Bioanalysis highlights 2014

TOXICOLOGICAL PROFILING BY CALUX PANEL

Behnisch PA, van der Burg B and Bram Brouwer

BioDetection Systems b.v.
Amsterdam, The Netherlands

BE-Basic

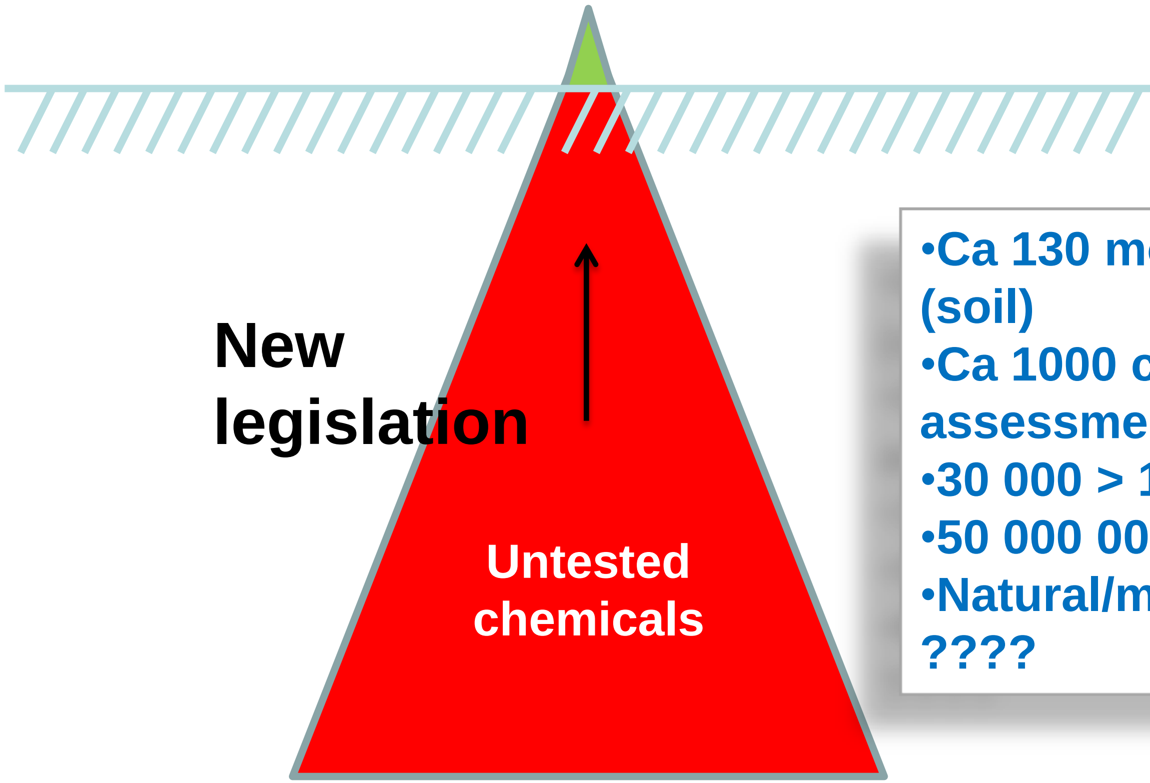
Questions

- How **in vitro toxic** are dioxins/PCBs compared to pesticides, EDCs, metals & others?
- How can we **speed up** to eliminate the in vitro toxic effects of pollutants ?
- How can we **know** more about the **unknown** *in vitro* effects of chemical pollutants ?

BE-Basic

Chemical monitoring alone is insufficient

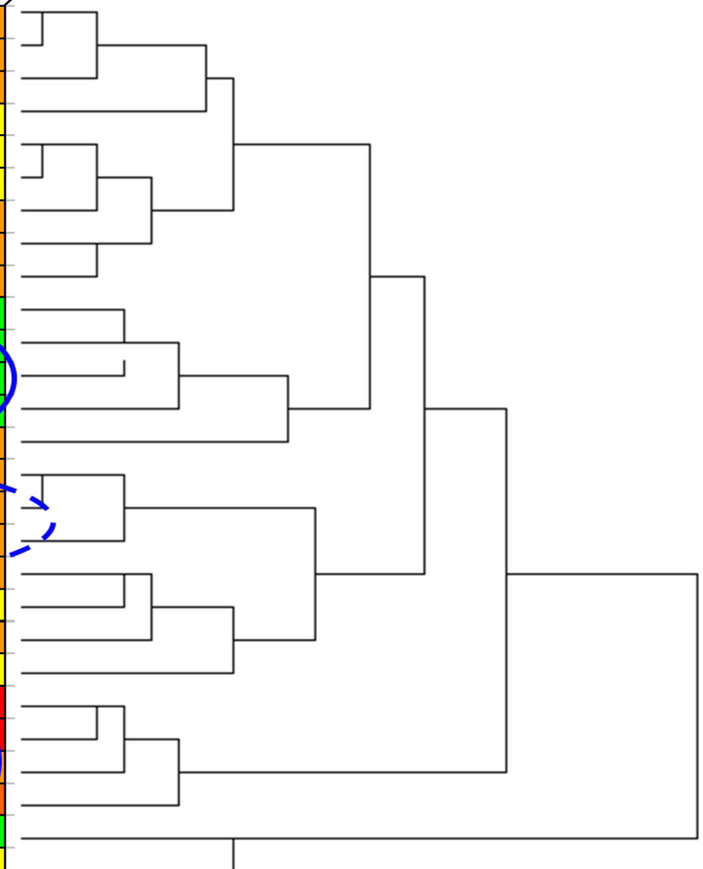
Tested chemicals → Monitored chemicals ↑↑



- **Ca 130 monitored (soil)**
- **Ca 1000 complete risk assessment**
- **30 000 > 1 ton**
- **50 000 000 CAS**
- **Natural/metabolites> ????**

Heat Map of BFRs (Hamers et al)

Compound	PR ago	AR ago	TScreen anta	TTR	E2SULT	ER ago	DR anta	ER anta	DR ago	TScreen ago	PR anta	AR anta
BDE39	1	1	1	1	1	1	2	1	1	1	2	3
BDE99	1	1	1	1	1	1	2	1	2	1	2	3
BDE127	1	1	1	2	2	1	3	1	1	2	2	3
BDE185	1	1	1	3	1	1	1	2	1	1	3	2
HBCDD TM	1	1	1	1	1	1	2	2	1	3	3	2
HBCDD beta	1	1	1	2	1	1	2	2	1	3	2	2
HBCDD gamma	1	1	1	1	1	1	2	3	1	4	3	3
BDE28	1	1	1	1	2	2	3	1	1	4	2	3
HBCDD alpha	1	1	1	2	1	1	3	1	1	4	3	3
BDE209	1	1	1	1	2	1	1	1	1	1	1	1
TBBPA-DBPE	1	1	1	3	3	1	1	1	1	1	1	1
BDE169	1	1	1	2	2	1	3	1	1	1	1	1
BDE206	1	1	3	1	3	1	1	1	1	1	1	1
BDE47	1	1	1	2	4	2	3	1	1	1	2	3
BDE190	1	1	1	3	3	1	1	3	3	1	3	3
6OH-BDE47	1	1	1	4	3	1	1	4	3	1	3	3
BDE181	1	1	1	3	1	1	1	3	3	1	3	3
BDE79	1	1	1	1	1	2	1	2	3	1	2	3
BDE153	1	1	1	1	1	1	1	1	4	1	3	2
BDE38	1	1	1	2	1	3	1	1	4	2	2	3
BDE183	1	1	1	1	2	1	1	3	3	3	3	2
BDE19	1	1	1	1	3	3	1	1	1	3	4	5
BDE100	1	1	1	1	2	3	2	1	1	4	3	5
BDE155	1	1	1	1	2	3	1	1	1	3	3	3
BDE49	1	1	1	2	3	3	2	1	2	5	3	4
TBBPA	1	1	1	5	5	1	1	1	1	3	1	1
246-TBP	1	1	1	5	4	1	1	3	1	1	2	2

