Development of a human cell-based screening panel for toxicological profiling: CALUX Highlights 2013

Behnisch PA and Brouwer B

BioDetection Systems b.v.
Amsterdam, The Netherlands

BE-Basic

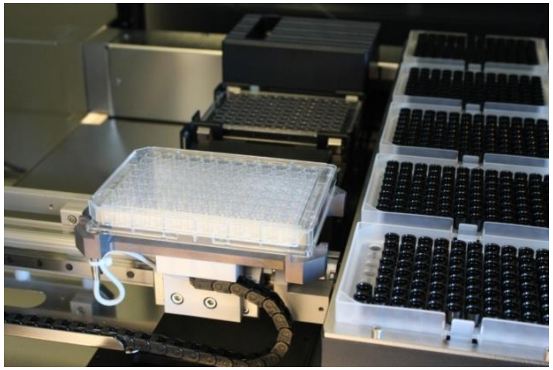
CALUX highlights 2013

CALUX highlights 2013

Automation of the CALUX assay

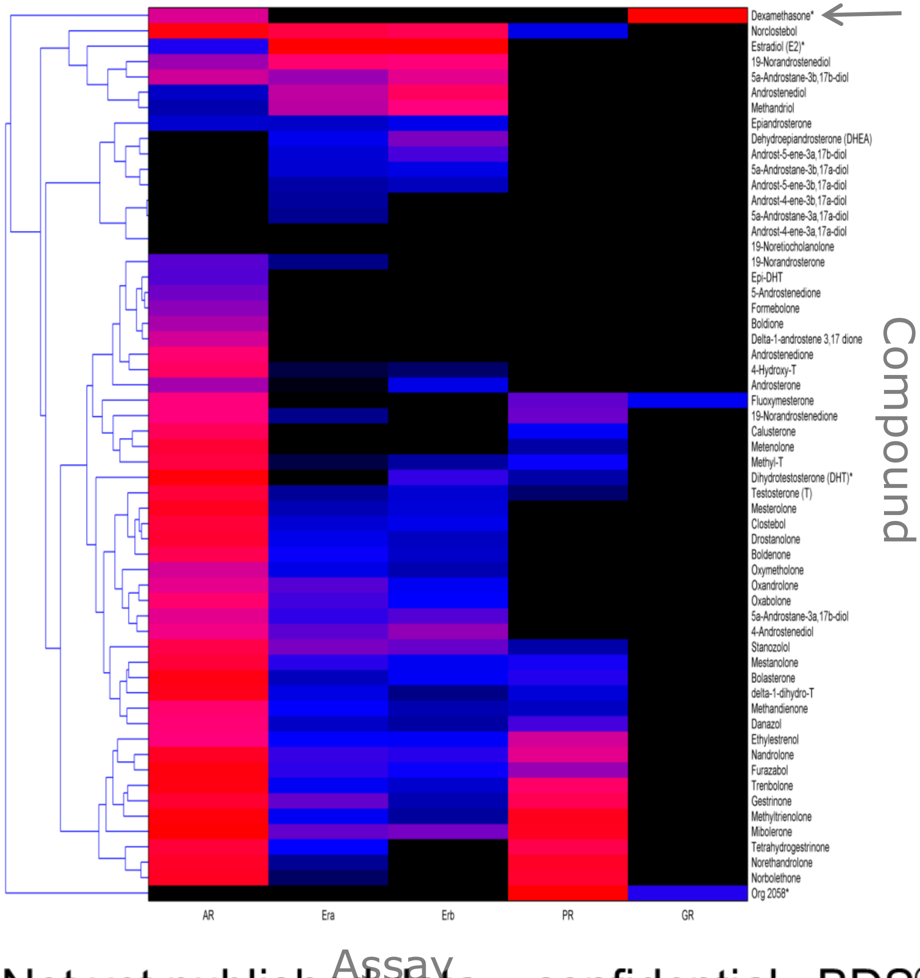
- For validation of the effect profiling principle, a lot of data is required!
  - *!Automate the CALUX assay to enable HighThroughput-screening!*

- Hamilton STARlet liquid handling robot coupled to a CO_2 incubator
- Reduced sample volume in 384-wells format
- 250 samples per week



Effect profiling of compounds with CALUX panel

- Each compound has a different *in vitro* effect profile
- *? Is it possible to relate these profiles to* in vivo *toxicity of the compounds?*



CALUX assays currently available:

Nuclear receptors		Signaling pathways	
name	**endpoint**	**name**	**endpoint**
DR CALUX	dioxins	**NFκB CALUX**	inflammation
PAH CALUX	PAHs	**p21 CALUX**	DNA damage
ERα CALUX	estrogens	**Nrf2 CALUX**	oxid. stress
ERβ CALUX	estrogens	**p53 CALUX**	DNA damage
AR CALUX	androgens	**TCF CALUX**	carcinogenesis
PR CALUX	progestins	**AP1 CALUX**	stress
GR CALUX	glucocortocoid	**HIF1α CALUX**	hypoxia
TRβ CALUX	thyroids	**ESRE CALUX**	ER stress
RAR CALUX	retinoids	**Cytotox CALUX**	cytotoxicity
PPARγ CALUX	obesogens		
PPARα CALUX	obesogens		
PPARδ CALUX	obesogens		
PXR CALUX	xenobiotics		
LXR CALUX	oxysterols		

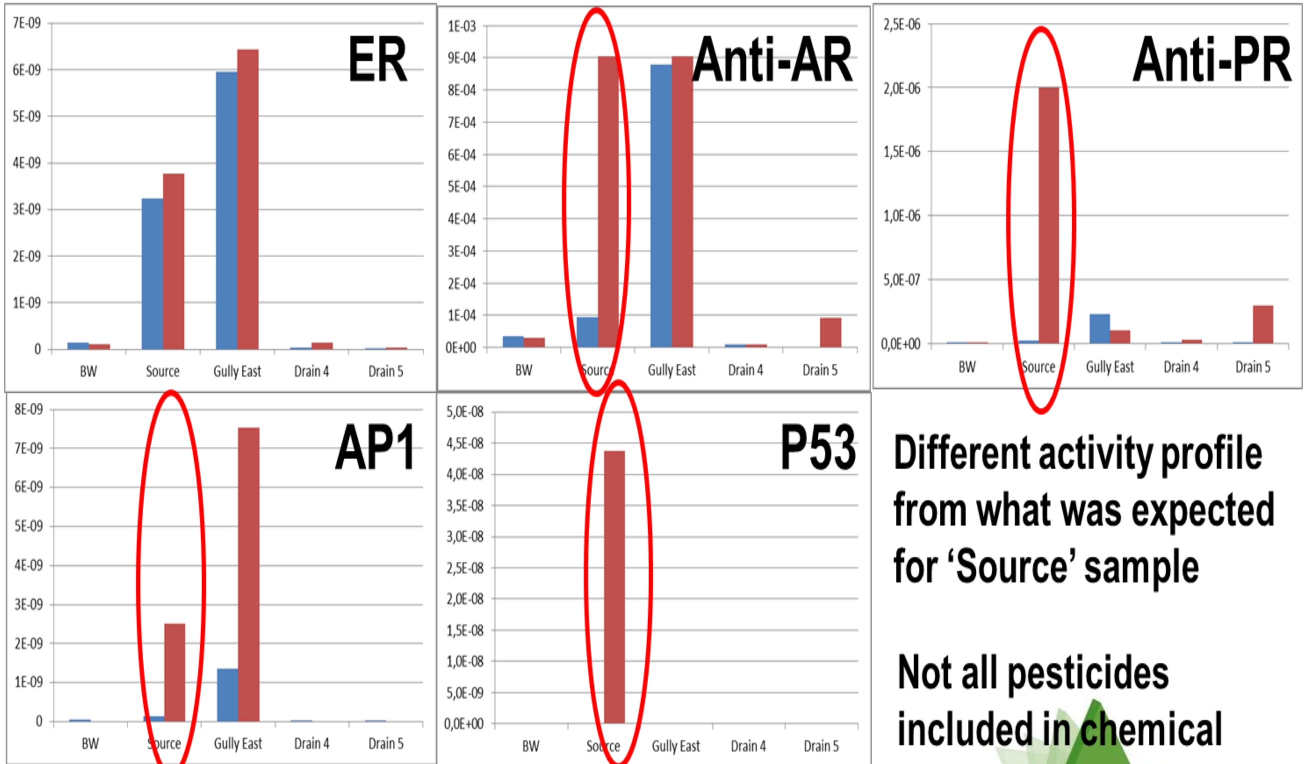
Studying effects of mixtures : pesticide dump in Tajikistan

	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

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!

Eco*Linc*

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												-8.5					-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

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	-

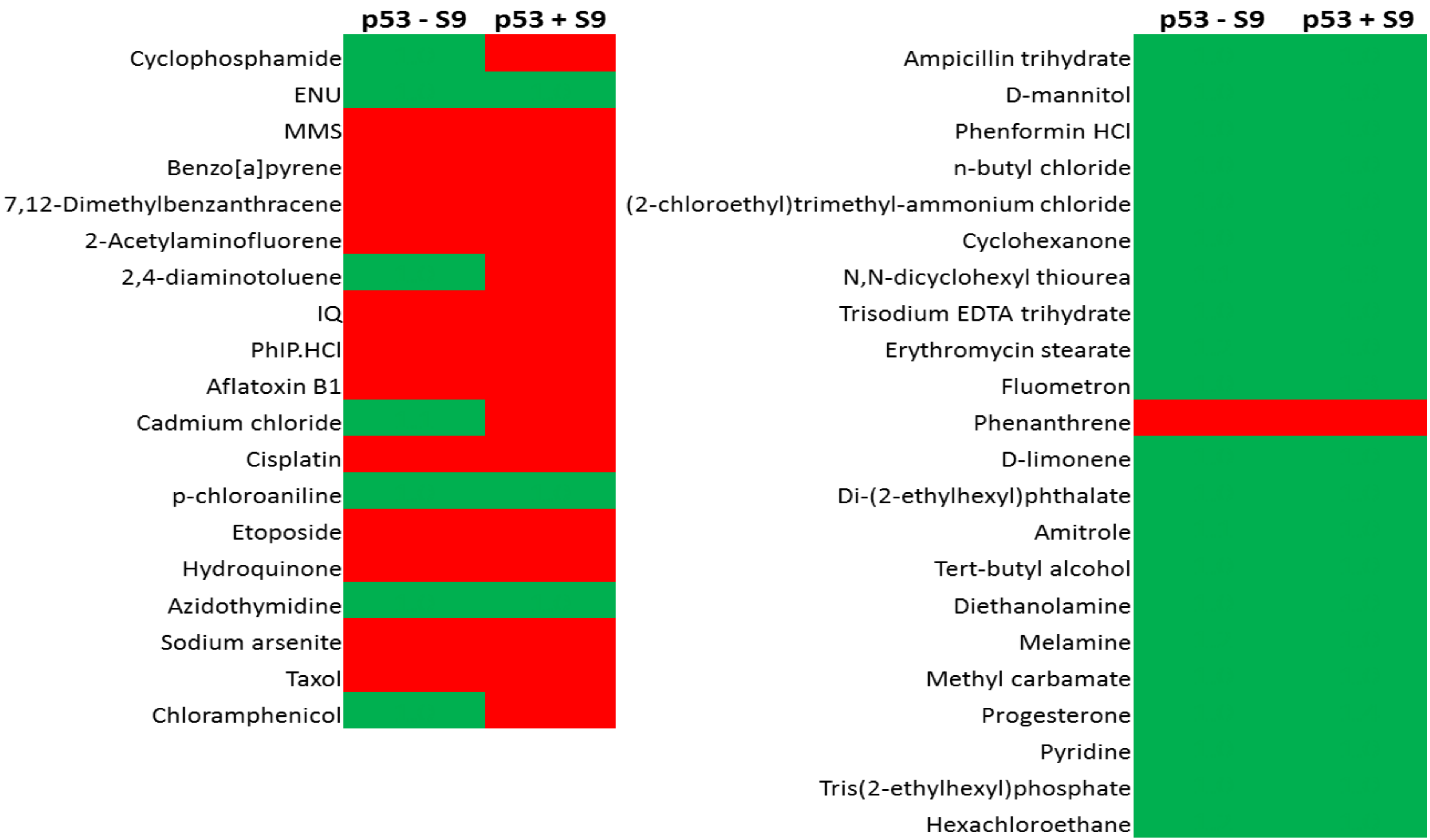
Sample	PAH CALUX-measured BEQ		Theoretical BEQ			Ratio measured BEQ) / Theoretical BEQ	
Synthetic mixtures	Concentration (mM)	Standard deviation (%)	REP-based concentration (mM)	TEF-based concentration (mM)	REP/ TEF	REP-based prediction	TEF-based prediction
Industrial soil, Sweden (41)	5.32	14	5.43	0.53	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

CALUX highlights 2013

BE-Basic

EcoLinc

New generation of Genotoxicity testing – Negative and positive results as expected..



