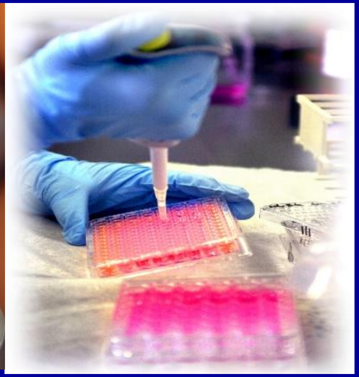
BioDetection Systems

Lebanon POPs Conference
Beirut, 9th May 2019

Bioanalysis for POPs & Endocrine Disrupters & their Diseases

Dr. Peter A. Behnisch,
BioDetection Systems,
Amsterdam

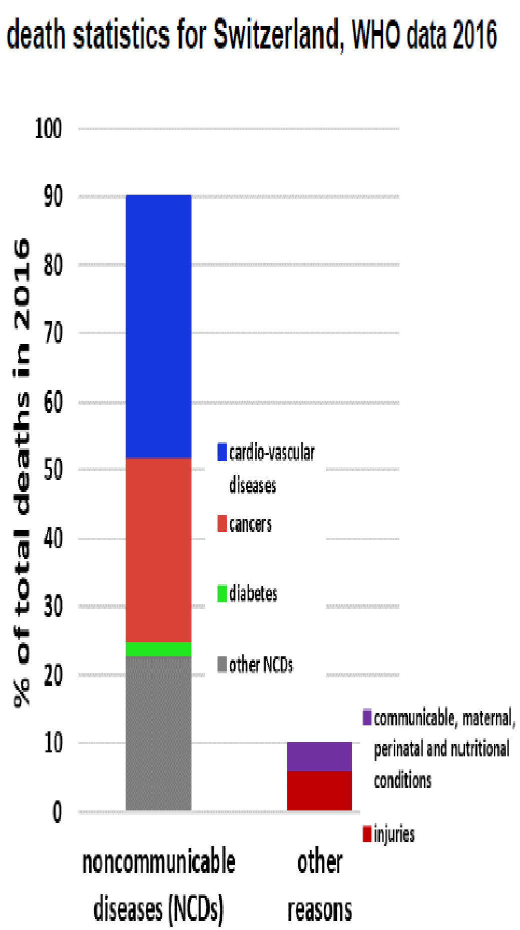


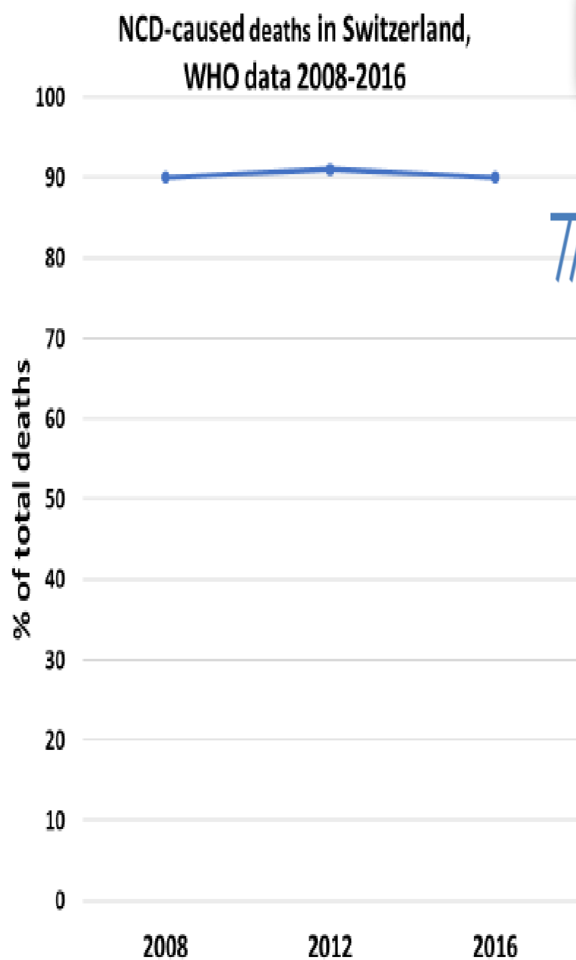


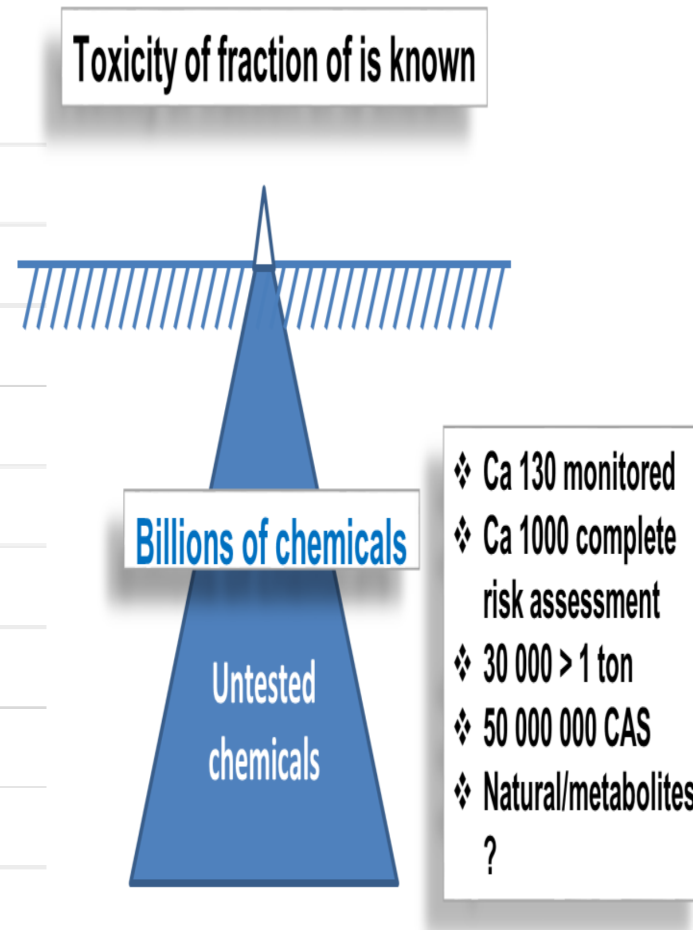
In the focus….

- What have Non-communicable disease to do with POPs & EDCs?
- How to analyse POPs & EDCs efficiently?
- Toxicity profiles of POPs & EDCs
- Examples of Dioxins/PCBs in the environment and their food impact
- Harbor Cities & Dioxins/PCBs
- Can consumer products and toys contain dioxins and EDCs?
- Tissue, blood and mother milk testing in wildlife and humans
- Take home message

Are Non-communicable diseases (NCDs) linked to thousand's of uncontrolled chemicals e.g. in plastic?





