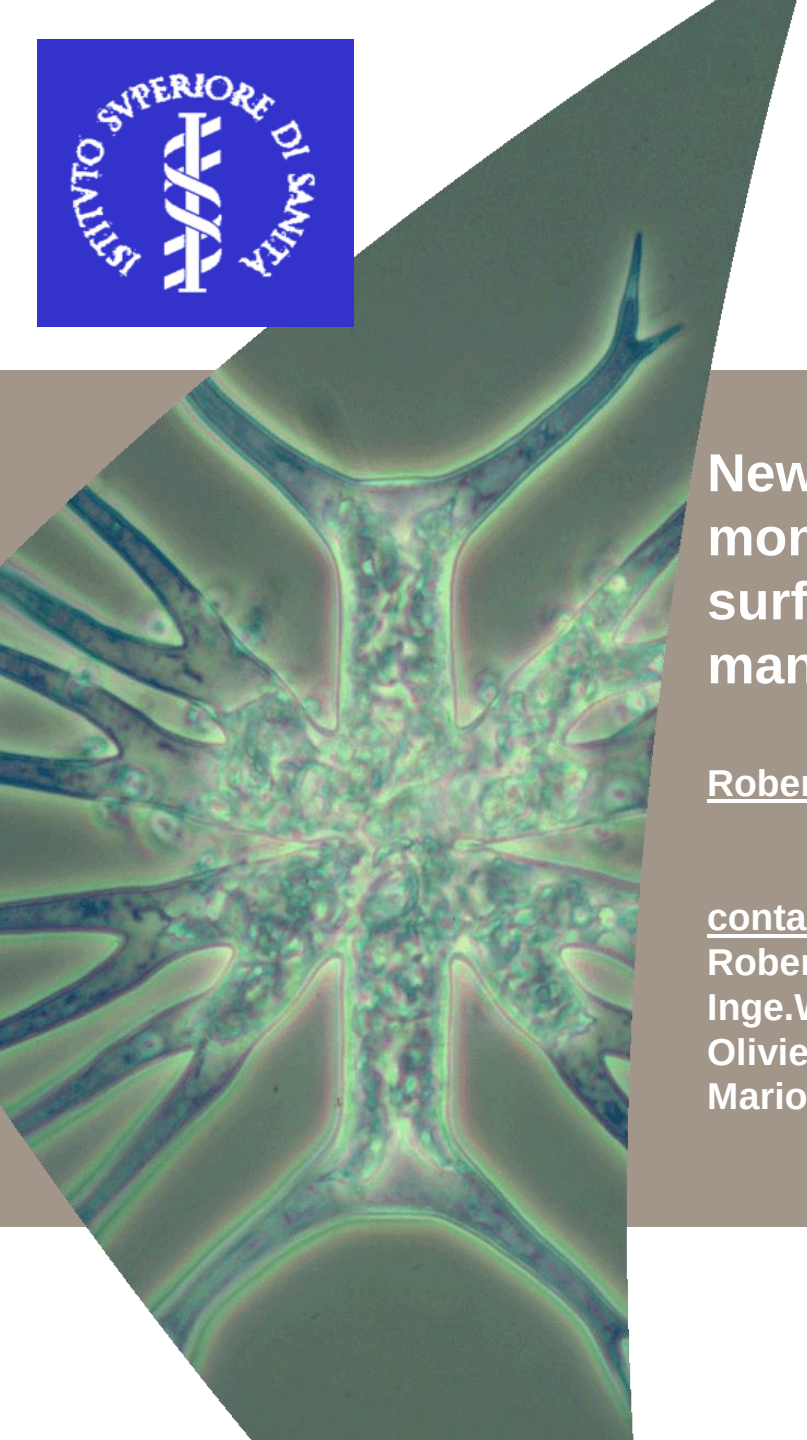

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Schweizerisches Zentrum für angewandte Ökotoxikologie
Centre Suisse d'écotoxigologie appliquée
Eawag-EPFL

New developments in estrogen and EDC monitoring and regulatory options for surface and waste water quality management

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Multilateral Meeting 11+12th April 2016, Rome, IT
and
9th Biodetecors Conference 14+15th April 2016 Lausanne, CH

Estrogen Monitoring project in the context of the WFD

Effect-based and chemical analytical monitoring for the steroidal estrogens: An international project to cope with a monitoring challenge

<u>This project is an applied follow up initiative of the:</u>
Science-Policy-Interface (SPI) and
Chemical Monitoring of Emerging Pollutants (CMEP-WFD) activity
with support of numerous project partners!!

<u>Primary aims</u>:
1) A project related to the watch list substances EE2 and E2, E1 with specific effect-based analytical methods can characterise their screening potential in combination with the best available chemical analytical methods.

2) To bridge the gap between conventional analytical and an effect-based monitoring

Structure

Background

Part I: Tools for risk assessment and risk characterisations

- Environmental Quality Standards (EQS) derivation, examples EE2 and E2
- Risk Assessment = Exposure Assessment / Hazard Assessment
- Sources and Risks: EU wide and for Switzerland
- Hormonally active substances and „endocrine disrupting“ effects
- Estrogenicity in treated wastewater & integrative effect assessments

Application

Part II: Estrogen Monitoring for WFD

- Effect-based and chemical analytical monitoring for the steroidal estrogens
- Project overview + prevalidation results
- Analytical and effect-based waste water risk assessment
- Effect-based and analytical sensitivity comparison
- Monitoring discussion and first surface water results with ER-Calux
- Short EDC relevance discussion
- Summary and outlook

Part I: Scope of Environmental Quality Standards (EQS)

EQS are based on reliable and relevant effect data.
The critical step is to identify and to evaluate them*.

• ***AA-EQS (Annual Average-EQS)*** **must be protective against the effects of long-term exposure**

- **Goal**:
  - protection against long-term exposure effects
- **Comparison**:
  - with the measured annual average concentration
- **Data**:
  - (sub)chronic effect data are preferred

• ***MAC-EQS (Maximum acceptable concentration)*** **must be protective against effects of short-term exposure.**

- **Goal**:
  - protection against short-term exposure effects
- **Comparison**:
  - with the measured or 95%ile concentration
- **Data**:
  - acute effect data are preferred

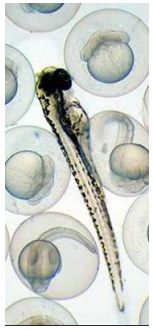
Because of the specific mode of action only an AA-EQS is proposed for E1, E2 and EE2 and Diclofenac,….

***More about data evaluation and «CREDibility» is available at:**
http://www.ecotoxcentre.ch/projects/risk-assessment/cred/

Options to derive an EQS

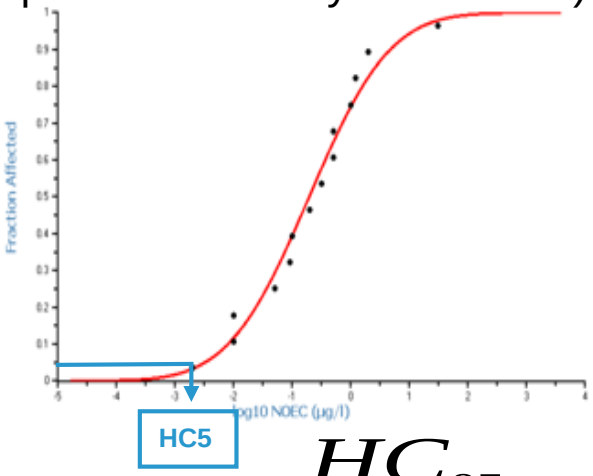
AF-method





$$\frac{EC_{50\,\min}}{AF} \quad \text{or} \quad \frac{NOEC_{\min}}{AF}$$

SSD
(Species Sensitivity Distribution)



