites | Yes | II | Q |
| DNA adducts | Yes | II | B-a |
| Liver pathology | Yes | I - Voluntary | Yes |
| | | | |
| **Metal Specific Biological Effects** | | | |
| Metallothionein | Yes | II | |
| ALA-D | Yes | II | |
| Oxidative stress | Yes | II | |
| **Endocrine disruption** | | | |
| Vitellogenin in cod | | | Yes |
| Vitellogenin in flounder | | | |
| Intersex in male flounder | | | B-a |

CEMP Cat I: QA in place; appropiate for Covention-wide assessments

CEMP Cat II: QA not in place; may be used for monitoring although with caution.

B=BEQUALM current; B-a= available via BEQUALM; Q= QUASIMEME current

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# OSPAR JAMP biological effects techniques – review and status

| Technique | JAMP | CEMP | AQC |
|---|---|---|---|
| | | cat/status | |
| **General Biological Effects** | | | |
| **INVERTEBRATES** | | | |
| Whole sediment bioassays | Yes | II | B |
| Sediment pore water bioassays | Yes | II | B |
| Sediment sea water elutriates | Yes | II | |
| Water bioassays OEB/ Tisbe | | II | B |
| | | | |
| Lysosomal integrity NRR mussel | | | B-a |
| MXR/MDR in mussel | | | |
| SFG in mussel | | | B-a |
| AChE in mussel | | | |
| MT in mussel | | | |
| Histopathology in mussel | | | |
| | | | |
| | | | |
| **FISH** | | | |
| AChE | | | |
| Lysosomal stability | Yes | II | B-a |
| CYP1A | Yes | II | B |
| Liver neoplasms | Yes | I - Voluntary | B |
| Externally visible fish diseases | Yes | I - Voluntary | B |
| Reproductive succes | Yes | II | B-a |

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# OSPAR JAMP biological effects technique – review and status

| Short-term bioassays and screening tools | AQC |
|---|---|
| **In vivo water / extracts of water or sediment** | |
| oyster embryo | B |
| mussel embryo | |
| *Tisbe* | B |
| *Daphnia* | B |
| *Nitocra* | |
| *Acartia* | |
| echinoderm embryo | |
| fish embryo | |
| algal growth | B |
| algal PAM | |

| | |
|---|---|
| **In vitro / extracts of water or sediment** | |
| Microtox | intercalibrated B-a |
| Mutatox | |
| YES | intercalibrated |
| YAS | |
| DR CALUX | intercalibrated? |
| ER CALUX | |
| Fish cell lines | |

B=BEQUALM current; B-a= available via BEQUALM; Q= QUASIMEME current

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# Package of methods relevant to monitoring for

## organotins

### Chemistry

Sediments and biota sampled at the same sites and time.

TBT, DBT, MBT in in sediments and in biota

TPhT, DPhT, MPhT in sediments and in biota where appropriate

Passive sampling?

### Biological effects techniques

#### Fish

No specific measurements available.

**General** – Liver histopathology, external fish diseases, reproductive success, lysosomal stability

#### Invertebrates (gastropods)

**Specific** occurrence/severity of imposex/intersex

**General** – Lysosomal stability, histopathology, Scope for Growth.

#### Bioassays

Lethal endpoint toxicity tests not useful.

Sediment assay with Nassarius. Other chronic *in vivo* tests may be relevant

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# Package of methods relevant to monitoring for

## Estrogenic substances

### Chemistry

Sediments/water and biota sampled at the same sites and time.

estrone, 17β-estradiol and 17α-ethynylestradiol, when suspected

alkylphenols and alkylphenol ethoxylates,

phthalates:DBP, DEHP

Passive sampling?

### Biological effects techniques

#### Fish

**'Specific'**
Bile estrogenicity
Plasma Vitellogenin
Gonadal intersex

**General** – reproductive success, lysosomal stability?

#### Invertebrates (mussel)

**Specific** – No specific biological effects measurements available.

**General** – Lysosomal stability?, histopathology, Scope for Growth?

#### Bioassays

Lethal endpoint toxicity tests not useful.

Some chronic *in vivo* tests with marine copepods and Mysids *in vitro* tests such as ER-CALUX, YES.

# Package of methods relevant to monitoring for

## PAH & alkylated PAH

### Chemistry

Sediments and biota sampled at the same sites and time.

PAH: anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1,2,3-cd]pyrene, pyrene and phenanthrene.

Alkylated PAH: C1-, C2-, and C3-naphthalenes, C1-, C2- and C3-phenanthrenes, and C1-, C2- and C3-dibenzothiophenes, dibenzothiophene.

Other PAHs may cause effects and would be useful to aid

Chemistry in sediments and for biota shellfish only.

Passive sampling?

### Biological effects techniques

#### Fish

**'Specific'** – hepatic CYP1A / EROD
PAH bile metabolites
DNA adducts, liver neoplasms, comet assay

**General** – Liver histopathology, external fish diseases, reproductive success, lysosomal stability

#### Invertebrates (mussel)

**Specific** – No specific biological effects measurements available.

**General** - Lysosomal stability, Comet assay, MXR, GST, histopathology, Scope for Growth.

#### Bioassays

Lethal endpoint toxicity tests not useful.