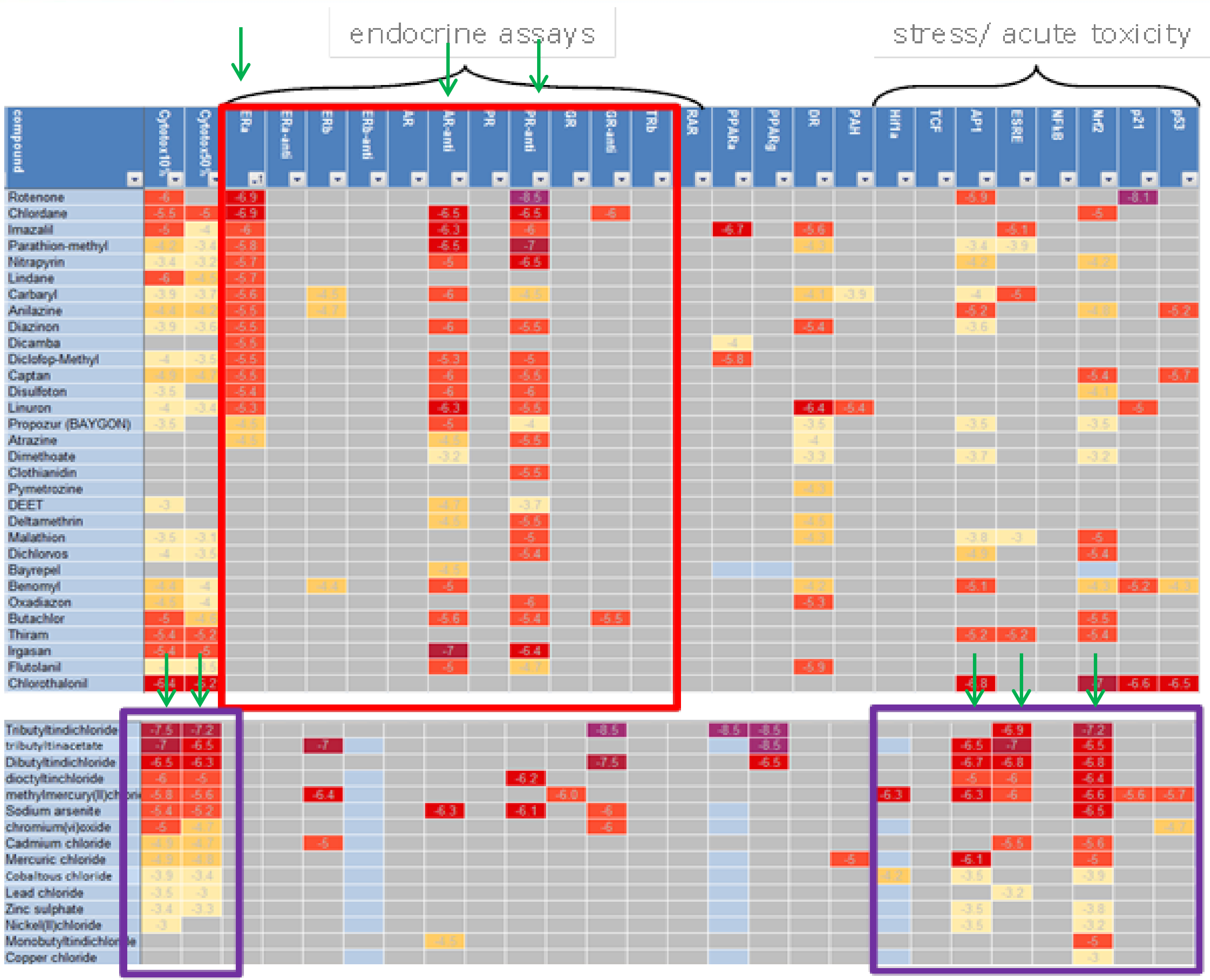


Heat Map of PFTs (Behnisch et al. DIOXIN 2012)

PFAA	Molecule	REP [1]	PPARα	PPARγ
Perfluoro-butanoic acid (PFBA)	$C_4F_7O_2^-$	0.26	0.17	< 2-fold induction
Perfluoro-pentanoic acid (PFPeA)	$C_5F_9O_2^-$	0.50	0.22	< 2-fold induction
Perfluoro-hexanoic acid (PFHxA)	$C_6F_{11}O_2^-$	0.41	0.38	< 2-fold induction
Perfluoro-heptanoic acid (PFHpPA)	$C_7F_{13}O_2^-$	0.89	2.1	< 2-fold induction
Perfluoro-octanoic acid (PFOA)	$C_8F_{15}O_2^-$	1	**1**	< 2-fold induction
Perfluoro-nonanoic acid (PFNA)	$C_9F_{17}O_2^-$	0.61	0.55	< 2-fold induction
Perfluoro-decanoic acid (PFDA)	$C_{10}F_{19}O_2^-$	0.37	Not active	< 2-fold induction
Perfluoro-undecanoic acid (PFUnDA)	$C_{11}F_{21}O_2^-$	0.15	Not active	< 2-fold induction
Perfluoro-dodecanoic acid (PFDoA)	$C_{12}F_{23}O_2^-$	Not active	Not active	< 2-fold induction
Perfluoro-tridecanoic acid (PFTrA)	$C_{13}F_{25}O_2^-$		Not active	< 2-fold induction
Perfluoro-tetradecanoic acid (PFTeDA)	$C_{14}F_{27}O_2^-$		Not active	< 2-fold induction
Perfluoro-butane-sulfonic acid (PFBS)	$C_4F_9O_3S^-$		Not parallel curve	< 2-fold induction
Perfluoro-hexane-sulfonic acid (PFHxS)	$C_6F_{13}O_3S^-$	0.41	< 2-fold induction	0.61
Perfluoro-octanesulfonic acid (branched-PFOS)	$C_8F_{17}O_3S^-$	0.26	< 2-fold induction	0.40
Perfluoro-octanesulfonic acid (linear-PFOS)	$C_8F_{17}O_3S^-$		< 2-fold induction	**1**

Dutch Be-Basic Project

Example CALUX profiling: pesticides vs heavy metals



Clearly different profiles!

pesticides:
- endocrine activity

heavy metals:
- acute toxicity
- general/oxidative stress

Example CALUX profiling:
REPs for CALUX panel of Dirty Dozen

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

Group	compound	ERa	AR-anti	PR-anti	GR-anti	DR	PAH
Pesticide POPs	Chlordane	8.7E-06	0.47	1.9E-04	5.0E-04		
Pesticide POPs	p,p'-DDT	3.5E-06	1.50	6.0E-05	1.6E-04		
Pesticide POPs	Dieldrin	6.9E-07	1.50	6.0E-04	5.0E-05		
Pesticide POPs	Endrin	3.5E-07	1.50	6.0E-04			
Pesticide POPs	Heptachlor	1.7E-05	1.50	6.0E-05			
Pesticide POPs	Hexachlorobenzene	3.5E-06	0.15	6.0E-05		5.0E-08	
Pesticide POPs	Mirex					7.9E-09	
Pesticide POPs	Toxaphene	3.5E-07	0.47	1.9E-04	1.6E-04		
Non-pest. POPs	PCB118		1.50	1.9E-04		6.3E-06	1.6E-03
Non-pest. POPs	PCB126		0.47	6.0E-05		1.6E-02	5.0
Non-pest. POPs	PCB128		1.50	1.9E-04			
Non-pest. POPs	PCB156	1.1E-06	0.15	6.0E-05		5.0E-06	3.2E-03
Non-pest. POPs	TCDD					1.0	500
Non-pest. POPs	Furan						1.3E-06

Clearly different profiles!

pesticides:
- endocrine activity

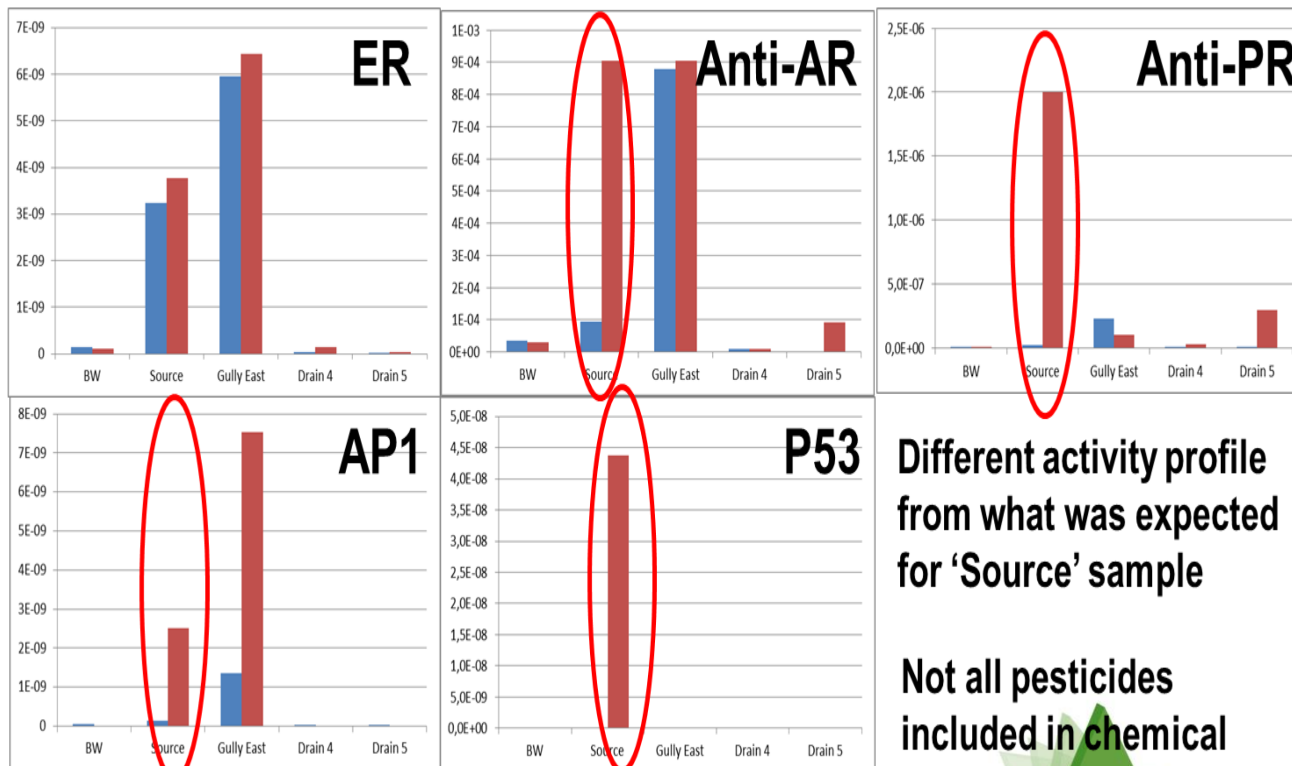
PCB/PCDD/Fs:
- AhR receptor activity
- anti-AR, anti-PR

