Effect-based tools for the assessment of priority and emerging contaminants: the EDA-EMERGE European Project

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On behalf of the EDA-EMERGE Marie Curie ITN, FP7-PEOPLE-2011

PRESENTATION OUTLOOK

Effect-based tools

- Water Framework Direct (WFD) context
- Effect-Directed Analysis (EDA) context

EDA-EMERGE project

- EDA-EMERGE project overview and strategy
- EDA: biological & chemical aspects and WFD context

Project outcomes

- Biotesting strategy in EDA & EDA case studies
- Bioassays on the regulatory level: WFD priority substances

Technical Report on **Aquatic Effect-Based Monitoring Tools**

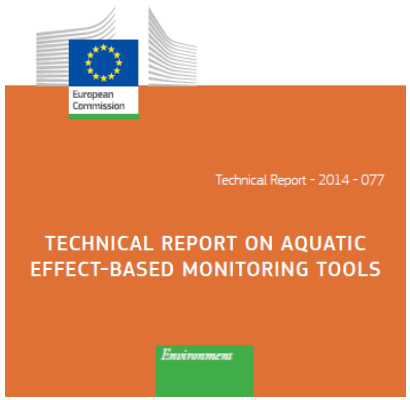
Technical Report on Aquatic Effect Based Monitoring Tools.

Technical Report 2014-077. EU Commission. doi: 102779/7260.

Effect-based tools described in three main groups:

- ✓ **Bioassays** *in vitro* and *in vivo* bioassays that measure the toxicity of environmental samples
- ✓ **Biomarkers** biological responses at individual level (or below) observed in field exposed organisms
- ✓ **Ecological indicators** biological responses at higher organisation levels, e.g. population and community

Technical Report on **Aquatic Effect-Based Monitoring Tools**



Technical Report on Aquatic Effect Based Monitoring Tools.

Technical Report 2014-077. EU Commission. doi: 102779/7260.

Objectives of effect-based tools in **WFD** context:

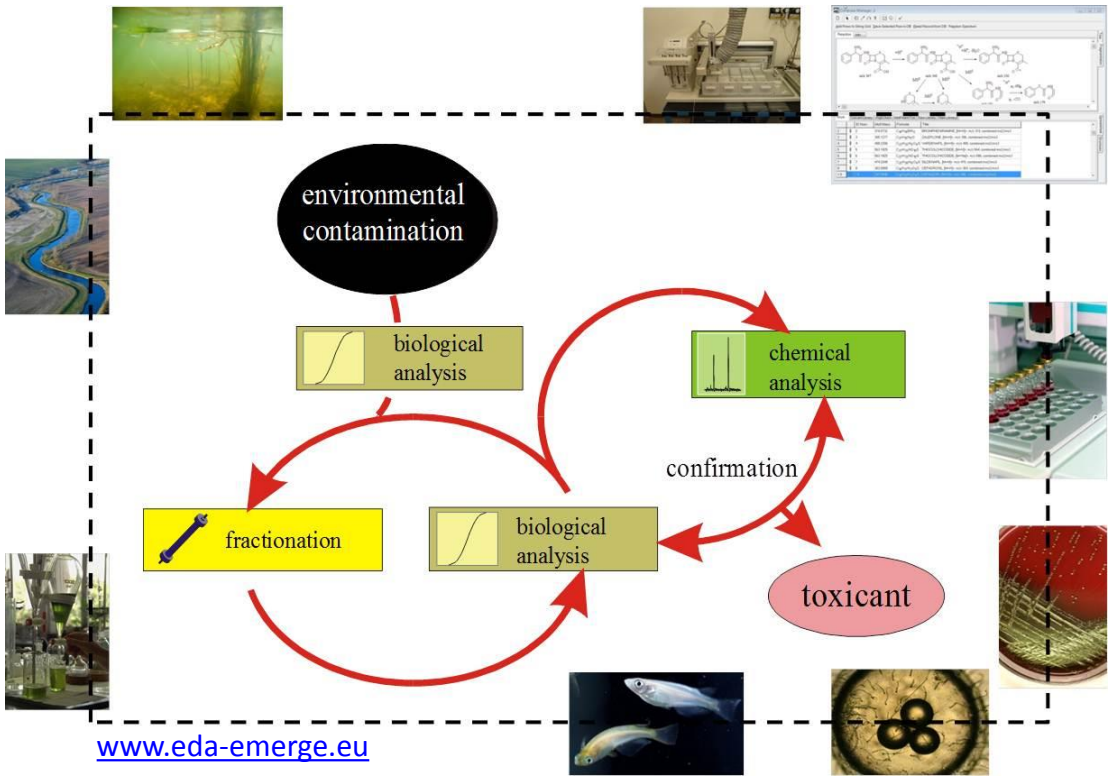
Screening tools: pressures & impacts **assessment**, **prioritise** water bodies investigation

