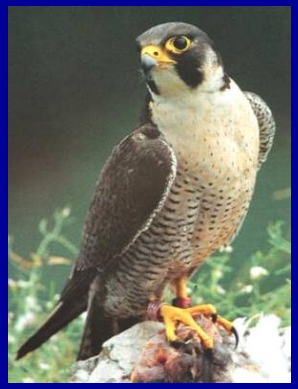
BioDetection Systems

Global Tour by CALUX – The last 365 days

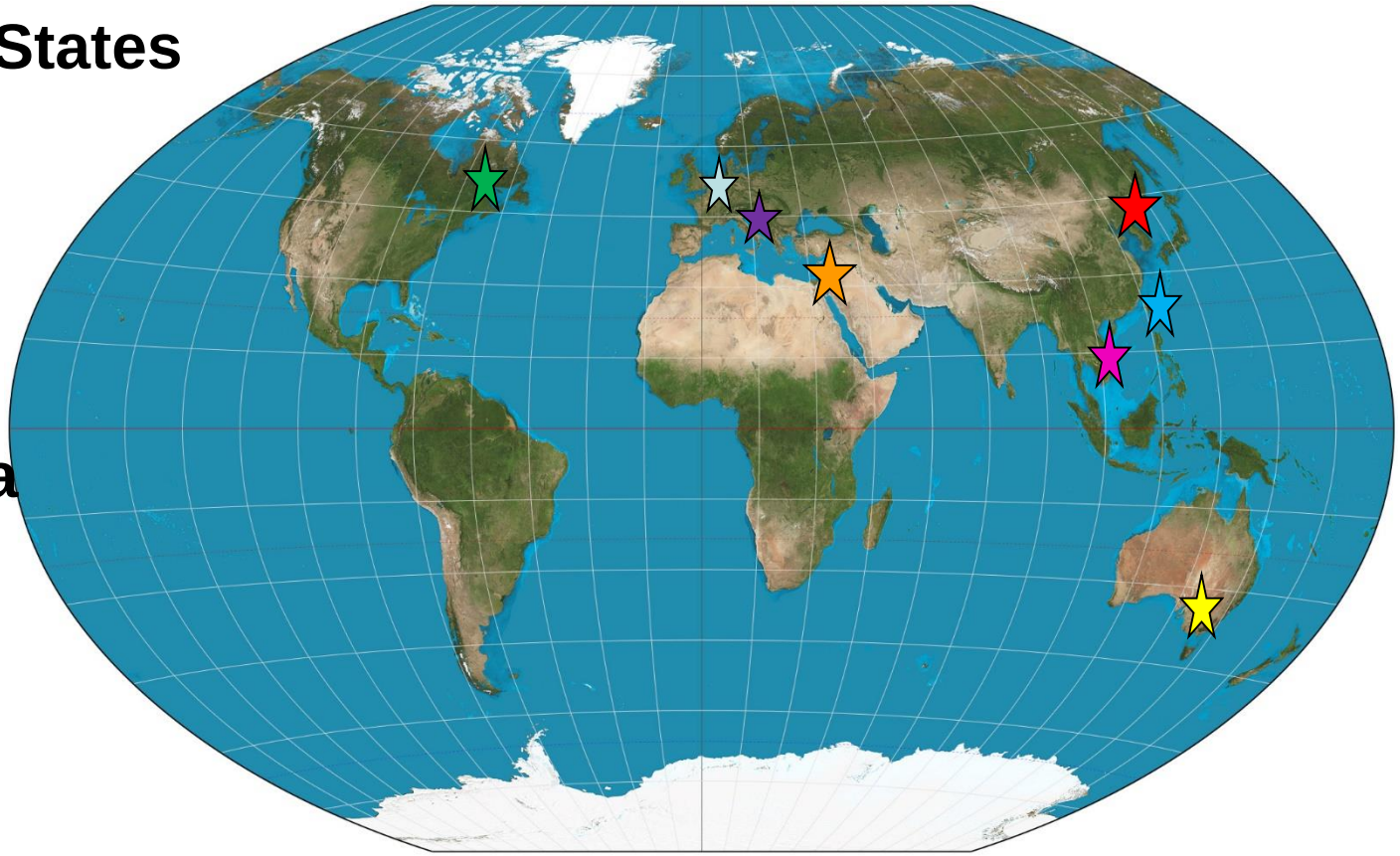


Dr. Peter A. Behnisch

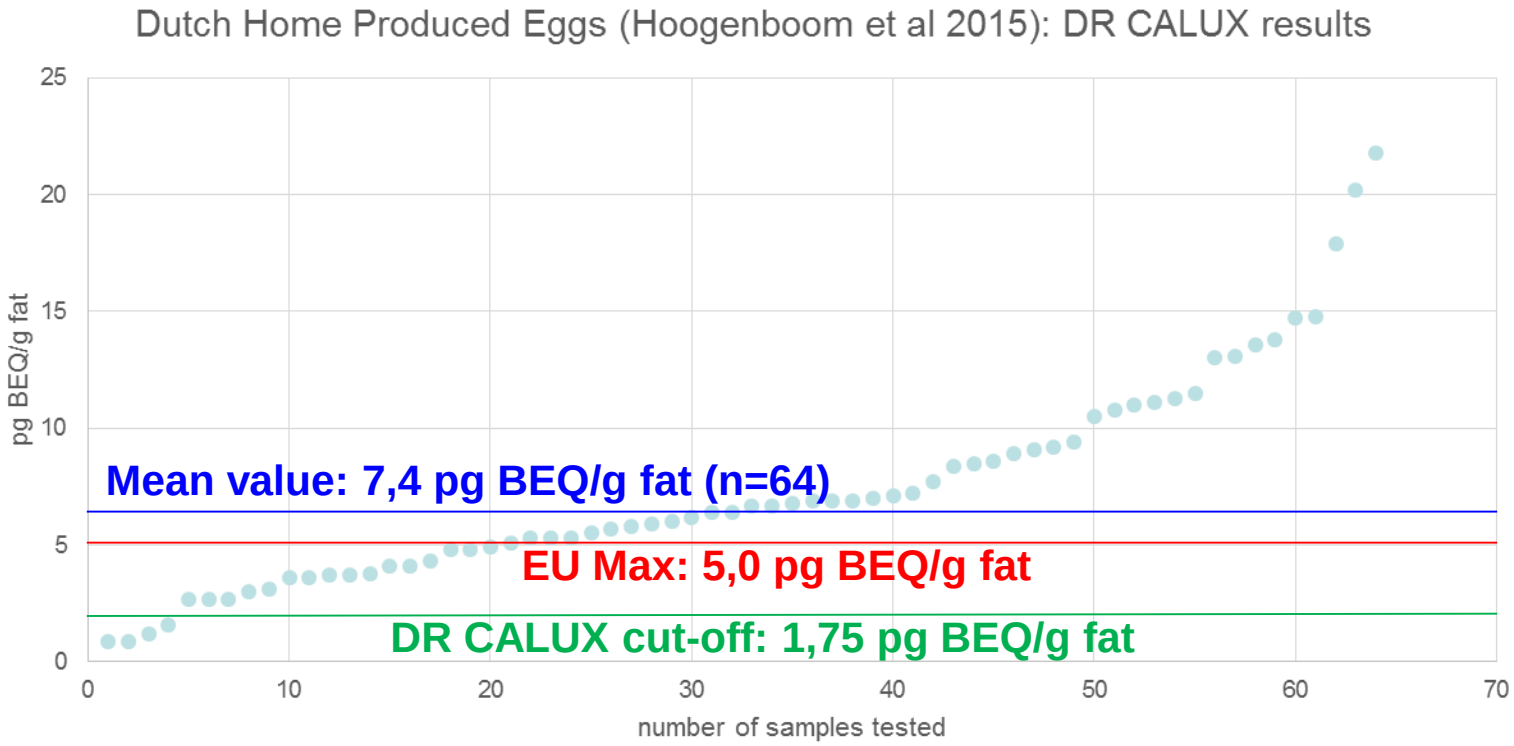
Director
Bio Detection Systems bv
Amsterdam

Global Tour 2015-2016

- NL, DE, CH
- China
- Balkan States
- Israel
- Taiwan
- Vietnam
- Australia
- Canada



The Netherlands: Home produced eggs > 60% suspect in DR CALUX



Hoogenboom et al (2015). DIOXIN 2015 oral presentation; Dioxins and PCBs in home produced egg. RIKILT report 2014-012

Germany: dl-PCBs in BIO eggs due to asbestos-cement fiber plates (ACP)

Table 1

BEQ- and TEQ-values of PCDD/Fs and dl-PCBs found in three organic-farmed egg samples and one pooled chicken sample determined by a screening with the DR-CALUX® bioassay and confirmed by GC–HRMS.