Some chronic *in vivo* and *in vitro* tests relevant i.e DR-CALUX.

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# Package of methods relevant to monitoring for

## PCBs, Polychlorinated dibenzodioxins and furans

### Chemistry

Sediments and biota sampled at the same sites and time.

PCBs - CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, and CB 180
Planar CBs (in biota) - CB 77, 126 and 169

PC dibenzodioxins and furans

Other CBs may cause effects therefore recommend co-planar CBs 105, 156.

Passive sampling?

### Biological effects techniques

#### Fish

**'Specific'** Hepatic CYP1A / EROD, comet assay, macroscopic liver neoplasms

**General** Liver histopathology, external fish diseases, reproductive success, lysosomal stability

#### Invertebrates (mussel)

**Specific** – No specific biological effects measurements available.

**General** - Lysosomal stability, Comet assay, MXR, GST, histopathology, Scope for Growth.

#### Bioassays

Lethal endpoint toxicity tests not useful.

Some chronic *in vivo* and *in vitro* tests relevant such as DR-CALUX.

# Package of methods relevant to monitoring for new emerging substances

Brominated flame retardants

- chemical methods for sediment and biota available (PBDEs, HBCD)
- no specific biological effects methods available. Thyroid hormone receptor assays in fish blood are relevant but lack field testing
- general biological effect measurements, such as induction of CYP1A/EROD activity, lysosomal stability and reproductive success may be appropiate

Perfluorooctane Sulphonate (PFOS)

- chemical methods for water, sediment and biota available
- no specific biological effects methods available
- some ED-relevant endpoints may be appropriate, such as reproductive success.

Nanoparticles

- both chemical analyses and biological effects methodologies are not available.
- general biological effect measurements, such as oxidative stress and embryo-larval bioassay may be appropriate.

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# Some conclusions

- Integrated packages of chemical and biological effects methods appropiate for monitoring specific groups of emerging substances in the marine environment are only partly available and coverage for fish and (selected) invertebrates is incomplete.

- Instead, “general” biological effects methods which are indicative of stress or the health status of organisms, or general toxicity bioassays that are likely to respond to these contaminants can be used.

- There is a need to develop specific biological effects techniques for several (groups of) emerging substances. These techniques when available should be validated and internationally standardized, and existing monitoring such as CEMP should be augmented.

# Passive sampling devices as time integrating tool (1)

A variety of passive sampling devices offer the potential for temporally integrated sampling of emerging Contaminants in water and assessment of their availability in sediments

**Principle of PS**

- Hydrophobic compounds in organisms are mainly accumulated in the body lipid
- Passive sampling mimics the body lipid and when deployed will passively accumulate dissolved compounds
- The higher concentration in the water the higher the uptake

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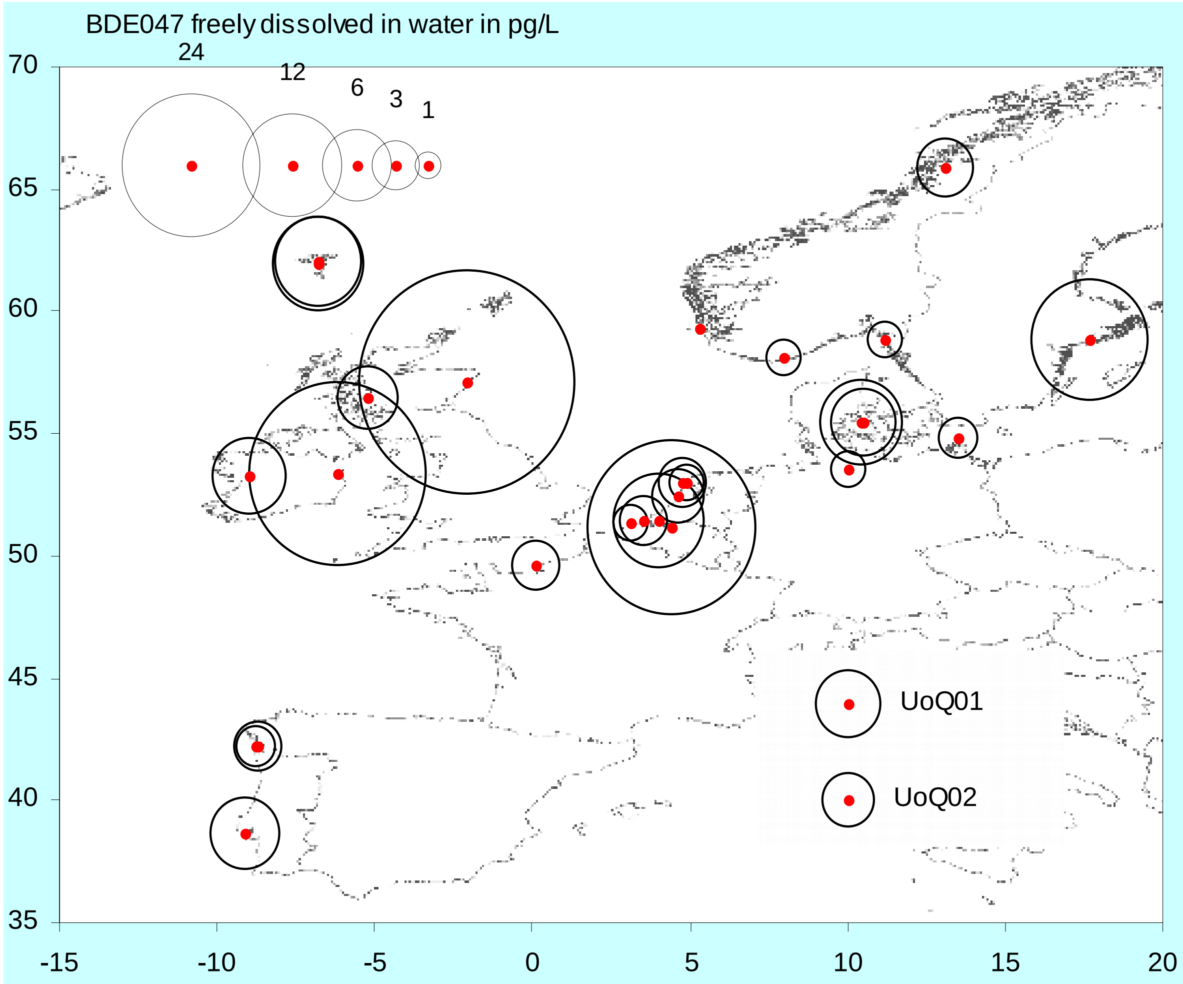
# Passive sampling of BDE047 in water (pg/l)




