CALUX tests for water testing:
Dioxins, Endocrine Disrupters, Genotoxicity, Obesity & PAHs

Dr. Peter A. Behnisch - BioDetection Systems BV, Amsterdam

Emerging problems associated with Endocrine Disrupting Chemicals

Decling fish populations due to EDCs..?

Where Have All *the* FISH Gone?

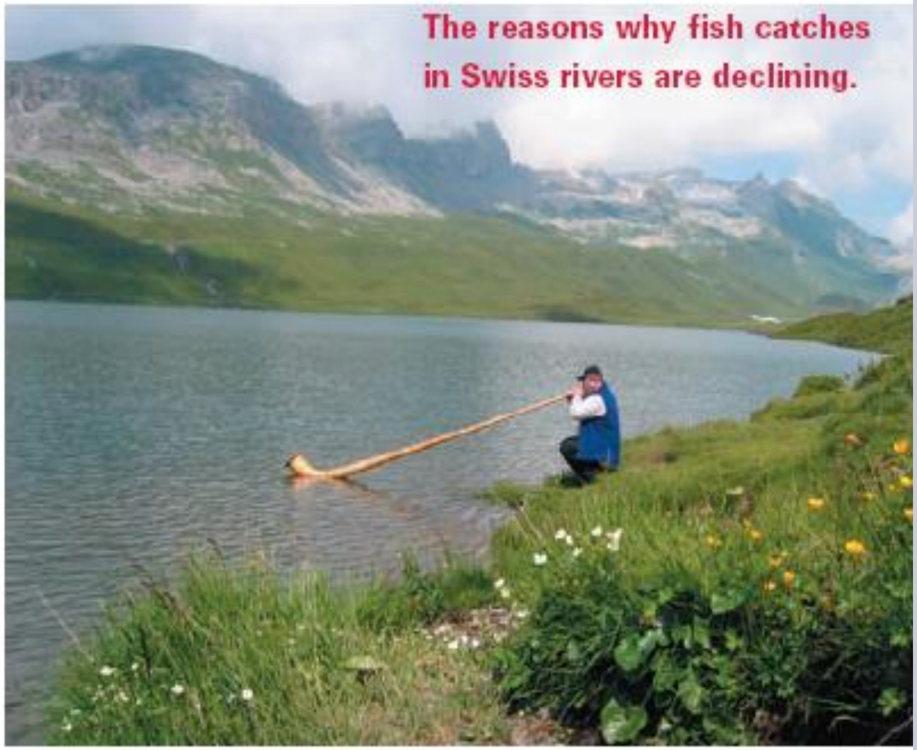


FIGURE 1

Trout catches in Switzerland

Catches have steadily declined since the 1980s, according to anglers' personal data records.

Health facilities flush estimated 100M kilos of drugs a year

Hospitals and other health institutes flush every year 113,4 milloen kg of un-used pharmaceuticals through the toilet. And this is a conservative estimate according to the *Associated Press*.

bron: Associated Press (via USA Today; 14-9-08)

Birth controll pill responsable for fish collapse: chronically dosed 3-4 ng EEQ/l already enough

Collapse of a fish population after exposure to a synthetic estrogen

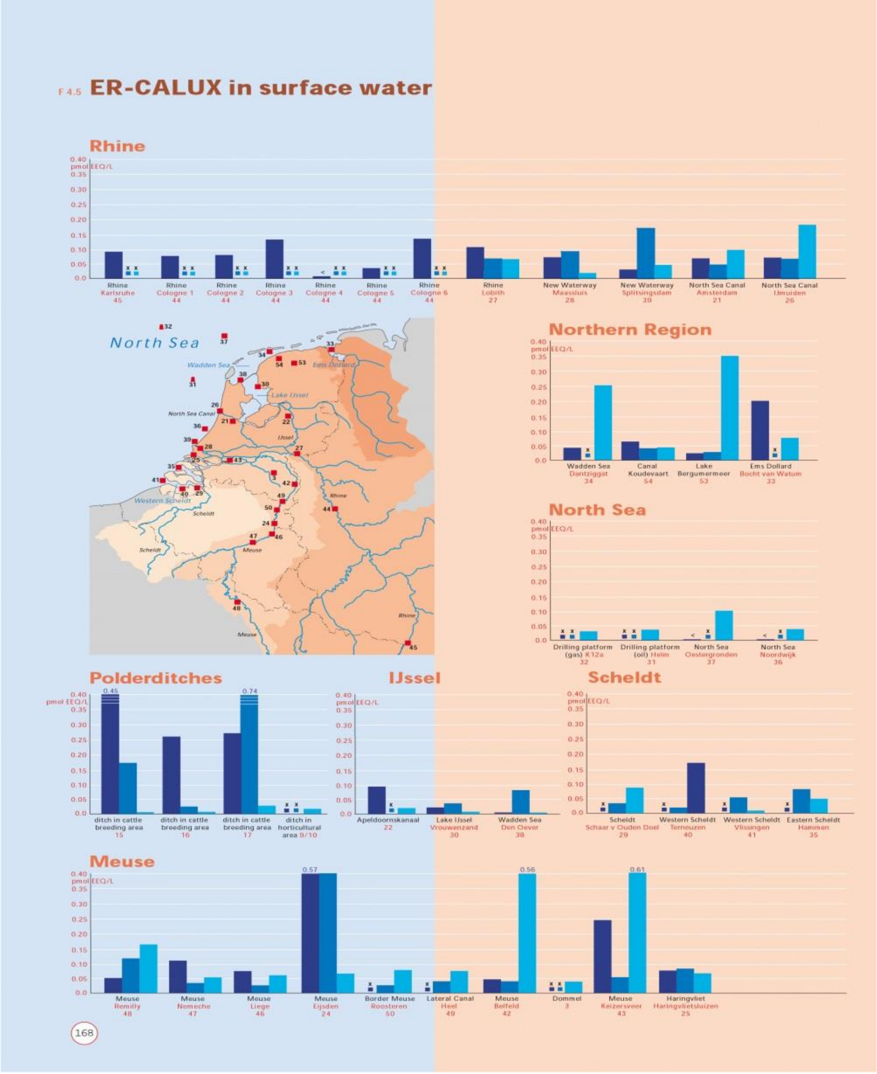
Karen A. Kidd*[†], Paul J. Blanchfield*, Kenneth H. Mills*, Vince P. Palace*, Robert E. Evans*, James M. Lazorchak[‡], and Robert W. Flick[‡]

*Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada R3T 2N6; and [‡]Molecular Indicators Research Branch, United States Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268

Edited by Deborah Swackhamer, University of Minnesota, Minneapolis, MN, and accepted by the Editorial Board March 29, 2007 (received for review October 27, 2006)

„Municipal wastewater are a complex mixture of estrogens…We conducted a 7 year, whole lake experiement in Ontario, and showed that chronic exposure of fathead minnow to low concentration (5-6 ng/L; which has a REP value of 0.75 = 3.75 ng EEQ/l water) of the birth control pill 17alpha ethinyl-estradiol lead to a near extiction of this species from this lake.“

Levels in Dutch Waste Water up to 151 ng EEQ/l water!



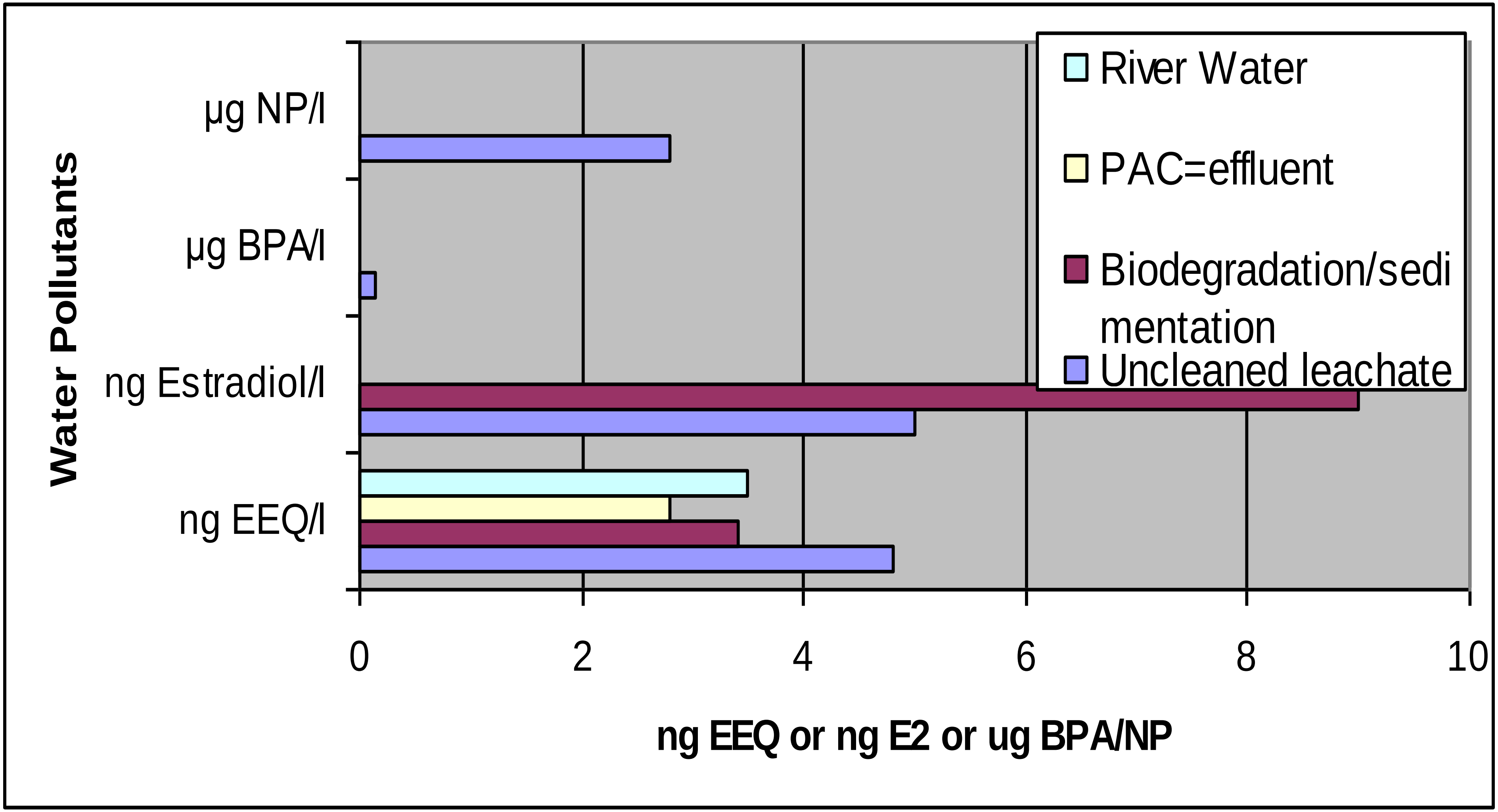
Estradiol equivalents (pmol EEQ/l)			
Compartment	*n*	Range (*n* > l.o.d.)	Median
Industrial wastewater:			
Effluent	3	0.2–9.5 (3)	0.9
Influent	5	5.8–560.4 (4[a])	317
Municipal wastewater:			
Effluent	10	<l.o.d.–2.2 (9)	0.3
Untreated influent	13	2.4–275.1 (13)	27.4
Surface water:			
Surface water	90	<l.o.d.–0.61 (85)	0.07
Polder ditches	11	0.003–0.74 (11)	0.03
Rainwater	3	0.01–0.22 (3)	0.13

River water: Dommel
highest ER CALUX and intersex in bream
Note: 1 pmol EEQ/l = 0,27 ng EEQ/l)

Landfill WWTPs (Nagoya, Japan)




Landfill leachates higher as 3.75 ng EEQ/l water!



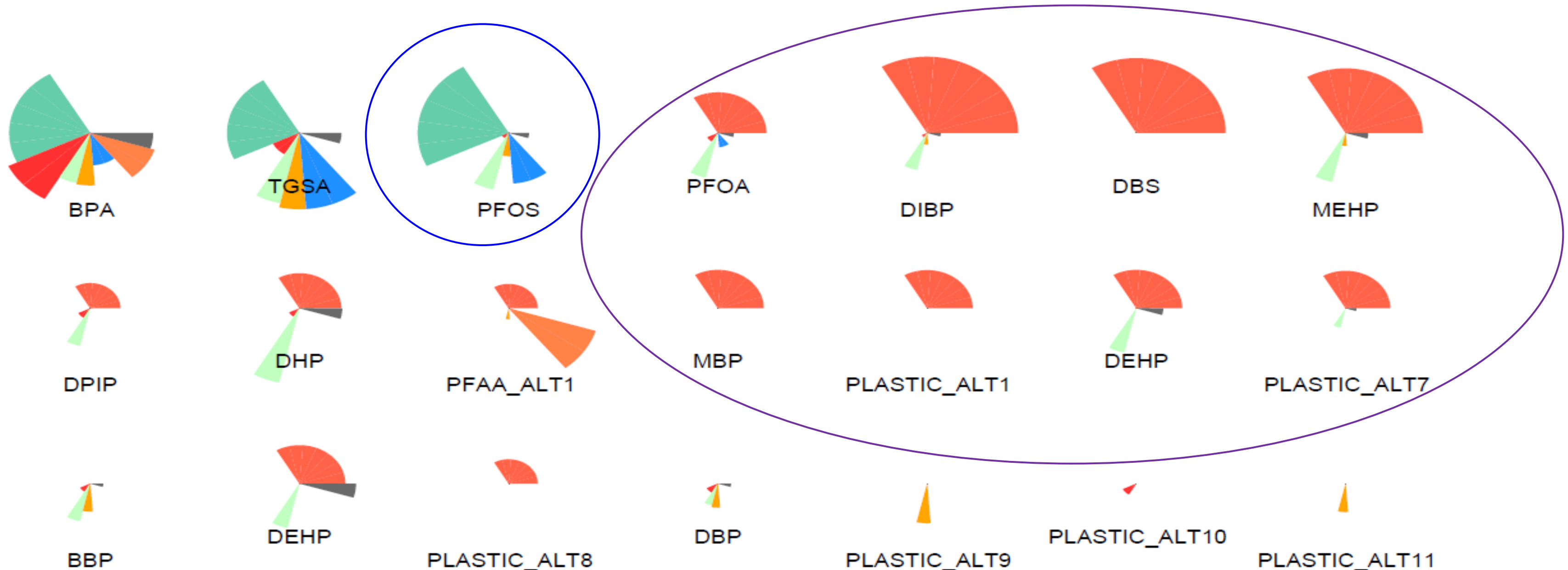
Behnisch, Sakai, et al. Chemosphere 43, 977 (2001)

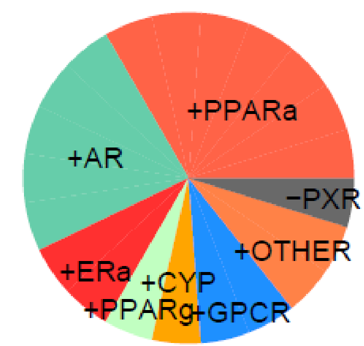
Potency of natural, phyto- and synthetic estrogens relative to estradiol in the ER-CALUX assay *(Murk et al 2002)*

Compound	Relative potency
Natural Estrogens:	
17ß-Estradiol	1
17a-Estradiol	5.6x10^{-2}
Estrone	1.6x10^{-2}
Estriol	1.0
Estradiol 3B-D-glucuronide	n.c.*
Phyto-estrogens:	
Genistin	2.6x10^{-4}
Diadzein	1.3x10^{-4}
Formononetin	1.1x10^{-4}
Biochanin A	5.3x10^{-4}
Genistein	6.0x10^{-5}
Pesticides:	
o,p'-DDT	9.1x10^{-6}
o,p'-DDE	2.3x10^{-6}
Methoxychlor	1.0x10^{-6}
Dieldrin	2.4x10^{-7}
Endosulfan	1.0x10^{-6}
Chlordane	9.6x10^{-7}
Simazine	n.c.*
Atrazine	n.c.*
Desethylatrazine	n.c.*
Deisopropylatrazine	n.c.*
Kepone	5.2x10^{-6}
Lindane	n.c.*

Compound	Relative potency
Synthetic estrogens:	
Ethynyl-estradiol	1.2
Diethylstilbestrol	0.1
Alkylphenols:	
4-nonylphenol (NP)	2.3x10^{-5}
4-octylphenol (OP)	1.4x10^{-6}
4-tert-pentylphenol	2.3x10^{-5}
NP1EO#	3.8x10^{-6}
NP2EO#	1.1x10^{-6}
NP4EO#	1.1X10^{-7}
NP10EO#	n.c.*
NP1EC#	n.c.*
NP2EC#	n.c.*
OP8/9EO#	n.c.*
Phthalates:	
dimethylphthalate	n.c.*
diethylphthalate	3.2x10^{-8}
dibutylphthalate	1.8x10^{-8}
butylbenzylphthalate	1.4x10^{-6}
di 2-ethylhexy phthalate	n.c.
dioctylphthalate	n.c.*
bisphenol A	7.8x10^{-6}
Benzo(a)pyrene	1.9x10^{-6}

Tox-profiling: plasticizers

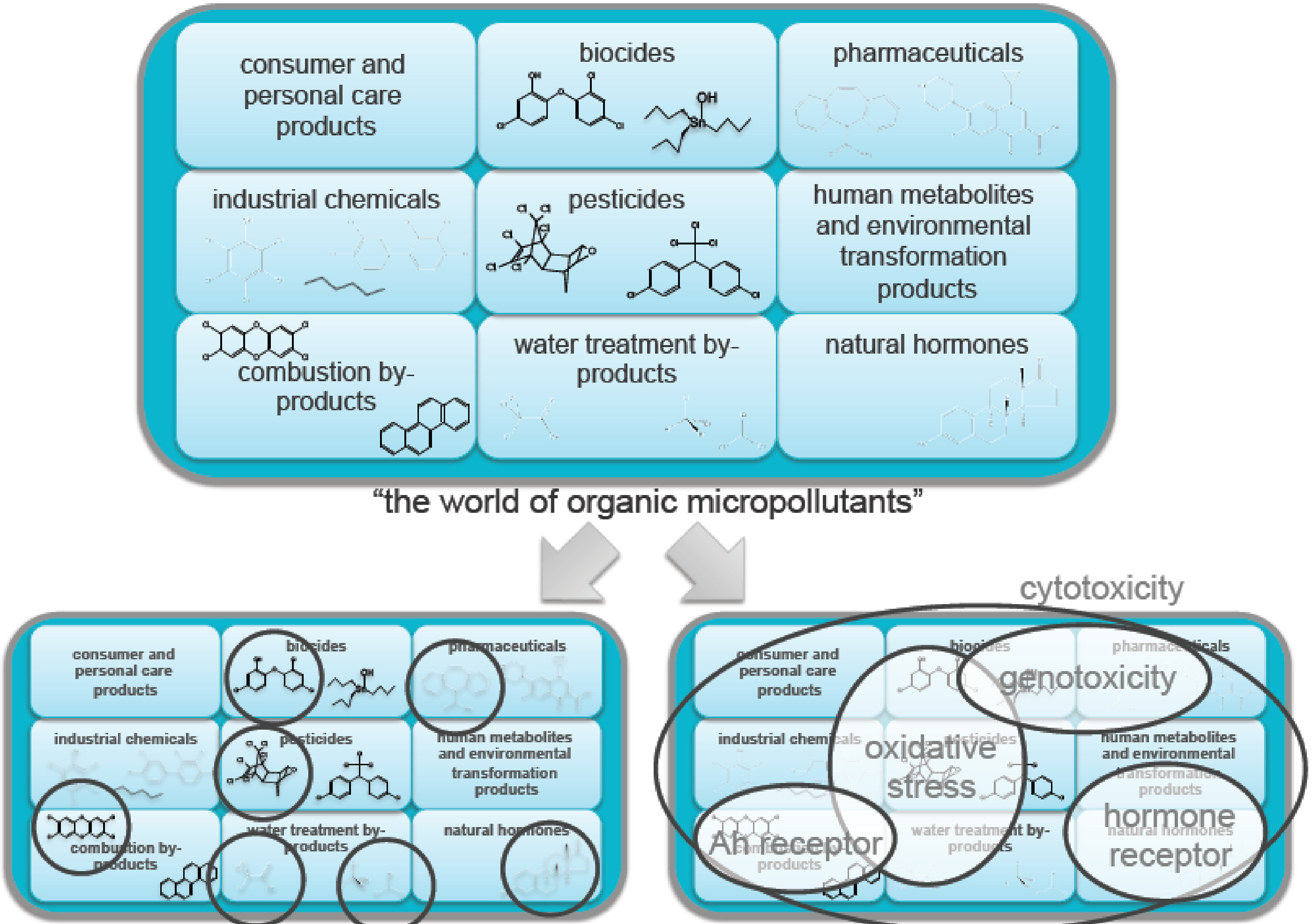




- PFOA and other plastic additives are PPARα active – need to be tested now..
- PFOS is not active in PPARα, but AR and PPARγ active

Biol Reprod. 2011 Aug;85(2):327-39. Epub 2011 May 12.
Predictive model of rat reproductive toxicity from ToxCast high throughput screening.
Martin MT, Knudsen TB, Reif DM, Houck KA, Judson RS, Kavlock RJ, Dix DJ.

Chemical versus biological analysis



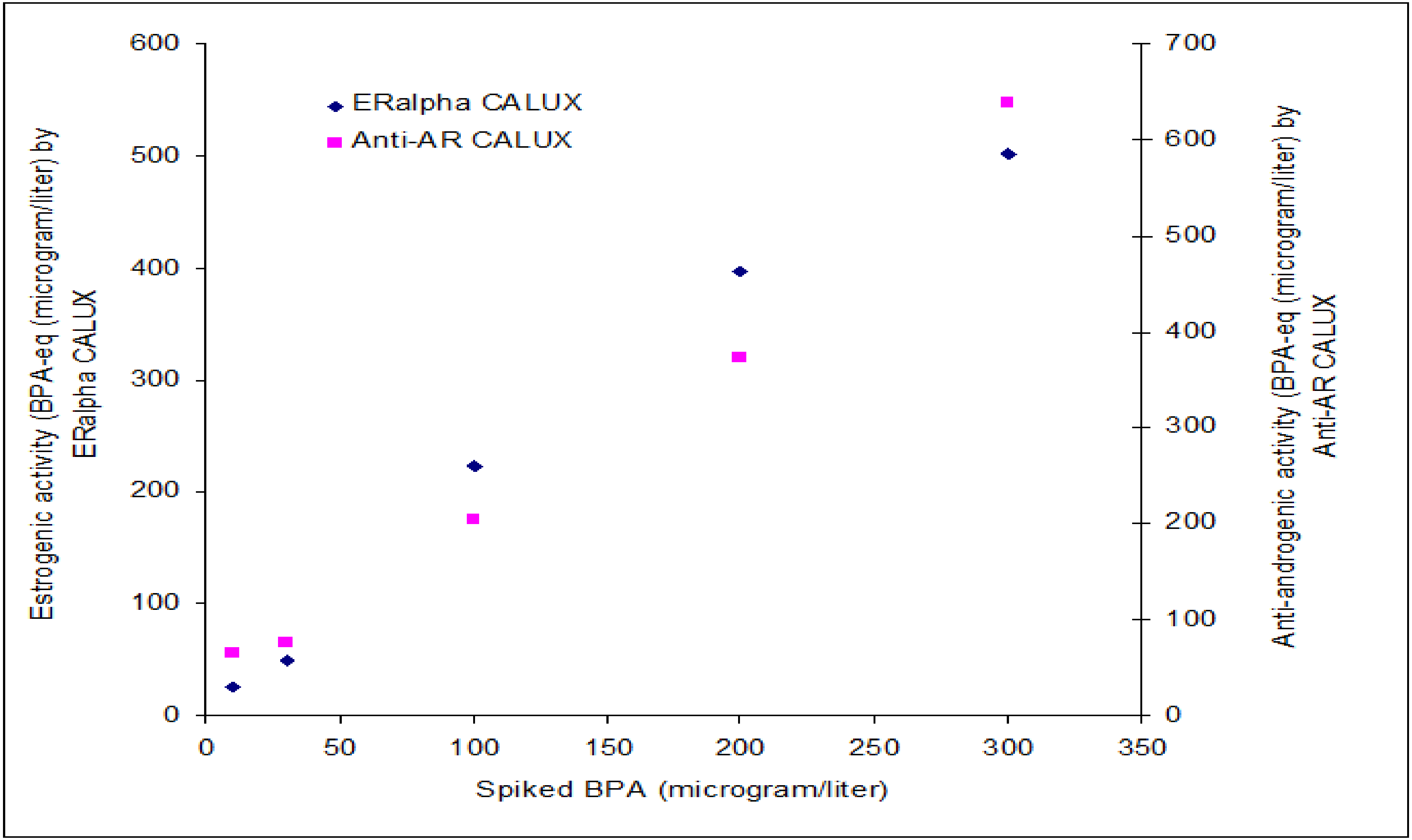
The “view” of an analytical chemist

The “view” of an environmental toxicologist

Escher and Leusch (2012)

ER and anti-AR CALUX correlates well with BPA in water samples

(Service Analysis for German EPA, Bad Dessau)



CALUX panel for pesticide polluted areas,
e.g. landfill in Tajikistan:

Effect-based in vitro CALUX analysis of a complex pesticide mixture/cocktail

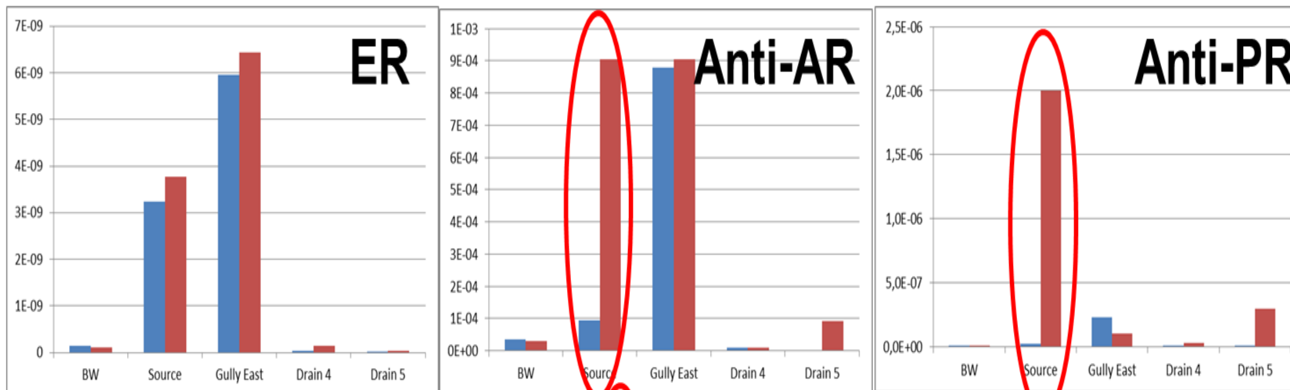
– how much toxicity can be really explained by chemical analysis of real world problems

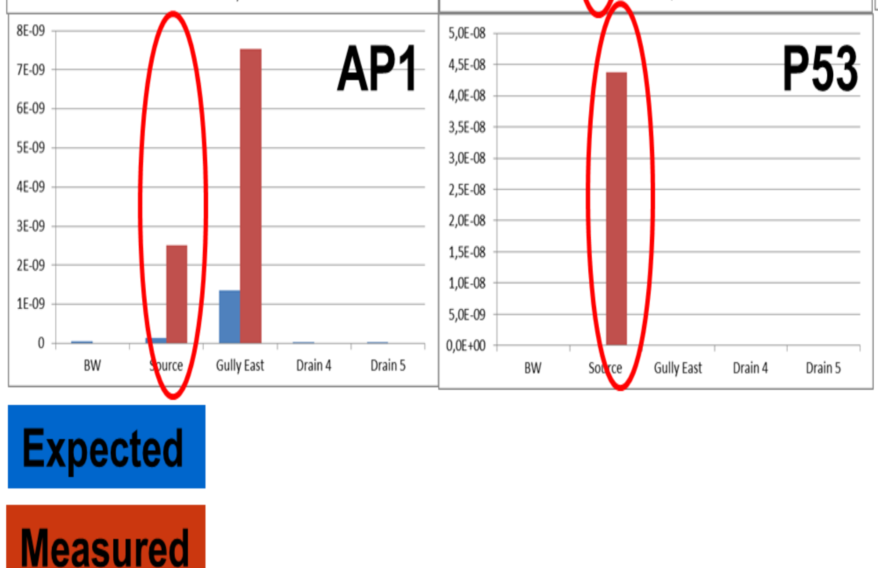
Studying effects of mixtures : pesticide dump in Tajikistan

	AR anti	PR anti	AP1	nrf2	p53	ER
Reference	flutamide	Ru486	TPA	curcumin	actinomycin D	17b-estradiol
EC10 ref compound	3,0E-08	5,0E-11	2,5E-10	3,2E-06	2,2E-09	2,0E-12
	REP	**REP**	**REP**	**REP**	**REP**	**REP**
Lindane	NA	NA	NA	NA	NA	1,0E-06
Aldrin	3,0E-02	5,0E-05	NA	NA	NA	4,0E-07
Dieldrin	**1,0**	NA	NA	NA	NA	4,0E-06
Endrin	**1,0**	NA	NA	NA	NA	1,6E-06
o,p-DDT	**0,3**	7,9E-05	1,0E-05	NA	NA	1,6E-05
p,p-DDT	**1,0**	2,5E-04	1,3E-05	NA	NA	2,0E-06
DDE	9,5E-03	5,0E-05	NA	NA	NA	4,0E-07

- Rapidly identify risks of single chemicals (for humans, environment)
- Measure chemicals in complex mixtures and link this to hazards
- Example pesticide dump side

	Dump 1	Dump 2
alpha-HCH	690,0	3,8
beta-HCH	120,0	13,0
gamma-HCH	8,3	570,0
delta-HCH	5,0	6,2
Aldrin	0,0	0,9
Dieldrin	1,4	0,0
Endrin	0,0	0,0
o,p-DDT	4,5	48,0
p,p-DDT	32,0	310,0
PCDDs, PCBs, PBDEs, PFTs		





Different activity profile from what was expected for 'Source' sample

Not all pesticides included in chemical analysis!

