Contribution of biodetection for water safety (Chair H. Hollert)

09.00-09.20	Future of water testing (H. Hollert, RWTH Aachen)
09.20-09.40	Bioactive compounds with EDA (C. Houtman, HWL Haarlem)
09.40-10.00	Bioactivities in drinking WTPs (A. Oskarsson, SLU Uppsala)
10.00-10.15	AquaNes Project (H. Besselink, BDS)
10.15-10.30	**Coffee Break and Poster Session**
10.30-10.50	WWTPs & Ozonation (H. Bielak, IWW Mülheim)
10.50-11.10	Safer advanced WWTPs (H. Schaar, TU Vienna)
11.10-11.30	Safety of pipeline migration water (R. Junek, UBA)
11.30-11.50	Ecotox & corrosion protection (E. Vermeirssen, Ecotox Centre)
11.50-12.10	Novel bioassays for antibiotics in water (T. de Boer, MLS)
12.10-12.30	Nationwide water quality assessment (M. de Baat UvA)
12.30-13.30	**Lunch & Presentation (QuoData, Tecan) & Posters**

11th BioDetectors Conference 2018

INSTITUT FÜR UMWELT-FORSCHUNG | RWTH AACHEN UNIVERSITY

Sponsored by

BDS BioDetection Systems · BERTHOLD TECHNOLOGIES · HAMILTON · LC Tech

Effect-based methods for evaluating Water and surface waters – Future of water testing

Henner Hollert,

Sarah Könemann, Thomas-Benjamin Seiler, Carolina Di Paolo, Sabrina Schiwy, Sarah Crawford, Werner Brack, Inge Werner, Robert Kase, Beate Escher & Mario Carere

Marie Curie ITN Project EDA Emerge
EU Integrated Project Solutions

BDS Conference 14.9.2018

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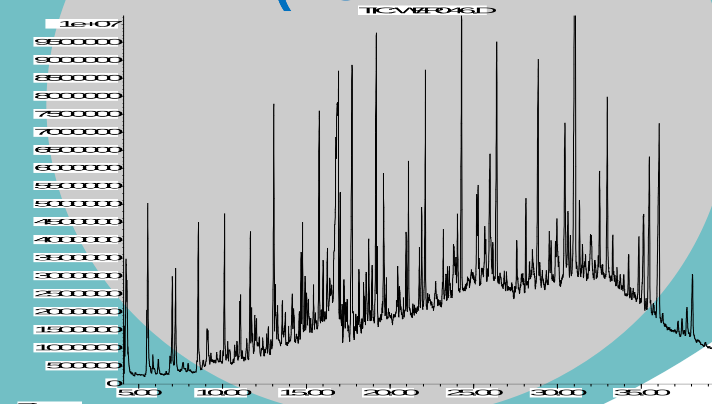
- Complex mixture
- Chemical Analysis of priority substances does often not explain the effects.
- Bioassays are suitable to evaluate effects of complex environmental mixtures

>143 Mio known chemicals

Over 1000 of chemicals with elevated concentrations

- **45 priority substances (EU-WFD**

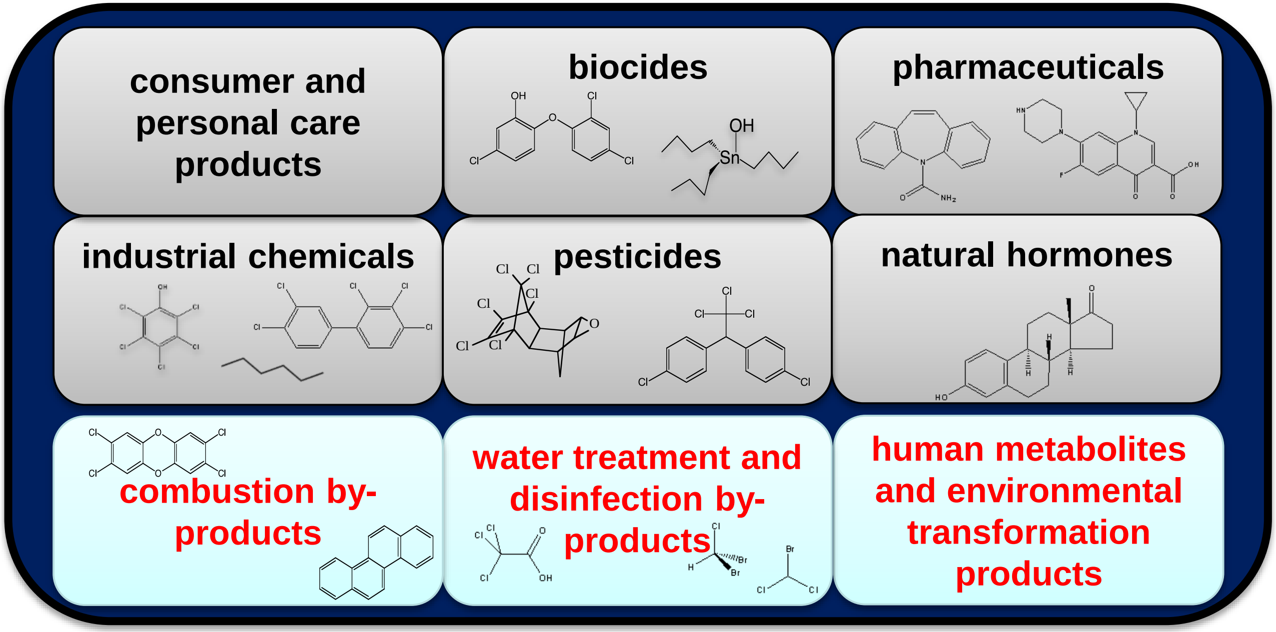


Is there a problem?

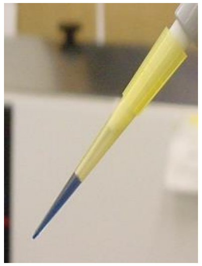
EU-WFD: EU-Water Framework directive

Brack et al. (2010) UWSF, Hecker & Hollert (2009): Environ Sci Poll Res, Hollert et al. (2009): UWSF

Anthropogenic organic chemicals **and** **transformation products** put pressure on ecosystems and drinking water resources



Courtesy by Beate Escher



mg/kg = mg/L = ppm = 10^6
µg/kg = µg/L = ppb = 10^9
ng/kg = ng/L = ppt = 10^{12}

Hollert et al., CS3 Meeting

Chemical and bio-analysis are complementary and deliver pieces of the puzzle

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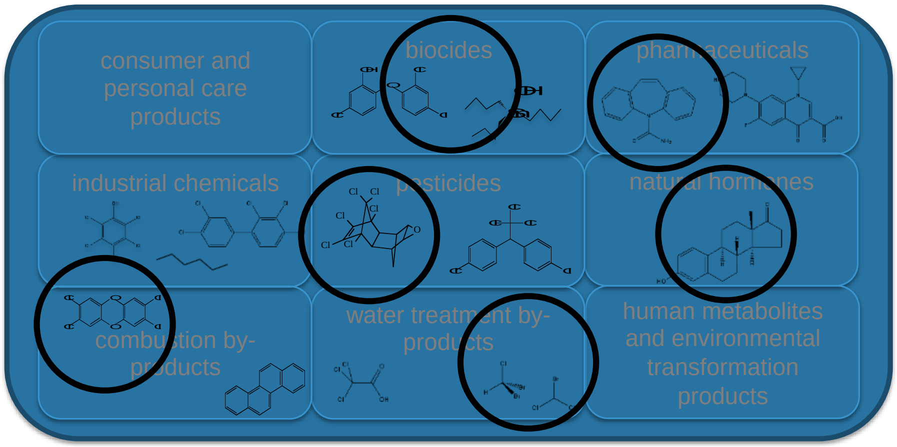
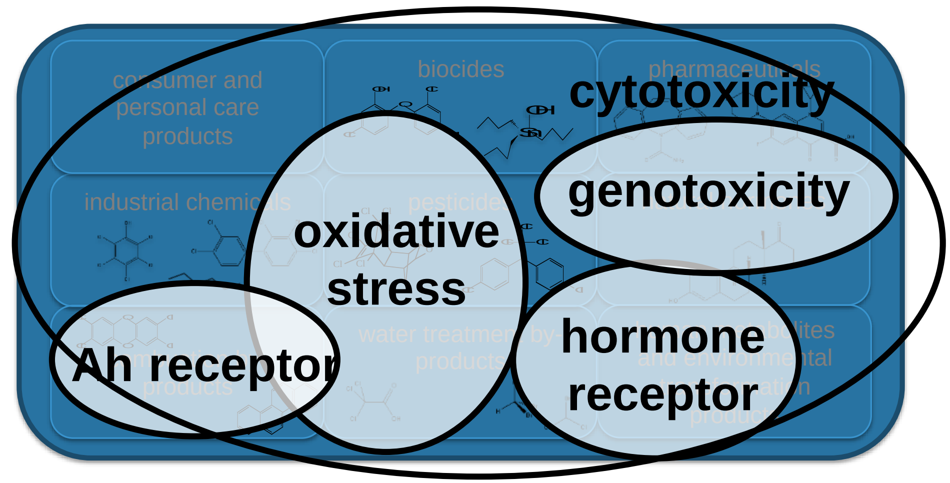
Chemical analysis:



- **Quantitative for key target chemicals**
- **Unknowns difficult and work-intensive to identify (non-target analysis)**

Bioanalytical tools:



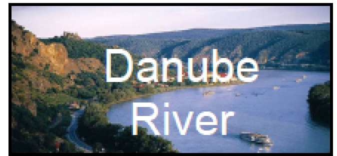
- **From fully integrative to summation of groups of chemicals with common mode of action**
- **Single chemicals cannot be resolved**

Tang, McCarty, Glenn, Neale, Warne, Escher, 2013. Water Res., 47: 3300-3314.