CALUX highlights 2013

Water testing for effects of chemicals/pharmaceuticals

or

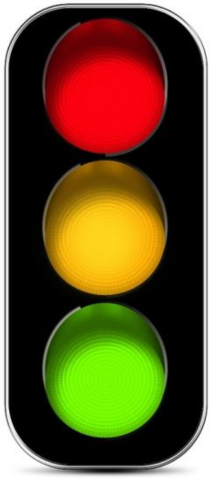
Endocrine disrupting chemicals

(Drinking, surface, ground, water treatment plants)

CALUX based trigger values for drinking water

CALUX > trigger value → more detailed examination warrented

CALUX < trigger value → health risks can be waived



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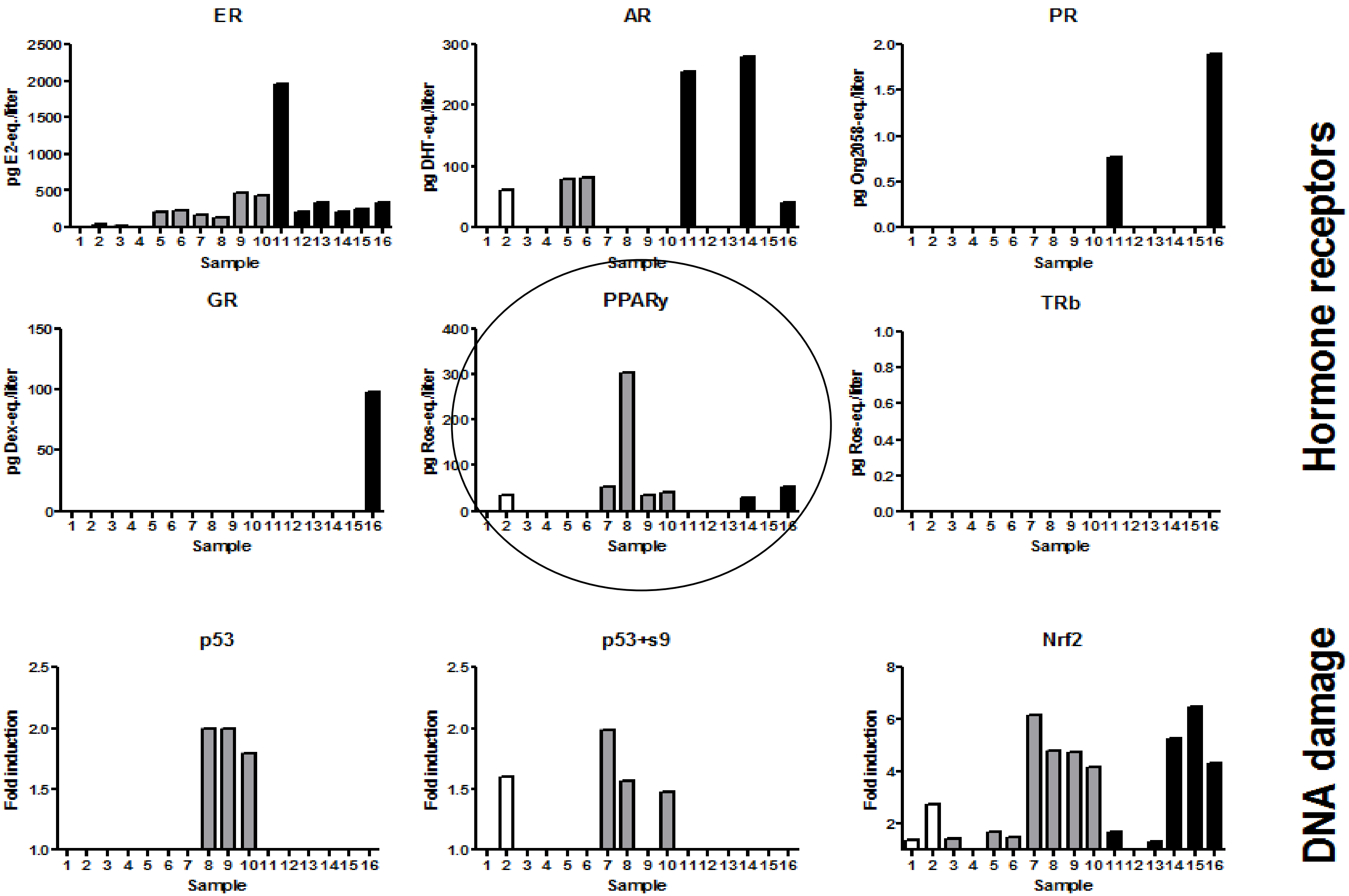
journal homepage: www.elsevier.com/locate/envint

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

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

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;

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

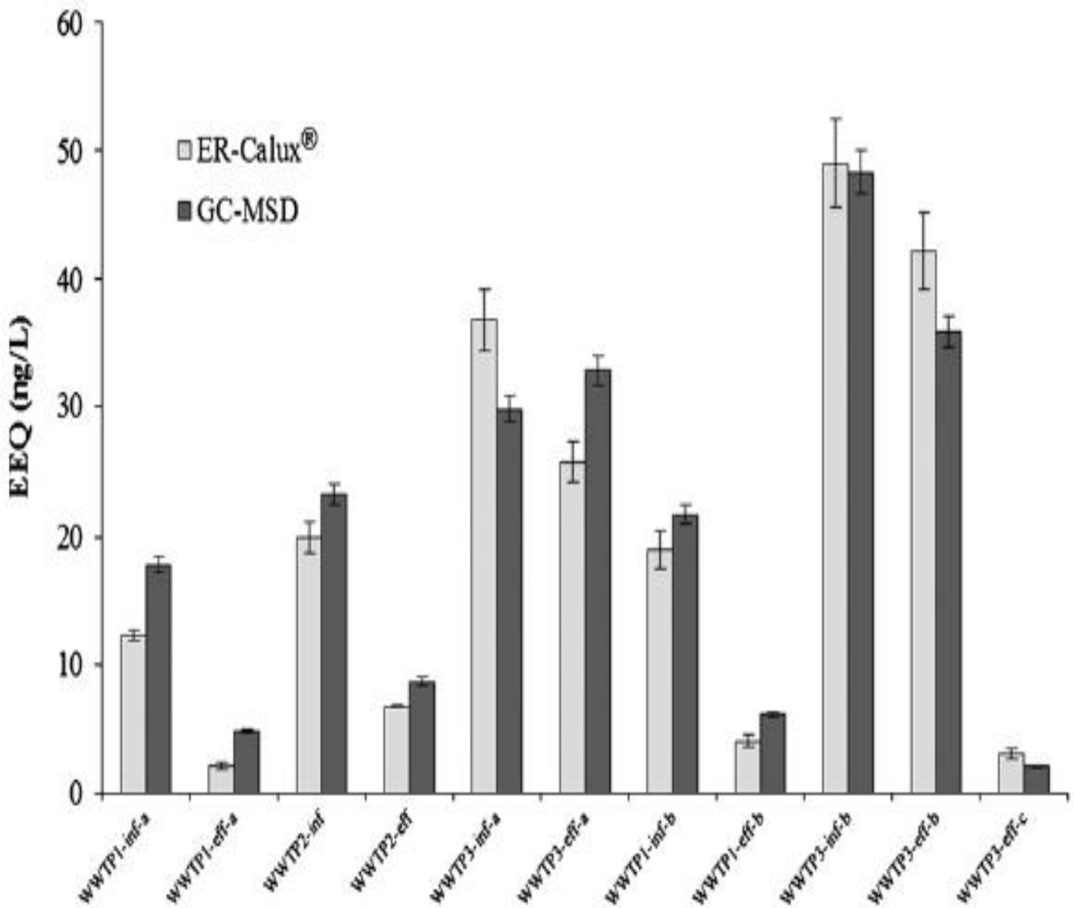
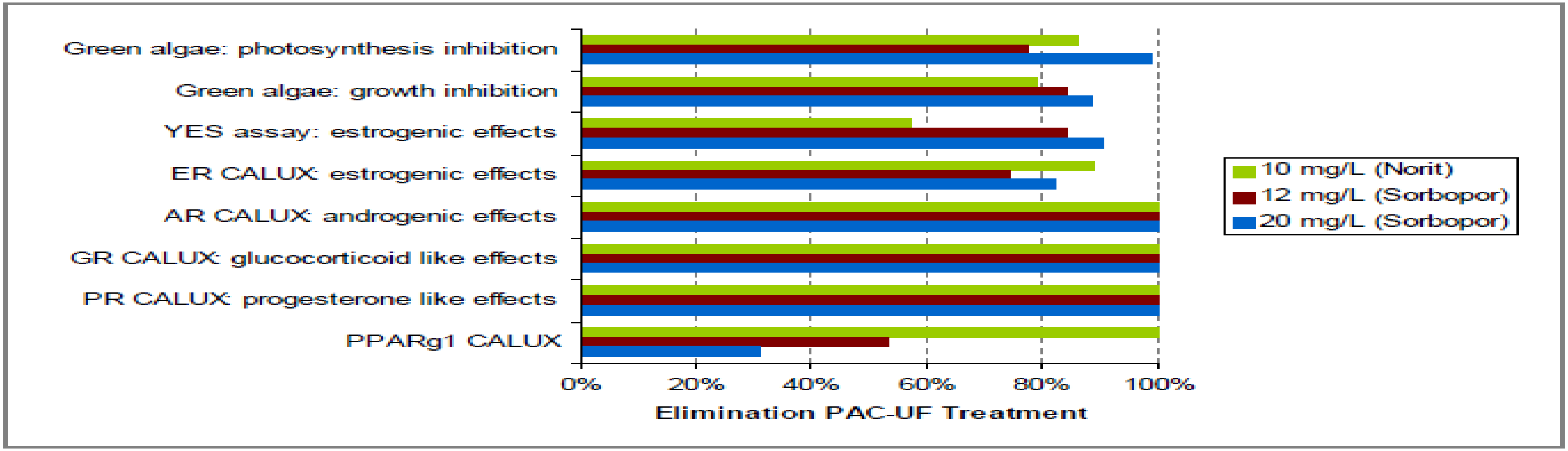
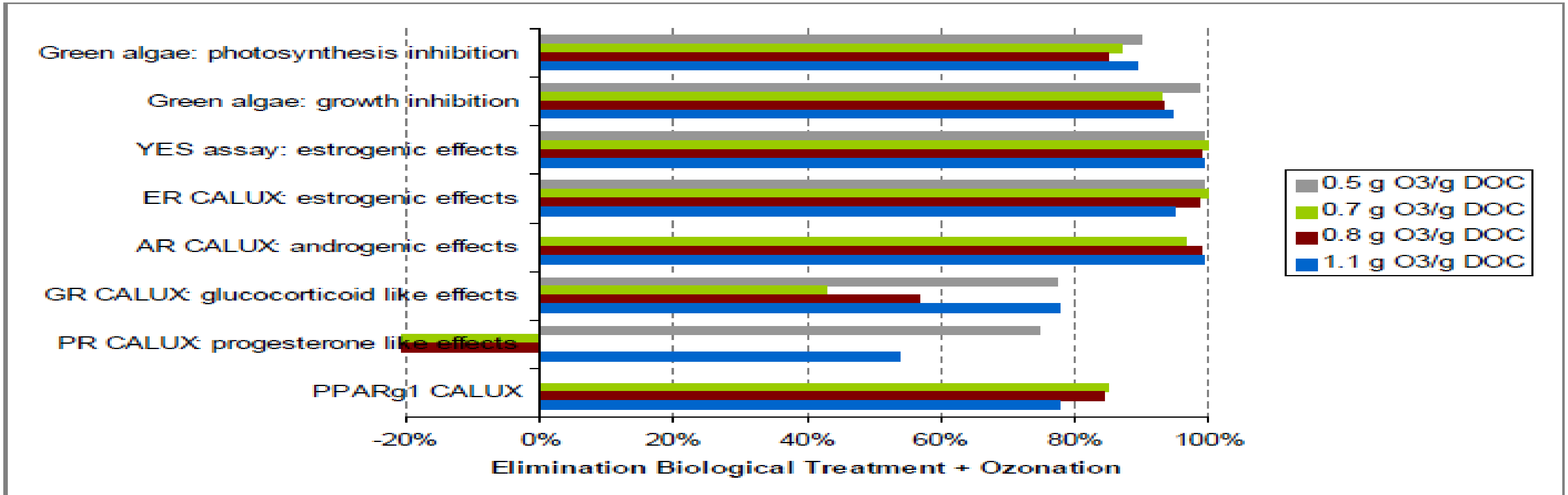