Source: ICES Passive sampling trial survey for water and sediment (PSTS) 2006 – 2007

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# Passive sampling devices as time integrating tool (2)

**Passive samplers:**

- do not metabolise
- toxic conditions - no mortality
- have no start concentration
- apply to all salinities
- no geographical limitations
- uptake varies with flow conditions

- OSPAR is currently considering some of these tools (e.g. silicone rubber) for application in its monitoring programmes.
- The potential role of passive samplers in WFD monitoring?

# Opportunities for biological effects measurements in WFD monitoring

Biological effects measurements are not required in WFD monitoring, but there are several opportunities

Monitoring in the WFD is an important tool to ensure that the good water status is reached in 2015.

The WFD monitoring programme will consist of both chemical parameters (priority substances, other relevant compounds) and ecological parameters.

The subsequent challenge to be met is two fold:

1. How can chemical and ecological information be linked into an overall insight in the quality of a water body?
2. how do we meet the monitoring requirements in a both cost-efficient and cost-effective way?

# The WFD requires three kinds of monitoring

1. **surveillance monitoring** (status and trend monitoring) assesses whether GES is being achieved. If this is not the case,
2. **operational monitoring** is needed to assess the degree to which the actual status deviates from GES and whether any measures taken have had an effect.
3. **investigative monitoring** (usually project-based). To identify causes and appropriate measures

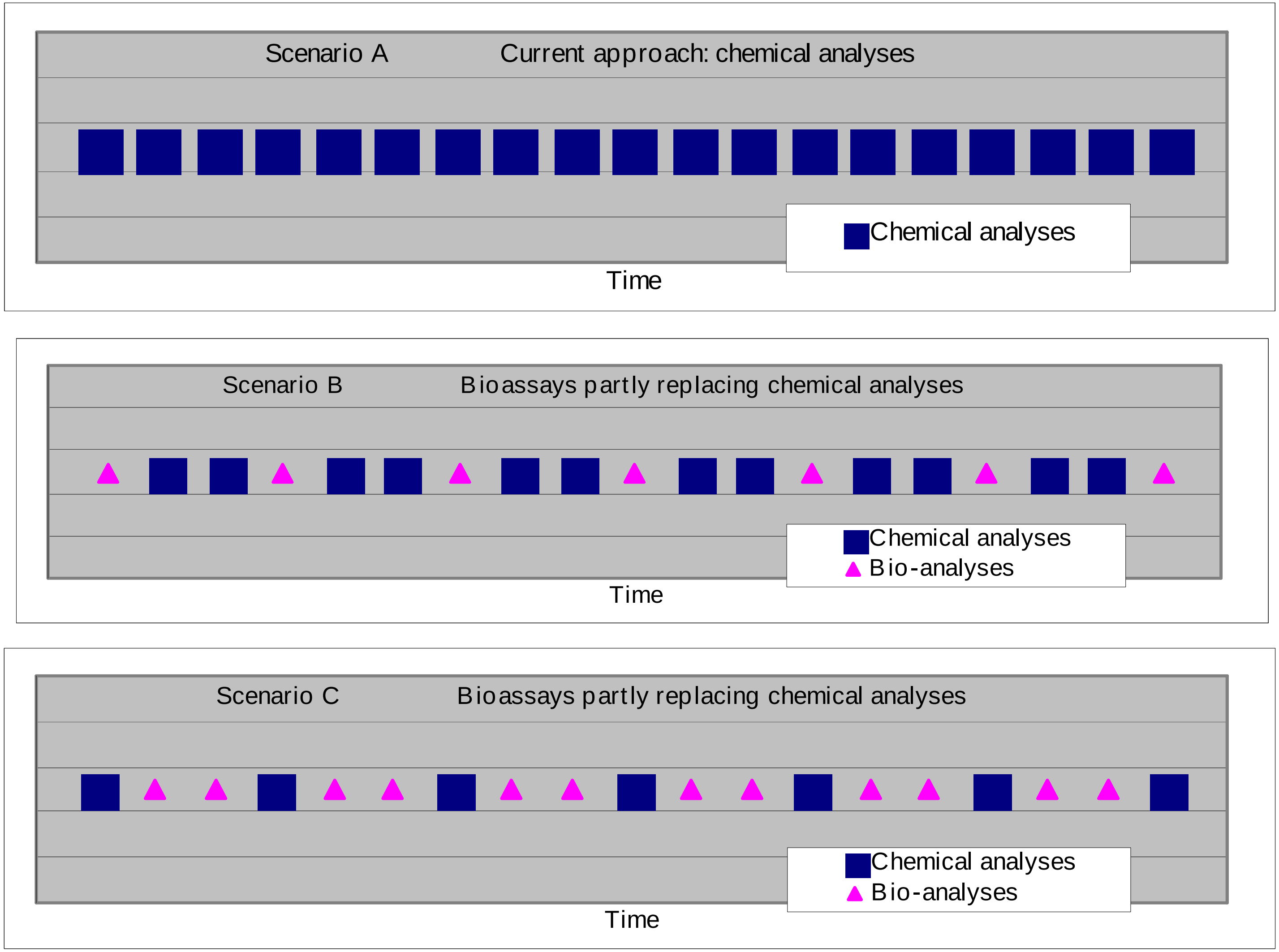
# What is bioanalysis?