$$\frac{HC_{05}}{AF}$$

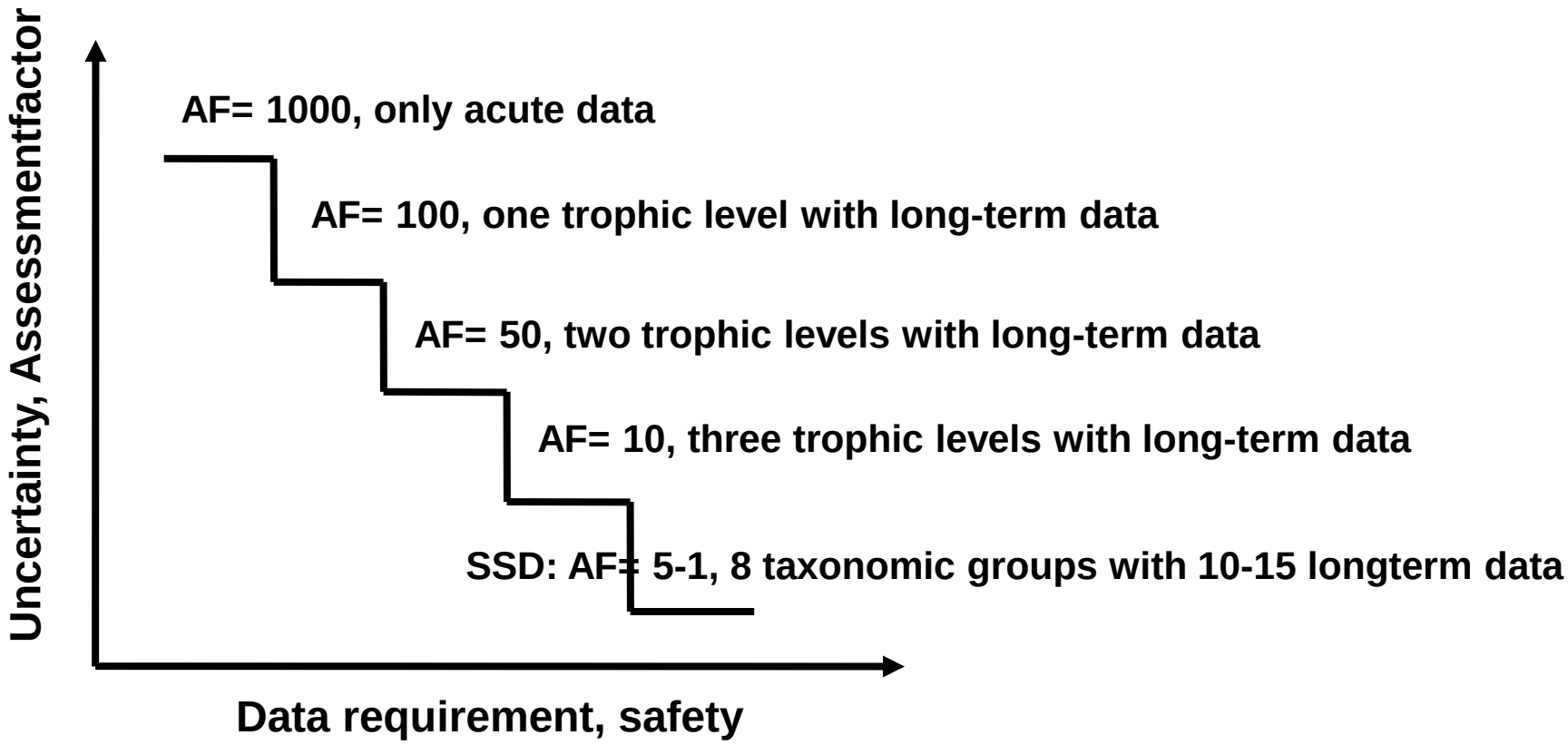
Micro- / Mesocosms

$$\frac{NOEC_{\min community}}{AF}$$

Ecological complexity

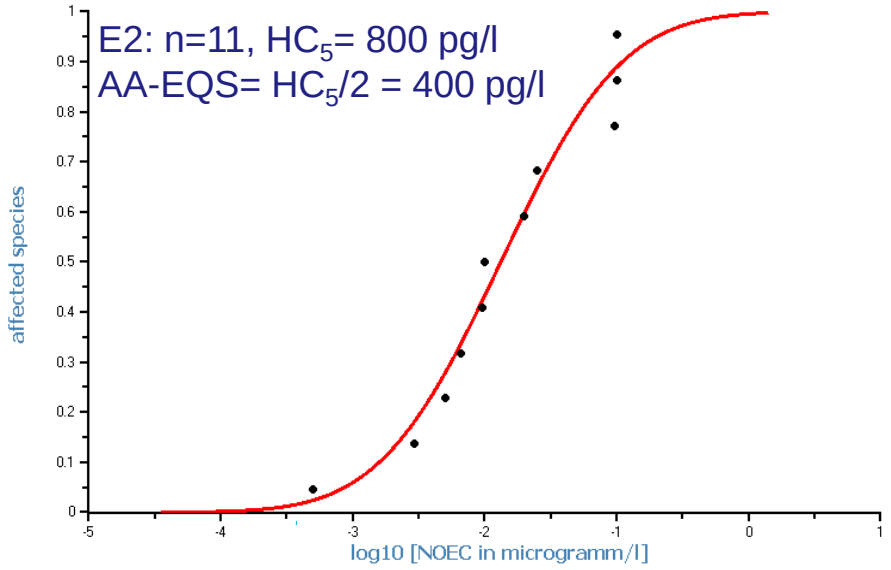
Assessmentfactor (AF)

Assessmentfactors in hazard assessment for AA-EQS derivation

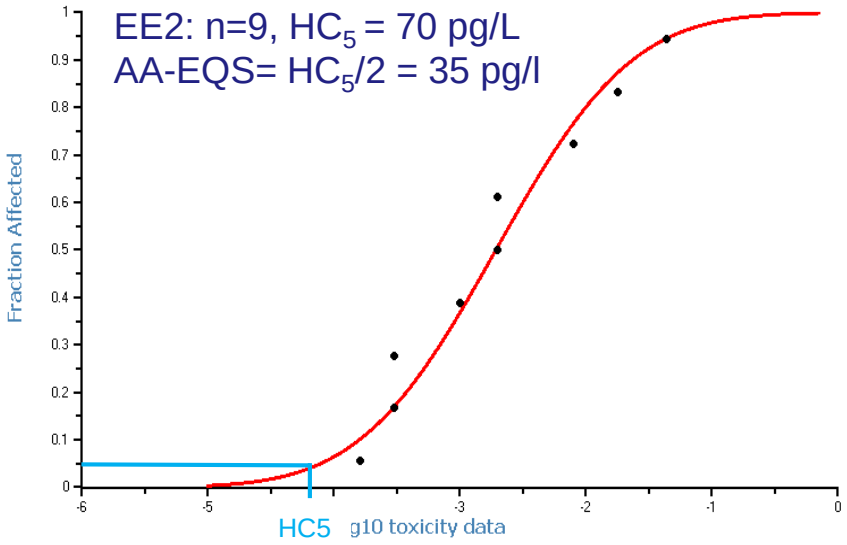


The monitoring challenge for E2 and EE2: Specific EU SSD approaches

SSD graph E2



SSD graph EE2



Both approaches consider population relevant effects in the most sensitive taxonomic groups (mainly fish species).

There was significant progress in the hazard assessment of EE2 and E2, but the monitoring of 35 pg/l EE2 and 400 pg/l E2 will require the best available analytical techniques and cannot be done with routine methods.

Risk Assessment = Exposure Assessment / Hazard Assessment

$$\text{Riskquotient (RQ)} = \frac{\text{MEC or TEQ}}{\text{QC}} = ?$$

<1 tolerable risk

>1 intolerable risk

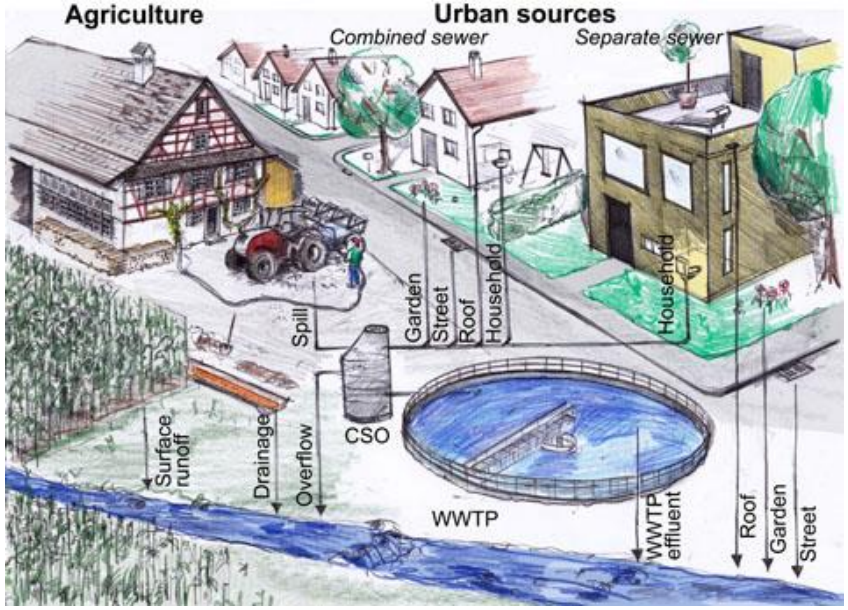
MEC= Measured environmental concentration, also usable PEC= Predicted environmental concentration
TEQ = Toxic Equivalent, in case of estrogen receptor activation EEQ Estradiolequivalents
QC= Quality criteria (in usual the AA-EQS)

The retrospective risk assessment is necessary because not all risks and substances can be covered in prospective risk assessment and unintended exposures are always possible.

main pathways of E2 and EE2 to water bodies

non-point sources

- Seasonal risks mainly by grasing of livestock
- Application of manure
- Edge of field water bodies
- Source reducing measures available



source: eawag.ch

point sources

- Continuous use and discharge
- Not all estrogens are removed by SWTPs
- For human-pharmaceuticals risk is currently not handled by authorisation
- Lifestyle is hard to influence

E2, E1, EE2 and more

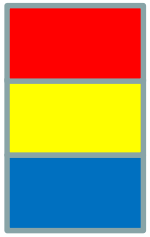
with some analytical challenges and the exposure is mainly modeled

To address the risk posed by EE2, E2 <u>and E1</u> were included in the EU watch list mechanism and <u>should be monitored at their EQS levels 35 pg/L, 400 pg/L, 3600 pg/L</u>

EE2 EU wide exposure prediction for surface water at median flow

Source: Johnson et al. 2013; EST

EE2 [pg/L]



>350

35-350

< 35

Predicted proportion of national river length with **EE2 EQS exceedance** in the range of: **<10% to >30%**

Only in a limited percentage of water bodies an EE2 related risk can be estimated, but we have a co-exposure of E2 and E1,….

So it should be **possible to identify hotspots** and introduce efficient **risk mitigation measures**

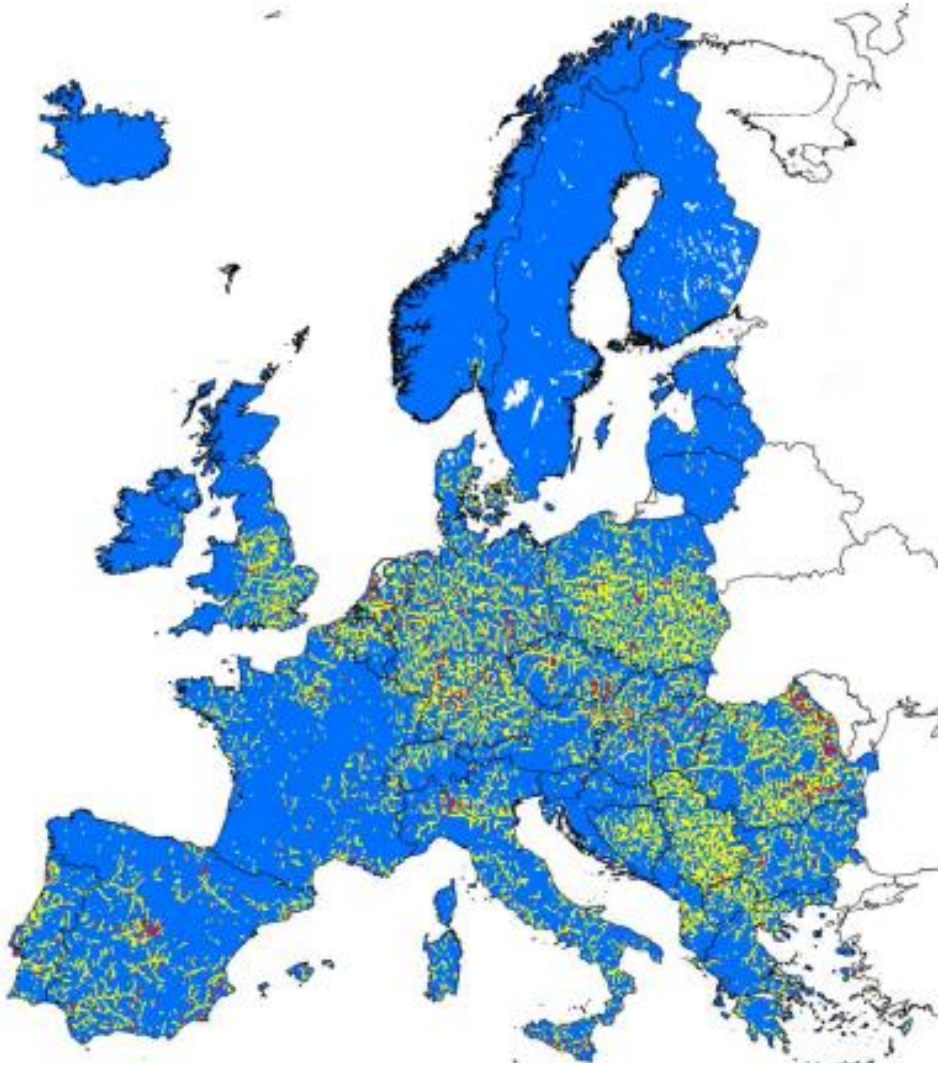
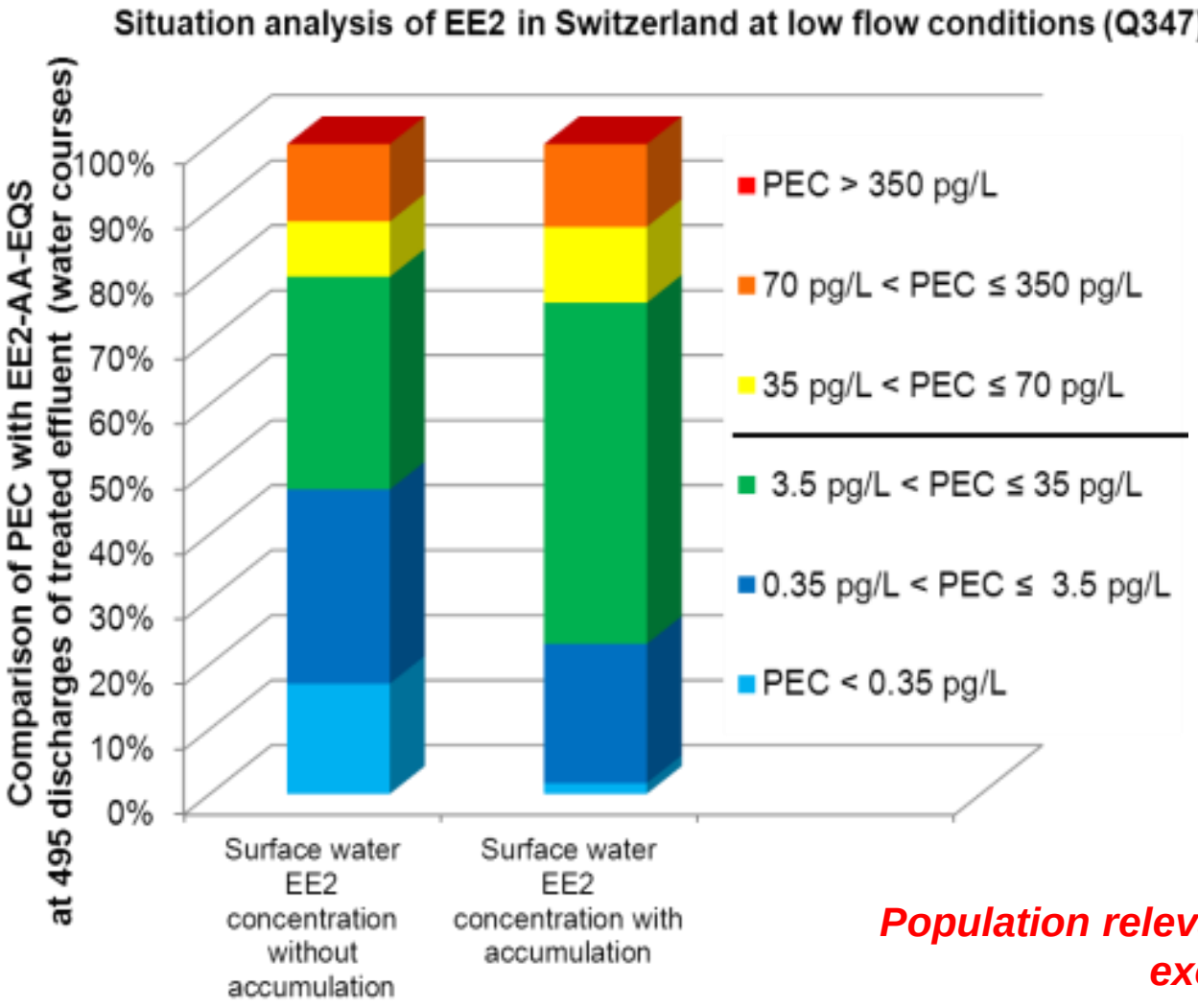


