Institute for Environmental Studies (IVM)

Toxicity profiling and use of bioassays in monitoring programs

Perspectives for development and implications for regulations

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Monitoring of (emerging) environmental pollutants

Chemical analysis

+ Identity known
+ Concentration known
- Not all compounds analyzed
- Compounds <DL not found
- Effect of mixture unknown
- Growing list of substances

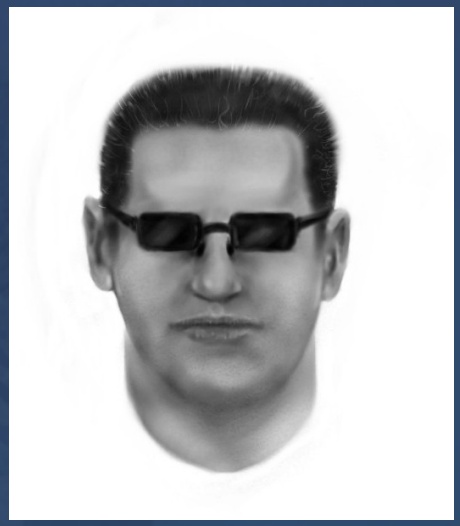
Bioassay

+ Effect of the mixture known
+ Mode of action known
- Identity responsible compounds unknown

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 (common) characteristics**
- **Identification of the “bad guys”**