Values indicate relative potency (REP) values compared to the reference compound activity. Yellow -> red = increasing relative potency. Reference compounds: ERa; E2. AR-anti; flutamide. PR-anti and GR-anti; Ru486. DR; TCDD. PAH; Benzo-a-pyrene.

Chemical pattern vs Tox patterns

- 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 xxxxxxxx	?????	??



Expected

Measured

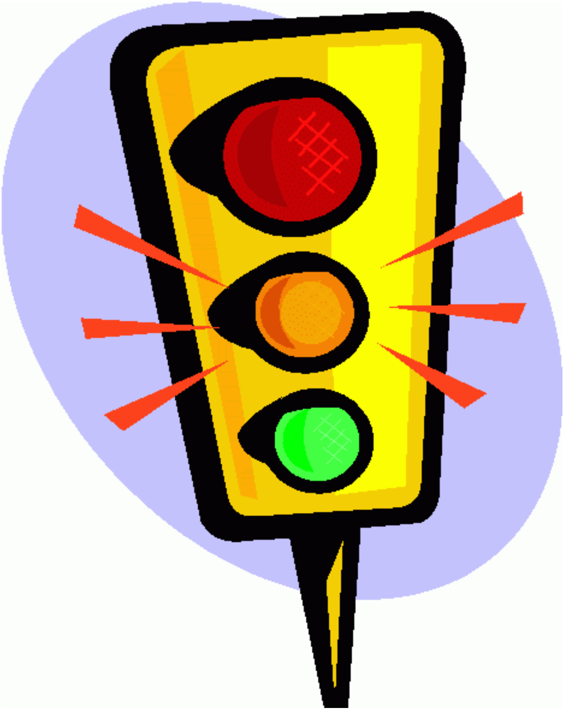
Different activity profile from what was expected for 'Source' sample

Not all pesticides included in chemical analysis!

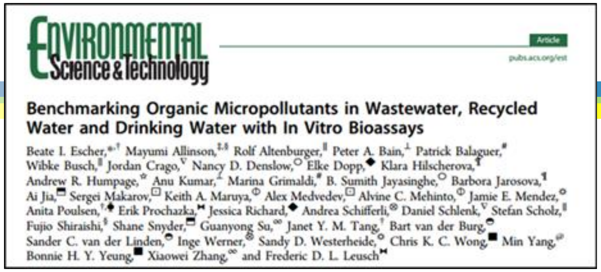
EU DEMEAU Project

DISSEMINATION – Bioassays in water quality monitoring

- **PRE-SCREENING technique → monitoring bioactive micropollutants**
- **COST-EFFECTIVE complementary tool to chemical analysis**
- **Incorporates MIXTURES and UNKNOWNS**



Encouraging examples


Environmental Science & Technology

Article

pubs.acs.org/est

Benchmarking Organic Micropollutants in Wastewater, Recycled Water and Drinking Water with In Vitro Bioassays

Beate I. Escher, Mayumi Allinson, Rolf Altenburger, Peter A. Bain, Patrick Balaguer, Wibke Busch, Jordan Crago, Nancy D. Denslow, Elke Dopp, Klara Hilscherova, Andrew R. Humpage, Anu Kumar, Marina Grimaldi, B. Sumith Jayasinghe, Barbora Jarosova, Ai Jia, Sergei Makarov, Keith A. Maruya, Alex Medvedev, Alvine C. Mehinto, Jamie E. Mendez, Anita Poulsen, Erik Prochazka, Jessica Richard, Andrea Schifferli, Daniel Schlenk, Stefan Scholz, Fujio Shiraishi, Shane Snyder, Guanyong Su, Janet Y. M. Tang, Bart van der Burg, Sander C. van der Linden, Inge Werner, Sandy D. Westerheide, Chris K. C. Wong, Min Yang, Bonnie H. Y. Yeung, Xiaowei Zhang, and Frederic D. L. Leusch

- Dutch & Australian case studies

Screening toolbox

Relevant endpoints	B. Escher et. al	BE-Basic case study
Xenobiotic metabolism	PXR activation AHR activation CAR	DR/PAH-CALUX
Hormone-mediated MoA	Estrogenicity Anti-androgenicity Glucocorticoid activity Progestagenic activity Thyroid activity	Erα-CALUX AR-CALUX PR-CALUX GR-CALUX TRβ-CALUX RAR-CALUX
Reactive MoA	Mutations (AMES, SOS) DNA repair (umuC) DNA damage response (Micronucleus)	P53-CALUX P53 S9+ CALUX (?)
Adaptive stress response	Oxidative stress pathway	Nrf2-CALUX
Developmental toxicity	Preimplantation toxicity Embryonic development Placenta	ZFET
Lipid metabolism	PPARα, PPARγ	PPARα, PPARγ, PPARδ
Photosynthesis	Photosynthesis	-
General response	Cytotoxicity Viability Vibrio fischeri (Microtox) Algae growth	Cytotoxicity Cytotoxicity S9+

Bottlenecks & solutions: first step
…to identify the relevant toxic endpoints of all relevant WFD compounds

➢ **Change conventional monitoring practice**

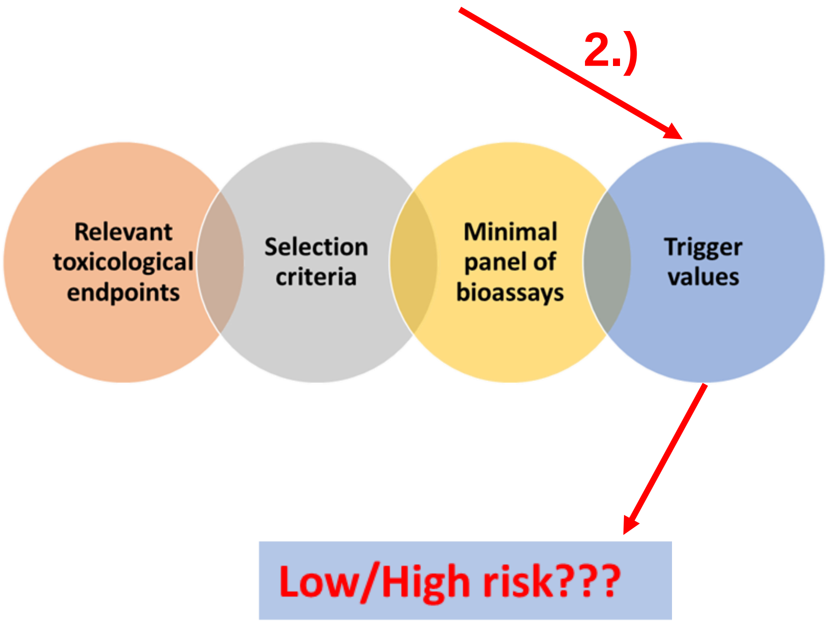
CALUX screening of the WFD compounds (n=33+8)