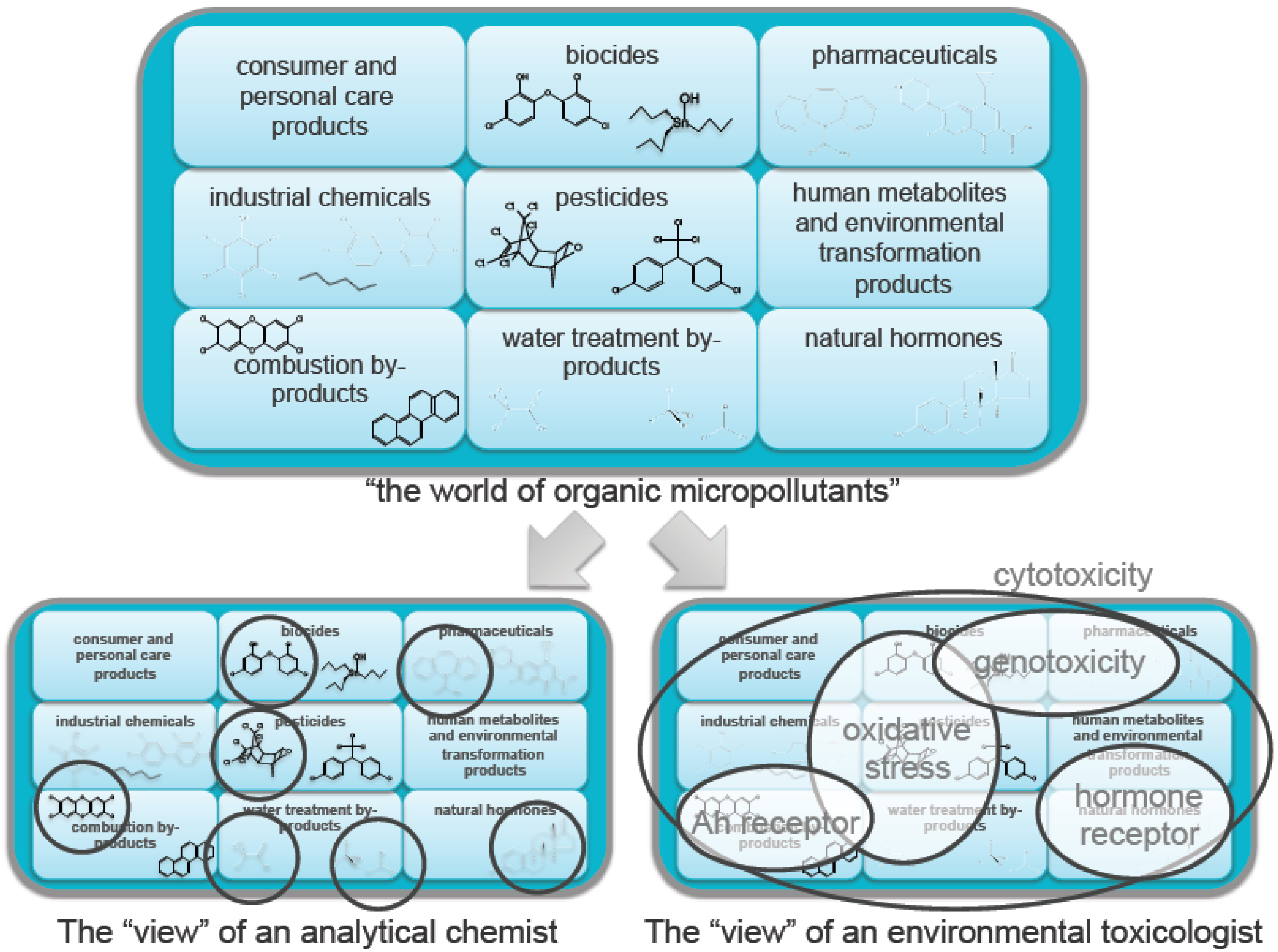


http://www.who.int/nmh/countries/en/

Policy and regulators for POPs & EDCs

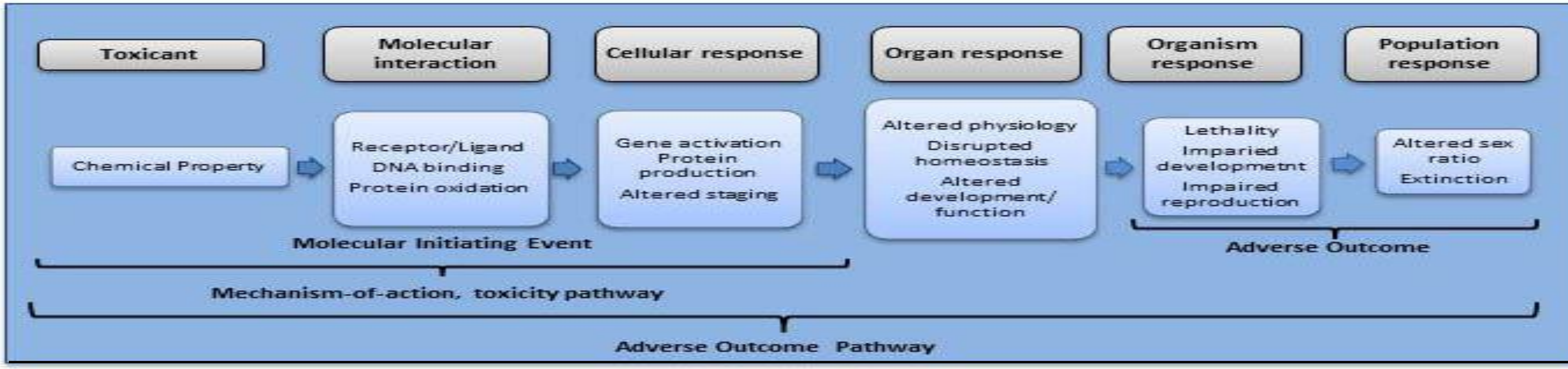
The medical cost of this serious public health issue has been recently estimated at €157 billion a year in the EU alone.

How to analyze them? Chemical versus biological analysis



Escher and Leusch (2012)

Cellular CALUX bioassays as marker for disease



Dioxins/PBDEs PCBs	AhR	DR CALUX	e.g. Chloracne Wasting Syndrom
BPA	Estrogen receptor	ER CALUX	Endocrine Disruption
Pesticide (e.g. DDT)	Inhibition androgen receptor	Anti-AR CALUX	Endocrine Disruption
PFOS/PFOA TBBPA	Thyroid TTR	TTR-TR CALUX	Thyroid Competition
PAHs	AhR	PAH CALUX	Mutagene/ Cancer

Diseases & In vitro Mode of Actions

Disease	Mechanism	Chemicals	CALUX Tests
Chloracne	AhR receptor	dioxins, dl-PCBs	**DR CALUX**
Obesity	PPAR receptors insufficiens, fat storage	TBT, PFOA/PFOS	**PPAR CALUX**
Breast cancer, Menopause	estrogen sensitive cells	E2, EE2, E3, BPA, Phthalate, Pesticides	**ER CALUX**
Prostate cancer	androgene sensitive cells	BPA, Anabolic steroids	**AR CALUX**
Infertility	endocrine dependent sex organ function/development	phthalate, BPA, NP, OP,	**ER, AR, TR, PR CALUX**
Hyper sensitivity	oxidative stress pathways	Pestizide	**Nrf2 CALUX**
Cancer General	genotoxicity, DNA repair mechanism	BaP, dioxins, PCBs	**P53, PAH CALUX, Dioxin CALUX**
Asthma, Allergy	glucocorticoid sensitive	dexamethason, Pharmaceuticals	**GR CALUX**
Thyroid gland	complex	T3	**TR CALUX**
Metabolic Syndrome	complex	dioxins, dl-PCBs, hormones	**DR and ER CALUX**
Leukemia	estrogen sensitive	dioxins	**DR CALUX**
Menopause	ER/AR pathways	hormones	**ER and anti-AR CALUX**

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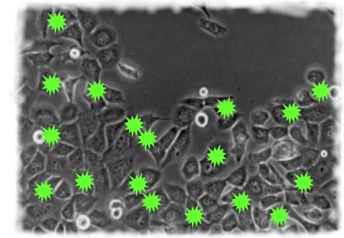
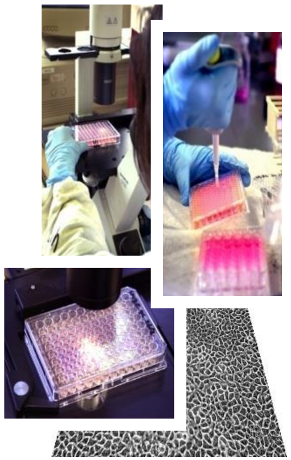
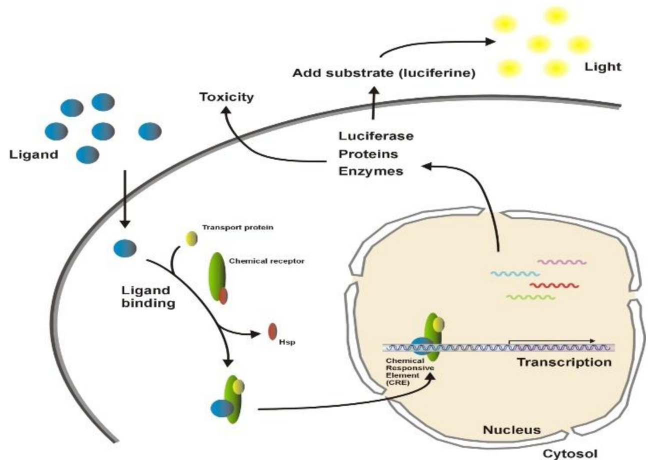
- Can consumer products and toys contain dioxins and EDCs?
- Relevance for wildlife and humans
- Take home message

How does CALUX® works?







SEEDING

Ready-to-seed cells in 96/384-well format

EXPOSURE

Dilution series (TCDD and sample)

DETECTION

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Example CALUX profiling: REPs for CALUX panel of Dirty Dozen

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		
	p,p'-DDT	3.5E-06	1.50	6.0E-05	1.6E-04		
	Dieldrin	6.9E-07	1.50	6.0E-04	5.0E-05		
	Endrin	3.5E-07	1.50	6.0E-04			
	Heptachlor	1.7E-05	1.50	6.0E-05			
	Hexachlorobenzene	3.5E-06	0.15	6.0E-05		5.0E-08	
	Mirex					7.9E-09	
	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
	PCB126		0.47	6.0E-05		1.6E-02	5.0
	PCB128		1.50	1.9E-04			
	PCB156	1.1E-06	0.15	6.0E-05		5.0E-06	3.2E-03
	TCDD					1.0	500
	Furan						1.3E-06

Clearly different profiles!

pesticides:
- endocrine activity

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

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.

THE "DIRTY DOZEND" POPS & OTHER POLLUTANTS: TOXICOLOGICAL PROFILING BY CALUX® PANEL

van Vugt-Lussenburg BMA*, Pieterse B, Middelhof I, Behnisch PA, van der Burg B and Bram Brouwer

Organohalogen Compounds Vol. 76, 1071-1073 (2014)

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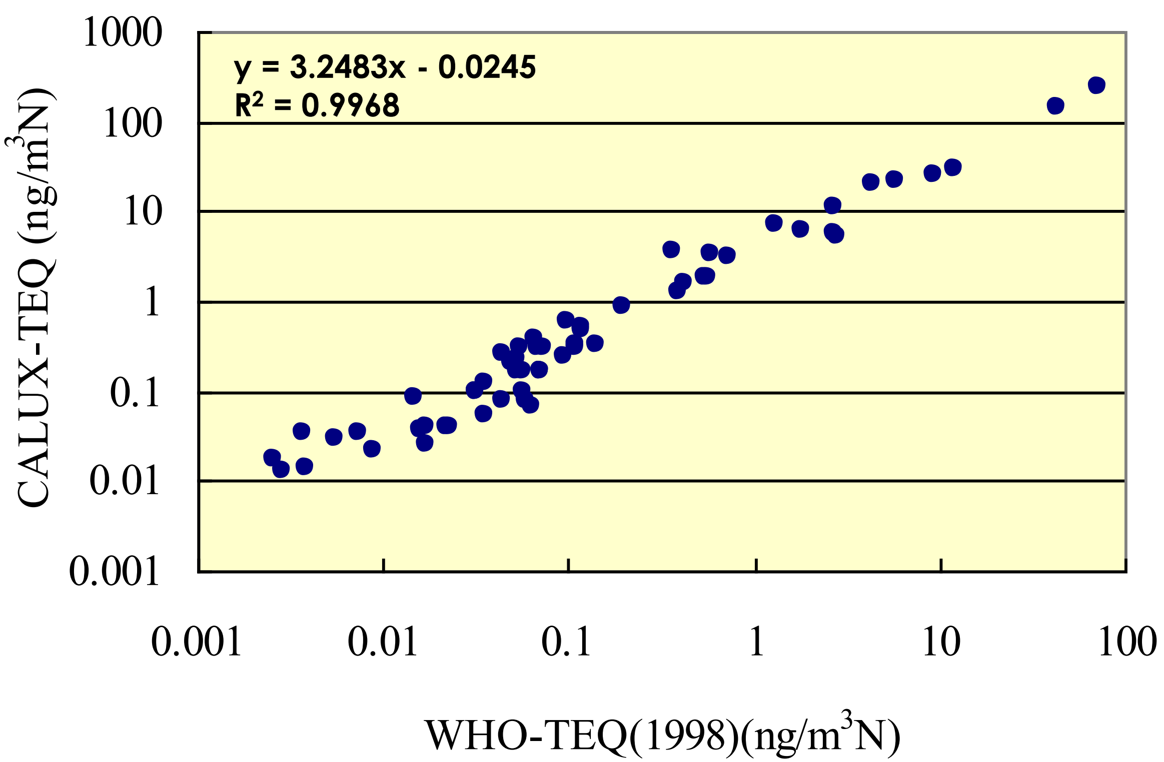
Japan (2001): DR CALUX vs GC/HRMS for emission gas samples (n=61)

