Effect based tools in a water and marine regulatory framework

– current use and future prospects

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Structure

1. Legislative framework
   - WFD, MSFD, CIS
2. Technical report on EBT
   - Content and conlusions about current WFD use in the 2014 report
3. New CIS EBT activity
   - Deliverables, challenges

- WFD: Water Framework Directive (2000/60/EC) – see also 2008/105/EC, revised by 2013/39/EU on EQS (environmental quality standards) for priority substances
- MSFD: Marine Strategy Framework Directive
- CIS: Common Implementation Strategy – see relevant guidance documents (e.g. no. 3, 7, 19, 25, 27, 28, 32, 33) and technical reports: http://ec.europa.eu/environment/water/water-framework/facts_figures/guidance_docs_en.htm
- EBT: Effect Based Tools

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1. Legislative framework

- Water Framework Directive and Environmental Quality Standards Directive.
  - Assessment of risk mostly done on a substance-by-substance basis. With some exceptions : markers (e.g. PAHs) / group EQS (e.g. dioxins - PBDE).
  - Watch list mechanism : includes groups of pollutants (e.g. neonicotinoids, antibiotics).
- Marine Strategy Framework Directive
  - Descriptors and Good Environmental Status (GES)

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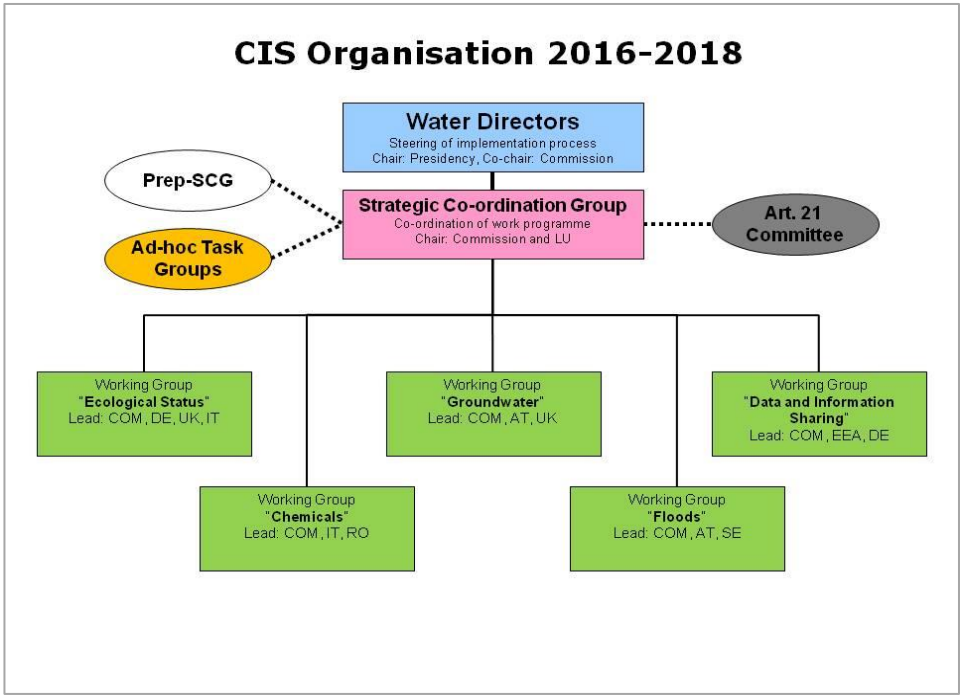
CIS: Common Implementation Policy (WFD)



WD meeting November Bratislava – proposal from COM

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Proposal for the WFD review as regards chemicals:

"In the WFD review, a more holistic approach, taking into account the presence of mixtures of chemicals acting together (for example through the use of effect-based tools in addition to group EQSs), could be considered, to provide a more accurate assessment of risks and a more appropriate targeting of monitoring and measures (see Annex II for further information)."