**Bio-analysis** is the use of a bioassay or a limited set of bioassays for the rapid screening of effects to indicate hazards of complex mixtures of toxic chemicals and not-analyzed toxicants.

Screenings assays, especially those based on chemical sum parameters, may, at least in part, replace chemical analysis and yield a broader coverage of ecologically relevant compounds than can be achieved by chemical analysis alone.

The purpose here is to reduce monitoring costs and a better indication of hazard.

# Scenarios for the use of bio-analysis in operational monitoring




Proposed model combining bio-analysis and chemical analysis of priority and other relevant compounds at large interval time points, with bioassays applied at smaller interval time points as trend monitoring.

# Selection of bioassays and associated toxic modes of action

| Bio-analysis | Toxic mode of action |
|---|---|
| Microtox® | Broad-spectrum acute toxicity – Cytotoxicity (narcosis) |
| Daphnia IQ | Broad-spectrum acute toxicity – Cytotoxicity (narcosis) |
| Daphnia 24-48 hr | Broad-spectrum acute toxicity – Neurotoxicity |
| Algal growth, 72 hr | Broad-spectrum acute toxicity – Cytotoxicity and specific toxicity mech. |
| Algal PAM | Mechanism-based – Photosynthetic toxicity |
| Umu-C, Mutatox® | Mechanism-based – Genotoxicity |
| DR-CALUX | Mechanism-based - Dioxin-like activity (AhR binding) |
| ER-CALUX | Mechanism-based – Estrogenic activity (ER binding) |

**Selection criteria**

- relevance of toxic modes of action
- duration (acute vs chronic)
- toxicity data
- relatively fast (0.5-72 h) and cheap (€ 40-250)

**Extraction procedure and toxicity testing**

- test run on water/SM extracts prepared for analytical chemistry after solvent exchange
- comparison with chemistry is valid and opens opportunities for EDA
- restricted to organic compounds