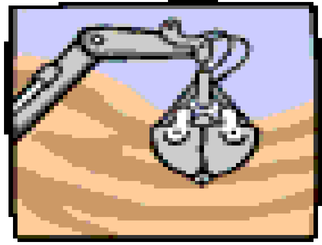


FN/FP ratio in comparison with the emission limit (0.1 ng-TEQ/m^{3}N)
FN: 0/61, FP: 19/61

FN/FP ratio in comparison with the emission limit (5 ng-TEQ/m^{3}N)
FN: 0/61, FP: 4/61

In courtesy of Dr. H Takigami, NIES, Japan

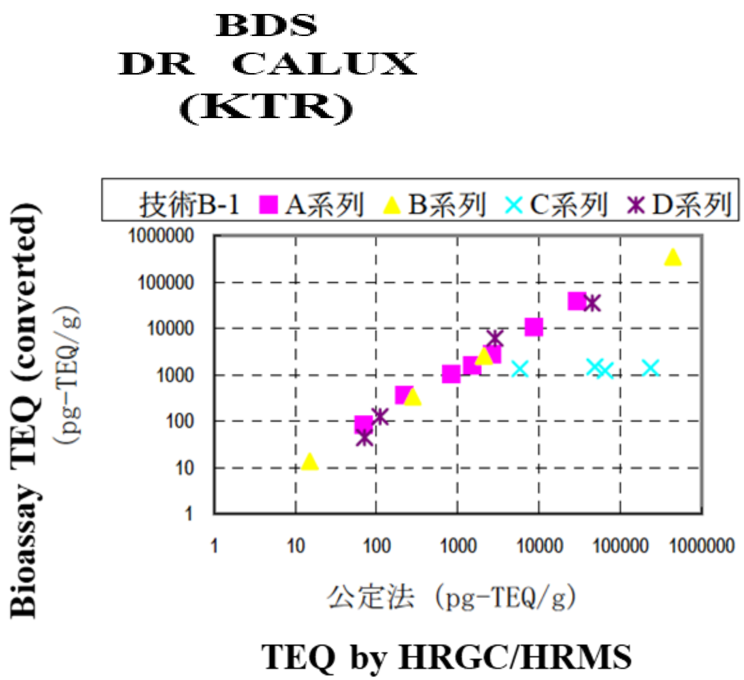
Japan (2005): DR CALUX in soil and sediment



Organizer: Dioxin Control Office, Ministry of the Environment, Japan
Time period: 2005-2006
Participants: 14 institutions
4 reporter gene assays

Samples for blind tests
19 soil samples consisting of four subgroups (TEQ: 15 – 450,000 pg/g)
18 sediment samples consisting of five subgroups (TEQ: 2.8-2,400 pg/g)

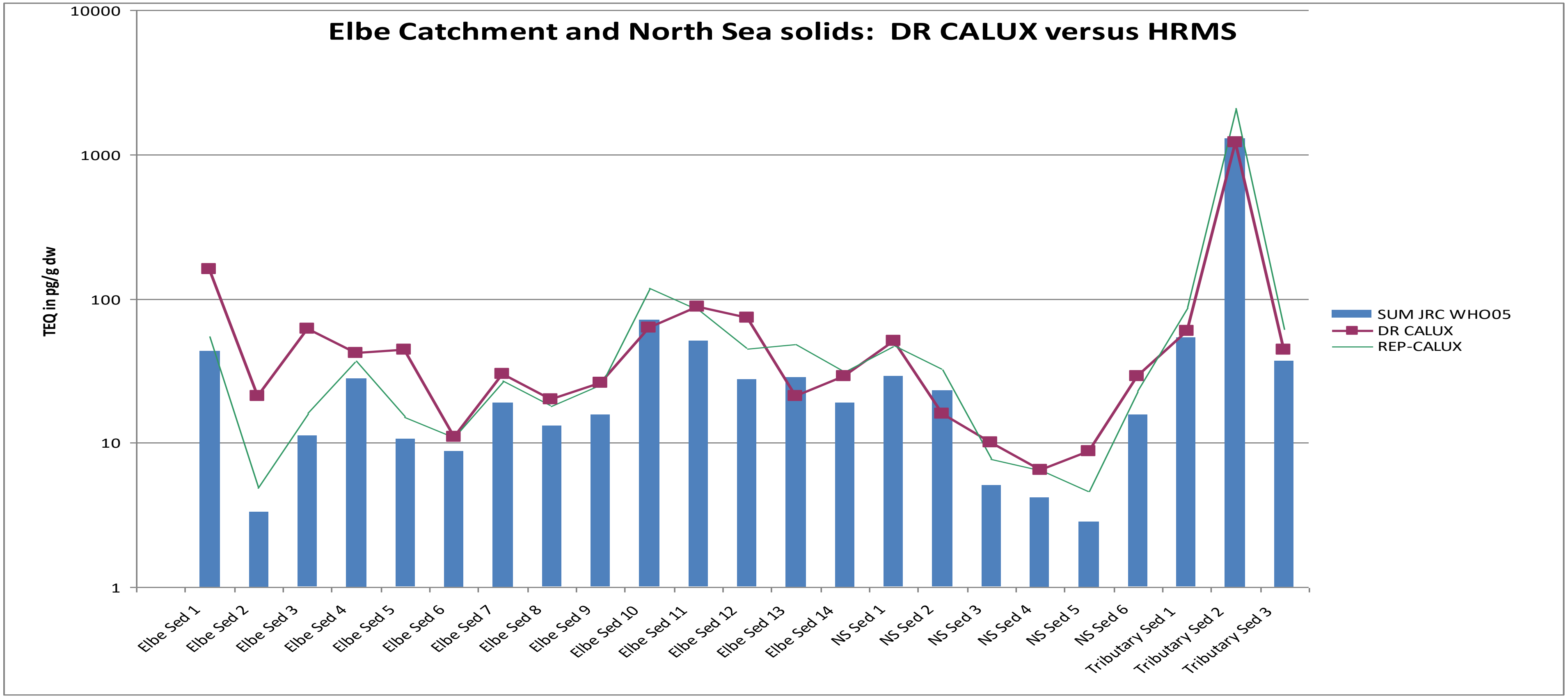
Obtained conclusions:
DR CALUX accepted as screening methods for TEQ measurement (resulting in guideline JIS 463, 2010).



A, B: Waste Incineration,
C: PCB spill, D: Pesticide

In courtesy of Dr. H Takigami, NIES, Japan

EU Reference Lab JRC, Ispra, Italy (2011): Sediments analysis by DR CALUX vs. HRGCMS Analysis



BIO/CHEMICAL ANALYIS OF SEDIMENTS FROM THE ELBE RIVER, THE NORTH SEA AND FROM SEVERAL TRIBUTARIES

Organohalogen Compounds Vol. 72, 645-647 (2010)

Peter A. Behnisch[1], Gunther Umlauf[2], Burkhard Stachel[3], Emiel Felzel[1], and Bram Brouwer[1]

Involved international Dioxin/PCB Crisis & Impact in feed/food chain

Country	Dioxin Source	Parameters	Economical damage
Brazil (1995)	Pesticides in Citrus Pellets	Dioxins	100 MIO EUR
Belgium (1998)	PCB Transformer Oils as feed	PCBs	625 MIO EUR (Wikipedia)
Australia (2005)	Harbor & pesticides	Dioxins	Ca. 5 MIO EUR
Chile (2013)	Feed addities	Dioxins, dl-PCBs	Ca. 10 MIO EUR
China (2014)	Industrial sides	Dioxins, dl-PCBs	No further action
Italy (since 2010)	Uncontrolled waste burning	Dioxins, dl-PCBs	Ca. 100 MIO EUR
Kuwait (2012)	National Food Monitoring	Dioxins, dl-PCBs	No further action
Thailand (since 2010)	National Food Monitoring	Dioxins, dl-PCBs	
Israel (since 2011)	Milk, egg, feed monitoring	Dioxins, dl-PCBs	
Germany (2011)	Liquid fat for feed	Dioxins	Ca. 100 MIO EUR
Turkey (2012)	National Food Monitoring	Dioxins, dl-PCBs	
Vietnam (since 2013)	Hot spots & decontamination	Dioxins, dl-PCBs	Ca. 100 MIO EUR
Brazil (since 2016)	PCP in recycled wood, since than National Monitoring by us	Dioxins, dl-PCBs	Ca. 100 MIO EUR

Dioxin problems Unsolved –
The old dirty dozen is more than ever alive..