Fig. 4. GC-MSD and ER-Calux® results of real waste water samples. The results of ER-Calux® assay are presented as mean ± SD calculated from three parallels. The results of GC-MSD are presented as determined concentrations of one measurement ± relative standard deviation of measurement by GC-MSD.

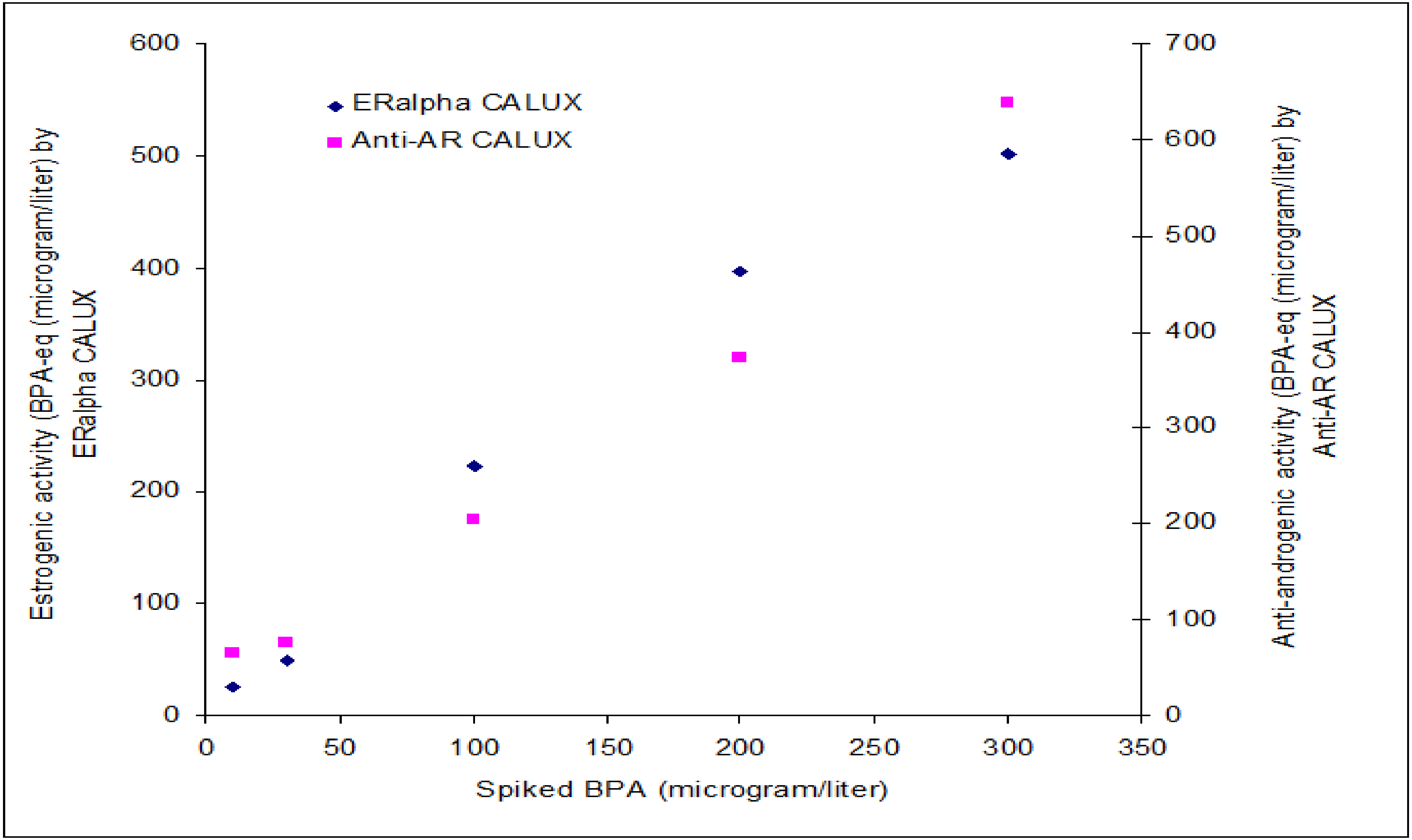
Water treatment plants treatment efficiency with active carbon or ozonation (Kienle et al, EAWAG 2012)





Plastic Migration of BPA/Phthalates: ER and anti-AR CALUX correlates well with BPA

(Service Analysis for German EPA, Bad Dessau)



SEVENTH FRAMEWORK PROGRAMME

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

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


Food risk & benefit

Bioactive estrogenic hormones in milk

(Behr et al 2011)

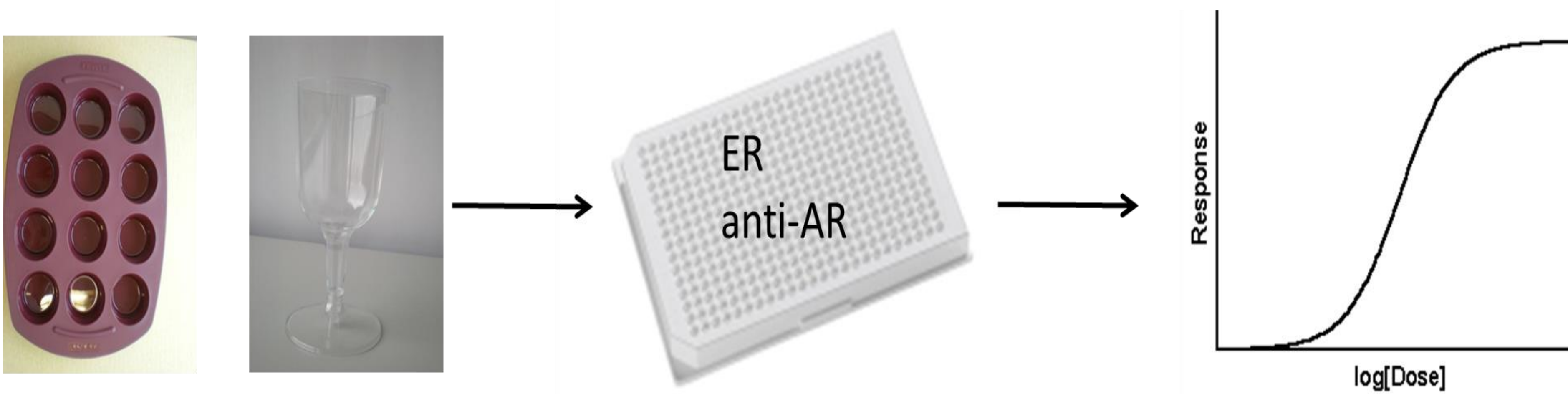
- Soya - based products had up to 1500ng EEQ/kg
- Soya-free product had between 10-40ng EEQ/kg
- Baby milk powder had 14-22ng EEQ/kg
- Soya lecithin was also strong estrogenic and therefore a main source of estrogenic activity
- the study concludes that estrogens are omnipresent in food and not only soya based products

Food Contact Materials testing by CALUX panel I

- <u>Germany: </u> **Testing of migration of plastic materials from baby food packaging in plastic (NaturNes) compared to glass materials (Hipp):**
- ***No difference observed between plastics and glass (ERα, anti-ERα, anti-AR, TRβ and PAH CALUX) (WDR Markt 2011).***

Food Contact Materials testing by CALUX panel II

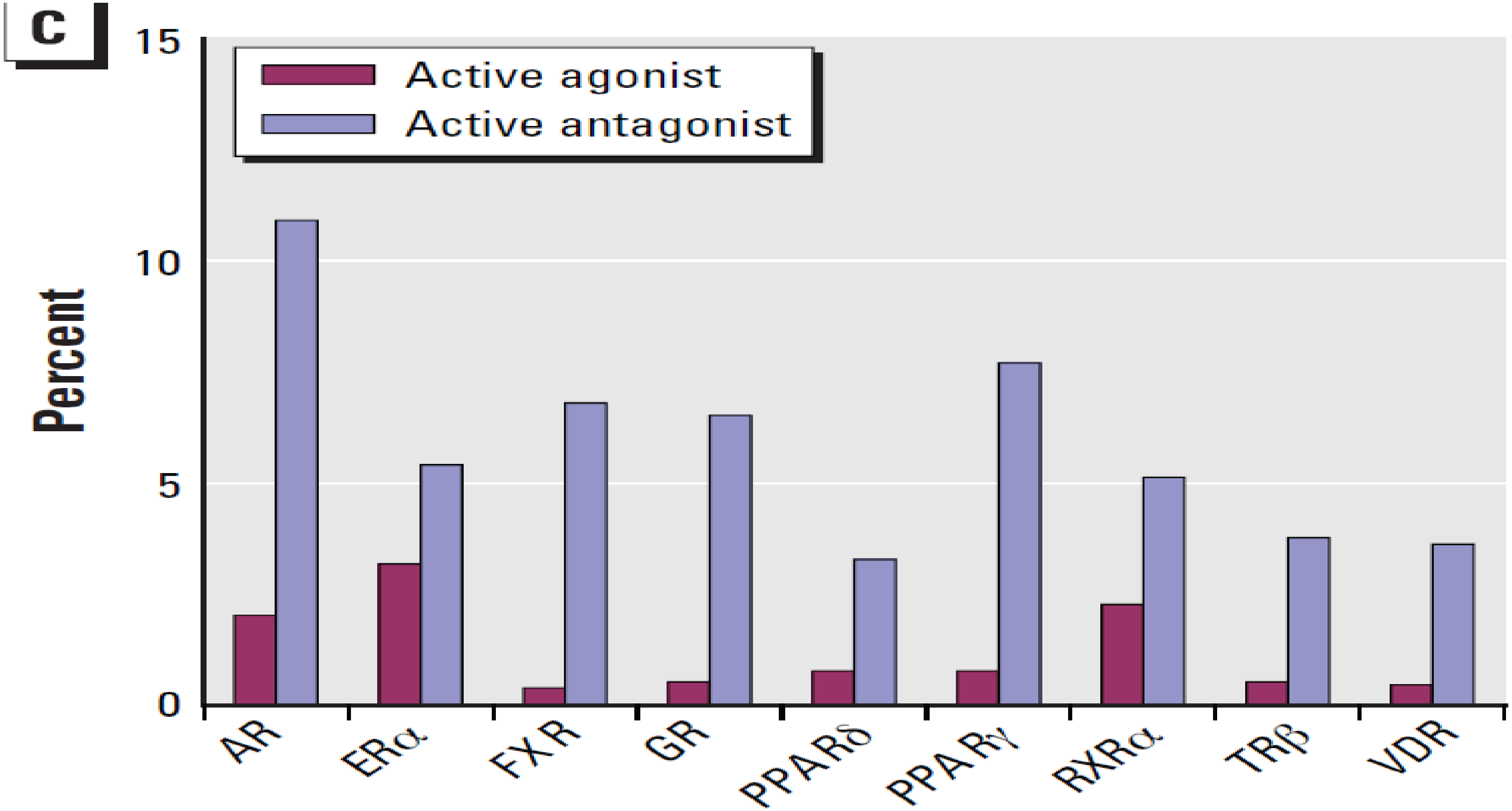
- **<u>Belgium: </u>Food packaging extracts were analysed for (anti)-estrogenic and (anti)-androgenic activity**
- ***Estrogenic activity was found in all flexible elastomers.***
- ***Anti-androgenic activity was found in 2 out of 3 polycarbonate samples (project with National Pack. Inst.)***