Sample	PCDD/Fs (pg TEQ/g)	dl-PCBs (pg TEQ/g)	Total TEQ (pg TEQ/g)	Total BEQ (pg BEQ/g)
Eggs 1	1.1	10.7	11.8	11.0
Eggs 2	0.72	5.7	6.4	6.2
Eggs 3	0.83	4.7	5.6	5.1
Chicken	0.57	10.6	11.2	11.0

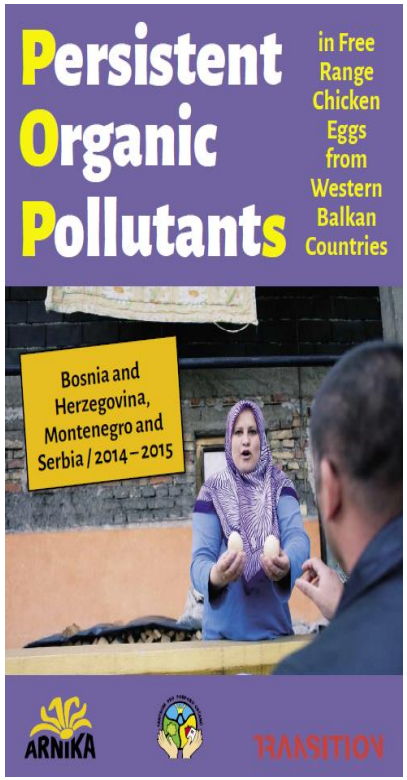
Table 2

TEQ-values of dl-PCBs and PCDD/Fs and concentrations of non-dl-PCBs for eggs and soil samples according to their distances to the ACP-cladding.

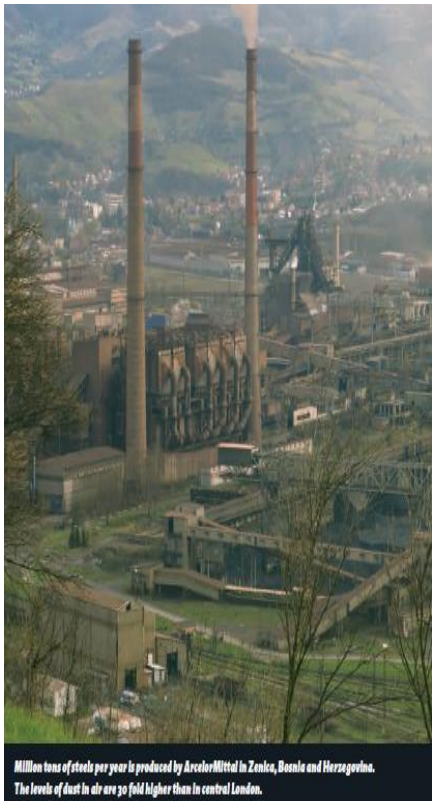
	Yard1[a]	Yard1	Yard2	Yard3	Yard4	Reference area
Distance to ACP (m)	0	0–1	2–8	1–5	4	35
Eggs						
dl-PCB-TEQ	–	10.7	5.7	5.4	2.4	–
Non-dl-PCBs	–	30,500	17,200	15,600	8700	–
PCDD/Fs-TEQ	–	1.1	0.6	0.7	0.5	–
Soil						
dl-PCB-TEQ	70.2	42.6	7.8	9.8	3.9	0.45
non-dl-PCBs	157,000	104,000	23,700	30,400	10,600	1200
PCDD/Fs-TEQ	3.5	3.8	1.5	1.8	2.1	1.3

J Winkler High levels of dioxin-like PCBs in organic-farmed eggs: a case study. Env Intern 80, 72-78 (2015)