Table 1: PCDD/Fs in free range chicken eggs samples from different regions – maximum levels measured in 2005 – 2018 (in pg WHO-TEQ/g of fat)Table 2: PCDD/Fs + dl-PCBs in free range chicken eggs samples from different regions – maximum levels measured in 2005 – 2018 (in pg WHO-TEQ/g of fat if not specified otherwise)

Country	Region	Date/ year	Locality	Measured level of PCDD/Fs	Exceed-ance of EU stan-dard	Potential source(-s)	Source of in-forma-tion
Ghana - Agbogbloshie (2018)	Africa	2018	Agbogbloshie	661	264	e-waste site	[18]
Ghana - Accra - hospital (2018)	Africa	2018	Accra - hospital WI	49	20	waste incineration	[18]
Cameroon - Yaounde (2018)	Africa	2018	Yaounde - hospital WI	4.6	2	waste incineration; open burning	[18]
Egypt (2010-2014)	Africa	2010-2014	not specified	4.5	2	metallurgical industry	[20]
Vietnam - Bien Hoa (2011)	Asia	2011	Bien Hoa	248	99	contaminated site (Agent Orange)	[21]
Thailand - Samut Sakhon (2015)	Asia	2015	Samut Sakhon	84	34	e-waste site and open burning	[22]
Indonesia - Kendalsari (2018)	Asia	2018	Kendalsari	49	20	secondary aluminium smelter	[23]
Taiwan - Chang-Hua County (2004-2005)*	Asia	2004-2005	Chang-Hua County	15.0	6	ash from metallurgical plant	[24]
China - Wuhan (2014)	Asia	2014	Wuhan	12.2	5	municipal waste incinerator	[25]
Turkey - Dilovasi (2008)	Asia	2008	Dilovasi, Kocaeli region	10.9	4	metallurgical industry	[26]
Kazakhstan - Balkhash (2013)	Asia	2013	Balkhash - west	9.8	4	car wrecks; metallurgical industry	[18]
Kazakhstan - Shabanbai BI (2014)	Asia	2014	Shabanbai Bi	9.3	4	PCBs oil contamination	[18]
Poland (2011)	CEE	2011	Not specified	29	12	PCP treated wood	[27]
Ukraine, Krivyi Ryh (2018)	CEE	2018	Krivyi Ryh	23	9	metallurgical industry	[17]
Czechia - Pitárne (2017)	CEE	2017	Pitarne	15.4	6	PVC recycling plant	[28]
Serbia - Grabovac (2015)	CEE	2015	Grabovac	11.1	4	chemical contamination	[16]
Poland - Malopolska (2017)	CEE	2017	Malopolska region	9.5	4	air pollution (general)	[29]
Armenia - Alaverdi (2018)	CEE	2018	Alaverdi	7.5	3	copper smelter	[12]
Bosnia and Herzegovina - Zenica (2015)	CEE	2015	Zenica	5.6	2	metallurgical industry	[16]
Czechia - Lhenice (2015)	CEE	2015	Lhenice	5.3	2	PCB contaminated site	[30]
Belarus - Gatovo (2014)	CEE	2014	Gatovo	4.3	2	car shredder	[31]
Portugal (2008)	Europe	2008	Not specified	61	25	PCP treated wood	[32]
Italy - Piedmont (2012)	Europe	2012-2013	Piedmont region	38	15	metallurgical industry	[33]
Belgium (2007)	Europe	2007	Not specified	20	8	not specified	[34]
Germany - Teningen (2014)	Europe	2014	Teningen	11.4	5	former PCB capacitors production (contaminated site)	[35]
Netherlands - Friesland (2014)	Europe	2014	Eastern part of Friesland	9.6	4	not clear	[36, 37]
Netherlands - Rijnmond (2014)	Europe	2014	Rijnmond and Rotterdam	9.6	4	industrialized area of Netherlands	[37]
Italy - Caserta	Europe	2014-2015	Caserta, Campania	6.2	2	open burning of waste	[38]
Netherlands - Harlingen (2013)	Europe	2013	Midlum, Harlingen	4.8	2	municipal waste incinerator	[39]
Uruguay, Minas	GRULAC	2009	Minas	23	9	PCBs burning cement kiln	[40, 41]
Brazil - Vespasiano (2014)	GRULAC	2014	Vespasiano, Bello Horizonte	7.4	3	fire in cement kiln (used tires burnt)	[42]
Peru - Zapallal (2010)	GRULAC	2010	Zapallal	4.4	2	ash from metallurgical workshops	[43]
Canada (2005-2006)	North America	2005-2006	not specified	10.6	4	PCP treated wood	[44]

Table 2: PCDD/Fs + dl-PCBs in free range chicken eggs samples from different regions – maximum levels measured in 2005 – 2018 (in pg WHO-TEQ/g of fat if not specified otherwise)

Country	Region	Date/ year	Locality	Levels of PCDD/ Fs + dl-PCBs	Exceed-ance of EU stan-dard	Potential source(-s)	Source of in-forma-tion
Ghana - Agbogbloshie (2018)	Africa	2018	Agbogbloshie	856	171	e-waste site	[18]
Ghana - Accra - hospital (2018)	Africa	2018	Accra - hospital WI	63	13	waste incineration	[18]
Tanzania - Arusha (2012)	Africa	2012	Arusha	20*	4	open burning of waste	[45]
Cameroon - Yaounde (2018)	Africa	2018	Yaounde - hospital WI	11.4	2	waste incineration, open burning	[18]
South Africa - Vanderbijlpark (2008-2009)	Africa	2008-2009	Vanderbijlpark	6.4	1	metallurgical industry	[46]
Vietnam - Bien Hoa (2011)	Asia	2011	Bien Hoa	249	50	contaminated site (Agent Orange)	[21]
Kazakhstan - Shabanbai BI (2015)	Asia	2015	Shabanbai Bi	155	31	PCBs oil contamination	[18]
Kazakhstan - Balkhash (2013)	Asia	2013	Balkhash - north	101*	20	metallurgical industry	[25]
Thailand - Samut Sakhon (2015)	Asia	2015	Samut Sakhon	96	19	e-waste site and open burning	[22]
Indonesia - Kendalsari (2018)	Asia	2018	Kendalsari	85	17	secondary aluminium smelter	[23]
China - Zheijang (2006-2015)	Asia	2006-2015	Zhejiang	37	7	municipal waste incineration, e-waste site	[47]
China - Beihai (2014)	Asia	2014	Beihai	37*	7	metallurgical industry	[14]
China - Wuhan (2014)	Asia	2014	Wuhan	16.0	3	municipal waste incinerator	[25]
Kazakhstan - Shetpe (2016)	Asia	2016	Shetpe	6.4	1	car wrecks, waste, cement kiln	[18]
Poland - Silesia (2018)	CEE	2018	Silesia	43	9	co-burning of PVC waste in household heating	[48]
Armenia - Nubarashen (2010)	CEE	2010	Nubarashen	37*	7	contaminated site	[49]
Ukraine, Krivyi Ryh (2018)	CEE	2018	Krivyi Ryh	36	7	metallurgical industry	[17]
Czechia - Pitárne (2017)	CEE	2017	Pitarne	32	6	PVC recycling plant	[28]
Poland (2011)	CEE	2011	Not specified	30	6	PCP treated wood	[27]
Armenia - Alaverdi (2018)	CEE	2018	Alaverdi	27	5	copper smelter	[12]
Belarus - Gatovo (2014)	CEE	2014	Gatovo	15.6	3	car shreder	[31]
Serbia - Grabovac (2015)	CEE	2015	Grabovac	13.5	3	chemical contamination	[16]
Czechia - Lhenice (2015)	CEE	2015	Lhenice	9.1	2	PCB contaminated site	[30]
Bosnia and Herzegovina - Zenica (2015)	CEE	2015	Zenica	8.7	2	metallurgical industry	[16]
Italy - Piedmont (2012)	Europe	2012-2013	Piedmont region	113	23	metallurgical industry	[33]
Belgium (2007)	Europe	2007	Not specified	95	19	not specified	[34]
Italy - Lombardia (2010)	Europe	2010	Lombardia	90	18	industrialized areas of Lombardia	[50, 51]
Netherlands (2012)	Europe	2012	not specified	80	16	asbestos fiber plates roof	[52]
UK - Bishop's Cleeve (2010)	Europe	2010	Bishop's Cleeve	55*	11	waste incineration ash	[25]
Germany - Teningen (2014)	Europe	2014	Teningen	36	7	former PCB capacitors produc-tion (contaminated site)	[35]
Netherlands - Friesland (2014)	Europe	2014	Eastern part of Friesland	18.9	4	not clear	[36, 37]
Italy - Naples	Europe	2014-2015	Naples, Campania	17.2	3	open burning of waste	[38]
Netherlands - Rijnmond (2014)	Europe	2014	Rijnmond and Rotterdam	14.2	3	industrialized area of Nether-lands	[37]
Italy (2013-2015)	Europe	2013-2015	not specified	12.7	3	not clear	[53]
Germany - State of Hesse (2013)	Europe	2013	State of Hesse	11.8	2	asbestos fiber plates roof	[54]
Netherlands - Harlingen (2013)	Europe	2013	Midlum, Harlingen	10.9	2	municipal waste incinerator	[39]
Germany - Eyller Berg (2014)	Europe	2014	Eyller Berg (near Kamp-Lintfort)	10.4	2	hazardous waste landfill	[35]
Italy - Caserta	Europe	2014-2015	Caserta, Campania	9.7	2	open burning of waste	[38]
Brazil - Vespasiano (2014)	GRULAC	2014	Vespasiano, Bello Horizonte	49	10	fire in cement kiln (used tires burnt)	[42]
Uruguay, Minas	GRULAC	2009	Minas	25	5	PCBs burning cement kiln	[40, 41]
Canada (2005-2006)	North America	2005-2006	not specified	12.8	3	PCP treated wood	[44]

*Mesured by DR CALUX® and expressed in pg BEQ (bioanalytical equivalent)/g of fat

a toxics-free future

https://english.arnika.org

www.ipen.org ipen@ipen.org @ToxicsFree

#StopToxicRecycling #StopPOPsWaste

Kuwait: National Food/Feed Monitoring (2014)

Ecotoxicology and Environmental Safety 100 (2014) 27–31

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

ELSEVIER

Screening for PCDD/Fs and dl-PCBs in local and imported food and feed products available across the State of Kuwait and assessment of dietary intake

CrossMark

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[a] Food and Nutrition Program, Environmental and Life Sciences Research Center, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, Kuwait
[b] Environment Management Program, Environmental and Life Sciences Research Center, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, Kuwait
[c] BioDetection Systems BV (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands

Table 1
DR-CALUX bioassay BEQs measured in food and feed samples from Kuwait markets.