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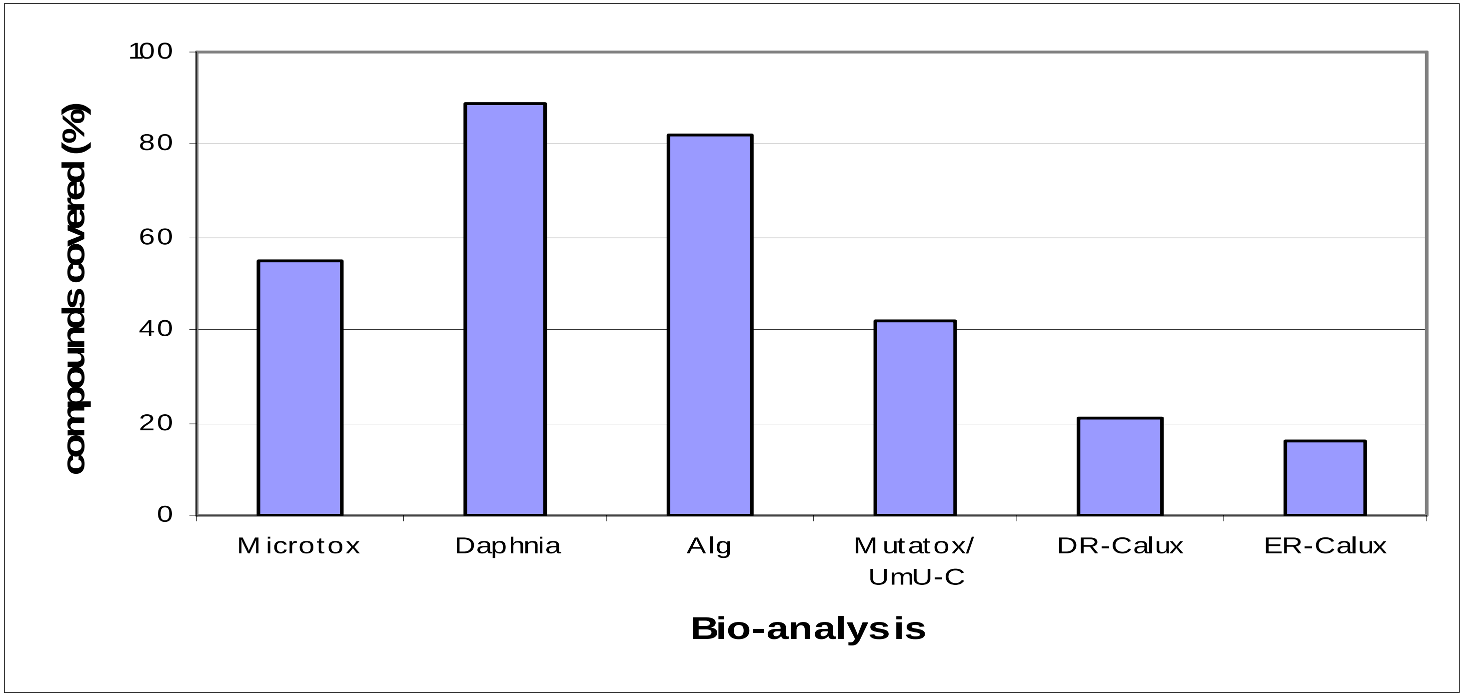
## Relevance of selected bio-analysis to WFD priority compounds




Total % of coverage of WFD priority compounds for each selected bio-analysis based on a compound/bioanalysis response matrix

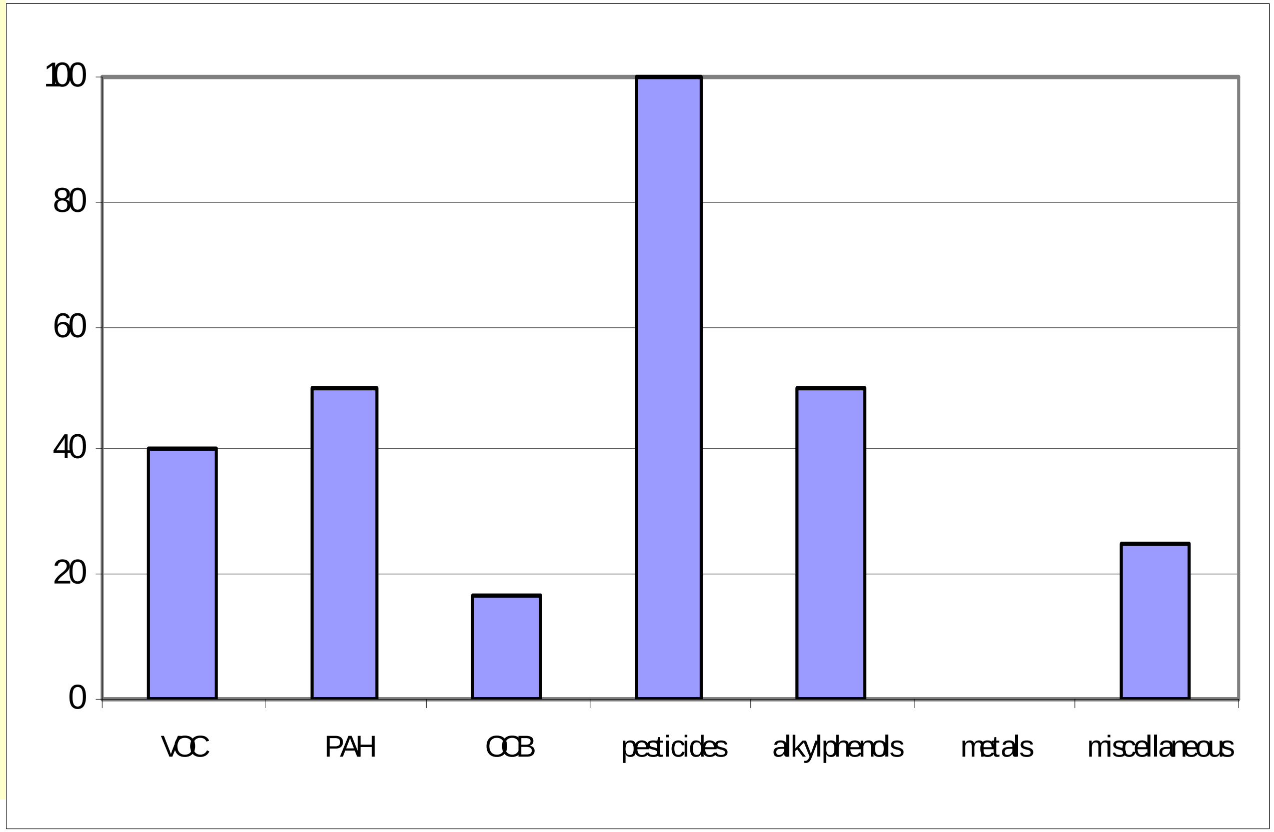
**Findings**

All the WFD priority compounds are covered by one or more of the selected assays. For mechanism-based bioanalysis, the coverage is small.