Group	Compound name	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
PRIORITY SUBSTANCES (n=20)	Alachlor																											
	Atrazine	-3	>-3.5	-5	>-3.5	>-3.5	>-3.5	>-3.5	-5	>-3.5	-6	>-3.5	>-3.5	>-3.5	>-3.5		>-3.5	>-3.5	-4	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5
	Benzene																											
	Chlorfenvinphos																											
	Chlorpyrifos-ethyl																											
	1,2-Dichloroethane																											
	Dichloromethane																											
	Di(2-ethylhexyl) phthalate (DEHP)	>-4	>-4	-6	>-4			>-4	>-4		-5																	
	Diuron																											
	Fluoranthene																											
	Isoproturon																											
	Lead and its comp.																											
	Naphthalene																											
	Nickel and its comp.																											
	4-n-octylphenol	-5	-4	-6	>-4.7			>-4.7	-6		-6																	
	4-tert-octylphenol	-6	-5	-8	>-5.5			>-5.5	-6		-6																	
	Pentachlorophenol	-5	-4	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	-6	>-4.3	>-4	>-4	>-4	>-4	>-4	>-4	>-4	>-4
	Simazine																											
	Trichlorobenzenes																											
	Trichloromethane																											
	Trifluralin																											
PRIORITY HAZARDOUS SUBSTANCES (n=13)	Anthracene dibenzo[a,h]	-4	>-4	>-5	>-5	-8		>-5	>-5	>-5	>-5	>-5	>-5	>-5	>-5			-5	-8	-10		>-4	>-4	>-4	>-4	>-4	>-4	>-4
	Pentabromo-diphenylether (6)																											
	Cadmium and its comp.																											
	C10-13-chloroalkanes																											
	Endosulfan	-5	-5	-7	>-5	>-5		>-5	-7	>-5	-7	>-5	>-5	>-5	>-5		>-5	>-5	>-5	>-5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5
	Hexachlorobenzene	>-5	>-5	-7	>-5	>-5	>-5	>-5	-6	>-5	-6	>-5	>-5	>-5	>-5	>-5	>-5	>-5	-5	>-5	>-5	>-5	>-5	>-5	>-5	>-5	>-5	>-5
	Hexachlorobutadiene																											
	Hexachlorocyclohexane																											
	Mercury																											
	Nonylphenol techn mix																											
	4-n-Nonylphenols	-5	-5	-5	>-4.9	-6	>-4.9	>-4.9	-7	>-4.9	-6	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.7	>-4.7	>-4.7	-5	>-4.7	>-4.7	>-4.7	>-4.7
	Pentachlorobenzene																											
	Benzo(a)pyrene	-3	>-3	-6	>-3	-4	>-3	>-3	-7	>-3	-6	>-3	>-3	>-3	>-3	>-3	>-3	>-3	-8	-10	>-3	>-3	>-3	-4	>-3	>-3	-5	-5
	Benzo(b)fluoranthene																											
	Benzo(g,h,i)perylene																											
	Benzo(k)fluoranthene																											
	Indeno(1,2,3-cd)pyrene																											
	Tributyltin-cation																											
SUBSTANCES UNDER REVIEW	AMPA																											
	Bentazon																											
	Bisphenol-A	-4	-4	-7	>-4	-7		>-4	-7	>-4	-6	>-4	-5	>-4	>-4		>-4	>-4	>-4	>-4	>-3.7	>-3.7	>-3.7	-4	>-3.7	>-3.7	>-3.7	>-3.7
	Dicofol																											
	EDTA																											
	Free cyanide																											
	Glyphosate																											
	Mecoprop																											
	Musk xylene																											
	PFOS																											
	Quinoxyfen																											
	TCDD	>-6.8	>-6.8	>-9	>-9	>-9		>-9	>-9	>-9	>-9	>-9	>-9	>-9	>-9			>-9	-12	-12		>-6.8	>-6.8	>-6.8	>-6.8	>-6.8	>-6.8	>-6.8
	PCB118	-5	>-4	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	-7	>-4.5	-7	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5	-7	-7	>-4	>-4	-4	>-4	>-4	>-4	>-4	>-4
	PCB126	-5	-4	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	-7	>-4.3	-6	>-4.3	>-4.3	>-4.3	>-4.3	>-4.3	-5	>-4.3	<-10.5	-10	>-4.4	>-4.4	>-4.4	-5		>-4.4	>-4.4	>-4.4
	PCB128	>-4	>-4	>-5	>-5	>-5		>-5	-7	>-5	-7	>-5	>-5	>-5	>-5			>-5	>-5	>-5		>-4	>-4	>-4	>-4	>-4	>-4	>-4
	PCB156	-5	-4	-6	>-5	>-5		>-5	-6	>-5	-6	>-5	>-5	>-5	>-5			>-5	-7	-7		>-4	-4	>-4	>-4	>-4	>-4	>-4

Identify the toxic endpoints → in line with the recent experiences (Dutch/Australian case) ??

Bottlenecks & solutions: 3nd step TEQ value approach to handle a complex mixture of endocrine disrupting chemical

- ➢ **Regulatory acceptance**



In vivo relevance / Health impacts

1.) TEQ value approach

Food safety (e.g. dioxins)
Learning from existing safety assessment practice

Environment International 55 (2013) 109–118

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]

[a] KWR Watercycle Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, The Netherlands
[b] BioDetection Systems B.V., Amsterdam, Science Park 406, 1098 XH Amsterdam, The Netherlands
[c] National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, PO Box 1, 3720 BA Bilthoven, The Netherlands

Assay	Trigger value
ER-CALUX	3.8 ng E2-eq / L
AR-CALUX	11 ng DHT-eq / L
GR-CALUX	3.8 ng DEX-EQ / L
PR-CALUX	3.8 ng Org2058-eq / L

Anu Kumar and Peter Bain from CSIRO, Australia: Endocrine activity in wastewater extracts during treatment

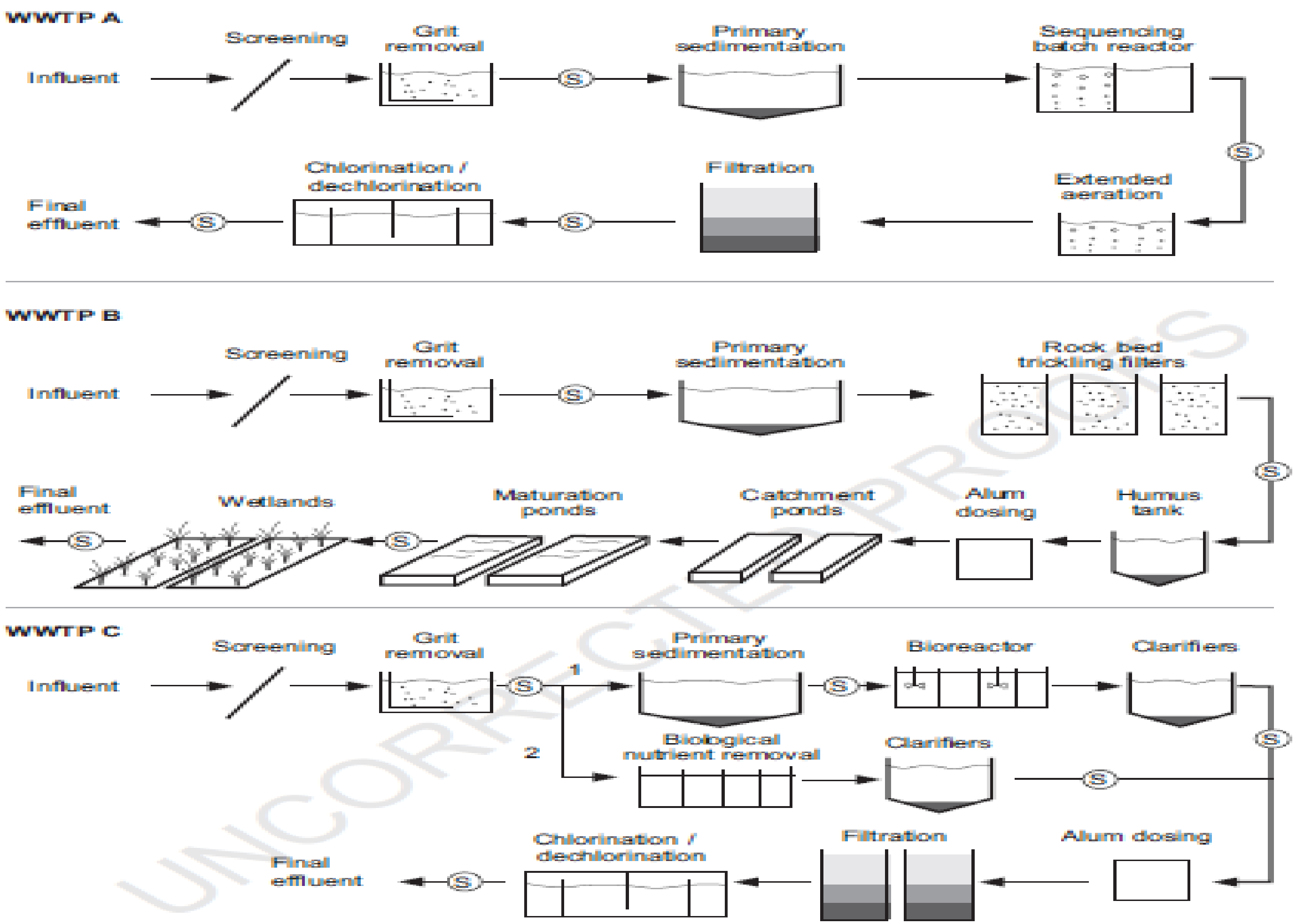
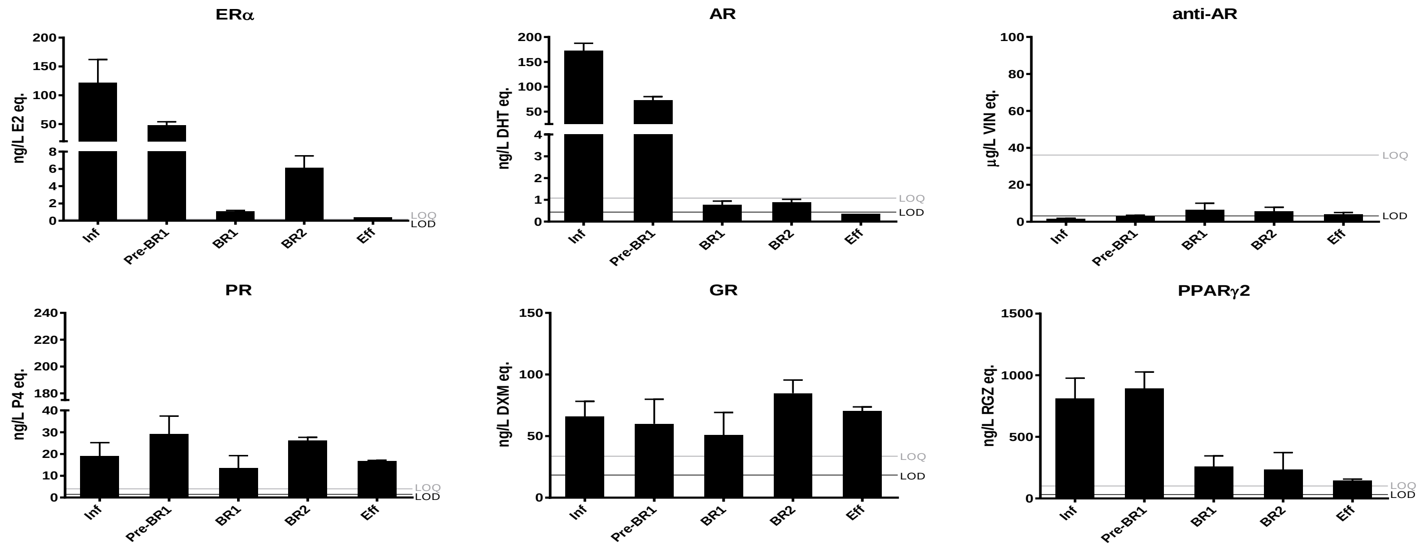