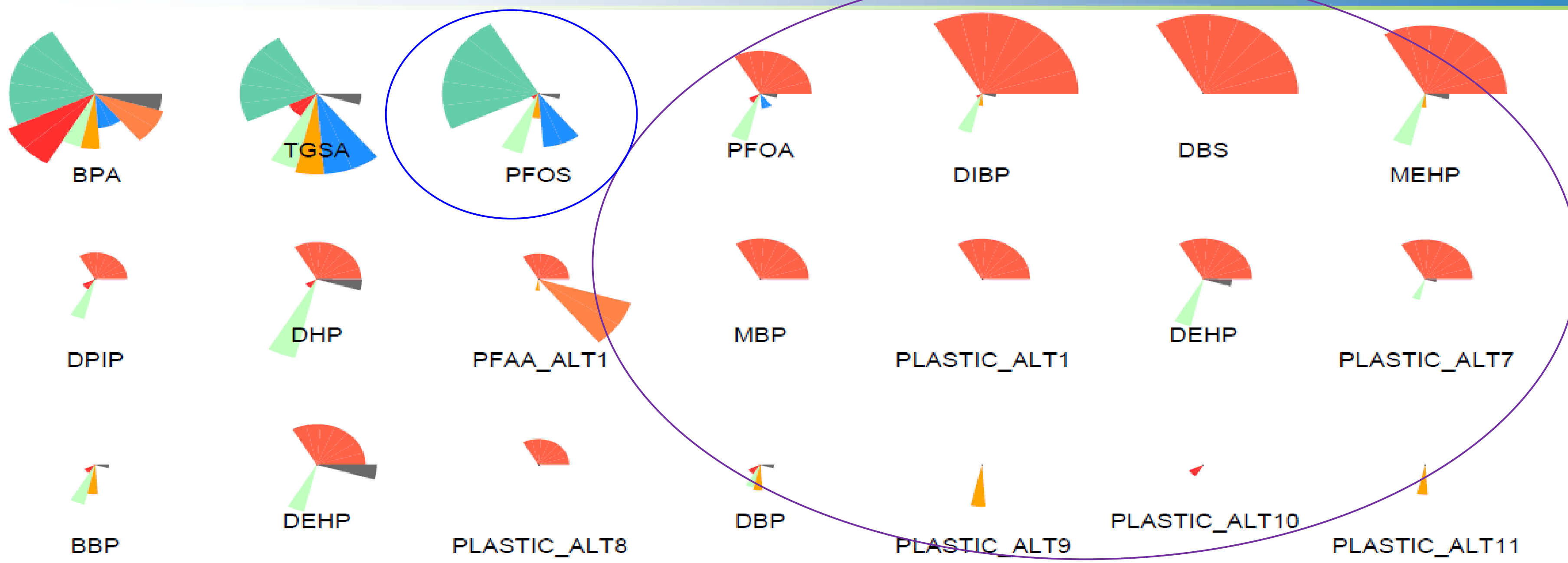
Why 56 CALUX tests? ToxCast project (USA-EPA) shows importance of anti-agonistic activities of many "Mode of Actions"

Chemical Genomics Profiling of Environmental Chemical Modulation of Human Nuclear Receptors

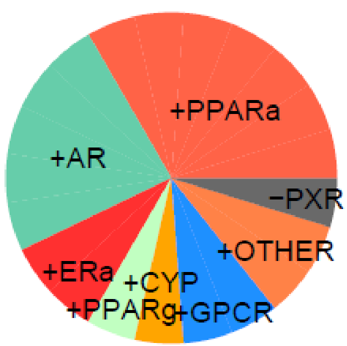
Ruili Huang,[1] Menghang Xia,[1] Ming-Hsuang Cho,[1] Srilatha Sakamuru,[1] Paul Shinn,[1] Keith A. Houck,[2] David J. Dix,[2] Richard S. Judson,[2] Kristine L. Witt,[3] Robert J. Kavlock,[2] Raymond R. Tice,[3] and Christopher P. Austin[1]



Why food contact materials ? ToxCast (US-EPA): ReproTox Predictions for Conventional and Alternative 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**

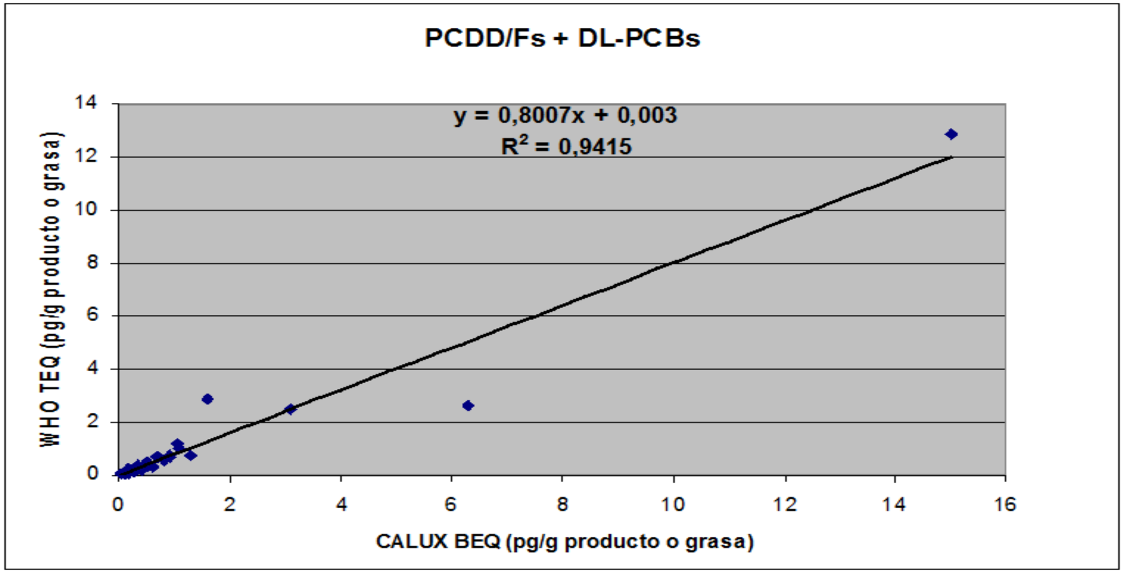


Biol Reprod. 2011 Aug;85(2):327-39. Epub 2011 May 12.
Predictive model of rat reproductive toxicity from ToxCast high throughput screening.
Martin MT, Knudsen TB, Reif DM, Houck KA, Judson RS, Kavlock RJ, Dix DJ.

CENTRO SUPERIOR DE INVESTIGACIÓN EN SALUD PÚBLICA

Dietary intake study for dioxins/dl-PCBs in Valencia

REQUIREMENTS	INTERNAL VALIDATION
PROCEDURE BLANK (<1PM)	0,6 pM
RECOVERY CERTIFIED REFERENCE MATERIAL / FORTIFIED SAMPLE.	91,45 % / 111,4 %
APPARENT BIOASSAY RECOVERY (30-130%)	83,16 %
SUPRESSION SIGNAL TEST (>75%)	118,81 %
REPRODUCIBILITY RSDR (<25%)	12,6 %
REPEATABILITY RSDr (<20%)	10,33 %
FALSE COMPLIANT RATE (<5%)	0 %



A total of 1270 composite samples were analysed corresponding to 189 individual food items that cover 90% of the adult and child diet.

CONSUMPTION DATA

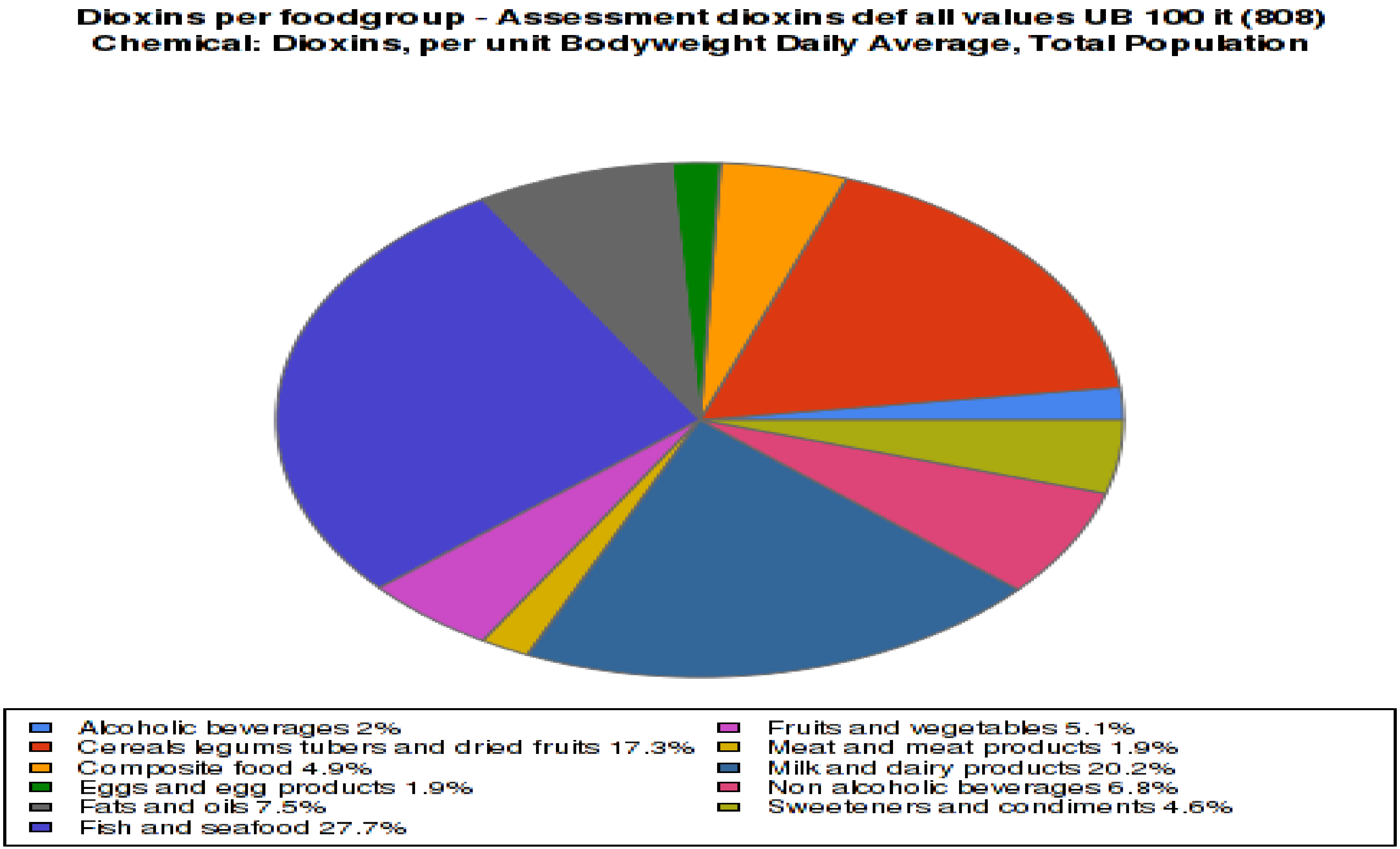
Consumption data were collected from a dietary survey using a 24-h recall and performed on 1478 subjects ranging from 6-70 years old.