# Sensitivity of bioanalysis to WFD compound groups

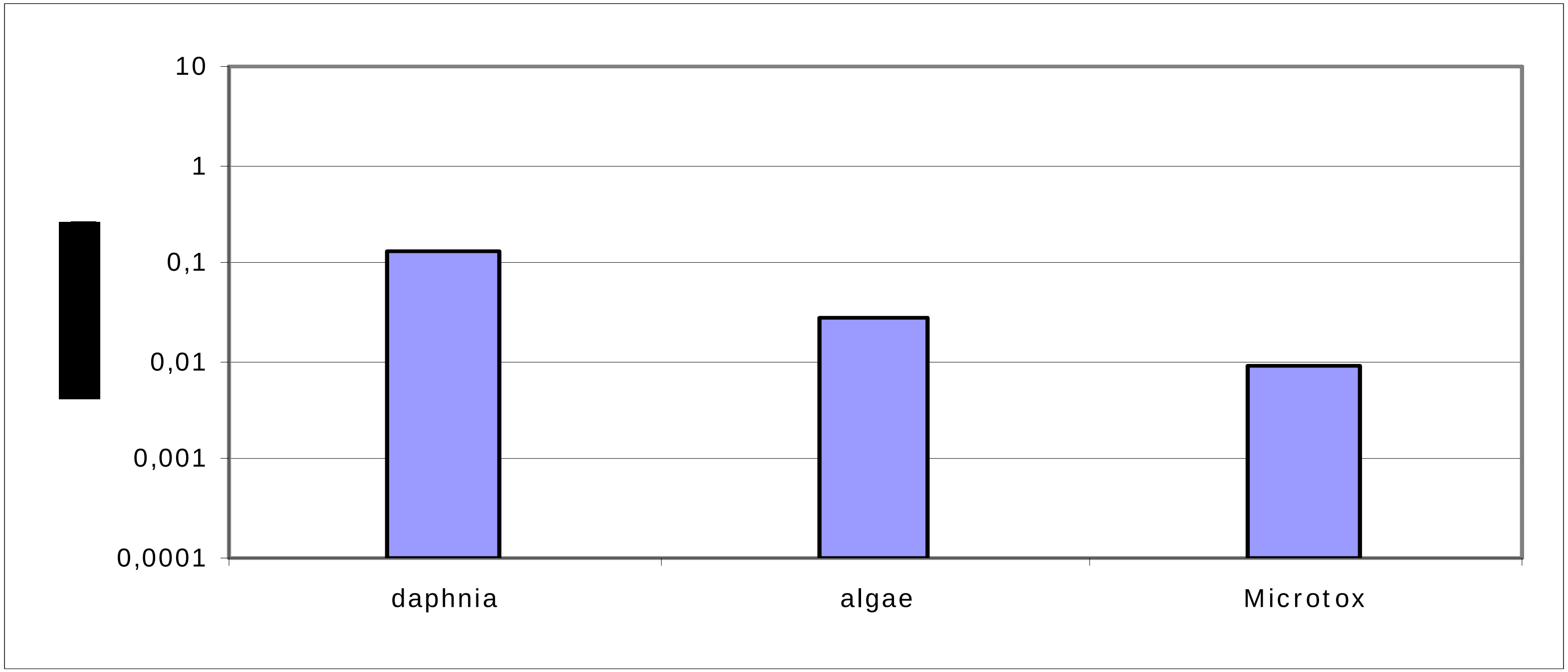
**Findings**

- Pesticides are the most likely group in which chemical analyses may be replaced by bio-analysis
- For some groups , eg alkylphenols, sampling procedure is inadequate
- The other compounds are undetectable due to low sensitivity or lack of available data.




% of WFD compounds that are detectable by bio-analyses using water samples after 1000-fold concentration. Based on Microtox, Daphnia (24-48 h) and algal growth (72-96 h) tests.

# Toxicity response in the artifical sample




Observed toxicity in the artificial sample containing WFD priority pollutants at their maximum permissible level.