Courtesy by Beate Escher

Danube case study: Large fraction of unspecific effects remains unexplained by detected chemicals

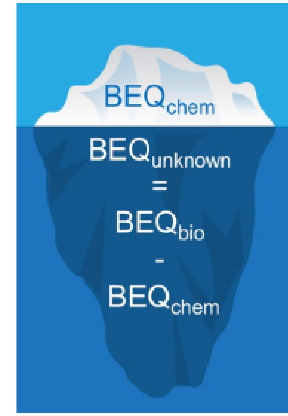
HV & LV SPE
Schulze et al. 2017 STOTEN



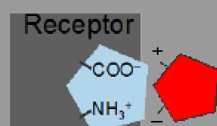
Chemical analysis (272 chemicals targeted)

BEQ_{bio} ↔ BEQ_{chem}

% contribution to effect?



First iceberg today ☺ !

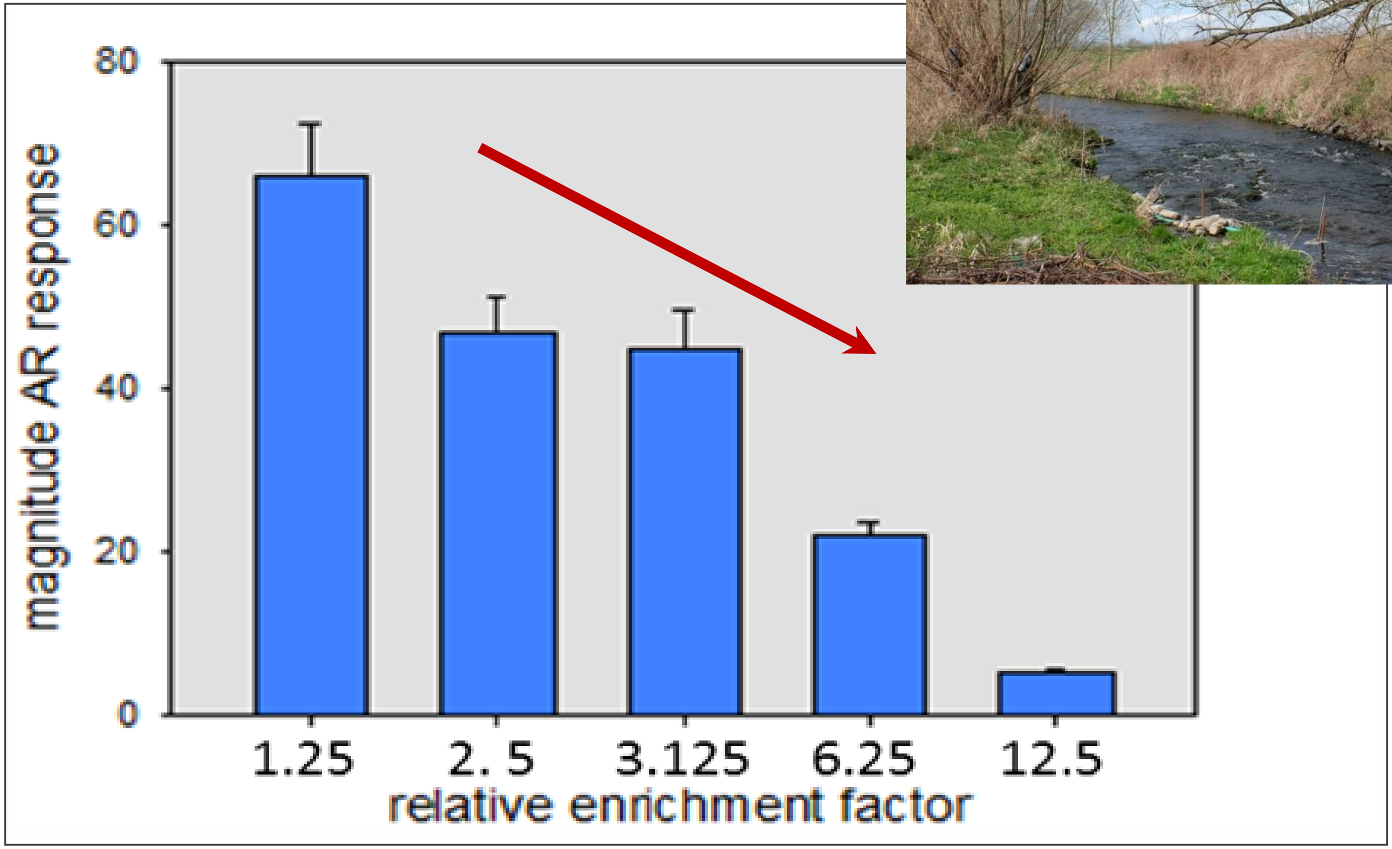
Metabolism	MIE	Key event	Cell	Organisms
AhR: 3-71% PXR: 0.003 to 0.1%	 ER <80%	Induction adaptive stress resp. ARE (ox. stress) <0.2% p53 (genotox) <0.1% nfkB (inflammation) 0%		Fish embryo toxicity 0.002 -0.2%

Neale, Ait-Aissa, Brack, Creusot,, Denison, Deutschmann, Hilscherova, Hollert, Krauss, Novak, Schulze, Seiler, Serra, Shao, Escher, (2015) ES&T 49: 14614-14624

Example: Anti-androgenic effects in River Holtemme, Germany

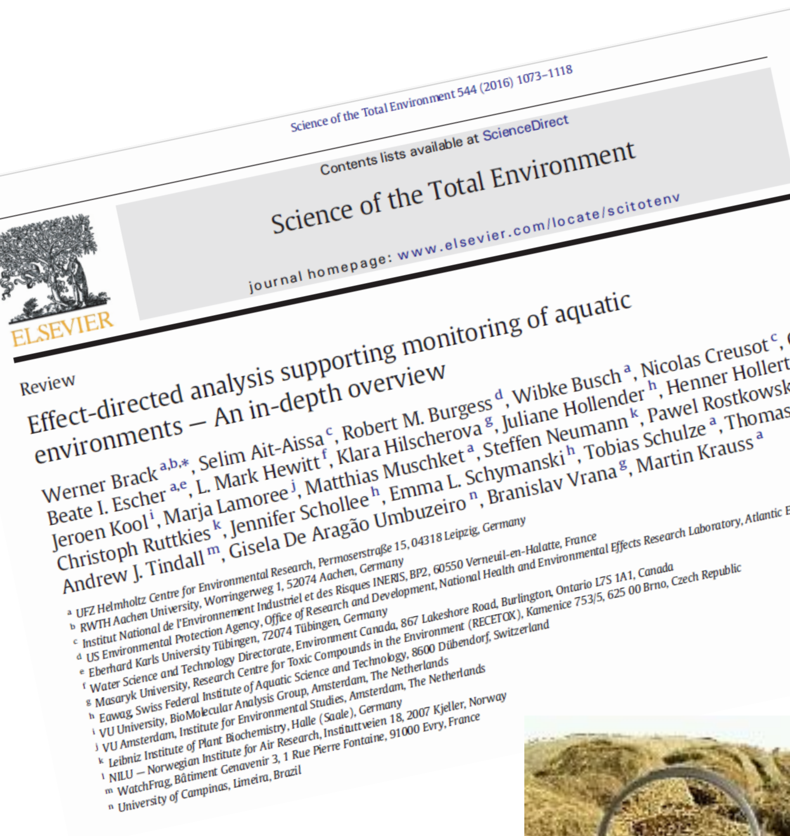
Institute for Environmental Research

AR CALUX:



Di Paolo … Muschket, Brack, Hollert & Seiler . 2016: Science of the Total Environment 826–833