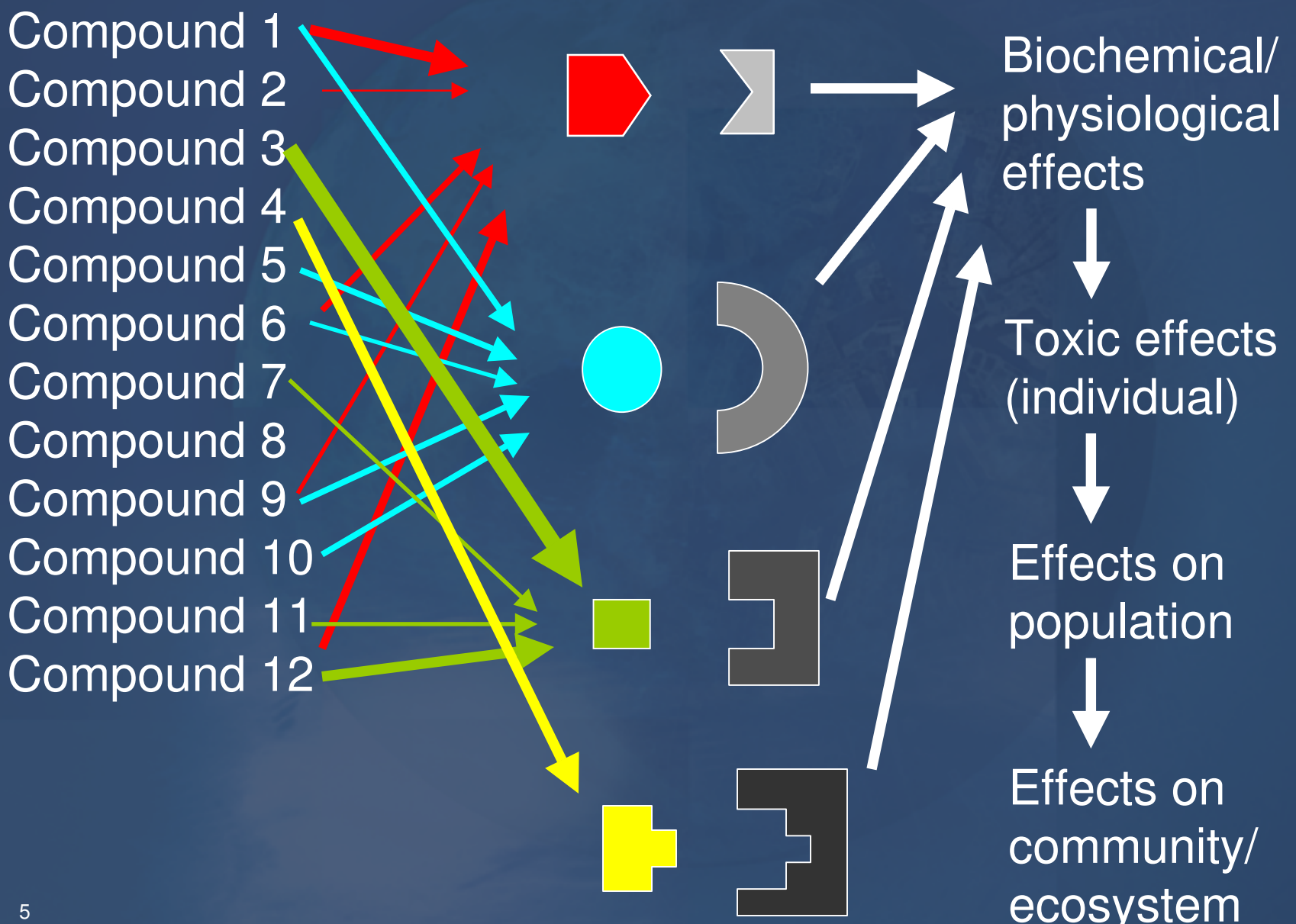
Profiling complex mixtures

E.g. Department C&B at IVM

- High Quality Contaminant Analysis
- Toxicity profiling
- Development of biomarkers and bioassays
- Effect Directed Analysis (EDA)
- Academic environment

- **Description**
- **Combination of (common) characteristics**
- **Hard to see individuals' contribution**
- **Total assessment of the mixture**
- **Useful for quality assessment**

Toxicity profiling: multiple characteristics



Biomonitoring using specific bioassays

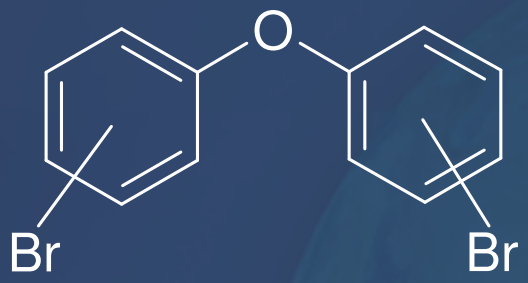
	MODE OF ACTION			
Compound 1	4	2		
Compound 2	1			
Compound 3			5	
Compound 4				4
Compound 5		2		
Compound 6	2	1		
Compound 7			1	
Compound 8				
Compound 9	1	2		
Compound 10		2		
Compound 11			1	
Compound 12	3		4	

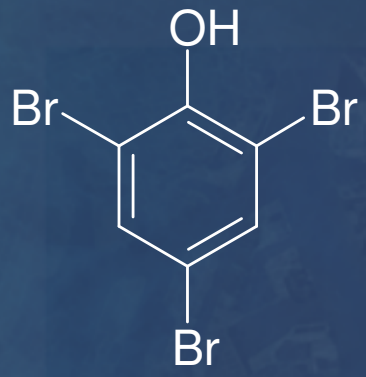
Mixture	3	2	2	2

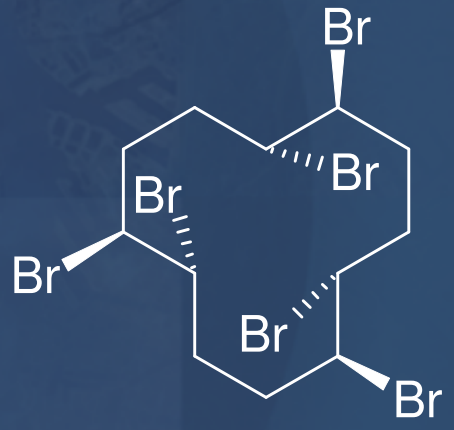
Toxicity profiling of individual compounds