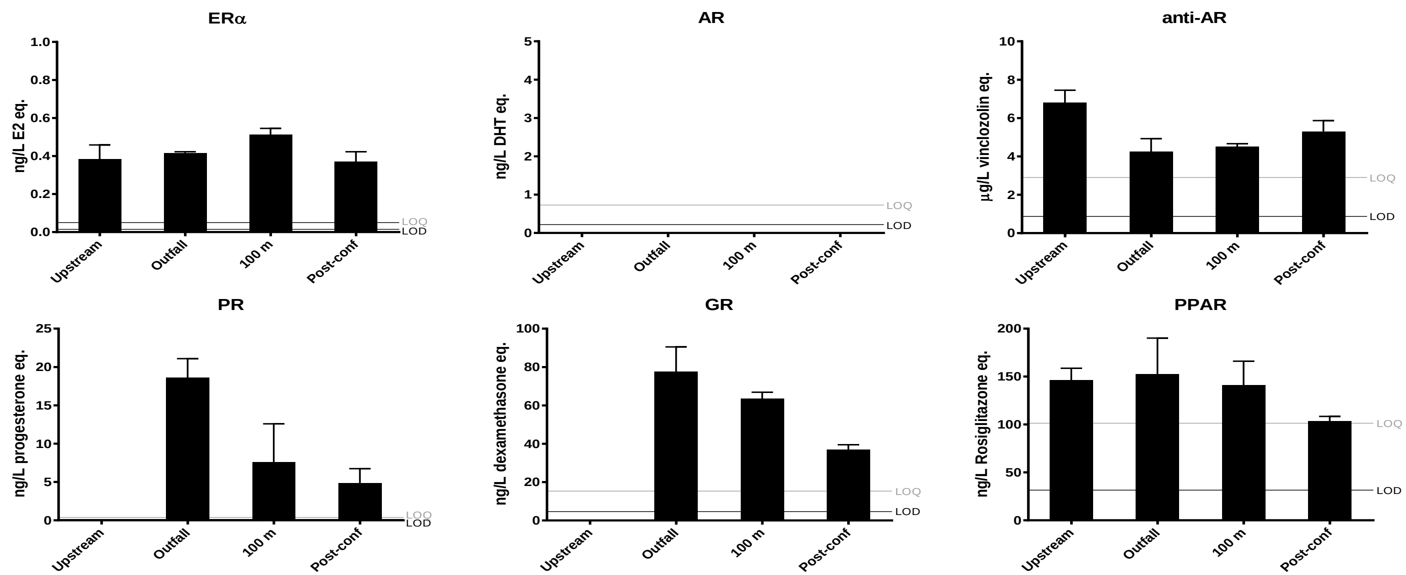
Figure 1. (A–C) Schematic diagrams showing the main treatment processes at each wastewater treatment plant (WWTP). Processing of solids has been omitted for clarity.

Anu Kumar and Peter Bain from CSIRO, Australia: Endocrine activity in wastewater extracts during treatment (CALUX bioassays)

WWTP C



WWTP B



ER CALUX vs Chemical analysis (E1, E2, EE2)

Table 4. Comparison of predicted and measured total estrogenicity at different stages of wastewater treatment[a]

WWTP	Predicted total estrogenicity (ng/L E2 equivalents)	Observed in vitro estrogenicity (ng/L E2 equivalents ± SD)	Proportion accounted for by predicted value % (range)
A			
Influent	30.3	49.0 ± 2.83	62 (58–66)
Bioreactor	3.83	1.55 ± 0.5	247 (187–363)
Filter	2.61	1.65 ± 0.07	158 (151–165)
Effluent	3.00	5.50 ± 4.7	55 (29–361)
B			
Influent	19.5	28 ± 22	70 (39–354)
Trickling filter	7.54	5.91 ± 0.89	128 (111–150)
Wetland	2.71	3.33 ± 3.0	81 (43–860)
Effluent	3.97	1.35 ± 0.21	294 (254–348)
C			
Influent	22.2	122 ± 40	18 (14–27)
Post bioreactor	10.9	47.5 ± 6.4	23 (20–27)
Aerobic digester	1.95	1.10 ± 0.07	177 (166–189)
Clarifier effluent	3.64	6.1 ± 1.4	60 (48–78)
Effluent	1.80	0.39 ± 0.01	463 (461–454)

Detection and Identification of Endocrine Active Substances in Food Packaging

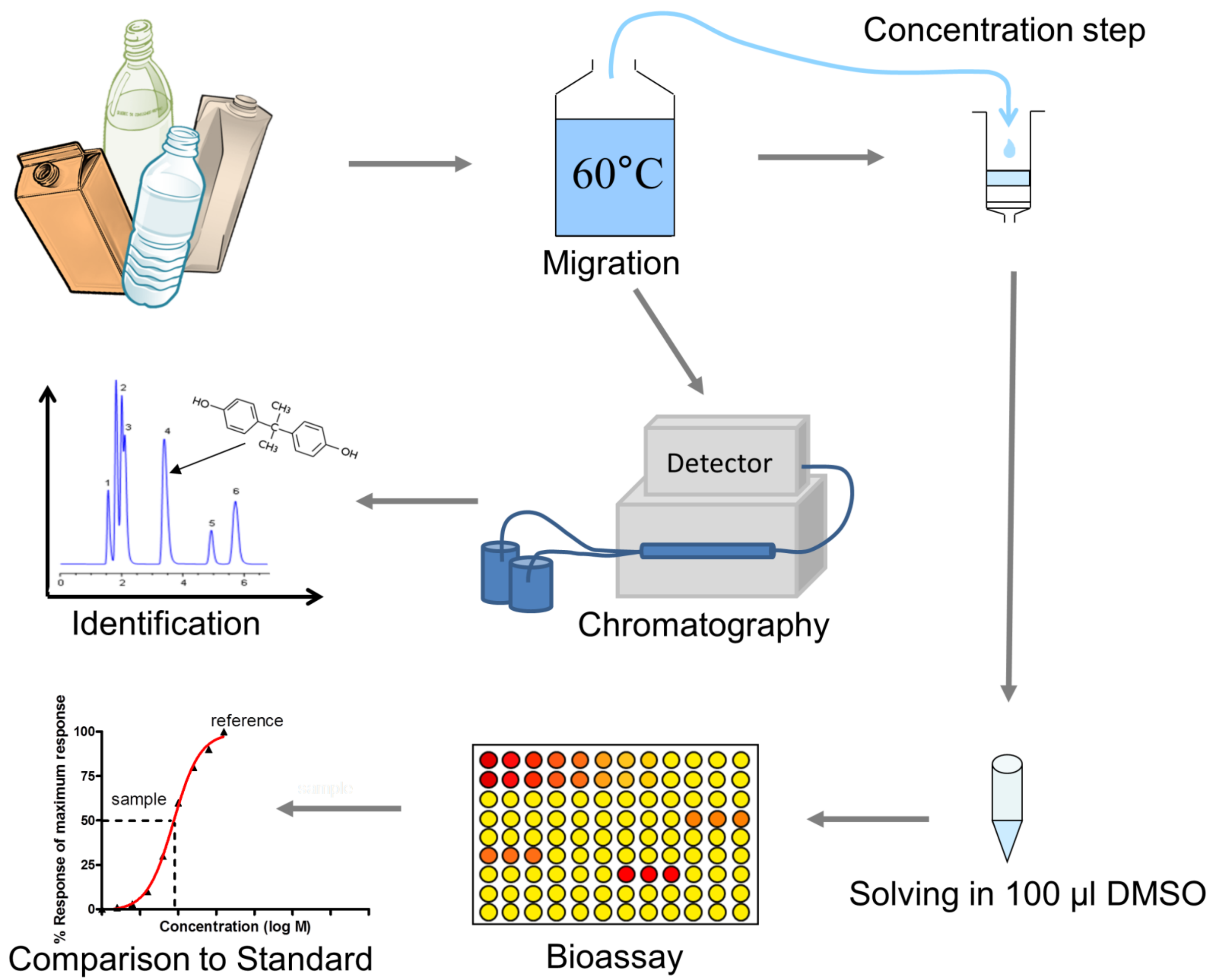
OFI Austrian Research Inst. for Chem. and Tech.