Early warning systems

Effects from **mixtures** and compounds **not evaluated by chemical analysis**

Additional support to **chemical** & **ecological** monitoring in water and sediment assessment

Effect-Directed Analysis (EDA): integrates **BIOASSAYS**, physico-chemical **FRACTIONATION** and **CHEMICAL ANALYSIS** in a sequential procedure to identify the unknown **TOXICANTS** in the sample that cause the main bioassay response



Brack *et al.* Env Sci Europe 2013, 25:18

Bioassays in EDA

- **Identification** of toxic samples
- **Guidance** of fractionation
- **Confirmation** of toxicity

Technical Report on **Aquatic Effect-Based Monitoring Tools**

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EDA (Effect-directed Analysis) in **WFD** context**:**

Investigative monitoring

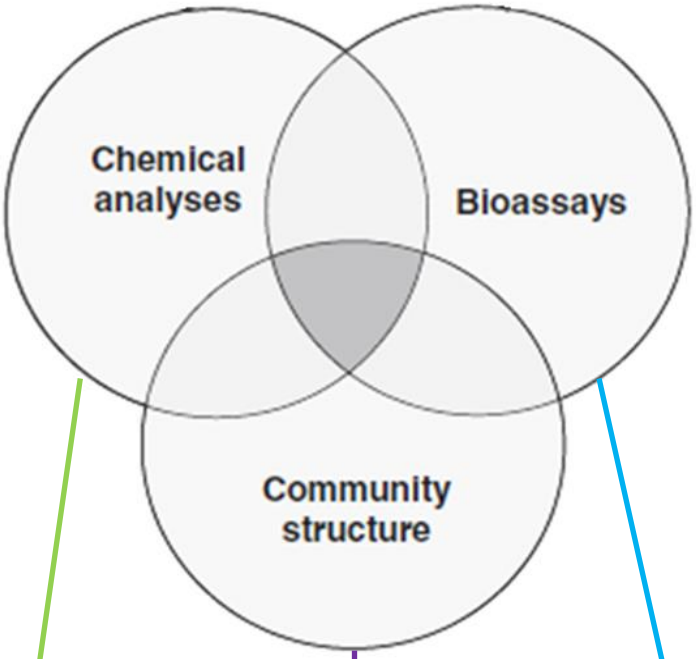
- at selected sites of particular interest or with conspicuous effects

Link ecological status with **contamination**

- establish **cause-effect** relationships
- **target mitigation** measures

EDA + Triad Approach

Hecker & Hollert 2009. Environ Sci Pollu Res 16: 607-613.



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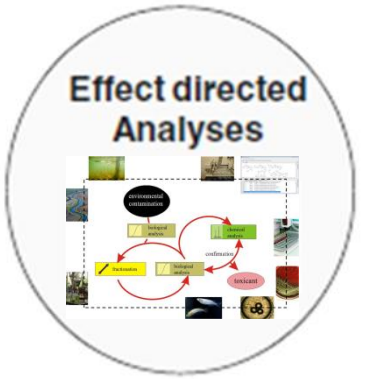


