Toxicity profiling: an effect-based integrative tool for sediment quality assessment

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norman

Deltares
Enabling Delta Life

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Toxicity profiling: a safety net to signal toxic potency

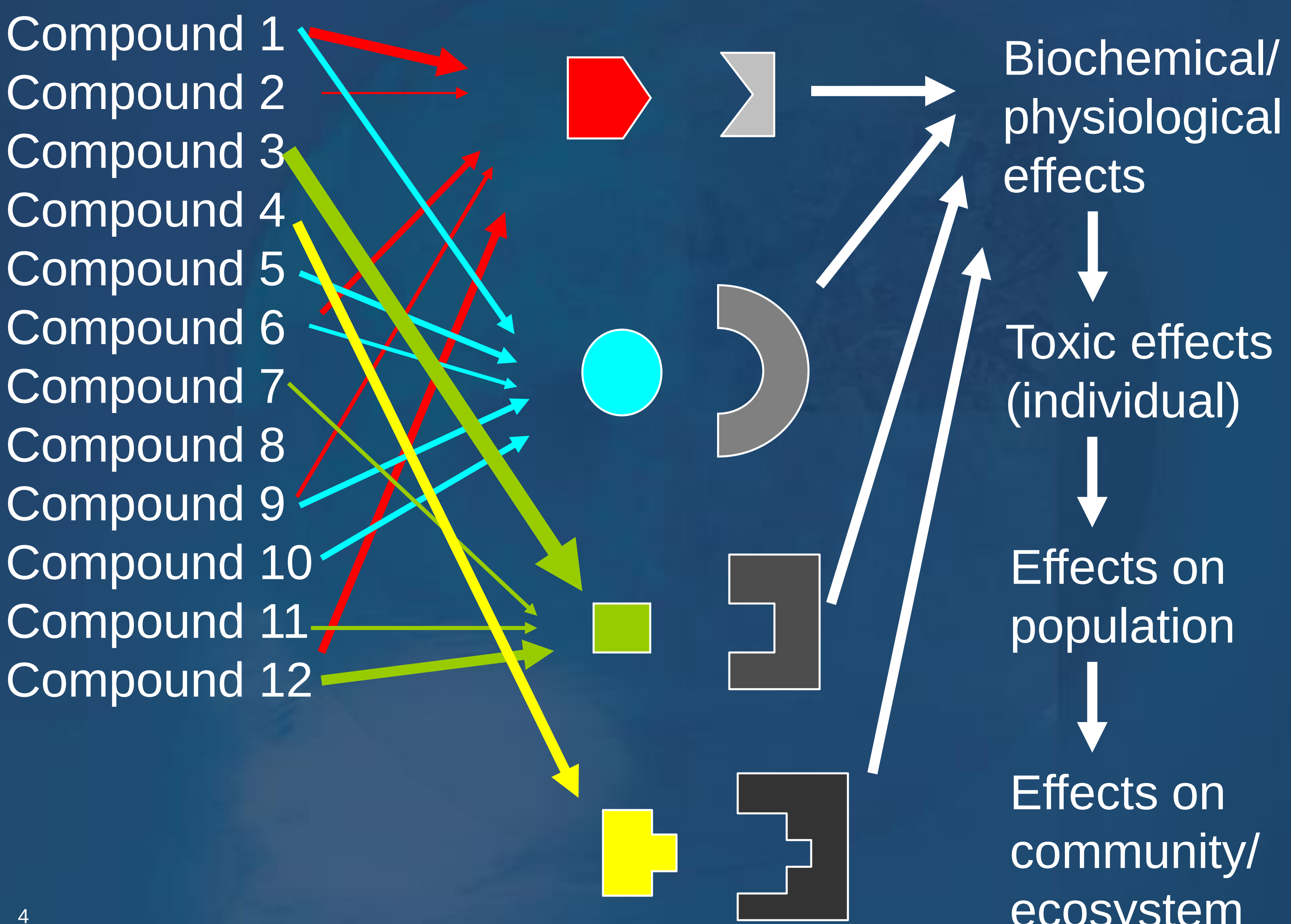
Profiling individual compounds

E.g. “suspect” in crime case

- Caucasian male
- 35-40 years old
- About 1.90 meter tall
- Robust physique
- Short, black hair
- Decent appearance
- Blue/grey blocked shirt with short sleeves
- Khaki-colored trousers
- Brown belt with chrome buckle
- Grey leather sandals
- Dark rectangular sunglasses

- **Description**
- **Combination of characteristics**
- **Identification of the “bad guys”**

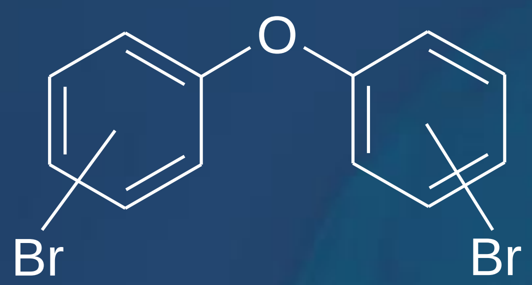
Toxicity profiling:
multiple characteristics of single compounds

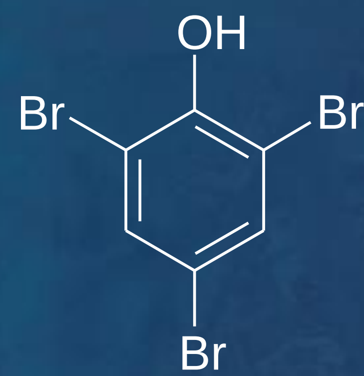


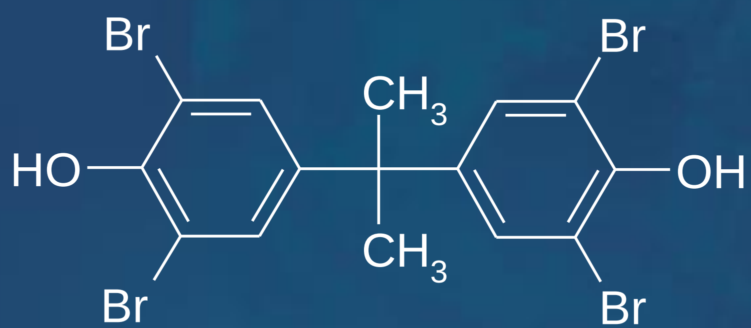
Biomonitoring using specific bioassays

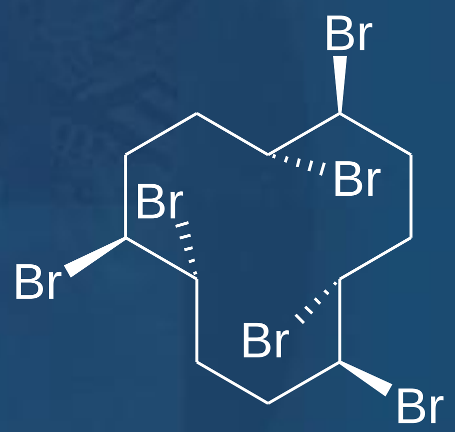
	MODE OF ACTION			
Compound 1	**4**	**2**		
Compound 2	**1**			
Compound 3			**5**	
Compound 4				**4**
Compound 5		**2**		
Compound 6	**2**	**1**		
Compound 7			**1**	
Compound 8				
Compound 9	**1**	**2**		
Compound 10		**2**		
Compound 11			**1**	
Compound 12	**3**		**4**	

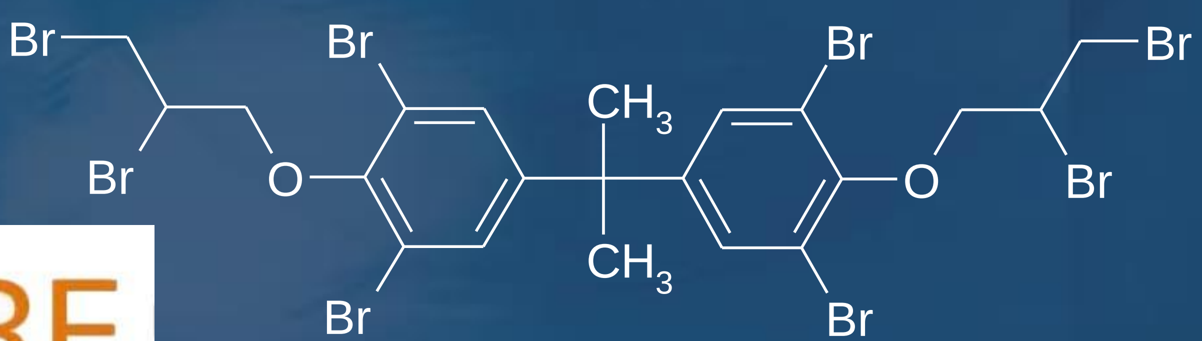
Toxicity profiling of brominated flame retardants (BFRs)