FIGURE 1. Location of European surface waters where EE2 concentrations are predicted to exceed 0.035 ng/L (yellow) and 0.35 ng/L (red) based on expected chemical discharge (mean excretion and mean sewage removal)

Situation analysis for EE2 in Switzerland



EE2 can exceed in 20-25% in surface water courses.

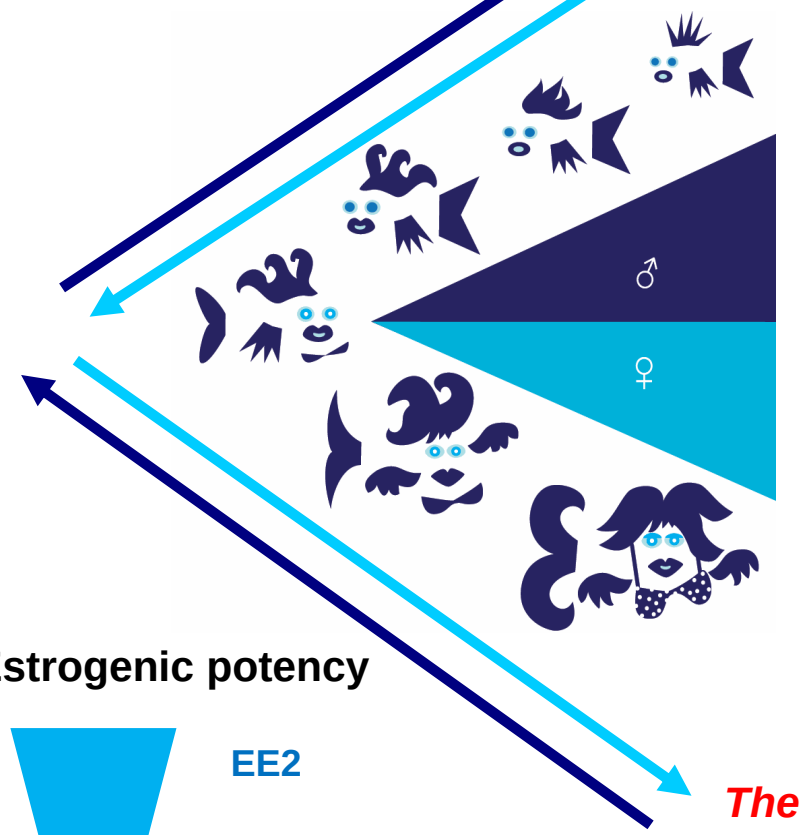
Additionally we have a cummulative risk (e.g. via EE2, E2, E1, NP, BPA, see Kase and Werner 2011)

Population relevant effects cannot be excluded.

Hormonally active substances and „endocrine disrupting“ effects

estrogenic effect
+ anti-androgenic effect

androgenic effect
+ anti-estrogenic effect



Estrogenic potency

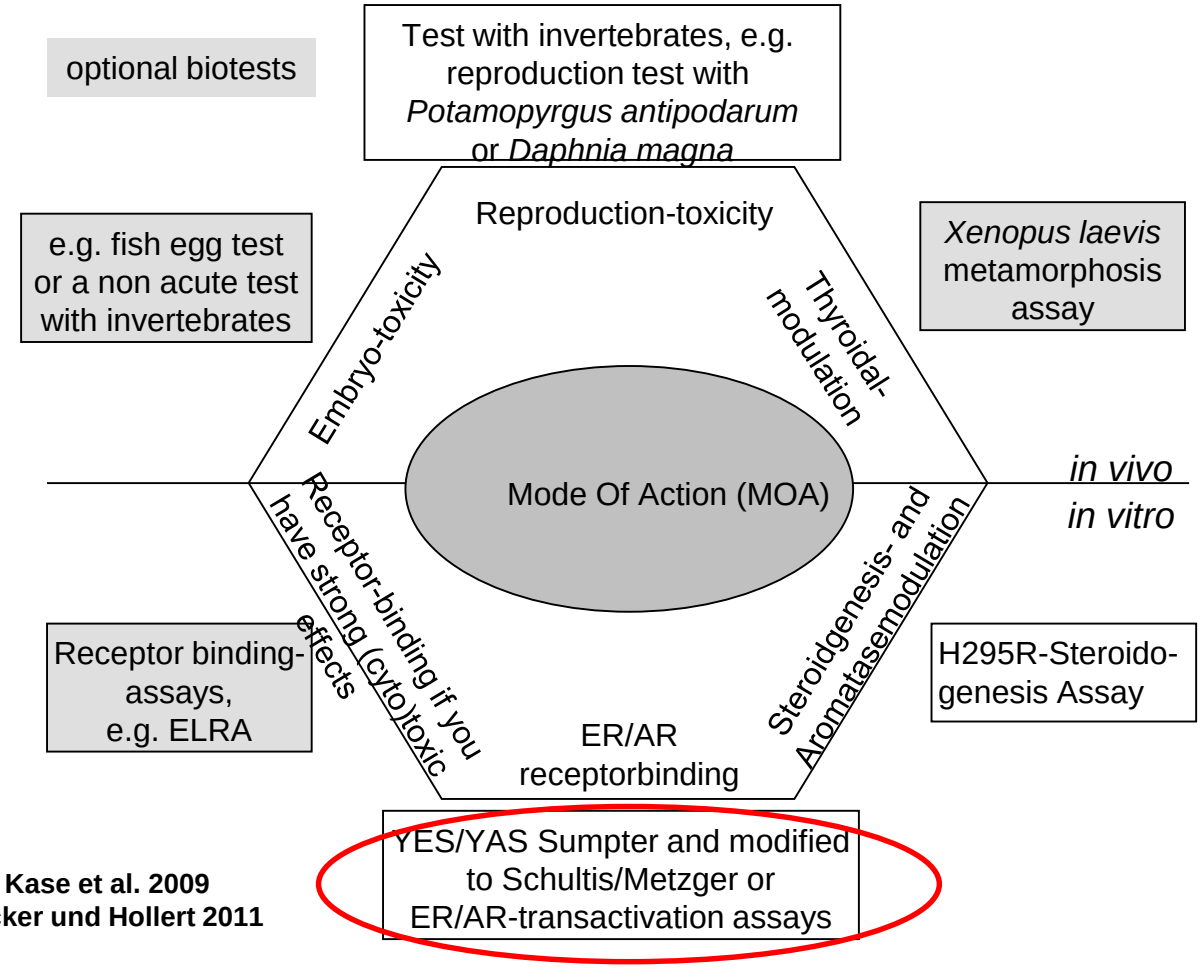
In both cases, the possible effects are:

- **changes in the behaviour**
- **EDCs can modulate the immune function→ higher susceptibility for pathogens→higher mortality**
- **changes in the fertility**
- **intersex**
- **and other population relevant effects**

Most effects are considered relevant according to the TGD for EQS

The steroidal hormone system is highly conserved among different taxa, so there is a widespread effect in animals and humans.

Can we detect multiple and EDC related stressor effects to protect aquatic organisms?



Source: Kase et al. 2009 and Hecker und Hollert 2011

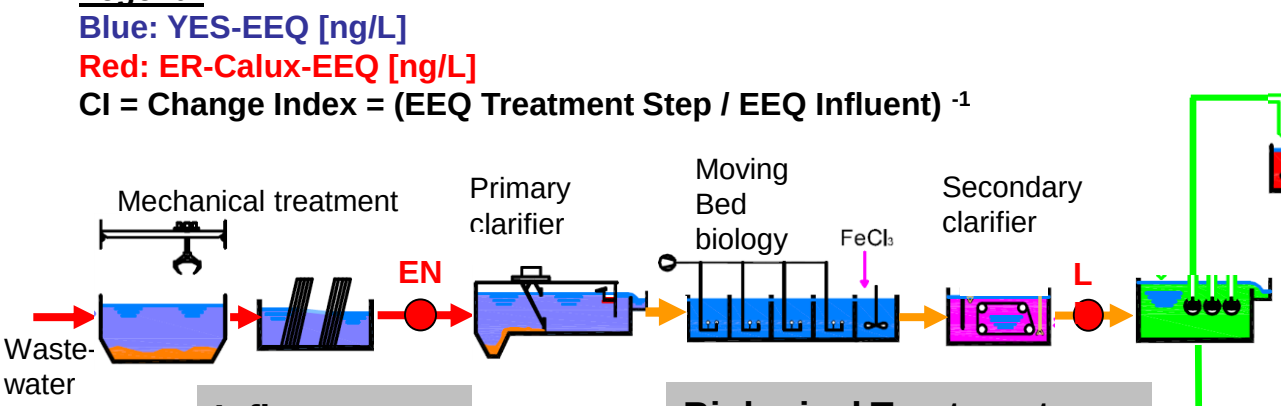
The modular system presented here allows the switching between test modules according to the continuously developing state-of-the-art of science and technology as well as the incorporation of novel developments.