Sample	N^a	Mean	Range	Maximum level[g,b,c]	Cut-off level[h]	Suspected samples
Beef[d]	50	2.00	0.10–5.50	4.0	2.67	10
Lamb[d]	18	1.90	0.40–2.90	4.0	2.67	3
Chicken[d]	100	1.60	0.10–3.90	3.0	2.0	26
Milk[d]	80	2.10	0.60–5.90	5.5	3.67	5
Eggs[d]	17	2.20	0.90–3.40	5.0	3.3	1
Fish[e]	18	0.30	0.10–0.90	6.5	4.3	–
Animal feed[f]	35	0.27	0.12–0.55	1.5	1.0	–

Summary

→ **318 (85 local and 233 imported) bovine and sheep meat, poultry meat, milk, eggs, and fish, were purchased from Kuwaiti supermarkets and local distributors**

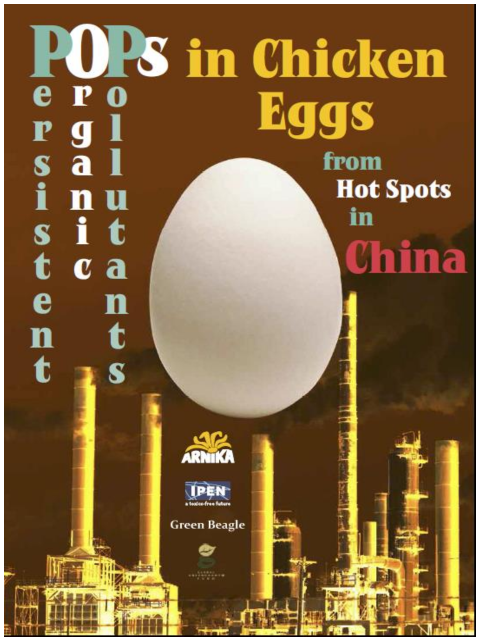
→ **26% chicken meat, 20% beef meat, 6% lamb meat, 6% milk and egg products very non-conform of EU guidelines**

→ **all fish and animal feed samples very conform EU guidelines**

https://english.arnika.org

www.ipen.org ipen@ipen.org @ToxicsFree
#StopToxicRecycling #StopPOPsWaste

China: free range chicken eggs from several industrial hot spots (2015)



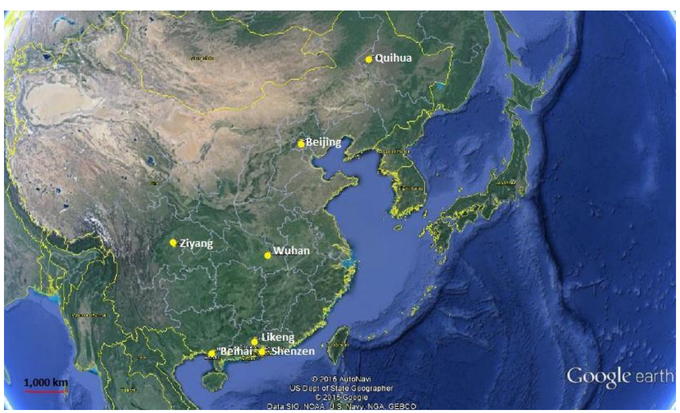
Beihei –metallurgic plant: 5-7 x EU Max

Likeng – Waste incinerator: 3 x EU Max

Quihua – PVC plant: 2 x EU Max

Shenzhen – Waste incinerator: above 1 x EU Max

Wuhan – waste incinerator: 1-7 x EU Max, with high PBDD/Fs levels



"This publication is part of Strengthening the capacity of pollution victims and civil society organisations to increase chemical safety in China"

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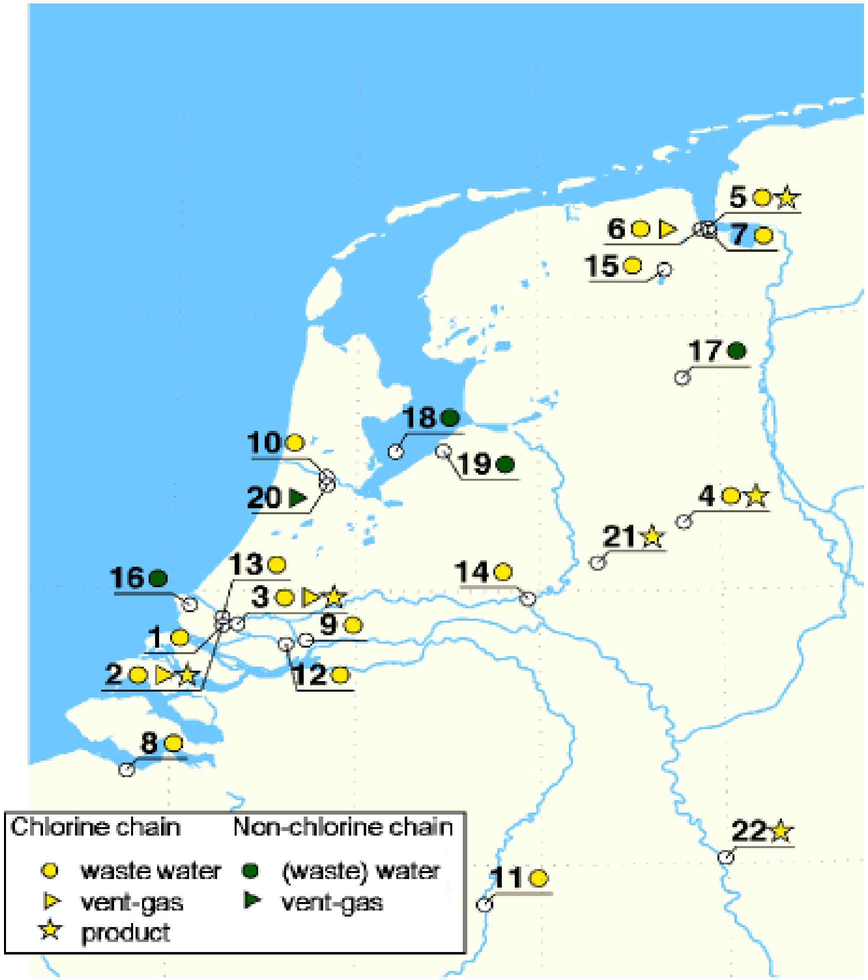
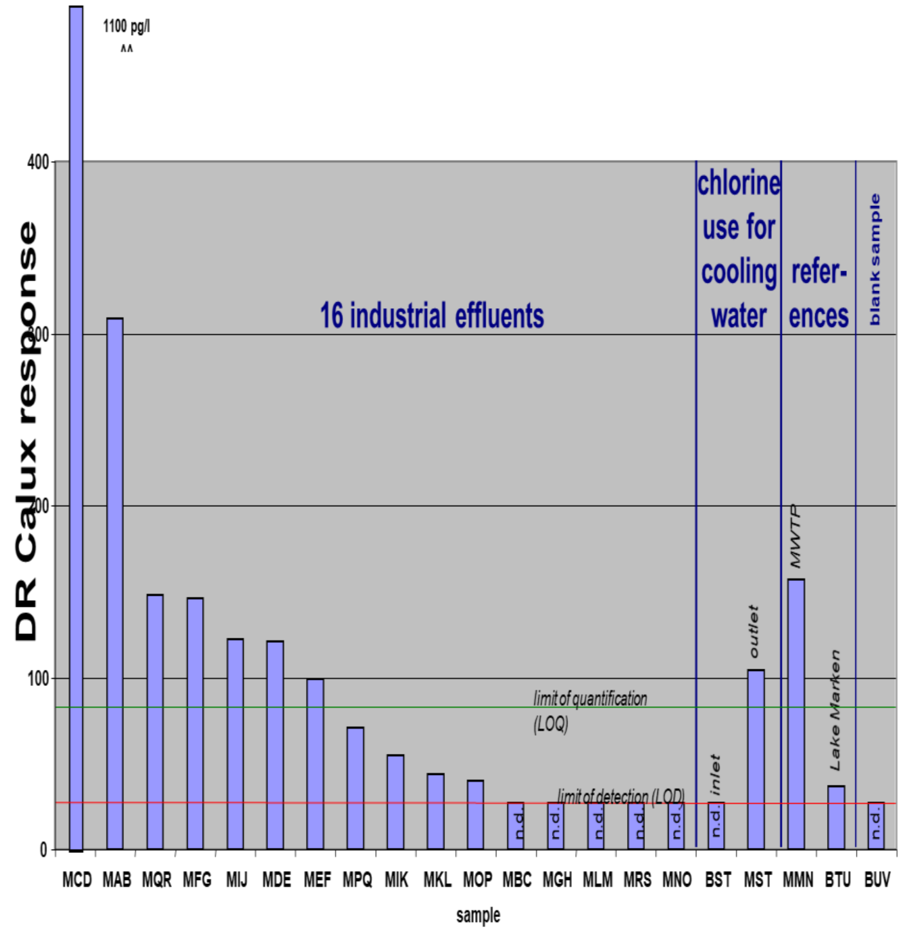
- Relevance for wildlife and humans
- Take home message

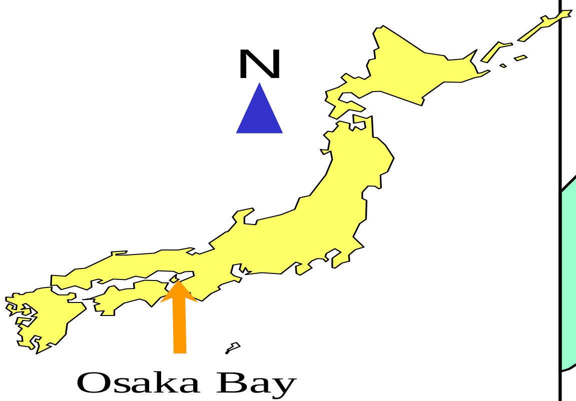
The Netherlands (OVOC, 2004): Industrial effluents from the Chlorine Chain with high dioxin levels due to chloroprene rubber, HCl and trichloroethylene