Br FIRE

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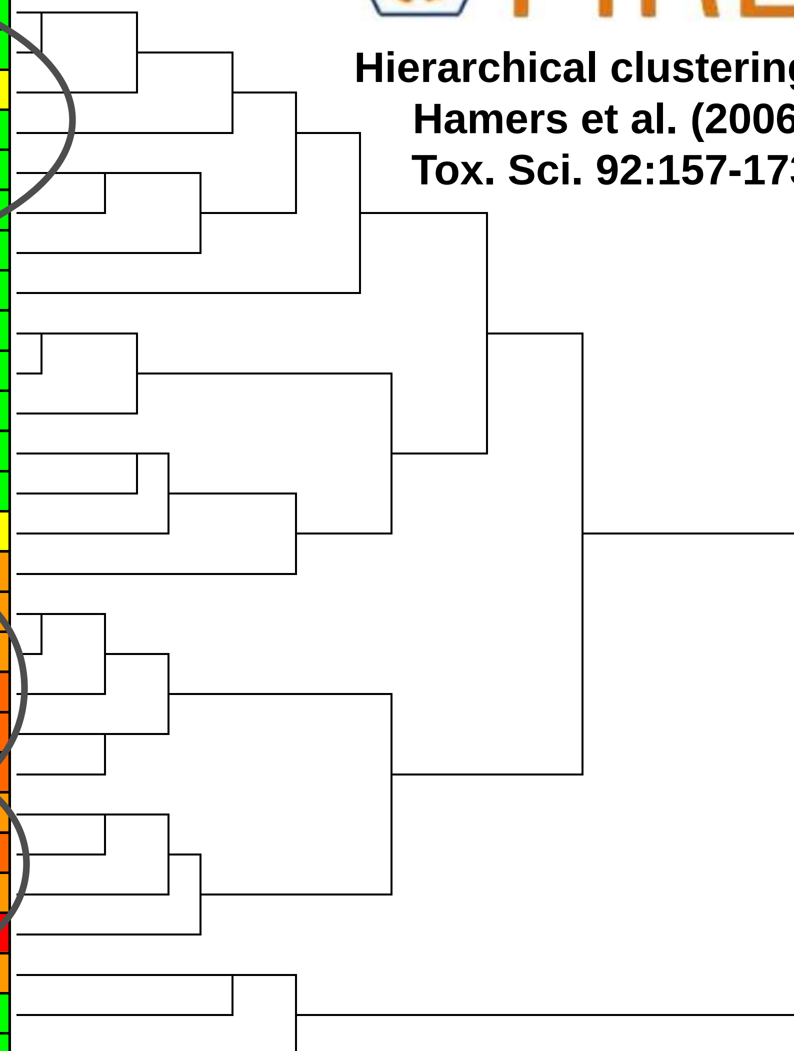
Toxicity profiling of brominated flame retardants (BFRs)

Compound	ERago	ERanta	TTR	DRago	DRanta	ARanta	PRanta	E2SULT	TSago	TSanta	ARago	PRago
BDE-19	3	1	1	1	1	5	4	3	3	1	1	1
BDE-28	2	1	1	1	3	3	2	2	4	1	1	1
BDE-38	3	1	2	4	1	3	2	1	2	1	1	1
BDE-39	1	1	1	1	2	3	2	1	1	1	1	1
BDE-47	2	1	2	1	3	3	2	4	1	1	1	1
BDE-49	3	1	2	2	2	4	3	3	5	1	1	1
BDE-79	2	2	1	3	1	3	2	1	1	1	1	1
BDE-99	1	1	1	2	2	3	2	1	1	1	1	1
BDE-100	3	1	1	1	2	5	3	2	4	1	1	1
BDE-127	1	1	2	1	3	3	2	2	2	1	1	1
BDE-153	1	1	1	4	1	2	3	1	1	1	1	1
BDE-155	3	1	1	1	1	3	3	2	3	1	1	1
BDE-169	1	1	2	1	3	1	1	2	1	1	1	1
BDE-181	1	3	3	3	1	3	3	1	1	1	1	1
BDE-183	1	3	1	3	1	2	3	2	3	1	1	1
BDE-185	1	2	3	1	1	2	3	1	1	1	1	1
BDE-190	1	3	3	3	1	3	3	3	1	1	1	1
BDE-206	1	1	1	1	1	1	1	2	1	3	1	1
BDE-209	1	1	1	1	1	1	1	2	1	1	1	1
TBBPA	1	1	5	1	1	1	1	5	3	1	1	1
246-TBP	1	3	5	1	1	2	2	4	1	1	1	1
6OH-BDE 47	1	4	4	3	1	3	3	3	1	1	1	1
HBCD TM	1	2	1	1	2	2	3	1	3	1	1	1
HBCD a	1	1	2	1	3	3	3	1	4	1	1	1
HBCD b	1	2	2	1	2	2	2	1	3	1	1	1
HBCD g	1	3	1	1	2	3	3	1	4	1	1	1
TBBPA-DBPE	1	1	3	1	1	1	1	4	1	1	1	1

Class	Criterium
1	Effect <20% at 10 µM
2	20% < effect < 50% at 10 µM
3	1 µM < EC_{50} < 10 µM
4	0.1 µM < EC_{50} < 1.0 µM
5	0.01 µM < EC_{50} < 0.1 µM

Toxicity profiling of brominated flame retardants (BFRs)

Cluster	Compound	AR ago	PR ago	TS anta	ER ago	DR anta	ER anta	DR ago	TTR	E2SULT	AR anta	PR anta	TS pot
V.2	BDE-39	1	1	1	1	2	1	1	1	1	3	2	1
V.2	BDE-99	1	1	1	1	2	1	2	1	1	3	2	1
V.2	BDE-127	1	1	1	1	3	1	1	2	2	3	2	2
V.2	BDE-185	1	1	1	1	1	2	1	3	1	2	3	1
V.1	BDE-206	1	1	3	1	1	1	1	1	2	1	1	1
V.1	BDE-209	1	1	1	1	1	1	1	1	2	1	1	1
V.1	BDE-169	1	1	1	1	3	1	1	2	2	1	1	1
V	BDE-47	1	1	1	2	3	1	1	2	4	3	2	1
IV.2	BDE-190	1	1	1	1	1	3	3	3	3	3	3	1
IV.2	6OH-BDE-47	1	1	1	1	1	4	3	4	3	3	3	1
IV.2	BDE-181	1	1	1	1	1	3	3	3	1	3	3	1
IV.1	BDE-79	1	1	1	2	1	2	3	1	1	3	2	1
IV.1	BDE-153	1	1	1	1	1	1	4	1	1	2	3	1
IV.1	BDE-38	1	1	1	3	1	1	4	2	1	3	2	2
IV.1	BDE-183	1	1	1	1	1	3	3	1	2	2	3	3
III	HBCD TM	1	1	1	1	2	2	1	1	1	2	3	3
III	HBCD beta	1	1	1	1	2	2	1	2	1	2	2	3
III	HBCD gamma	1	1	1	1	2	3	1	1	1	3	3	4
III	BDE-28	1	1	1	2	3	1	1	1	2	3	2	4
III	HBCD alpha	1	1	1	1	3	1	1	2	1	3	3	4
II	BDE-19	1	1	1	3	1	1	1	1	3	5	4	3
II	BDE-100	1	1	1	3	2	1	1	1	2	5	3	4
II	BDE-155	1	1	1	3	1	1	1	1	2	3	3	3
II	BDE-49	1	1	1	3	2	1	2	2	3	4	3	5
I	TBBPA	1	1	1	1	1	1	1	5	5	1	1	3
I	TBBPA-DBPE	1	1	1	1	1	1	1	3	4	1	1	1
I	246-TBP	1	1	1	1	1	3	1	5	4	2	2	1