Effect-directed analysis

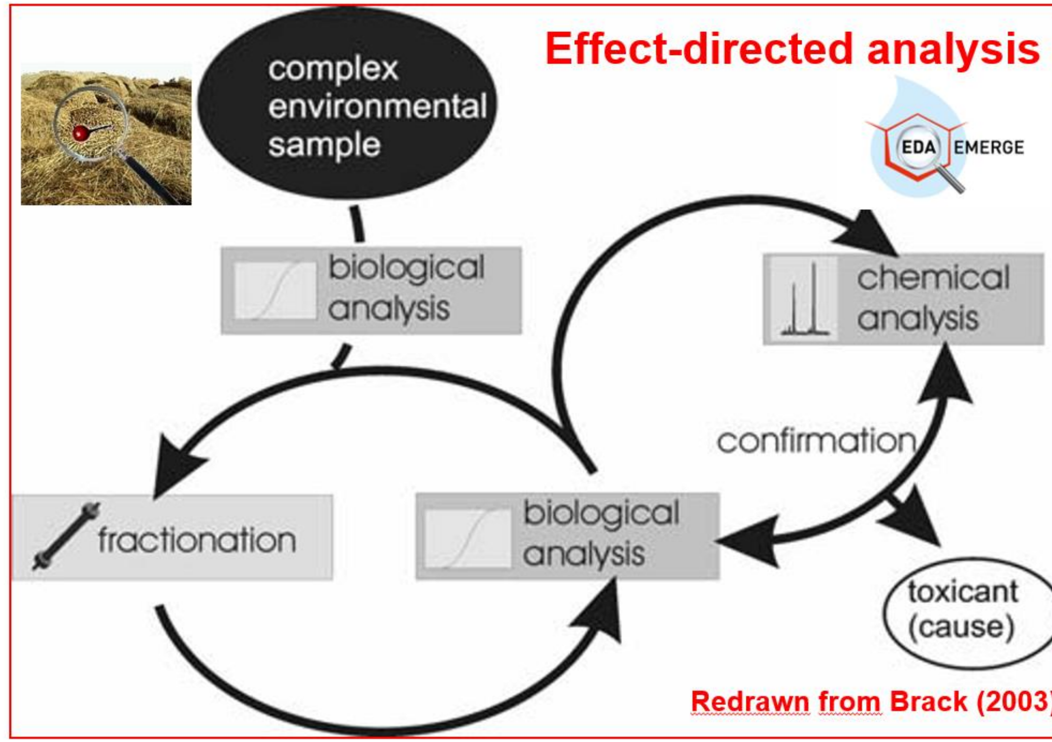

Science of the Total Environment 544 (2016) 1073–1118
Contents lists available at ScienceDirect
Science of the Total Environment
journal homepage: www.elsevier.com/locate/scitotenv
ELSEVIER

Review

Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview

Werner Brack [a,b,*], Selim Ait-Aissa [c], Robert M. Burgess [d], Wibke Busch [a], Nicolas Creusot [c], C
Beate I. Escher [a,e], L. Mark Hewitt [f], Klara Hilscherova [g], Juliane Hollender [h], Henner Hollert [b]
Jeroen Kool [i], Marja Lamoree [j], Matthias Muschket [a], Steffen Neumann [k], Paweł Rostkowski
Christoph Ruttkies [k], Jennifer Schollee [h], Emma L. Schymanski [h], Tobias Schulze [a], Thomas-
Andrew J. Tindall [m], Gisela De Aragão Umbuzeiro [n], Branislav Vrana [g], Martin Krauss [a]

[a] UFZ Helmholtz Centre for Environmental Research, Permoserstraße 15, 04318 Leipzig, Germany
[b] RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
[c] Institut National de l'Environnement Industriel et des Risques INERIS, BP2, 60550 Verneuil-en-Halatte, France
[d] US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ec
[e] Eberhard Karls University Tübingen, 72074 Tübingen, Germany
[f] Water Science and Technology Directorate, Environment Canada, 867 Lakeshore Road, Burlington, Ontario L7S 1A1, Canada
[g] Masaryk University, Research Centre for Toxic Compounds in the Environment (RECETOX), Kamenice 753/5, 625 00 Brno, Czech Republic
[h] Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland
[i] VU University, BioMolecular Analysis Group, Amsterdam, The Netherlands
[j] VU Amsterdam, Institute for Environmental Studies, Amsterdam, The Netherlands
[k] Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany
[l] NILU — Norwegian Institute for Air Research, Instituttveien 18, 2007 Kjeller, Norway
[m] WatchFrag, Bâtiment Genavenir 3, 1 Rue Pierre Fontaine, 91000 Evry, France
[n] University of Campinas, Limeira, Brazil



Hecker & Hollert (2009) Effect-directed analysis (EDA) in aquatic ecotoxicology: state of the art and future challenges, Environ Sci Poll Res, 16:607–613

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Effect-directed analysis

W. Brack et al. / Science of the Total Environment 544 (2016) 1073–1118 1077

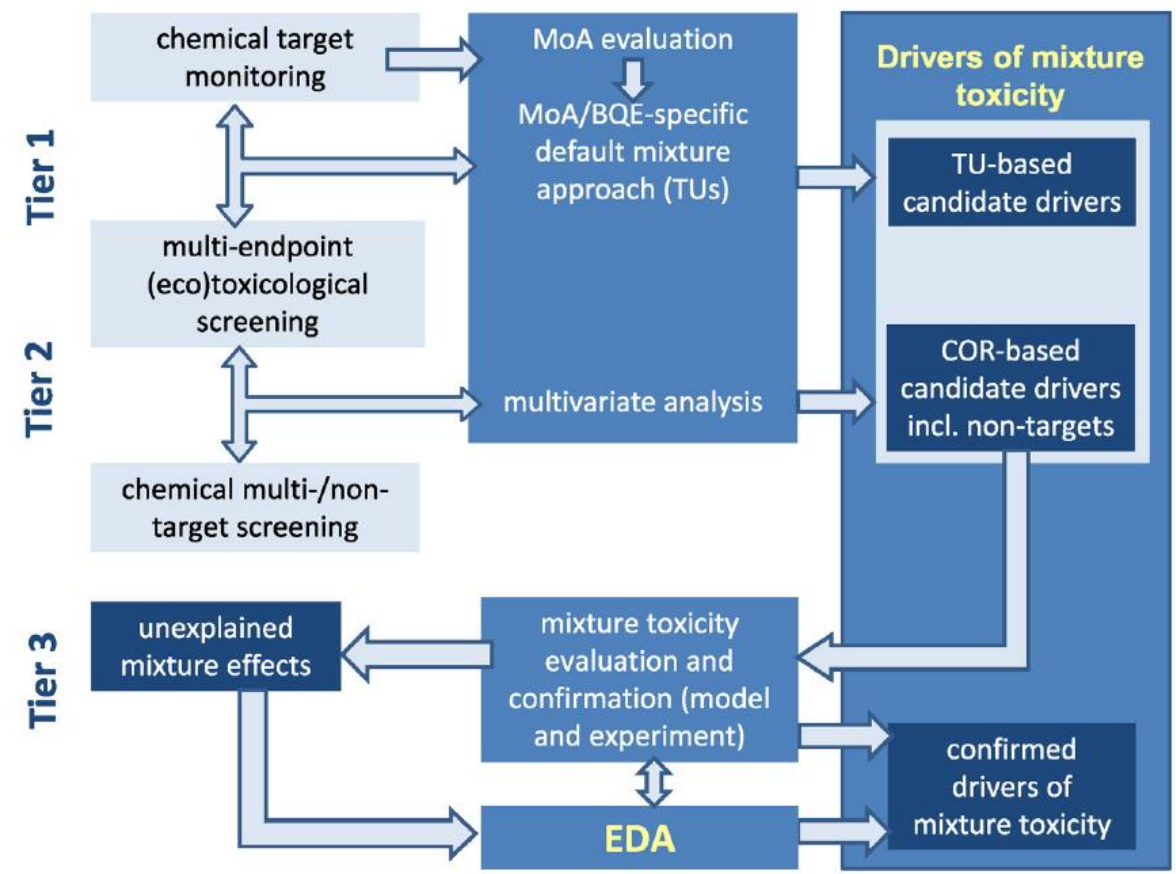

Fig. 2. Scheme of a conceptual framework for monitoring-based identification of drivers of mixture toxicity and the role of EDA in that framework. MoA = mode of action, BQE = Biological Quality Element, TU = toxic units, COR = correlation (modified from Altenburger et al., 2015).