→**chemical** contamination

→**ecological** status

→**(eco)toxic** potential

→**identification** of contaminants responsible for effects

WFD monitoring

Effect-based tools

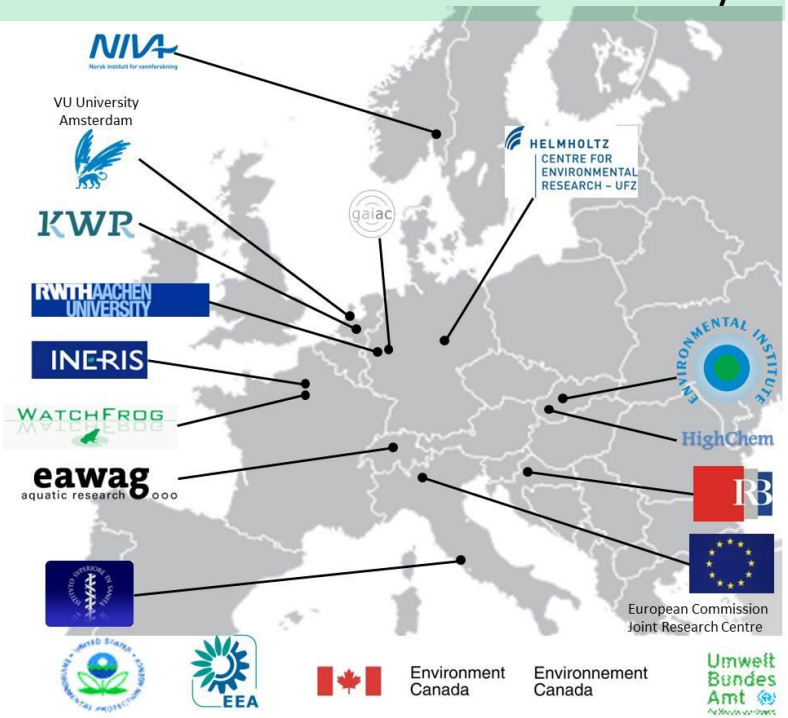
EDA-EMERGE - Novel Tools in Effect-Directed Analysis to Support the Identification and Monitoring of Emerging Toxicants on a European Scale

Marie Curie FP7 Initial Training Network (ITN) coordinated by Dr. Werner Brack, UFZ Leipzig

www.eda-emerge.eu

"EDA-EMERGE helps meet the **requirements** of the EU **Water Framework Directive** (WFD) and creates a **basis** for **future regulations** to protect and use water resources sustainably"

Aim: "to train a new generation of scientists with the **interdisciplinary skills** that meet the major **challenges** in **monitoring, assessment and management** of pollution in EU rivers"



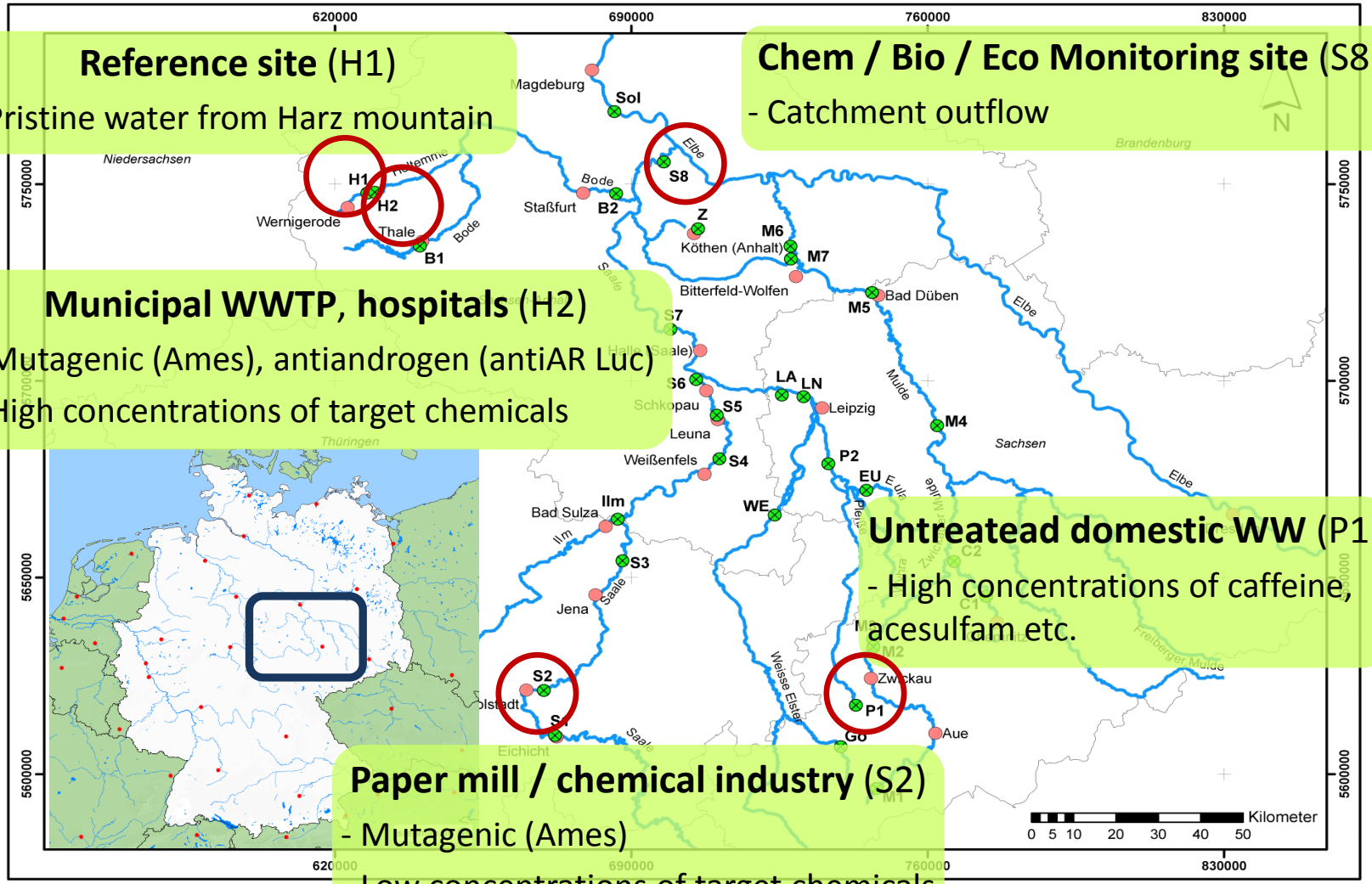
Brack *et al.* 2013. Env Sci Europe 2013, 25:18