Br FIRE

Hierarchical clustering Hamers et al. (2006) Tox. Sci. 92:157-173

Metabolite profiling

	PARENTS													METABOLITES											
Compound	ERago	ERanta	TTR	DRago	Dranta	ARanta	PRanta	E2SULT	Tscreenago	Tscreenanta	ARago	PRago	Metabol	ERago	ERanta	TTR	DRago	Dranta	ARanta	PRanta	E2SULT	Tscreenago	Tscreenanta	ARago	PRago
BDE 19	3	1	1	1	1	5	4	3	3	1	1	1	5	1	1	4	1	3	3	2	4	CT?	4	1	1
BDE 28	2	1	1	1	3	3	2	2	4	1	1	1	4	CT?	3?	4	CT	CT	3	2	4	CT?	4	1	1
BDE 38	3	1	2	4	1	3	2	1	2	1	1	1	5	3	1	4	CT	CT	3	2	4	CT	CT	1	1
BDE 39	1	1	1	1	2	3	2	1	1	1	1	1	2	3	1	4	1	3	3	2	4	CT?	2	1	1
BDE 47	2	1	2	1	3	3	2	4	1	1	1	1	2	2	1	3	1	3	3	2	4	CT?	2	1	1
BDE 49	3	1	2	2	2	4	3	3	5	1	1	1	5	CT?	1	4	CT	CT	4	3	4	CT	CT	1	1
BDE 79	2	2	1	3	1	3	2	1	1	1	1	1	2	2	1	3	1	3	3	2	2	CT	CT	1	1
BDE 99	1	1	1	2	2	3	2	1	1	1	1	1	2	1	1	3	1	1	3	2	1	1	1	1	1
BDE 100	3	1	1	1	2	5	3	2	4	1	1	1	2	1	1	3	1	1	4	3	2	2	1	1	1
BDE 153	1	1	1	4	1	2	3	1	1	1	1	1	1	1	1	2	1	2	2	3	1	2	1	1	1
BDE 155	3	1	1	1	1	3	3	2	3	1	1	1	3	2	2	4	2	1	3	3	3	2	1	1	1
BDE 185	1	2	3	1	1	2	3	1	1	1	1	1	2	4	1	3	1	1	2	3	2	4	1	1	1
BDE 209	1	1	1	1	1	1	1	2	1	1	1	1	1	3	1	1	1	1	1	1	1	2	1	1	1
TBBPA	1	1	5	1	1	1	1	5	3	1	1	1	2	2	1	4	1	1	1	1	5	5	1	1	1
246-TBP	1	3	5	1	1	2	2	4	1	1	1	1	3	4?	1	4	1	1	2	2	4	2	1	1	1
6OH-BDE 47	1	4	4	3	1	3	3	3	1	1	1	1	4	1	3	4	1	1	3	3	3	CT	1	1	1
HBCD TM	1	2	1	1	2	2	3	1	3	1	1	1	3	1	1	1	1	1	2	3	1	4	1	1	1
HBCD alpha	1	1	2	1	3	3	3	1	4	1	1	1	2	1	1	1	1	2	3	3	2	4	1	1	1
HBCD beta	1	2	2	1	2	2	2	1	3	1	1	1	4	2	1	1	1	2	2	2	3	2	1	1	1
HBCD gamma	1	3	1	1	2	3	3	1	4	1	1	1	4	1	1	1	1	1	3	3	1	5	1	1	1
TBBPA-DBPE	1	1	3	1	1	1	1	4	1	1	1	1	0	3	1	2	1	1	3	1	1	3	1	1	1

Hamers et al. (2008)
Mol Nutr Food Res 52:284-298

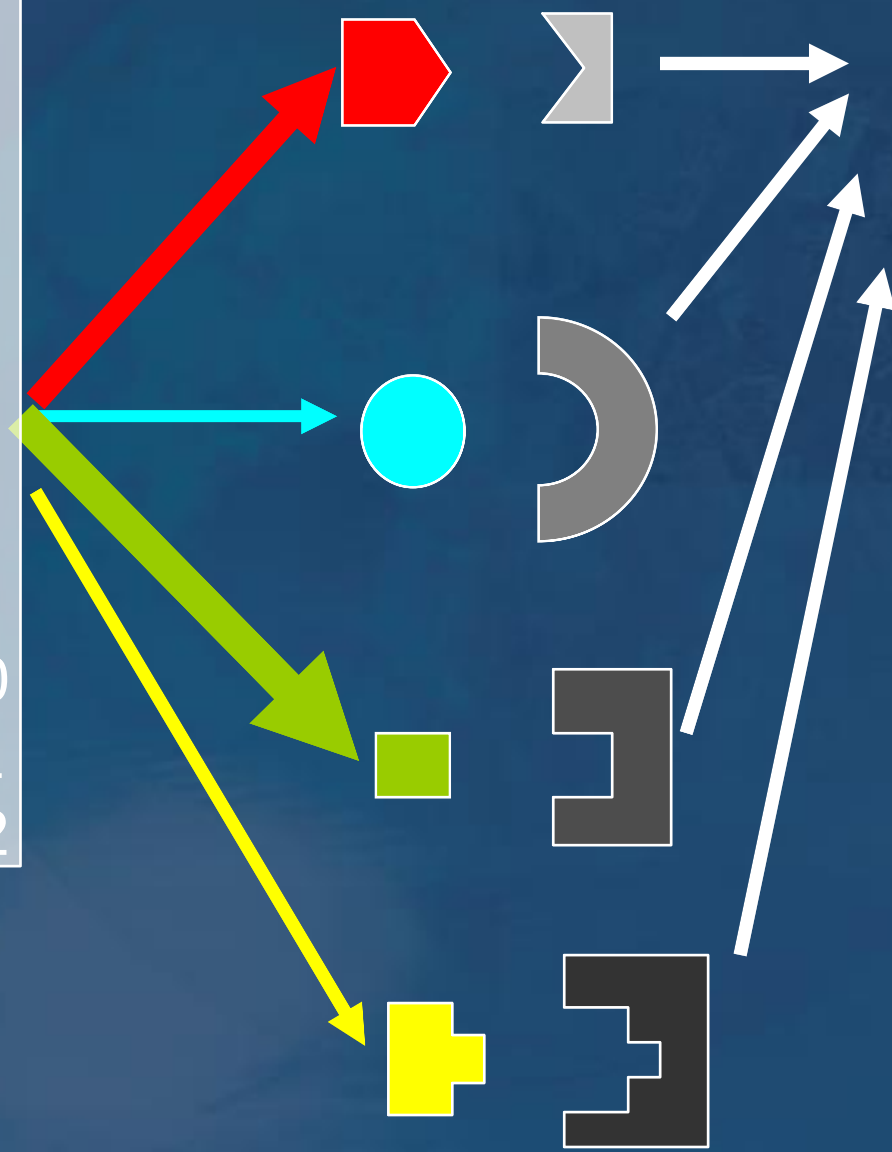
Profiling complex mixtures

E.g. Chemistry & Biology Department at IVM

- High Quality Contaminant Analysis
- Toxicity profiling
- Development of biomarkers and bioassays
- Effect Directed Analysis (EDA)
- Toxicogenomics
- Teaching
- Academic environment
- **Description**
- **Combination of characteristics**
- **Hard to see individuals' contribution**
- **Total assessment of the mixture**
- **Useful for quality assessment**

Toxicity profiling: multiple characteristics of a complex mixture

Compound 1
Compound 2
Compound 3
Compound 4
Compound 5
Compound 6
Compound 7
Compound 8
Compound 9
Compound 10
Compound 11
Compound 12



Biochemical/ physiological effects

Toxic effects (individual)