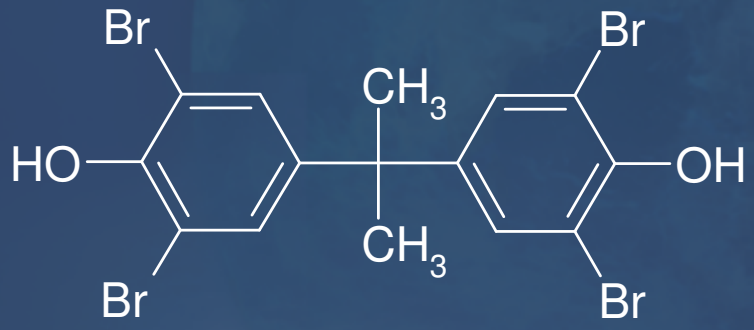
… and their metabolites

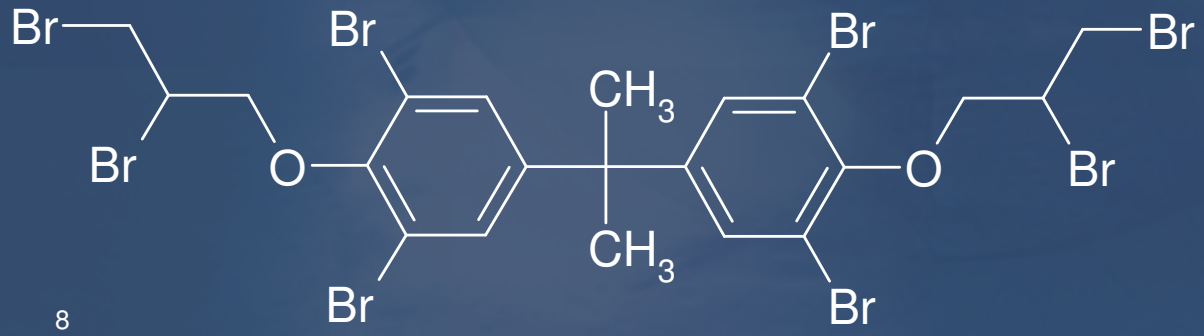
Toxicity profiling of brominated flame retardants (BFRs)









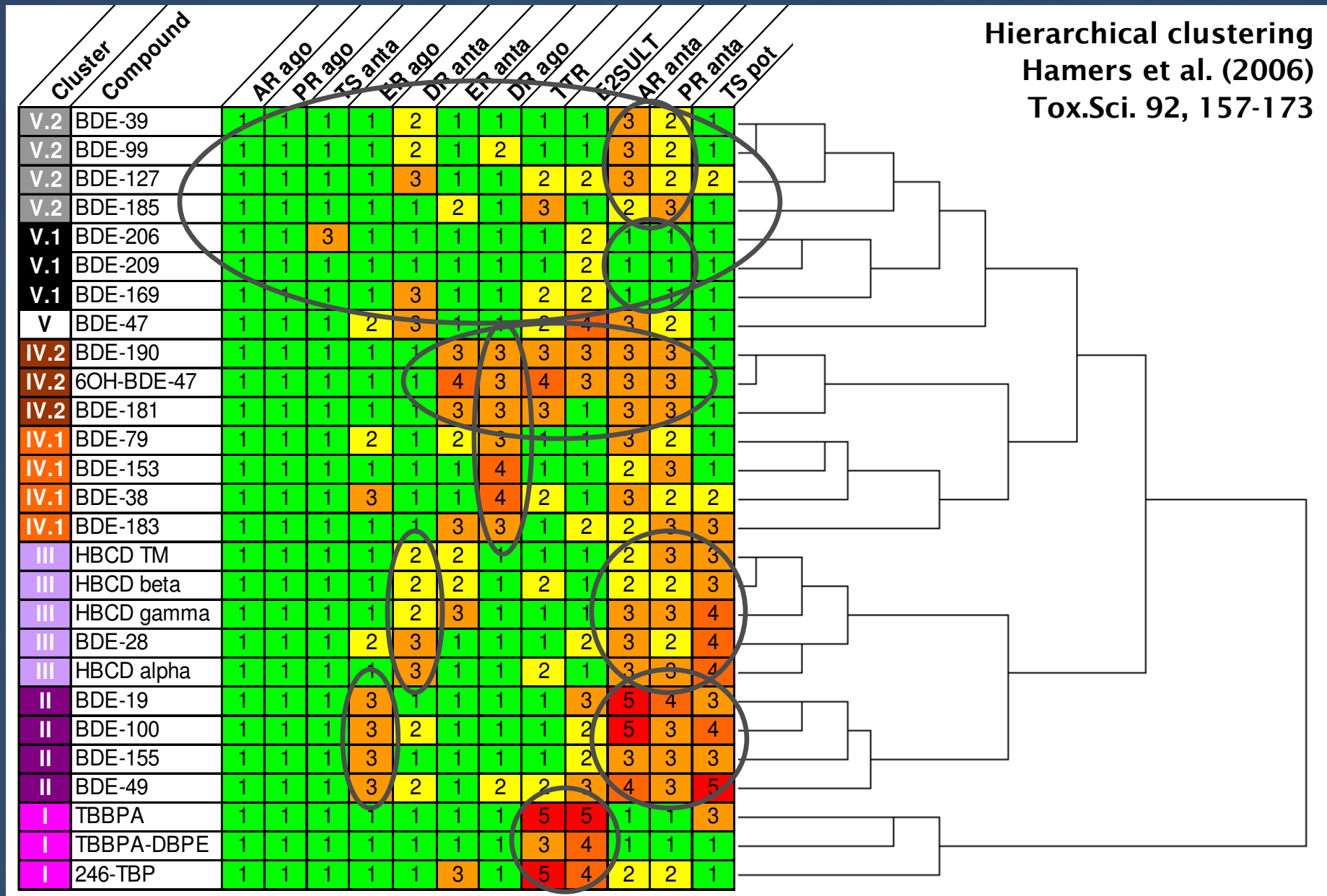


Toxicity profiling of brominated flame retardants (BFRs)