Christian Kirchnawy

and

Johannes Mertl

Mitglied bei:
AUSTRIAN COOPERATIVE RESEARCH



Concentration step
60°C
Migration
Detector
Chromatography
Identification
Solving in 100 µl DMSO
Bioassay
reference
sample
% Response of maximum response
Concentration (log M)
Comparison to Standard

More than 300 food packaging analysed for hormone activity:

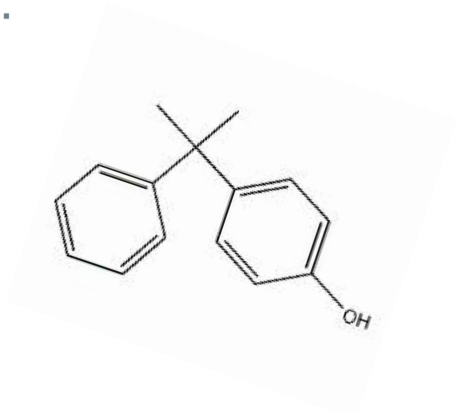
- PET, PP, PE, PS, composite films, paper, food cartons
- About 85% of analysed packaging did not show any hormone activity!
- Erα CALUX®: about 15% estrogen active, activities lower than expected, based on studies on mineral water
- No sample was tested androgen or thyroid hormone active
- Some of the estrogen active migrates were also antiandrogen active
- 8 out of 70 samples showed very low activities in the PPARγ CALUX®

Mostly non-intentionally added substances (NIAS):

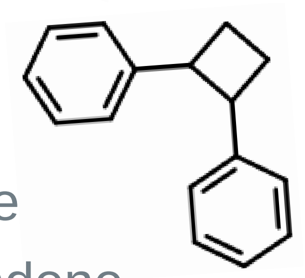
Identification by GC-MS and HPLC-MS difficult !

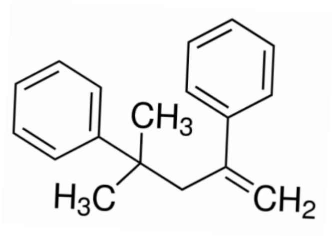
Substances identified that cause hormone activity:

- Degradation products of antioxidants
  - 2,4-dicumylphenol
  - 4-cumylphenol

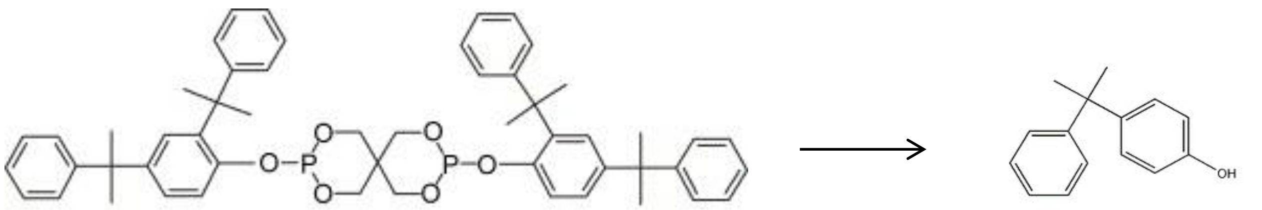


- By-products of polystyrene polymerization ?
  - 1,3-diphenylpropane
  - trans-1,2-diphenylcyclobutane
  - 1,1,3-trimethyl-3-phenyl-2H-indene
  - 2,4-diphenyl-4-methyl-1-pentene



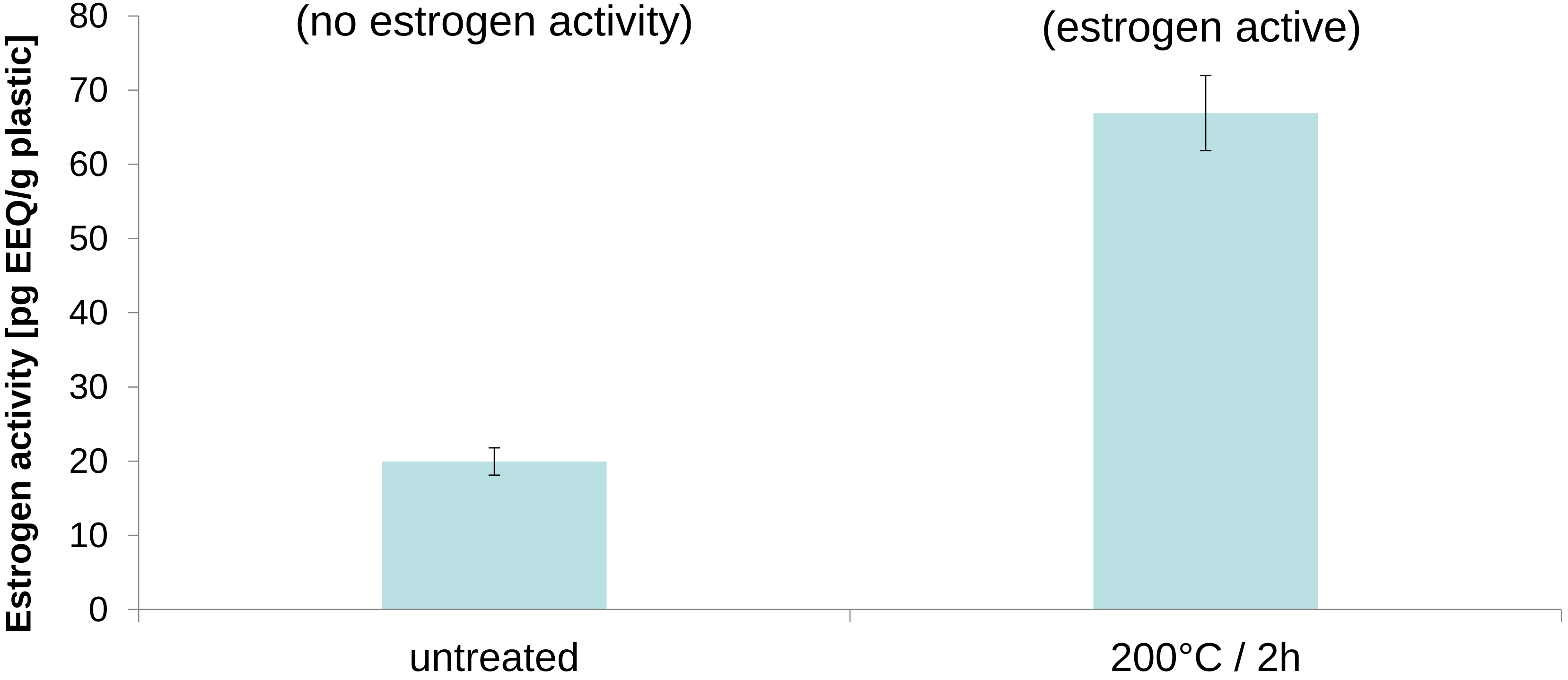


PP-Sample: Effect of processing on endocrine activity



Antioxidant Alkanox 28
(no estrogen activity)