Effects on population

Effects on community/ ecosystem

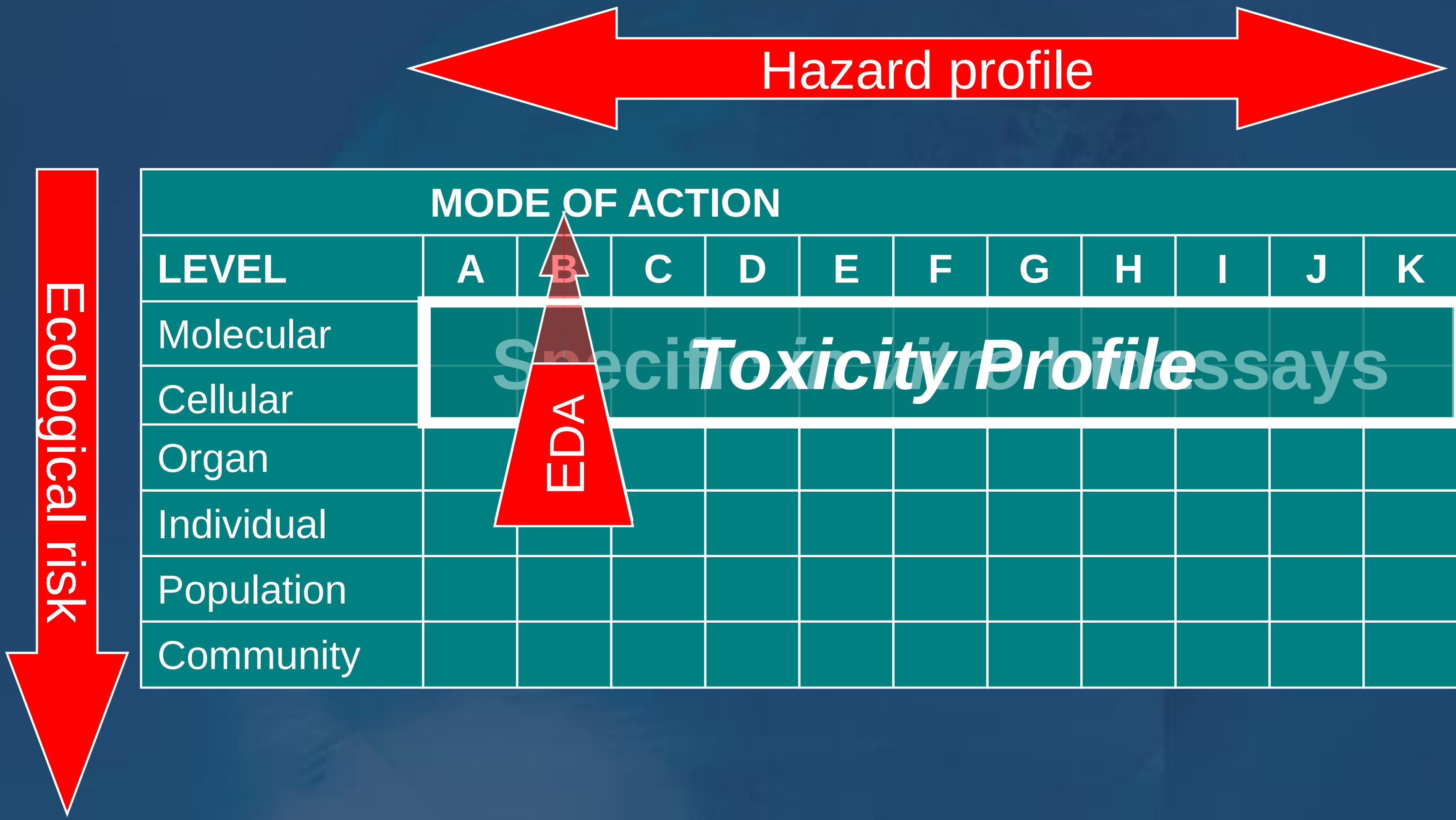
Biomonitoring using specific bioassays

	MODE OF ACTION			
	(red)	(cyan)	(green)	(yellow)
Mixture 1	**4**	**2**		
Mixture 2	**1**			
Mixture 3			**5**	
Mixture 4				**4**
Mixture 5		**2**		
Mixture 6	**2**	**1**		
Mixture 7			**1**	
Mixture 8				
Mixture 9	**1**	**2**		
Mixture 10		**2**		
Mixture 11			**1**	
Mixture 12	**3**		**4**	

Chemical analysis vs Toxicity test of a mixture

	Chemical analysis	Toxicity test
+	•Identity is known •Concentration is known	•Activity of the mixture is known •All compounds contribute
-	•Not all compounds are analyzed •Compounds <DL are not recognized •Activity of the mixture is unknown •Growing list of compounds	•Composition of the mixture is unknown •Responsible compounds are unknown

Biomonitoring using specific in vitro bioassays



From Toxicity Profile to Hazard Profile

Hazard profile

MODE OF ACTION											
LEVEL	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Molecular											
Cellular											

Toxicity Profile

Toxicity profiles of European river sediments

MODE OF ACTION

	TA98-S9 rev/g	TA98+S9 rev/g	TAMix-S9 rev/g	TAMix+S9 rev/g	UmuC -S9 4NQO eq ng/g	ABC ERY eq ng/g	Anti-YAS FLU eq ng/g	ARago DHT eq pmol/g	Aranta FLU-eq nmol/g	YES E2 eq ng/g	T4-TTR T4-eq pmol/g	DRago TEQ pg/sed
E1	454	65	-	23	-	<3.3	NR	-	-	-	205	150
E2	854	43	-	28	24	<3.3	7	-	[illegible]	-	538	450
E3	374	83	-	47	-	<3.3	9	-	23	0.07	667	600
L1	16	25	-	-	-	8	[illegible]	-	-	-	182	80
L2	60	23	-	-	-	[illegible]	4	1.2	-	0.03	294	90
L3	-	59	-	-	-	9	14	2.1	-	0.48	418	75
SF1	-	50	17	59	-	3	16	0.92	-	0.38	175	460
SF2	-	454	[illegible]	83	-	<18.5	67	-	98	0.59	-	4150
SM1	120	214	-	80	-	7	22	-	12	0.15	92	1160
SM2	-	57	18	43	NR	1	57	-	-	0.25	93	900

What is high and what is low?