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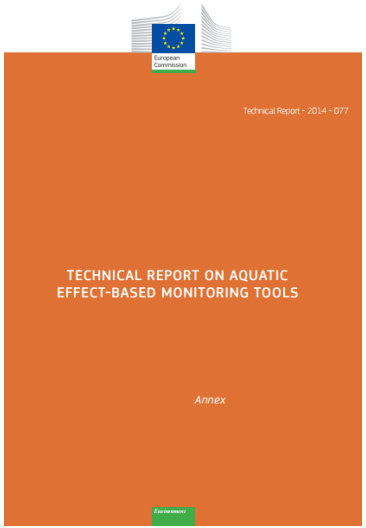
CIS Work Programme 2016-2020

- Among the main tasks of the WG Chemicals :
  - Dedicated activity on effect-based tools (EBT)
  - Main objective: examine and further document the possible implementation of effect-based tools/methods for monitoring and assessment in the WFD context, bearing in mind their possible application under the MSFD. Also includes consideration of links between ecological and chemical status.
  - Link with the technical report on aquatic effect-based monitoring tools published in 2014 under the CIS, and past and on-going projects.

2. Technical report on EBT + annex (2014)



- Aim of the report:

*Describe state of the art of **aquatic** effect-based **monitoring** tools; from a Water Framework Directive perspective.*

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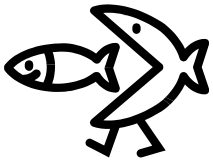
Why Effect Based Tools?



- **Chemistry (>EQS?, Trend?)**
  - Risk based (also secondary poisoning, human health)
  - Which substances? Bioavailable? Combined exposure?
- **Toxicology**
  - Risk based, combined exposure, Bioavailable
  - Which stressor/s? Human health? Sec poisoning?
- **Ecology (structure/function)**
  - Combined exposure, Bioavailable
  - Which stressor/s? ”Late response” Human health? Sec poisoning?

Different categories of tools (ch 1.2.; ch 4, 5)

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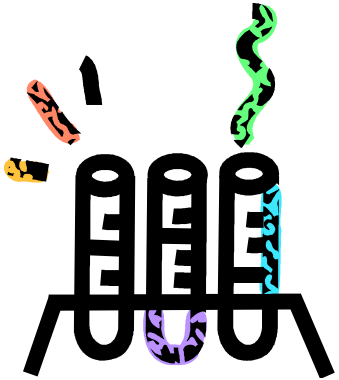
Bioassays, measure the toxicity of environmental samples

- in vitro
- in vivo

Biomarkers, biological responses at individual level or below, observed in field exposed organisms

- exposure-effect
- specific- general

Ecological indicators, (BQE, biological quality elements); higher biological organisation levels (population, community)



In vitro assays, general points

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- Related to certain **mode of action (MoA)**
  - Examples: estrogenicity, androgenicity, Ah (”dioxin receptor”) activation, thyroid hormone disruption, genotoxicity
- Results generally expressed on **chemical equivalent** basis
  - Examples: TCDDeq, E2 eq, benzo(a)pyrene eq…
- **Standards** available
  - Examples: Ames, EROD, umuC, Micronucleus, VTG, Estrogenicity assays
- **Low costs, small scale, short duration, any samples can be analysed**:

High throughput applications, screening (of surface water and effluents)

Table 4.1. *In vitro* assays that were nominated for monitoring purposes during a Swedish Workshop (W), recommended for WEA assessments by COHIBA (C) or OSPAR (O), and initially selected for evaluation regarding high throughput screening and EDA purposes in the MODELKEY project (M[39]). The table also includes information about the type of compounds (mode of action) the assay responds to.

Name/s of assay	Workshop/ COHIBA/Modelkey	Mode of action/endpoint
AR CALUX (anti-)	W, **M**	Androgen receptor (activation or blocking)
DR CALUX	W, **M**	AH receptor binding
ER CALUX[40] (anti-)	W, **M**	/Alpha and beta/ oestrogen receptors
GR CALUX (anti-)	W	Glucocorticoid receptor
PAH CALUX	W, M[41]	AH receptor binding
PR CALUX	W	Progesterone receptor
Acetylcholinesterase inhibition assay	W	Inhibition of acetylcholinesterase activity
Carboxylesterase inhibition assay	W	Inhibition of carboxylesterase activity
Ames	W, **M**, O	Genotoxicity: Mutations[42]
umuC	W, **M**, C	SOS response to DNA damage[43]
TTR-binding	W, **M**	Competition with thyroid hormone for binding to TTR (transport protein)
TRb CALUX	W	Thyroid receptor beta
EROD	C	EROD induction
YES	C, **M**	ER receptor
YAS	C, **M**	AR receptor
P-53 accumulation	(M)[44]	Genotoxicity
Green screen	(M)[45]	Genotoxicity
RYA	**M**	ER receptor
ABC assay	**M**	Antibiotic activity