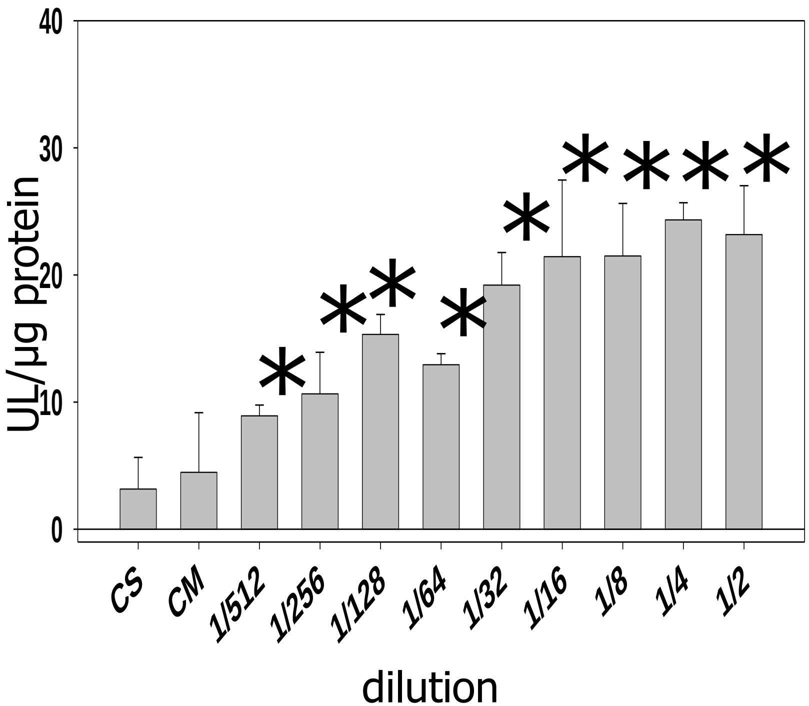
4-Cumylphenol
(estrogen active)



High oil spil activity observed in DR CALUX
(By Dr. J. Navas Team, INIA, Madrid)

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

DOSE RESPONSE CURVE IN DR-CALUX



ED50= 1/47

NOED<1/512

Summary:

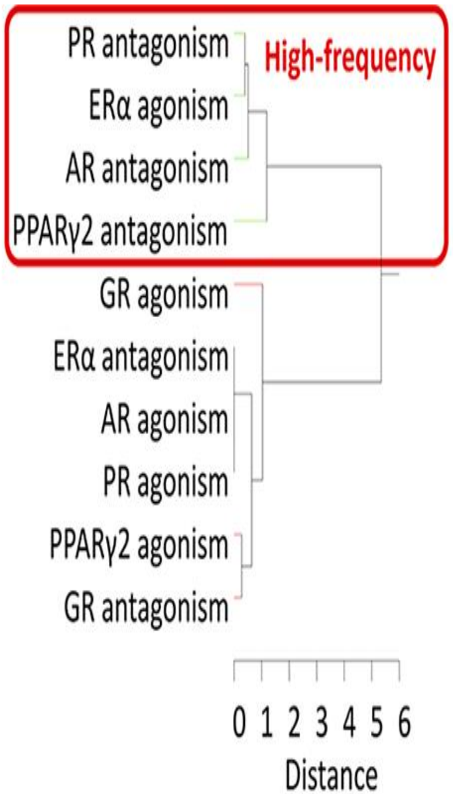
- Water soluble fraction of Prestige´s fuel induces CYP1A and activates AhR
- Such AhR activation can been associated with disruption of the estrogen receptor (ER) mechanisms of action, which can probably lead to reductions of the reproductive performance.

Monitoring of endocrine disrupting chemicals in our daily life:

House dust as marker for emerging endocrine disrupters in households

Indoor house dust (5 countries) as marker for "household EDCs and obesogens"

(Suzuki et al EST 2013)

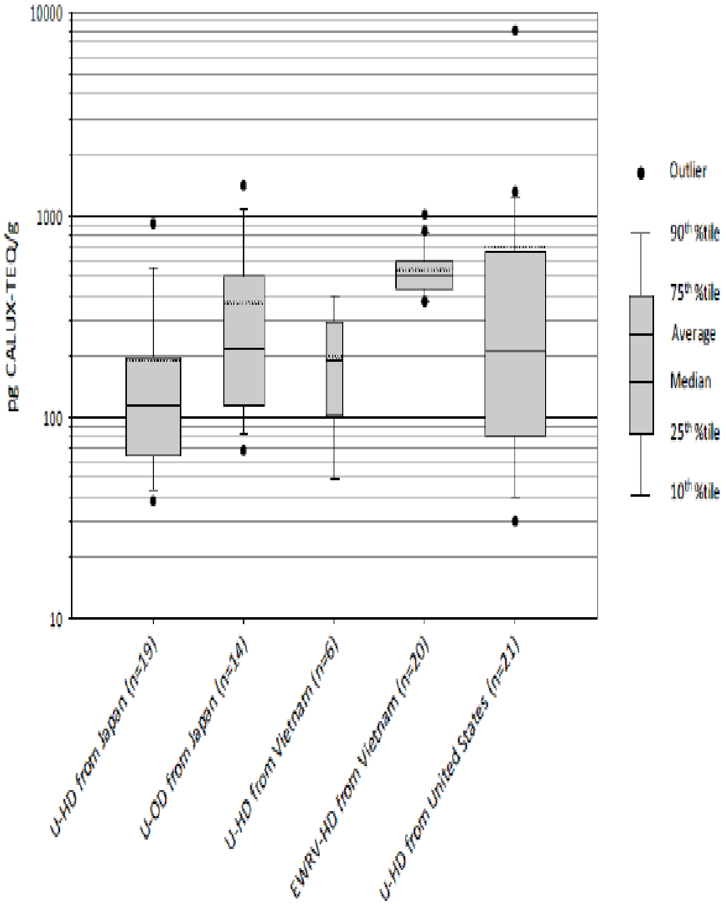


	dose eliciting agonistic effects[a]			dose eliciting antagonistic effects[b]			
dust extract	ERα	GR	PPARγ2	AR	PR	GR	PPARγ2
JPN HD	12	NE	120	120	39	120	120
JPN OD	12	NE	NE	120	38	120	120
US HD1	38	110	NE	38	38	NE	11
US HD2	38	NE	NE	38	38	NE	110
US HD3	12	40	NE	38	38	NE	120
US HD4	39	100	NE	120	120	NE	120
VN HD1	110	NE	NE	110	110	NE	NE
VN HD2	110	NE	NE	110	39	NE	NE
PHL HD1	70	NE	NE	70	23	NE	70
PHL HD2	69	NE	NE	69	69	NE	69
PHL HD3	72	NE	NE	72	72	NE	72
IND HD1	140	NE	NE	140	140	NE	NE
IND HD2	NE	NE	NE	NE	NE	NE	NE

Many compounds in house dust & many CALUX effects = many more R&D studies needed

	agonistic effects (M)				antagonistic effects (M)									
	ERα		PPARγ2		AR		ERα		PR		GR		PPARγ2	
compounds	REC_5	EC_{50}	REC_5	EC_{50}	RIC_{20}	IC_{50}	RIC_{20}	IC_{50}	RIC_{20}	IC_{50}	RIC_{20}	IC_{50}	RIC_{20}	IC_{50}
E2	1.0×10^{-12}	4.2×10^{-12}	—	—	—	—	—	—	—	—	—	—	—	—
ROS	—	—	1.0×10^{-8}	5.2×10^{-8}	—	—	—	—	—	—	—	—	—	—
FLU	—	—	—	—	1.0×10^{-7}	2.8×10^{-7}	—	—	—	—	—	—	—	—
TAM	—	—	—	—	—	—	1.0×10^{-7}	3.3×10^{-8}	—	—	—	—	—	—
RU486	—	—	—	—	—	—	—	—	7.3×10^{-11}	7.9×10^{-11}	1.0×10^{-9}	2.2×10^{-9}	—	—
GW9662	—	—	—	—	—	—	—	—	—	—	—	—	3.0×10^{-10}	8.4×10^{-10}
BDE-47	1.0×10^{-6}	NC	1.0×10^{-5}	NC	3.0×10^{-7}	5.2×10^{-7}	NE	NC	3.0×10^{-7}	1.2×10^{-6}	NE	NC	NE	NC
BDE-99	3.0×10^{-6}	NC	NE	NC	3.0×10^{-7}	9.7×10^{-7}	NE	NC	3.0×10^{-7}	1.1×10^{-6}	NE	NC	NE	NC
BDE-100	1.0×10^{-6}	5.1×10^{-6}	NE	NC	3.0×10^{-8}	9.8×10^{-8}	NE	NC	3.0×10^{-7}	3.7×10^{-7}	NE	NC	NE	NC
BDE-183	NE	NC	NE	NC	3.0×10^{-6}	3.3×10^{-6}	NE	NC	1.0×10^{-6}	1.5×10^{-6}	NE	NC	NE	NC
BDE-209	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
γ-HBCD	NE	NC	NE	NC	1.0×10^{-6}	NC	NE	NC	3.0×10^{-7}	3.8×10^{-7}	NE	NC	NE	NC
TBBPA	1.0×10^{-5}	NC	1.0×10^{-5}	NC	1.0×10^{-5}	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	NE	NC
TMP	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
TEP	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
TPrP	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
TNBP	1.0×10^{-5}	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	3.0×10^{-6}	4.2×10^{-6}	NE	NC	NE	NC
TCEP	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
TCIPP	NE	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	3.0×10^{-6}	1.1×10^{-5}	NE	NC	NE	NC
TDCIPP	3.0×10^{-6}	NC	NE	NC	1.0×10^{-6}	1.9×10^{-6}	NE	NC	3.0×10^{-7}	8.5×10^{-7}	NE	NC	NE	NC
TBOEP	NE	NC	NE	NC	NE	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	NE	NC
TPHP	1.0×10^{-6}	3.3×10^{-6}	NE	NC	3.0×10^{-6}	5.8×10^{-6}	NE	NC	1.0×10^{-6}	1.9×10^{-6}	NE	NC	NE	NC
TEHP	NE	NC	NE	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	1.0×10^{-5}	NC	NE	NC
TMPP	1.0×10^{-6}	NC	NE	NC	3.0×10^{-6}	4.1×10^{-6}	NE	NC	3.0×10^{-7}	1.4×10^{-6}	NE	NC	NE	NC
2,6-TXP	1.0×10^{-8}	8.3×10^{-8}	NE	NC	1.0×10^{-6}	2.2×10^{-6}	NE	NC	1.0×10^{-6}	1.5×10^{-6}	NE	NC	NE	NC
TOP	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC	NE	NC
2-TIPPP	NE	NC	NE	NC	1.0×10^{-6}	3.5×10^{-6}	3.0×10^{-6}	NC	1.0×10^{-6}	3.0×10^{-6}	1.0×10^{-5}	NC	NE	NC
3-TIPPP	NE	NC	NE	NC	1.0×10^{-5}	NC	NE	NC	1.0×10^{-6}	3.5×10^{-6}	NE	NC	NE	NC
4-TIPPP	NE	NC	NE	NC	1.0×10^{-5}	NC	1.0×10^{-5}	NC	3.0×10^{-6}	NC	1.0×10^{-5}	NC	1.0×10^{-6}	2.9×10^{-6}