PAH CALUX

How to assess a complex mixture of PAHs in soil, sediments, water or oil spills by chemical analysis and effect-based PAH CALUX

PAH	Accession number	MW	REP (M/M)	List	IARC classification	TEF
naphthalene	91-20-3	128	<0.0001	EPA	2B	0.001
acenaphtylene	208-96-8	152	<0.0001	EPA	-	0.001
acenaphpthene	83-32-9	154	<0.0001	EPA	3	0.001
fluorene	86-73-7	166	<0.0001	EPA	3	0.001
phenanthrene	85-01-8	178	<0.0001	EPA	3	0.001
anthracene	120-12-7	178	<0.0001	EPA	3	0.01
fluoranthene	206-44-0	202	<0.0001	EPA	3	0.001
pyrene	129-00-0	202	<0.0001	EPA	3	0.001
benzo[c]fluorene	205-12-9	216	<0.0001	EU	3	-
benzo[g,h,i]perylene	191-24-2	276	<0.0001	EPA, EU	3	0.01
cyclopenta[c,d]pyrene	27208-37-3	226	0.0003	EU	2A	-
dibenzo[a,l]pyrene	191-30-0	302	0.002	EU	2A	-
dibenzo[a,h]pyrene	189-64-0	302	0.2	EU	2B	-
dibenzo[a,i]pyrene	189-55-9	302	0.2	EU	2B	-
dibenzo[a,e]pyrene	192-65-4	302	0.3	EU	2B	-
benz[a]anthracene	56-55-3	228	0.3	EPA, EU	2B	0.1
chrysene	218-01-9	228	0.8	EPA, EU	2B	0.01
benzo[a]pyrene	50-32-8	252	1	EPA, EU	1	1
benzo[j]fluoranthene	205-82-3	252	1.3	EU	2B	-
dibenz[a,h]anthracene	53-70-3	278	1.3	EPA, EU	2A	5
indeno[1,2,3-cd]pyrene	193-39-5	276	1.3	EPA, EU	2B	0.1
5-methylchrysene	3697-24-3	242	1.4	EU	2B	-
benzo[k]fluoranthene	207-08-9	252	3.7	EPA, EU	2B	0.1
benzo[b]fluoranthene	205-99-2	252	5.0	EPA, EU	2B	0.1
2,3,7,8-TCDD	1746-01-6	322	5.0		1	-

Sample	PAH CALUX-measured BEQ		Theoretical BEQ			Ratio measured BEQ) / Theoretical BEQ	
Synthetic mixtures	Concentration (mM)	Standard deviation (%)	REP-based concentration (mM)	TEF-based concentration (mM)	REP/ TEF	REP-based prediction	TEF-based prediction
Industrial soil, Sweden (41)	5.32	14	5.43	0.53	10.2	1.0	10.2
Industrial soil, Sweden 2 (41)	5.10	7	6.79	1.58	2.2	0.8	1.7
Industrial soil, France (42)	7.40	9	10.05	3.06	6.4	0.7	4.7
Industrial soil, Germany (42)	11.87	3	9.15	1.86	4.9	1.3	6.4
Industrial soil, Portugal (42)	6.43	30	5.01	1.07	4.7	1.3	6.0
Roadside, India (40)	1.41	14	13.51	0.76	17.1	1.0	18.3
Urban soil, United Kingdom (39)	1.14	3	11.39	1.32	8.1	1.1	8.7
Reference samples	**Concentration (µmol/kg)**	**Standard deviation (%)**	**REP-based concentration (µmol/kg)**	**TEF-based concentration (µmol/kg)**	**REP/ TEF**	**REP-based prediction**	**TEF-based prediction**
Sewage sludge (LGC9182)	101	17	33.0	3.5	9.4	3.1	28.9
River sediment (LGC6288)	138	4	32.2	5.9	5.5	4.3	23.4
Industrial soil (BCR524)	2160	10	442	55.6	8.0	4.9	38.9

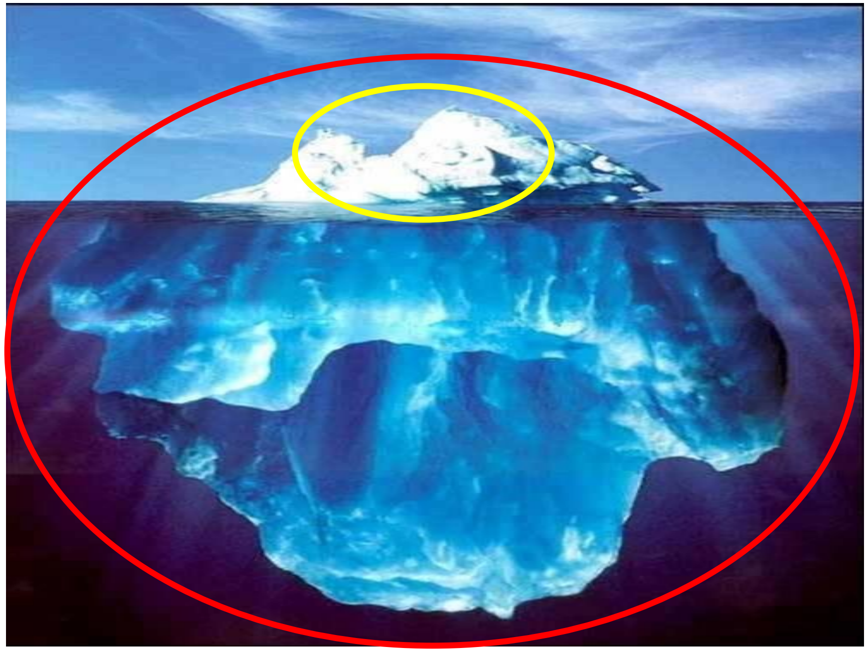
Toxicology for the twenty-first century

Thomas Hartung

The testing of substances for adverse effects on humans and the environment needs a radical overhaul if we are to meet the challenges of ensuring health and safety.

The simplest testing strategy would combine two different approaches, such as a screening approach (a method to identify 'suspicious' substances with less effort and allowing false-positive results) and a confirmatory one (which may be more sophisticated and specifically identifies hazards with higher certainty). All substances that test positive

Effect based bioanalysis – more than only the top of the mountain from chemical analysis (compound specific analysis)

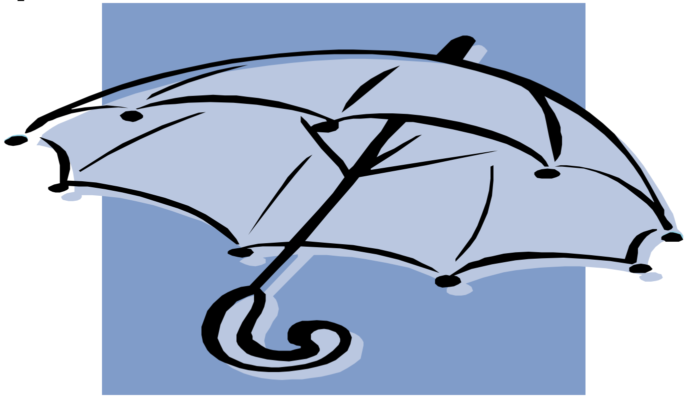


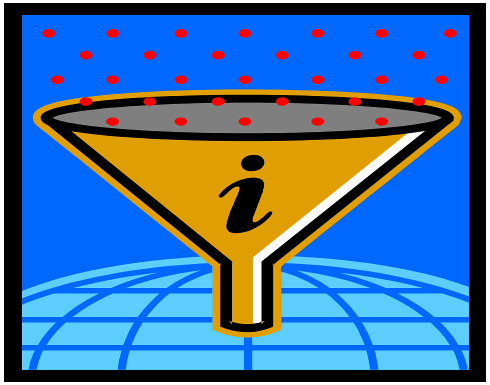
- Substances:
  - selected priority pollutants
- Effects:
  - General toxicity: effects of total mixture of pollutants
  - Specific toxicity: effects of substances with a similar mechanism of toxic action
  - Unknown cause of effect (TIE needed)

More reliable risk assessment by use of toxic bioanalytical screening prior to relevant chemical analyses

Dilemma and Solution in Safety Management

Dilemma → **More and more compounds to be tested…. How to manage risks from complex mixtures**



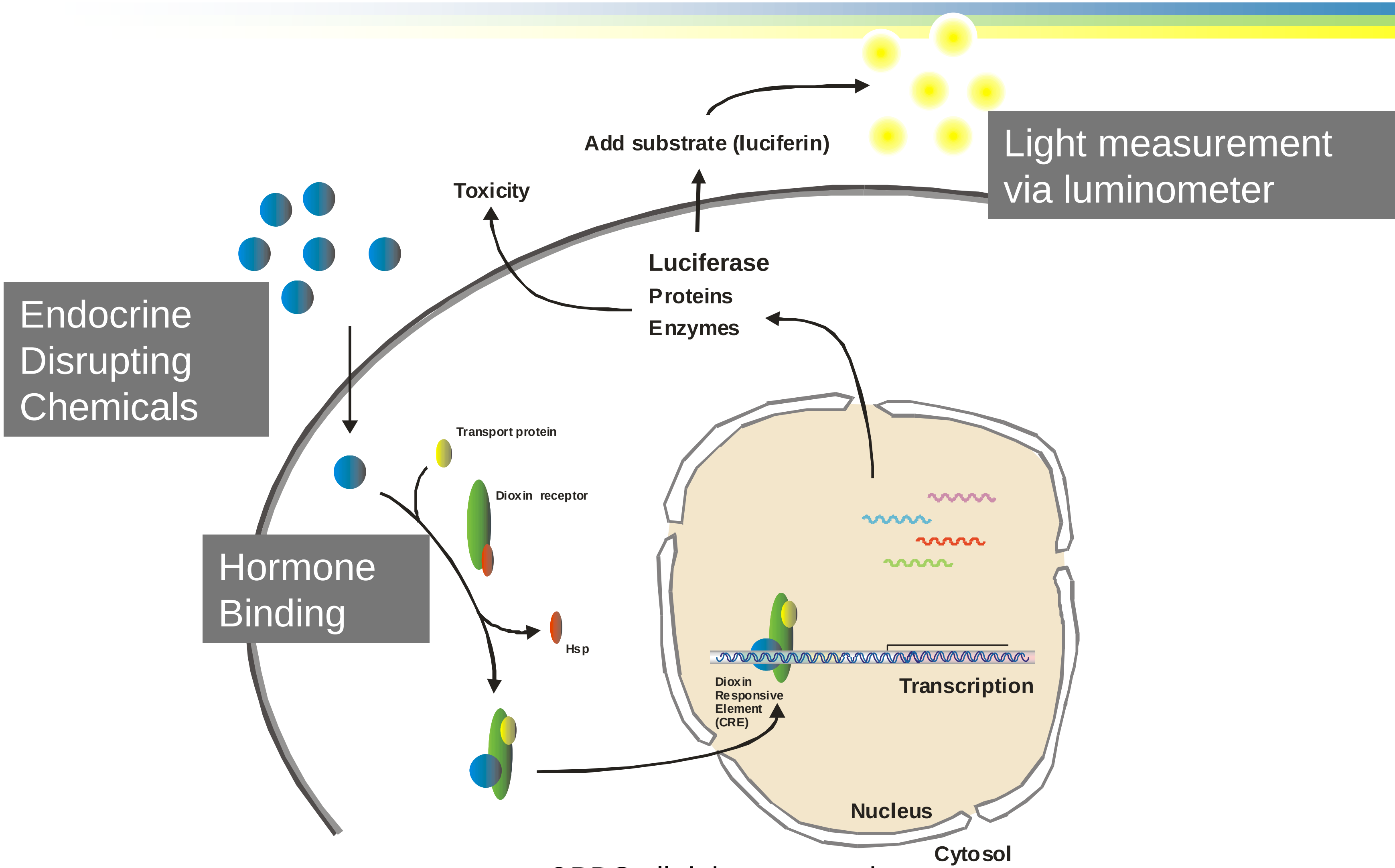


Solution → **Paradigm shift → from compound → to effect oriented analysis**

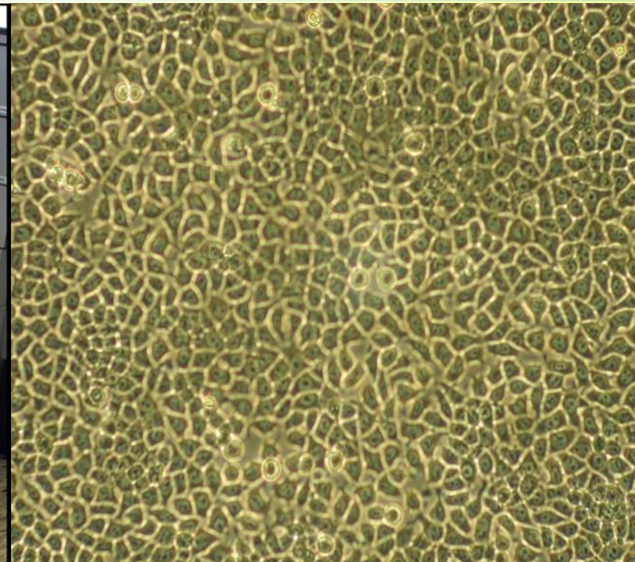
Screening technologies applied in EC monitoring and R&D projects

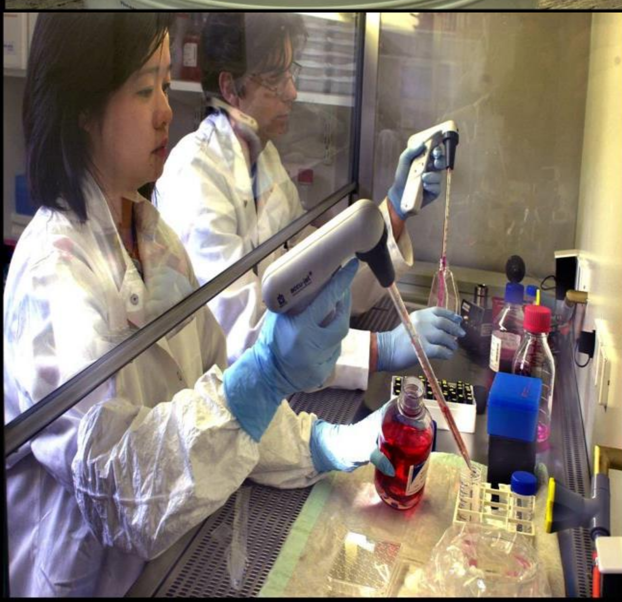
- ## Food and Feed (safety/functional foods)
  - EU Project DIFFERENCE – dioxin/PCB screening in food/feed
  - EU project Plantlibra- beneficial food ingredients
  - Dutch Food and Nutrition project-tests for beneficial food ingredients
- ## Water
  - Technological collaboration project Economic affairs – genomics-based biodetection
  - EU Project TECHNEAU – water safety
  - EU Project ACE – what to do with complex mixtures of pollutants?
  - Dutch project Genes for Water- water safety
- ## Environment
  - Dutch Projects Ecogenomics – healthy soil, DNA barcoding
  - EU Project FACE IT – early warning oil spill biotests
  - EU Project HORIZONTAL – dioxin/PCB screening in soil, sludge/biowaste
  - Belgium DISCRISET Project – rapid testing for hazardous waste
  - Japanese MILLENIUM Project for safe waste recycling technologies
  - Swiss Project: Global warming – how to make car exhaust gas safer?
- ## Chemicals and biologicals (safety/discovery)
  - EU Project FIRE: brominated flame retardants
  - EU Project REPROTECT – non animal testing for REACH
  - EU project METAEXPLORE- metagenomics
  - EU project CHEMSCREEN- non animal testing for REACH
  - Netherlands Toxicogenomics Centre- genomics and non animal testing for chemical safety
- ## Human health (clinical/epidemiology/doping)
  - Wada project- antidoping
  - EU Project NEW GENERIS – Baby/mother health biomarkers
- ## Pharmaceuticals (safety/discovery)
  - Dutch Projects EcoLinc – metagenomics approaches
  - Top Institute Pharma project – tests for advarse drug reactions/metabolism
  - Netherlands Toxicogenomics Centre- genomics and non animal testing for drug safety

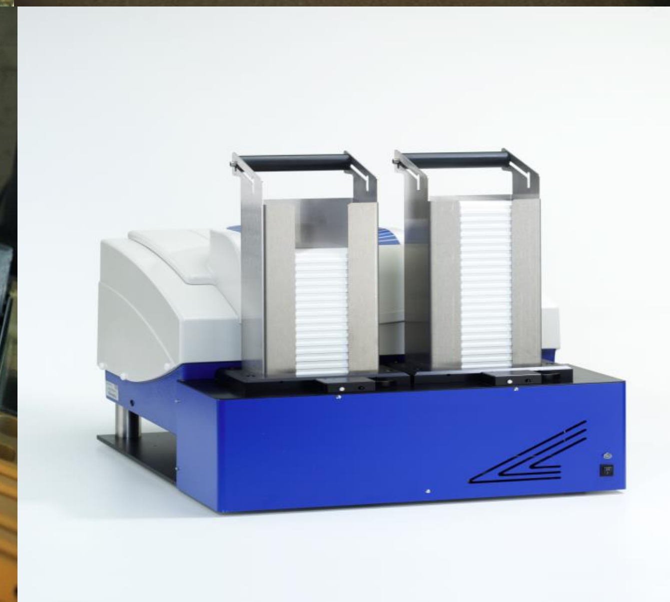
CALUX® Principles



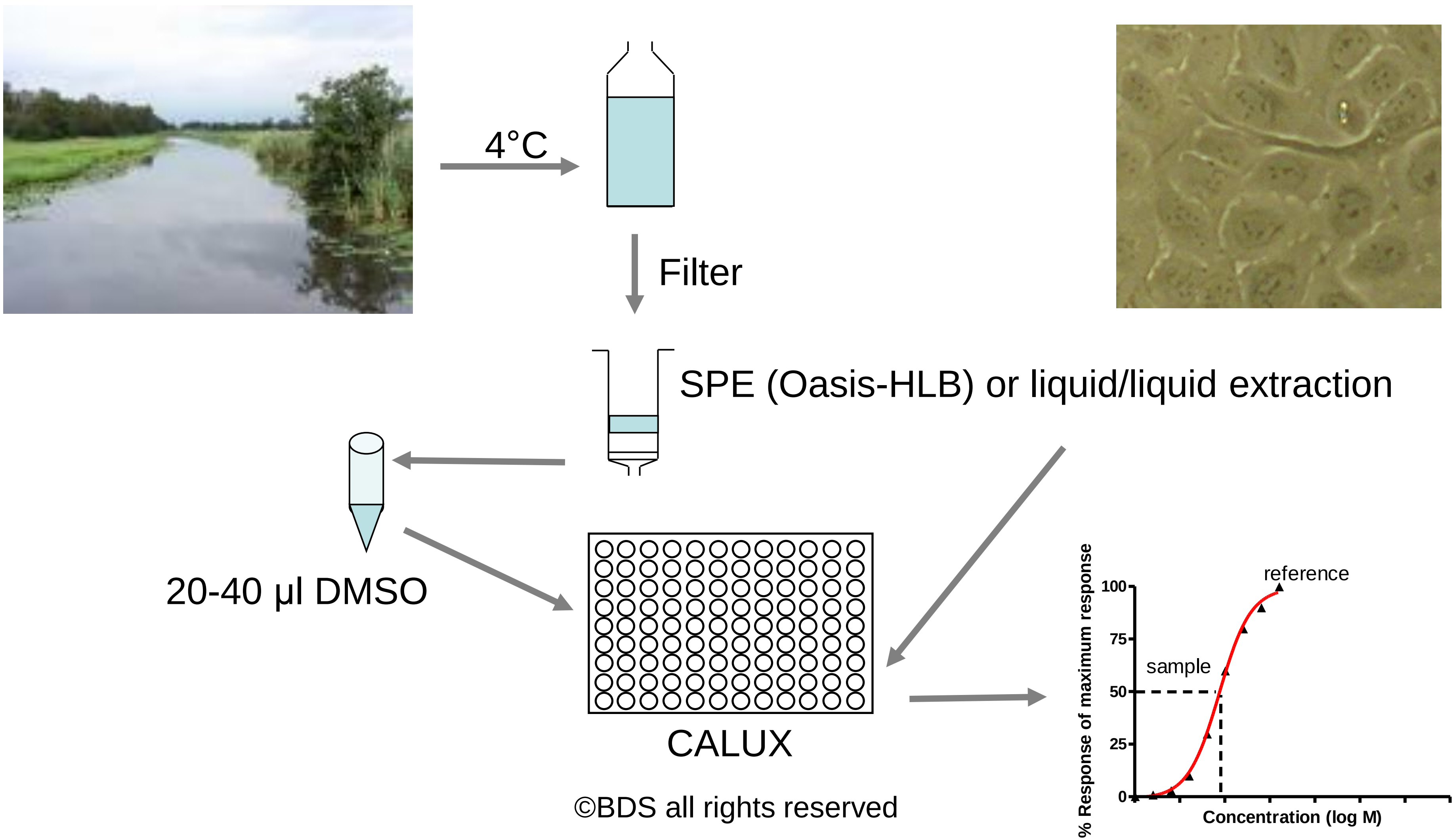
EDC testing & robotics





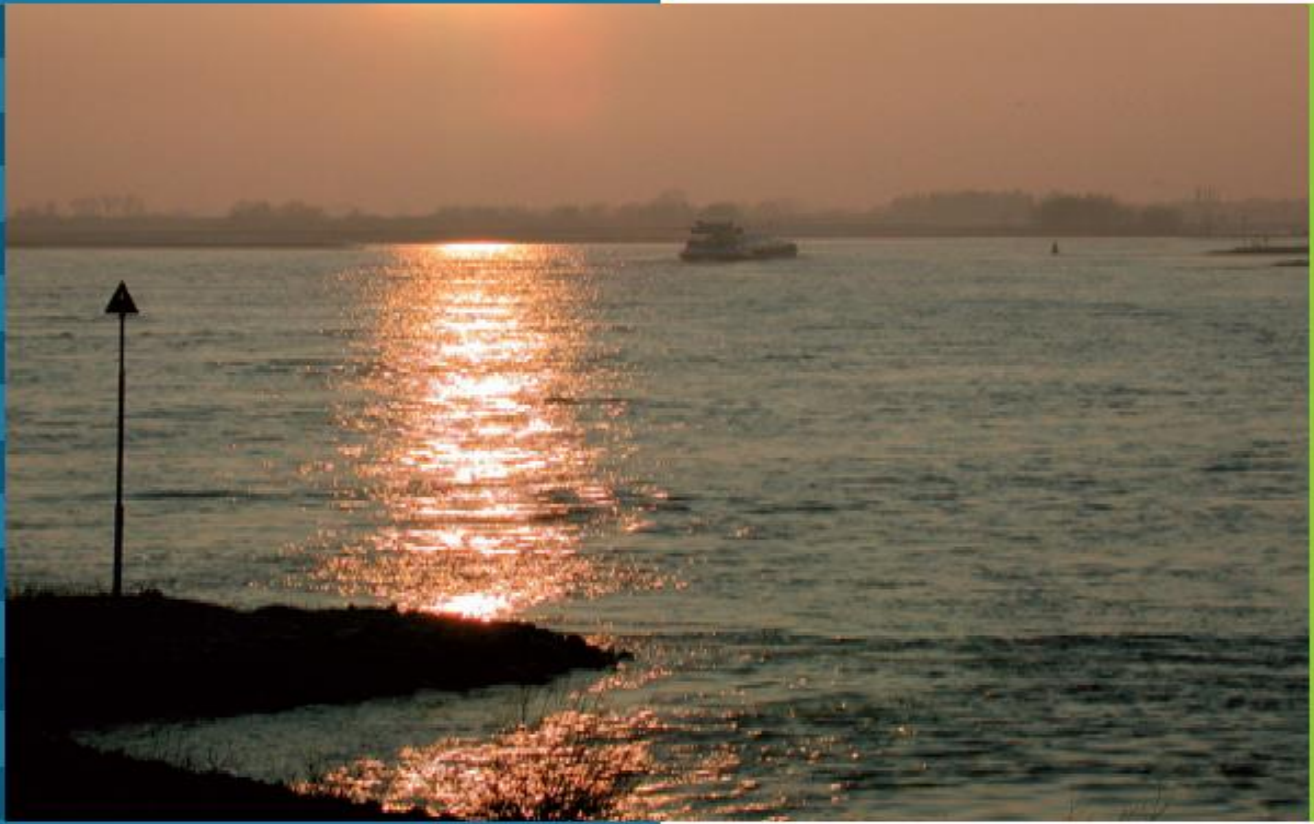


Water Hormone - Bioanalysis by CALUX



Temporal variation in multiple hormonal activities of surface waters located in the Dutch part of the Rhine basin

RIWA
Rhine Water Works
The Netherlands



Appendix 2. Raw data of the present study (location Lobith), as equivalents of the given reference compound.