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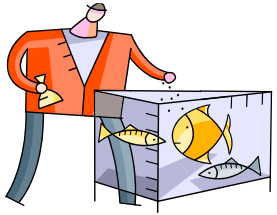
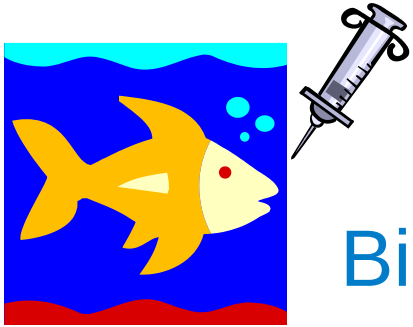


In vivo assays

- Whole organism level response (lethality, growth, reproduction…)
  - More complex system studied
- Short/Long term exposures
  - Short term: requires sample preconcentration (surface water), certain effects excluded
  - Long term: increase in costs
- Large number of standards available (but primarily developed for chemicals testing)

Toxic pressure (areas/trends), Contaminated sediment (tier II)



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Biomarkers

Exposure:
Early warning

Effect: higher ecological relevance

Specific:
indicates cause

General:
covers more substances/ pressures

Batteries are generally better,
*Combine with biota monitoring for **integrated monitoring***
*Relation in vitro assays – biomarkers (**source identification possible**)*

Biomarker	Description	Responds to	Marine assessment criteria available (ICES)	Integrated monitoring component (ICES)	Indicator (Regional Seas Conventions)
EROD activity	Biotransformation enzyme induced by planar hydrocarbon	PCBs, PAHs and dioxin-like compounds	BAC	Core in fish	OSPAR cand
Acetylcholinesterase activity (AChE)	Enzyme implicated in nervous transmission	Organophosphates, carbamates and similar molecules	BAC and EAC (both mussels and fish)	Core in fish and mussels	
Vitellogenin (VTG) in male fish	A precursor of egg yolk, normally synthesized by female fish	Oestrogenic endocrine disrupting compounds	BAC	Core in fish	
Metallothionein (MT)	Metal scavenger implicated in protection against oxidative stress	Heavy metals and inducer of oxidative stress	BAC (mussels only)	Additional in mussels	
Amino-levulinic acid deshydratase (ALAD)	Enzyme implicated in amino-acid metabolism	Lead exposure	NO	NO	
Lysosomal stability	General health, lysosomes play a key role in liver injury caused by various xenobiotics	Several classes of pollutants, including PAH, inducer of oxidative stress, metals, organochlorines	BAC and EAC[59]	Additional in fish, core in mussels	OSPAR cand, HELCOM preCore
DNA adducts	Alteration of DNA structure able to disturb DNA function	Genotoxic compounds including PAHs and other synthetic organic	BAC and EAC	Additional in fish	

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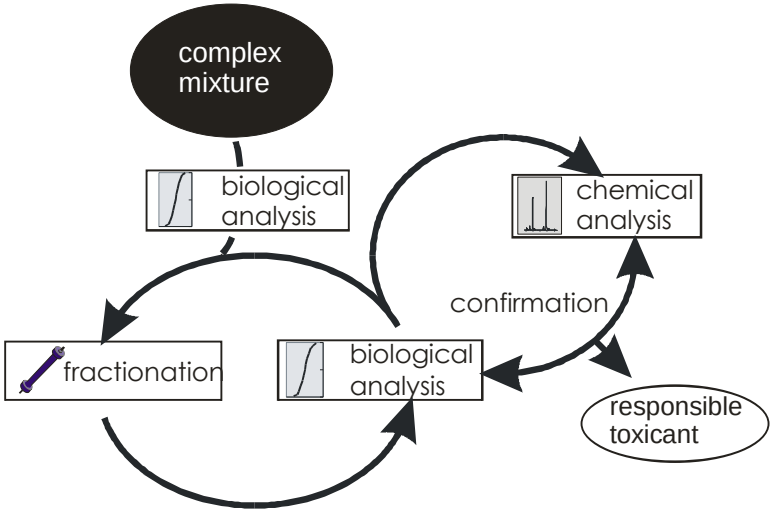
Chemistry+Toxicology: - to identify cause/s