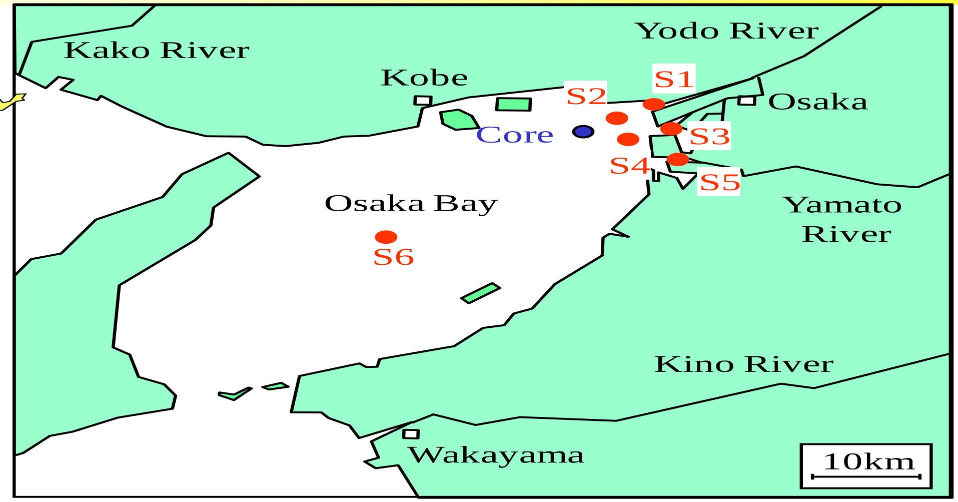


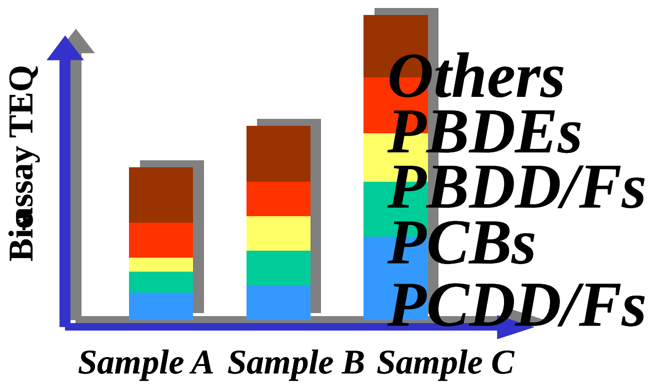


Van Hattum et al., 2004

Osaka Bay, Japan:
DR CALUX covers also brominated dioxins



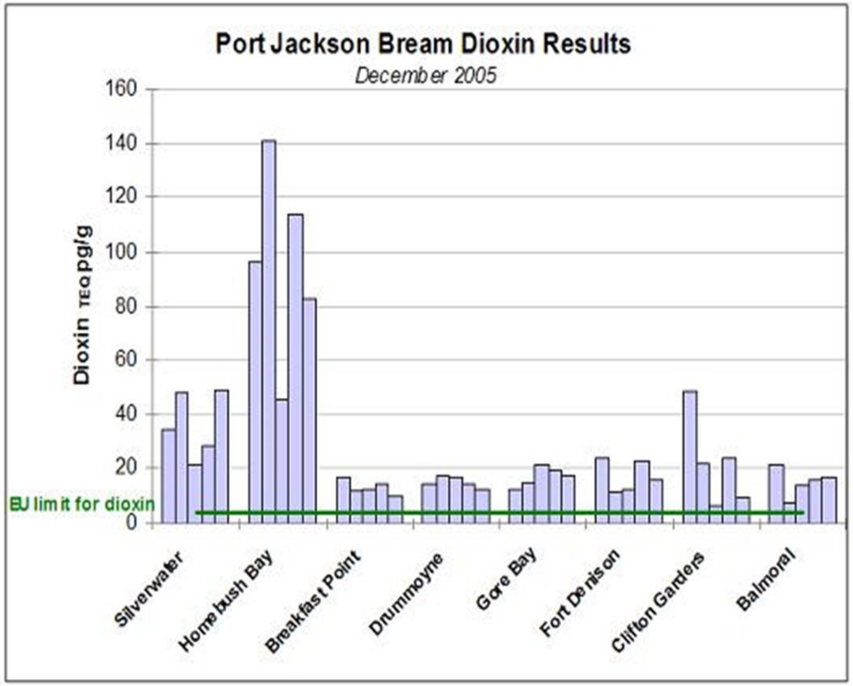


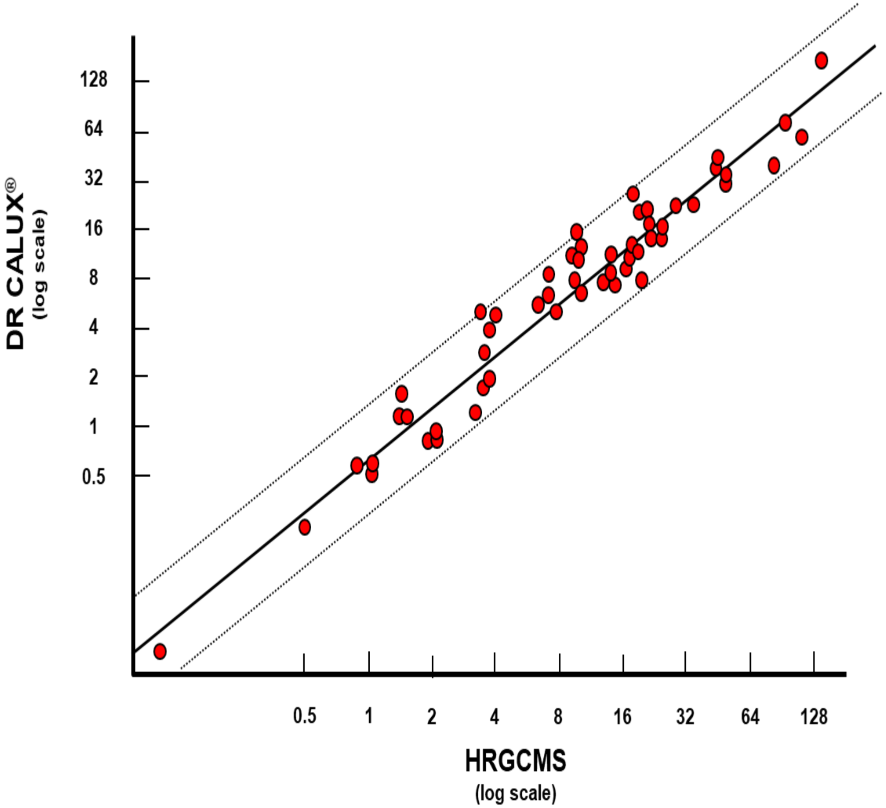


In courtesy of Dr. H Takigami, NIES, Japan

Sydney Harbour, Australia 2006:
Many fish dioxin polluted through pesticide producer

NSW DEPARTMENT OF
PRIMARY INDUSTRIES





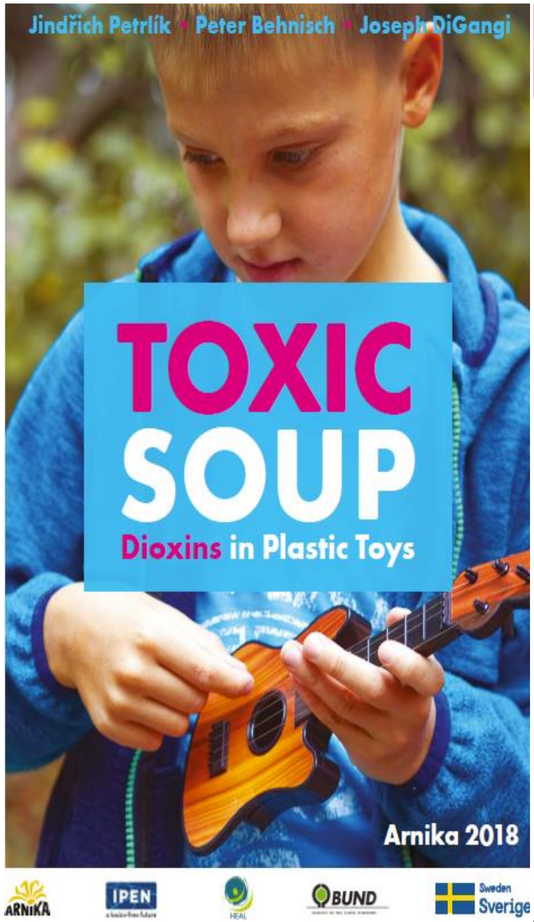
Summary:
→ **Good correlation between DR CALUX and GC/HRMS**
→ **National acceptance in a few weeks**

In courtesy of

In the focus….

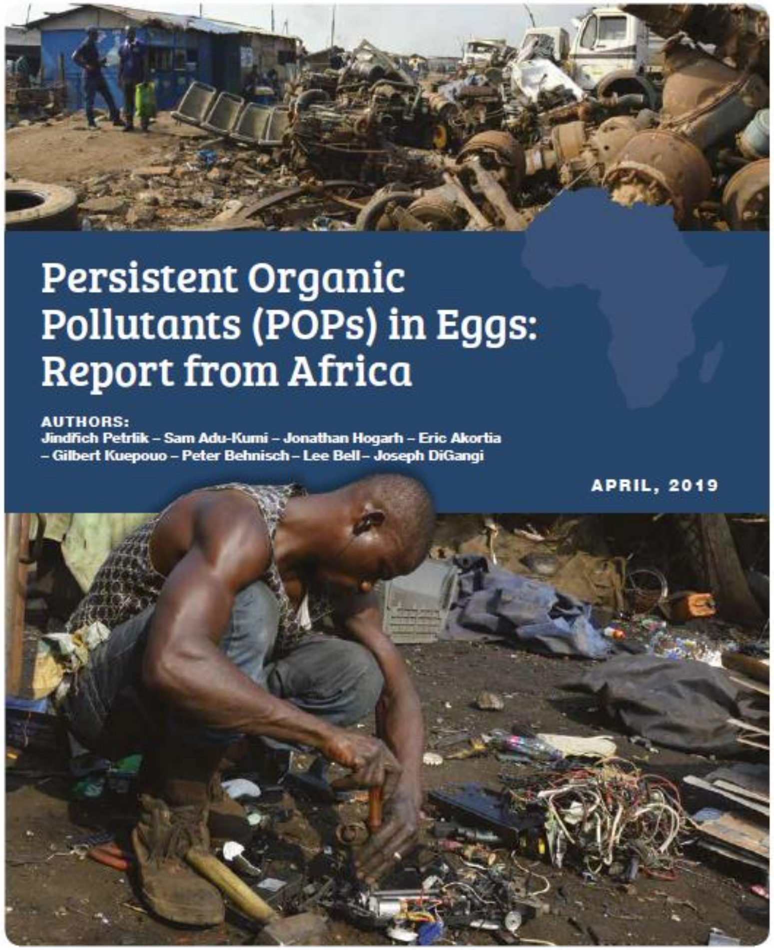
- What have Non-communicable disease to do with POPs & EDCs?
- How to analyse POPs & EDCs efficiently?
- Toxicity profiles of POPs & EDCs
- Examples of Dioxins/PCBs in the environment and their food impact
- Harbor Cities & Dioxins/PCBs
- **Can consumer products and toys contain dioxins and EDCs?**
- Relevance for wildlife and humans
- Take home message