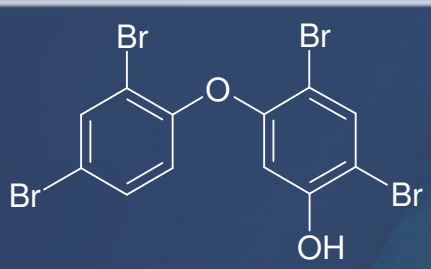
Compound	ERago	ERanta	TTR	DRago	DRanta	ARanta	PRanta	E2SULT	TSago	TSanta	ARago	PRago
BDE-19	3	1	1	1	1	5	4	3	3	1	1	1
BDE-28	2	1	1	1	3	3	2	2	4	1	1	1
BDE-38	3	1	2	4	1	3	2	1	2	1	1	1
BDE-39	1	1	1	1	2	3	2	1	1	1	1	1
BDE-47	2	1	2	1	3	3	2	4	1	1	1	1
BDE-49	3	1	2	2	2	4	3	3	5	1	1	1
BDE-79	2	2	1	3	1	3	2	1	1	1	1	1
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

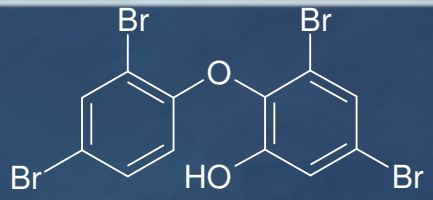
Toxicity profiling of brominated flame retardants (BFRs)



Parent compound vs metabolites: example of BDE-47

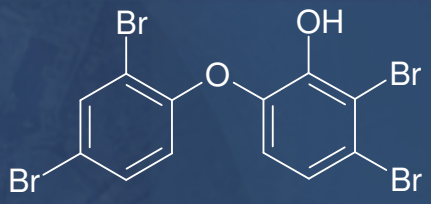


5OH-BDE-47 (4%)
IC50 (TTR) 25 nM
IC50 (E2SULT) 110 nM

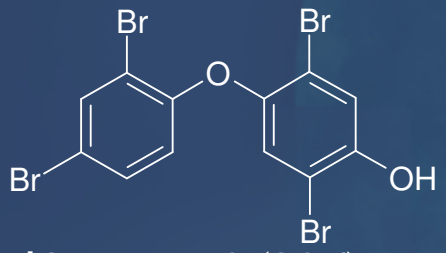


6OH-BDE-47 (1%)
IC50 (TTR) 150 nM
IC50 (E2SULT) 400 nM

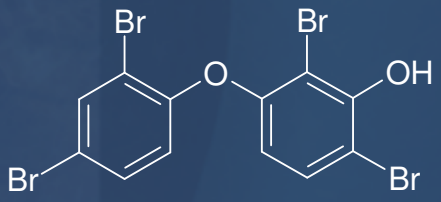
Hamers et al. (2008)
Mol.Nutr.FoodRes. 52, 284-298



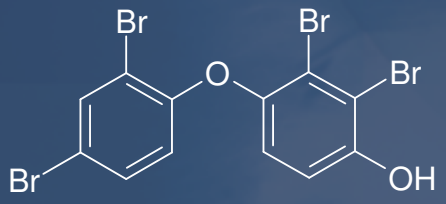
2'OH-BDE-66 (16%)
IC50 (TTR) 170 nM
IC50 (E2SULT) 1800 nM



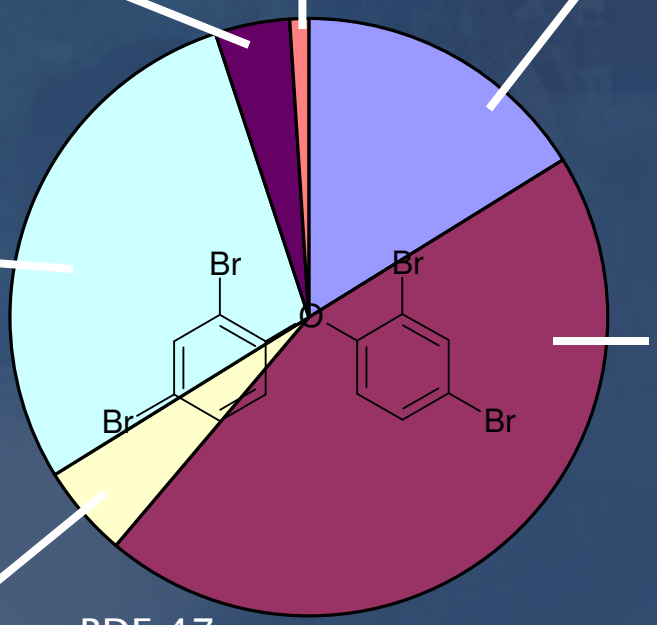
4'OH-BDE-49 (29%)
IC50 (TTR) 19 nM
IC50 (E2SULT) 18 nM