genotoxic

antibiotic

hormone disrupting

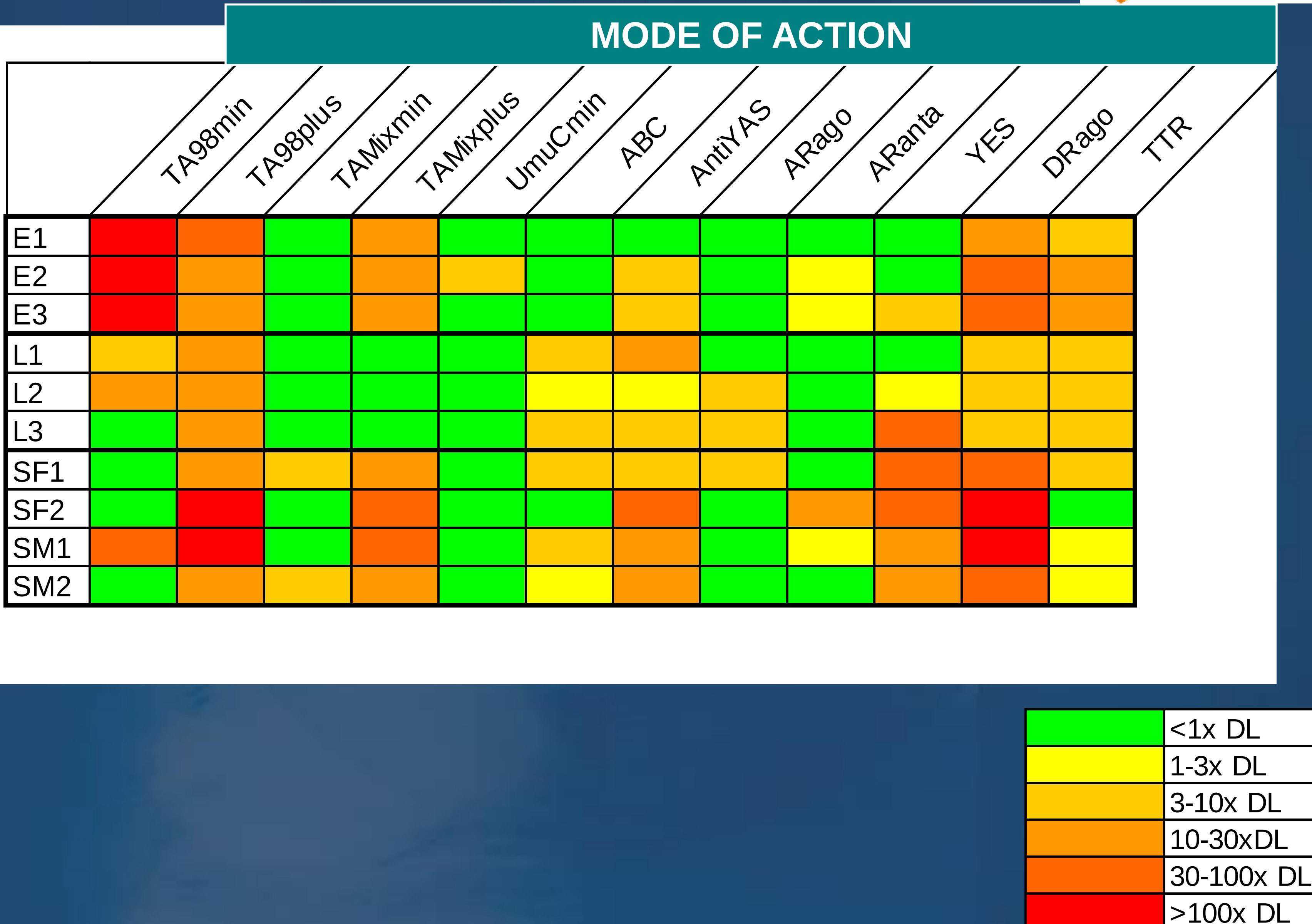
dioxin-like

River Elbe

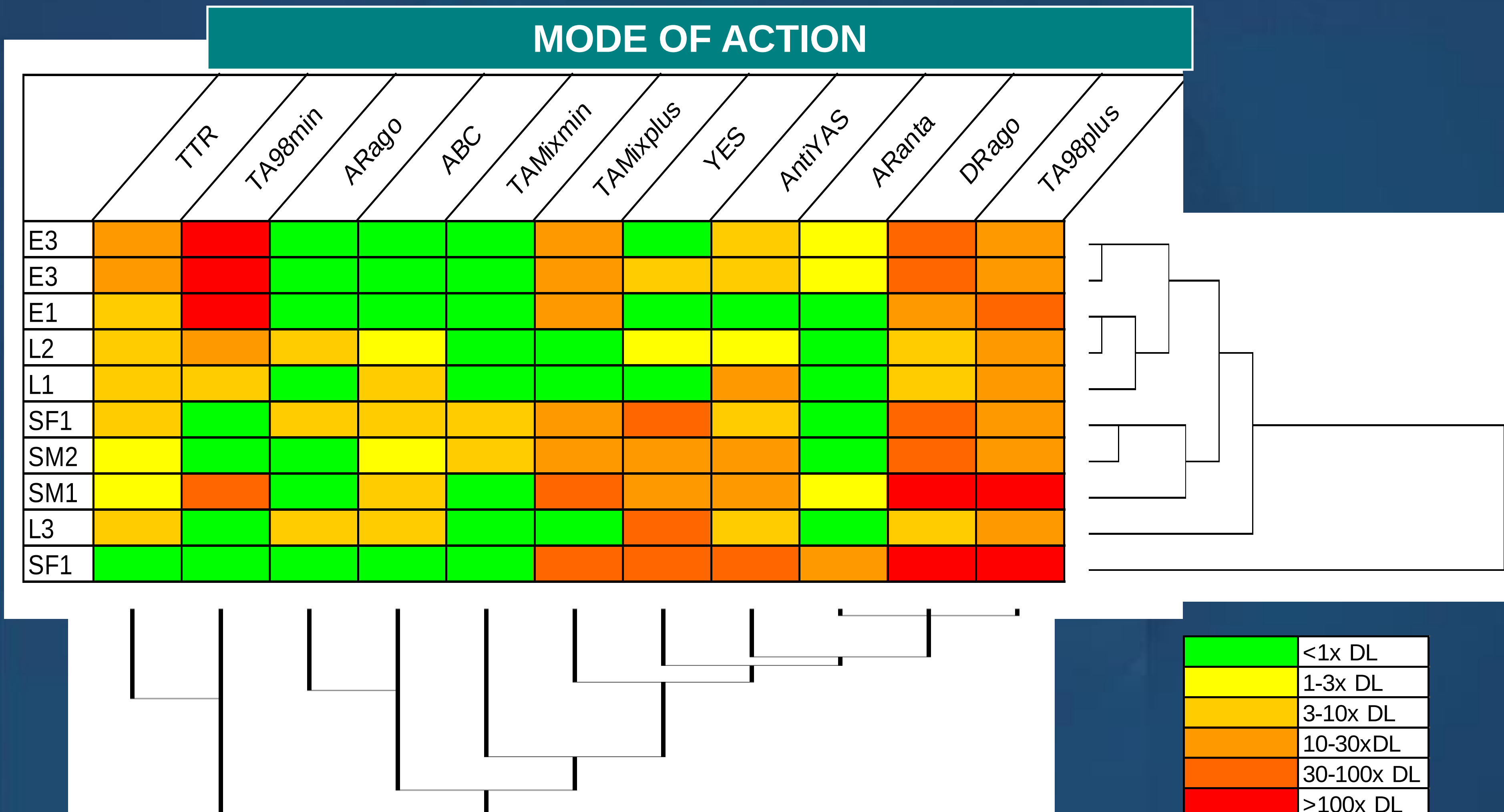
River Llobregat

River Scheldt (fresh/marine)

Ratio response:detection limit



Hierarchical clustering DL-based hazard profile



Toxicity profiles of harbor sediments

MODE OF ACTION

Location	DR-CALUX total (pg TEQ/g dw)	DR-CALUX stable (pg TEQ/g dw)	ER-CALUX total (pg EEQ/g dw)	ER-CALUX stable (pg EEQ/g dw)	TTR stable (pmol T4-eq/g dw)	Microtox total (1/EC20) (1/mg dw)	Microtox stable (1/EC20) (1/mg dw)	umu-S9 total (ng 4NQOEQ/g dw)	umu+S9 total (ng 2AAEQ/g dw)
Oosterschelde 1	10	2	5	2	3	0.2	0.02	13	87
Oosterschelde 2	153	4	34	2	3	0.2	0.02	13	87
Zierikzee buiten	621	32	11	2	13	2	25	28	87
Zierikzee binnen	11305	158	126	2	3	2	0.02	13	259
Veerse Meer	844	20	182	2	7	33	0.05	81	461
Haringvliet	9150	108	340	2	16	6	0.02	13	87
Bruinisse	664	116	124	2	8	1	0.02	13	453
Dintel Sluizen	4651	92	92	2	13	3	0.02	13	87
Moerdijk	10986	134	65	2	6	10	0.02	13	255
Nieuwe Maas	1130	18	15	2	3	0.3	0.02	13	87
Nieuwe Waterweg	881	3	10	2	3	0.3	0.02	35	87
Rotterdam IJssel Haven	3659	163	76	2	7	4.0	0.02	13	87
Rotterdam 2e Petroleumhaven	8827	112	128	4	9	1.9	0.31	13	87
Biesbosch 1	3770	101	45	2	3	2.6	0.02	27	87
Biesbosch 2	4053	74	40	2	3	2.0	0.02	52	87

What is high and what is low?

dioxin-like (DR-CALUX) | hormone disrupting (ER-CALUX, TTR) | decreased respiration (Microtox) | genotoxic (umu)

Houtman et al. (2004)
ET&C 23:32-40

Response ratios location:watersystem-specific reference

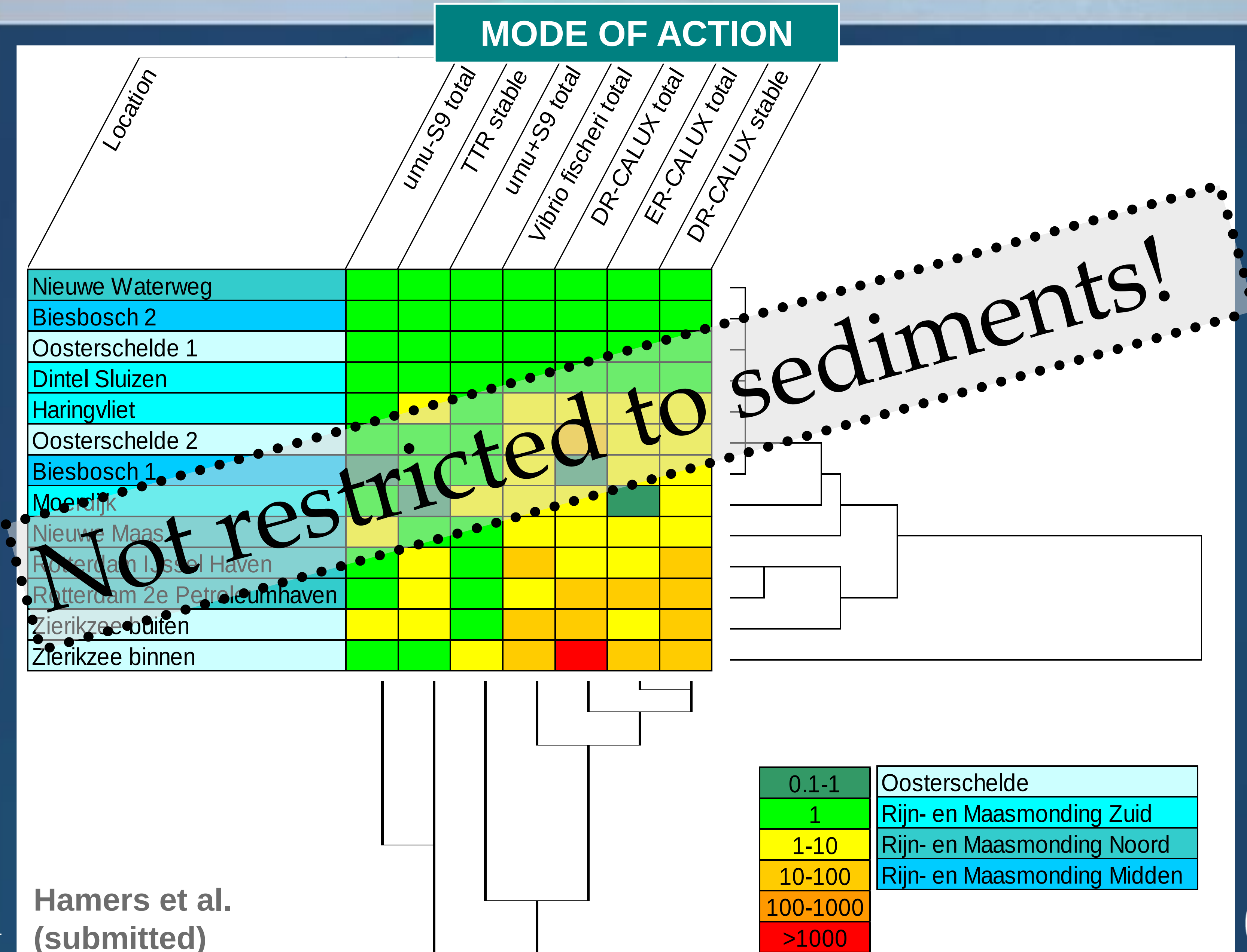
Location	DR-CALUX total	DR-CALUX stable	ER-CALUX total	ER-CALUX stable	TTR stable	Vibrio fischeri total	Vibrio fischeri stable	umu-S9 total	umu+S9 total	
	MODE OF ACTION									
Oosterschelde 1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	reference
Oosterschelde 2	16	2.8	6.7	1.0	1.0	1.3	1.0	1.0	1.0	
Zierikzee buiten	65	20	2.1	1.0	4.5	13	1111	2.1	1.0	
Zierikzee binnen	1178	99	25	1.0	1.0	13	1.0	1.0	3.0	
Haringvliet	2.0	1.2	3.7	1.0	1.2	1.8	1.0	1.0	1.0	
Dintel Sluizen	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	reference
Moerdijk	2.4	1.4	0.7	1.0	0.5	2.9	1.0	1.0	3.0	
Nieuwe Maas	1.3	5.9	1.5	1.0	1.0	1.2	1.0	2.7	1.0	
Nieuwe Waterweg	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	reference
Rotterdam IJssel Haven	4.2	52	7.8	1.0	2.2	14	1.0	1.0	1.0	
Rotterdam 2e Petroleumhaven	10	36	13	2.0	3.1	6.9	14	1.0	1.0	
Biesbosch 1	0.9	1.4	1.1	1.0	1.0	1.3	1.0	0.5	1.0	
Biesbosch 2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	reference