Brack W, Ait-Aissa S, Burgess RM, Busch W, Creusot N, Di Paolo C, Escher BI, Mark Hewitt L, Hilscherova K, Hollender J, Hollert H, Jonker W, Kool J, Lamoree M, Muschket M, Neumann S, Rostkowski P, Ruttkies C, Schollee J, Schymanski EL, Schulze T, Seiler TB, Tindall AJ, De Aragao Umbuzeiro G, Vrana B, Krauss M (2016): Effect-directed analysis supporting monitoring of aquatic environments--An in-depth overview. Sci Total Environ 544, 1073-118

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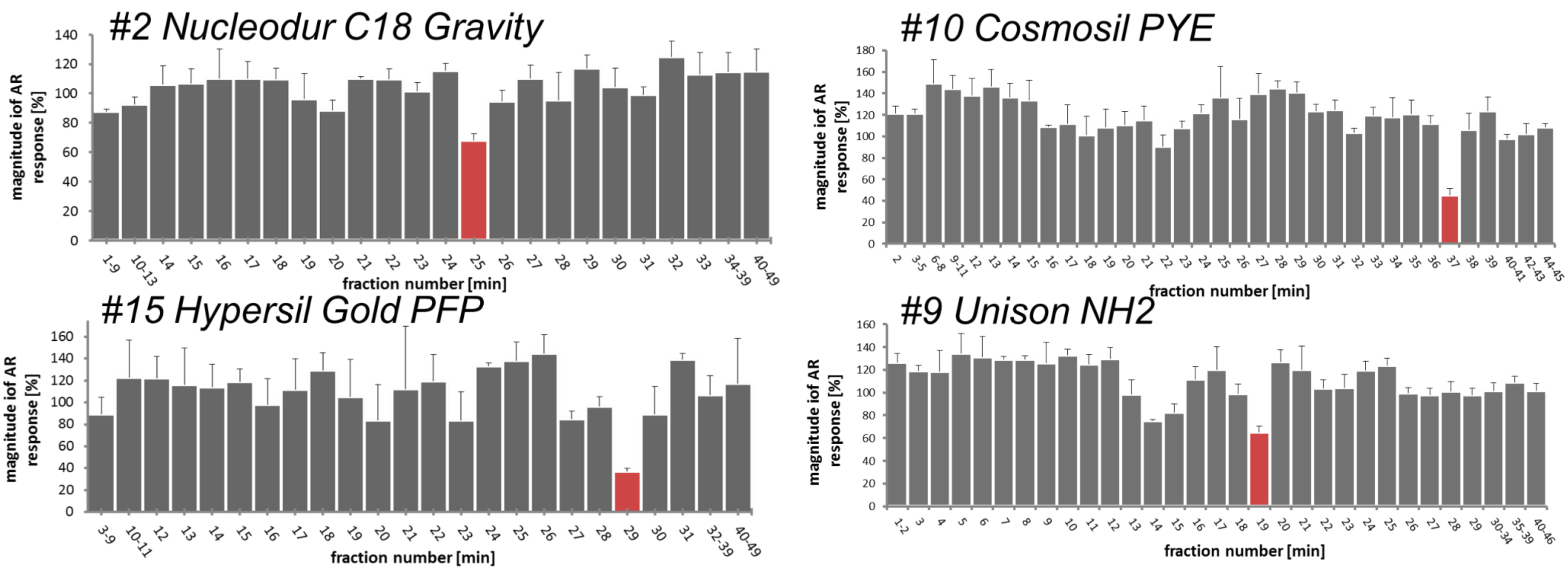
Example: Anti-androgenic effects in River Holtemme, Germany

Parallel RP-LC fractionation on orthogonal columns and testing of fractions:



Each fractionation procedure provides one active fraction:

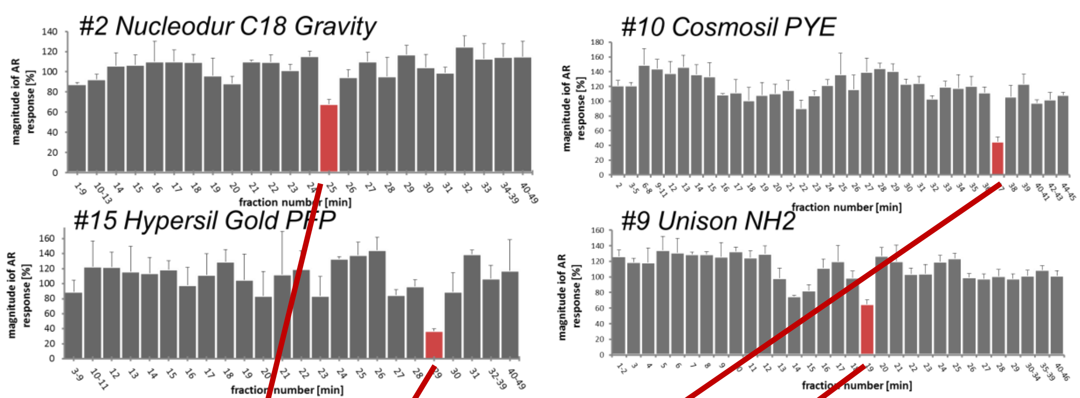
⇒ **Searching for the peaks they have in common**

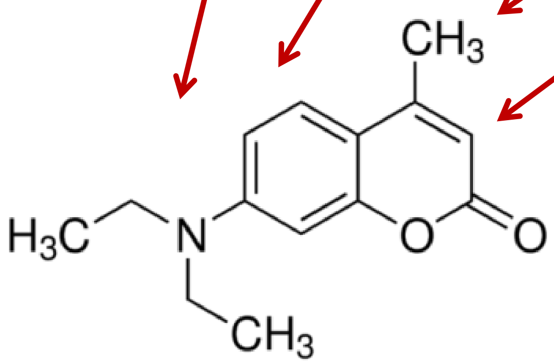
⇒ **Identification with LC-HRMS**

Muschket M, Di Paolo C, Tindall AJ, Touak G, Phan A, Krauss M, Kirchner K, Seiler TB, Hollert H, Brack W (2018): Environmental science & technology 52, 288-297

Higher tier EDA

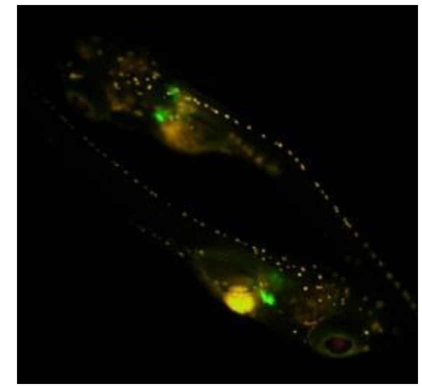
Example: Anti-androgenic effects in River Holtemme, Germany





4-methyl-7-diethyl-aminocoumarin and two metabolites

Quantitative confirmation as cause of the effect *in vitro*



Confirmation of effect *in vivo* in transgenic Medaka larvae

Muschket M, Di Paolo C, Tindall AJ, Touak G, Phan A, Krauss M, Kirchner K, Seiler TB, Hollert H, Brack W (2018): Environmental science & technology 52, 288-297

We started as a consortium of 17 partners …

We now have 70+ members !!!

The NORMAN network on emerging environmental substances

Valeria Dulio, INERIS
Executive Secretary of the NORMAN Association
Valeria.dulio@ineris.fr

Henner Hollert, RWTH Aachen University
Head Working Group 2 on Bioassays of the NORMAN Association

Network of reference laboratories, research centers and related organisations for monitoring of emerging environmental substances

Working Groups

Mission:

- **Exchange information** on emerging substances
- Improve **data quality**
- Promote **synergies** among research teams and **more effici**