3OH-BDE-47 (45%)
IC50 (TTR) 17 nM
IC50 (E2SULT) 23 nM



4OH-BDE-42 (5%)
IC50 (TTR) 19 nM
IC50 (E2SULT) 23 nM



BDE-47
IC50 (TTR) 36000 nM
IC50 (E2SULT) 4000 nM

Toxicity profiling of environmental samples (complex mixtures)

Example of Harbor Sediments

Toxicity profiles of harbor sediments

Locatie	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 mg 4NQOEQ/g dw)	umu+S9 total mg AAEQ/g dw)
Oosterschelde 1	10	2	5	2	3	0.2			
Oosterschelde 2	153	4	34	2	3				
Zierikzee buiten	621	32	11	2					87
Zierikzee binnen	11305	158	126						259
Veerse Meer	844	20						81	461
Haringvliet	9150						0.02	13	87
Bruinisse						1	0.02	13	453
Dintel Sluizen					13	3	0.02	13	87
Moerdijk				2	6	10	0.02	13	255
			15	2	3	0.3	0.02	13	87
		3	10	2	3	0.3	0.02	35	87
R	3659	163	76	2	7	4.0	0.02	13	87
R	8827	112	128	4	9	1.9	0.31	13	87
Bie	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?

PAHs dioxines PCBs

Estrogens as DDT, nonylphenol, phthalates

Decreased respiration, e.g. carbaryl, nonylphenol, (metals)

Genotoxic compounds as PAHs

Competition with thyroid hormone: e.g. phenols

Houtman et al. (2004) Environ.Toxicol.Chem. 23, 32-40

Toxicity profiling of harbor sediments: Comparison to watersystem-specific reference

Locatie	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	
Oosterschelde										
Rijn- en Maasmonding Zuid										
Rijn- en Maasmonding Noord										
Rijn- en Maasmonding Midden										
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

Legend

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

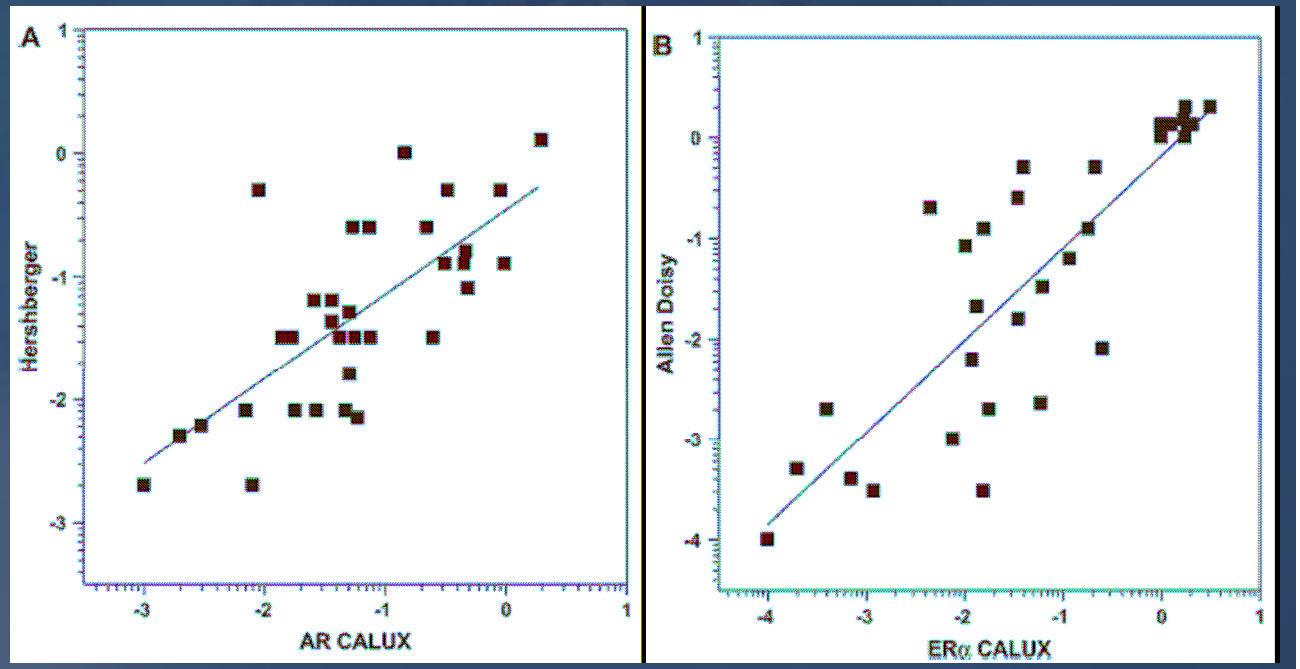
Ecological validation of toxicity profiles