Location groups:
- Oosterschelde
- Rijn- en Maasmonding Zuid
- Rijn- en Maasmonding Noord
- Rijn- en Maasmonding Midden

Legend

0,1-1
1
1-10
10-100
100-1000
>1000

Hierarchical clustering: reference based hazard profiles



Hamers et al. (submitted)

Toxicity Profiling and Climate Change related events

- Impact of Climate Change on the Quality of Urban and Coastal Waters - Diffuse Pollution (DiPol): www.interreg-dipol.de

- Toxicity profiling of suspended particulate matter
  – Before or after an event (reference)
  – During an event
- Climate Change related Events
  – Run-off (urban, highway, industrial, agricultural)
  – Sewer overflow
  – Flooding events

European Union

The Interreg IVB North Sea Region Programme

Investing in the future by working together for a sustainable and competitive region

The DiPol-Project is partly funded by the European Regional Development Fund.

From toxicity to hazard and ecological risk

Comparison to a reference toxicity profile

- Detection limit
- Reference profile
  - On beforehand appointed
  - Based on “lowest” toxicity profile
  - Based on “good ecological quality”
- Ecologically relevant effect levels

From Toxicity Profile to Ecological Risk

Ecological risk

LEVEL
Molecular
Cellular
Organ
Individual
Population
Community

The famous “so-what?” question

Bioassay response threshold

MODE OF ACTION

Location	DR-CALUX total (pg TEQ/g dw)	DR-CALUX stable (pg TEQ/g dw)	ER-CALUX total (pg EEQ/g dw)	ER-CALUX stable (pg EEQ/g dw)	TTR stable (pmol T4-eq/g dw)	Microtox total (1/EC20) (1/mg dw)	Microtox stable (1/EC20) (1/mg dw)	umu-S9 total (ng 4NQOEQ/g dw)	umu+S9 total (ng 2AAEQ/g dw)
	9150.2	108.1	340	1.9	16.2	6.25	0.0225	13	86.5

LEVEL
Molecular
Cellular
Organ
Individual
Population
Community

Ecological relevance

Ecological relevance

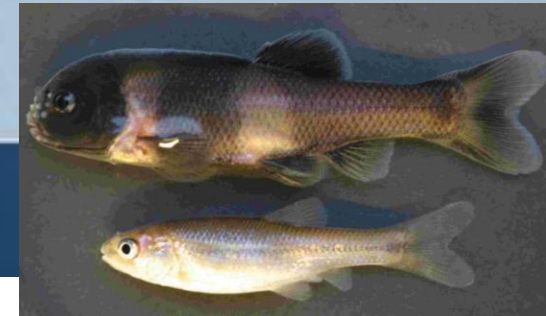
? ?

Example: threshold for estrogenic potency in sediments

- Fish are sensitive species
- Most studies with ethynylestradiol (EE2)
- Three key studies identified
  - Multiple generation study (zebrafish)
  - Lifecycle study (medaka)
  - Field study (fathead minnow)



Effect EE2 on fathead minnow population



- EE2 5 ng/l, during summer season

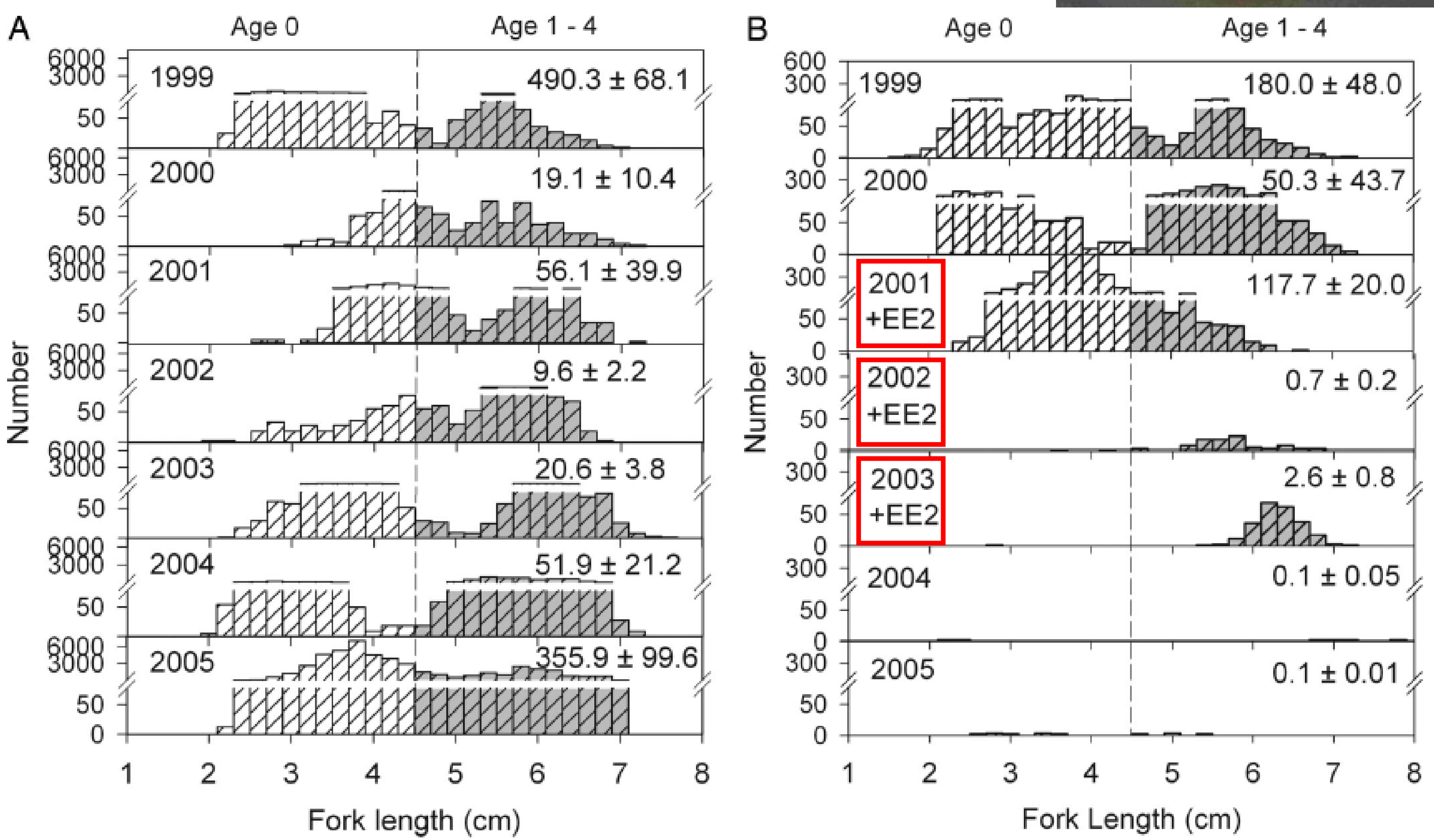


Fig. 3. Length frequency distributions of fathead minnow captured in trap nets in reference Lake 442 (*A*) and Lake 260 (*B*) (amended with 5–6 ng·L^{-1} of EE2 in 2001–2003) during the fall of 1999–2005. Distributions for each fall have been standardized to 100 trap-net days. Mean ± SE daily trap-net CPUE data for adults and juveniles for the fall catches are shown in the panels.