EDA-EMERGE STRATEGY

- New generation of **EDA approaches** →identify emerging toxicants in surface & drinking water
- Integration of **innovative bioanalytical tools** with **powerful fractionation and cutting edge analytical and computational structure elucidation tools**
- Extensive training courses
- Joint **European demonstration program (EDP)** and higher tier **EDA case studies**

(adapted from Zuzana Rabova, EI, SK)

Selection of sampling sites (Germany)



Reference site (H1)

- Pristine water from Harz mountain

Chem / Bio / Eco Monitoring site (S8)

- Catchment outflow

Municipal WWTP, hospitals (H2)

- Mutagenic (Ames), antiandrogen (antiAR Luc)
- High concentrations of target chemicals

Untreatead domestic WW (P1)

- High concentrations of caffeine, acesulfam etc.

Paper mill / chemical industry (S2)

- Mutagenic (Ames)
- Low concentrations of target chemicals

(adapted from Tobias Schulze, UFZ, DE)

European Demonstration Program & EDA case studies

Selection of sampling sites

Sampling & pre-fractionation

Chemical analysis

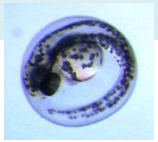
Bioassay battery

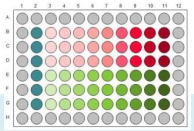
Evaluation of chemical & bioanalytical data

Effects related to:
- Target chemicals?
- Priority substances?

Unknown toxicant(s)
→ **EDA case study**
Newly developed bioanalytical, fractionation, analytical and structure elucidation tools

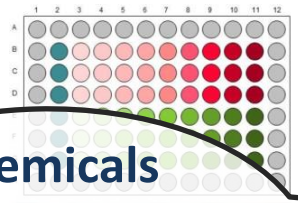
Algae test	Freshwater algal growth inhibition test with unicellular green algae
FET test	Fish Embryo Acute Toxicity (FET) Test with *Danio rerio*
(anti)ER-Luc assay	Cell-based receptor reporter gene assays: estrogen (ER), androgen (AR) and glucocorticoid (GR)
(anti)AR-Luc assay	
GR-Luc assay	
Ames	Ames fluctuation assay
AChE inhibition assay	Acetylcholinesterase inhibition assay