EDA Effects Directed Analys

TIE Toxicity Identification Evaluation

- 3 tiered process;
- 1st: rough characterisation

High throughput tests necessary to reduce costs

Biomarker effects need first to be confirmed in bioassays

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Ecological indicators (BQE)

- Four (of totally 300) registered tools reported to respond to hazardous substances (see also Birk et al 2012):
  - Infaunal Quality Index (IQI; UK)
  - Quality Index Ver2 (DK)
  - Multivariate AZTI Marine Biotic Index (M-AMBI Spain; Muxika et al. 2007)
  - Benthic Opportunistic Annelida Amphipoda Index/Benthic Opportunistic Polychaete Amphipoda Index (FR; Dauvin and Ruellet, 2007; 2009).
- Some novel tools:
  - Multimetric index (functional traits; non specific)
  - SPEARpesticides, SPEARorganic, NemaSPEAR
  - PICT (community function; highly specific)

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Annex includes e.g.

- case studies
- native *vs* pretreated water samples, passive sampling
- Standards/Guidance available
- fact sheets for certain biomarkers and *in vitro* assays
- **biomarkers and *in vitro* assays *vs* mode of action**
- assessment criteria for effect based tools
- overview of existing DNA microarrays

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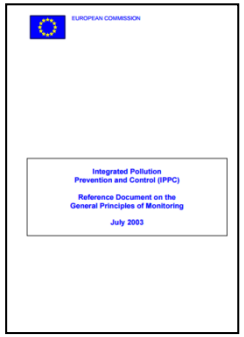
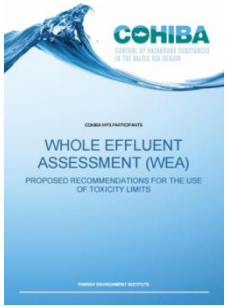
Current European use of EBTs and main drivers

- Characterisation of complex mixtures (pressures – risk assessment)
  - WEA (campaigns)
  - dredged sediment
  - contaminated sediment/sites
- Cover many substances at lower costs and sometimes better LOQ
  - Screening (e.g. Ah receptor binding, estrogenicity)
- Assess quality
  - identify regions of decreased quality (incl operator recipient control)
  - early and immediate warning (alarm system) for drinking water protection (”biomonitors”)
  - early but long term warning (reference areas – trend studies)
  - MSFD indicators (marine status)





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WEA

- BREF (2003) on monitoring: *"During the last few years biological test methods/systems have raised more and more interest. Fish/fish egg test, daphnia test, algae test and luminescent bacteria test are all common test methods for the toxicity assessment of complex waste water streams. They are often used to obtain additional information to the information that can be gained from sum parameter measurements (COD, BOD, AOX, EOX...). With toxicity tests it is possible to asses the possible hazardous character of waste water in an integrated manner and to asses all synergistic effects which may occur because of the presence of a lot of different single pollutants. Apart from the possibility of using the toxicity tests to estimate potential hazardous effects on the ecosystem/surface water these tests can help to protect or to optimise biological waste water treatment plants. Toxicity tests, when used in combination with direct measurements of specific substances and with the measurements of sum parameters, are increasingly becoming a set part of any Whole Effluent Assessment strategy (WEA)."* http://eippcb.jrc.ec.europa.eu/reference/BREF/mon_bref_0703.pdf

- COHIBA (2010): *"Unfortunately, controlling whole effluent toxicity or combined effects is not a common practice. There are a few exceptions to this. The German legislation has set whole effluent toxicity limits values for several industrial sectors. In Sweden and Denmark there are guidelines for utilization of WEA in environmental permits of larger industrial plants, although the Danish guidelines are not statutory. In Finland WEA has been applied in few environmental permits, but this is not a common practice. In the entire Baltic Sea region, municipal discharges are not subjected to whole effluent toxicity control."* http://www.cohiba-project.net/identification/recommendations/en_GB/recommendations/

Tools to investigate toxicity (WEA)

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Table 4: Mutagenicity and genotoxicity

	Test	Applied by: Belgium	France	Portugal	Germany	Netherlands	Sweden	UK	Common or optional toolbox	
In vitro										
ISO 13829:2000	Determination of the genotoxicity of water and waste water using the *umu*-test				R	RD				optional
ISO 16240:2005	Determination of the genotoxicity of water and waste water -- Salmonella/microsome test (Ames test)				RD					optional
MUTATOX test	*Vibrio fischeri (Photobacterium phosphoreum)* non luminescent variant			RD						optional
VITOTOX test	*Salmonella typhimurium (SOS* bioluminescence variant)	RD								optional
ISO/DIS 21427-2	Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 2: "Mixed population" method using the cell line V79				RD					
In vivo										
ISO/FDIS 21427-1	Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 1: Evaluation of genotoxicity using amphibian larvae (ISO/DIS accepted)	RD	RD							optional