Kidd et al. (2007) PNAS 104:8897-8901

Example: threshold for estrogenic potency in sediments

No effect concentration in water (0.35 ng EE2/l)

↓ Freundlich isotherms

No effect concentration in sediment (64-1700 pg EE2/g dw)

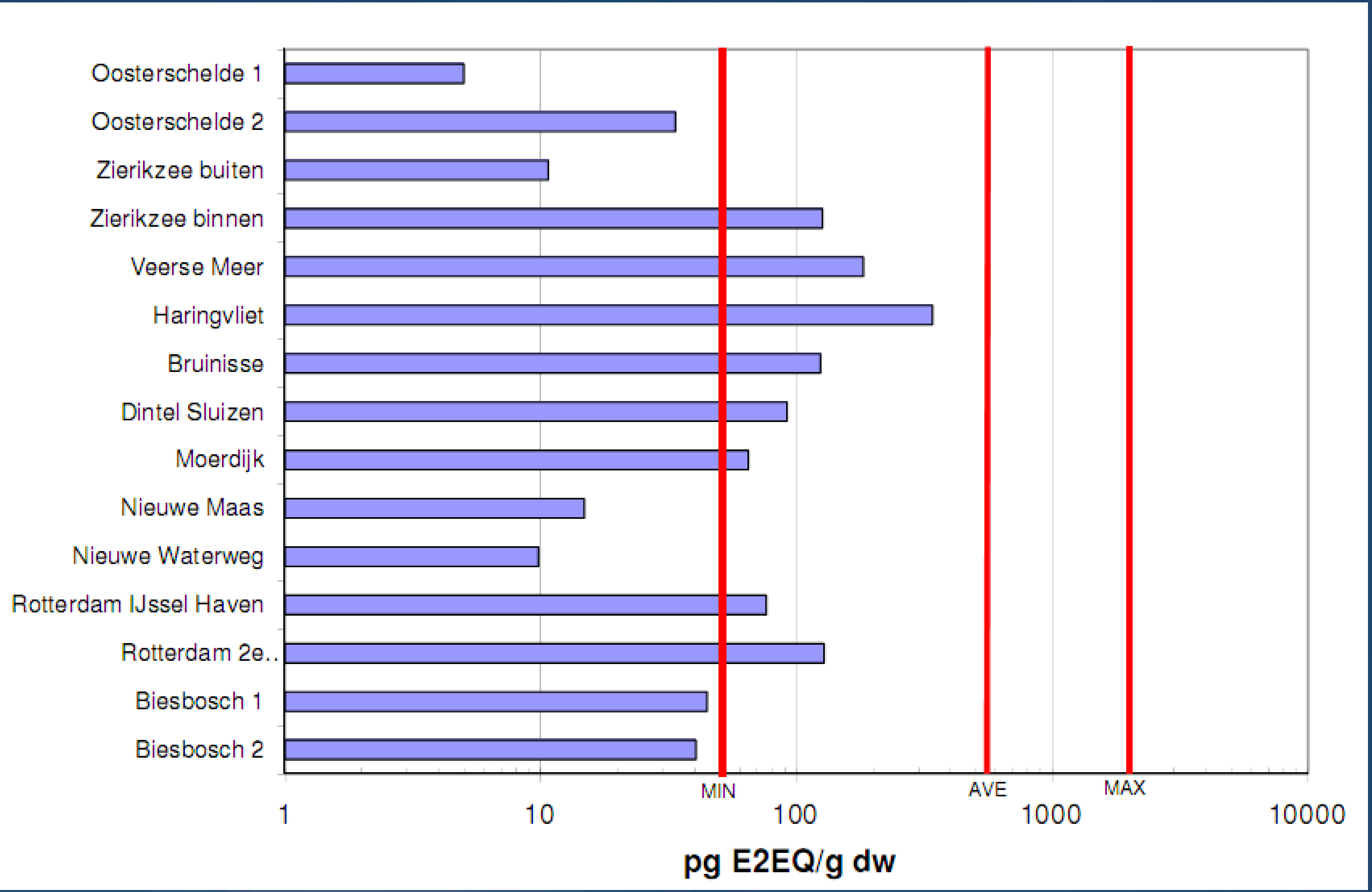
↓ Relative potency EE2:E2

No effect concentration in bioassay (71-2000 pg E2/g dw)

EE2 is “worst case” reference compound

- Persistent in the environment
- Hardly metabolized by fish
- Steep Freundlich isotherm

Example: threshold for estrogenic potency in sediments



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From Toxicity profiles to hazard and risk profiles

Toxicity profile
Equivalent concentrations

Hazard-profile
Reference location

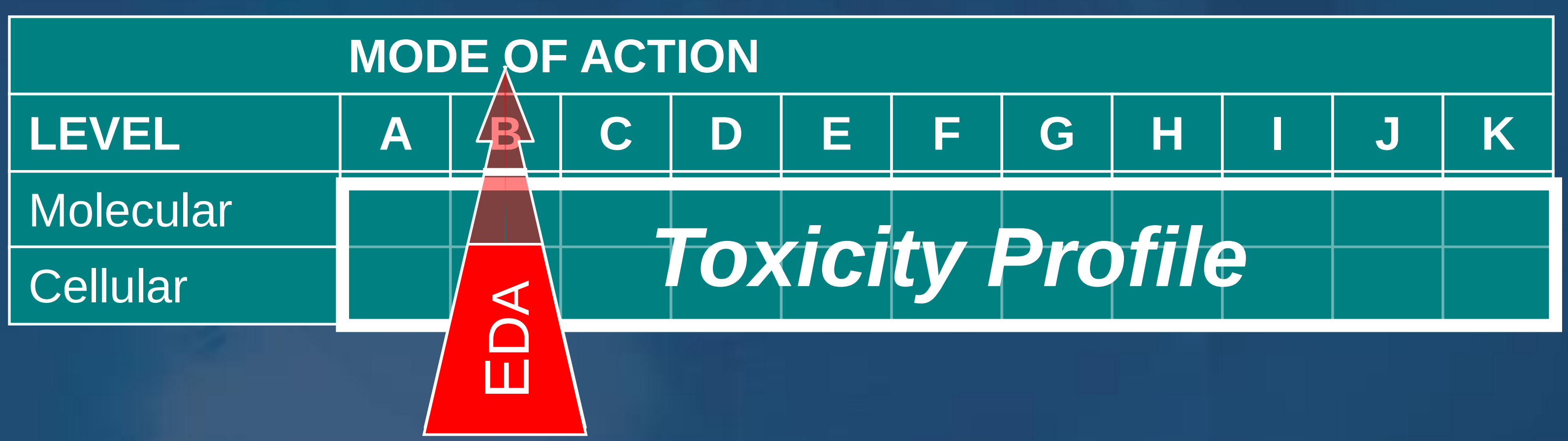
Risk-profile
Threshold value

DR-CALUX stable Reference	ER-CALUX total Reference	Location	DR-CALUX stable (pg TEQ/g dw)	ER-CALUX total (pg EEQ/g dw)	DR-CALUX stable Common tern	ER-CALUX total fathead minnow
1	1	Oosterschelde 1	2	5	0.1	0.01
3	7	Oosterschelde 2	4	34	0.2	0.05
20	2	Zierikzee buiten	32	11	1.6	0.02
99	25	Zierikzee binnen	158	126	7.9	0.19
12	36	Veerse Meer	20	182	1.0	0.27
68	68	Haringvliet	108	340	5.4	0.51
72	25	Bruinisse	116	124	5.8	0.19
58	18	Dintel Sluizen	92	92	4.6	0.14
84	13	Moerdijk	134	65	6.7	0.10
11	3	Nieuwe Maas	18	15	0.9	0.02
2	2	Nieuwe Waterweg	3	10	0.2	0.01
102	15	Rotterdam IJssel Haven	163	76	8.1	0.12
70	26	Rotterdam 2e Petroleumhaven	112	128	5.6	0.19
63	9	Biesbosch 1	101	45	5.1	0.07
46	8	Biesbosch 2	74	40	3.7	0.06