•of research findings to **policy-makers**

1) Prioritisation

2) Bioassays

3) Effect-Directed Analysis

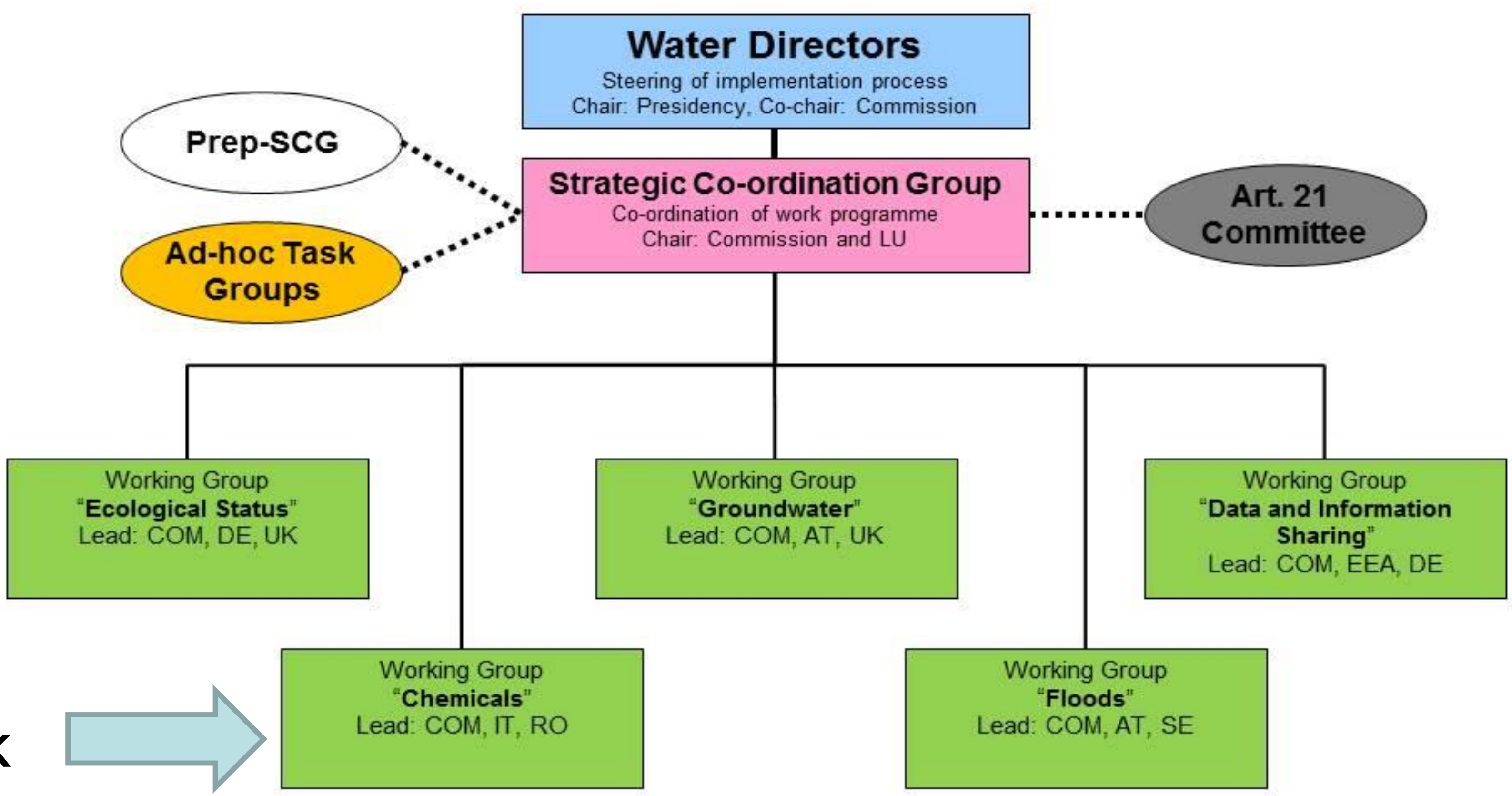
4) Engineered Nanoparticles

5) Wastewater reuse

6) Indoor environment

+ 2 Cross-WG: Passive sampling and NT screening

CIS Organisation 2016-2018



EBM TASK

COMMON IMPLEMENTATION STRATEGY

CMEP (Chemical Monitoring and Emerging Pollutants) Activity-Task on Effect-Based Tools (2010-2012) in the context of WG Chemicals.

Technical Report on Aquatic Effect Based Tools Published (2014)

RWTH AACHEN UNIVERSITY
Institute for Environmental Research

Wernersson *et al. Environmental Sciences Europe* (2015) 27:7
DOI 10.1186/s12302-015-0039-4

Environmental Sciences Europe
a SpringerOpen Journal

RESEARCH Open Access

The European technical report on aquatic effect-based monitoring tools under the water framework directive

Ann-Sofie Wernersson[1], Mario Carere[2*], Chiara Maggi[3], Petr Tusil[4], Premysl Soldan[4], Alice James[5], Wilfried Sanchez[5], Valeria Dulio[5], Katja Broeg[6], Georg Reifferscheid[7], Sebastian Buchinger[7], Hannie Maas[8], Esther Van Der Grinten[9], Simon O'Toole[10], Antonella Ausili[3], Loredana Manfra[3], Laura Marziali[11], Stefano Polesello[11], Ines Lacchetti[2], Laura Mancini[2], Karl Lilja[12], Maria Linderoth[12], Tove Lundeberg[12], Bengt Fjällborg[1], Tobias Porsbring[1], DG Joakim Larsson[13], Johan Bengtsson-Palme[13], Lars Förlin[13], Cornelia Kienle[14], Petra Kunz[14], Etienne Vermeirssen[14], Inge Werner[14], Craig D Robinson[15], Brett Lyons[16], Ioanna Katsiadaki[16], Caroline Whalley[17], Klaas den Haan[18], Marlies Messiaen[19], Helen Clayton[20], Teresa Lettieri[21], Raquel Negrão Carvalho[21],

… validation and harmonization of methods for the chemical and biological monitoring of emerging contaminants (eg. Wernersson et al. 2015).

solutions

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Joint Danube Survey 3

Marie Curie ITN Project EDA Emerge
EU Integrated Project Solutions

...ectors of the European Union, Candidate and EFTA Countries
...th and 5th of December 2013

Informal meeting of Water and Marine Directors of the European Union, Candidate and EFTA Countries
Vilnius, 4th and 5st of May 2013

Final Synthesis

Introduction

The Water and Marine Directors of the European Union[1] (EU) and EFTA countries[2] met on 4th and 5st of December 2013.

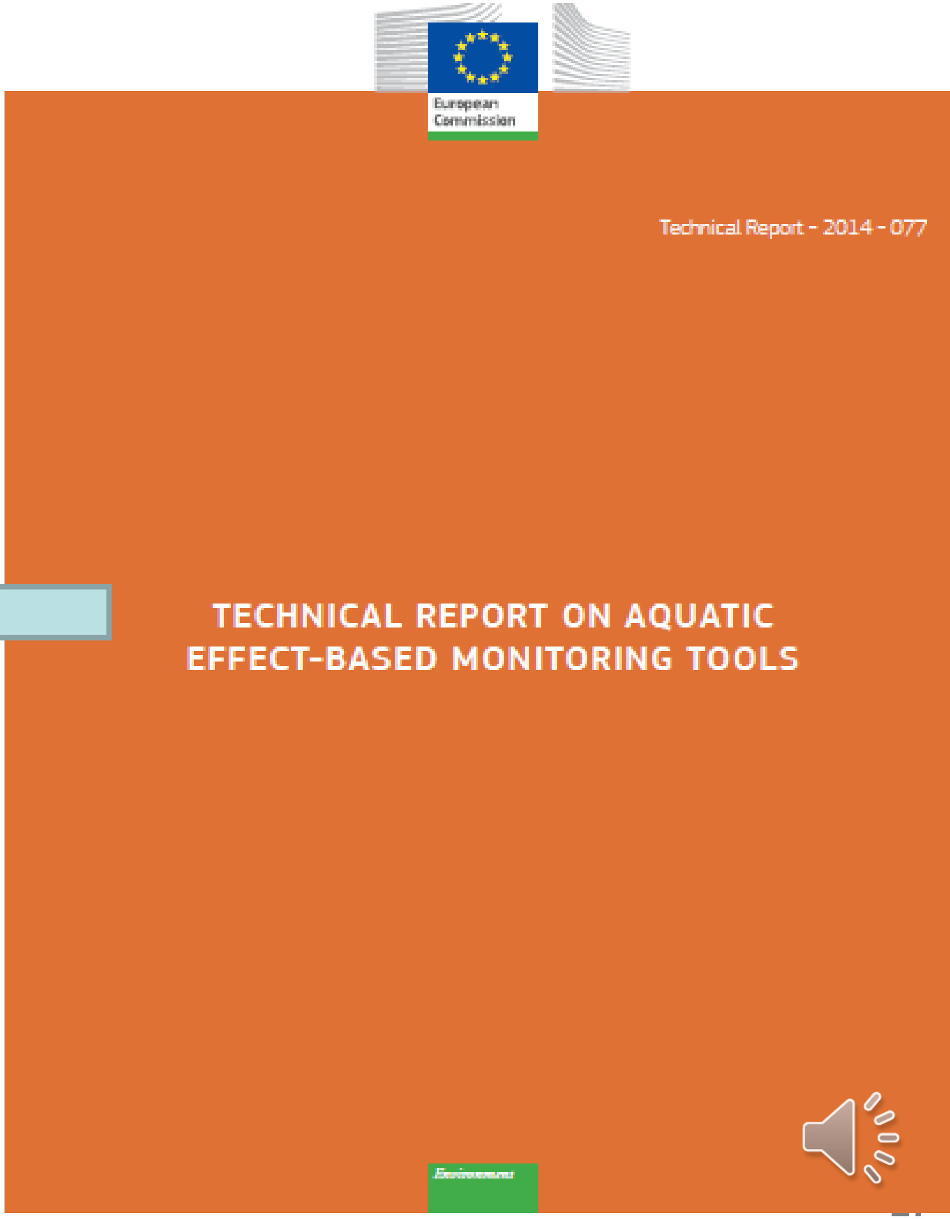
The Water Directors

- received information about a number of issues (CIS progress report, preparation of Commission report on the progress of the WFD Programme of Measures, the recently published reports on the implementation of Nitrates and the Urban Waste Water Treatment Directives, the review of the Annexes of the Groundwater Directive, the