Balkan states: free range chicken eggs from several industrial hot spots

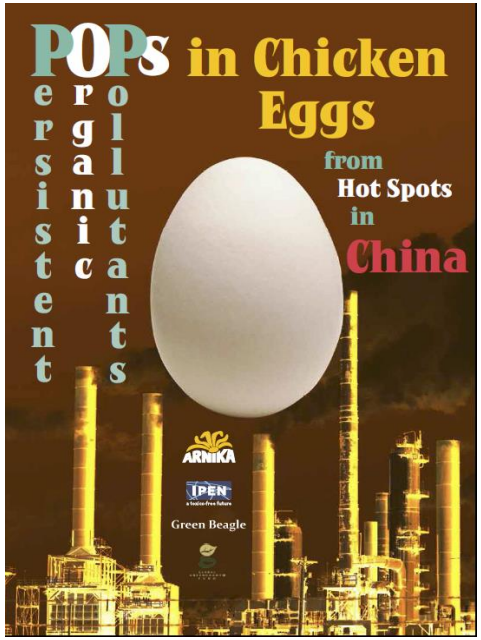


Sample	Locality	Country	PCDD/Fs and DL PCBs (DR CALUX)	PCDD/Fs (DR CALUX)
ZEN-1sh	Gračanica (Zenica)	Bosnia and Herzegovina	12	8.8
BiH-E-01	Divkovići I (Tuzla)	Bosnia and Herzegovina	7.7	5.6
BiH-E-02	Divkovići II (Tuzla)	Bosnia and Herzegovina	6.5	4.3
PLZ-E1+E2+E3	Plužine – Orah	Montenegro	0.98	0.34
SRB-ECG-02 and 03	Grabovac II (Obrenovac)	Serbia	7.0	5.2
SRB-ECG-04, 05 and 06	Ušće – Gorjača – Cola Bara (Obrenovac)	Serbia	4.4	3.2



J Petrlik (Arnika) and P. Behnisch (BDS), Persistent Organic Pollutants (POPs) in free range chicken eggs from hot spots in three Balkan states (2015)

China: free range chicken eggs from several industrial hot spots



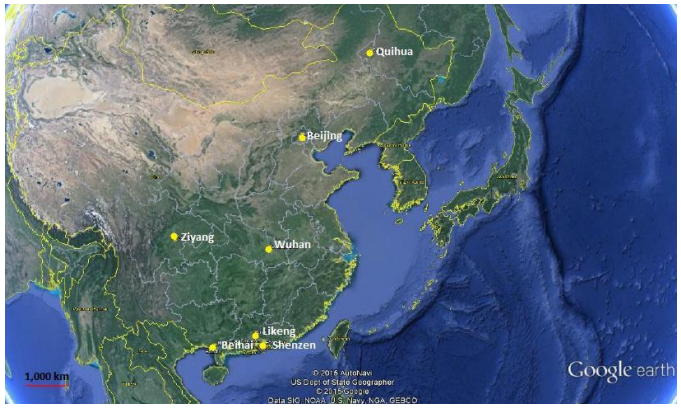
Beihei –metallurgic plant: 5-7 x EU Max

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”

In courtesy by J Petrlik (Arnika)

Taiwan: Soil surveillance for dioxins

- **800 soil samples were analysed by DR-CALUX**
- **with a mean dioxin level of 36 pg-BEQs/g.**
- **Soil dioxin-BEQs were higher in northern Taiwan (62 pg-BEQ/g) than in central, southern, and eastern Taiwan (8-25 pg-BEQ/g).**
- **Soil samples collected in northern Taiwan, and especially**
  - **in Bade City,**
  - **soils near industrial areas, and**
  - **soils with darker color may contain higher dioxin-BEQ level**

Ding-Yan Lin et al. Int. J. Environ. Res. Public Health **2014**, 11

Israel: Sediments and fish livers from the Mediterranean and Red Sea coasts analysed by DR CALUX

Table 3
Comparison of TEQ values evaluated by CALUX and HRGC/HRMS in sediment and fish extracts.

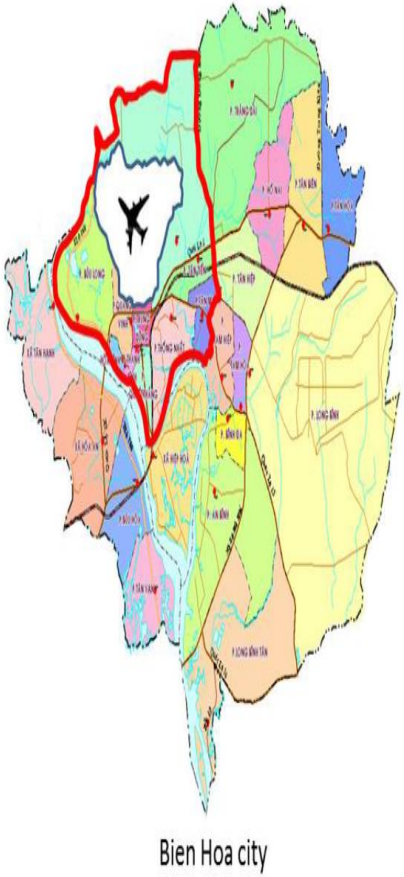
Sample or fish designation[a]	CALUX TEQ (pg/g ± SD)[b,e]	CALUX LOD (pg/g)[c]	CALUX LOQ (pg/g)[d]	HRGC/HRMS TEQ (pg/g ± SD)[f]
1	48.9 ± 0.98	0.56	1.37	18.29
2	27.47 ± 0.95	0.42	1.05	24
20	20.02 ± 1.24	0.8	2.1	16.06
F1	5.90 ± 0.56	0.50	0.70	7.53