**Findings**

ER-CALUX response very low (0.052 EEQ)

no response for UmU-C and Mutatox

DR-CALUX was not performed

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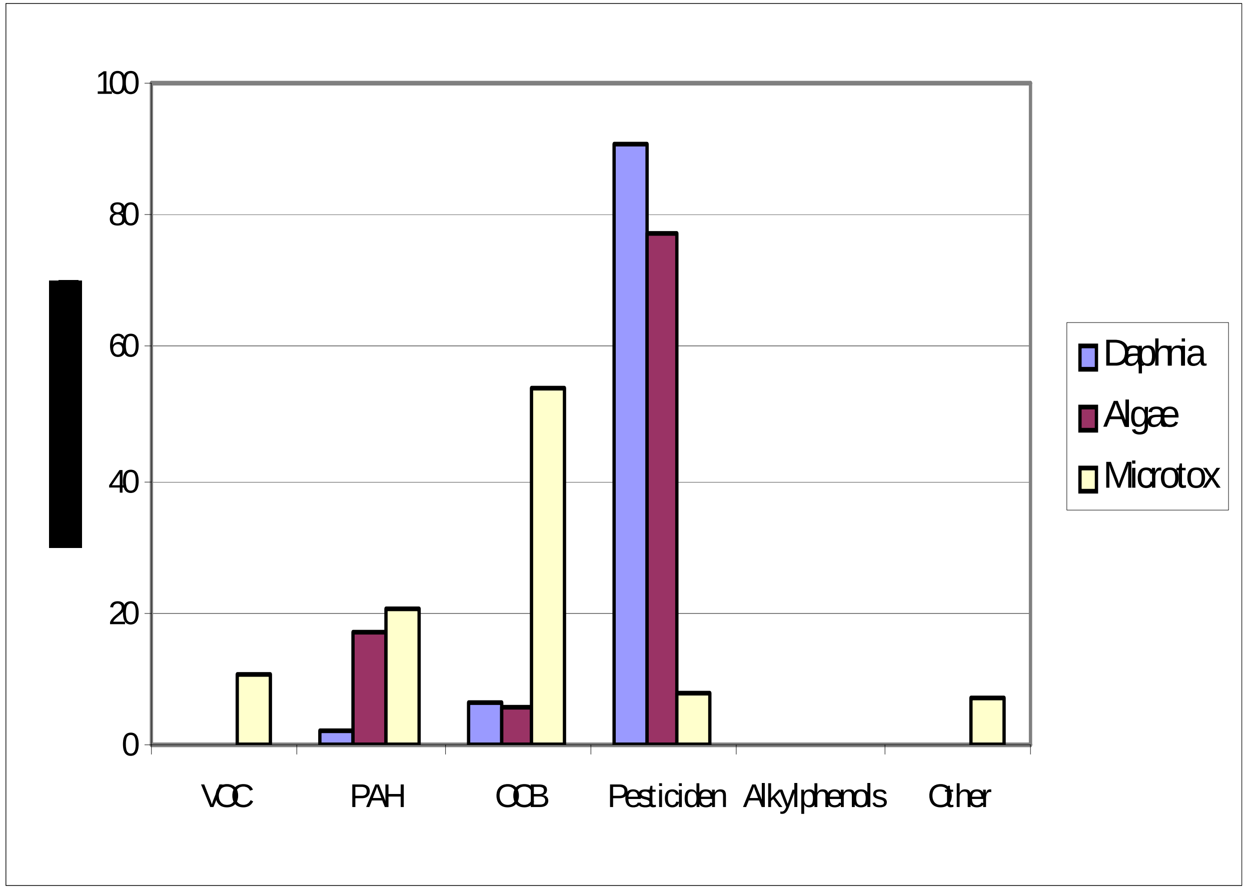
# Sensitivity of bio-analysis to the extracted priority pollutants