In short: **In principle yes**, but far too expensive for routine monitoring, therefore a focus on screening of the most investigated mode of actions of EDC is necessary.

Example: Estrogenicity during wastewater treatment steps

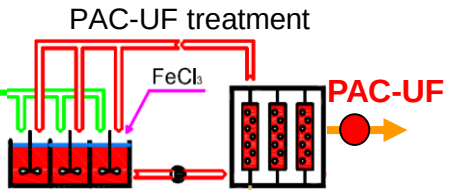
Results of the pilot study at the STEP de Vidy Lausanne with the YES and ER-Calux in 17β-estradiol-equivalents (EEQ) (4th measurement campaign):

Legend:
Blue: YES-EEQ [ng/L]
Red: ER-Calux-EEQ [ng/L]
CI = Change Index = (EEQ Treatment Step / EEQ Influent) $^{-1}$



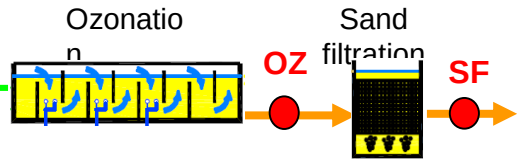
Influent
37.15 ng/L
9.8 ng/L

Biological Treatment
3.03 ng/L; $CI_{LF/EN}$ = 12
0.90 ng/L; $CI_{LF/EN}$ = 11



PAC-UF Treatment
0.29 ng/L; $CI_{PAC/EN}$= 126
0.16 ng/L; $CI_{PAC/EN}$= 61

AA-EQS proposal for E2 = 0.4ng/L



Ozonation + SF
0.23 ng/L; $CI_{OZ+SF/EN}$= 160
0.15 ng/L; $CI_{OZ+SF/EN}$= 65

Compared to the conventional biological treatment a 6-13 times lowered receptor binding estrogenicity results via additional waste water treatment steps.

Options are developed where to proceed?
It can make a difference if you release 10% receptor mediated estrogenicity or only 1%

Why effect-based methods?

Chemical pressures are normally occuring from a sum of known and unknown substances:

1) For environmental samples with an unknown composition (unknown mixtures) the effect-based tools are the only methods to detect specific hormonally and endocrine disruptive effects.

2) Using specific effect-based methods samples can be divided into critical and unpolluted ones if they contain substances clearly related to an effect (e.g. E2 and EE2). This allows a reduction and optimized use of analytical resources.

3) Specific effect-based methods can be used to identify other EDCs and support the implementation of national and EU EDC strategies.

Technical agreement was already generated in an international expert workshop 2013, considering, different national needs + EU CMEP and SPI needs:
http://www.bafg.de/DE/05_Wissen/02_Veranst/2013/2013_02_27_votum_en.pdf?__blob=publicationFile

Part II: Estrogen Monitoring project

A very dynamic and growing project,

which is based on regulatory Science to Policy Interface (SPI) needs and Chemical Monitoring of Emerging Pollutants (CMEP) activities

Included project 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), Federal Ministry for the Environment (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 25 institutes, agencies and 12 nations are involved.

A very multi-national project including expertise from various agencies and institutes. This participation shows the high level of interest.

We are very grateful that you indicated your collaboration and participation.

And last but not least our special thanks to the NORMAN-Network (www.norman-network.net) for their collaboration and support

European Commission

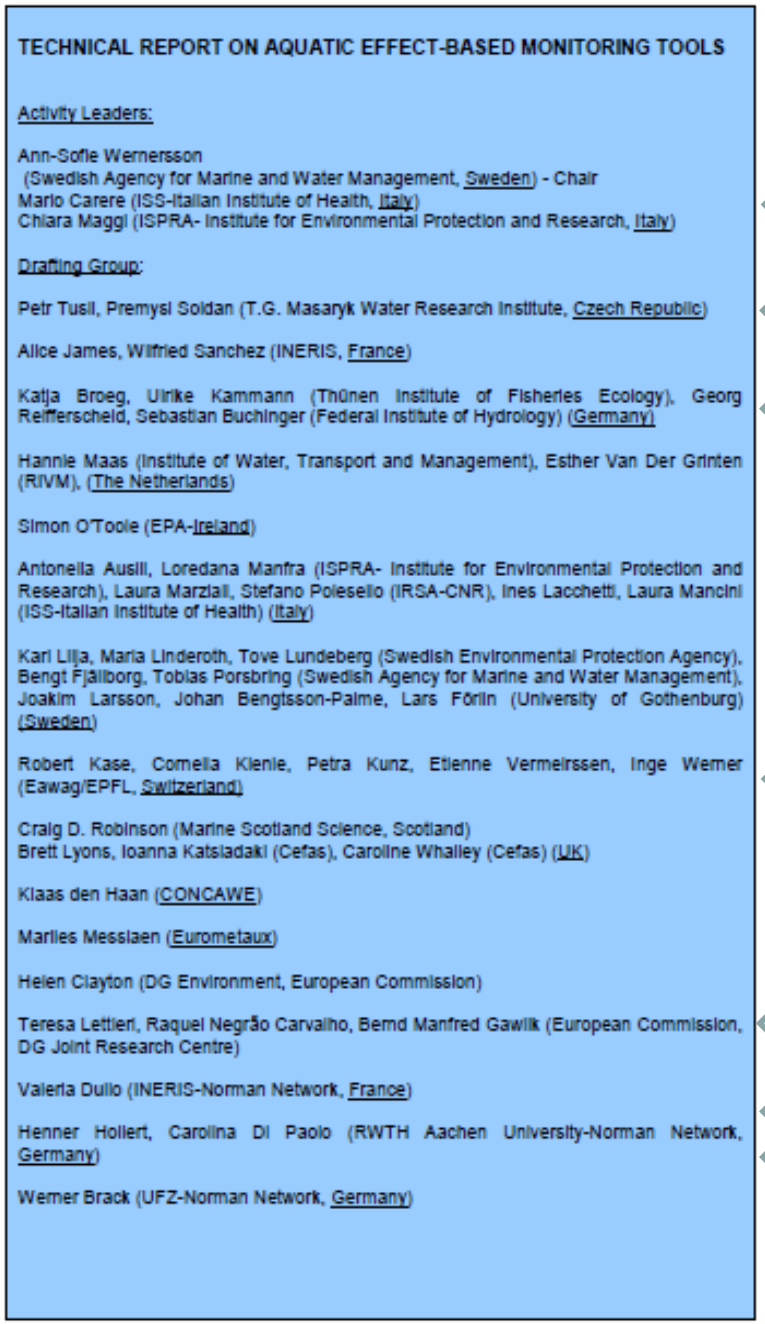
Technical Report - 2014 - 077

Published in: Wernersson Ann-Sofie; Carere Mario, et al. (2015): The European technical report on aquatic effect-based monitoring tools under the water framework directive. **Environmental Sciences Europe**, 2015; 27 (1) DOI: 10.1186/s12302-015-0039-4. http://www.enveurope.com/content/pdf/s12302-015-0039-4.pdf

TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS

In this project:
«We think it is time to demonstrate their application potential in an applied international collaboration project. To bridge the gap between chemical analytical and effect-based analysis for the future.»

Environment



TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS

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Werner Brack (UFZ-Norman Network, Germany)

1

Schedule 2015-2017

Most of the project information is available at:
http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/

Drafting group results: Sampling, Extraction, Data Evaluation, Screening and Risk Assessment

Q3+Q4 2015: Sampling & extraction (parallel to the EU watch list mechanism)

15.+16. February: 3rd project meeting at ONEMA, Paris, FR

Q1+Q2 2016: Chemical analytical and effect-based measurements of samples extracts

Q2 2016-Q1 2017: Data evaluation and reporting
(2 publications and 1 SPI WG Chemicals report)

Q1 2017: Final project meeting at JRC (tbc), IT

Main Tasks in WG Chemicals 2016-2018 WFD

- New Priority Substances review: SG-R re-established in 2014; experts contributing to JRC technical work. Possible de-listing of PS will be considered. Short-list of substances will be needed in 2016.

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

- Passive sampling: exchange of information on latest developments; discussion of application in context of WFD.

- Review of the watch list.

Sampling overview

Samples	AT	BE	CZ	DE	ES	FR	IT	Sum
Waste-water	3/3	2/2	2/2	4/4	2/2	1/1	3/3	17/17
Surface-water	1/1	2/2	2/2	4/4	1/1	1/1	5/5	16/16
Sum	**4**	**5**	**4**	**8**	**4**	**2**	**8**	**33**

Preparation of sampling material and shipment

Kindly provided by INERIS (Fabrizio Botta)

Impressions from waste water sampling in BE and CZ

BE

CZ

Our warmest thanks to Carole, Aurore, Petr, Premysl, Manfred, Christoph, Lomig, Francesca, Sara, Stefano, Isabel, Julia and many other colleagues !!

Included methods

Detection methods covered:

- **High end chemical analysis (JRC, BfG, Swiss Centre for Applied Ecotoxicology)**
- **ER-Calux (BDS)**
- **MELN (INERIS)**
- **BG1Luc4E2 + ER-GeneBLAzer (UFZ)**
- **Hela 9903 (RECETOX)**
- **Yeast Estrogen Screen assays (BfG)**
- **T47D-Kbluc assay (RWTH Aachen)**

3 x high end chemical analysis +7 x effect-based analysis, some of them are in OECD validation processses or already in ISO standardisation

All of the screening methods have shown their applicability for single substances, artificial mixtures or environmental samples in different projects.

5 screening methods are already compared in a prevalidation project with single substances and mixtures (Kunz et al. in prep.)

Now we will have in 2016 the chance to compare and charaterise all the methods with realistic environmental samples + control samples.