E. Millán, O. Pardo, V. Yusà, Public Health Research Center

Dietary intake study in Valencia

Foodgroup	Mean	Minimum	Maximum
Alcoholic beverages	0.030	0.010	0.056
Cereals legums tubers and dried fruits	0.187	0.048	2.670
Composite food	0.230	0.014	0.810
Eggs and egg products	0.082	0.041	0.136
Fats and oils	0.367	0.132	0.627
Fish and seafood	0.686	0.124	2.590
Fruits and vegetables	0.023	0.001	0.845
Meat and meat products	0.033	0.003	0.176
Milk and dairy products	0.307	0.014	1.750
Non alcoholic beverages	0.016	0.001	0.151
Sweeteners and condiments	0.229	0.100	0.900

Dietary intake study of dioxins/dl-PCBs in Valencia



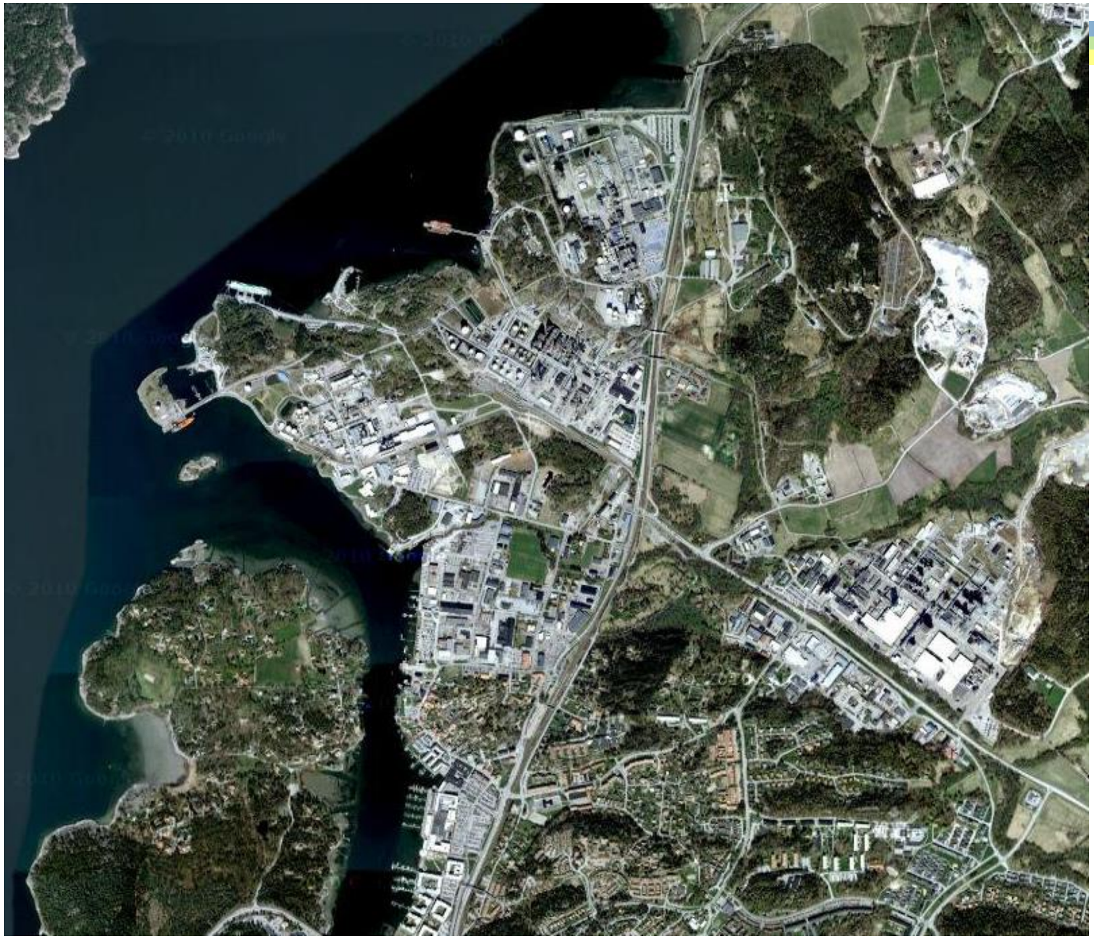
Dietary intake study for PCDD/Fs in Valencia

- **For adults, the average daily intake was estimated as 1.38 and 1.56 pg WHO-TEQ/kg b.w ·day for the lower bound (LB) and upper bound (UB) scenarios, respectively.**
- **For children, the average intake was estimated as 2.43 and 2.73 pg WHO-TEQ/kg b.w·day for the LB and UP scenarios, respectively.**
- **The estimated intakes show that 14 % (LB) or 17 % (UB) of the children population and 4 % (LB) or 5 % (UB) of the adult population exceed the tolerable daily intake (TDI) recommended by the WHO.**


Wildlife Health


Public Health

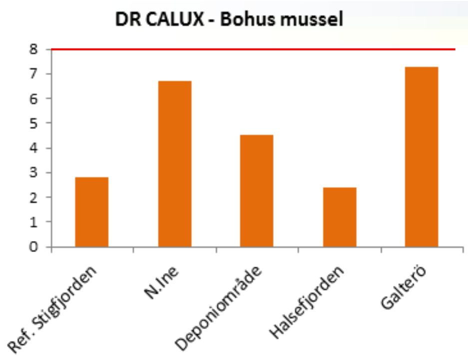
Mussel Monitoring e.g., Bohus coast, Sweden

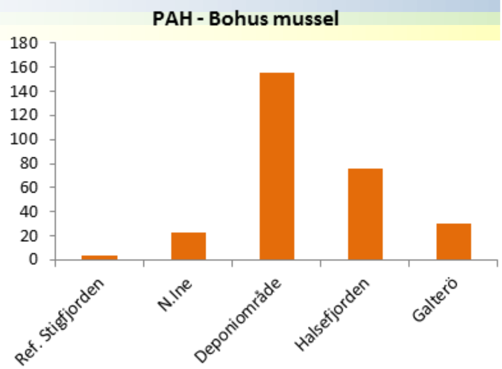


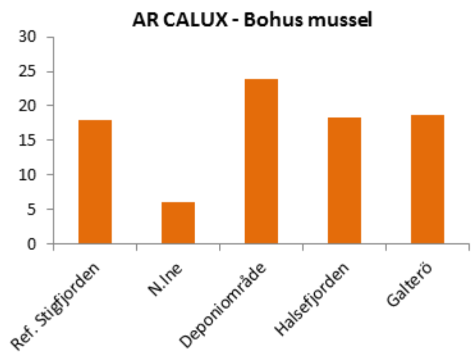
Screenshot from google maps:

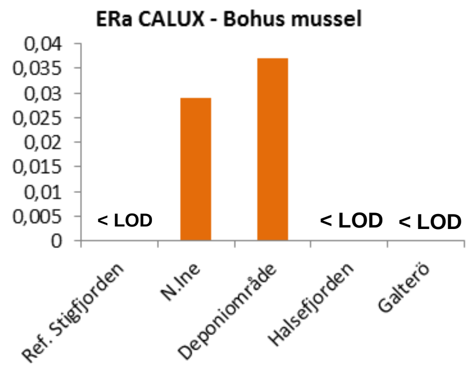
Large chemical industries

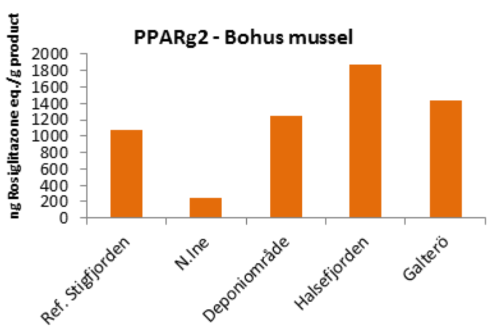
Mussel analyses, **Bohuskusten (coast)**