The famous “so-what?” question

In vivo validation of in vitro bioassays

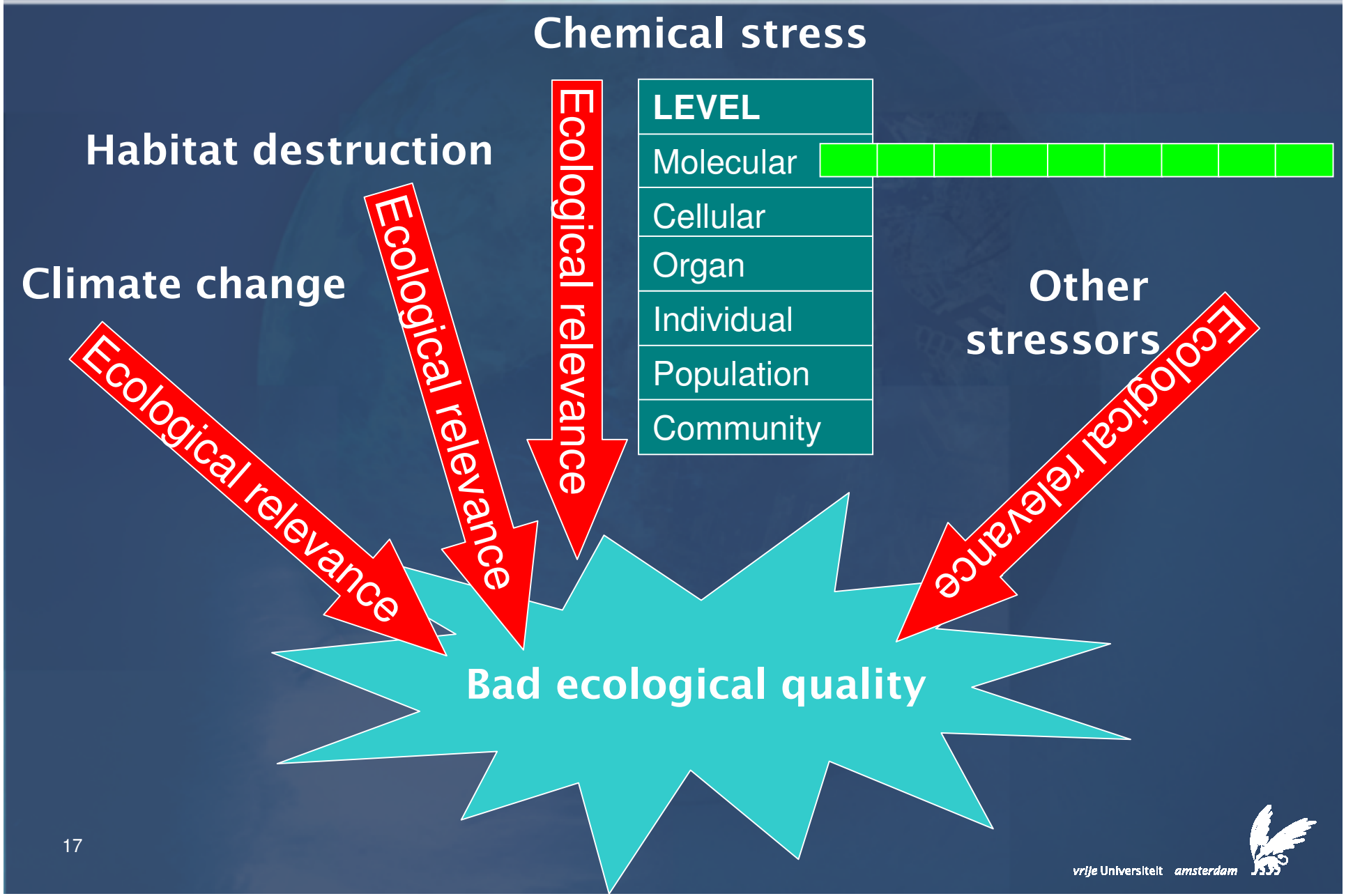
Ecological relevance

LEVEL
Molecular
Cellular
Organ
Individual
Population
Community
Ecosystem

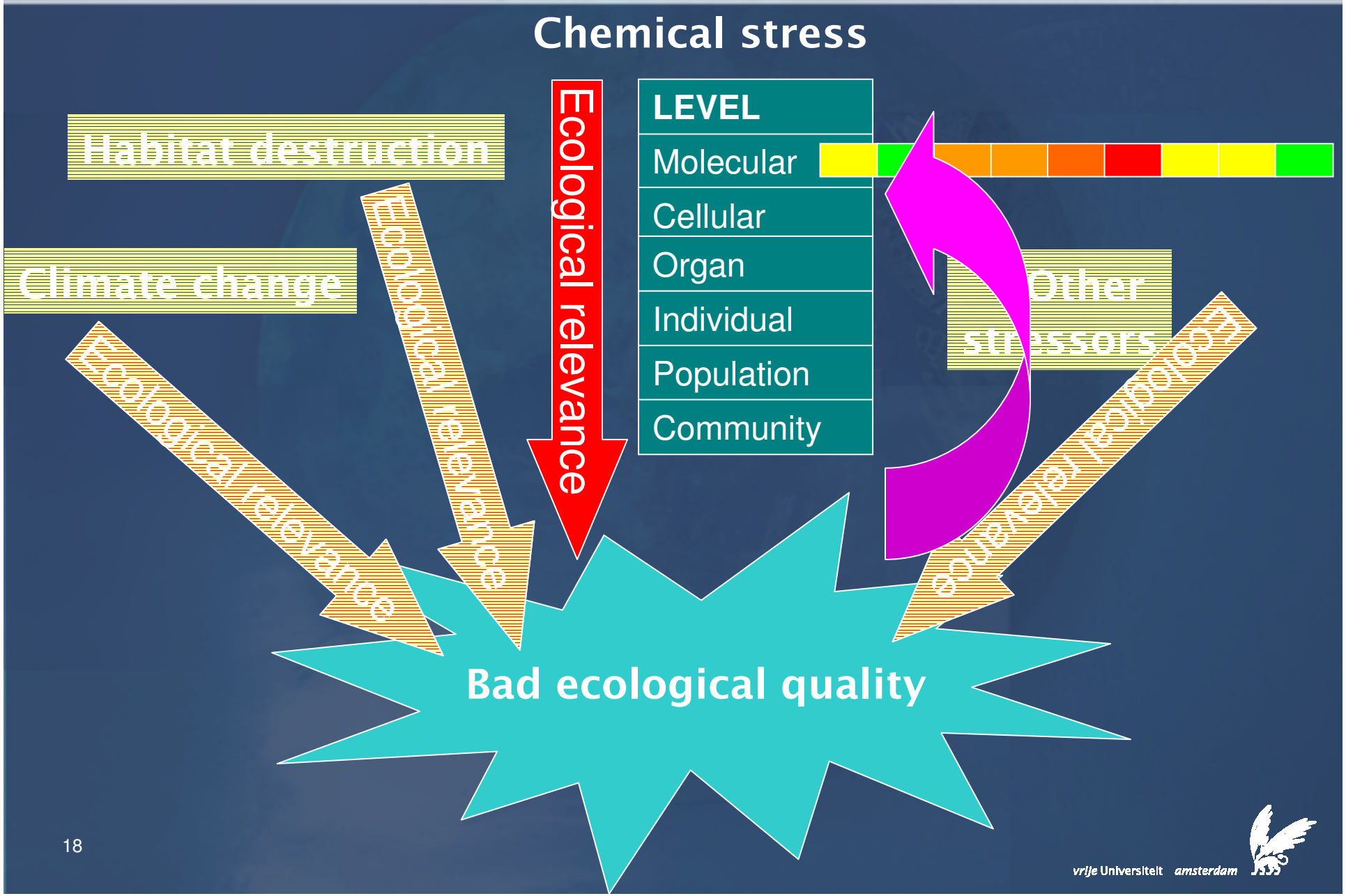


Sonneveld et al. (2006)
Toxicol.Sci. 89, 173-187

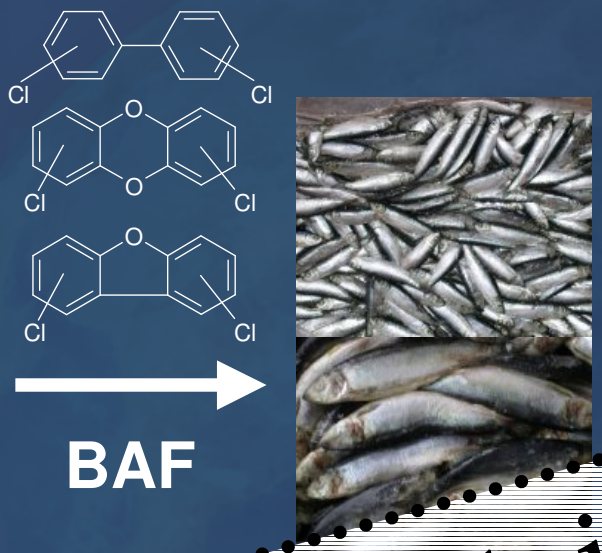
Ecological validation of in vitro bioassays

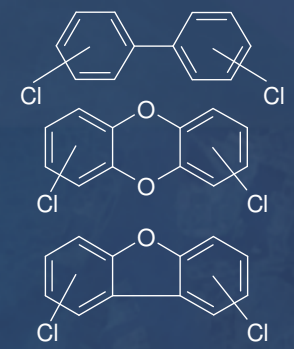


Ecological validation of in vitro bioassays



Example 1: Derive safe value for dioxin-like compounds



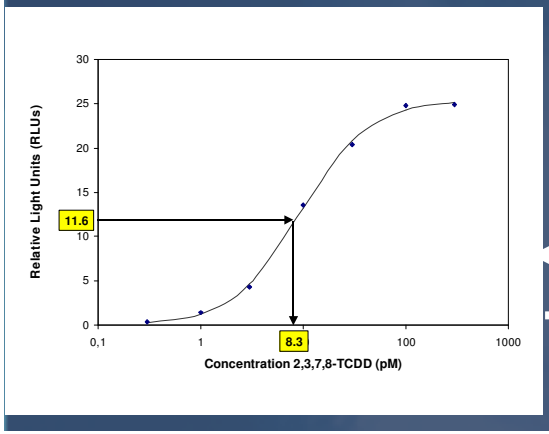


BAF

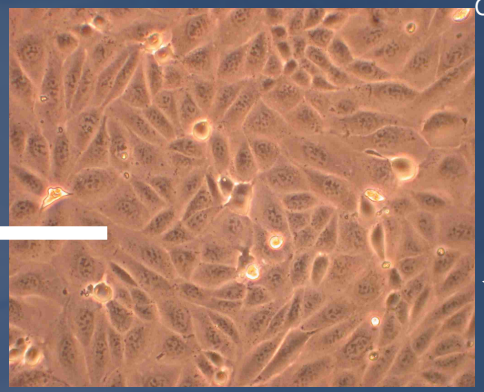
BSAF

Uncertainty???

K_f



TEQ



REP

Example 2: NORMAN working group 2

The use of bioassays in monitoring programmes: interpretation of results

- Definition and standardization of the interpretation of the results of monitoring with bioassays