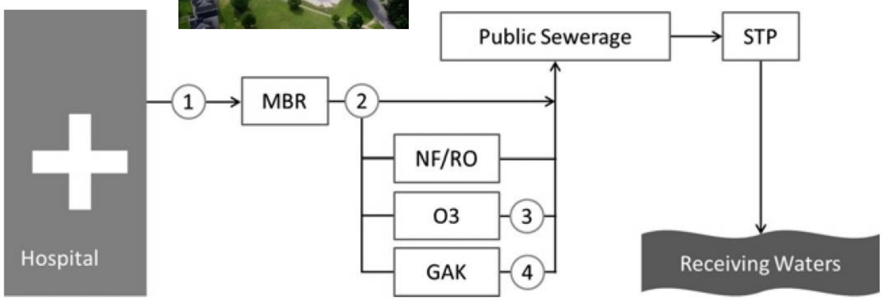
In vitro assays in Europe

Table 1 *In vitro* assays and their modes of action

Name/s of assay	Mode of action/endpoint
AR CALUX (anti-)	Androgen receptor (activation or blocking)
DR CALUX	AH receptor binding
ER CALUX (anti-)	Alpha and beta/estrogen receptors
GR CALUX (anti-)	Glucocorticoid receptor
PAH CALUX	AH receptor binding
PR CALUX	Progesterone receptor
Acetylcholinesterase inhibition assay	Inhibition of acetylcholinesterase activity
Carboxylesterase inhibition assay	Inhibition of carboxylesterase activity
Ames	Mutagenicity
umuC	Primary DNA damage
TTR-binding	Competition with thyroid hormone for binding to TTR (transport protein)
TRb CALUX	Thyroid receptor beta
EROD	EROD induction
YES	ER receptor
YAS	AR receptor
P-53 accumulation	Genotoxicity
Green screen	Genotoxicity
RYA	ER receptor
ABC assay	Antibiotic activity



- ER-CALUX
- L-YES
- H295r assay



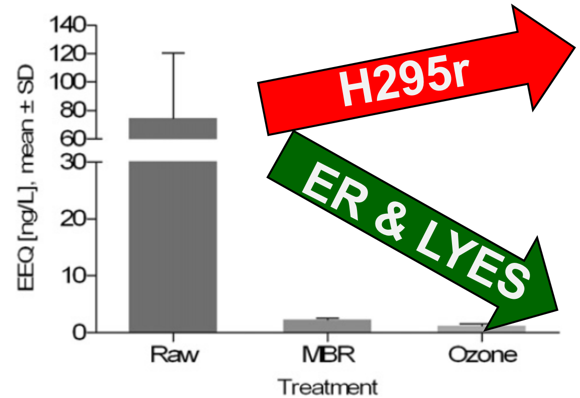


Fig. 2 – Comparison of the Estradiol Equivalents (EEQs) of the three different sewage samples raw (raw sewage before treatment), MBR (sewage after membrane bioreactor treatment) and Ozone (sewage after MBR and ozone treatment) in the LYES. Bars represent mean values of three independent sampling dates with SEM (error bars).

Maletz, S., Floehr, T., Beier, S., Klümper, C., Brouwer, A., Behnisch, P., Higley, E., Giesy, J.P., Hecker, M., Gebhardt, W., Linnemann, V., Pinnekamp, J., Hollert, H. 2014 Water Research

The Utility of Exposure and Effect-Based Analysis in the Ecotoxicological Assessment of Transformation Products

Y. Müller,[1,3] L. Zhu,[1,2,3] S. E. Crawford,[*,1,3] S. Küppers,[2] S. Schiwy,[1] and H. Hollert[1]

Drewes and Letzel; Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening Strategies and ...
ACS Symposium Series; American Chemical Society: Washington, DC, 2016.

Intra- and inter-day variability of EEQ measurements

Institute for Environmental Research

Water Research 110 (2017) 378–388

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Effect-based tools for monitoring estrogenic mixtures: Evaluation of five *in vitro* bioassays

Petra Y. Kunz [a, 1, 2], Eszter Simon [a, 1], Nicolas Creusot [b], B. Sumith Jayasinghe [c], Cornelia Kienle [a], Sibylle Maletz [d], Andrea Schifferli [a], Christine Schönlau [d, 3], Selim Aït-Aïssa [b], Nancy D. Denslow [c], Henner Hollert [d], Inge Werner [a], Etiënne L.M. Vermeirssen [a, *]

[a] *Swiss Centre for Applied Ecotoxicology Eawag-EPFL, 8600 Dübendorf, Switzerland*
[b] *INERIS, Institut National de l'Environnement Industriel et des Risques, Unité ECOT, Verneuil en Halatte, France*
[c] *University of Florida, Center for Environmental and Human Toxicology, Gainesville, FL, USA*
[d] *RWTH Aachen University, Institute for Environmental Research, Aachen, Germany*

- The aim of this study was to compare the intra- and inter-day variability of EEQ measurements using five different ERTAs (YES, ER-CALUX, MELN, T47D-KBluc and GeneBLAzer-ER) with regard to their applicability as effect-based tools in environmental monitoring.
- Of the five ERTAs, ERa-CALUX had the best precision and repeatability (overall CV of 13%).

NORMAN Estrogen Monitoring Project

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Institute for Environmental Research

Trends in Analytical Chemistry 102 (2018) 225–235

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

ELSEVIER

TrAC

Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive

Check for updates

Sarah Könemann [a, b, *, 1], Robert Kase [b, 1], Eszter Simon [b], Kees Swart [c], Sebastian Buchinger [d], Michael Schlüsener [d], Henner Hollert [a], Beate I. Escher [e, f], Inge Werner [b], Selim Aït-Aïssa [g], Etienne Vermeirssen [b], Valeria Dulio [g], Sara Valsecchi [h], Stefano Polesello [h], Peter Behnisch [c], Barbora Javurkova [i], Olivier Perceval [k], Carolina Di Paolo [a], Daniel Olbrich [b], Eliska Sychrova [i], Rita Schlichting [e], Lomig Leborgne [l], Manfred Clara [m], Christoph Scheffknecht [n], Yves Marneffe [o], Carole Chalon [o], Petr Tušil [p], Přemysl Soldàn [p], Brigitte von Danwitz [q], Julia Schwaiger [r], Maria Isabel San Martín Becares [s], Francesca Bersani [t], Klara Hilscherová [i], Georg Reifferscheid [d], Thomas Ternes [d], Mario Carere [u]

… the NORMAN Estrogen Monitoring Project is aiming to further increase the acceptance of effect-based methods as a screening tool for the monitoring programmes under the Water Framework Directive (WFD)

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oekotoxzentrum
centre ecotox

INSTITUTE FOR ENVIRONMENTAL RESEARCH | RWTH AACHEN UNIVERSITY

ISTITUTO SUPERIORE DI SANITÀ

Partners…

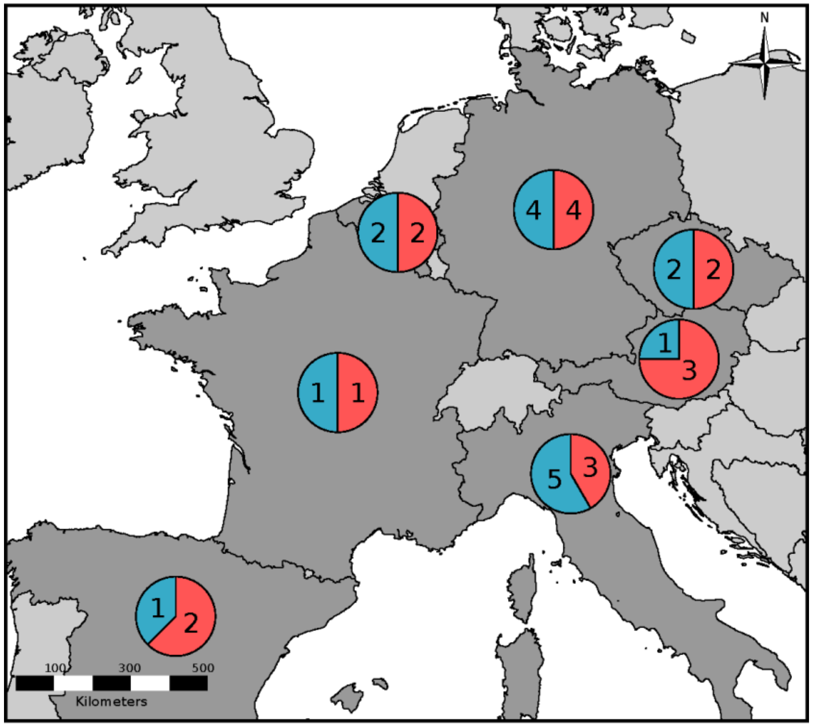
Joint Research Centre (EC), ONEMA (FR), INERIS (FR), Bio Detection Systems (NL), Swiss Centre for Applied Ecotoxicology (CH), Federal Institute of Hydrology (DE), Federal Environment Agency (DE), RWTH Aachen (DE), RECETOX (CZ), NORMAN-Network, Helmholtz Centre for Environmental Research-UFZ (DE), IRSA-CNR (IT), Italian Institute of Health (IT), University of Leon (ES), Water Research Institute T.G.Masaryk (CZ), Bavarian State Office for Environment (DE), LANUV (DE), Environment Agency Austria (AT), ISSeP (Scientific Institute of Public Service) Wallonia (BE), SMAT (IT), Agence de l'eau Adour-Garonne (FR), Ontario Ministry of the Environment and Climate Change (CAN), McGill University (CAN), Environmental Institute (SK).