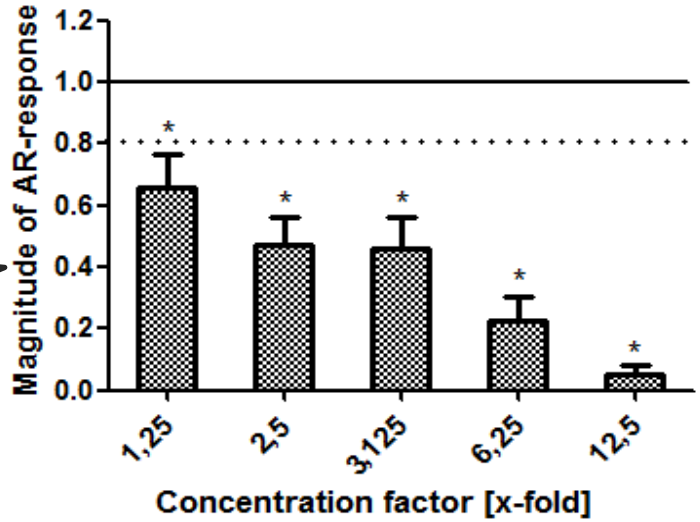


ANTIANDROGENICITY EDA CASE STUDY

Holtemme river ⟶ antiAR Luc cells & cytotoxicity ⟶ **antiAR hotspot**

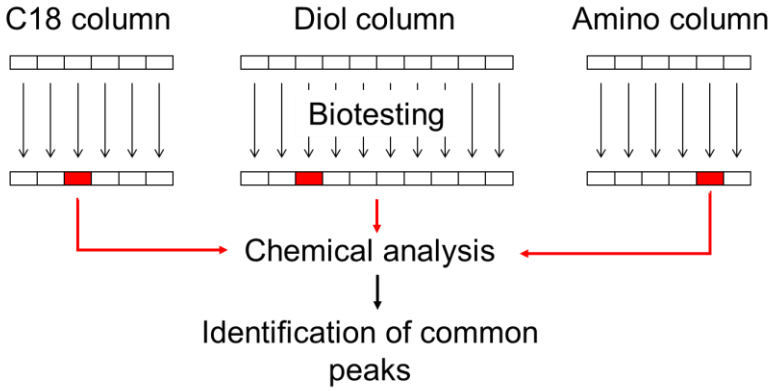


Chemicals causing antiandrogenic effect…?



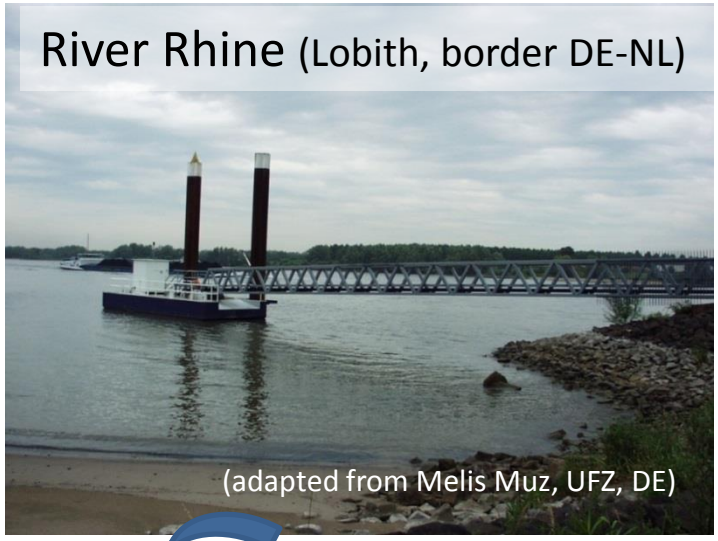
Antiandrogenicity EDA case study (ongoing work)

- **Bioassay miniaturization & biotesting strategy** - antiAR-CALUX assay and cytotoxicity assays (RWTH)
- **Fractionation** - parallel orthogonal separation; **Chemical analysis** - LC-HRMS/MS; **non-target workflow** (UFZ)



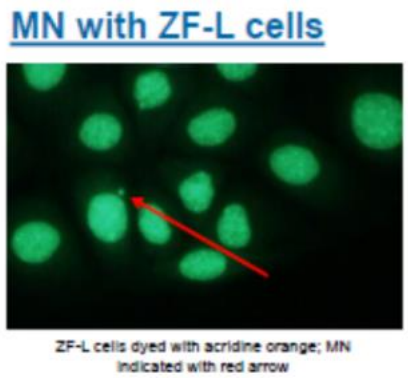
(adapted from Matthias Muschket, UFZ, DE)