- Validate relationship between biological quality (WFD species lists) and effects measured with bioassays or biomarkers
- Standardize and scientifically underpin interpretation of biomonitoring results
- Uncertainty analysis

Perspectives for development and implications for regulations

Perspectives for development & implications for regulations

Individual compounds

- Get GRIP (Grouping, Ranking, Prioritization)
  - QSARs, read-across
  - Reduced animal testing
  - REACH
- Include metabolization of chemicals
  - NORMAN annual workshop 2
- Expanding test set with *-omics*
- In vitro – in vivo validation is a bottle-neck

Perspectives for development & implications for regulations

Complex environmental mixtures

- Get GRIP (Grouping, Ranking, Prioritization)
  - Reference profiles for locations with different use
  - Safety net to select samples for EDA
  - Prioritize sampling locations ("hot spots")
- Use as tool for investigative monitoring in WFD
  - Relationship between bad ecological quality and chemical stress
- Expanding test set with *-omics*
- Bioassay – ecosystem validation is a bottle-neck
  - NORMAN working group 2

Tasks for Norman 2009-2010

Expert Group Toxicity Profiling

- Meeting November 2009 (Amsterdam)
- Position paper on use and interpretation of toxicity profiles
  - Profiles as such (GRIP)
  - In combination with EDA
  - Ecological relevance?

Working Group Bioassays in Monitoring

- Inventory of bioassays (specific and non-specific)
- Discussions on
  - Interpretation of results
  - Requirements for implementation
- Develop strategic plan
  - Validation Study
  - Final workshop
- Position paper