Prevalidation publication in preparation, adapted from Cornelia Kienle 2015

Effect-based tools for monitoring (xeno)estrogens in surface waters: Evaluation of 5 different in vitro assays and two approaches for EEQ-derivation*

Petra Y. Kunz[1], Eszter Simon[1], Selim Aït-Aïssa[2], Nicolas Creusot[2], Nadzeya Homazava[1], B. Sumith Jayasinghe[3], Cornelia Kienle[1], Sibylle Maletz[4], Andrea Schifferli[1], Christine Schönlau[3], Nancy D. Denslow[3], Henner Hollert[4] and Inge Werner[1]

[1] Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Dübendorf, Switzerland
[2] INERIS, Institut National de l'Environnement Industriel et des Risques, Verneuil en Halatte, France
[3] University of Florida, Center for Environmental and Human Toxicology, Gainesville, Florida, USA
[4] RWTH Aachen University, Institute for Environmental Research, Aachen, Germany

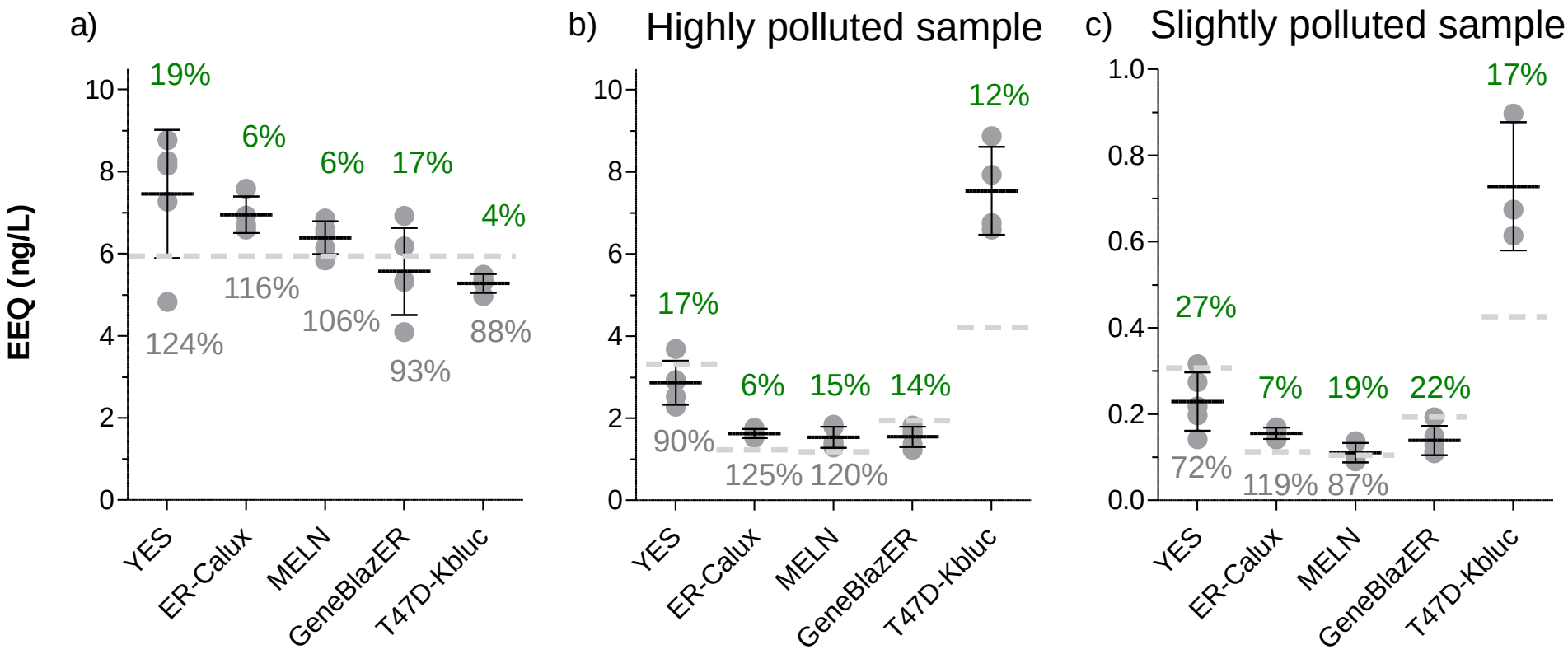
This research has received funding from the European Union's Seventh Framework Programme under the grant agreement no. 308339.

*title might change

What was found?

17β-Estradiol

„Effluent-receiving water“



Overall variability (%)

Calculated EEQs

Recovery (%)

Summary:

Variability range: 4 - 27 %

Recovery range: 72 – 125 %

Do we have risks in our sampled waste waters?

$$\text{Riskquotient (RQ)} = \frac{\text{MEC or TEQ}}{\text{QC}} = ?$$

<1 tolerable risk

>1 intolerable risk

MEC= Measured environmental concentration, also usable PEC= Predicted environmental concentration
TEQ = Toxic Equivalent, in case of estrogen receptor activation EEQ Estradiolequivalents
QC= Quality criteria (in usual the AA-EQS)

AA-EQS EE2= 35 pg/L

AA-EQS E2= 400 pg/L

AA-EQS E1= 3600 pg/L

All following **analytical data** are kindly provided and adapted from Michael Schluesener from Federal Institute of Hydrology, Koblenz, DE.

All following **bioanalytical data** are kindly provided and adapted from **Peter Behnisch and Kees Swart**, Bio Detection Systems, Amsterdam, NL.

Analytical results: Mixed stressors and mixed risks

Known risks of steroidal estrogens in our waste water samples:

	E1 [ng/L]	E1 LOD [ng/L]	E2 [ng/L]	E2 LOD [ng/L]	EE2 [ng/L]	EE2 LOD [ng/L]	Single E1-RQ	Single E2-RQ	Single EE2-RQ	Cumulative E1, E2, EE2 RQ
Sample_2	12	0.03	< LOD	0.5	< LOD	0.1	3.3333			3.3333
Sample_4	0.21	0.03	< LOD	1	< LOD	0.1	0.0583			0.0583
Sample_5	1.9	0.03	< LOD	1	< LOD	1	0.5278			0.5278
Sample_9	4.7	0.03	< LOD	0.5	< LOD	1	1.3056			1.3056
Sample_12	11	0.03	0.43	0.3	< LOD	0.5	3.0556	1.0750		4.1306
Sample_13	5.5	0.03	< LOD	1	< LOD	0.1	1.5278			1.5278
Sample_14	0.81	0.03	< LOD	0.5	< LOD	0.5	0.2250			0.2250
Sample_16	3.3	0.03	< LOD	0.5	< LOD	0.5	0.9167			0.9167
Sample_17	0.25	0.03	< LOD	0.3	< LOD	0.1	0.0694			0.0694
Sample_19	2.5	0.03	< LOD	0.3	< LOD	0.1	0.6944			0.6944
Sample_20	18	0.03	1.2	0.3	4	0.1	5.0000	3.0000	114.2857	122.2857
Sample_21	0.46	0.03	< LOD	0.3	< LOD	0.1	0.1278			0.1278
Sample_23	7.2	0.03	0.22	0.2	9.4	1	2.0000	0.5500	268.5714	271.1214
Sample_26	0.056	0.01	< LOD	0.1	< LOD	0.03	0.0156			0.0156
Sample_29	0.096	0.01	< LOD	0.1	< LOD	0.03	0.0267			0.0267
Sample_31	0.13	0.03	< LOD	0.3	< LOD	0.5	0.0361			0.0361
Sample_33	12	0.03	0.35	0.3	< LOD	0.5	3.3333	0.8750		4.2083
									Mean	24.1536

7 x E1 EQS exceedance ; 2 x E2 EQS exceedance ; 2 x EE2 EQS exceedance

Analytical results: Mixed stressors and mixed risks

Matrix effects in waste water are leading to analytical problems and higher LODs up to 1 ng/L for EE2, E2.

In 76% (13 of 17) waste water samples E2 was not quantified.

In 88% (15 of 17) waste water samples EE2 was not quantified.

It is unlikely that they are at zero concentration, normally they occure and act together.

Mixture risk assessment for steroidal estrogens

Minimal: What is the mixed known risk (RQ)?

$$\sum RQ_{EE2,\ E2,\ E1} = \sum (MEC_{EE2,\ E2,\ E1} / AA\text{-}EQS_{EE2,\ E2,\ E1})$$

+ Additional unquantified and **unknown risks are somewhere among 0→LOD/2→LOD**

Likely: What is the mixed known + LOD/2 risk (RQ)?

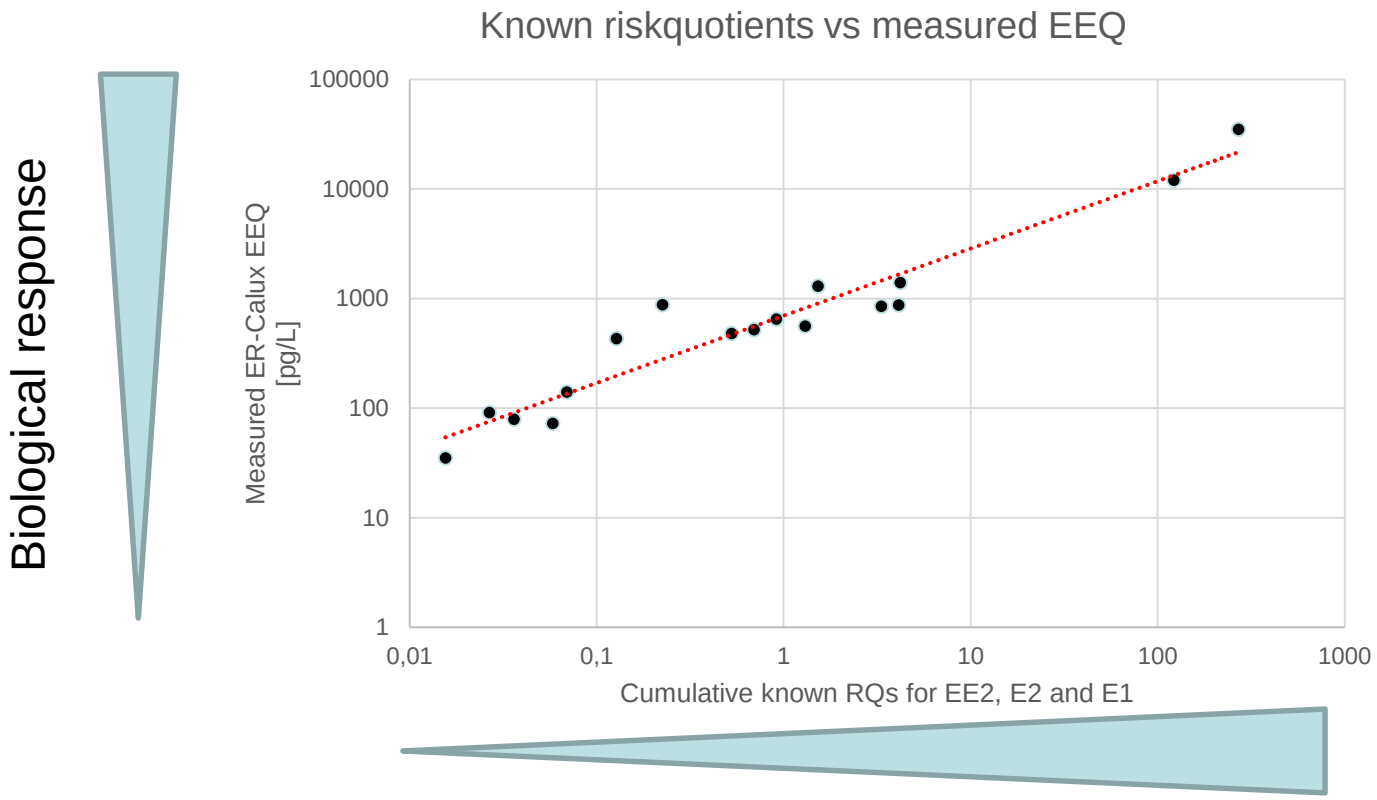
$$\sum RQ_{EE2,\ E2,\ E1} = \sum (MEC_{EE2,\ E2,\ E1} \text{ or } LOD/2_{EE2,\ E2,\ E1} / AA\text{-}EQS_{EE2,\ E2,\ E1})$$

It is unlikely that LOD/2 is in every case safe? Therefore the LOD should be also tested…

Maximal: What is the mixed known + LOD risk (RQ)?

$$\sum RQ_{EE2,\ E2,\ E1} = \sum (MEC_{EE2,\ E2,\ E1} \text{ or } LOD_{EE2,\ E2,\ E1} / AA\text{-}EQS_{EE2,\ E2,\ E1})$$