High persistent dioxin-like activity in house dust



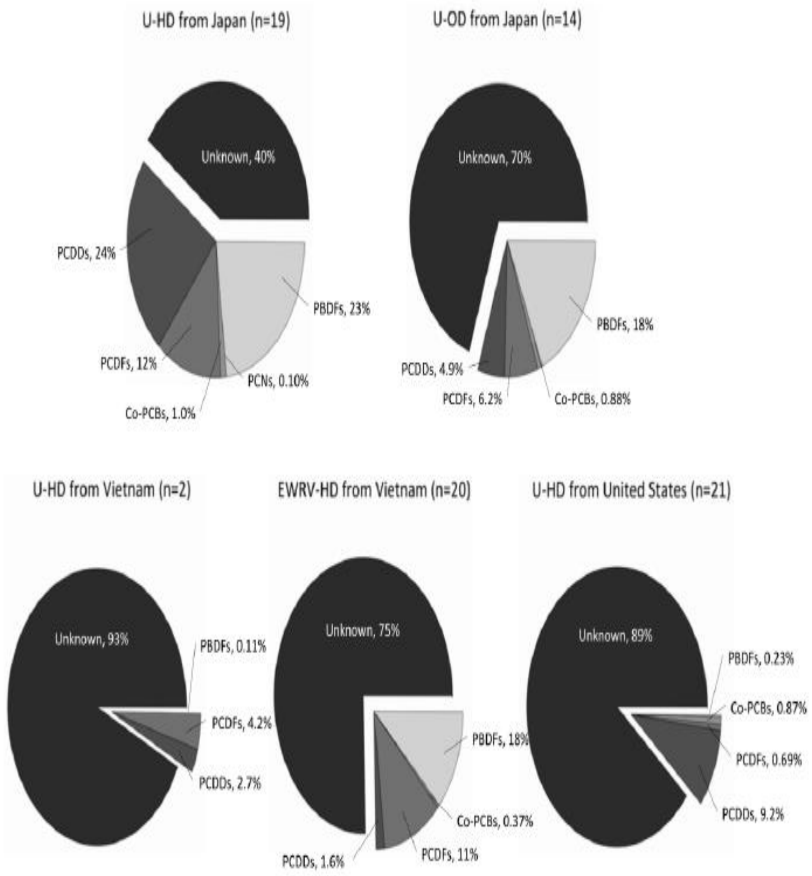


Fig (1). Comparison of dioxin-like activity (pg CALUX-TEQ/g) found in indoor dusts collected from Japan [51], Vietnam [57] and United States [58]. U-HD, urban house dust; U-OD, urban office dust; EWRV-HD, E-waste recycling village house dust.

Suzuki et al (2014), Current Organic Chemistry 18

Ant-agonistic effects more relevant than agonistic effects

(a) Composite crude extracts of indoor dusts

Compounds	Agonistic reults (REC_5, µg-dust)					Antagonistic results (RIC_{20}, µg-dust)				
	AR	ERα	PR	GR	PPARγ2	AR	ERα	PR	GR	PPARγ2
JPN U-HD	NE	12	NE	NE	120	120	NE	39	120	120
JPN U-OD	NE	12	NE	NE	NE	120	NE	38	120	120
US U-HD1	NE	38	NE	110	NE	38	NE	38	NE	11
US U-HD2	NE	38	NE	NE	NE	38	NE	38	NE	110
US U-HD3	NE	12	NE	40	NE	38	NE	38	NE	120
US U-HD4	NE	39	NE	100	NE	120	NE	120	NE	120
VN U-HD1	NE	110	NE	NE	NE	110	NE	110	NE	NE
VN U-HD2	NE	110	NE	NE	NE	110	NE	39	NE	NE
PHL U-HD1	NE	70	NE	NE	NE	70	NE	23	NE	70
PHL U-HD2	NE	69	NE	NE	NE	69	NE	69	NE	69
PHL U-HD3	NE	72	NE	NE	NE	72	NE	72	NE	72
IND U-HD1	NE	140	NE	NE	NE	140	NE	140	NE	NE
IND U-HD2	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE

(b) Flame retardants (FRs)

Compounds	Agonistic reults (REC_5, M)					Antagonistic results (RIC_{20}, M)				
	AR	ERα	PR	GR	PPARγ2	AR	ERα	PR	GR	PPARγ2
BDE-47	NE	1.0E-06	NE	NE	1.0E-05	3.0E-07	NE	3.0E-07	NE	NE
BDE-99	NE	3.0E-06	NE	NE	NE	3.0E-07	NE	3.0E-07	NE	NE
BDE-100	NE	1.0E-06	NE	NE	NE	3.0E-08	NE	3.0E-07	NE	NE
BDE-183	NE	NE	NE	NE	NE	3.0E-06	NE	1.0E-06	NE	NE
BDE-209	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
γ-HBCD	NE	NE	NE	NE	NE	1.0E-06	NE	3.0E-07	NE	NE
TBBPA	NE	1.0E-05	NE	NE	1.0E-05	1.0E-05	NE	1.0E-05	NE	NE
TMP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TEP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TPrP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TBP	NE	1.0E-05	NE	NE	NE	1.0E-05	NE	3.0E-06	NE	NE
TCEP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TCIPP	NE	NE	NE	NE	NE	1.0E-05	NE	3.0E-06	NE	NE
TDCIPP	NE	3.0E-06	NE	NE	NE	1.0E-06	NE	3.0E-07	NE	NE
TBEP	NE	NE	NE	NE	NE	NE	NE	1.0E-05	NE	NE
TPhP	NE	1.0E-06	NE	NE	NE	3.0E-06	NE	1.0E-06	NE	NE
TEHP	NE	NE	NE	NE	NE	NE	1.0E-05	NE	1.0E-05	NE
TCP	NE	1.0E-06	NE	NE	NE	3.0E-06	NE	3.0E-07	NE	NE
2,6-TXP	NE	1.0E-08	NE	NE	NE	1.0E-06	NE	1.0E-06	NE	NE
TOP	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
2-TIPP	NE	NE	NE	NE	NE	1.0E-06	3.0E-06	1.0E-06	1.0E-05	NE
3-TIPP	NE	NE	NE	NE	NE	1.0E-05	NE	1.0E-06	NE	NE
4-TIPP	NE	NE	NE	NE	NE	1.0E-05	1.0E-05	3.0E-06	1.0E-05	1.0E-06

Fig (4). Agonistic and antagonistic potencies on AR, ERα, PR, GR, and PPARγ2 of (a) composite crude extracts of indoor dusts collected from Japan (JPN), United States (US), Vietnam (VN), the Philippine (PHL) and Indonesia (IND) and (b) flame retardants (FRs) [84]. REC_5: Agonist concentration indicating 5% induction. RIC_{20}: Antagonist concentration indicating 80% induction. NE: No effect at about 70 µ g-dust in well for PHL and about 100 µ g-dust in well for JPN, US, VN and IND for indoor dusts and at 1.0E-5 M for FRs. U-HD, urban house dust; U-OD, urban office dust.