CALUX	ERa	PR	GR	AR	TRB
LOBITH	E2 (ng/L)	Org2058 (ng/L)	Dex (ng/L)	DHT (ng/L)	T3 (ng/L)
1-aug-07	0.029	0.031	<LOD	<LOD	<LOD
14-aug-07	0.027	0.020	<LOD	<LOD	<LOD
29-aug-07	0.026	0.027	0.92	0.017	<LOD
12-sept-07	0.73	0.039	1.7	0.034	<LOD
26-sept-07	0.04	0.038	1.4	0.025	<LOD
10-oct-07	0.032	0.028	1.4	<LOD	<LOD
24-oct-07	0.041	0.032	1.6	<LOD	<LOD
7-nov-07	0.031	0.042	2.2	0.017	<LOD
21-nov-07	0.1	0.046	2.4	0.031	<LOD
5-dec-07	0.068	0.049	2.6	0.051	<LOD
19-dec-07	0.044	0.055	1.1	<0.05	<LOD
2-jan-08	0.042	0.078	2.2	<0.05	<LOD
16-jan-08	0.075	0.068	2	<0.05	<LOD

Available CALUX® assays for many "mode of actions"

Nuclear receptors			Signaling pathways			Controls		
name							atus	cell
DR CALUX							✓.	U2OS
PAH CALUX							✓.	all
ER CALUX							✓.	all
ERalpha CALUX							✓.	all
ERbeta CALUX								
ERalpha CALUX								
ERbeta CALUX								
AR CALUX								
PR CALUX								
GR CALUX								
TR CALUX								
RAR CALUX								
PPARγ1 CALUX								
PPARγ2 CALUX								
PPARα CALUX	✓.	U2OS	STAT CALUX	✓.	U2OS			
PPARδ CALUX	✓	[illegible]	[illegible]	✓	[illegible]			
LXR CALUX								
PXR CALUX								
VDR CALUX								
MR CALUX	✓.	U2OS						

- **Acute toxicity**
- **Oxidative stress/cell repair**
- **Dioxins/dl-PCBs/PAHs**
- **Endocrine effects/EDCs**
- **Obesogens (TBT, PFOA/PFOS)**
- **Reproductive effects**
- **Genotoxicity/carcinogenicity**
- **Metabolism**
- **etc**

CALUX: n=28

Agonist/antagonist: 25x2=56 assays

OECD: Validation reporter gene assays

OECD/OCDE 457

Adopted:
2 October 2012

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists

- **“me-too” validation**
- **intra-laboratory validation**
- **inter-laboratory validation**

Validation – intra-laboratory

- Detailed protocol
- Agonims: 22 test items; antagonism: 10 test items
- Reference compound, positive control, negative control
- Accuracy
- Sensitivity
- Specificity
- Predictivity

Validation – test items

Agonism

No.	Chemicals	CAS	Mw (g/mol)
1	Etyl paraben	120-47-8	166.17
2	Kaempferol	520-18-3	286.24
3	Butylbenzyl phthalate	85-68-7	312.36
4	p,p'-methoxychlor	72-43-5	346
5	19-Nortestosterone	434-22-0	274.4
6	Bisphenol A	80-05-7	228.29
7	Kepone	143-50-0	490.6
8	4-Cumylphenol	599-64-4	212.29
9	Genistein	446-72-0	270.24
10	Coumestrol	479-13-0	268.22
11	4-tert-Octylphenol	140-66-9	206.32
12	17a-Estradiol	57-91-0	272.38
13	Norethynodrel	68-23-5	298.42
14	Diethylstilbestrol	56-53-1	268.35
15	meso-Hexestrol	84-16-2	270.37
16	17a-Ethinyl estradiol	57-63-6	296.4
17	Atrazine	1912-24-9	215.68
18	Corticosterone	50-22-6	346.46
19	Linuron	330-55-2	249.09
20	Spironolactone	52-01-7	416.57
21	Ketoconazole	65277-42-1	531.43
22	Reserpine	50-55-5	608.68
reference compound	17b-estradiol	50-28-2	272.38
positive control	17a-methyltestosterone	58-18-4	302.45
negative control	Corticosterone	50-22-6	346.46

Antagonism

No.	Chemicals	CAS	Mw (g/mol)
1	Tamoxifen	10540-29-1	371.51
2	4-Hydroxytamoxifen	68047-06-3	387.51
3	Raloxifen HCl	82640-04-8	510.04
4	17a-Ethinylestradiol	57-63-6	296.4
5	apigenin	520-36-5	270.24
6	Chrysin	480-40-0	254.24
7	Coumesterol	479-13-0	268.22
8	Genistein	446-72-0	270.24
9	Kaempferol	520-18-3	286.24
10	Resveratrol	501-36-0	228.24
reference compound	Tamoxifen	10540-29-1	371.51
positive control	4-Hydroxytamoxifen	68047-06-3	387.51
negative control	Resveratrol	501-36-0	228.24

Validation – intra-laboratory

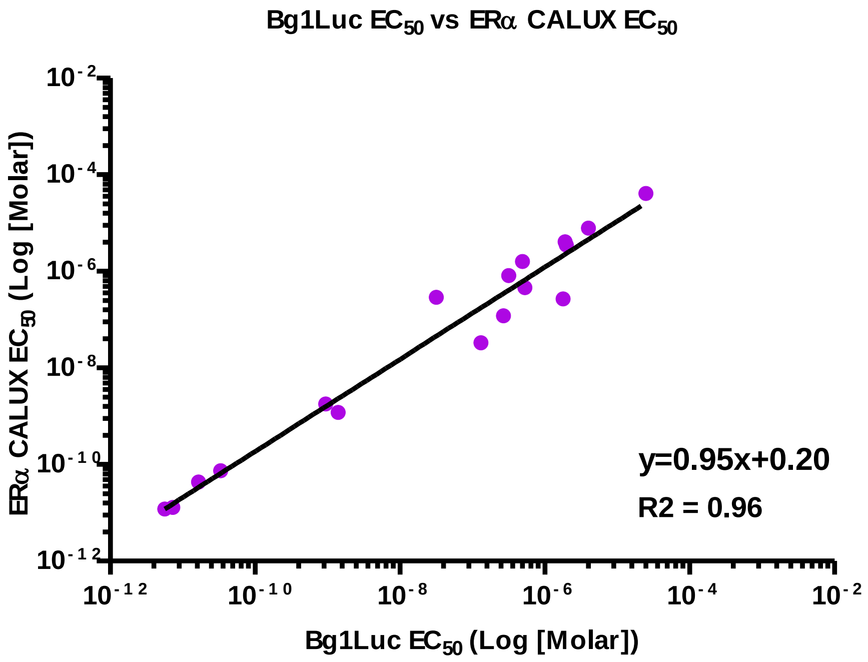
Test item	CAS no.	Erα CALUX Classification	ICCVAM Classification
17β-estradiol	50-28-2	Pos	Pos
Etyl paraben	120-47-8	Pos	Pos
Kaempferol	520-18-3	Pos	Pos
Butylbenzyl phtalate	85-68-7	Pos	Pos
p,p'-methoxychlor	72-43-5	Pos	Pos
19-Nortestosterone	434-22-0	Pos	---
Bisphenol A	80-05-7	Pos	Pos
Kepone	143-50-0	Pos	Pos
4-Cumylphenol	599-64-4	Pos	Pos
Genistein	446-72-0	Pos	Pos
Coumestrol	479-13-0	Pos	Pos
4-tert-Octylphenol	140-66-9	Pos	Pos
17a-Estradiol	57-91-0	Pos	Pos
Norethynodrel	68-23-5	Pos	Pos
Diethylstilbestrol	56-53-1	Pos	Pos
meso-Hexestrol	84-16-2	Pos	---
17a-Ethinyl estradiol	57-63-6	Pos	Pos
Atrazine	1912-24-9	Neg	Neg
Corticosterone	50-22-6	Neg	Neg
Linuron	330-55-2	Neg	Neg
Spironolactone	52-01-7	Neg	Neg
Ketoconazole	65277-42-1	Neg	Neg
Reserpine	50-55-5	Neg	Neg

		Era CALUX		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	15	0	15
	Negative	0	6	6
	Total	15	6	21

RESULTS - intra-laboratory validation

Overall accuracy	100%	21/21
Sensitivity	100%	15/15
Specificity	100%	6/6
False positive	0%	0/6
False negative	0%	0/15
Positive predictivity	100%	15/15
Negative predictivity	100%	6/6

Validation – intra-laboratory



Test items	ERa CALUX EC50 (M)	BG1Luc EC50 (M)
17β-estradiol	1.2E-11	5.63E-12
Etyl paraben	4.1E-05	2.48E-05
Kaempferol	7.9E-06	3.99E-06
Butylbenzyl phthalate	3.5E-06	1.98E-06
p,p'-methoxychlor	4.1E-06	1.92E-06
19-Nortestosterone	2.7E-07	1.80E-06
Bisphenol A	4.6E-07	5.33E-07
Kepone	1.6E-06	4.91E-07
4-Cumylphenol	8.1E-07	3.20E-07
Genistein	1.2E-07	2.71E-07
Coumestrol	3.3E-08	1.32E-07
4-tert-Octylphenol	2.9E-07	3.19E-08
17a-Estradiol	1.2E-09	1.40E-09
Norethynodrel	1.8E-09	9.39E-10
Diethylstilbestrol	7.4E-11	3.34E-11
meso-Hexestrol	4.3E-11	1.65E-11
17a-Ethinyl estradiol	1.3E-11	7.31E-12

WFD: ISO guideline in development

Document ISO/TC 147/SC 5/WG 9 N 76

NA 119-01-03 AA N 3177
NA 119-01-03-05-01 AK N 1180
NA 119-01-03-05-09 AK N 61

Mit der Bitte um Stellungnahme
bis 2014-04-04

NEW WORK ITEM PROPOSAL	
Closing date for voting 2014-04-11	Reference number (to be given by the Secretariat)
Date of circulation 2014-01-11	ISO/TC 147 / SC 5 N 826
Secretariat DIN	☐ Proposal for new PC

A proposal for a new work item within the scope of an existing committee shall be submitted to the secretariat of that committee with a copy to the Central Secretariat and, in the case of a subcommittee, a copy to the secretariat of the parent technical committee. Proposals not within the scope of an existing committee shall be submitted to the secretariat of the ISO Technical Management Board.

The proposer of a new work item may be a member body of ISO, the secretariat itself, another technical committee or subcommittee, or organization in liaison, the Technical Management Board or one of the advisory groups, or the Secretary-General.

The proposal will be circulated to the P-members of the technical committee or subcommittee for voting, and to the O-members for information.

IMPORTANT NOTE: Proposals without adequate justification risk rejection or referral to originator.
Guidelines for proposing and justifying a new work item are contained in Annex C of the ISO/IEC Directives, Part 1.

☒ The proposer has considered the guidance given in the Annex C during the preparation of the NWIP.

Proposal (to be completed by the proposer)

Title of the proposed deliverable.
(in the case of an amendment, revision or a new part of an existing document, show the reference number and current title)

English title: ISO/NP 19040-3 "Water quality - Determination of the estrogenic potential of water and waste water - Part 3: In vitro human cell-based reporter gene assay"

French title (if available)

Scope of the proposed deliverable.

This International Standard specifies a method for the determination of the estrogenic activity of water and waste water by means of a reporter gene assay utilizing stably transfected human cells.
This method is applicable to:

- fresh water;
- waste water;
- aqueous extracts and leachates;
- eluates of sediments (fresh water);
- pore water;
- aqueous solutions of single substances or of chemical mixtures;
- drinking water.

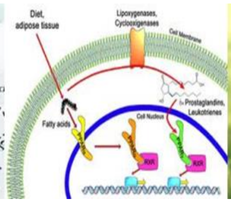
Purpose and justification of the proposal.

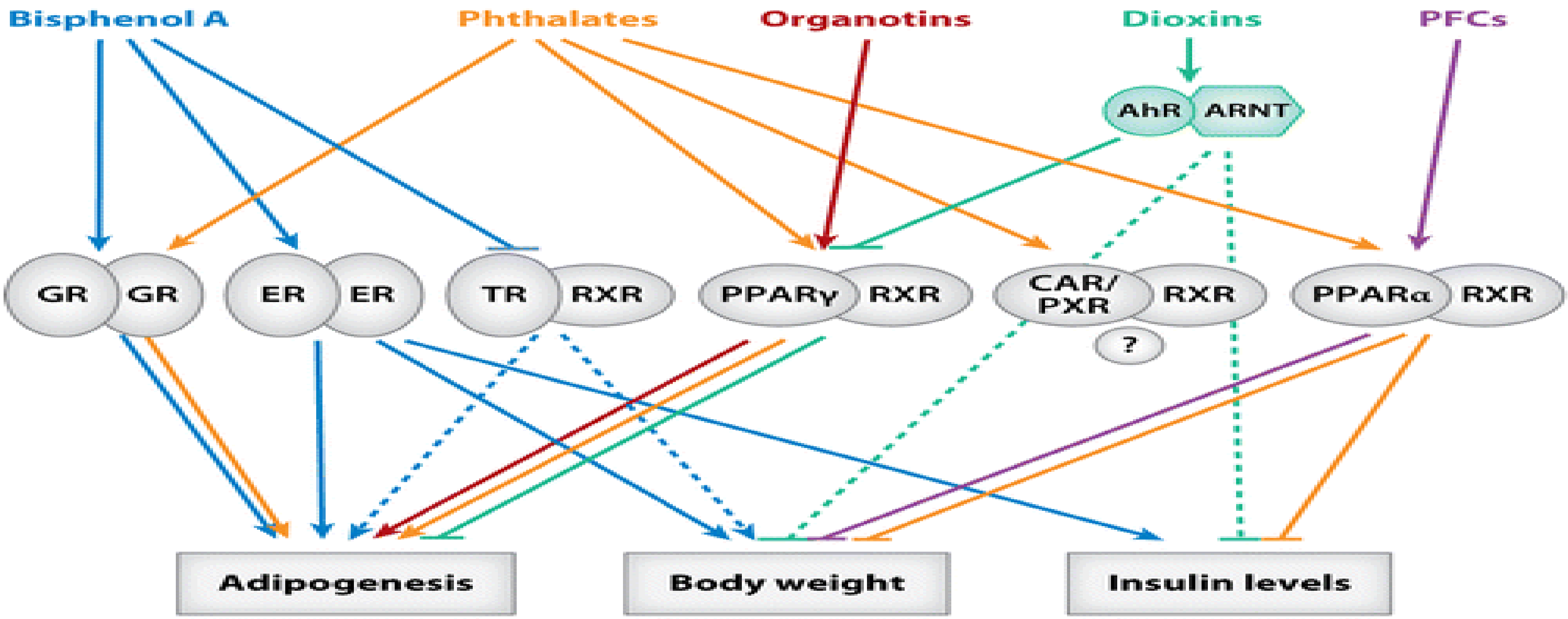
The estrogenic activity of water and waste water is an relevant endpoint for the quality assessment of (waste)water.

The relevance of estrogenic compounds is reflected in the EU-WFD Annex X and the "watch list" as agreed upon by the EU-Commission in August 2013 [2013/39/EU] with obligation to monitor the estrogen receptor agonists EE2 and E2.

The here described protocol based on human cells is a robust and highly sensitive bioanalytical method for the determination of estrogenic activity in (waste) water.

Why a panel of in vitro CALUX tests?
Link from important chemicals to important health risks



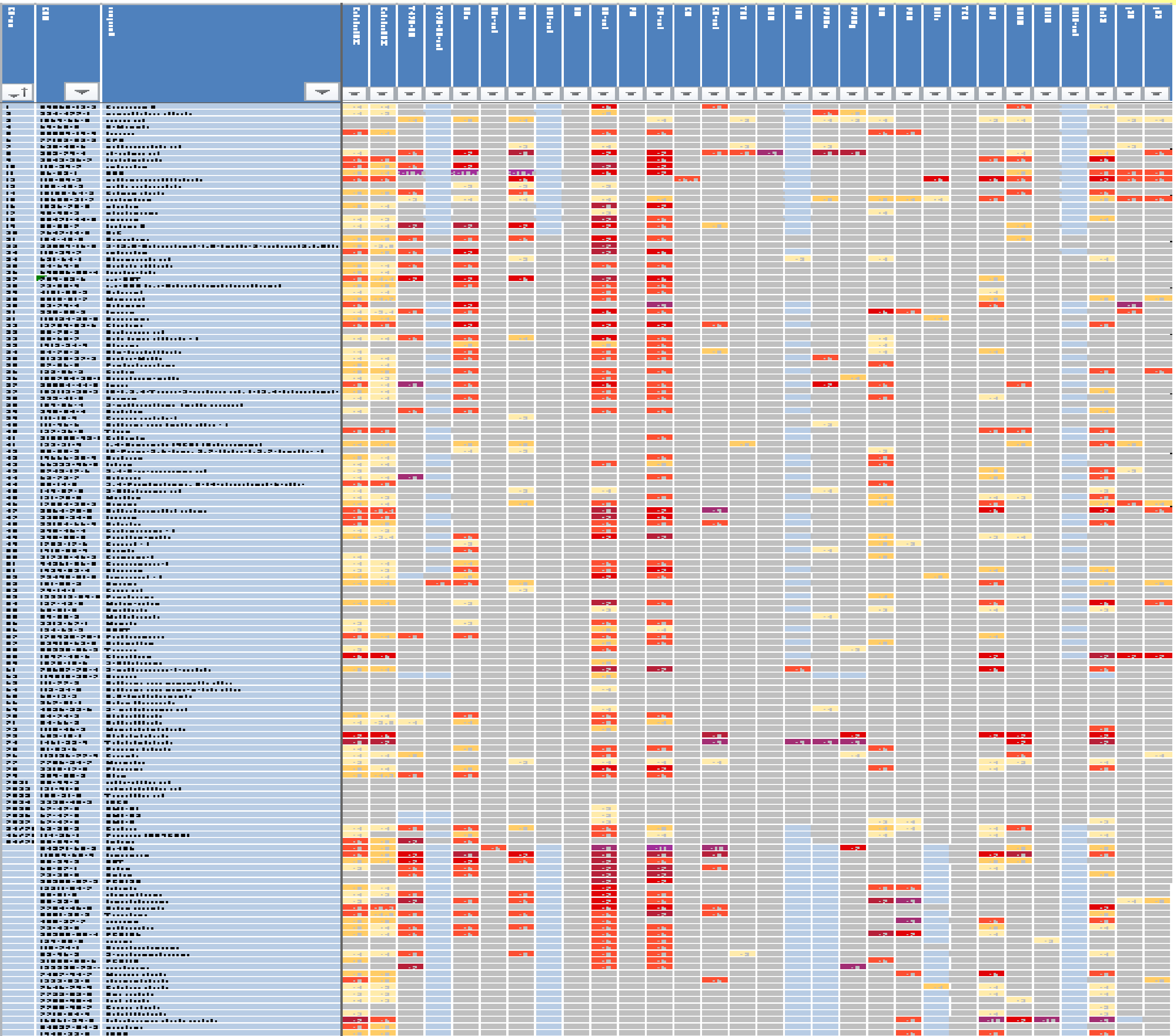


Casals-Calas C, Desvergne B. 2011 Annu. Rev. Physiol.73:135-62

Top 10 chemicals: ER CALUX “umbrella”

Compound	Rel Potency EC50
17β-Estradiol	1,0
17α-EE2	1,1 – 1,9
Estrone (E1)	0,1-0,4
Estriol (E3)	0,04- 0,01
Bisphenol A	$2,5 \times 10^{-5}$
Nonylphenol	$4,6 \times 10^{-5}$
4-t-Octylphenol	$1,4 \times 10^{-6}$
Benzyl butyl phthalate	0,0000014
Nonylphenol ethoxylates	0,0000038
Dimethyl-phthalate	0,000011

FP7 ChemScreen Project (2011-2015):
In vitro CALUX (n=30) profiling of ca. 250 chemicals



ChemScreen

www.chemscreen.eu

CALUX results of prioritised compounds

no activity
EC10 = 1E-3M
EC10 = 1E-7M

	compound	Cytotox10%	Cytotox50%	ERa	ERa-anti	ERb	ERb-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	RAR	LXR	PPARa	PPARg	DR	PAH	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53
Dirty Dozen POPs	Chlordane	-5.5	-5	-6.9					-6.5		-6.5		-6													-5		
	DDT	-4.5	-4.2	-6.5		-5.8			-7		-6		-5.5				-5						-4.7	-4.5				
	Dieldrin	-3		-5.8					-7		-7		-5				-5.5											
	Endrin			-5.5					-7		-7																	
	Heptachlor	-5	-4.5	-7.2					-7		-6																	
	Hexachlorobenzene			-6.5					-6		-6								-5									
	Mirex																		-4.2									
	Toxaphene	-5	-4.8	-5.5		-5.5			-6.5		-6.5		-5.5													-6.1		
	PCB118	-4.5							-7		-6.5								-7.1	-6.5			-4.1					
	PCB126	-4.8	-4.4						-6.5		-6						-5		<-10.5	-10.2				-4.5				
	PCB128								-7		-6.5																	
	PCB156	-4.5	-4	-6					-6		-6								-7	-6.8			-4					
	TCDD																		-11.5	-12								
	Furan																			-3.4						-3		
Additional POPs	dibenzo[a,h]anthracene	-4				-7.5												-5	-8	-10								
	dibenzo[a,h]pyrene			-7															-8.9	-9.1			-6.2	-5.5		-6	-6	
	benzo[a]pyrene	-3		-6		-3.9			-6.5		-6								-8.1	-9.7				-4			4.6	-4.5
	tributyltinacetate	-7	-6.5			-7												-8.					-6.5	-7		-6.5		
	methylmercury(II)chloride	-5.8	-5.6			-6.4						-6.0									-6.3		-6.3	-6		-6.6	5.6	-5.7
Heavy metals	Lead chloride	-3.5	-3																					-3.2				
	Mercuric chloride	-4.9	-4.8																				-5.3			-5.5		
	Cadmium chloride	-4.9	-4.7																				-5			-5.3		
	Cobaltous chloride	-3.9	-3.4																-4.2		-4.2		-3.8			-4.5		
	Copper chloride																									-3		
	copper sulfate	-3.4	-3.2																-3.5		-4.5					-5		
	Zinc sulphate	-4.3	-4.1																				-4.1					
	Sodium arsenite	-5.4	-5.2						-6.3		-6.1		-6													-6.5		-4.9
	Nickel(II)chloride	-3.5	-3																		-3.4		-3.1			-4.2		
	chromium(vi)oxide	-5	-4.7																									-4.7

Dirty Dozen POPs: endrocine activity, dioxin receptor (dioxins/PAHs)

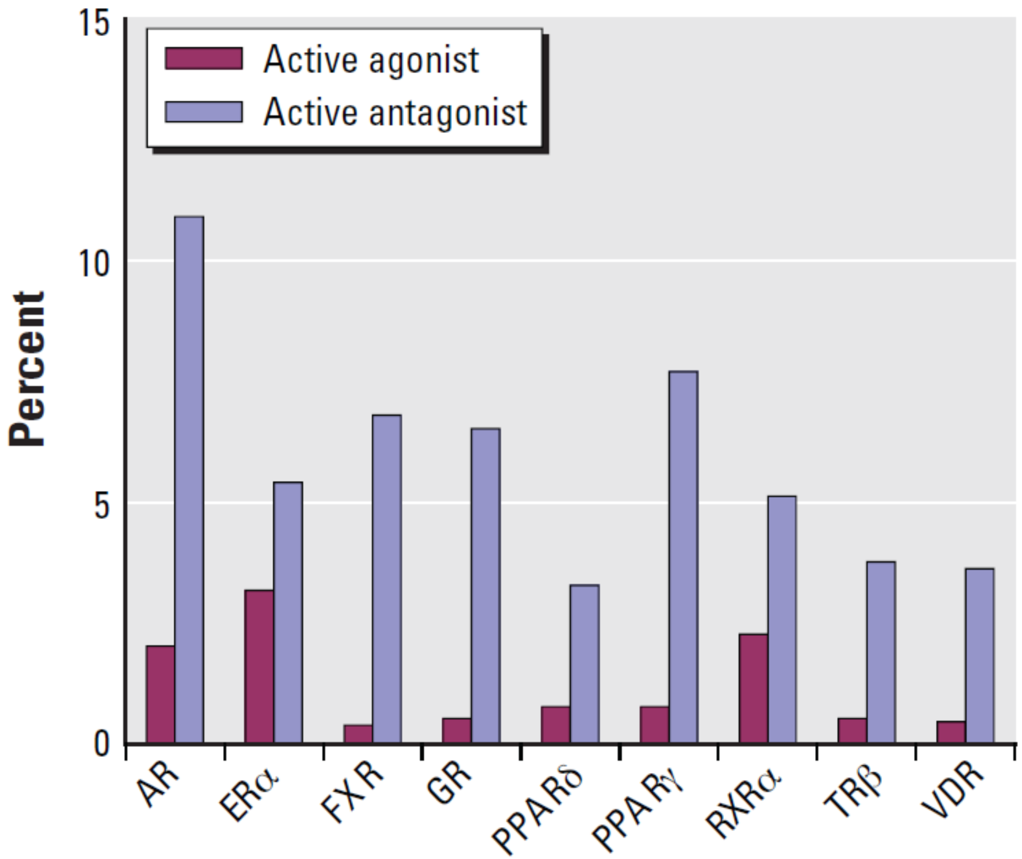
Additional POPs: dioxin receptor (PAHs), stress pathways