Around 65 colleagues from 24 institutes, agencies and 12 nations are involved, moreover 3 pharmaceutical companies joined the project in 2016.

Special thanks to the NORMAN-Network (www.norman-network.net) and Helen Clayton and Stéphanie Schaan DG Environment of EU Commission for their collaboration and support.

SAMPLING, CLEAN-UP, ANALYSES…

- **17 waste water and 16 surface water samples**
- **Cleaned up and extracted**
- **Analysed with**
  - **3 high-end chemical analytical methods**
  - **5 different *in vitro* bioassays**



Number of surface (blue) and waste water (red) samples taken in each country. (Könemann et al. 2018)

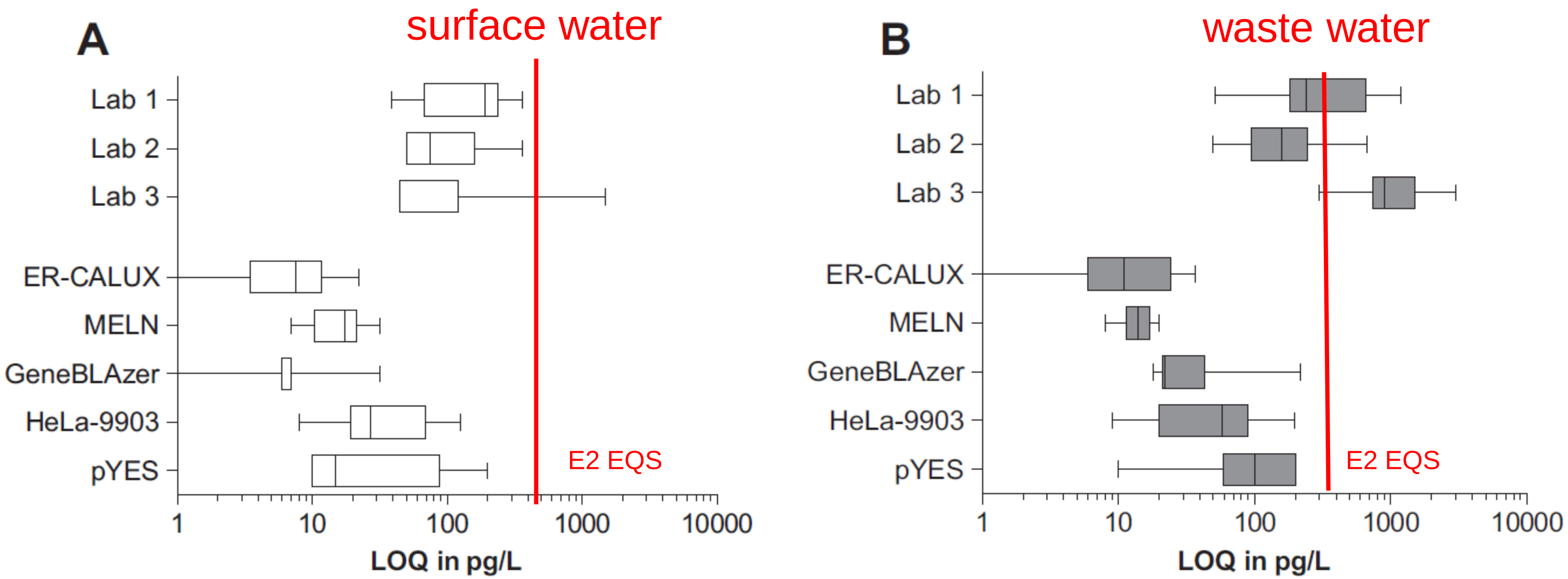
RESULTS – CHEMICAL ANALYSES

Percentage of LOQs (n=3) below the proposed EQS for E1, E2 and EE2. SW = 16 surface water samples, WW = 17 waste water samples.

	E1	E2	EE2
SW	100%	96%	19%
WW	100%	59%	0%

- E1 can be quantified at EQS level
- Quantification accuracy for E2 is suffucient for surface water
- EE2 cannot be sufficiently quantified at EQS level

Sample-dependent LOQs in surface and waste water extracts

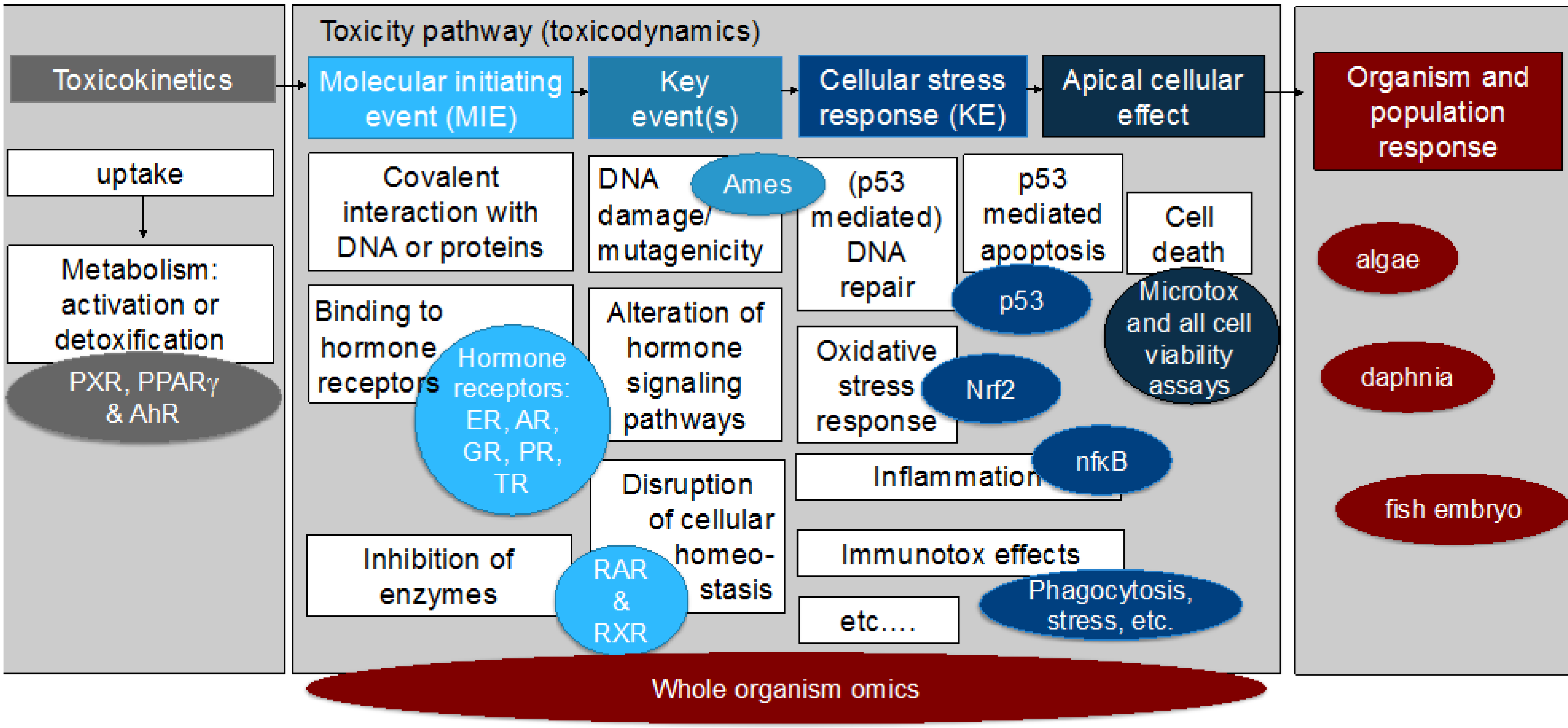


LOQs for E2 (reference compound) in surface water (A) and waste water (B) extracts for each bioassay and chemical analytical method.