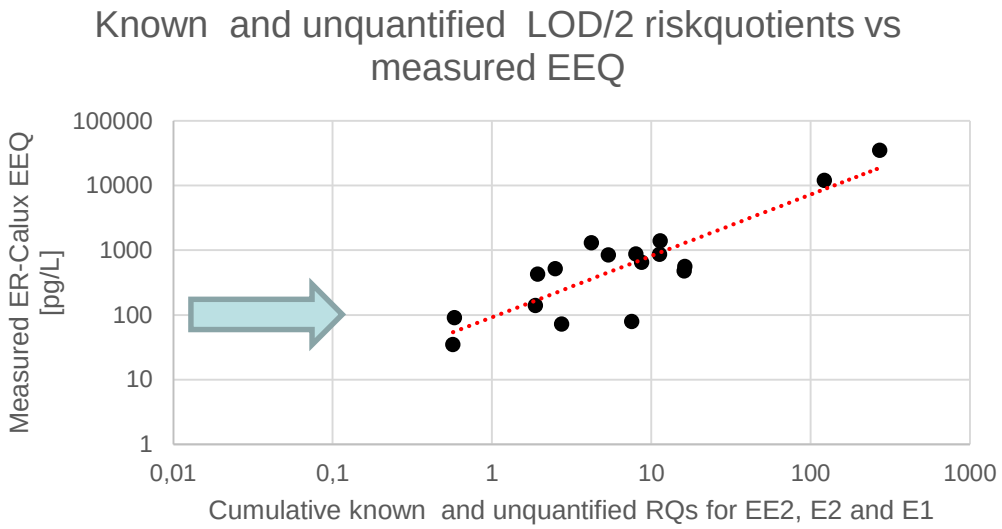
Known risks vs measured EEQ

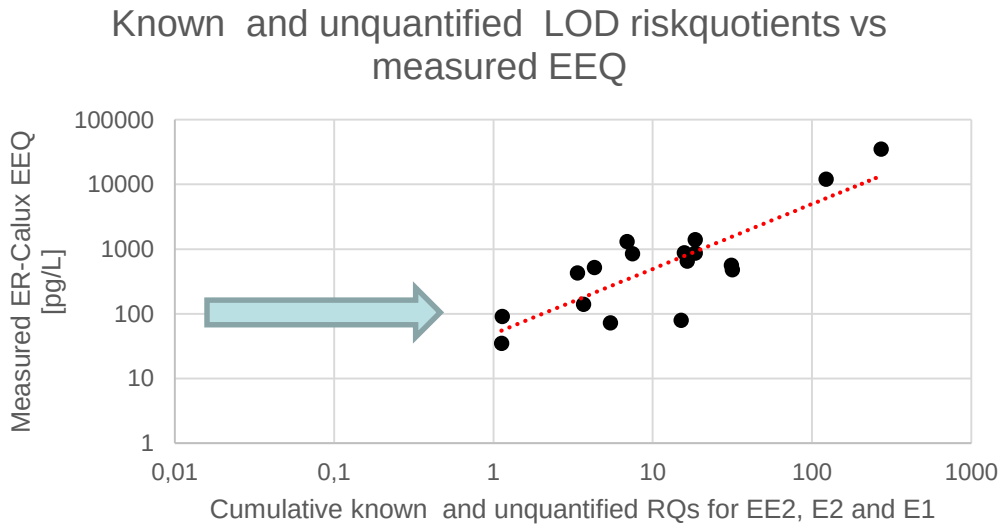


Promising: Mixture risk quotients (based on measurements and EQS) is highly correlated to the measured integrative EEQ ER-Calux values.

Effect-based methods can address chemical pressures and mixture risks.

Likely and maximal risk scenarios





In every case EEQ correspond to risks.

How indicative and safe are the EEQ trigger values?

Effect-based trigger values to compare for risk analysis

First need of choice of published trigger values (TV):

a) Jarosova et al. 2014: TV = 0.3 ng/L EEQ

b) Loos R 2012, Kunz et al. 2014: TV = 0.4 ng/L EEQ

c) Ron van der Oost (not published): TV =0.5 or 1 ng/L EEQ

We will compare with the medium option b, related to the E2-EQS.

Proof of concept

	Cumulative RQ				Trigger value 400 pg/L indicates risk		
Sample	known	known or LOD/2	known or LOD	measured EEQ ER-Calux [pg/l]	known	known + LOD/2	known + LOD
2	3.33	5.39	7.44	850	yes	yes	yes
4	0.06	2.74	5.42	72		no	no
5	0.53	16.06	31.60	480		yes	yes
9	1.31	16.22	31.13	560	yes	yes	yes
12	4.13	11.27	18.42	870	yes	yes	yes
13	1.53	4.21	6.88	1300	yes	yes	yes
14	0.23	7.99	15.76	880		yes	yes
16	0.92	8.68	16.45	649		yes	yes
17	0.07	1.87	3.68	140		no	no
19	0.69	2.50	4.30	520		yes	yes
20	122.29	122.29	122.29	12000	yes	yes	yes
21	0.13	1.93	3.36	430		yes	yes
23	271.12	271.12	271.12	35000	yes	yes	yes
26	0.02	0.57	1.12	35			no
29	0.03	0.58	1.13	91			no
31	0.04	7.55	15.07	79		no	no
33	4.21	11.35	18.49	1400	yes	yes	yes
				Risk indication accuracy:	**100%**	**82 %**	**70%**

Specific effect-based tools can indicate known and unquantifiable risks in water samples for EE2, E2 and E1 with a high risk indication accuracy.

Effect-based tools should be applied as screening tools to identify polluted water bodies, because they are the only tools to address unknown mixture risks.

Bridging many gaps - to stop toxic ignorance

These findings allow us to bridge following gaps for waste water

- From *in vitro* results to population relevant risk assessments, EEQ ≈ RQ
- From single substance to mixture assessments, RQ→∑ RQ
- From known mixture assessment to unknown mixture assessment, $\sum RQ_{known} \rightarrow \sum RQ_{known + unknown}$
- From Screening to Risk Assessment, because it will not matter if other substances like genistein can generate in specific cases a positive EEQ result, afterwards you will find it out with an improved analytical approach (mini EDA, see Kunz et al. 2014).
- For the organism it does not matter which substance binds to the ER receptor and causes the effect.

Comparing highly sensitive chemical analytical and effect-based methods

Chemical analytical (BfG)	E1	E2	EE2
LOD	3 pg/L	30 pg/L	10 pg/L
LOQ	10 pg/L	100 pg/L	35 pg/L

Advantage: You can quantify each analyte

Effect-based ER-Calux (BDS)	E1	E2	EE2
LOD	260 pg/L	5.2 pg/L	4.3 pg/L
LOQ	850 pg/L	17 pg/L	14.2 pg/L

Advantage: You can quantify the receptor activation→ more sensitivity in screening

Preliminary conclusions for effect-based methods

Most prevalidation results of effect-based methods underline the reliability, accuracy, robustness and sensitivity.

<u>Additionally they are cost-efficient high throughput methods:</u>

Installation cost of high end chemical analytical device > 300k Euro

Laboratory equipment for effect-based methods < 30k Euro

Why not to use the advantages of effect-based and chemical analysis in combination?

Status of high end chemical analysis, adapted from Michael Schluesener and Arne Wick 2015, Federal Institute of Hydrology, Koblenz, DE

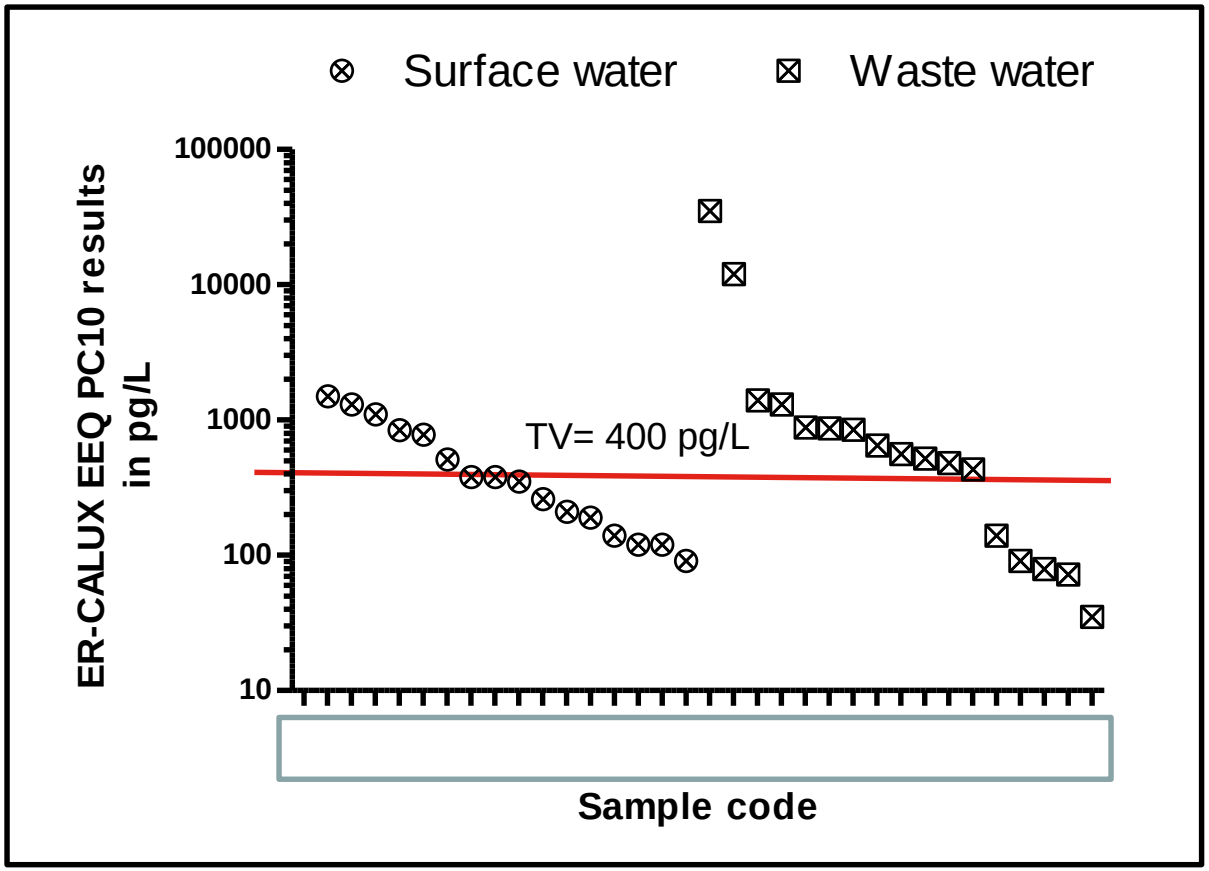
Real world samples

	E1	E2	EE2
Rhine (Koblenz)	290 pg/L	<LOD	<LOD
LOD	3 pg/L	30 pg/L	10 pg/L
LOQ	10 pg/L	100 pg/L	35 pg/L

<u>Good news:</u>
In principle it is now possible to quantify steroidal estrogens in surface water at their EQS levels.
But only few institutes in Europe are capable to measure at these low concentrations.
So it would be nice to know in advance where to find chemical pressures to reduce the monitoring load.

Which level of estrogenicity was found in European surface and waste waters?