R: Regulatory purposes RD: Research and Development

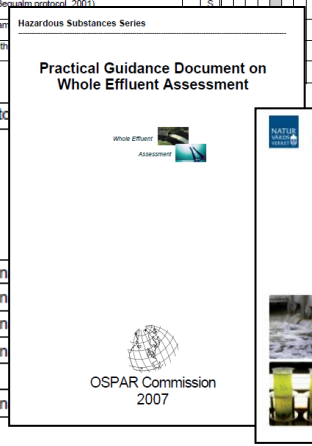
It should be noted that far more tests are being and have been used in research and development projects some of which have been described[26] References:

Table 2: Chronic toxicity

Trophic level	Test and guideline	Fresh water or Salty water	Applied by: Belgium	Finland	Portugal	Germany	Netherlands	Sweden	UK	Arkema	Cancelas	Common or optional toolbox	
		Fresh water / Salty water											
Bacteria	*Pseudomonas putida* growth inhibition test (Pseudomonas cell multiplication inhibition test) (ISO 10712:1995)	F											Optional
	Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms (ISO 15522:1999)	F / S											Optional
Algae	Freshwater algal growth inhibition test with unicellular green algae (ISO 8692:2004) [23] or Growth inhibition of *Desmodesmus subcapitata* (DIN 38412-33:1991)	F										Common	
	Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum* (ISO 10253:2006)	S										Common	
Crustacean	*Daphnia magna*, Reproduction Test (OECD 211) or Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea) (ISO 10706:2000)	F										Common	
	Harpacticoid Copepod development and reproduction test (*Nitocra sp.*) (OECD draft)	S											Optional
Fish	Short term toxicity test on Embryo and Sac-Fry Stages (OECD 212) or Determination of the acute toxicity of wastewater to zebrafish *Danio rerio* eggs (ISO/DIS 15088)	F										Common	
	Determination of toxicity to embryos and larvae of freshwater fish – Semi-static method (ISO 12890:1999)	F										Common	
	Subchronic toxicity to fish	F / S											Optional
Other	Determination of the chronic toxicity to *Brachionus calyciflorus in 48 h* (ISO/CD 20666)	F											
	Oyster larvae development *Crassostrea gigas* (Beguelin protocol, 2001)	S										Common	
	Toxicity to eggs and larvae of *Mytilus edulis* (Gram												Optional
	Fertilization and Embrionic Development Test with												Optional
												Common	

Table 5: Endocrine disruption

	Test		Applied by: Germany	Netherlands	Sweden	Common/Optional to
In vitro	*YES test*	*Yeast modified*	RD	RD	R	Option
	YAS test	*Yeast modified*			R	Option
	E-screen Assays with MCF-7cell line		RD			Option
	ER-calux test	*Human cell line, modified*		RD		Option
	Estrogenic and androgenic effects [27]	*Modified yeast cells*			R	
In Vivo	*Zebrafish, two generation*	*Fish test*			R	Option



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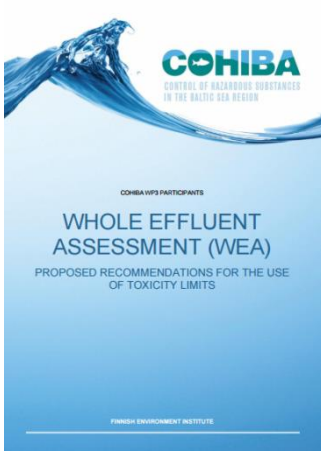
Emission limit values based on toxicity (WEA use)



- ECx=Effective Concentration x% (EC50=20% means 50% of the test organisms were affected when the effluent concentration was 20%)
- NOEC=No Observed Effect Concentration

- LID=Lowest Ineffective Dilution (Germany: 1/NOEC)
- TU=Toxic Unit (USA: acute toxicity TU_A = 100/EC50 and chronic toxicity TU_C= 100/NOEC)
- Some take dilution into account, some not.