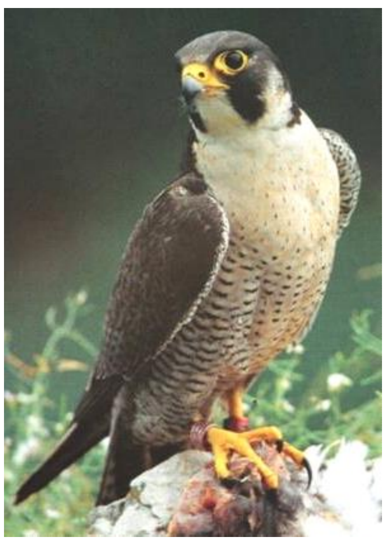


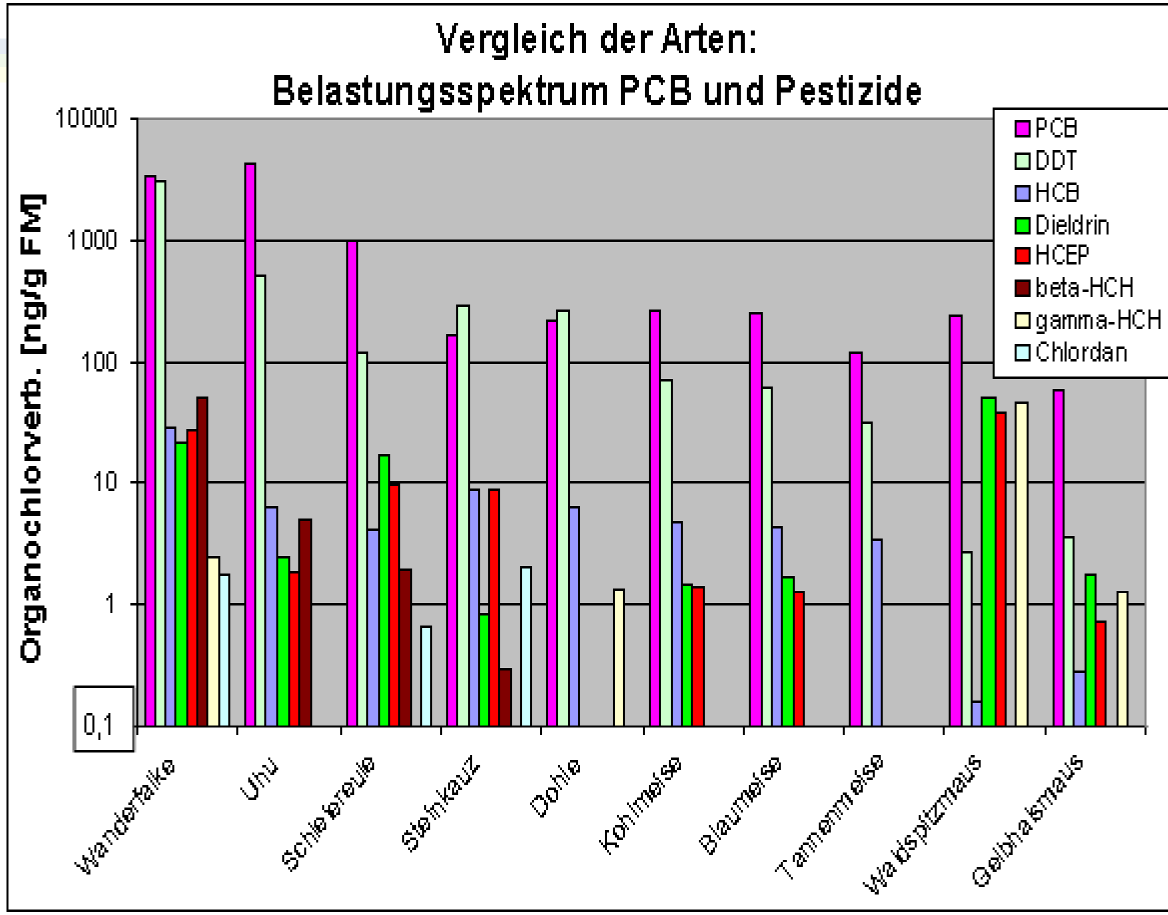
AR_CALUX1	µg flutamide eq./g product
DR_CALUX1	pg 2,3,7,8-TCDD TEQ/g product
Era_CALUX1	ng 17ß-estradiol eq./gr product
PAH_CALUX1	ng B(a)P eq./gr product
PPARg2	ng Rosiglitazone eq./g product

Terrestrial (Avian) Food Chain

Organochlorine Pollution of Eggs (2001):

Peregrine Falcon >
Eagle Owl >
Barn Owl >
Little Owl ≈ Jackdaw
➢Great Tit ≈ Blue-Bonnet
➢> Coal Tit





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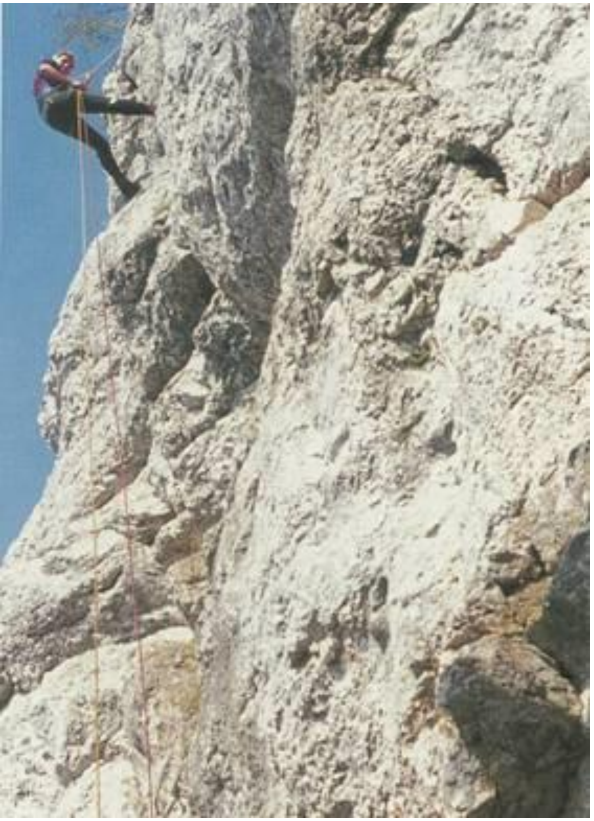
Failed peregrine falcons eggs

Years of catching and numbers in the state of Baden-Württemberg

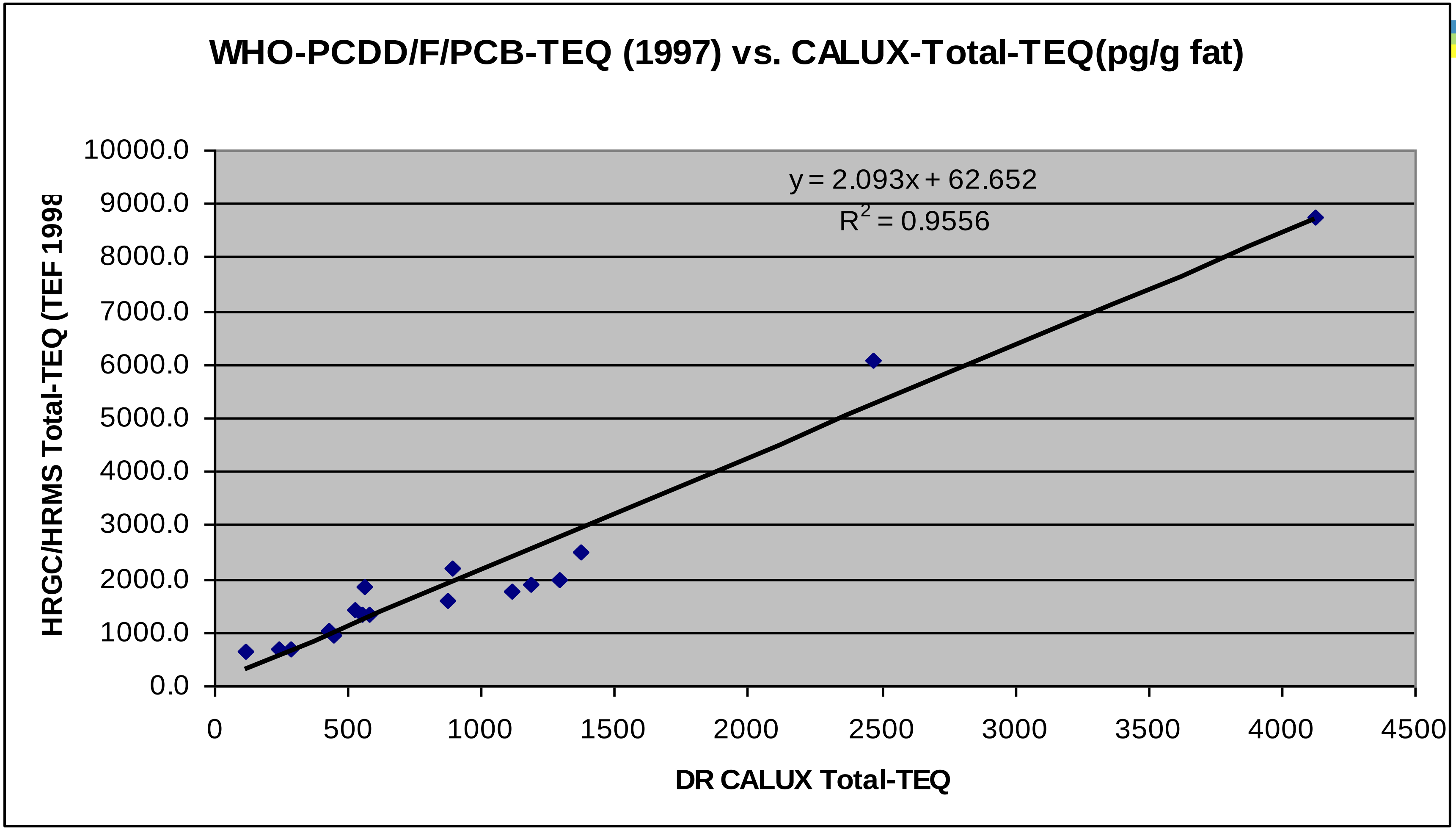


Catch the rest eggs (Foto: F. Schilling)

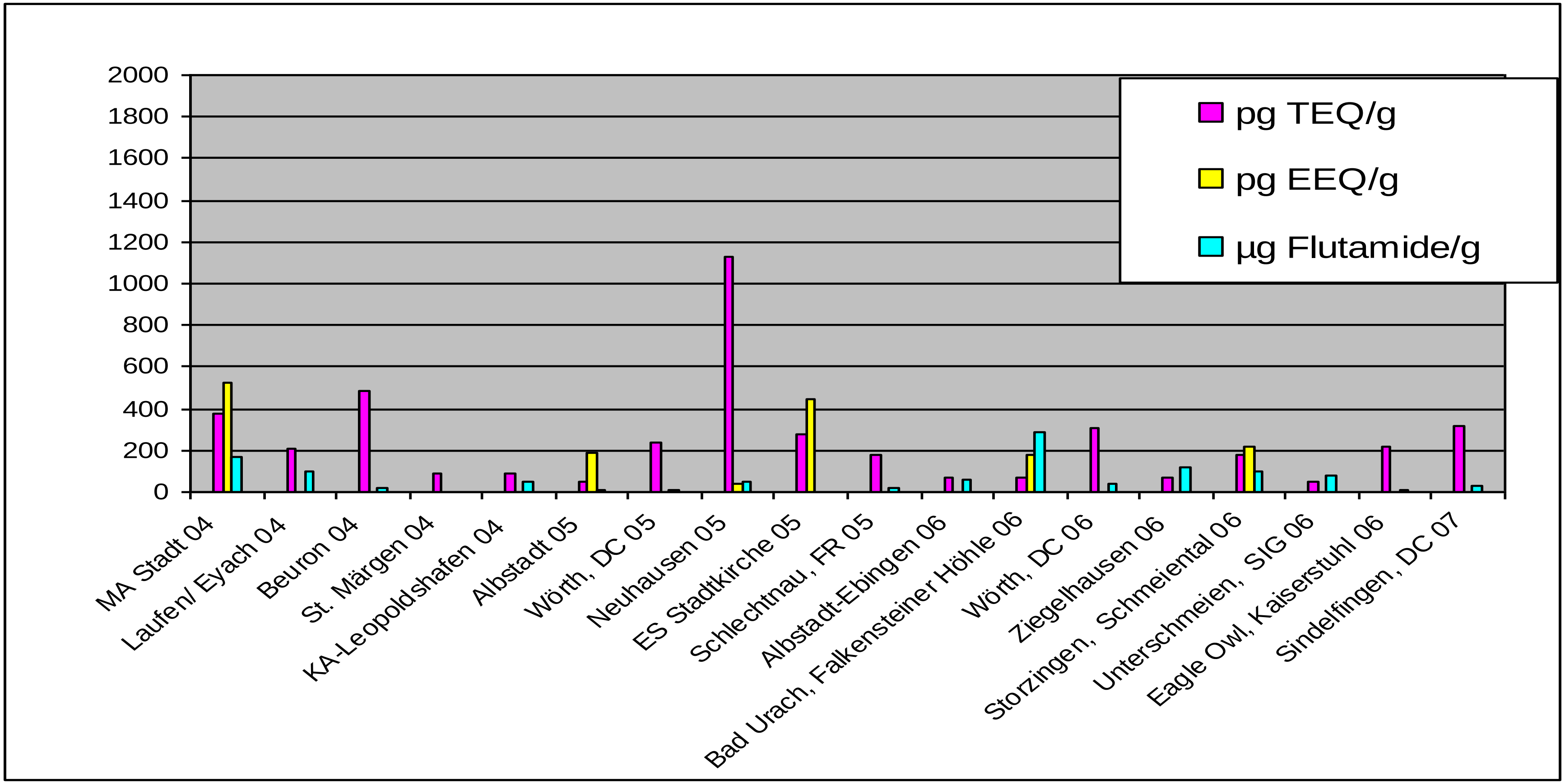
Jahr	n	Jahr	n
1967	1		
1968	3		
1969	11		
1970	4		
1971	7	1991	37
1972	-	1992	28
1973	6	1993	30
1974	14	1994	-
1975	16	1995	9
1976	14	1996	4
1977	9	1997	9
1978	11	1998	5
1979	12	1999	28
1980	9	2000	31
1981	8	2001	42
1982	17	2002	36
1983	21	2003	35
1984	18	2004	41
1985	20	2005	29
1986	24	2006	27
1987	24	2007	28
1988	18		
1989	40		
1990	25	Σ	7 5 1