Toys & consumer products with high levels of brominated dioxins



Country / Sample	Type	PBDD/Fs (pg TEQ/g)[1]	DR CALUX (pg TEQ/g)	PBDEs (ug/g)[2]	HBCD (ug/g)	TBBPA (ug/g)
Argentina ARG_04	Rubik's-like cube	727	1,200	708	1	na[3]
Brazil BRZ-T-7A	Toy, car	750	590	169	0.2	8
Cambodia KAM-H-1	Hair diadem	1,950	1,500	358	0.3	10
Canada CA-H-1C	Hair rack	1,500	1,300	718	< 0.01	1
Czechia JI_11	Cube	2,159	17,000	2,614	91	na[3]
Czechia SIX_02	Hairclip	60	210	1,623	8	na[3]
France FR-T-3	Toy revolver	2,058	520	1,077	1	314
Germany D-TO7	Key fob	3,821	820	511	2	307
India IND_11	Rubik's-like cube	690	1,300	593	2	na[3]
Japan JP-O-1	Smart phone holder	1,200	560	693	0.5	37
Nigeria NIG_06	Rubik's-like cube	860	4,800	1,780	9	na[3]
Nigeria NIG_11	Rubik's-like cube	56	370	1,218	8	na[3]
Portugal PT-T-10a	Toy small guitar	1,137	270	3,318	2	37

Dioxins & other POPs in chicken eggs at E-waste recycling sides in Africa



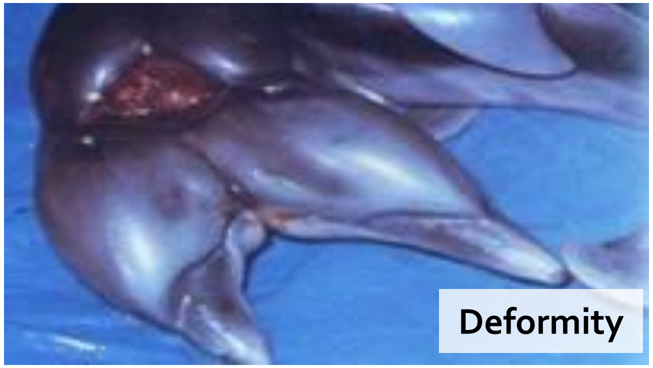
PCDD/F/PBDD/F/DL-PCB-TEQ (pg TEQ/g fat)	Yaoundé -hospital	Accra – Agbogbl.	Accra - hospital	Kumasi - hospital	Accra- super- market	EU stand. /limits
DR CALUX	9.6	840	56	5.2	1.2	
GC/HRMS	11.4	1150	63	2.6	0.56	5.00
Other POPs						
HCB	1.4	25.1	3.63	0.76	< 0.2	-
PeCB	0.35	22.4	2.88	< 0.2	< 0.2	
6 PCB	30	168	7.8	< 1.2	< 1.2	40.00
sum HCH	2.5	< 0.6	< 0.6	< 0.6	< 0.6	
sum DDT	22	9.7	79	0.82	< 1.2	

In the focus….

- What have Non-communicable disease to do with POPs & EDCs?
- How to analyse POPs & EDCs efficiently?
- Toxicity profiles of POPs & EDCs
- Examples of Dioxins/PCBs in the environment and their food impact
- Harbor Cities & Dioxins/PCBs
- Can consumer products and toys contain dioxins and EDCs?
- **Tissue, blood and mother milk testing in wildlife and humans**
- Take home message

Can we relate effects in wildlife with in vitro effects

Relation with contaminants?



Dr. Go SUZUKI

Center for Marine Environmental Studies, Ehime University, Japan

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

Suzuki, G.[1,*], Tue, N.M.[1], van der Linden, S.[2], Someya, M.[1], Takahashi, S.[1], Brouwer, A.[2,4], van der Burg, B.[2], Lamoree, M.[3], van Velzen, M.[3], Isobe, T.[1], Tajima, Y.[5], Yamada, T.[5], Tanabe, S[1]

[1]Center for Marine Environmental Studies, Ehime University, Matsuyama 790-8577, Japan, [2]BioDetection Systems b.v., 1098 XH Amsterdam, the Netherlands, [3]Institute for Environmental Studies, VU University, 1081 HV Amsterdam, the Netherlands, [4]Faculty of Earth and Life Sciences, VU University, 1081 HV Amsterdam, the Netherlands, [5]National Museum of Nature and Science, Tokyo 110-8718, Japan

Agonistic activity / Antagonistic activity

AR-CALUX

	Agonistic: Persistent fraction	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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	2.6E-03	2.3.E-03	NA	1.0E-03	4.5E-04	2.6E-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	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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	2.6E-03	2.3.E-03	3.4.E-04	1.0.E-03	4.5E-04	2.6E-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	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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

	Agonistic: Persistent fraction	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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

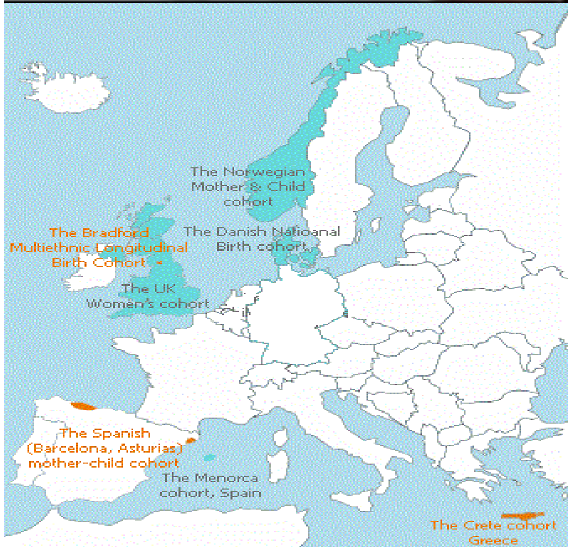
	Agonistic: Persistent fraction	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
Baikal seal (2005)-Blubber	4.3.E-04	1.1E-03	7.1.E-03	[illegible]	3.6.E-04	4.5.E-04	NA	NA	NA	NA
Baikal 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

	Agonistic: Persistent fraction	Agonistic: Strong	Moderate	Mild	Weak	Antagonistic: Persistent fraction	Antagonistic: Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction					Crude hydrophobic fraction			
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	1.5.E-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

EU: Best biomarkers for EDCs for mother- newborn baby ?



1st case: 5 EU countries

•NEW GENERIS project from 2006 to 2009

•approx. 1000 mother- newborn baby cohortes

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

•cord and motherblood was taken at day of birth

•Analysed by DR-, ER and AR CALUX

CALUX responses are correlated to several health effects

Effect	Publikation	CALUX Test	Country
Leukemia	Cancer Epidemiol Biomarkers Prev 2012;21:1756-1767 & others	DR/ER CALUX	Global
Leukemia	Environ Health Perspect 122:193–200	DR/ER/AR CALUX	Spain
Birth weight	Science of the Total Environment 484 (2014) 121–128	DR CALUX	Spain/Greek
Childrens development	Environment International	DR, ER AR CALUX	Denmark

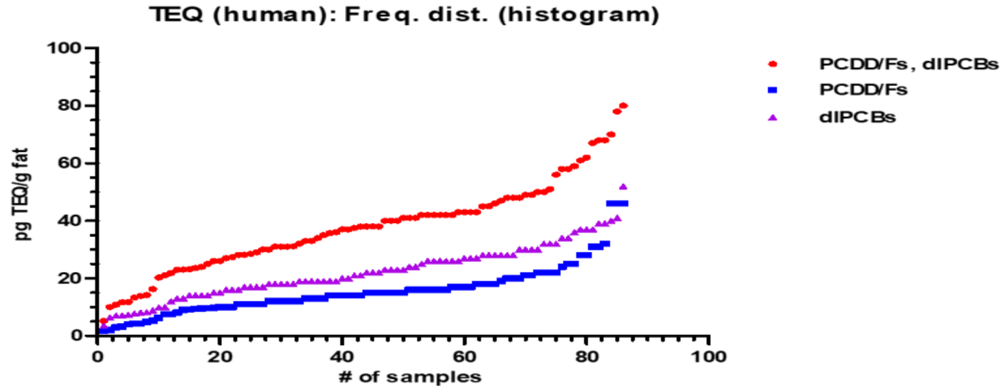


<u>2nd case:</u>

Neighbors living nearby an industrial land fill in Kamp-Lintfort in Germany

Table 1 and Figure 1: Minimum, median, maximum, 95%/5% percentile and mean value of the sum of PCDD/PCDF/dl-PCB/dl-compounds-BEQ, PCDD/F-BEQ and dl-PCB/other dl-like compounds data analysed by DR CALUX® from the human plasma samples from 86 neighbours living near an industrial landfill in the city of Kamp-Lintfort.

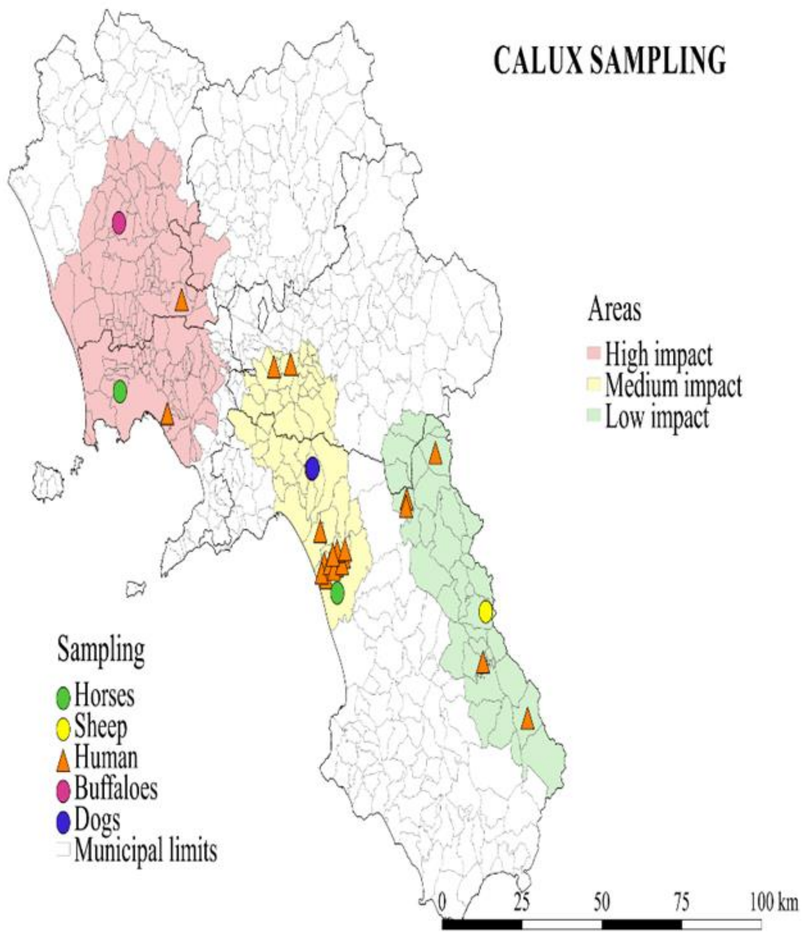
	DR CALUX®		
	PCDD/Fs, dl-PCBs	PCDD/Fs	dl-PCBs
Number of values	86	86	86
minimum	5.2	1.7	3.5
median	38	15	22
maximum	80	46	52
5% percentile	12	3.5	7.2
95% percentile	68	32	39
mean	38	16	22
SEM	1.7	0.94	1