- **DR CALUX and chemical analysis did show similar results**
- **Only 1 of 22 sediment samples were above international guided level of 100 ng TEQ/kg**
- **Still 3 of 12 fish samples were above EU trigger value for fish of DR CALUX (4,3 pg BEQ/g)**

Yana Yudkovski et al. Marine Pollution Bulletin 98 (2015) 295–300

Vietnam: monitoring of 200 residents by the Vietnam Military Medical University in Hanoi

Location: Da Nang and Bien Hoa



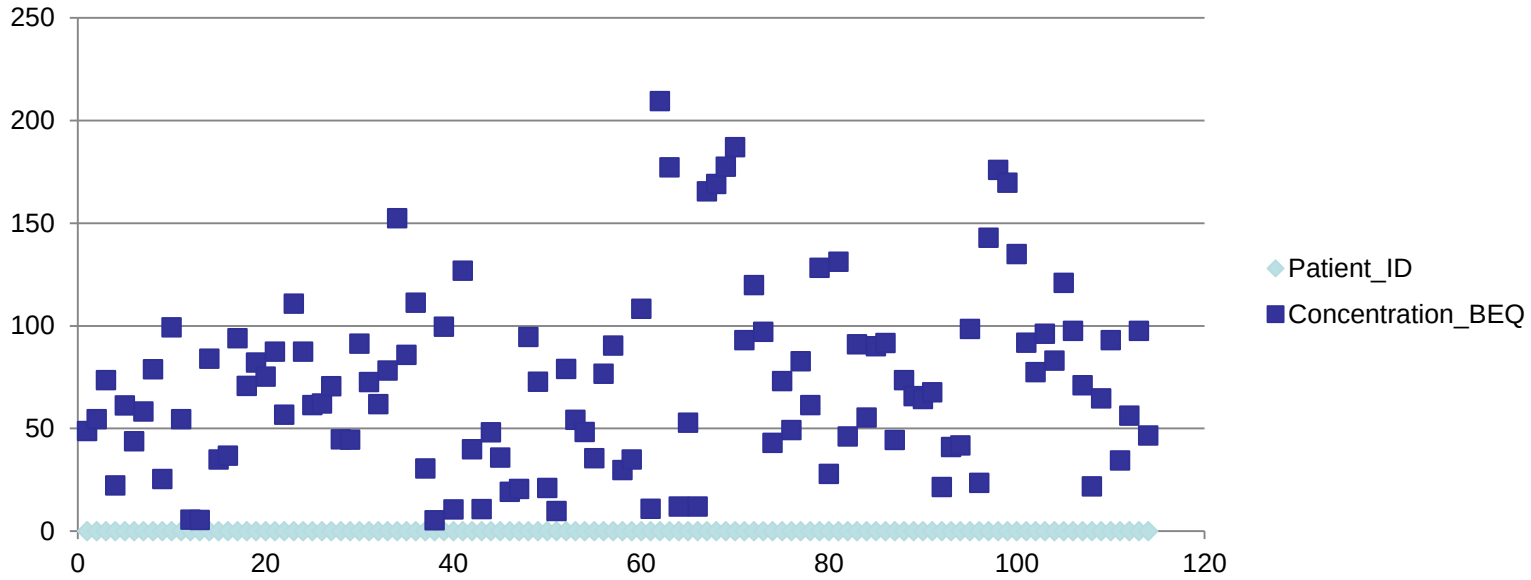
CROSS CHECKING

Blinded 14 samples coded: Cross 1-14 sent from BDS, Armsterdam, The Netherlands for cross checking

Results at VMMU are consistent with the result from BDS

Dioxin levels in human plasma by DR CALUX

Chart of BEQ distribution of using DR CALUX (n =114)