Die Schalen der Jahrgänge 1999, 2000 und 2001 teils ausgeblasen, teils geöffnet (Foto: F. Schilling)

Total-WHO-TEQ vs Total DR CALUX TEQ



Dioxin, estrogen- and anti-androgen equivalents in falcon eggs by CALUX serie



EC NEW GENERIS Projekt – 6 EU countries and 1200 mothers/newborn babies: DR CALUX as biomarkers for baby health

- **NEW GENERIS Projekt from 2006 to 2010**

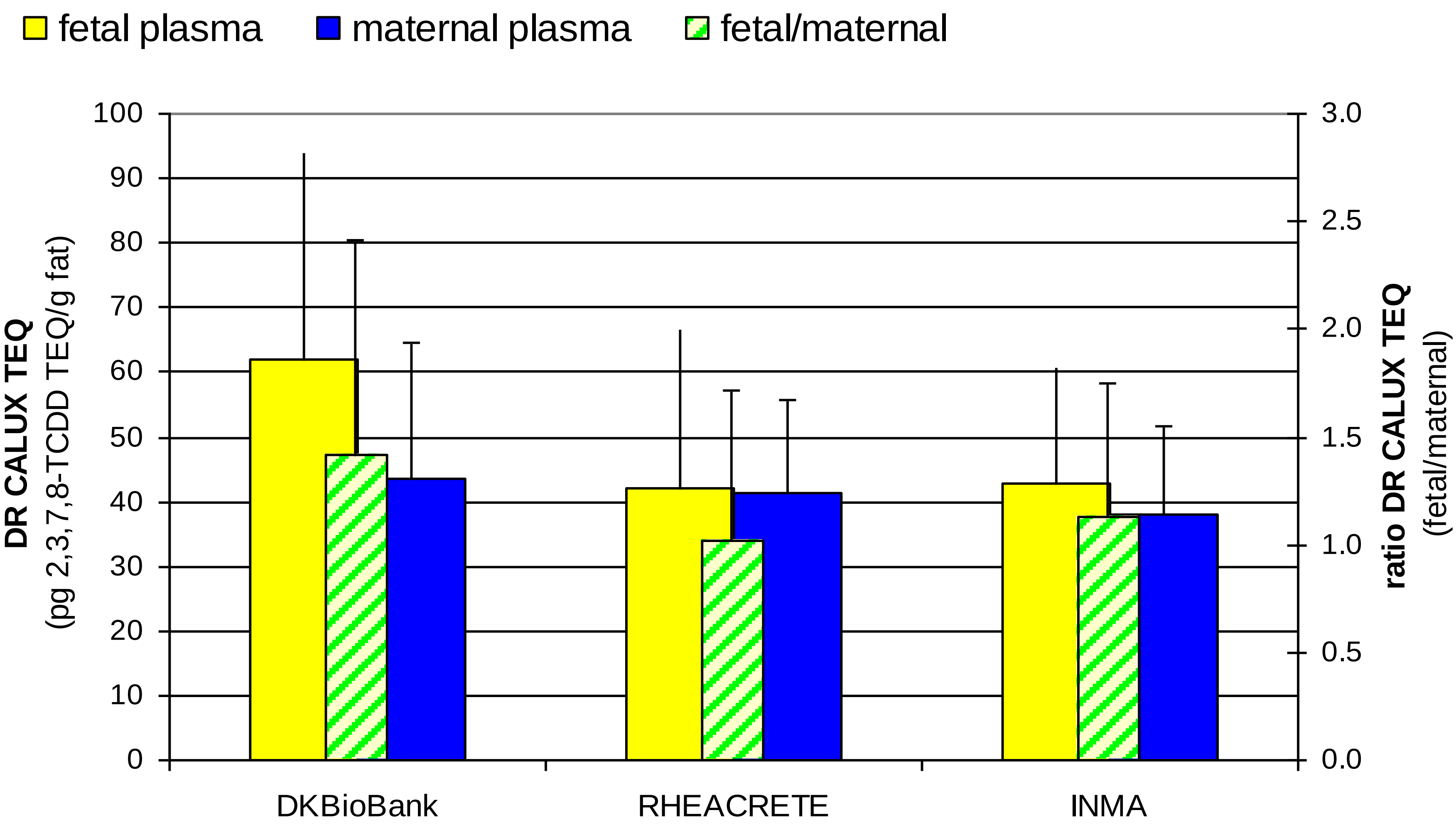
•ca. 2500 samples from mother/baby cohortes

•from 5 different EU counrties (Denmark, UK, Norway, Greek, Spain)

•Cord blood from the day of birth and blood of the mother analysed via ER, AR and DR CALUX

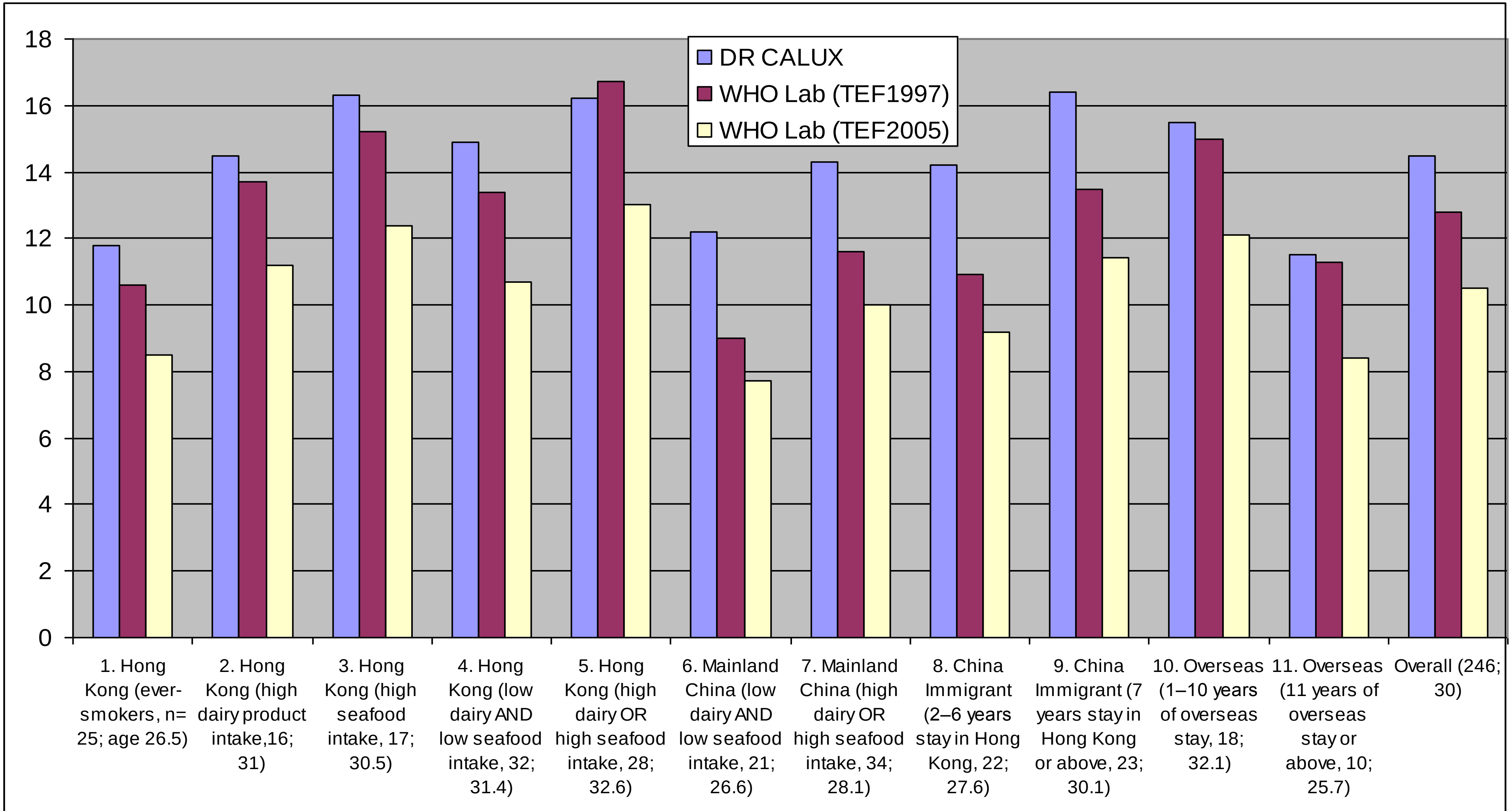
Conclusion: Effects from von bioactive stabile Dioxins/PCBs on head dimension and therefore development of the newborn baby is in discussion

Which effects have bioactive stabile Dioxine/PCBs for new born babies?