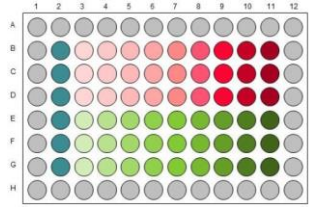
MUTAGENICITY EDA CASE STUDY



Significant mutagenicity Ames TA98+S9

⇒ Hypothesis: Aromatic Amines



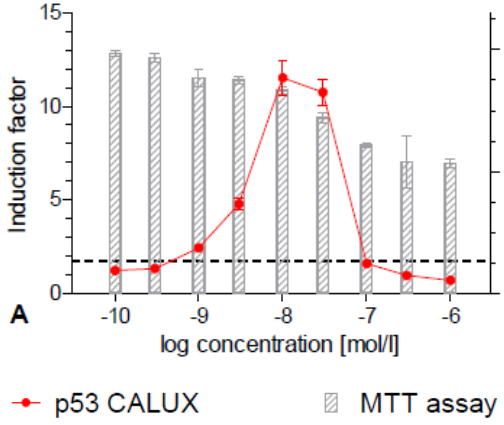


Mutagenicity EDA case study (ongoing work)

- **Biotesting strategy**

- Ames Fluctuation Assay different strains (UFZ)
- Micronucleus in V79 and in zebrafish cell lines (RWTH)
- p53-CALUX cell-based reporter gene assay (RWTH)

- RP- **Fractionation** ; **Chemical analysis** and **non-target workflow** (UFZ)

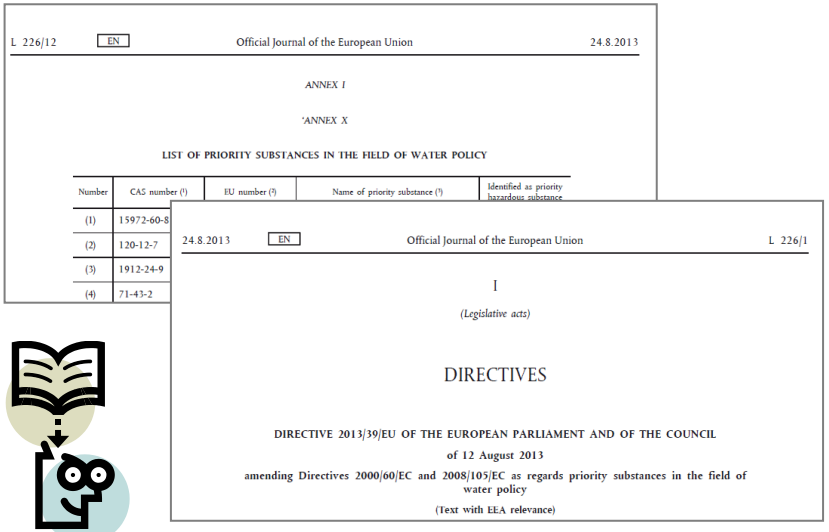


REVIEW: BIOASSAYS VS WFD PRIORITY SUBSTANCES

What is the contribution of **bioassays** for the **monitoring of priority substances ?**

Literature review: Toxic effects of **WFD priority substances** identified by **mechanism-specific** bioassays

→**Support** the integration of **mechanism-specific** bioassays in water quality monitoring


L 226/12 EN Official Journal of the European Union 24.8.2013

ANNEX I

'ANNEX X

LIST OF PRIORITY SUBSTANCES IN THE FIELD OF WATER POLICY

Number	CAS number (1)	EU number (2)	Name of priority substance (3)	Identified as priority hazardous substance
(1)	15972-60-8			
(2)	120-12-7			
(3)	1912-24-9			
(4)	71-43-2			

24.8.2013 EN Official Journal of the European Union L 226/1

I

(Legislative acts)