Bioassays especially useful for the screening and risk assessment (Kase et al. 2018, TRAC)

Bioassays

- Cover all steps of the AOP (adverse outcome pathway)
- Link between cellular and apical *in vivo* effects



Note: Some endpoints are missing in the consortium (e.g. neurotoxicity)

Neale PA, et al. (2017). Water research 123, 734-750

NORMAN: Drafting of a common position within the wider scientific community on how to use bioassays for water quality monitoring (chemical status):

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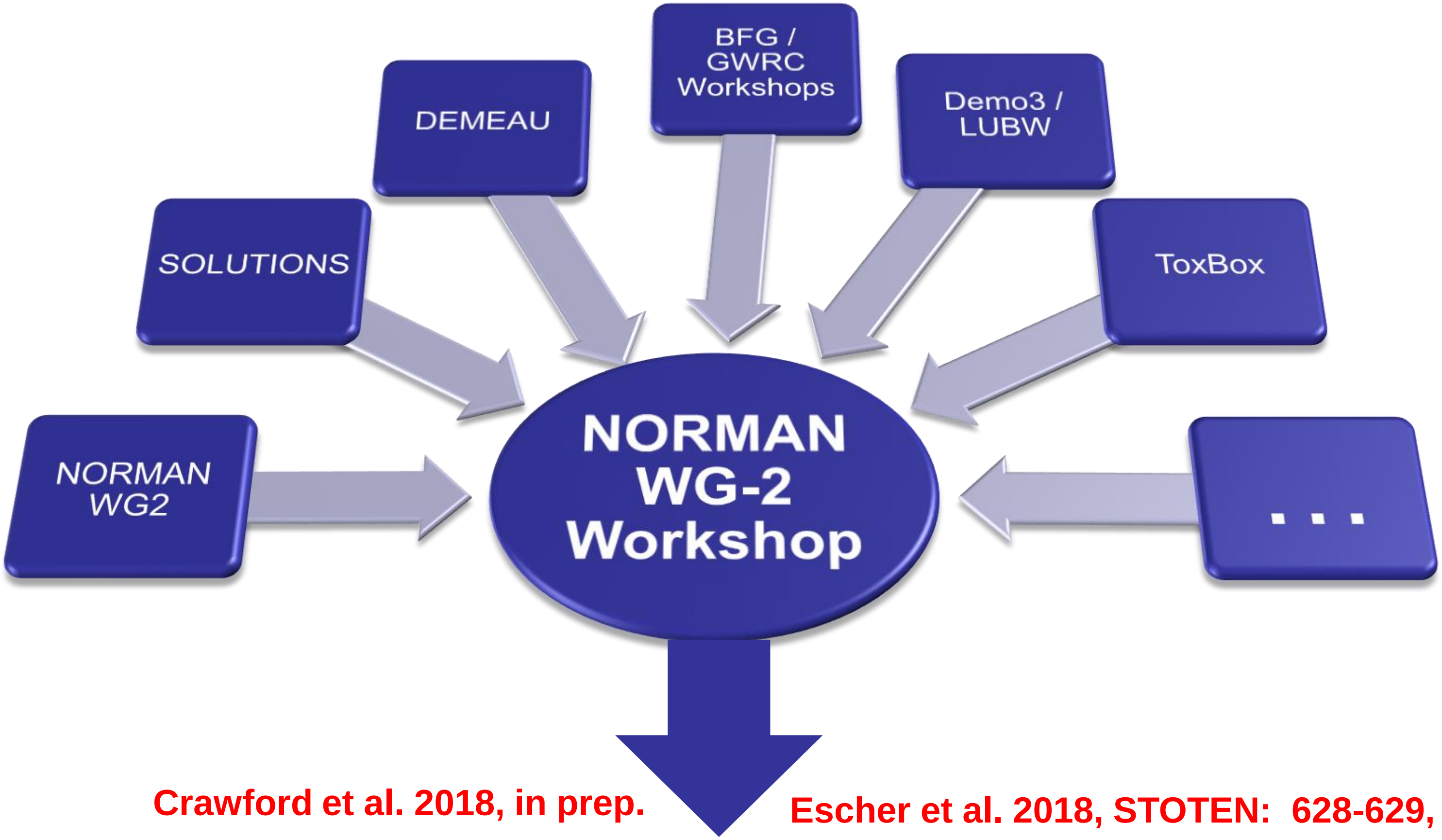

Crawford et al. 2018, in prep.

Escher et al. 2018, STOTEN: 628-629, 748-765

Joint Scientific Publication:
- Recommendations for a common battery of bioassays
- Agreement on a check list of quality / perfomance criteria for the benchmarking of bioassays

Development of
effect-based trigger values (EBT)
Proposed Collaboration
NORMAN-SOLUTIONS

RWTH AACHEN UNIVERSITY
Institute for Environmental Research

Aims of this action:

(1) to write a joint manuscript on neurotoxicity as an emerging MOA for water quality monitoring

(2) to organize a follow-up workshop on aquatic neurotoxicity, increasing the awareness on the topic and the collaboration between interested stakeholders; and

(3) to develop an interlaboratory activity in 2018 to demonstrate the performance and usefulness of the bioassays on neurotoxicity.

You are invited to join the JPA !
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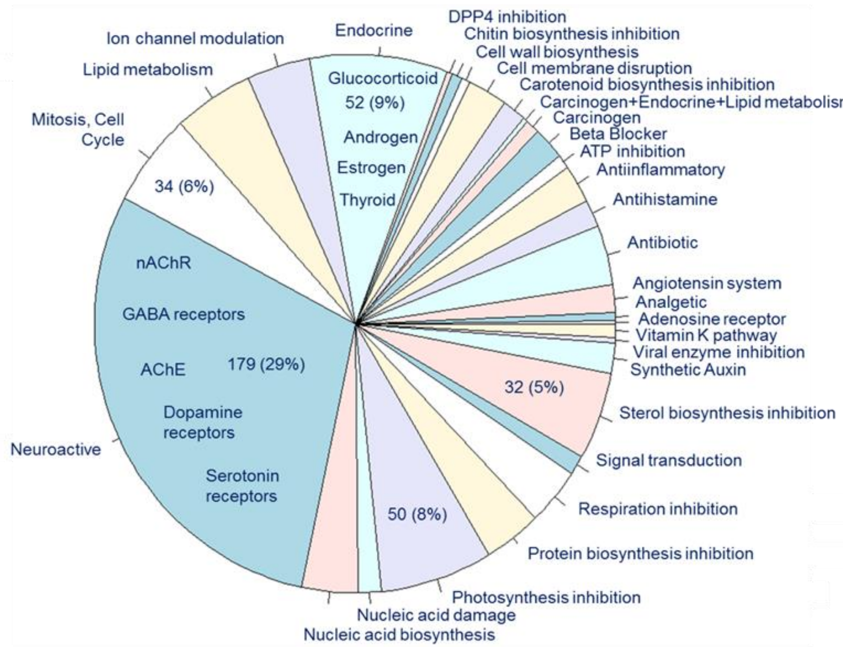

Bioassays that cover MOAs of known environmental pollutants

Busch et al. (2016). Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring. *Environ.Toxicol. Chem.* 35: 1887-1899.

CMEP (Chemical Monitoring and Emerging Pollutants) Activity-Task on Effect-Based Tools (2010-2012) in the context of WG Chemicals.

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Technical Report on Aquatic Effect Based Tools Published (2014)

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WG Chemicals Mandate 2016-2018 ("*Effect-based assays; links between chemical and ecological status; mixtures. Possible follow-up of estrogen-screening project. Exchange of information on innovative techniques, approaches and potential application in WFD context*")

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Water Directors Endorsement (November 2016) for a new approach for the chemical status assessment

Expert Group of WG chemicals «Effect-based Methods»-Objectives

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- Identification and Selection of relevant modes of Actions of Chemical Substances
- Identification and Selection of «Effect based methods» for the detection of the relevant Modes of Actions
- Selection of «effect based methods» to detect the effect of complex mixtures
- Identification of«trigger values/assessment criteria»
- Evaluation of methods connected with the Ecological Status
- Link with Marine strategy
- Use in the WFD and Identification of sources
- Feasibility of the approach

ACTIVITY: EFFECT-BASED METHODS

for WG Chemicals as part of the Water Framework Directive CIS Work Programme (2016-2018) endorsed by the Water Directors

"Effect-based assays; links between chemical and ecological status; mixtures. Possible follow-up of estrogen-screening project. Exchange of information on innovative techniques, approaches and potential application in WFD context"

TERMS of REFERENCE

Introduction

In the aquatic environment[1] thousands of chemical substances are discharged daily, from point and

Marie Curie ITN Project EDA Emerge
EU Integrated Project Solutions

Thank you for your attention!

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Effect-Based Trigger Values developments

Environmental quality standard (EQS) proposals **- single substances**

EQS (ng / L)	E1	E2	EE2
	3.6	0.4	0.037
	-	0.4	0.035

Median of several test specific EBT

Effect-based trigger values (EBT) **- integrated effects**

EBT (ng / L)	Based on	Reference
0.3	PNEC, REP, wastewater composition	Jarosova et al. 2014
0.4	  EQS	Loos et al. 2012, Kunz et al. 2014
0.5	SSD, scenario-based	van der Oost et al. 2017

all EBT proposals were tested in Estrogen Monitoring project