ER-Calux PC10 results in pg/L EEQ for 16 surface waters and 17 waste-waters

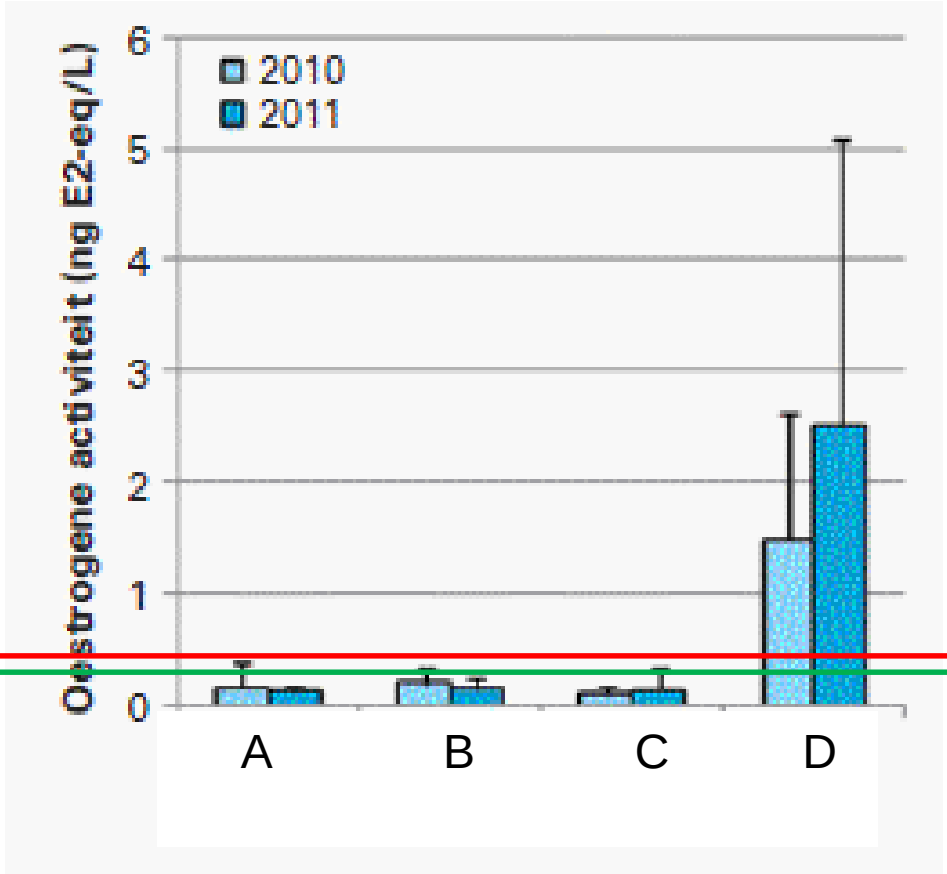


Surface water sample code	ER-Calux EEQ in pg/L	Waste water sample code	ER-Calux EEQ in pg/L
	1500		35000
	1300		12000
	1100		1400
	840		1300
	780		880
	510		870
	380		850
	380		650
	350		560
	260		520
	210		480
	190		430
	140		140
	120		91
	120		79
	91		72
			35

Data provided by BDS

Monitoring load can be reduced: SW 6 x above TV proposal and WW 12 x above TV proposal. Please keep in mind we have mainly asked for potentially polluted samples.

Example: Where do you would like to invest monitoring ressources?



From RIWA 2012: Mean estrogenicity activity in Rhine at Lobith, Lek at Nieuwegein, Amsterdam Rhinechannel and Maas at Keizersveer

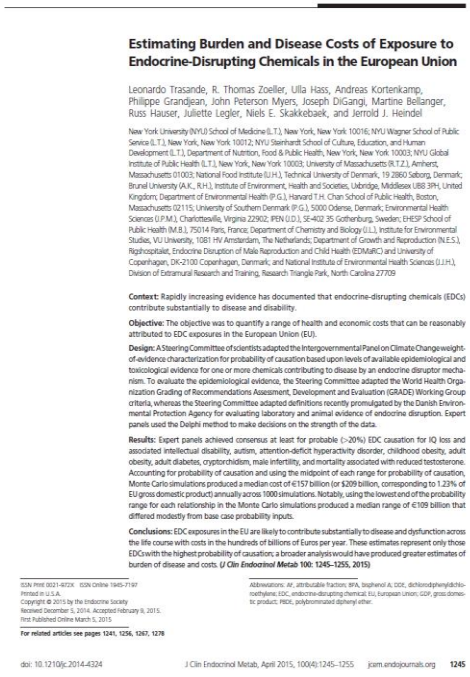
TVs:

AA-EQS for E2 at 0.4 ng/L (Loos et al. 2012)

EEQs-SSE 0.3 ng/L for municipal waste water (Jarosova et al. 2014)

Aim: General effect-based trigger values are proposed, it would be necessary to characterize them in comparison with analytical EE2, E2 and E1 monitoring data for polluted samples !! ➔ test specific trigger values can be elaborated which could allow a more reliable and specific screening

Does EDC matter also for us ?



ORIGINAL ARTICLE

Estimating Burden and Disease Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union

Leonardo Trasande, R. Thomas Zoeller, Ulla Hass, Andreas Kortenkamp, Philippe Grandjean, John Peterson Myers, Joseph DiGangi, Martine Bellanger, Russ Hauser, Juliette Legler, Niels E. Skakkebaek, and Jerrold J. Heindel

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Context: Rapidly increasing evidence has documented that endocrine-disrupting chemicals (EDCs) contribute substantially to disease and disability.

Objective: The objective was to quantify a range of health and economic costs that can be reasonably attributed to EDC exposures in the European Union (EU).

Design: A Steering Committee of scientists adapted the Intergovernmental Panel on Climate Change weight-of-evidence characterization for probability of causation based upon levels of available epidemiological and toxicological evidence for one or more chemicals contributing to disease by an endocrine disruptor mechanism. To evaluate the epidemiological evidence, the Steering Committee adapted the World Health Organization Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group criteria, whereas the Steering Committee adapted definitions recently promulgated by the Danish Environmental Protection Agency for evaluating laboratory and animal evidence of endocrine disruption. Expert panels used the Delphi method to make decisions on the strength of the data.

Results: Expert panels achieved consensus at least for probable (>20%) EDC causation for IQ loss and associated intellectual disability, autism, attention-deficit hyperactivity disorder, childhood obesity, adult obesity, adult diabetes, cryptorchidism, male infertility, and mortality associated with reduced testosterone. Accounting for probability of causation and using the midpoint of each range for probability of causation, Monte Carlo simulations produced a median cost of €157 billion (or $209 billion, corresponding to 1.23% of EU gross domestic product) annually across 1000 simulations. Notably, using the lowest end of the probability range for each relationship in the Monte Carlo simulations produced a median range of €109 billion that differed modestly from base case probability inputs.

Conclusions: EDC exposures in the EU are likely to contribute substantially to disease and dysfunction across the life course with costs in the hundreds of billions of Euros per year. These estimates represent only those EDCs with the highest probability of causation; a broader analysis would have produced greater estimates of burden of disease and costs. (*J Clin Endocrinol Metab* 100: 1245–1255, 2015)

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Abbreviations: AF, attributable fraction; BPA, bisphenol A; DDT, dichlorodiphenyldichloroethylene; EDC, endocrine-disrupting chemical; EU, European Union; GDP, gross domestic product; PBDE, polybrominated diphenyl ether.

For related articles see pages 1241, 1256, 1267, 1278

Conclusions: EDC exposures in the EU are likely to **contribute substantially to disease and dysfunction across the life course with costs in the hundreds of billions of Euros per year**. These estimates represent only those EDCs with the highest probability of causation; a broader analysis would have produced greater estimates of burden of disease and costs. **(*J Clin Endocrinol Metab* 100: 1245–1255, 2015)**

Available at: http://press.endocrine.org/doi/pdf/10.1210/jc.2014-4324

Comment from project-partner: “It is time for our politicians to act against EDs...”.

Simple answer: YES, largely

In our project we are investigating the most known but most underregulated Mode of Action of Endocrine Disruption of ER receptor activation. Some of these ER activating substances enter the food chain, and if we protect the environment we will also protect us!!
Example: EE2 bioconcentration in fish (BCF) ~ 600

Mixed risks from EDCs

Prospective EU EDC regulation (REACh, PPP, Biocides, etc.) is delayed, again.

Nevertheless, we know that Endocrine Disruption results from different EDCs with different exposure patterns.

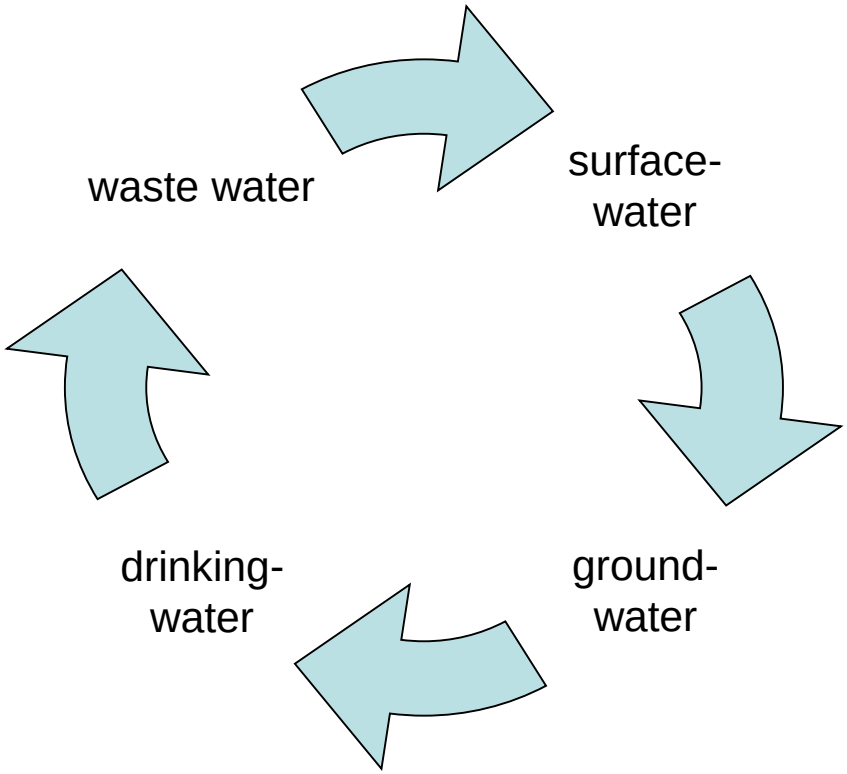
In our project we are focusing on steroidal estrogens and receptor activating substances and mainly on aquatic risks.

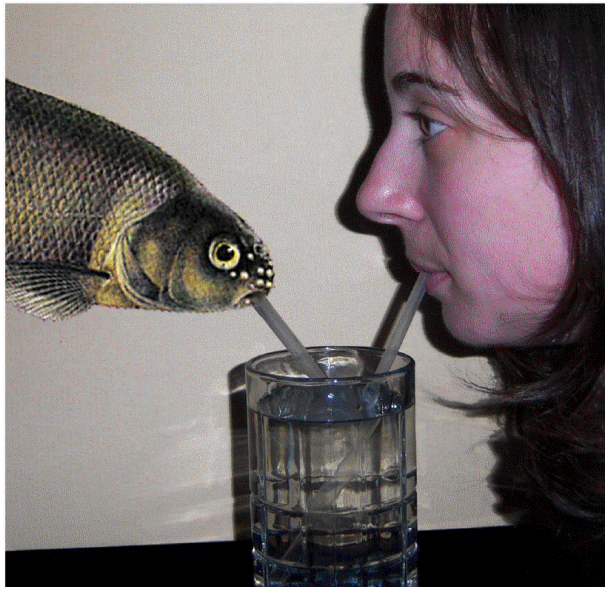
How strong is the combined risk of EE2, E2, E1, Nonylphenol, BPA, Phthalates, Myco-, Phytoestrogens, Pesticides?