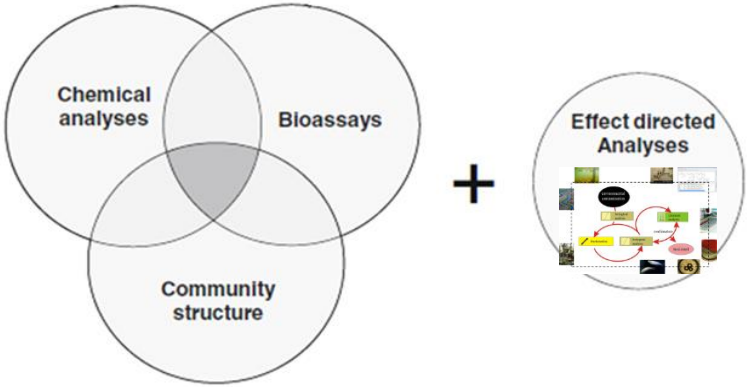
DIRECTIVES

DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 12 August 2013

amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy

(Text with EEA relevance)

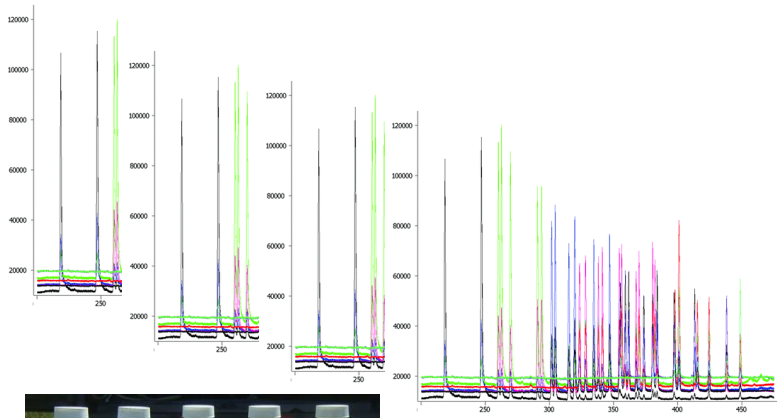


Review: Bioassays vs WFD priority substances

Example of substances → Dioxins

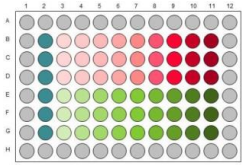
37) Dioxins and dioxin-like compounds

(9) This refers to the following compounds:
7 polychlorinated dibenzo-p-dioxins (PCDDs):
10 polychlorinated dibenzofurans (PCDFs):
12 dioxin-like polychlorinated biphenyls (PCB-DL):

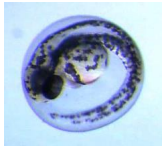


Focant 2004. Analyst.

Toxic effects and respective bioassays



- Mediated via **aryl hydrocarbon receptor** →cell-based reporter gene assay
- Modulation of **cytochrome CYP induction** → EROD assay & CYP expression
- **Teratogenic effects** in fish → fish embryo and early life stage toxicity tests
- Furthermore, some dioxins are suspected **endocrine disruptors** and present evidence of **carcinogenicity /mutagenicity**



→ Bioassays account for **mixture effects** and total **toxicity equivalent** of samples

OECD TG 236 Fish Embryo Acute Toxicity (FET) test (2013)

The OECD TG 236 should be used for generating information on acute fish toxicity whenever possible and, accordingly, be included into the respective pieces of legislation and guidance documents

Strategy to **replace, reduce and refine** the use of fish in aquatic toxicity and bioaccumulation testing. JRC EURL ECVAM. 2014.

Opportunity → Zebrafish embryo bioassays can identify **acute** and **mechanism-specific toxic** effects caused by emerging and priority water contaminants:

- **Acute toxicity**: almost all priority substances
- **Growth:** diclofenac, PFOS, dioxins
- **Skeletal malformations**: dichlorvos, PFOS, dioxins
- **Cardiovascular effects**: brominated diphenylethers, dioxins, PFOS, dichlorvos
- **Neurotoxicity**: atrazine, brominated diphenylethers, cadmium, chlorpyrifos, cypermethrin

- ✓ **Organism-level effects**
- ✓ **Mixture toxicity**
- ✓ **Ecologically relevant toxic effects**

SUMMARY

Effect-based tools

+ WFD:

- Screening, early warning and investigative monitoring
- Able to identify priority substances and emerging pollutants
- Account for mixture and matrix effects
- Information on the mechanism-specific total toxicity equivalent of sample

+ EDA:

- Identification of emerging compounds based on combined chemical analysis and biotesting approaches

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Thank you! ☺ Questions?

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