Heavy metals: acute toxicity, stress pathways

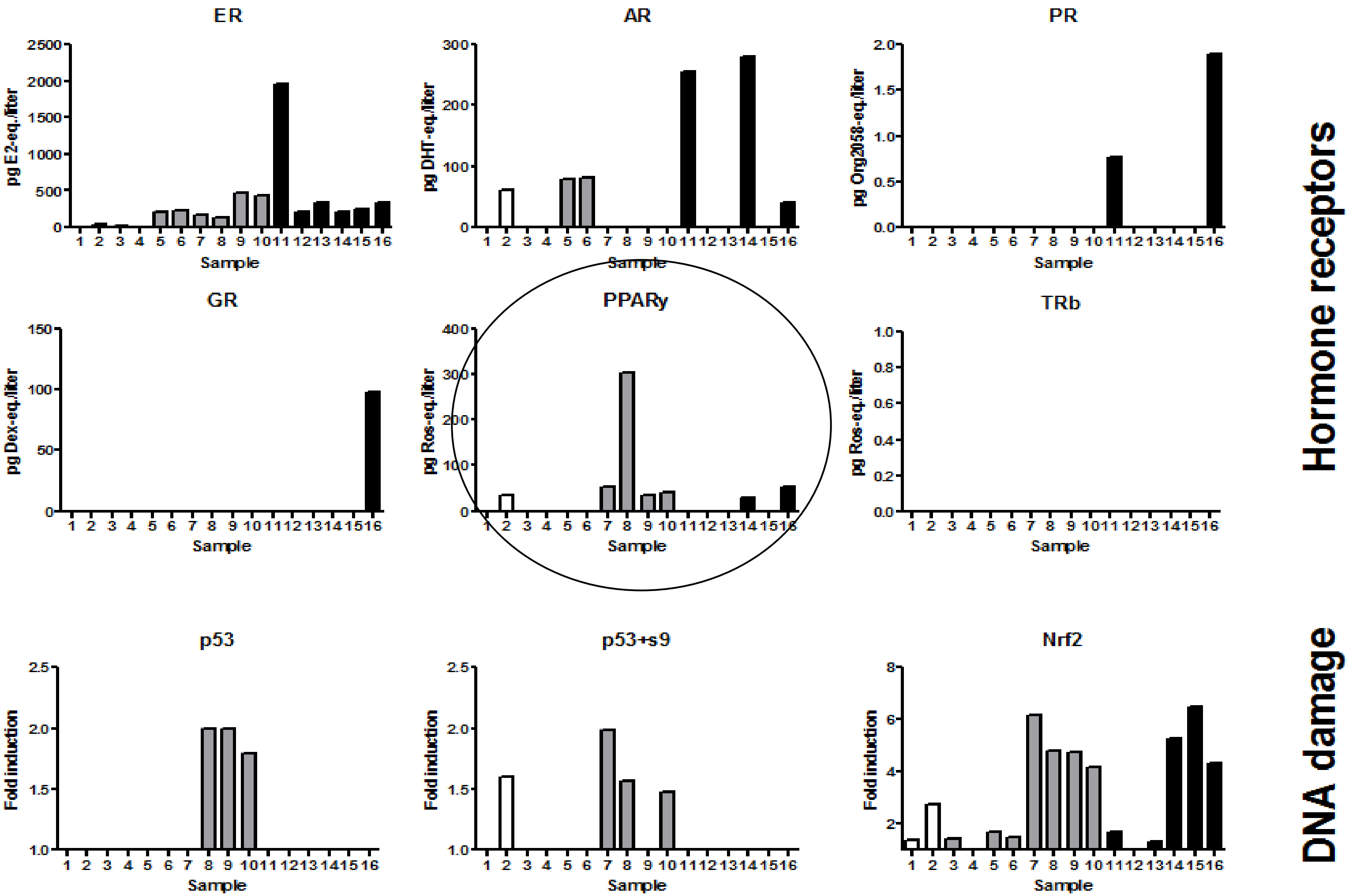
ToxCast project: testing of ± 2500 pesticides

Chemical Genomics Profiling of Environmental Chemical Modulation of Human Nuclear Receptors

Ruili Huang,[1] Menghang Xia,[1] Ming-Hsuang Cho,[1] Srilatha Sakamuru,[1] Paul Shinn,[1] Keith A. Houck,[2] David J. Dix,[2] Richard S. Judson,[2] Kristine L. Witt,[3] Robert J. Kavlock,[2] Raymond R. Tice,[3] and Christopher P. Austin[1]



Which types of MODE OF ACTIONS are detected?
PPAR, Nrf2 and p53 +/- S9 significant like other EDCs!



White bars– drinking water

Grey bar- surface water

Black bar – waste water

How to test EDCs in water?
JRC, Ispra, Italy (2013 report)

J R C T E C H N I C A L R E P O R T S

Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD)

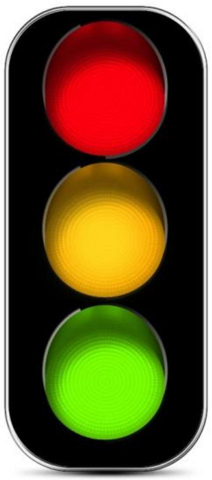
Compound	EQS	LOQ LC/MS	LOQ YES Yeast cells	LOQ CALUX Human cells
17-alpha-Ethinylestradiol	0,035 ng/l	1-2 ng/l	0,2 ng/l	0,03 ng/l
17-beta-Estradiol	0,4 ng/l	1-2 ng/l	0,2 ng/l	0,03 ng/l
Umbrella: BPA, NP, phthalates				

Summary: Using effect-based tools will reduce the high costs of the few currently available analytical "high end" methods for the measurement of E2 and EE2 and provide reliable information on the endocrine disrupting potential of water samples.

CALUX based trigger values for drinking water

CALUX > trigger value → more detailed examination warrented

CALUX < trigger value → health risks can be waived



Contents lists available at SciVerse ScienceDirect

Environment International

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

Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays

Walter Brand [a,*,1], Cindy M. de Jongh [a,1], Sander C. van der Linden [b], Wim Mennes [c], Leo M. Puijker [a], Cornelis J. van Leeuwen [a], Annemarie P. van Wezel [a], Merijn Schriks [a,**], Minne B. Heringa [a,2]

Bioassay	Trigger value
ERα CALUX	3.8 ng E2-eq./L
AR CALUX	11 ng DHT-eq./L
GR CALUX	21 ng Dex-eq./L
PR CALUX	333 ng Org2058-eq./L
…. CALUX	….. eq./L

Status box

Water Directors meeting (Vilnius 4 December 2013)

Agenda point: 1.c (Batch endorsement of documents)

Document: WD/2013-2/4 (see also separate annex)

Title: **TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS**

Version no: 7 Date: 20 Nov 2013

Activity Leaders:
Ann Sofie Wernersson (Swedish Agency for Marine and Water Management, Sweden) - Chair
Mario Carere (ISS-Italian Institute of Health, Italy)
Chiara Maggi (ISPRA, Italy)

DR CALUX

The Dioxin Responsive (DR) CALUX® comprises rat hepatoma cell lines (H4IIE), incorporating the firefly luciferase gene coupled to Dioxin Responsive Elements (DREs) as a reporter gene for the presence of dioxins (PCDDs) and dioxin-like compounds (e.g. furans (PCDFs) and dioxin-like PCBs (dlPCBs)). Following binding of dioxins and/or dioxin-like compounds to the cytosolic Arylhydrocarbon receptor (AhR), the ligand-receptor complex binds the DRE. Cells that are exposed to dioxins or dioxin-like compounds not only express proteins that are under normal circumstances associated to DRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds (2,3,7,8-TCDD). DR CALUX bioassays report total 2,3,7,8-TCDD TEQs for environmental matrices and total BEQs for food/feed matrices.

- **What is analysed (endpoint; unit):** ng 2,3,7,8-TCDD equivalents/kg sample processed
- **Test duration:** 24h
- **Method used**: Marine Quality Assurance procedures available in the future through between particular independent laboratories (Davies & Vethaak 2012)
- **Positive control used**: 2,3,7,8-TCDD
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample, but the substances that the assay responds to are in the aquatic environment primarily found accumulated in e.g. sediments and biota (tissues).
- **Cells examined:** Rat liver cell line
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ) (see below).
- **What /type of/ substances does the assay respond to:** Ah receptor active compounds, e.g. Polyhalogenated dioxins/furans, dioxin like PCBs, and if using other pretreatment of samples also PAHs (see PAH CALUX).
- **Sensitivity (LOD/Q):** . The bioassays' LOQ is 1 pg 2,3,7,8-TCDD equivalents per amount of material processed. For example, if 5 grams of dried soil/sediment or 1 liter of water is processed, an LOQ of 0.2 ng 2,3,7,8-TCDD equivalents per gram of soil/sediment or 1 ng 2,3,7,8-TCDD equivalents per liter of water is obtained respectively.
- **Variability (e.g. CV for single substance tests) if known:** <20%
- **Influence by cytotoxicity/risk of false positives/negatives:** As the sample is cleaned up by a sulphuric acid treatment and afterwards with an additional step to separate dl-PCBs from PCDD/Fs, cytotoxicity is rarely occurring. In case of false positive/false negative guided levels has to be established to compare it with. In case of the EC project HORIZONTAL no false positive or false negative samples occurred. For such methods usually a false positive and negative ratio of 5% is reasonable.
- **Complexity/learning period:** 2 weeks of training
- **Costs:** Low[56], especially compared to chemical analysis of dioxins and dioxin-like compounds. Generally not depending on matrix studied.
- **Commercial availability:** Commercial ISO 17025 accredited performers are available

ERα CALUX (agonistic/antagonistic)

The ERα Responsive (ERα) CALUX® comprises a human bone marrow cell line (U2OS), incorporating the firefly luciferase gene coupled to Estrogen Responsive Elements (EREs) as a reporter gene for the presence of estrogens and/or estrogen-like compounds. Following binding of estrogens or estrogen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds the ERE. Cells that are exposed to estrogens and/or estrogen-like compounds not only express proteins that are under normal circumstances associated to ERE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds 17β-estradiol. ERα CALUX bioassays report total 17β-estradiol equivalents for environmental matrices.

- **What is analysed (endpoint; unit):** pg 17β-estradiol equivalents/g sample processed
- **Test duration:** 24h
- **Method used**: Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; Australian Water Commission; Ongoing evaluations at the ISO-TC 147 standardisation group led by BFG-Germany; EPA California; China National Water Quality Monitoring in Jinan.
- **Positive control used**: 17β-estradiol (E-2)
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Cells examined:** Human bone marrow cell line
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ) (see below).
- **What /type of/ substances does the assay respond to** Binding to the Estrogen receptor (alpha and beta for original ER CALUX and only alpha for ERalpha CALUX)
- **Sensitivity (LOD/Q):** The bioassays' LOQ is 35 pg 17β-estradiol equivalents per amount of material processed. For example, if 5 grams of dried soil/sediment or 1 liter of water is processed an LOQ of 7 pg 17β-estradiol equivalents per gram of soil/sediment or 35 pg 17β-estradiol equivalents per liter of water is obtained respectively. Original ER CALUX: 0.1 ng EEQ/l water (see e.g. Leusch, 2008).
- **Variability (e.g. CV for single substance tests) if known:** <20%
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as type of water matrix.
- **Complexity/learning period:** 1 week of training
- **Costs:** Low[58]. Costs are generally not depending on matrix studied.
- **Commercial availability:** Commercial ISO 17025 accredited performers available
- **WFD relevance:** This bioassay analysis is more sensitive than most chemical analyses (lowest LOD reported by Loos 2012 is e.g. 0.1 ng/l for a chemical analysis of EE-2 and E-2, if using USEPA method 1698; in practice the LOQ that is possible to reach by regular laboratories is generally higher). The assay could therefore be very valuable on a screening level to identify water bodies at risk due to the combined exposure to a large number of estrogenic substances that could constitute RBSPs (see case studies "Laxsjön – investigating sediment contamination, using chemical and in vitro bioassay approach") and to lower the frequency of analytical high end monitoring in water bodies for E2. EE2 and E2 are also suggested to be included in 2008/105/EC. Because EE2 is significantly (about 10-25 times) more potent *in vivo* than E2, but only 3 times more potent in ER CALUX, this should be taken into account if evaluating data in an absolute manner (comparison with EQS), when considering the need for additional studies. In vivo studies of oestrogenic response, or using precautionary EE2 equivalents can be considered, if the presence of EE2 is likely, e.g. via high ratio of municipial waste water. The EU-EQS proposal for E2 is based on a SSD approach of the most sensitive aquatic organisms, and concludes that an

FP7 Project DEMEAU: How to move forward with human cell-based bioassays in regulatory and global usage

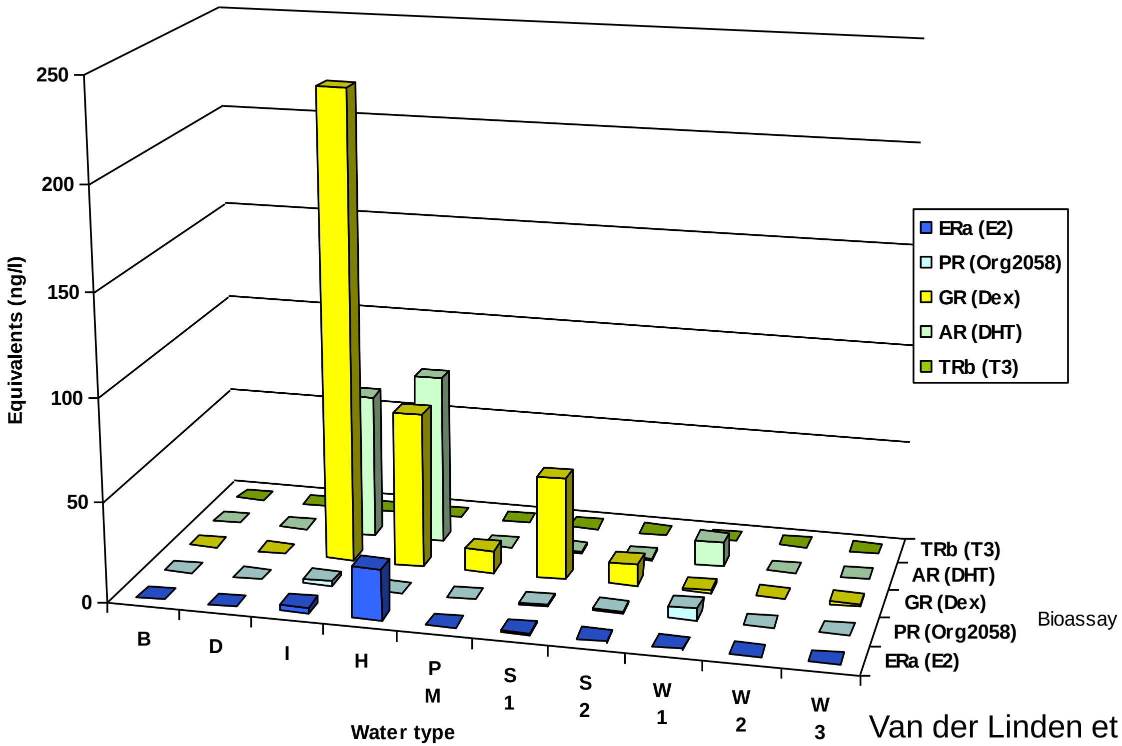
SEVENTH FRAMEWORK PROGRAMME

Demonstration of promising technologies to address emerging pollutants in water and waste water

KWR Watercycle Research Institute
KOMPETENZZENTRUM WasserBerlin
eawag aquatic research
IWW
n|w Fachhochschule Nordwestschweiz
VEOLIA ENVIRONNEMENT Recherche & Innovation
Van Remmen UV Techniek
CORDOUAN Technologies
Rwb waterservices
CETaqua
eco logic
BDS BioDetection Systems
aquatune
AMPHOS 21
SIGRIST PROCESS-PHOTOMETER
Quantis Sustainability counts
HYDOR

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

High levels of glucocorticoids found in hospital waste water by GR CALUX®

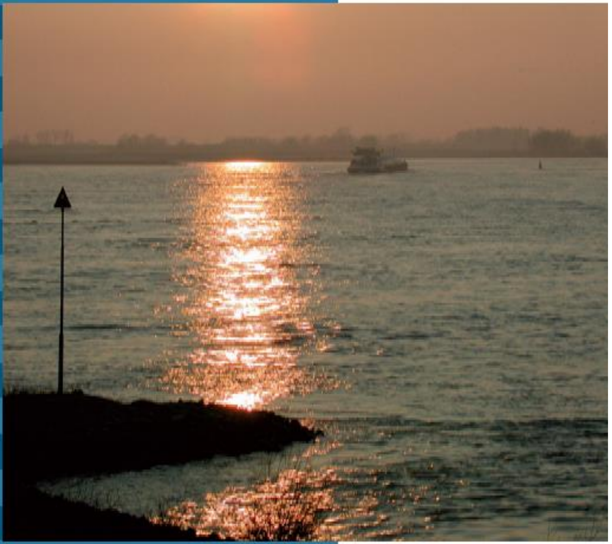


Van der Linden et al. Env. Sci. Techn. 2008, 42, 5814–5820

- Several hormonal activities found in waste and surface water
- Glucocorticoids are new problems, especially in hospital waste water?
- Profile shows hotspots of compound classes to focus further on

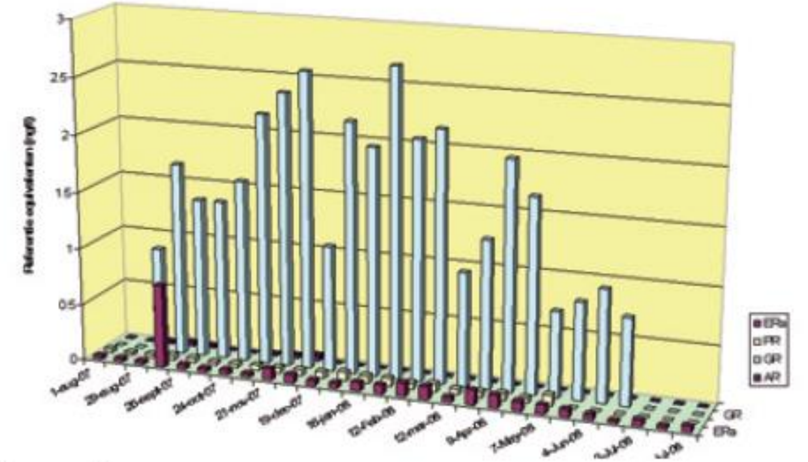
Rhine Monitoring of endocrine effects (ER, AR, TR, GR) in time

Temporal variation in multiple hormonal activities of surface waters located in the Dutch part of the Rhine basin

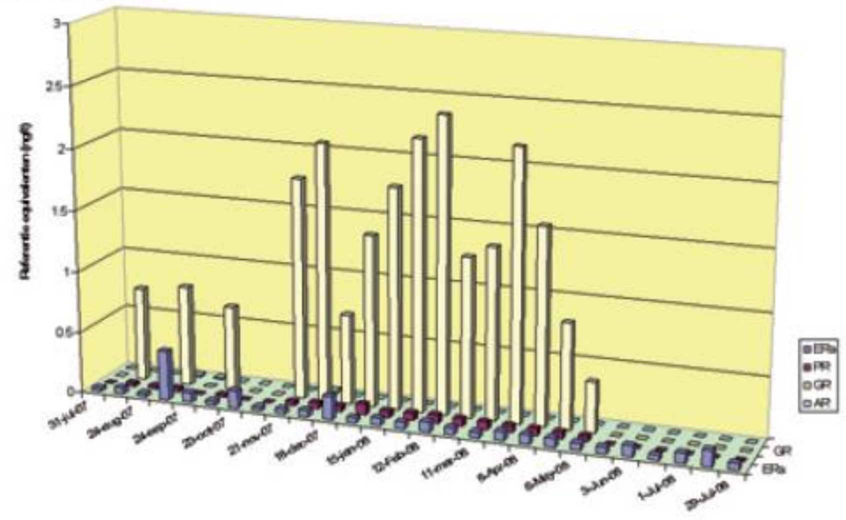


RIWA
Rhine Water Works
The Netherlands

A Lobith



B Nieuwegein



Identification of new class of pollutants: glucocorticoids (dexamethason-like)

Environ. Sci. Technol. 2010, 44, 4766–4774

High-Resolution Mass Spectrometric Identification and Quantification of Glucocorticoid Compounds in Various Wastewaters in The Netherlands

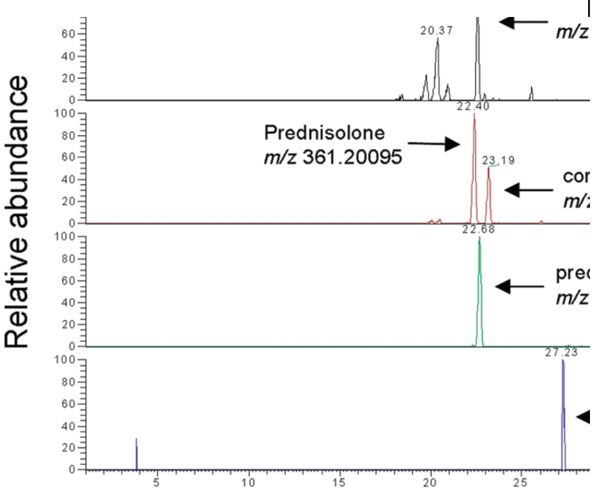
MERIJN SCHRIKS,*,† JAN A. VAN LEERDAM,† SANDER C. VAN DER LINDEN,‡ BART VAN DER BURG,‡ ANNEMARIE P. VAN WEZEL,† AND PIM DE VOOGT†,§

KWR Watercycle Research Institute, Nieuwegein, The Netherlands, BioDetection Systems B.V., Amsterdam, The Netherlands, and Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

glucocorticogenic activity can be explained to a fairly large extent by their contribution.

Introduction

The presence of xenobiotic compounds in our environment has become a topic of worldwide concern, especially since some of them may disrupt hormone-dependent (physiological) processes, such as vertebrate fetal development (*1*). Much attention has been directed to anthropogenic compounds that target the estrogen receptor such as 4-nonylphenol, bisphenol A, phthalate plasticizers (*2, 3*), and the natural and synthetic hormones 17α-(ethynyl) estradiol, 17β-estradiol, estriol, and estrone (*4, 5*). In the last decades it has become clear that these compounds may enter the aquatic



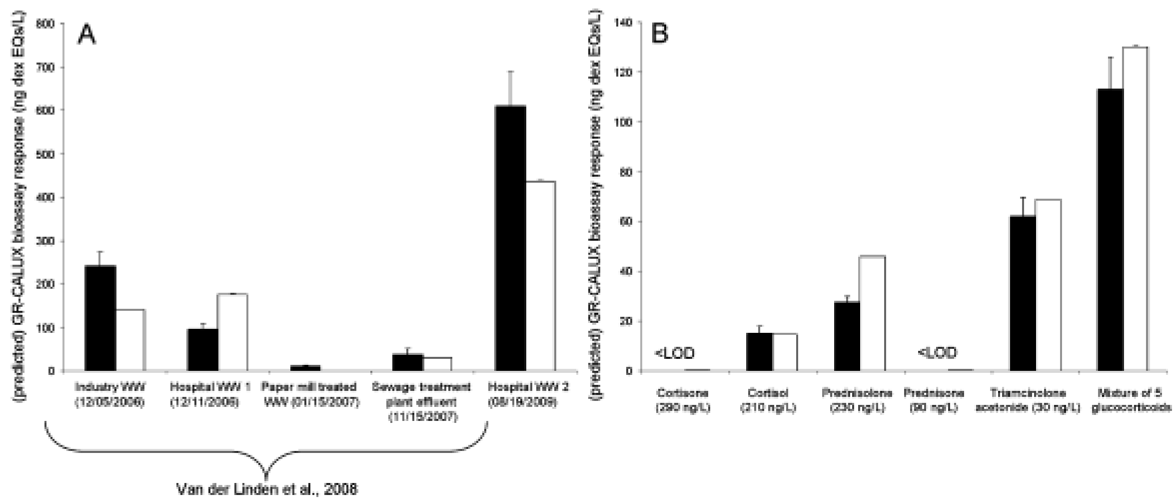
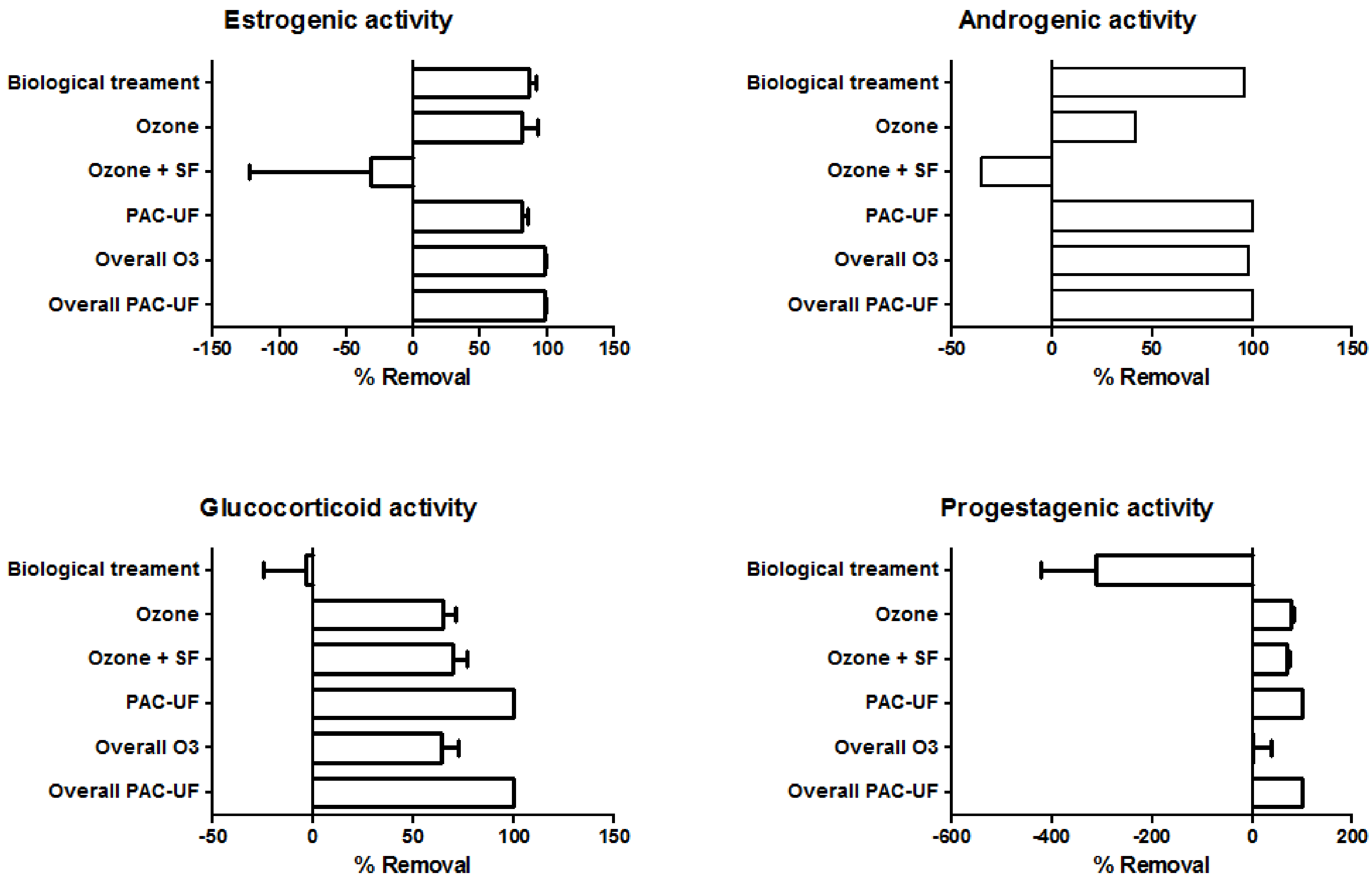


FIGURE 5. Measured GR CALUX bioassay response (black bars) versus "predicted GR CALUX bioassay response" (white bars, corrected for individual compound recoveries in wastewater) elicited by (A) the various wastewater (WW) extracts and (B) by extracts of five individual glucocorticoid standards and a mixture (at the same concentration levels as the individual glucocorticoid standards). GR CALUX bioassay responses of the extracts were interpolated into a dexamethasone standard curve (0.03–100 nM) and response magnitude is presented as nanogram of dexamethasone equivalents/L (ng dex EQs/L).