We have currently some limited estimates.

But at least we can measure the combined chemical pressures in water bodies for one of the most investigated Mode of Action of EDCs.

Remember What's Good for the Fish is Good for Us Also





Source: US-EPA, Lazorchak J 2010

It makes sense to begin somewhere!!!

Summary and outlook

With a comparison of screening EEQ values with analytical based risk-quotients for steroidal estrogens, we are able to:

1) Increase the monitoring efficiency for steroidal estrogens

2) To bridge the gap between conventional analytical and an effect-based monitoring

3) Lowering costs for monitoring & providing risk management options for EDCs and pharmaceutical strategies

Please feel free to exchange ideas, observations, suggestions and questions:

Robert Kase (Robert.Kase@oekotoxzentrum.ch)
Mario Carere (Mario.Carere@iss.it)

Thank you for your time and attention !!!

More info at:
http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/

Appendix I: Expected benefits and options for effect-based methods

Maybe some of you are still asking: WHY?

The combination of analytical and effect-based methods would offer more options:

10 points after workshop brainstorming 2013

taking into account analytical and financial restraints

Appendix I: Expected benefits and options for effect-based methods

1. Effect-based monitoring tools have reached a level of maturity that they can be implemented into WFD at various levels of application (e.g. screening or investigative monitoring).

2. They have the potential to reduce the high cost of specific analytical measurements. Thus, they provide reliable information for EDCs relevant mode of action.

3. For environmental screening purposes they have already proven their regulatory applicability, e.g. in case of river-basin specific pollutants.

4. Using specific effect-based methods samples can be divided into critical and unpolluted ones if they contain substances clearly related to an effect (e.g. E2 and EE2). This allows a reduction and optimized use of analytical resources.

5. Specific effect-based methods shall be fit-for-purpose, freely accessible and deliver comparable results of defined quality.

Appendix I: Expected benefits and options for effect-based methods

6. Pre-validation will characterize the performance in terms of sensitivity, robustness, reliability, relevance and reproducibility.

7. The process of pre-validation should be linked to the CIS process and a further standardization could be envisaged. (fortunately a new ISO work item was launched in 2013)

8. Specific effect-based methods can be used to identify other EDCs and support the implementation of the EU EDC strategy.

9. For environmental samples with an unknown composition (unknown mixtures) the effect-based tools are the only methods to detect specific hormonally and endocrine disruptive effects.

10. An effect-based monitoring as a component for the watchlist mechanism could helpful to identify unknown environmental pollutants (prioritization issue, else you will only find or not find what you are looking for)

Appendix II: Effect-based methods linked to CIS work programme 2013-2015 for WG Chemicals

I. Tasks related to new legislation

«Preparation of watch list monitoring ,… agreeing on technical specifications for monitoring them» Comment: For some substance classes, like the steroidal estrogens technical agreement is already available in expert recommendations:
http://www.bafg.de/DE/05_Wissen/02_Veranst/2013/2013_02_27_votum_en.pdf?__blob=publicationFile

II. Tasks required to implement existing legislation

« Developing approaches to the quantification of pressures from chemical pollution, ….»
Comment: Pressures are normally occuring from a sum of known and unknown substances. Effect-based tools are the only tools which can address unknown mixtures for a specific mode of action.

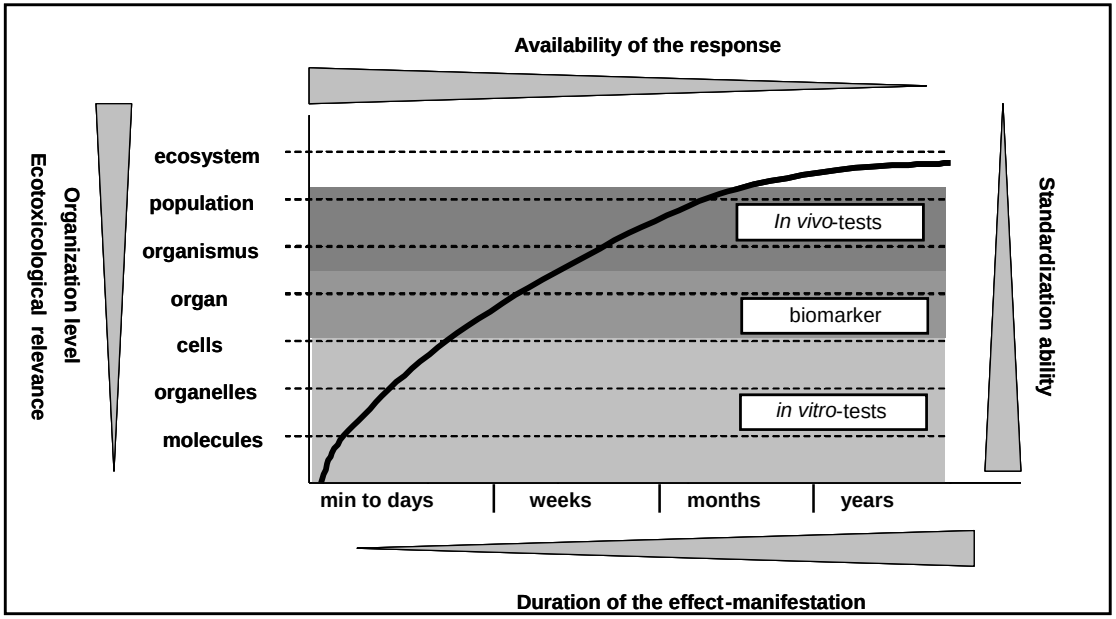
III. Tasks related to future developments

« Identification of best available techniques not entailing excessive costs»
Comment: Effect-based tools as screening tools can lower the costs:

a) they are normally cheaper and more sensitive than the high end analytics for several compounds

b) as screening tools they can reduce the number of samples which have to be monitored by high end analytics

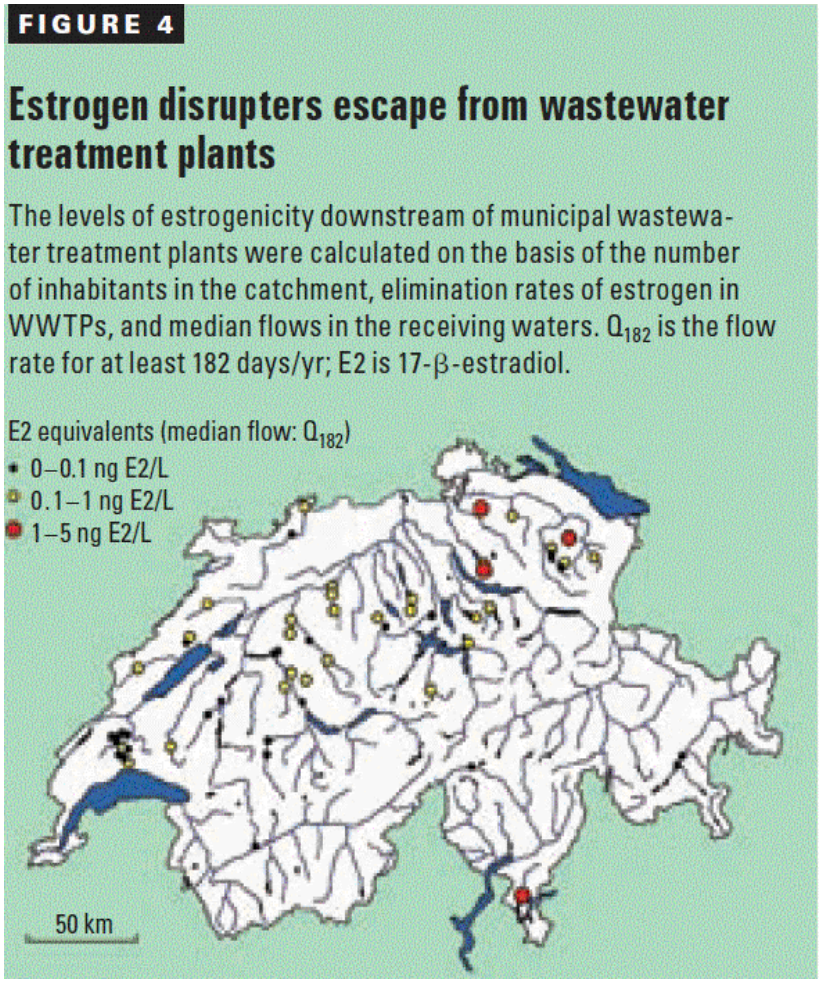
Appendix III: Need to bridge gaps - from *in vitro* results to population relevant EQS and mixtures



Ecotoxicological effect and organization levels and duration of effect manifestation of biotests (adapted from Braunbeck 1993 and Kase et al. 2009)

This can work: If we can correlate biological responses with population relevant EQS.

Appendix III: What about the combined risks of steroidal estrogens at median flow



Source: Burkhard-Holm et al. 2005

- **The SSD based EQS for E2 will be at 0.4 ng/l** (**derived from 11 population relevant chronic NOECs of different fish species**)
- The **most sensitive study** is done **with rainbow trout** (Lahnsteiner et. al. 2006) → **NOEC of 0.5 ng/L** (for endpoints: fertilization success, sperm density and volume).
- The **E2, the metabolite estrone (E1)** and the **pharmaceutical 17-alpha ethinylestradiol (EE2) contribute additionally to** the estrogen receptor mediated **estrogenicity, so we have a cummulative risk.**
- Also industrial chemicals like **BPA, Nonylphenol, Octylphenol** and some **Phthalates** could have weaker estrogenic impacts and have lower receptor binding potentials. (Kase and Werner 2011)

It is highly likely that different steroidal estrogens (EE2, E2 and E1) and some estrogenic industrial chemicals have an impact at environmental relevant concentrations on fish populations

Appendix III: Are combination effects in fish likely?

- **YES:** Estrogenic impacts, histopathologic effects and immunotoxic **effects** are likely caused **by a variety of chemical stressors in fish**.

- **YES**: **Endogenic factors**, **seasonal cycles** and **environmental factors** influence the biological stress response in sensitive aquatic organisms. E.g. immunomodulations(e.g. Cuklev et al. 2011), can act together with other factors (temperature) to cause **adverse effects**.

- **YES**: Estrogenic substances have the potential to cause immunomodulations, e.g. the **susceptibility** for pathogens is **increased by exposure to estrogens** (Casanova-Nakayama et al. 2011).

- **YES**: Substances that impair **reproductive success** such as nonylphenol (Lahnsteiner et al. 2005) **can inhibit the recovery of fish populations**

Can we detect multiple and EDC related stressor effects to protect aquatic organisms?

Appendix III: Expected challenges for the watch list substances EE2 and E2

If you want to monitor an exposure related risk for EE2 and E2:

- **A worst case could be a monitoring dataset full of non-detects due to insufficient detection limits (imagine a LOD of 100 pg/L for EE2 risk or no risk?)**
- **The stability of the samples is a critical point in estrogen analysis**
- **Methodical choices and variability will strongly influence the comparability of results**
- **For the relatively low EQS of EE2 and E2 in the sub ng/L range (the best available methods in combination are needed**

Fortunately we have now a promising set of best available chemical analytical and effect-based analytical methods in our project to improve the monitoring and detection

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