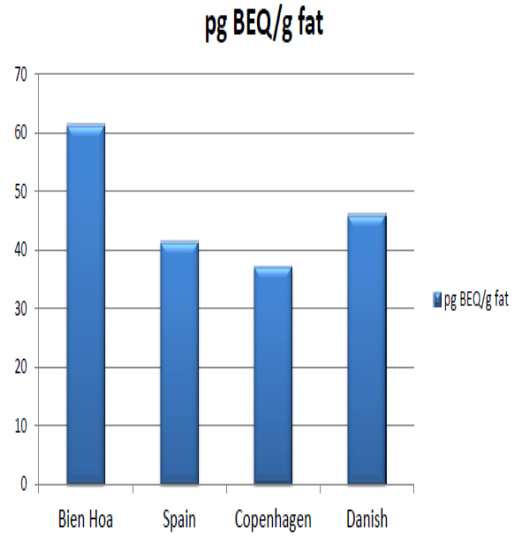


Conclusion: Wide dynamic range of dioxin-BEQs (5.4- 210 BEQ/g fat)

Table 2. Dioxin levels by DR CALUX® method

	Men (n=44)		Women (n=148)		
	Mean	SD	Mean	SD	P
Dioxin level (pg BEQ /g fat)	74.4	33.5	61.4	28.9	0.01

P, by Independent-Samples T Test



Dioxin level (by DR CALUX) in plasma of women in Bien Hoa and around the world

Papadopoulou E et al., 2013. Sci Total Eviron.
Pederson M et al., 2010. Environ Int.
Halldorsson TI et al., 2009. Environ Res.

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Fig 2. Correlation between Dioxin and cortisol in wome