Comparison GR CALUX-EQs vs. chemical analysis for different water samples (Schriks et al., EST 2010)

Water sample	GR-CALUX (ng dex EQs/L) *(vd Linden et al., 2008)*	Detected glucocorticoids (LC-MS/MS)	Conc. LC-MS/MS [ng/L]	REP
Industry wastewater	243	**•Prednisolone** **•Dexamethasone** **•Cortisone** **•Cortisol** ***•Fluocortin/fluprednidene*** ***•Hydrocortisone aceponate***	**180** **80** **20** **10** ***Not confirmed*** ***Not confirmed***	**41** **80** **0.07** **0.7** **---** **---** *∑ 122*
Hospital wastewater	96	**•Cortisone** **•Prednisolone** **•Cortisol** **•Prednisone** **•Triamcinoloneacetonide** ***•Fluocortin/fluprednidene*** ***•Hydrocortisone aceponate***	**290** **230** **210** **90** **30** ***Not confirmed*** ***Not confirmed***	**0.2** **52.7** **15.5** **0.2** **67.8** **---** **---** *∑ 136*
Paper mill treated WW	11	No compounds detected	**---**	**---**
STP effluent	38	**•Triamcinoloneacetonide** ***•Hydrocortisone aceponate***	**10** ***Not confirmed***	**23** **---** *∑ 23*

Removal efficiency by water treatment steps

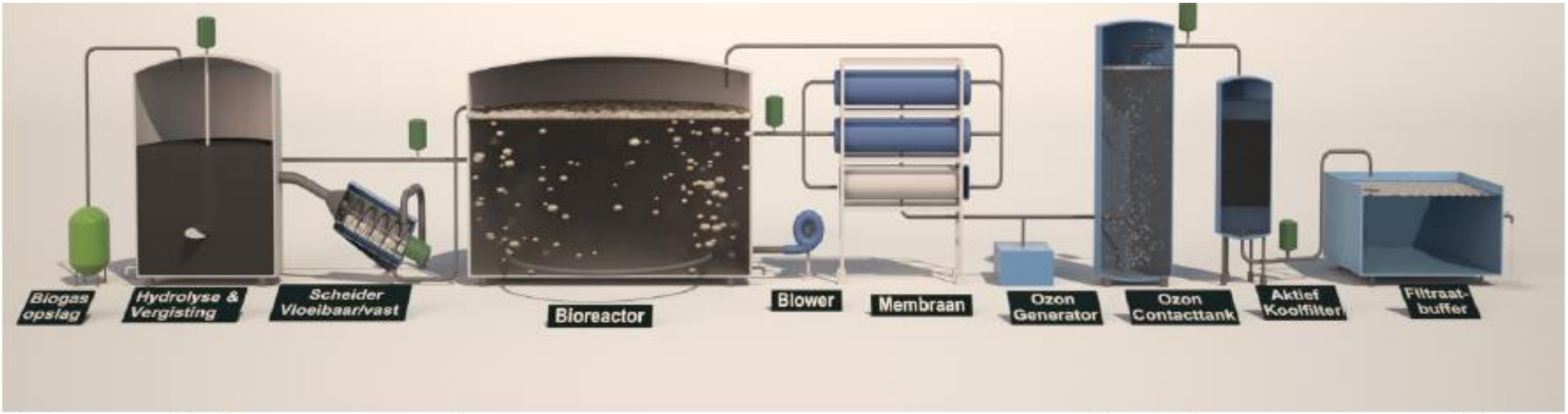


Dutch Project Pharmafilter: 99,99% pharmaceuticals removed by hospital WWTPs analyzed by CALUX panel and chemical analysis

- IWA Newsletter No. 33, Sept 2011

<u>Hospital waste water treatment: test and full scale WWTPs:</u>

- Water treatment by membrane bioreactor, ozonization and activated carbon
- Medicines were removed below LOD
- Removal rate analyzed by ER-, AR- and GR-CALUX was 99,99%



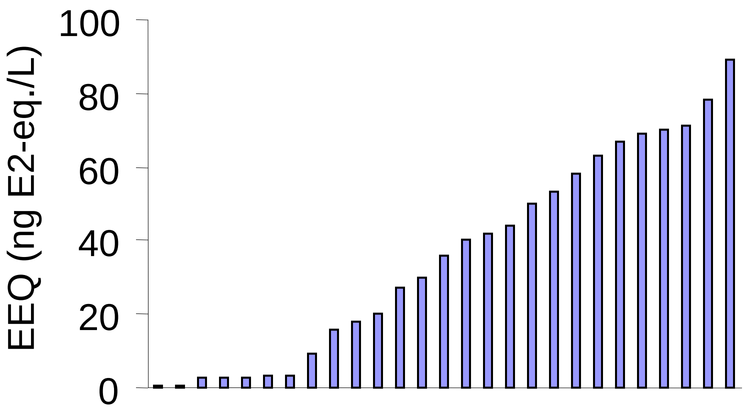
Scheme of the waste- and waste water system: gas system, digestion, membrane bioreactor, ozonation and GAC-filtration

Estrogenic activity during drinking water treatment

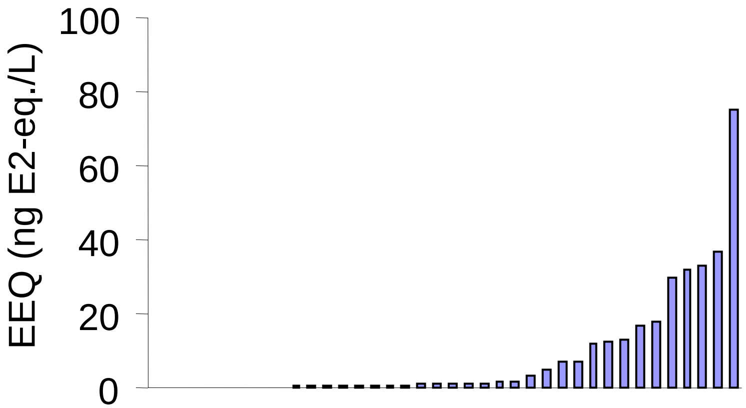
Estrogenic activity	ng/l EEQ
• Surface water river Meuse:	0,762
• Intake Brakel:	0,328
• Rapid sand filtration	0,092
• Dune infiltration	0,068
• Activated carbon filtration	< 0,020
• Slow sand filtration	< 0,020
• Tap water	< 0,020
• Blanc water: Evian	< 0,020

Modern WWTPs (e.g. PAC, O_3) can clean up efficient more than 99% of EDCs

Influent by ER CALUX



Effluent by ER CALUX



Emission of pharmaceuticals from Dutch care institutions into wastewater: chemical en effect monitoring

Barry Pieters[1], Lideke Vergouwen and Stefan Kools

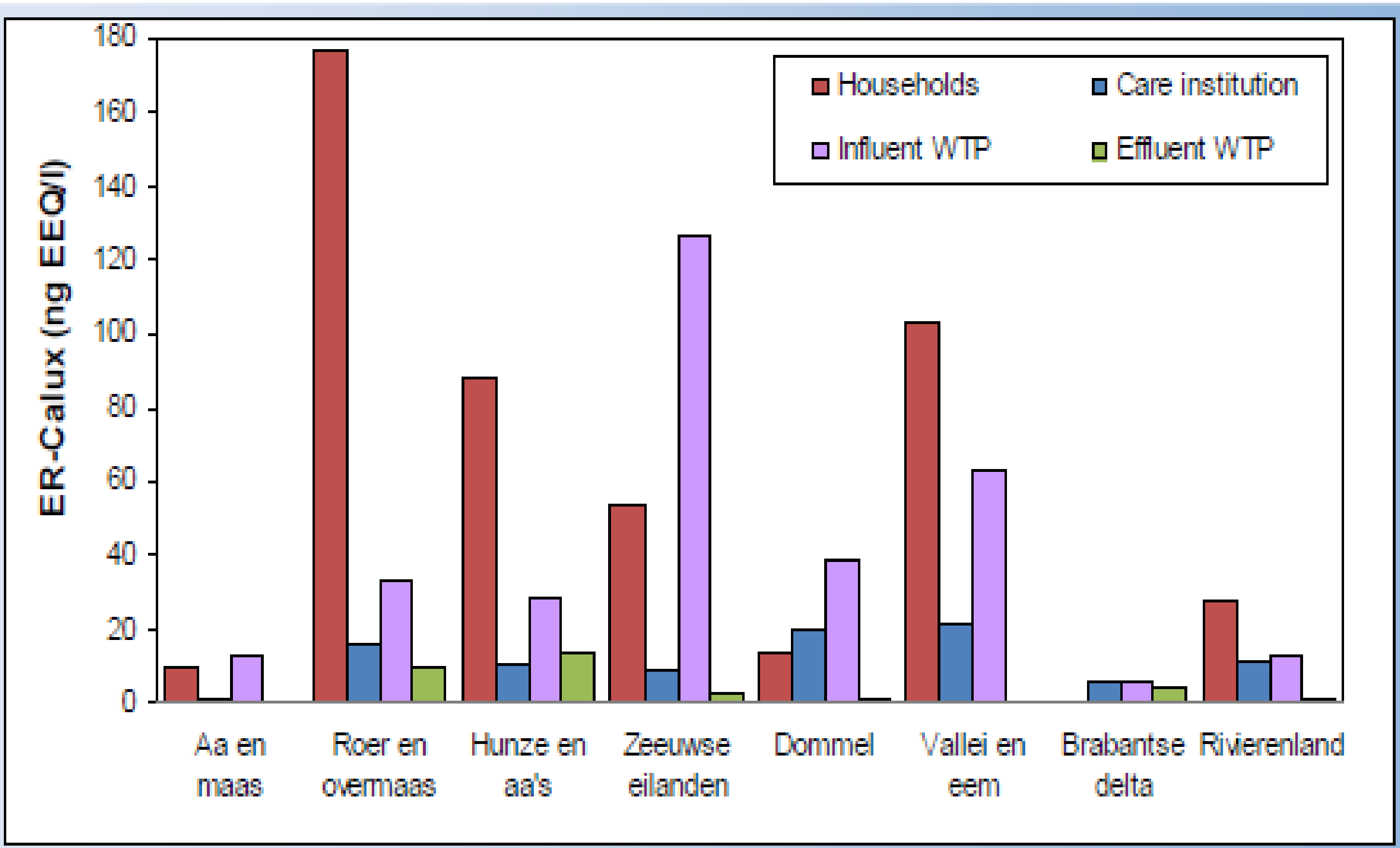
[1]Grontmij, Amsterdam, The Netherlands, barry.pieters@grontmij.nl

Grontmij

stowa

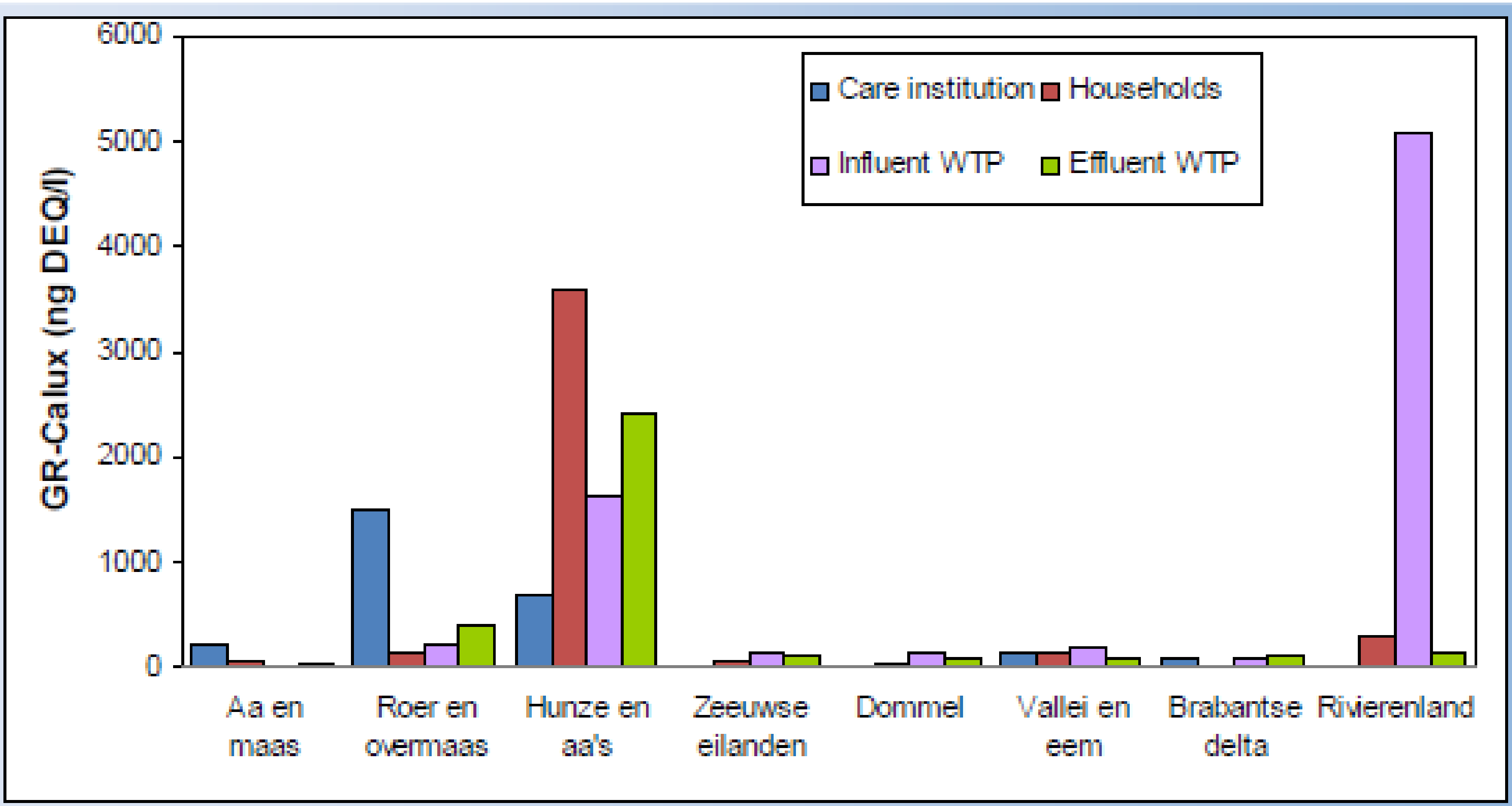
planning connecting respecting the future

ER-Calux: estrogen activity



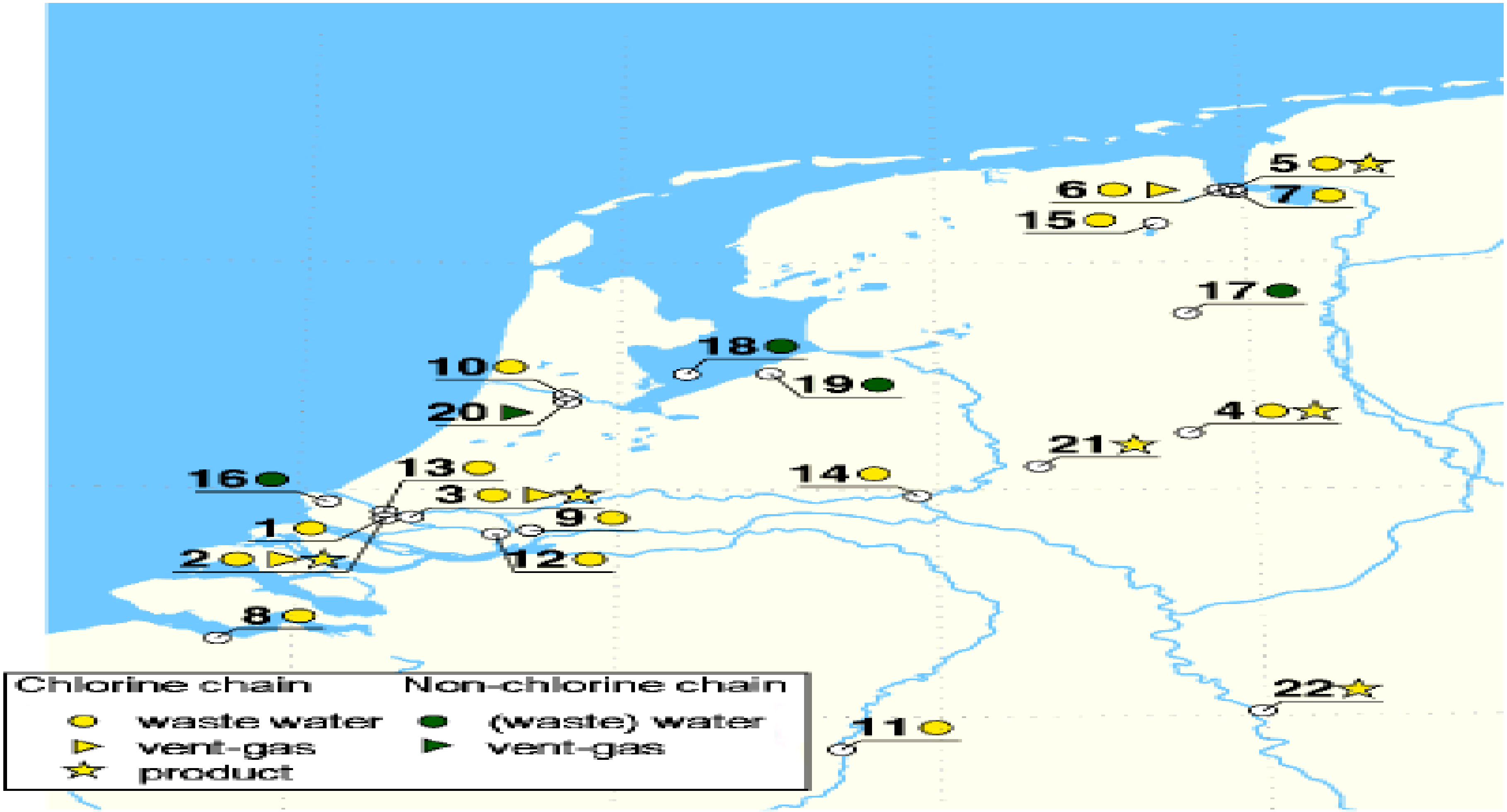
- high removal rates in WTP
- care institutions: except ZE, lower activity than households
- households: RO, HA and VE high activity

GR-Calux: glucocorticoid activity



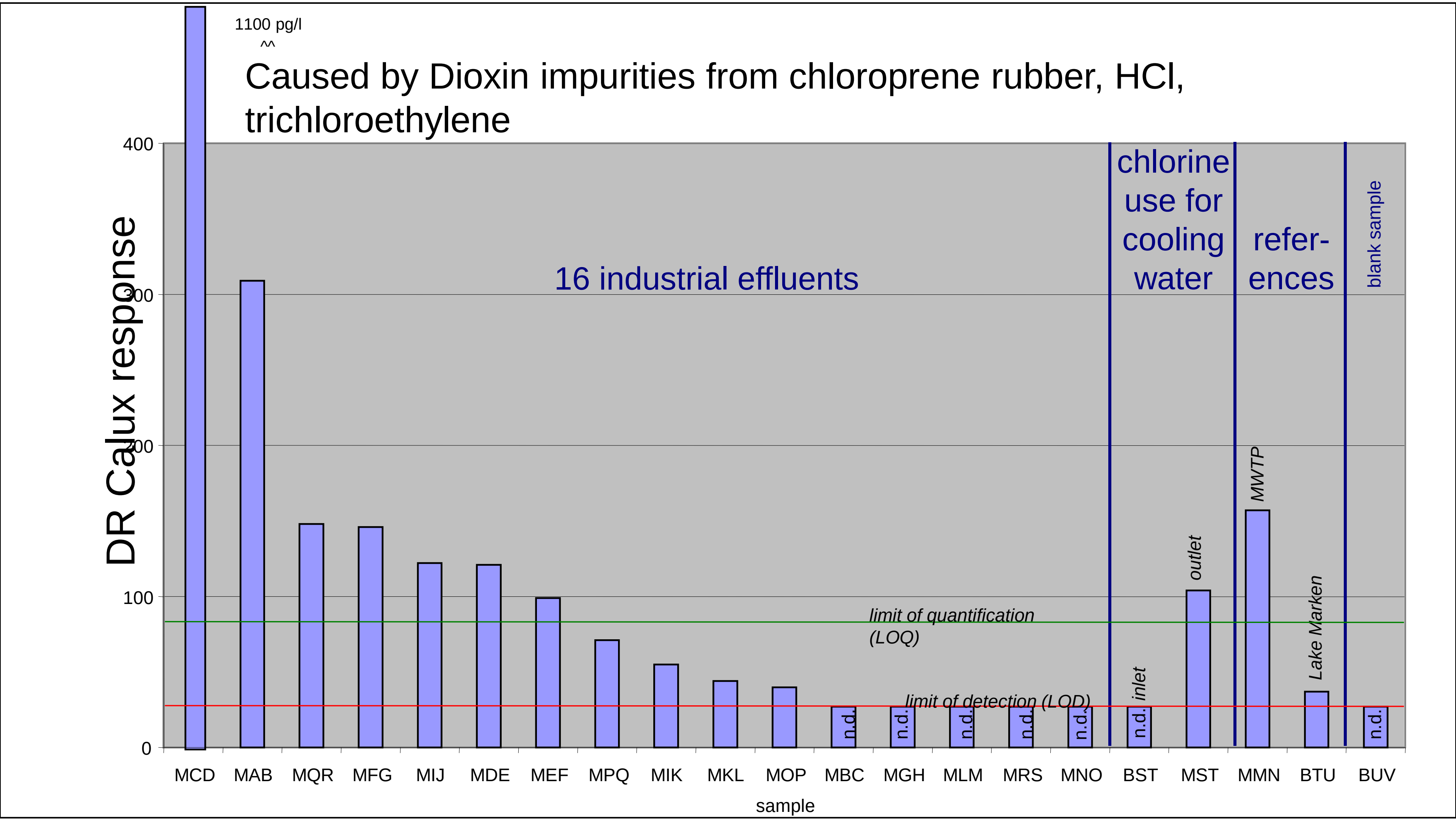
- differing removal rates in WTP
- care institutions: RO an HA high activity
- households: HA high activity

Dioxins/dl-PCBs in effluents from WWTPs of the Chlorine Industry (OVOC Project 2002)



Van Hattum et al., 2004

OVOC (2005, NL): Dioxin-like activity in effluents from the chlorine industry

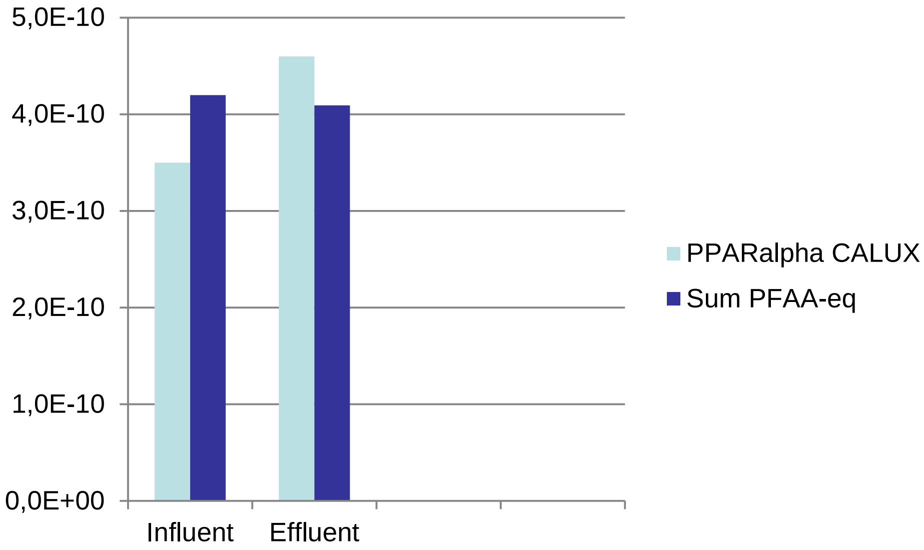


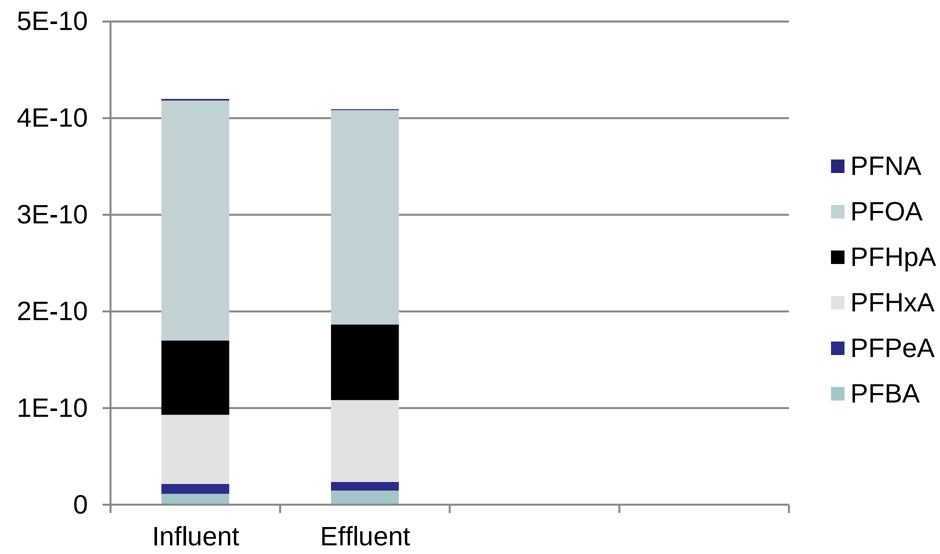
Van Hattum et al., 2004

Landfill in Amsterdam with high PFOA/PFOS concentrations:

High activites in the PPAR CALUX for obesity

Landfills influents and effluents – PPARα CALUX vs. chemical analysis in GW7674-EQs





<u>Summary for landfill samples:</u>

- PPARα CALUX results are in the same range of concentrations as chemical PFAA-EQs
- based on concentration and potency, PFOA, PFHpA and PFHxA are responsible for the PPAR α activity

<u>Sweden</u>

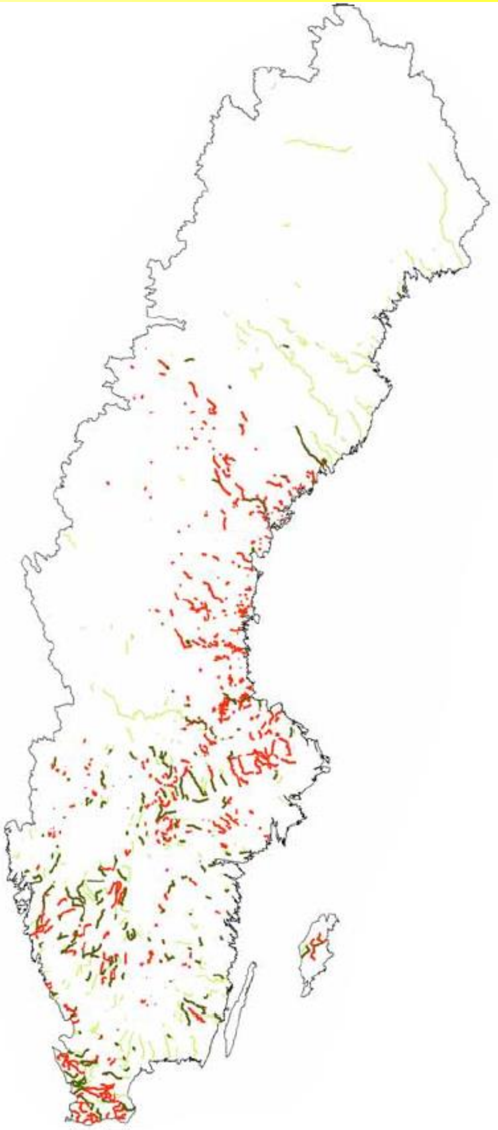
Local EPA in Västra Götaland

National monitoring programs of priority emerging pollutants

Red: At risk without monitoring

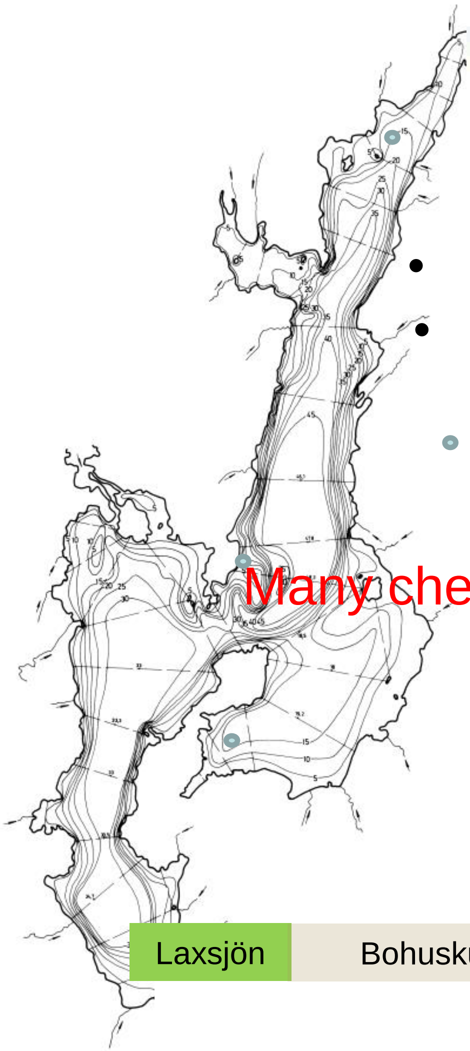
Green: At risk with monitoring

Light green: Monitored water bodies

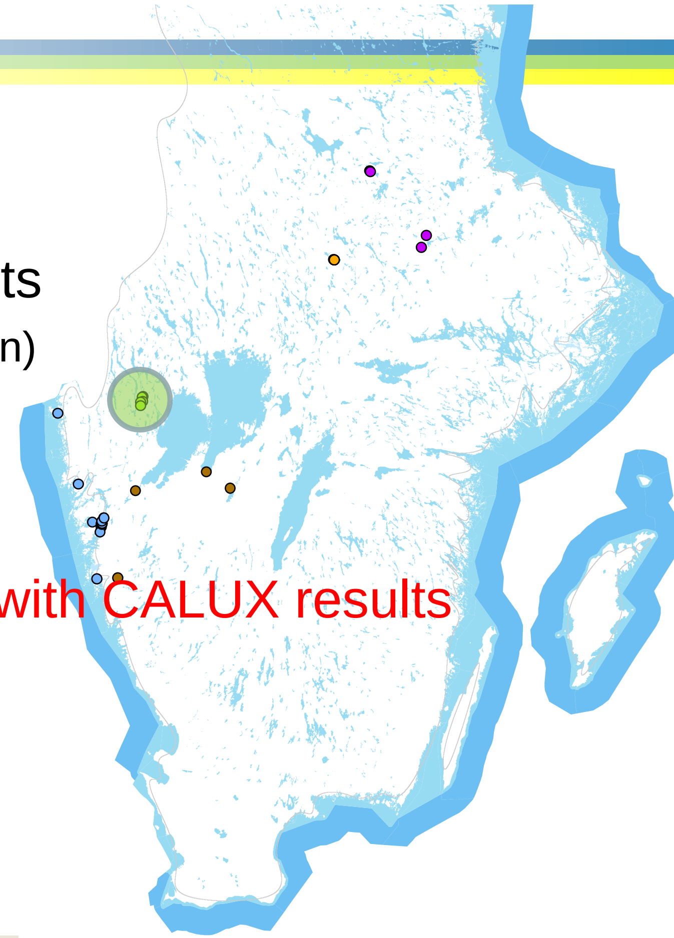


73 % of the water bodies at risk, are not monitored in the national program!

Focus on diverse polluted sides



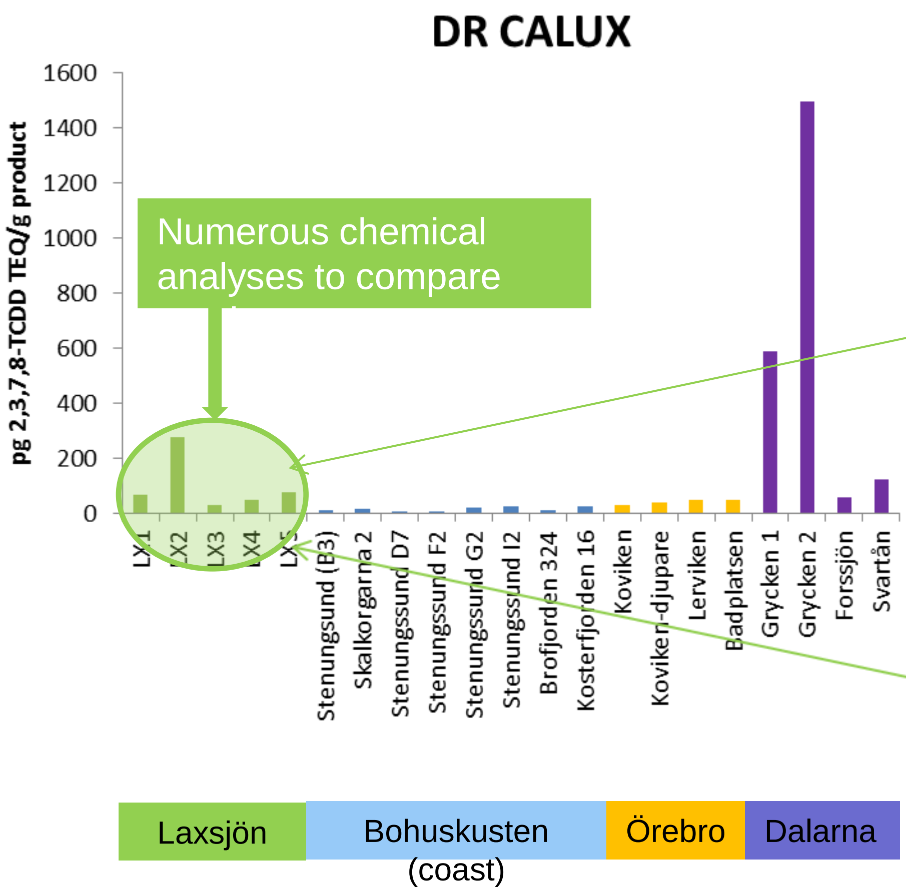
- Paper mills
- Waste water treatment plants
- Lumber mills (with impregnation)
- Historical pollution

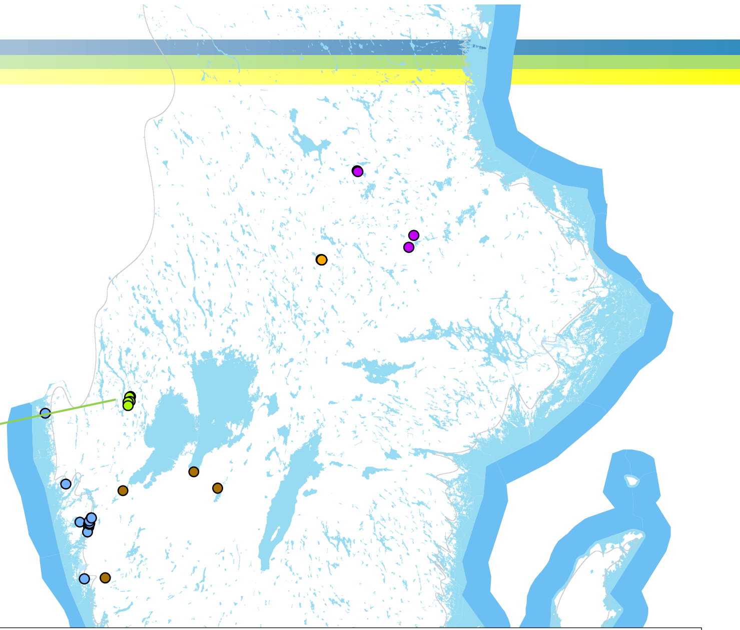


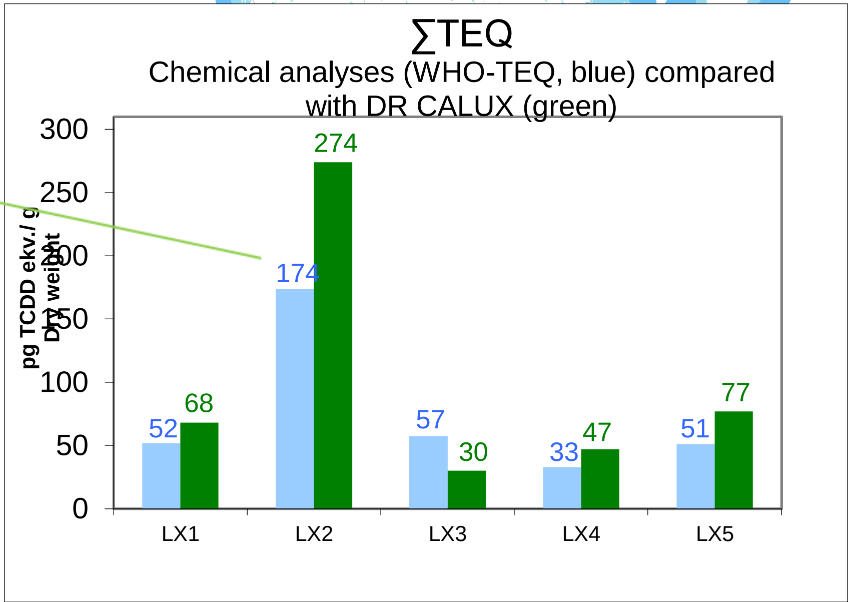
Many chemical analyses to compare with CALUX results

Laxsjön	Bohuskusten (coast)	Leachate	Örebro	Dalarna

Dioxins/dl-PCB (POPs):
Chemical results confirm DR CALUX screening

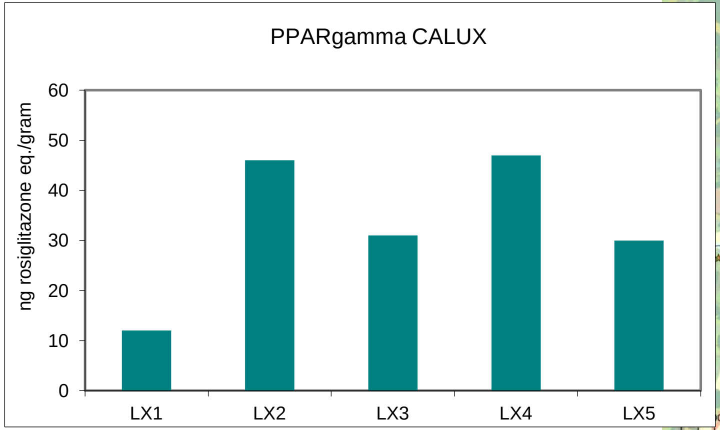


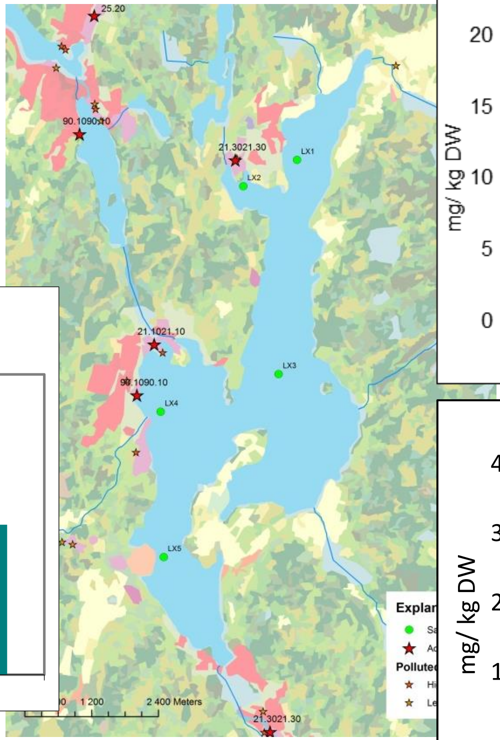


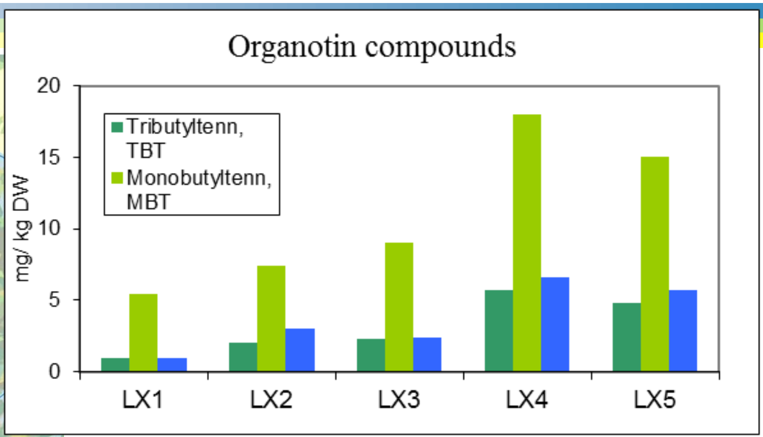


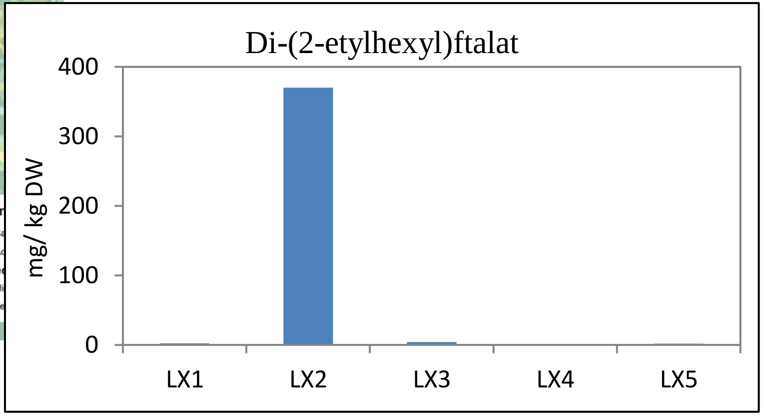
High obesity activity confirmed by organotin/plastic additives

Comparing chemical results with PPAR CALUX results
(sediment)







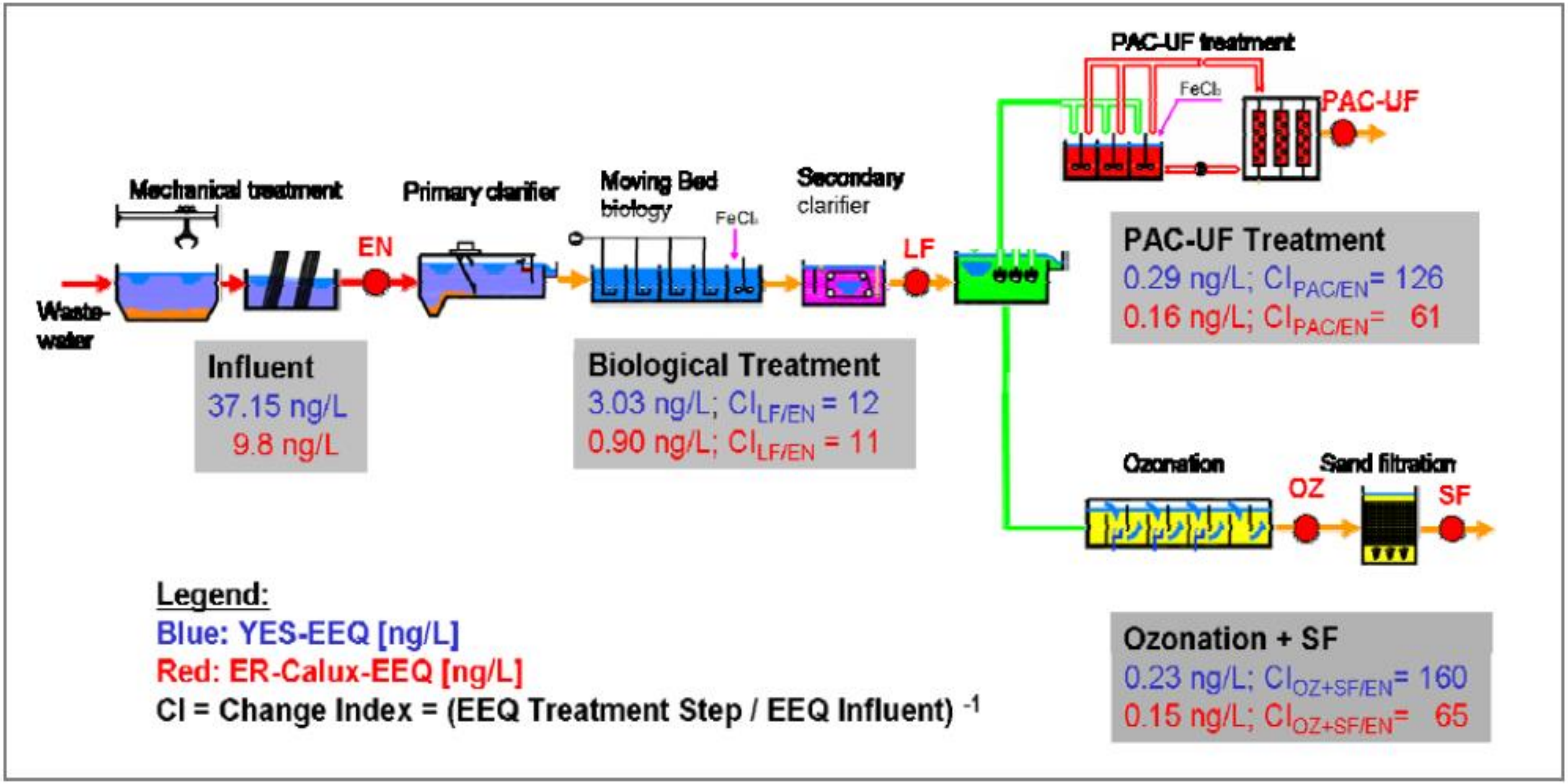


Compounds with obesogenic properties: TBT & phtalates

Switzerland

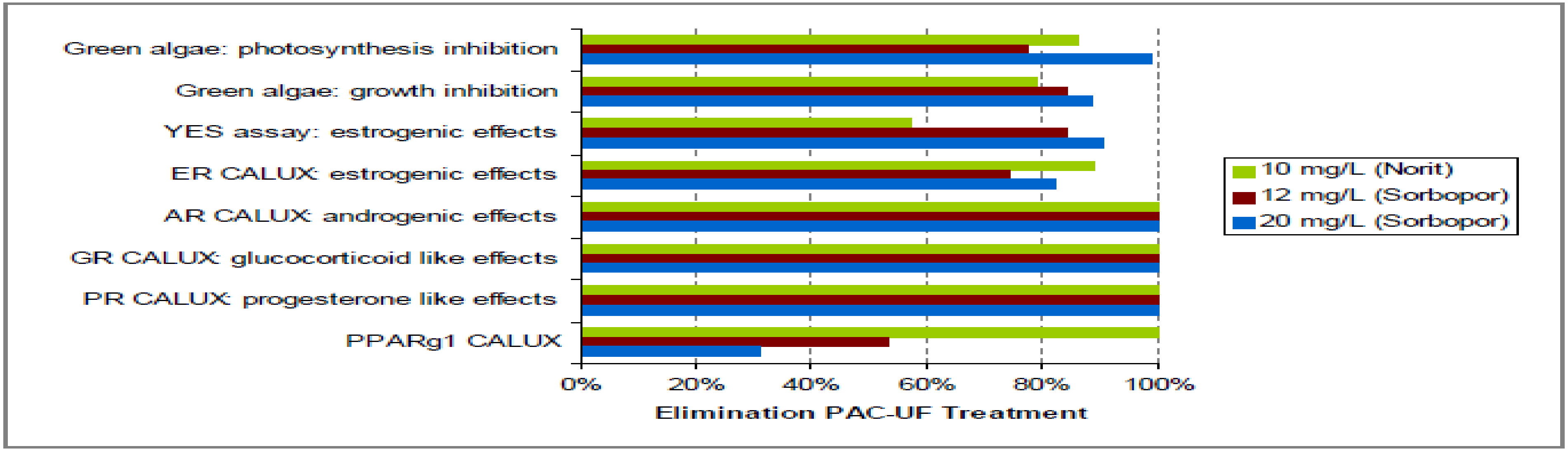
Oekotox centre/EAWAG

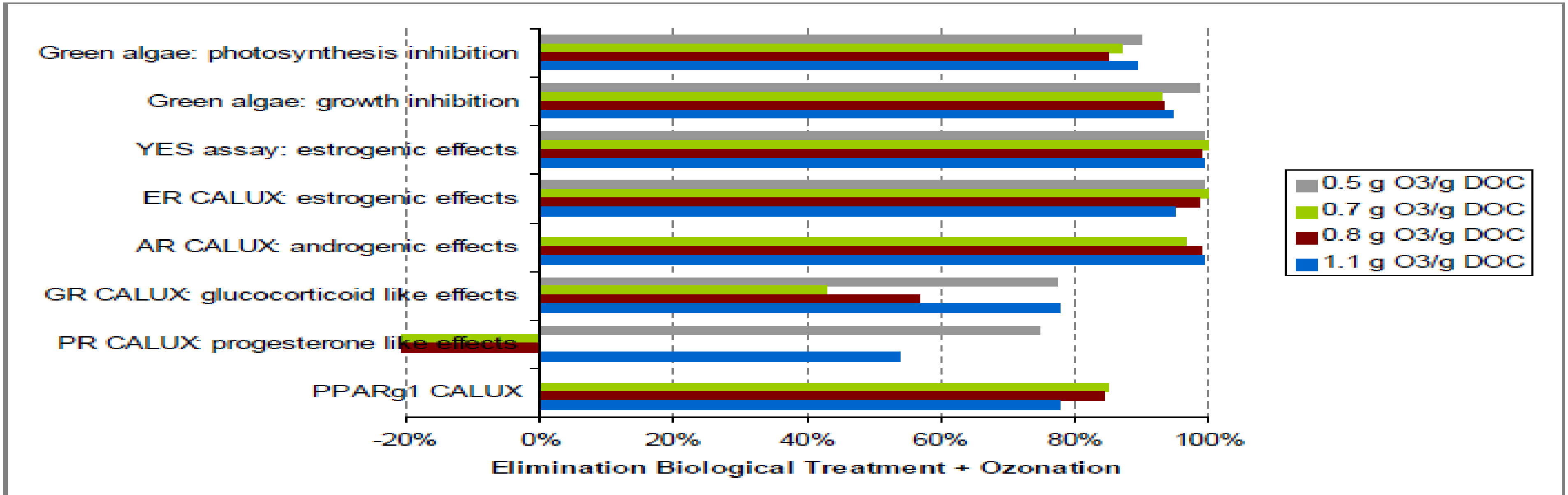
Micropol project: Lausanne Pilot WWTPs: PAC/UF and Ozon eliminates EDCs



Kienle et al (2012)

Water treatment plants treatment efficiency with active carbon or ozonation (EAWAG 2012)





<u>**Germany**</u>

University of RWTH Aachen

EPA Germany

WWTPs effluents treated with ozone and bio membrane shows low estrogenic activity (by ER CALUX and LC/MS)

Table 3b – Calculated[a] chemEEQs based on results from earlier studies with the ER CALUX® (Table 1) and the analytical data (Table 2) compared to the data calculated with the ER CALUX®.