1
1-10
10-100
100-1000
>1000

0.01-0.03
0.03-0.10
0.10-0.30
0.30-1.0
>1.0

From Toxicity Profile to Compound Identification



Effect directed analysis (EDA) with in vitro bioassays

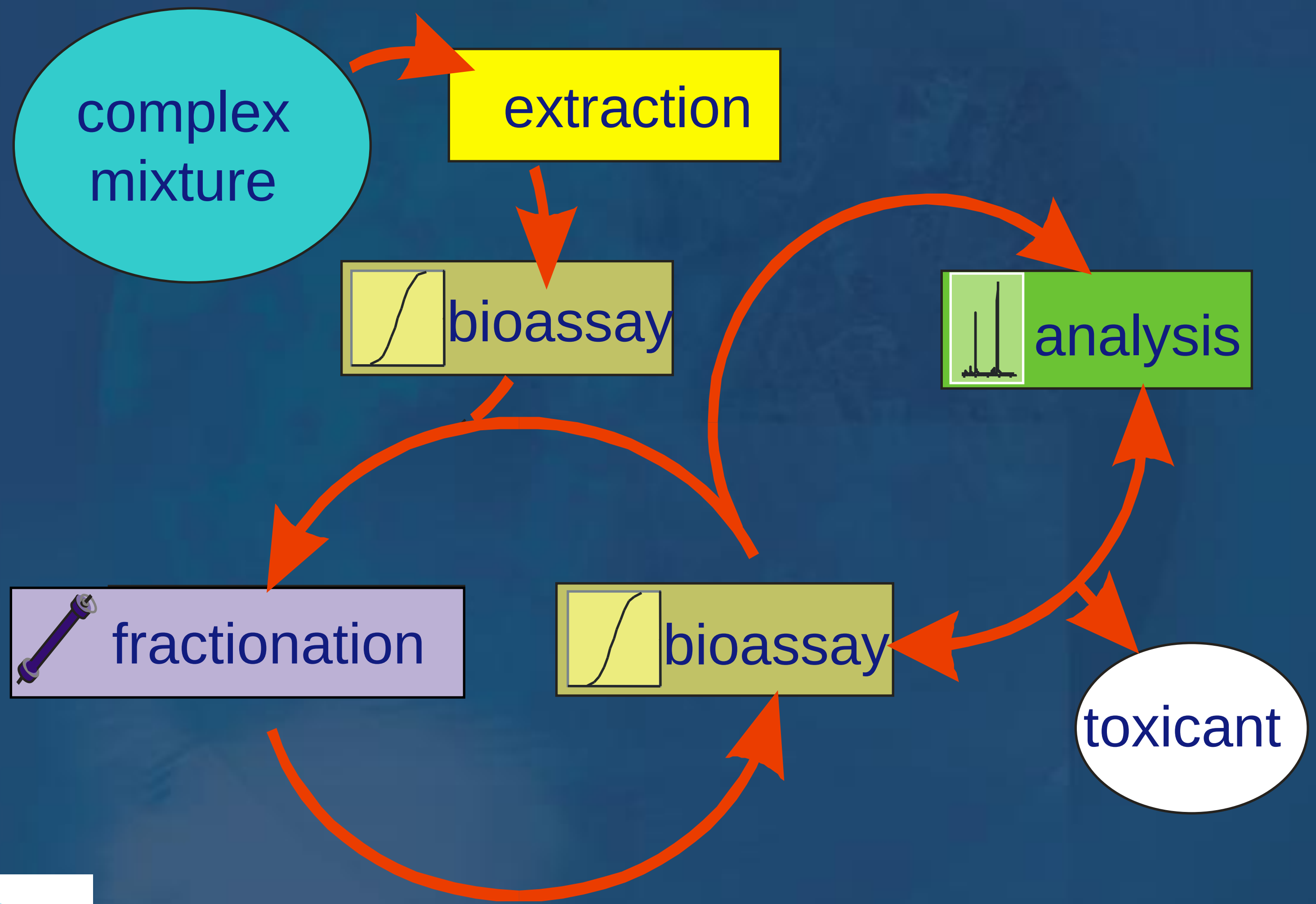
Locatie	DR-CALUX total	DR-CALUX stable	ER-CALUX total	ER-CALUX stable	TTR stable	Microtox total	Microtox stable	umu-S9 total	umu+S9 total
Oosterschelde 1	1	1	1	1	1	1	1	1	1
Oosterschelde 2	16	3	7	1	1	1	1	1	1
Zierikzee buiten	[illegible]	20	[illegible]	1	4	13	1100	2	1
Zierikzee binnen	1178	99	25	1	1	13	1	1	3
Veerse Meer	88	12	36	1	2	204	2	6	5
Haringvliet	[illegible]	68	68	1	5	38	1	1	1
Bruinisse	[illegible]	72	25	1	3	9	1	1	5
Dintel Sluizen	485	58	18	1	4	21	1	1	1
Moerdijk	[illegible]	84	13	1	2	61	1	1	3
Nieuwe Maas	[illegible]	11	[illegible]	1	1	2	1	1	1
Nieuwe Waterweg	92	2	[illegible]	1	1	2	1	3	1
Rotterdam IJssel Haven	381	102	15	1	2	24	1	1	1
Rotterdam 2e Petroleumhaven	919	70	[illegible]	2	3	12	14	1	1
Biesbosch 1	393	63	9	1	1	16	1	2	1
Biesbosch 2	422	46	8	1	1	12	1	4	1

EDA

Compound identification?

Source identification???

Identification of active compounds: Effect-Directed Analysis



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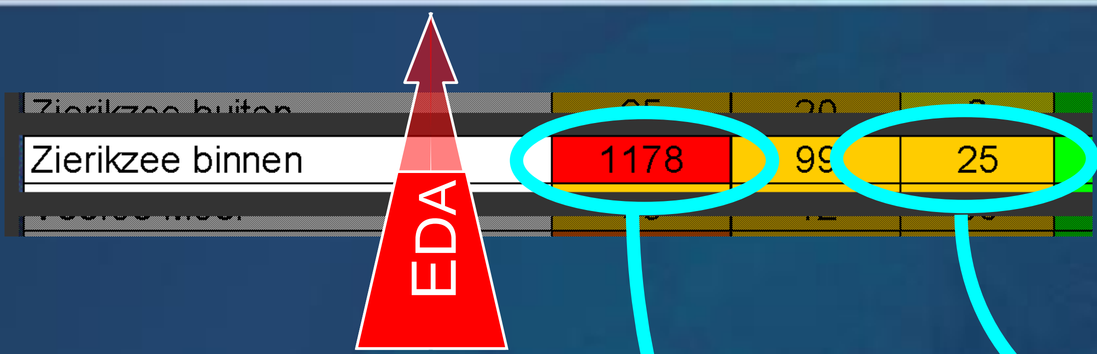
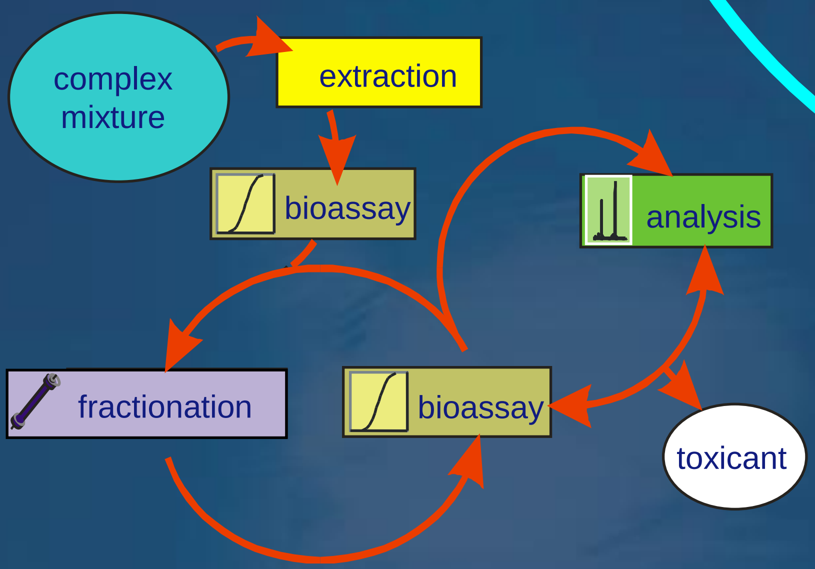
EDA in sediment from Zierikzee inner harbour



76% of estrogenic response by natural hormones

38% of dioxin-like response by PAHs



Houtman et al. (2006)
Chemosphere 65:2244-2252

Future perspectives in Toxicity Profiling

- Expansion of the test battery
  - Other toxicity syndromes
  - Multiple endpoints: -omics
  - High-throughput screening

- Improved ecological relevance
  - **B**ioavailability aspects
  - Toxicokinetics models
  - In vivo bioassays?

NORMAN Expert Group Meeting - October 9, 2009

Some “expert judgments”…

- Cover as many syndromes as possible
- Cover as many compounds as possible
- Avoid false negatives rather than false positives
- Accept confounders and deal with them
- Try to connect to WFD and its terminology
  - combination with chemical and ecological monitoring
- Good demonstration studies can demonstrate that toxicity profiling improves weight of evidence for
  - existing toxic hazard
  - cause / source of toxic hazard
  - need for measures
  - (cost) effectiveness of measures

Toxicity profiling: a first step to get GRIP on mixtures

- **<u>G</u>roup** locations with similar hazard profiles
- **<u>R</u>ank** locations based on “distance” to reference profile
  - Assigned
  - Good Ecological quality
  - Risk threshold
- **<u>I</u>dentify**
  - Important modes of action
  - Responsible compounds (EDA)
- **<u>P</u>rioritize**
  - Hot Spots
  - Compounds of interest

Profiling: unique combination of typical characteristics