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Dredged sediment

- Pore water
- Sediment extracts

- In vivo (e.g. Tisbe, Crassostrea, Skeletonema; TU<1.0)
- In vitro (e.g. dioxin activity; TEQ/kg)

(KLIF, 2011)

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Current (regulatory) use

	Whole Effluent Assessment, dredged sediment etc	Receiving water	/Emission/ limit values
Chemical	Suspected ”listed” substances	Suspected ”listed” substances	µg/l etc.
Toxicological	Toxicity tests (in vivo, in vitro)	Biomarkers Bioassays	ECX, NOEC, LID, TU, Substance Equivalents

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Potential WFD use (today)

- Pressure and impact assessment (WFD Annex II 1.4. and 1.5.):
  - screening tools
  - *early warning*
- To *support compliance* checking (e.g. tier II sediment)
- Identify new potential River Basin Specific Pollutants (RBSPs)
  - EDA

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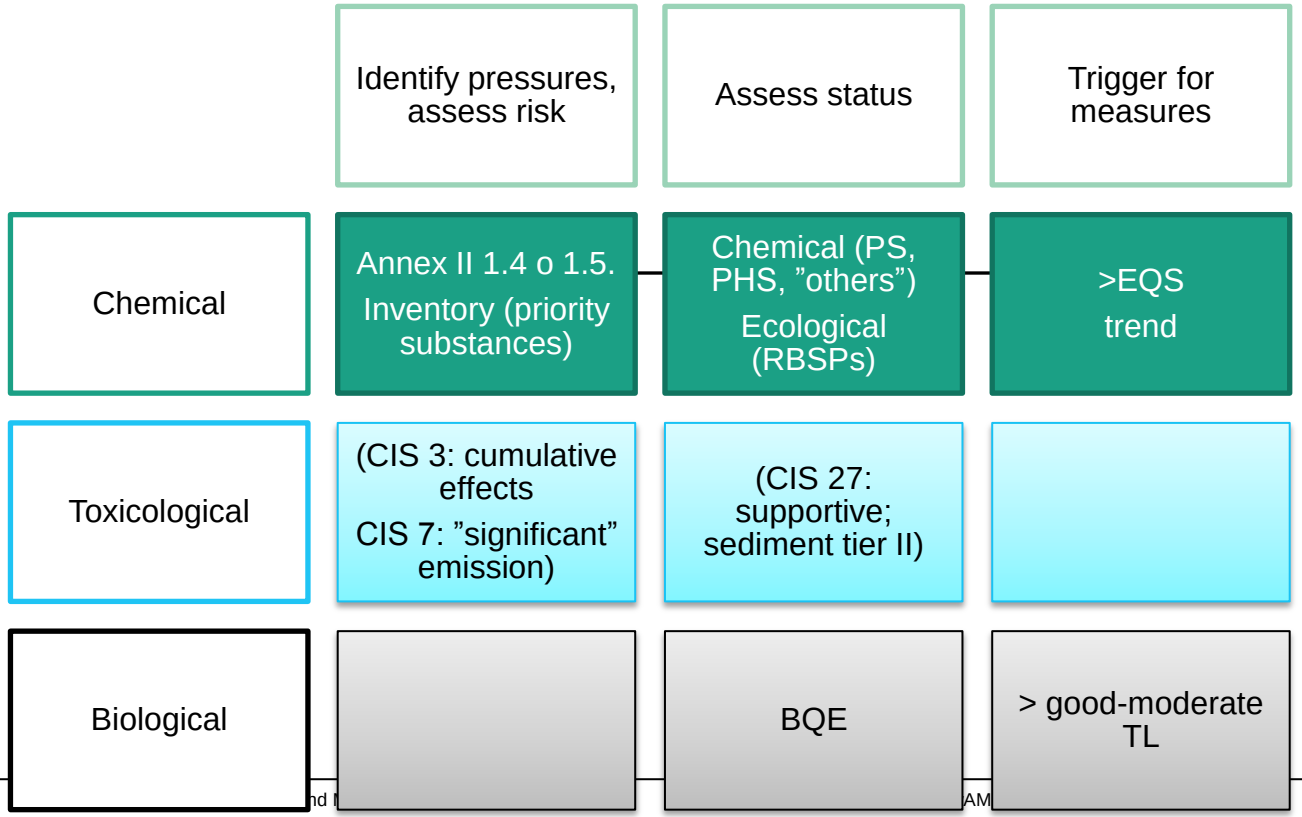
WFD



CIS: Common Implementation Strategy; numbers refer to guidance documents

MSFD

Chemical	Criteria 8.1. & 9.1
Toxicological	Criteria 8.2.
Biological	Criteria 8.2.