Treatment	Active agent [EEQ ng/L]										ER CALUX® [EEQ ng/L]
	E1	E2	E2-ac	EE 2	E3	BPA	t-NP	MPro-ac	Total[b]		
MBR A	0.29	<5	n.a.	<9.2	<0.18	0.0015	0.080	n.a.	0.37	=	0.37+/−0.09
AO	<0.08	<5	n.a.	<9.2	<0.18	0.011	0.078	n.a.	0.09		0.06+/−0.06
MBR B	0.26	<5	n.a.	<9.2	<0.18	0.0013	0.097	n.a.	0.36		0.83+/−0.06
BO	<0.08	<5	n.a.	<9.2	<0.18	0.011	0.054	n.a.	0.07		n.d.
MBR C	0.19	<5	n.a.	<9.2	<0.18	0.0011	0.097	n.a.	0.29		1.23+/−0.24
CO	<0.08	<5	n.a.	<9.2	<0.18	< 0.0007	0.062	n.a.	0.06		n.d.
MBR A-C									0.34		0.81+/−0.43
OZ A-C									0.07		0.02+/−0.04

a Calculated Concentrations of EEQ = Relative estrogenic potency x concentration [ng/L].

b "Total" calculated only from values that lay above the limit of quantification; n.a. = data not available; n.d. = value not detectable; E1 = estrone; E2 = 17β-estradiol; E2-ac = 17β-estradiol acetate; EE2 = 17α-ethinylestradiol; E3 = estriol; BPA = bisphenol A: t-NP = nonylphenol; MPro-ac = medroxyprogesterone acetate.

Sibylle Maletz, T. Floehr, J. Pinnekamp & H. Hollert; RWTH Aachen University;

Study of metabolite formation during the use of ozone in municipal waste water treatment plants

Project management : IWW, Mülheim an der Ruhr

On behalf of the:

Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the German State of North Rhine-Westphalia (MKULNV) for the financial support of the project.

Investigated municipal WWTP

WWTP Bad Sassendorf (Lippeverband)

- 12,000 PE.
- Post treatment dosing of ozone to the effluent of conventional biological treatment. Polishing pond.

WWTP Schwerte (Ruhrverband)

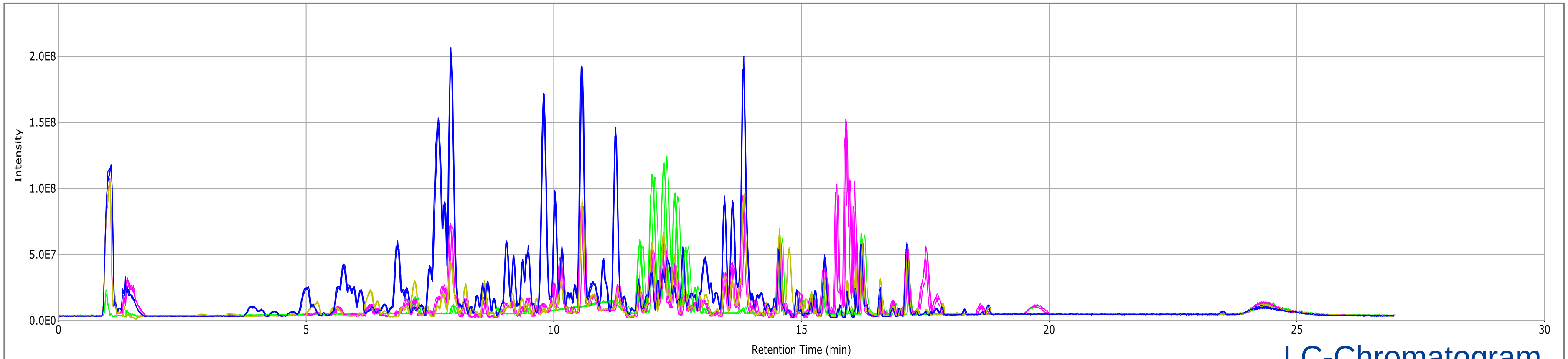
- 50,000 PE.
- Consists of two separated lines. Ozone and/or powdered activated carbon are applied. Recirculation process can be operated.

WWTP Duisburg-Vierlinden (Wirtschaftsbetriebe Duisburg AöR)

- 30,000 PE.
- Two parallel lines have been installed to compare ozone dosage by diffusor or by injector. The wastewater outline is fed to an additional biological stage (fluidised bed reactor).

GC- and LC-MS Screening

WWTP Bad Sassendorf
5 mg/L Ozone



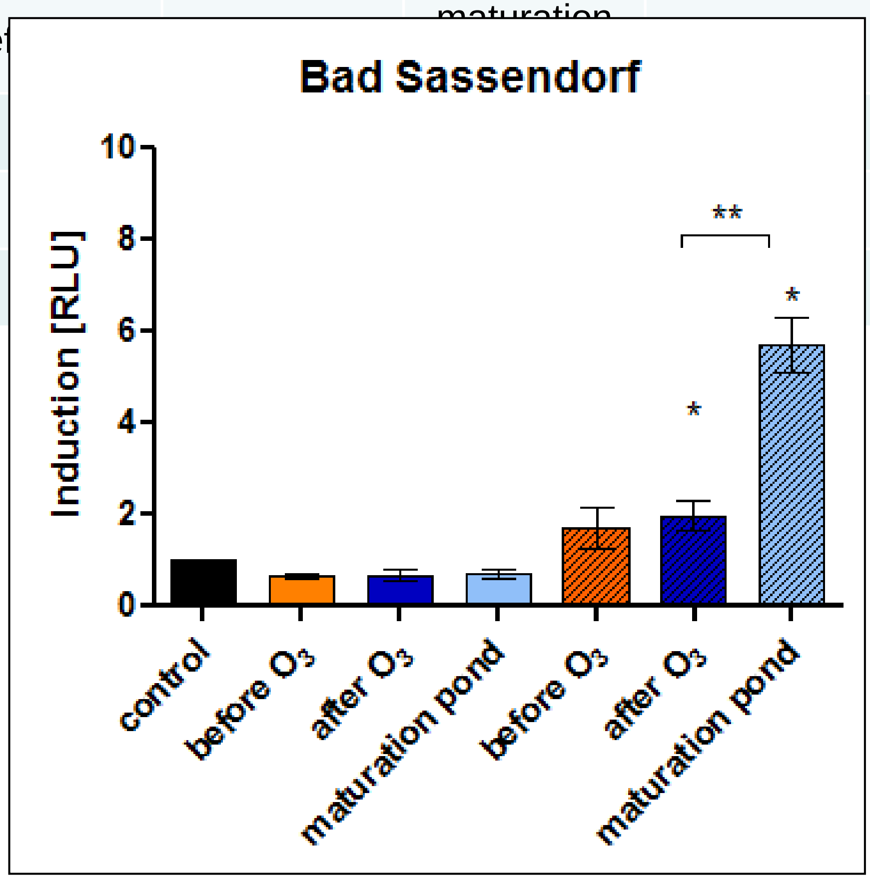
LC-Chromatogram

Control sample*
Before Ozonation
After Ozonation
After Maturation pond

* Internal Standard: ~ 120 substances

Estrogenicity WWTP Bad Sassendorf

Date of sampling	O_3 z-spec.	Sample					
		original			*extract*		
		bef		maturation		after O_3	maturation pond
02.08.2013	0.7					1.1 ng/L	cytotoxic
16.08.2013	0.7					n.d.	9.9 ng/L
30.08.2013	0.9					1.2 ng/L	5.8 ng/L

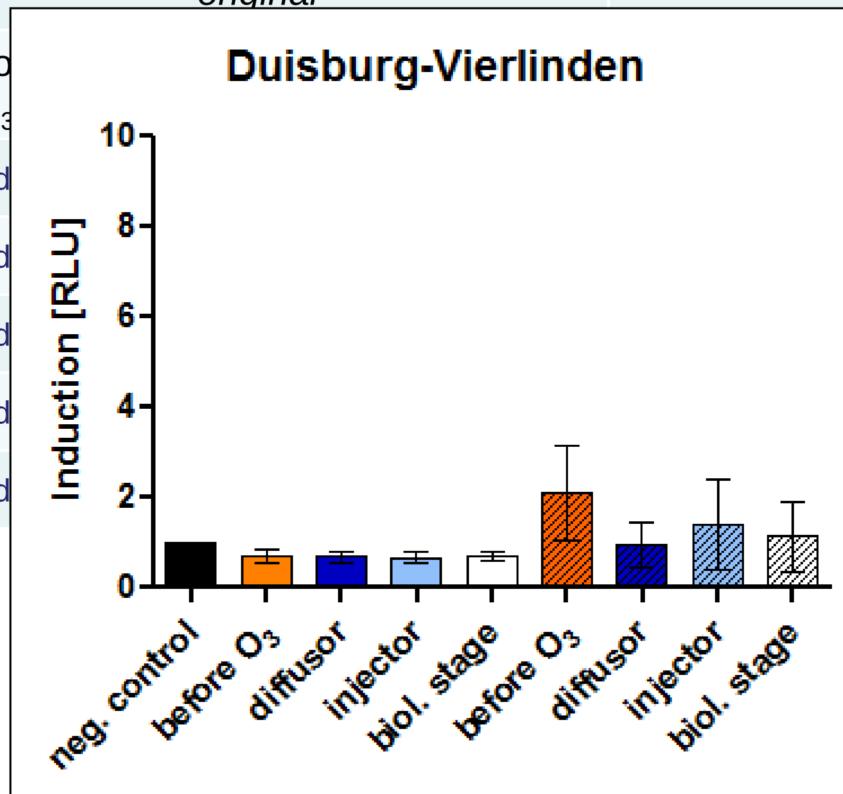


n.d. = not detected

* statistically significant compared to neg. control
** statistically significant compared to previous treatment step

Estrogenitcity WWTP Duisburg-Vierlinden

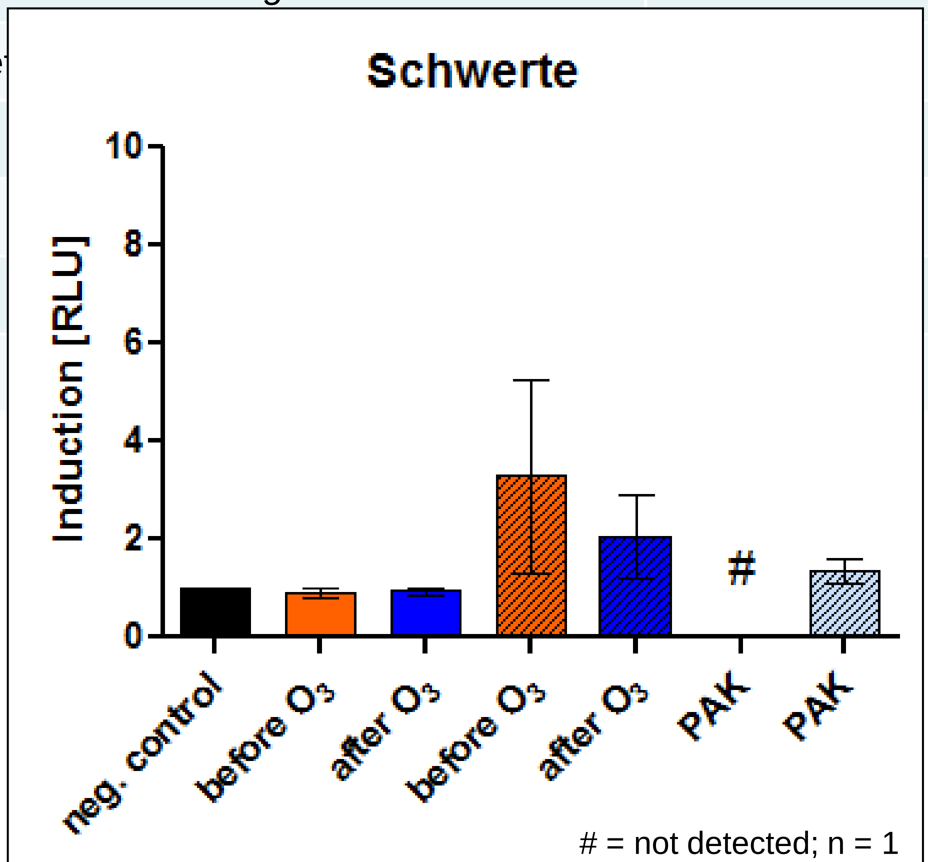
Date of sampling	O_3 z-spec.	Sample			
		original	*extract*		
		befo O_3	ffusor	injector	biol. stage
13.09.2012	0.5	n.d	n.d.	-	n.d.
20.09.2012	0.5	n.d	n.d.	0.7 ng/L	0.3 ng/L
25.10.2012	0.7	n.d	n.d.	n.d.	0.3 ng/L
31.10.2012	0.7	n.d	n.d.	n.d.	n.d.
16.11.2012	0.9	n.d	n.d.	n.d.	n.d.



n.d. = not detected;
- = not tested

Estrogenicity WWTP Schwerte

Date of sampling	O_3 z-spec.	Sample			
		original		*extract*	
		be[fore]		after O_3	PAK
29.11.2012	0.9			1.5 ng/L	-
07.12.2012	0.9			3.0 ng/L	-
12.12.2012	0.5			1.6 ng/L	-
12.03.2013	0.9			21.1 ng/L	1.4 ng/L



n.d. = not detected;
- = not tested

Conclusions

- **Estrogenicity only detected in extracts**
  - Bad Sassendorf → increase in estrogenicity after ozonation (e.g. through phytoestrogens, matrix effects)
  - Duisburg Vierlinden → varying results, partial loss of estrogenicity
  - Schwerte → slight decrease in estrogenicity, but not statistically significant

- **High variation of effluent composition**
  - General statement on estrogenicity for one WWTP not possible

- **Efficiency of ozonation is dependent on WWTP effluent composition**

Comparison of different genotoxicity tests *in vitro* for assessment of surface water quality

E. Dopp, J. Richard, S. Zander-Hauck

7th BioDetector Conference (November 7 – 8, 2013)

IWW

Institute affiliated with the
UNIVERSITÄT DUISBURG ESSEN
Open-Minded

Possible endpoints for biological effects

In vivo

Algae growth (*Desmodesmus Subspicatus)*

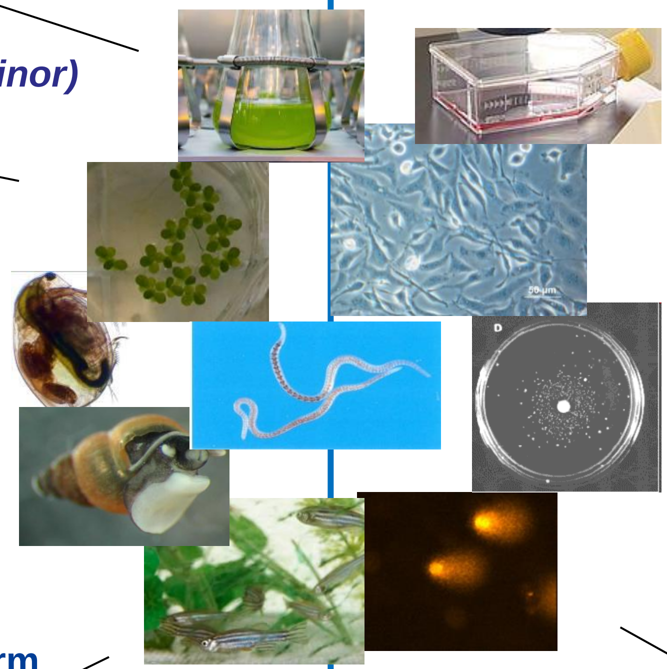
Duckweed growth *(lemna minor)*

Toxicity to water flea
(Daphnia magna)

Estrogenicity to snails
(Potamopyrgus antipodarum)

Growth inhibition to gloss worm
(Lumbriculus variegatus)

Toxicity to fish eggs (Danio rerio)



In vitro

General cell damage (*Cytotoxicity)*

DNA damage (*Genotoxicity)*

Health oriented guided value:
0.3 µg/L if not genotoxic
0.1 µg/L if genotoxic

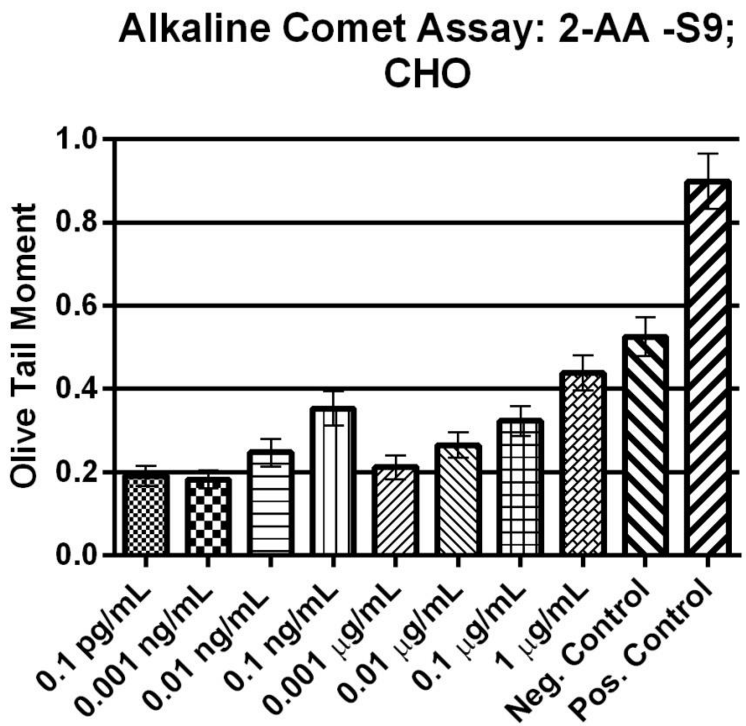
Inheritable DNA damage
(*Mutagenicity)*

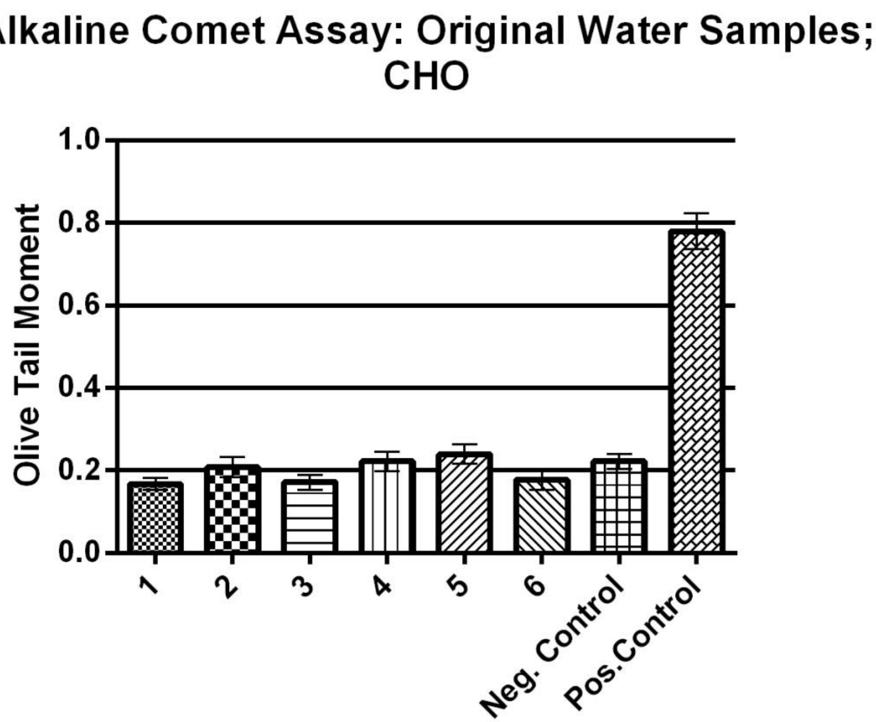
Estrogenic effects (*Estrogenicity)*

	MTT-Test	Comet-Assay	Umu-Test	MN-Test	P53-Calux®
ENU	100 µg/ml	100 µg/ml	-	No genotox up to 100 µg/ml	100 µg/ml
4-NQO	3 µg/ml	No genotox up to 0.3 µg/ml	-	No genotox up to 0.3 µg/ml	0.3 µg/ml
Mitomycin C	No cytotox up to 20 µg/ml	20 µg/ml	-	2 µg/ml	0.5 µg/ml
2-AA	1 µg/ml	No genotox up to 1 µg/ml	<0.1 µg/ml	No genotox up to 1 µg/ml	No genotox up to 0.1 µg/ml

P53 Calux® and Umu-test are able to detect genotoxic effects at concentrations ≤ 0.5 µg/ml. Comet and MN assay require higher substance concentrations.

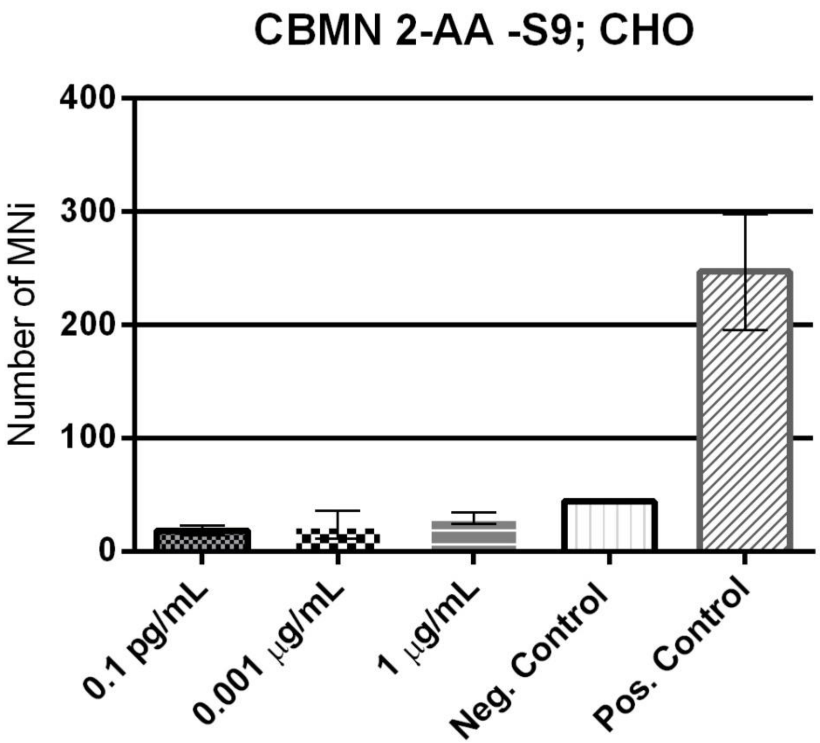
Results: Comet Assay

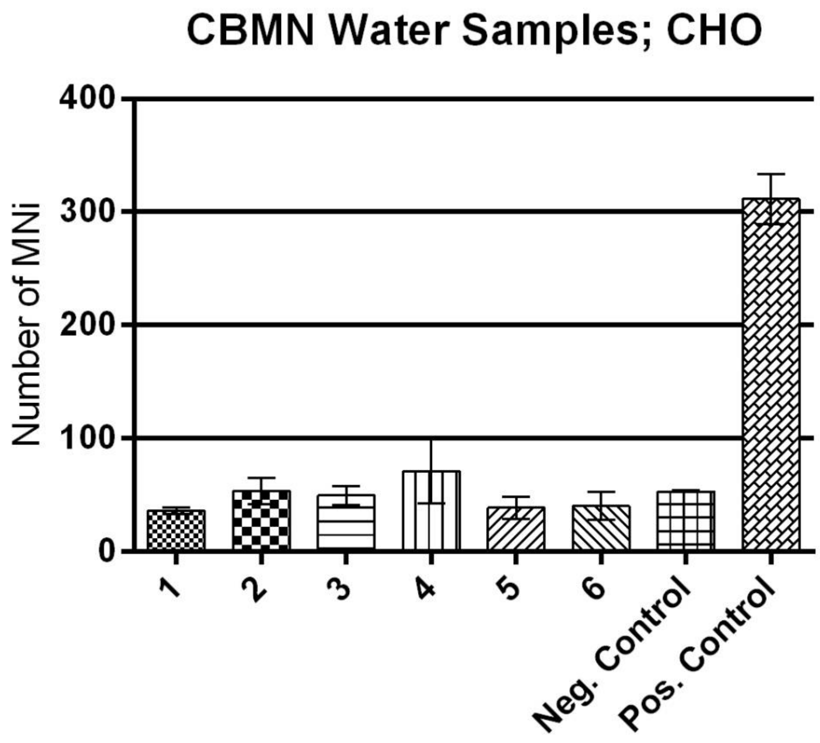




No genotoxic effects were detectable with the Comet Assay and the UMU-test (data not shown) in original and concentrated water samples (2.8x and 4x).