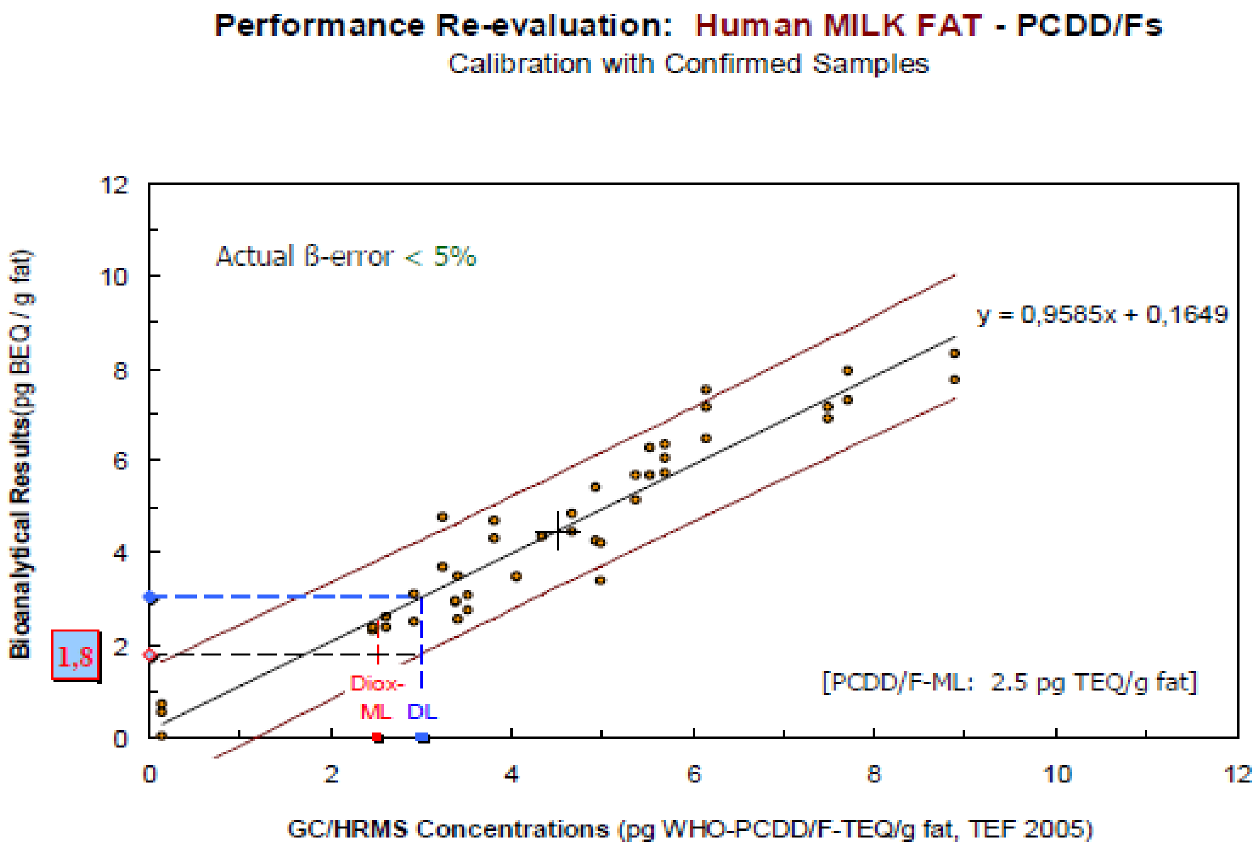


Mother milk in Hong Kong and China

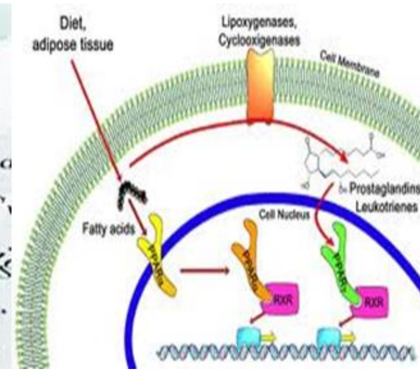
Comparison DR CALUX-Total-TEQ **(BDS)** and HRGC/HRMS WHO-Total-TEQ **(WHO Reference lab)** for pooled breast milk samples (pg/g fat) from Hui et al. Chemosphere 69, 1287 (2007)



Re-evaluation data from human mother milk (data from EU-RL CVUA Freiburg)



Obesity testing by PPAR CALUX panel



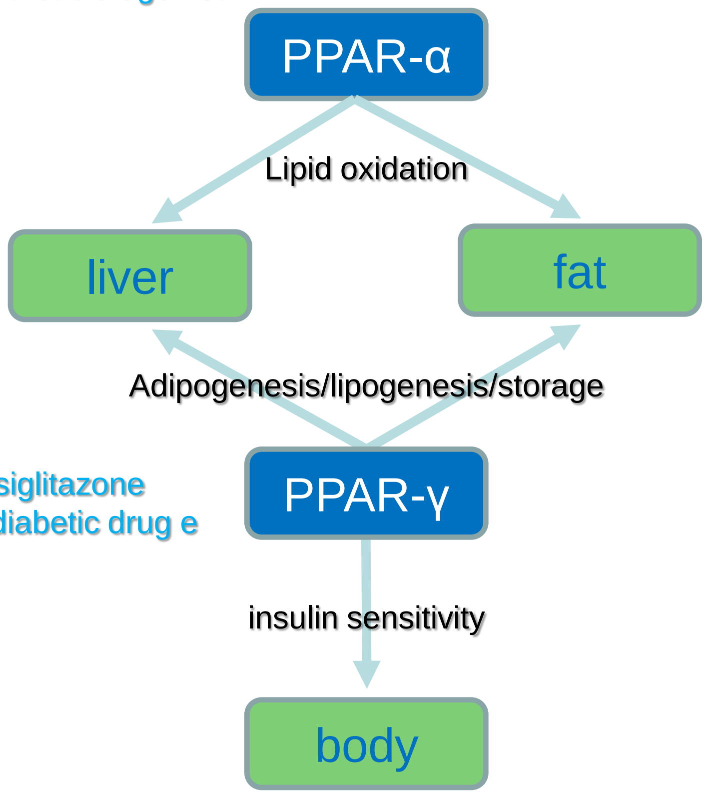
PPAR Isoform	Organ specificity	Function
PPARα	Liver	Fatty acid metabolism
PPARγ	Adipose tissue	Lipid storage
PPARδ	ubiquitous	Energy homoeostasis

→ PFOA activates PPARα and PPARγ (but not PPARδ)
Vanden Heuvel et al. (2006) Toxicol Sci 92: 476-489

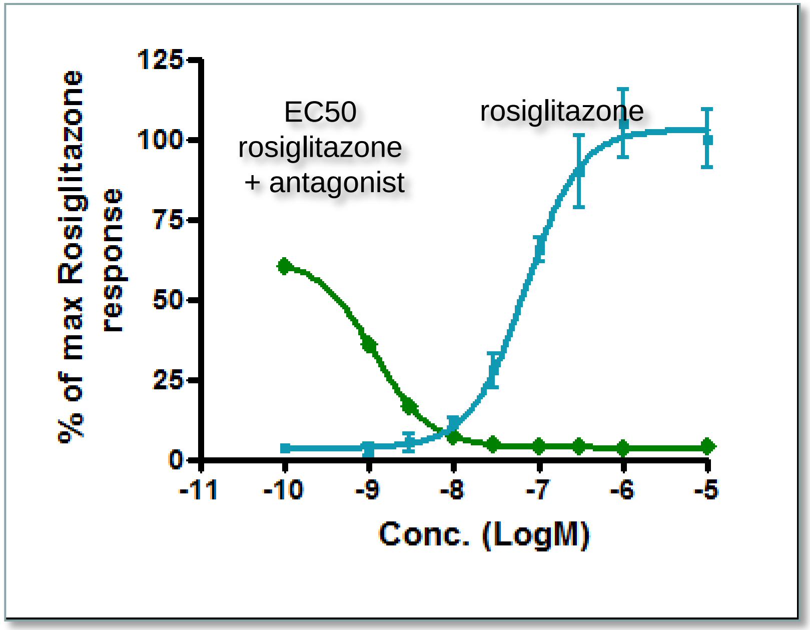
→ PFCAs activate PPARα
positive correlation between carbon chain length and the level of PPARα activation
Wolf et al. (2008) Toxicol Sci 106: 162-171

PPAR (α, , δ and γ) CALUX: obesity and insulin sensitivity

Reference compounds:
SGW7674 =
Synthetic α agonist



Rosiglitazone
= anti-diabetic drug e

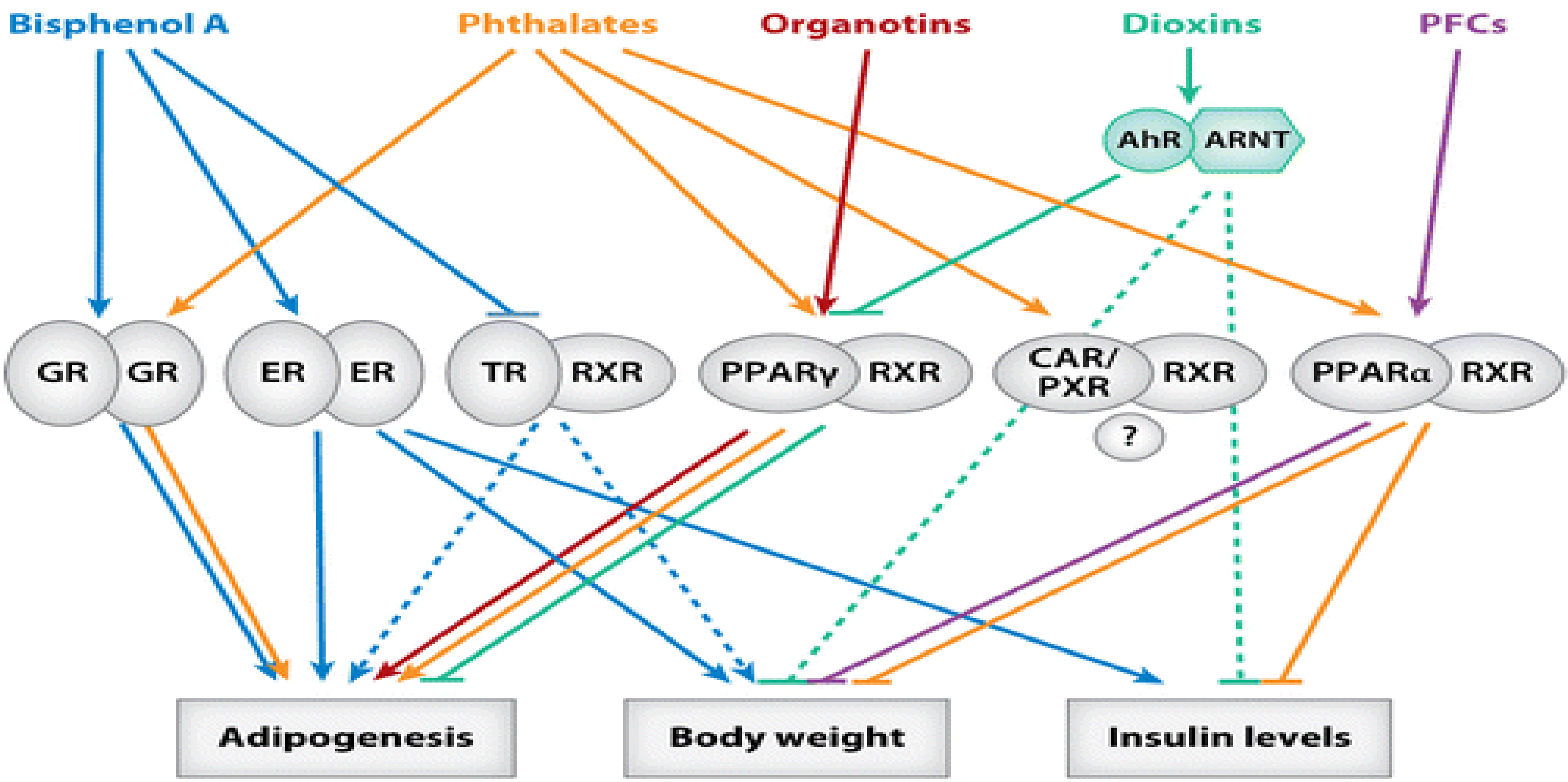


Gijsbers et al., 2011 Analytical Biochemistry 414:77-83

WAGENINGEN UR
For quality of life

syngenta

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

PPARα and PPARγ CALUX :
Toxic EQs compared to GW7674 and Rosiglitazone

Compounds	Molecule	PPARα	PPARγ
GW7674 (PPARα CALUX Standard)		1	-
Rosiglitazone (PPARγ CALUX Standard)		-	1
Tributyltin acetate		***Not determined***	***4.8***
Monoethylhexylphtalate		***1.7E-05***	***8.0E-04***
Perfluoro-butanoic acid (PFBA)	$C_4F_7O_2^-$	5.3E-06	
Perfluoro-pentanoic acid (PFPeA)	$C_5F_9O_2^-$	6.9E-06	
Perfluoro-hexanoic acid (PFHxA)	$C_6F_{11}O_2^-$	1.2E-05	
Perfluoro-heptanoic acid (PFHpPA)	$C_7F_{13}O_2^-$	6.7E-05	
Perfluoro-octanoic acid (PFOA)	$C_8F_{15}O_2^-$	3.1E-05	
Perfluoro-nonanoic acid (PFNA)	$C_9F_{17}O_2^-$	1.7E-05	
Perfluoro-hexanesulfonic acid (PFHxS)	$C_6F_{13}O_3S^-$		5.5E-05
Perfluoro-octanesulfonic acid (branched-PFOS)	$C_8F_{17}O_3S^-$		3.6E-05
Perfluoro-octanesulfonic acid (linear-PFOS)	$C_8F_{17}O_3S^-$		9.1E-05

Thanks….Questions..???

Do you want to know more about POPs & EDCs cocktails……???