Blood plasma monitoring of contaminants in humans and domestic animals using a panel of CALUX® bioassays: three case studies

Behnisch PA[1], Besselink H[1], Malonek L[2], Limone A[3], Pizzolante A[3], Pierri A[3], Ferro A[3], Gallo A[3], Buonerba C[3], Pierri B[4], Di Stasio A[3], Cerino P[3], Durward-Akhurst SA[5], Schultz NE[5], Norton EM[5], Rendahl AK[6], Geor RJ[7], Mickelson JR[8], McCue ME[5], Brouwer A[1,9]

Abstracts Book of the Dioxin 2018 : 38th International Symposium on Halogenated Persistent Organic Pollutants & 10th International PCB Workshop, 26-31 August 2018, Kraków, Poland



3rd case:

"Land of fire", wider area, Napoli, Italy:

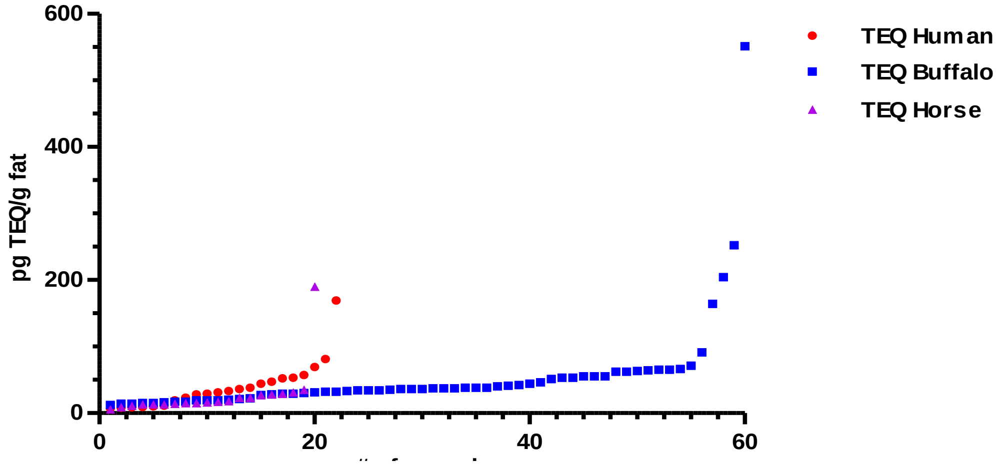
Blood testing of humans and animals by CALUX panel (buffalo, horse, dogs, human)

Human plasma testing for dioxins and dioxin-like compounds in Campania region, Italy

Table 2 and Figure 2: Minimum, median, maximum, mean, 95% and 5% percentile value of the PCDD/PCDF/dl-PCB/dl-compounds-TEQ analysed by DR CALUX® from human, buffalo and horse plasma samples in several locations in the Campania region, Italy (e.g. Avellino, Salerno, Caserta and Napoli area).

	DR CALUX®		
	Human	**Buffalo**	**Horse**
Number of values	22	60	20
minimum	7.3	12	5.5
median	32	37	17
maximum	169	551	190
5% percentile	7.5	14	5.7
95% percentile	160	200	180
mean	39	55	27
SEM	7.6	10	8.7

TEQ (human, buffalo, horse) Freq. dist. (histogram)



Blood plasma monitoring of contaminants in humans and domestic animals using a panel of CALUX® bioassays: three case studies

Behnisch PA[1], Besselink H[1], Malonek L[2], Limone A[3], Pizzolante A[3], Pierri A[3], Ferro A[3], Gallo A[3], Buonerba C[3], Pierri B[4], Di Stasio A[3], Cerino P[3], Durward-Akhurst SA[5], Schultz NE[5], Norton EM[5], Rendahl AK[6], Geor RJ[7], Mickelson JR[8], McCue ME[5], Brouwer A[1,9]

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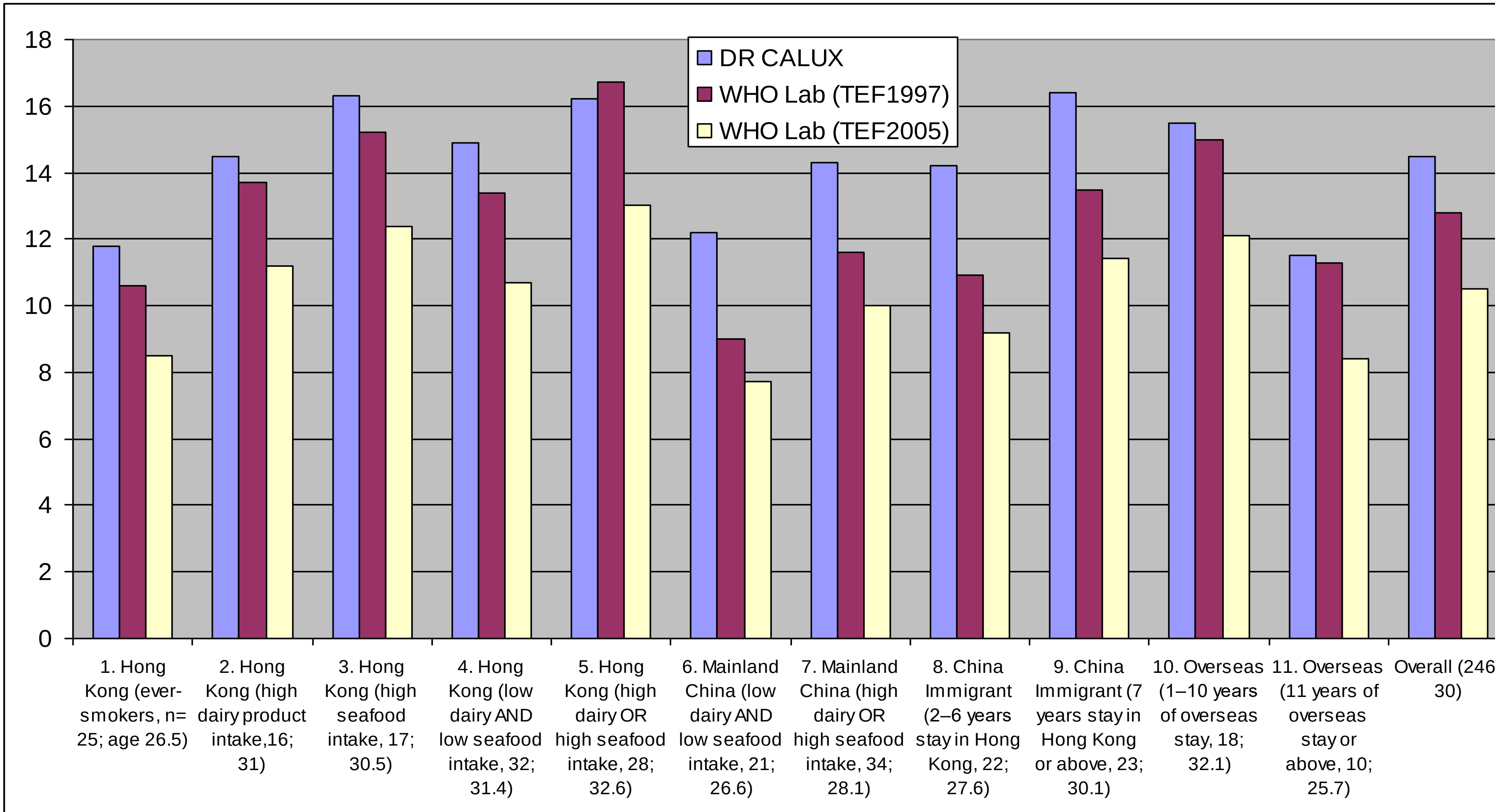
Harbor City Istanbul, Turkey (Yilmaz et al 2014)
Mother milk biomonitoring by DR CALUX

- **Investigation of breast milk of 48 healthy lactating mothers who live in Istanbul**
- **There was significant correlation between DR-CALUX values and genotoxicity by comet assay scores ($p<0,001$).**
- **Also a significant correlation was seen between Body Mass Index (BMI) and DR-CALUX results.**
- **The highest DR-CALUX values (13 and 12 pg PCDD/F/dl-PCB-BEQ/g fat) were detected for the samples taken from mothers with the highest BMI scores**
- **Less than 10% of breast milk samples have been above 10 pg PCDD/F/dl-PCB-BEQ/g fat.**
- **These values are comparable to other CALUX studies in breast milk e.g. China (mean value around 14 pg TEQ/g fat, Lui et al 2007 or Kayama et al 2003).**

Hong Kong/China (2007): Mother milk testing by DR CALUX and GC/HRMS [WHO- and EU Reference lab CVUA Freiburg]

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)



More safety –
through covering also the unknown

- **Monitoring of POPs & EDCs in water, food & blood**
- **Providing robust, easy, high capacity and low cost state-of-the art de-risking solutions**
- **Prevent consumer scares through monitoring of environmental sources, food and human uptake**
- **POPs & EDCs as well as other toxic pathways**

We are ready to go!

Invitation for 12th BioDetectors Conference
Vienna, 26/27th Sept 2019

Vienna University of Technology