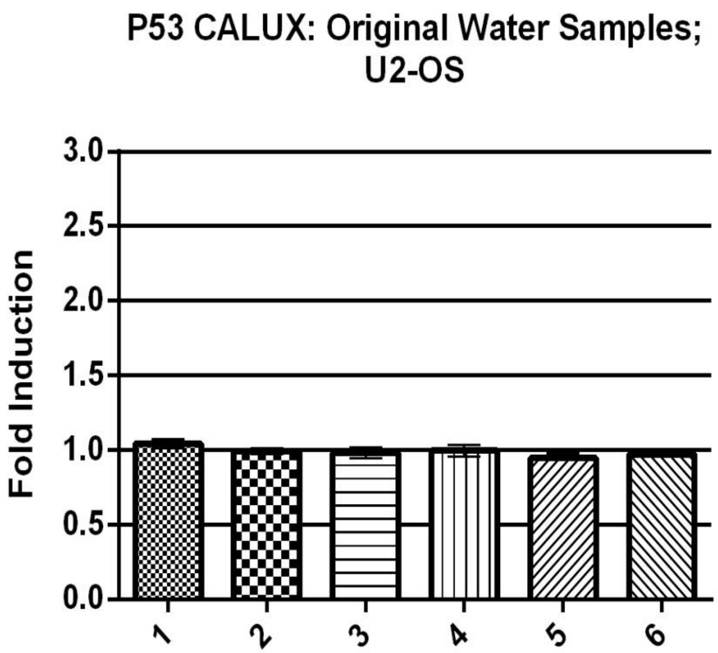
Results: Micronucleus Assay

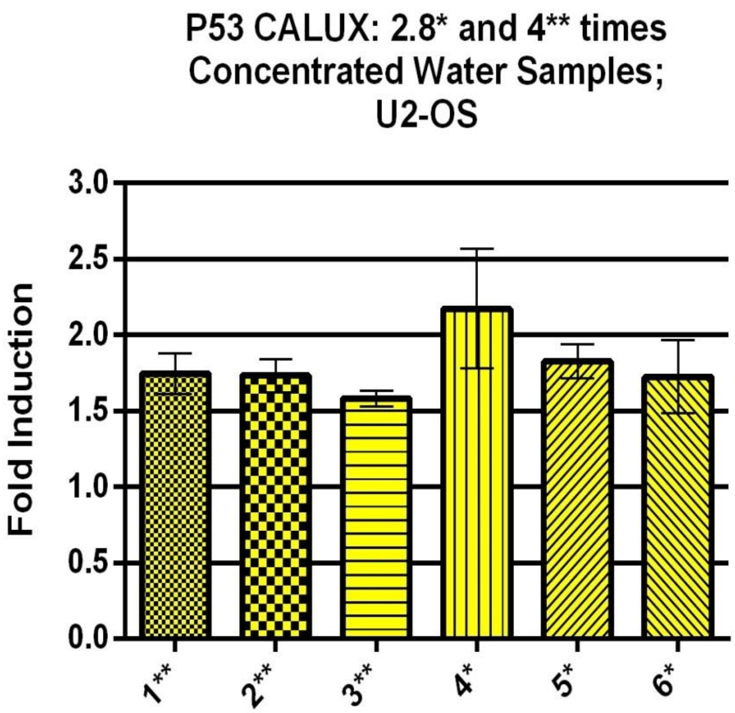




No genotoxic effects were detectable with the Micronucleus Assay in original and concentrated water samples (2.8x and 4x).

Results: p53 Calux®





Significant genotoxic effects were detected with the P53 Calux® in concentrated water samples (2.8x and 4x).

Slovenia

National Institute of Biology

Waste Water samples: Good EEQ correlations between ER CALUX vs GC-MS

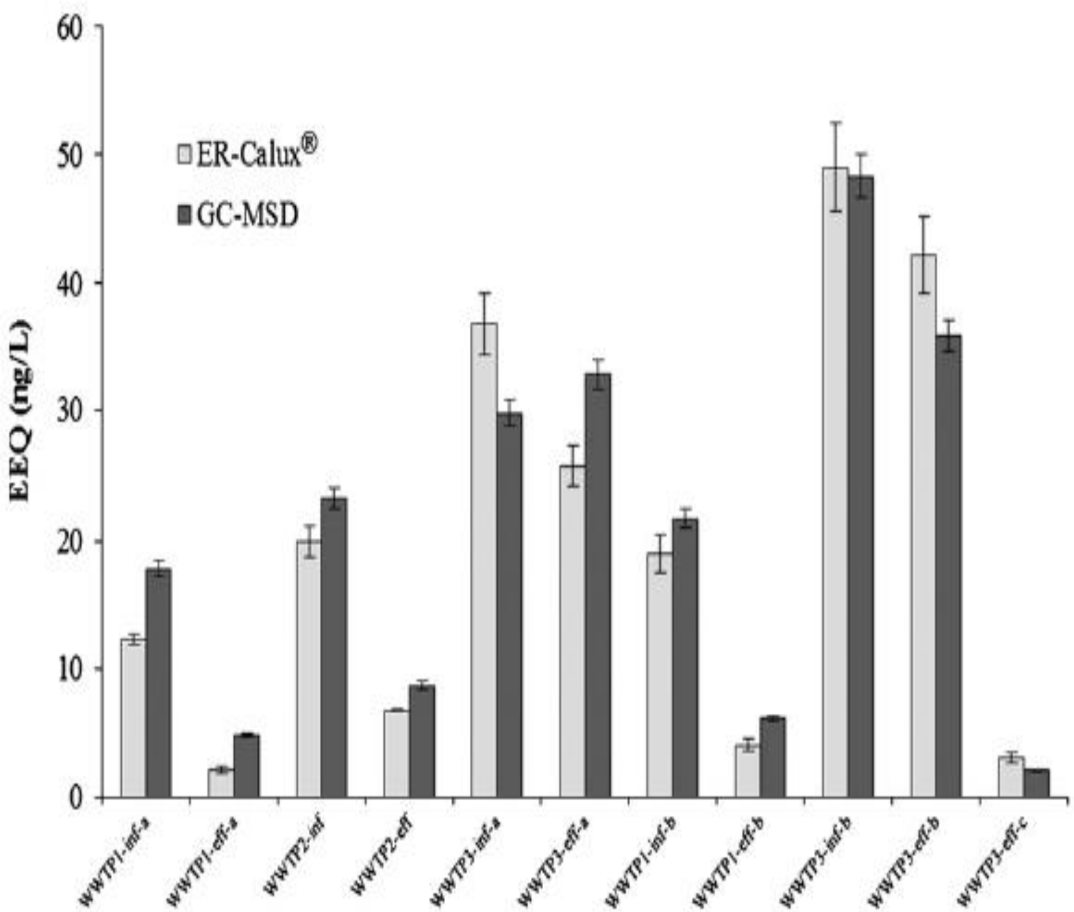


Fig. 4. GC-MSD and ER-Calux® results of real waste water samples. The results of ER-Calux® assay are presented as mean ± SD calculated from three parallels. The results of GC-MSD are presented as determined concentrations of one measurement ± relative standard deviation of measurement by GC-MSD.

BDS

Effect-based identifications of anti-androgens in environmental media and human tissues.

Elizabeth M. Hill
Pawel Rostkowski.

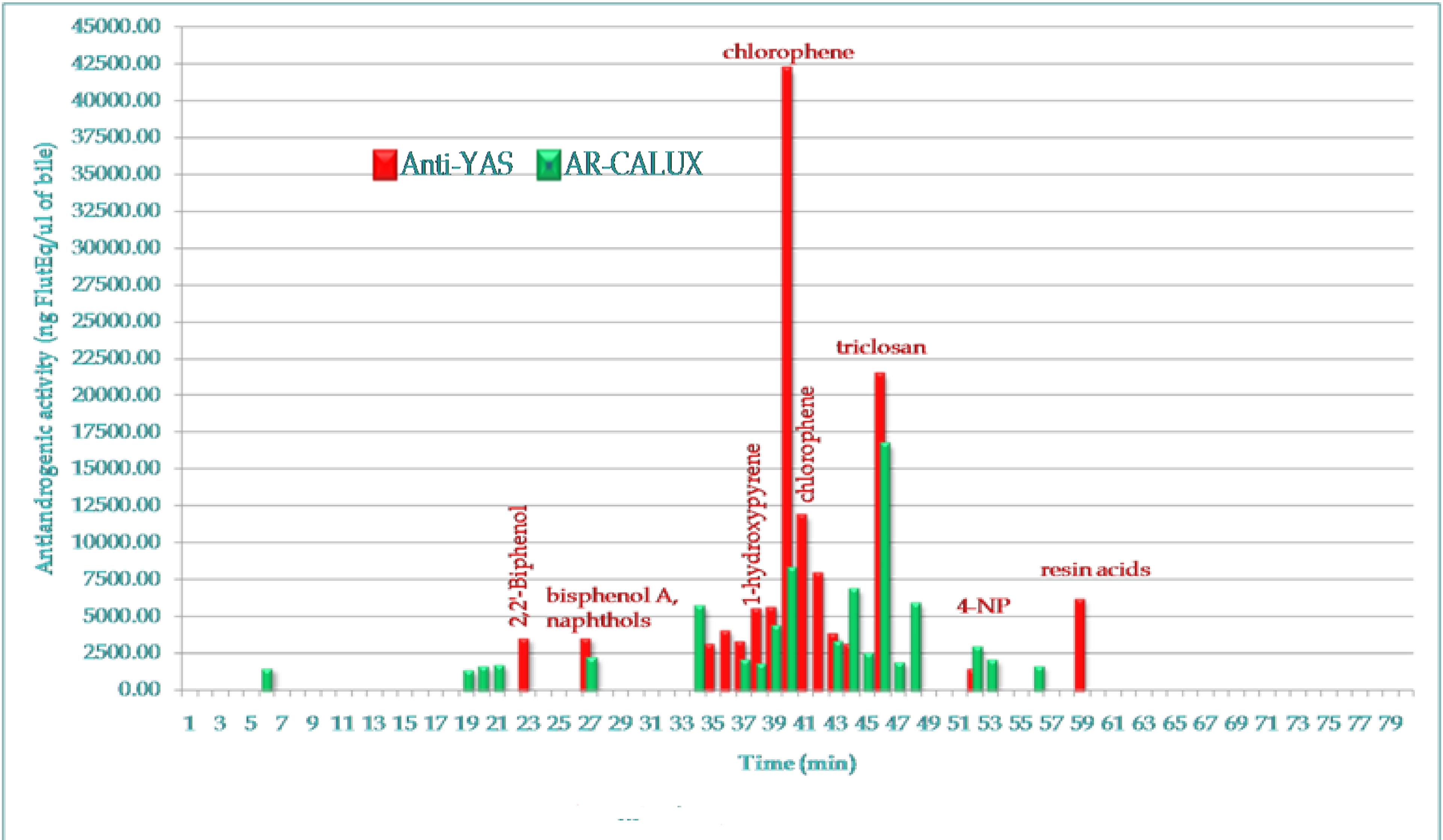
School of Life Sciences,
University of Sussex, UK.

University of Sussex

NATURAL ENVIRONMENT RESEARCH COUNCIL

contamed

Comparison from anti-YAS and anti-AR CALUX: Results in fish bile



Comparison from anti-androgenic compounds in the anti- YAS and anti- AR-CALUX

Compound	Potency Anti-YAS	Potency AR-CALUX
Flutamide	**1 (IC_{50} 1.2 mg/L)**	**1 (IC_{50} 0.12 mg.L)**
dichlorophene	**3.4-6.0**	**0.2**
chlorophene	**12.9-13.3**	**0.7**
triclosan	**2.8-5.2**	**0.6**
hydroxypyrene	**4.7-19.5**	**0.2**
4-nonylphenol	**0.2-0.3**	**0.4**
bisphenol A	**0.6-0.7**	**0.5**
abietic acid	**3.5-4.4**	**Incomplete curve**

Australia

„A national approach to health risk assessment, risk communication and management of chemical hazards from recycled water"

Chapman, Leusch, Prochazka, Cumming, Ross

Griffith University

Humpage, Froscio, Laingam

Australian Water Quality Centre

Khan, Trinh, McDonald

UNSW Water Research Centre

Waterlines Report 2011

Recycled Water: EDC via panel of CALUX tests:
a) Evaluation of steroid profiles of to be expected chemicals

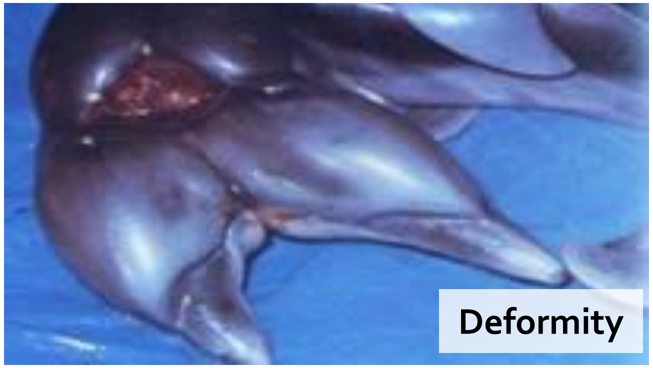
	HepCYP1A2	CALUX						
		AR +	AR -	ERα +	ERα -	GR	PR	TRβ
	BaP	DHT	Flutamide	βE2	Tamoxifen	Dexa	Org 2058	T3
17β-Estradiol (βE2)	ND (<-1.39)	-4.40	1.86	0.00	Agonist	ND (<-5.03)	ND (<-5.56)	ND (<-4.32)
Estrone (E1)	ND (<-0.69)	ND (<-4.56)	1.17	-1.85	Agonist	ND (<-4.34)	ND (<-4.74)	ND (<-3.63)
17α-Estradiol (αE2)	ND (<-1.39)	ND (<-5.26)	1.14	-2.69	Agonist	ND (<-5.03)	ND (<-5.44)	ND (<-4.32)
Estriol (E3)	ND (<-1.06)	ND (<-5.21)	-0.26	-1.77	Agonist	ND (<-4.71)	ND (<-5.11)	ND (<-4.00)
17α-Ethynylestradiol (EE2)	ND (<-1.35)	ND (<-5.22)	1.96	0.73	Agonist	ND (<-5.00)	-4.75	ND (<-4.29)
Mestranol	ND (<-1.03)	ND (<-4.81)	0.30	-3.21	Agonist	ND (<-4.67)	ND (<-5.08)	ND (<-3.97)
Testosterone	ND (<-1.36)	-0.77	Agonist	-5.78	Agonist	ND (<-5.01)	ND (<-5.15)	ND (<-4.30)
5α-Dihydrotestosterone (DHT)	ND (<-1.36)	0.00	Agonist	-4.81	Agonist	ND (<-5.00)	-5.28	ND (<-4.31)
17β-Trenbolone	ND (<-1.39)	-0.30	Agonist	-4.26	Agonist	ND (<-5.04)	-2.59	ND (<-4.33)
Levonorgestrel	ND (<-1.33)	-0.56	Agonist	-5.54	Agonist	-4.79	-0.36	ND (<-4.26)
Bisphenol A (BPA)	ND (<-1.47)	ND (<-5.34)	-0.67	-4.84	Agonist	ND (<-5.11)	ND (<-5.51)	ND (<-4.40)
4-Nonylphenol (4NP)	ND (<-1.48)	ND (<-5.35)	-0.53	-4.04	Agonist	ND (<-5.12)	ND (<-5.53)	ND (<-4.42)
4-t-Octylphenol (4tOP)	ND (<-1.51)	ND (<-5.38)	-0.39	-4.91	Agonist	ND (<-5.15)	ND (<-5.12)	ND (<-4.45)
Atenolol	ND (<-1.40)	ND (<-5.27)	-1.03	ND (<-7.16)	ND (<-2.06)	ND (<-4.47)	ND (<-5.47)	ND (<-4.35)
Caffeine	ND (<-1.54)	ND (<-5.68)	ND (<-1.04)	ND (<-7.29)	ND (<-2.19)	ND (<-5.18)	ND (<-5.58)	ND (<-4.49)

Recycled Water: EDC via panel of CALUX tests:
a) Evaluation of steroid profiles of several water samples

	HepCYP1 A2	CALUX						
		AR +	AR -	ERα +	ERα -	GR	PR	TRβ
	BaP Eq μg/L	DHT Eq ng/L	Flu Eq μg/L	βE2 Eq ng/L	TMX Eq μg/L	Dexa Eq ng/L	Org2058 Eq ng/L	T3 Eq ng/L
Treated sewage								
WRP 1	<19	ND (<2)	ND (<25)	0.39 ± 0.21 (<0.05 – 1.51)	Agonist	ND (<12)	ND (<0.004)	ND (<25)
WRP 2	43 ± 8.1 (<19 – 58)	ND (<2)	ND (<25)	0.83 ± 0.45 (<0.05 – 2.58)	Agonist	28 ± 4.8 (<24 – 40)	0.30 ± 0.29 (<0.004 – 1.66)	ND (<25)
WRP 3	19 ± 7.4 (<19 – 30)	ND (<2)	ND (<25)	4.22 ± 1.15 (2.40 – 4.73)	Agonist	69 ± 10 (52 – 81)	1.41 ± 0.46 (1.00 – 2.16)	ND (<25)
Class A recycled water								
WRP 1	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	<2	ND (<12)	ND (<0.004)	ND (<25)
WRP 3	37 ± 5.5	ND (<2)	ND (<25)	1.90 ± 0.77	Agonist	62 ± 13	0.64 ± 0.38	ND (<25)
RO recycled water								
WRP 2	ND (<19)	ND (<2)	ND (<25)	0.17 ± 0.15 (<0.04 – 0.87)	<2	ND (<12)	ND (<0.004)	ND (<25)
WRP 5	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	2.3 ± 0.9 (<2 – 8)	ND (<12)	ND (<0.004)	ND (<25)
WRP 6	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	4.4	ND (<12)	ND (<0.004)	ND (<25)
Other miscellaneous								
Bottled water	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	ND (<2)	ND (<12)	ND (<0.004)	ND (<25)
Tap water	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	ND (<2)	ND (<12)	ND (<0.004)	ND (<25)
Rainwater	ND (<19)	ND (<2)	ND (<25)	ND (<0.05)	ND (<2)	ND (<12)	ND (<0.004)	ND (<25)
Field blank	ND (<19)	ND (<2)	ND (<25)	<0.05	<2	ND (<12)	ND (<0.004)	ND (<25)

Japan
Decline of aquatic biodiversity/increase diseases – pollutants in aquatic wildlife (2008-2011)

Relation with contaminants?



Dr. Go SUZUKI

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Evaluation of potential bioaccummulative compounds exerting endocrine-disrupting activities in wild animals using *in vitro* bioassays and chemical fractionation

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Agonistic activity / Antagonistic activity

AR-CALUX	Agonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	NA	7.1.E-03	1.1.E-03	1.1.E-03	4.3.E-04	1.1.E-03	7.1.E-03	1.1.E-03	1.1.E-03
Baikal seal (1992)-Blubber	4.5.E-04	NA	2.3.E-03	3.4.E-04	1.0.E-03	4.2.E-04	[illegible]	2.3.E-03	3.4.E-04	1.0.E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-03	7.3.E-03	5.8.E-03	5.0.E-03	1.5.E-02	1.5.E-03	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	2.8.E-03	1.2.E-03	1.6.E-03	1.2.E-03	9.3.E-03	2.8.E-03	1.2.E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	1.2.E-02	1.9.E-03	1.2.E-03	9.3.E-03	2.8.E-04	1.2.E-02
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	2.8.E-03	1.2.E-02	1.9.E-03	1.2.E-02	9.3.E-03	8.3.E-04	1.2.E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02	1.9.E-03	4.1.E-04	9.3.E-03	8.3.E-04	1.2.E-02

ERa-CALUX	Agonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	1.1E-02	7.1.E-03	NA	1.1E-03	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	2.0E-03	2.3.E-03	NA	1.0E-03	4.5E-04	2.0E-03	7.7E-03	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-03	7.3.E-03	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	1.2E-02	NA	NA	1.2.E-03	1.9E-03	1.2E-02	3.1E-03	[illegible]	1.2E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	3.4E-03	1.9E-03	1.2E-03	9.3E-03	2.8E-04	NA
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	NA	2.2E-03	1.9E-03	1.2E-02	9.3E-03	[illegible]	NA
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	4.1E-03	1.9E-03	4.1E-04	9.3E-03	2.8E-04	NA

GR-CALUX	Agonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	1.1E-02	7.1.E-03	1.1.E-03	1.1.E-03	4.5E-04	1.1E-02	2.1E-02	1.1E-03	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	2.0E-03	2.3.E-03	3.4.E-04	1.0.E-03	4.5E-04	2.0E-03	2.3E-02	1.0E-03	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	1.5E-02	1.5E-02	4.9.E-03	7.3.E-03	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	4.1E-03	NA	2.8.E-03	NA	1.9E-03	4.1E-03	3.1E-03	8.3E-04	4.1E-04
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	1.2.E-02	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9.E-03	4.1E-03	NA	2.8.E-03	1.2.E-02	1.9E-03	4.1E-03	9.3E-03	8.3E-04	1.2E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02

PR-CALUX	Agonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	NA	7.1.E-03	NA	1.1E-03	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	NA	2.3.E-03	NA	NA	4.5E-04	7.7E-04	2.3E-02	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	NA	NA	1.8E-03	5.0E-03	1.5E-02	4.9E-04	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	NA	NA	1.9E-03	1.2E-03	3.1E-03	[illegible]	[illegible]
Common cormorant-Liver	1.9.E-03	NA	NA	2.8.E-03	NA	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9.E-03	NA	NA	NA	1.2E-02	1.9E-03	4.1E-03	3.1E-03	[illegible]	1.2E-02
Finless porpoise-Liver	1.9.E-03	NA	NA	NA	1.2E-02	1.9E-03	4.1E-04	9.3E-03	[illegible]	1.2E-02

DR-CALUX	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	[illegible]	[illegible]	NA	NA	[illegible]	NA	NA	2.1.E-02	3.6.E-04	NA
Baikal seal (1992)-Blubber	[illegible]	[illegible]	NA	NA	1.0E-03	NA	NA	7.7.E-03	3.4.E-04	NA
Baikal seal (2005)-Liver	[illegible]	[illegible]	5.0.E-03	NA	2.2E-03	NA	NA	NA	4.9.E-04	NA
Baikal seal (1992)-Liver	[illegible]	[illegible]	NA	NA	1.2E-04	NA	NA	3.1.E-03	[illegible]	NA
Common cormorant-Liver	[illegible]	[illegible]	NA	NA	NA	NA	NA	9.3.E-03	[illegible]	1.2.E-02
Raccoon dog-Liver	[illegible]	[illegible]	NA	NA	NA	NA	NA	3.1.E-03	[illegible]	1.2.E-02
Finless porpoise-Liver	[illegible]	NA	9.3.E-04	[illegible]	4.1E-03	NA	[illegible]	NA	NA	NA

PPARg1-CALUX	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	1.1E-03	7.1.E-03	[illegible]	3.6.E-04	4.5.E-04	NA	NA	NA	NA
Baikal seal (1992)-Blubber	4.5.E-04	[illegible]	2.3.E-03	[illegible]	3.4.E-04	4.5.E-04	NA	NA	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	1.5E-03	1.5.E-02	4.9.E-04	[illegible]	1.8.E-03	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	4.1.E-04	3.1.E-03	[illegible]	[illegible]	1.9.E-03	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	[illegible]	9.3.E-04	[illegible]	4.1.E-03	1.9.E-03	NA	NA	NA	NA
Raccoon dog-Liver	1.9.E-03	1.2E-03	3.1.E-03	[illegible]	1.2.E-02	1.9.E-03	NA	NA	NA	NA
Finless porpoise-Liver	1.9.E-03	[illegible]	3.1.E-03	[illegible]	1.2.E-03	1.9.E-03	NA	NA	NA	NA

PPARg2-CALUX	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak	Persistent fraction	Strong (Crude hydrophobic fraction)	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	1.1E-03	7.1.E-03	[illegible]	[illegible]	NA	NA	NA	NA	NA
Baikal seal (1992)-Blubber	4.5.E-04	[illegible]	7.7.E-04	[illegible]	[illegible]	NA	NA	NA	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	1.5E-03	5.0.E-03	[illegible]	[illegible]	NA	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	[illegible]	9.3.E-04	[illegible]	[illegible]	NA	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	[illegible]	3.1.E-04	[illegible]	1.2.E-03	NA	NA	NA	NA	NA
Raccoon dog-Liver	1.9.E-03	4.1E-04	3.1.E-04	[illegible]	4.1.E-03	NA	NA	NA	NA	NA
Finless porpoise-Liver	1.9.E-03	[illegible]	3.1.E-04	[illegible]	4.1.E-04	NA	NA	NA	NA	NA

NA: Not analyzed due to ago/antagonistic response	Response at more than 1.0E-02 g-wet/well
Not detected at indicated dose	Response at 1.0E-2 to 1.0E-03 g-wet/well
Cytotoxicity at indicated dose	Response at 1.0E-3 to 3.0E-04 g-wet/well
Synergistic response at indicated dose	Response at less than 3.0E-04 g-wet/well

Netherlands
UV crèmes used of tourists
ER, AR and PR CALUX

TOXICOLOGICAL SCIENCES 83, 264–272 (2005)
doi:10.1093/toxsci/kfi035
Advance Access publication November 10, 2004

Interaction of Polycyclic Musks and UV Filters with the Estrogen Receptor (ER), Androgen Receptor (AR), and Progesterone Receptor (PR) in Reporter Gene Bioassays

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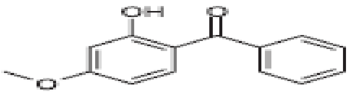
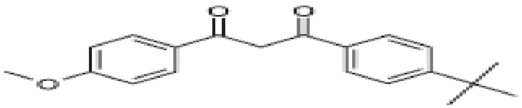
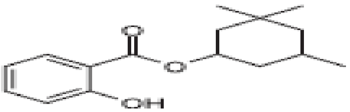
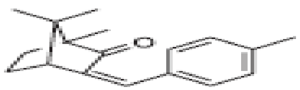
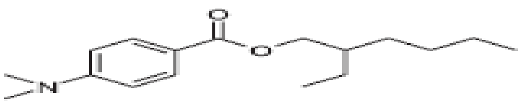
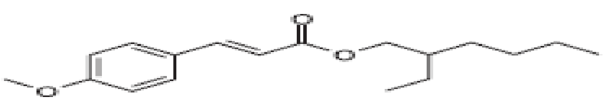
ER, AR and PR CALUX results in UV-filters

UV-filters	
Benzophenone-3 (Bp-3)	
3-Benzylidene camphor (3-BC)	
Butyl methoxydibenzoylmethane (B-MDM)	
Homosalate (HMS)	
4-Methylbenzylidene camphor (4-MBC)	
Octyl dimethyl-*p*-aminobenzoic acid (OD-PABA)	
Octyl-methoxycinnamate (OMC)	

Conclusions about UV filters

- Some UV filters exert effects in different receptors
- The UV filters have been found to be mainly ER agonists and AR/PR antagonists
- The here tested UV filters are found already in mother milk and maybe also found in breast tissue or breast cancer tissue
- In case of anti-AR the effects on wildlife are known of DDE, DDT or vinclozolin
- In case of PR not much is so far known and will need further investigation
- Also effects regarding TR, GR or PPARγ haven’t been so far investigated…

Take home message

- Multiple biodetectors or Effect based analysis tools have been evaluated in many countries and various projects for many environmental applications

- Endocrine disrupters are not only female hormones (estrogens):
  Male and other important ED endpoints needs more focus

- International issues with complex mixture cocktails and multi-pollutants effects are increasing

- ….no effect levels in state-of-the art WWTPs can be achieved for 56 CALUX tests and their mode of actions….please try your WWTPs..

- ..and now we are open for any discussions with you...

Invitation for the8th BioDetectors Conference in Torino, Italy on 25/26th Sept 2014

Questions?