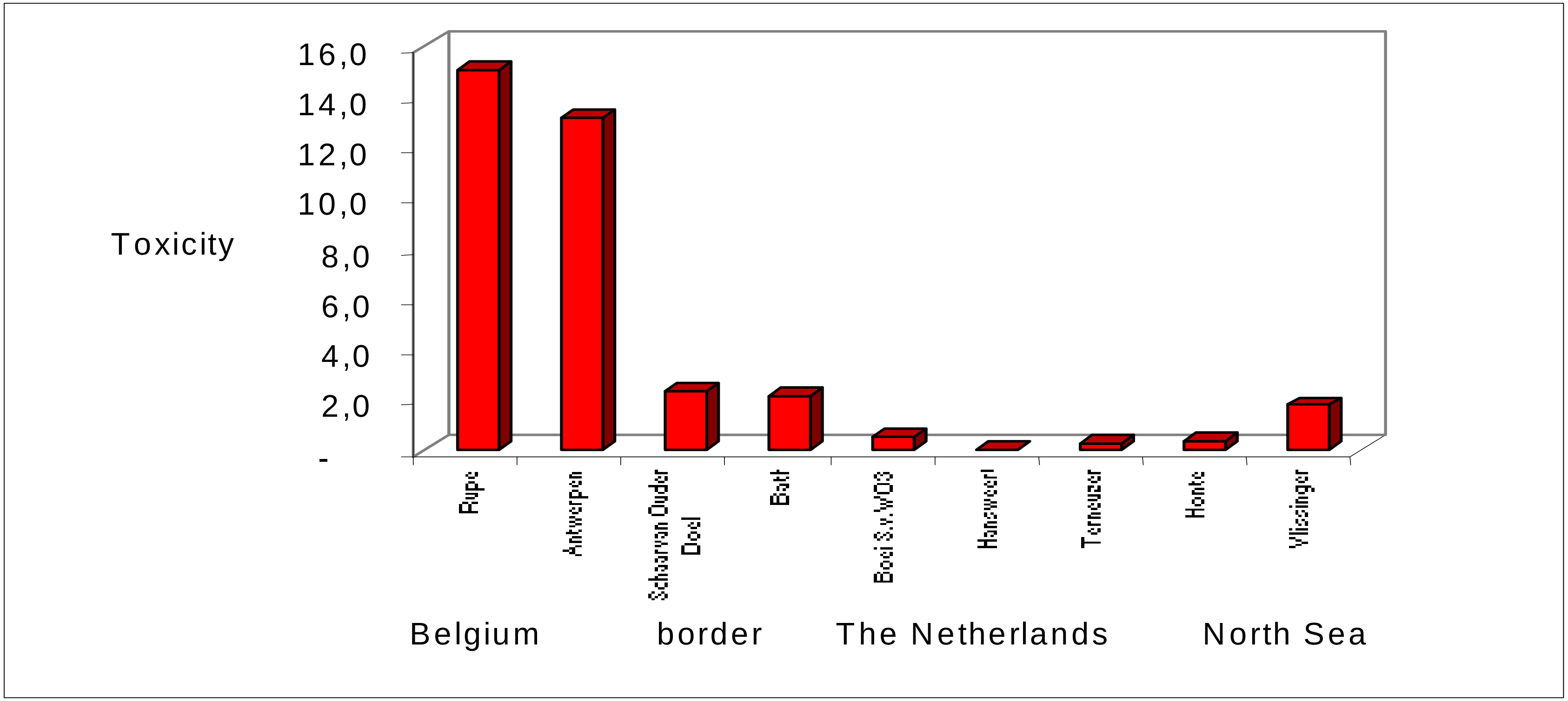
**Findings**

- Pesticides are responsible for the main response by daphnia and algae, but PAHs and OCBs also contribute
- The response of Microtox is divided over various pollutants, except alkylphenols




Comparative responses for Daphnia, algae and Microtox® when exposed to the different groups of priority pollutants in the artificial water concentrate. Based on measured concentrations in XAD concentrate and toxicity data from literature.

# Comparison with field data




Overall toxicity in the Scheldt estuary measured by bioanalysis using Daphnia IQ, Algae PAM and microtox (De Groot et al., 2004).

**Conclusion:** concentrations of priority compounds in water mostly below d.l.
>>> other compounds besides priority pollutants must be responsible

# Preliminary effect assessment using bioanalysis

| location | | Cf (ECf50)* | | | Cf (MTE) (from PAF5) |
|---|---|---|---|---|---|
| | | Daphnia | Algae | Micro-tox | |
| Vlissingen | | 416 | 52 | 15 | 2.0 |
| | | 180 | 56 | 38 | 3.2 |
| | | 403 | 28 | 57 | 4.0 |
| | | 243 | 16 | 84 | 17.2 |
| | | 271 | 15 | 97 | 3.2 |
| | | 271 | 9 | 52 | 1.8 |
| | | 92 | 9 | 50 | 1.6 |
| Antwerpen | | 144 | 2 | 23 | 0.4 |

**expected chronic effect**:
green = NE
yellow = NE<effect< MPE
red = SE

Indication of toxicity in surface water of the Western Scheldt estuary on basis of 3 different bioassay responses allowing a preliminary effect assessment (Maas et al., 2003)

# Opportunities for bio-analysis - summary

- Several selected bio-analyses are sensitive enough to measure effects of priority pollutants and can be used in cost effective monitoring
- Combined bioanalysis and chemical assessment enable the effects of compounds other than the selected priority pollutants to be monitored
- Bioanalysis can be applied to all salinities and have no geographical limitations
- Bioanalysis is logistically and technically feasible, but some further work is needed (eg. extraction methodology)

Reference

Opportunities for bio-analysis in WFD chemical monitoring using bioassays

by Maas and van den Heuvel-Greve. RIZA 2005.053X.

## Conclusions and future challenges

- Integrated packages of chemical and biological effects methods appropiate for monitoring specific groups of emerging substances in the marine environment are only partly available and provide incomplete coverage for fish and invertebrates.
- This underpines the need for an “overall” integrated monitoring package with high effect level stress indicators and different ecosystem components.
- The combined use of bioanalysis and chemical measurements can be used for preliminary assessment and identification of hazard of complex mixtures of toxic and not-analysed toxicants.
- There is a need for consistent pan-European screening programmes designed for hazard assessment, including the application of bioanalysis, passive samplers, novel sensors and micro-arrays, when they become available, and instrumental methods to identify causal compounds.
- In this connection, NORMAN should promote to the European Commission, the additional value of passive samplers and bioanalysis in WFD and their potential role as connective link between WFD and the Marine Strategy Directive. Both methods are generic and can be applied to a wide variety of environments.

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