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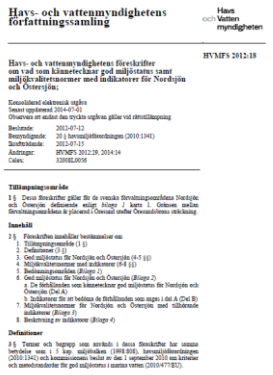


Havs- och vattenmyndighetens författningssamling

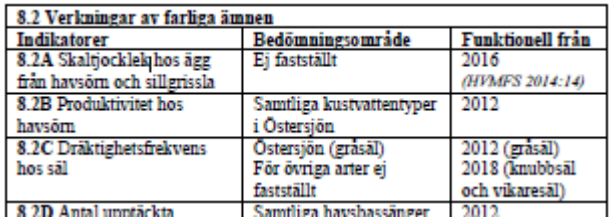
HVMFS 2012:18

Havs- och vattenmyndighetens föreskrifter om vad som kännetecknar god miljöstatus samt miljökvalitetsnormer med indikatorer för Nordsjön och Östersjön;

Tillämpningsområde

Innehåll

Definitioner



8.2 Verkningar av farliga ämnen		
Indikatorer	**Bedömningsområde**	**Funktionell från**
8.2A Skaltjockleklhos ägg från havsörn och sillgrissla	Ej fastställt	2016 *(HVMFS 2014:14)*
8.2B Produktivitet hos havsörn	Samtliga kustvattentyper i Östersjön	2012
8.2C Dräktighetsfrekvens hos säl	Östersjön (gråsäl) För övriga arter ej fastställt	2012 (gråsäl) 2018 (knubbsäl och vikaresäl)
8.2D Antal uppträckta	Samtliga havsbassänger	2012

Also EBTs for birds, marine mammals… (secondary poisoning)

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3. CIS EBT activity

- Support and interest from MS and stakeholders, incl NORMAN.
- 4 co-leads : IT (Mario Carere), JRC (Teresa Lettieri), SE (Ann-Sofie Wernersson, Niklas Hansson), CH (Robert Kase).
- ToR final (March 2017).
- Start up April 2017.
- Final report end 2018. (Review of WFD in 2019)
- Not a new technical report – but will build on it.

From ToR:

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- Identify relevant MoAs and available EBTs for these MoAs (1, 2)
- Trigger values (4)
- Assess level of maturity, robustness, reliability etc – listing, prioritizing, selecting tools. Use within WFD, MSFD (3, 5, 7)
- BQEs and OMICS - evaluation (6)
- Approaches to identify the underlying causes to identify sources of emissions and facilitate measures (8)
- Assess the practical feasibility and cost effectiveness of using EBTs alongside chemical approach; advantages and disadvantages compared to current WFD approach (9).

Selecting the tools

Feedback/recomendations from You?

- ? How to best use EBTs alongside chemical tools to develop an approach to assess risk from chemicals (including mixtures) in and via the aquatic environment and also facilitate measures? Conceptual line.
- ? Which tools work the best and for which purpose? Costs? Availability of labs? Standards? Comply with QA/QC? MoA covered? Substances covered? Risks (QS) covered? Assessment criteria? Material needed? Compartment/matrix?
- ? Are there tools to also assess indirect toxicity – secondary poisoning (food chain)? Human health risks (drinking water, via fish and seafood)?
- ? Provide case studies to illustrate how to identify the underlying causes to identify sources of emissions and facilitate measures (objective no 8)? Identify ”suspect sources” or ”substances”?
- ? How to go from toxicity in field samples to toxicity of emissions and vice versa (to control or assess emissions)? Methodology.

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	Pressures (e.g. WEA)	Status	Emission limits
Chemical	PBT substances T-substances not covered by T	PBT substances T-substances not covered by T Trend	Conc (individual and sum) Amount
Toxicological	Estrogenicity Ah receptor activity etc (in vitro)	Estrogenicity Ah receptor activity etc (in vitro, biomarker)	Conc equivalents
Biological	Reproduction etc (in vivo)	BQE (”effect” biomarkers?)	TU (in vivo)

THANK YOU!!