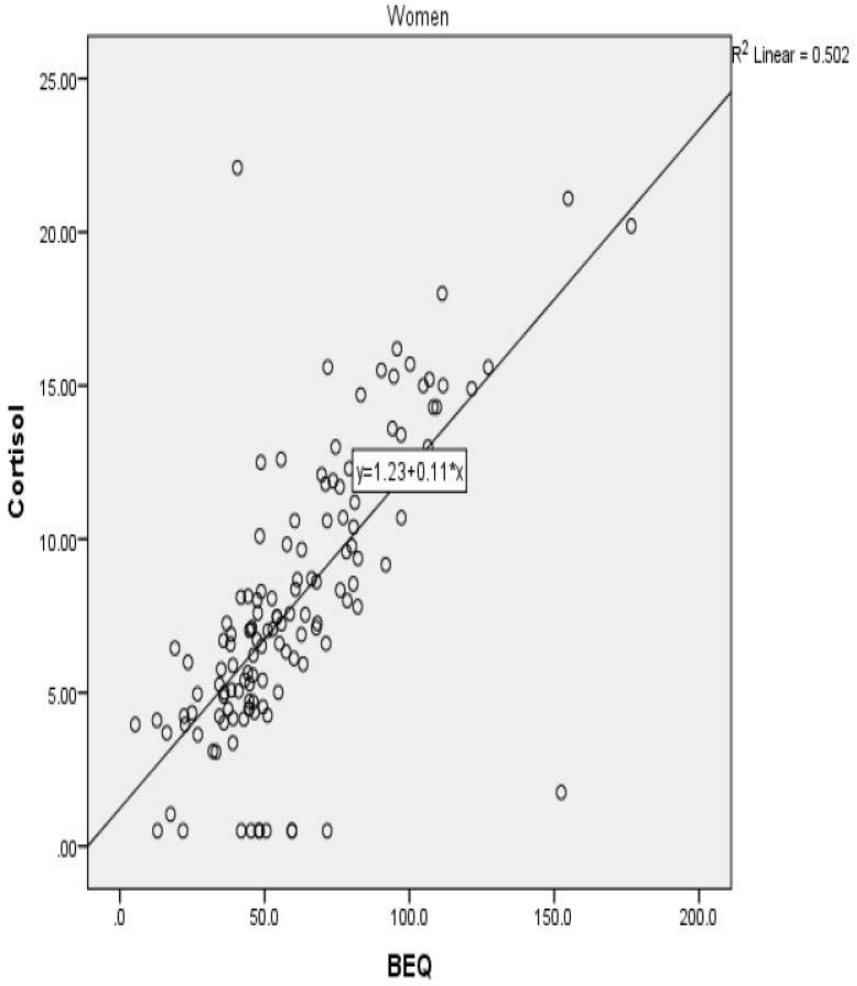

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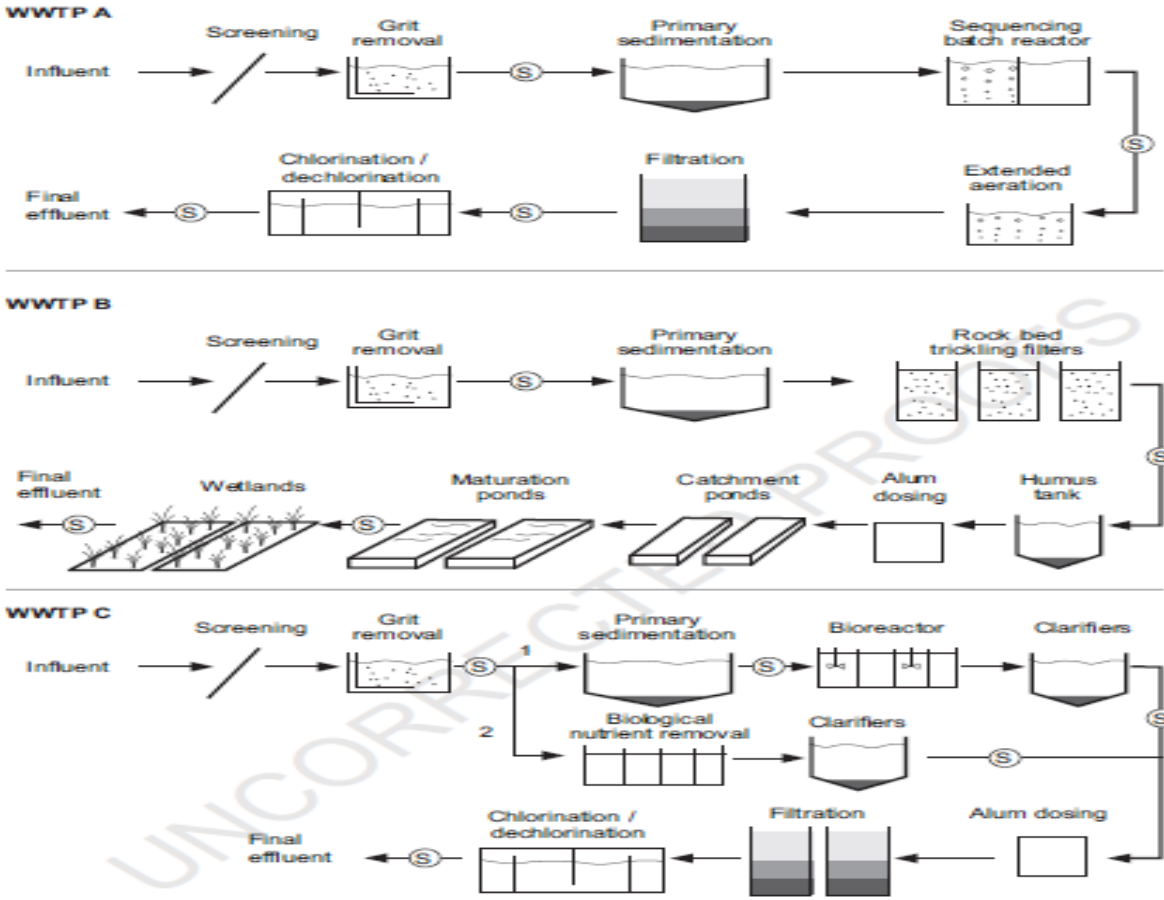
Table 2. Abnormal thyroid hormone levels by BEQ category

		High < 69.16 BEQ/g	Low > 69.16 BEQ/g	Total
		n (%)	n (%)	n (%)
TSH	Normal	8 (14.04%)	10 (17.54%)	18 (15,79)
	Abnormal	49 (85.96%)	47 (82.46%)	96 (84.21%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR=0.767, CI: 0.279-2.111, χ^2=0,2639, p=0.607		
T3	Normal	**18 (31.58%)**	**30 (52.63%)**	48 (42.11%)
	Abnormal	**39 (68.42%)**	**27 (47.37%)**	66 (57.37%)
	Total	**57 (100%)**	**57 (100%)**	114 (100%)
		OR=2.407, CI: 1.222-5.163, χ^2=0,5.19, p=0.023		
FT3	Normal	25 (43.86%)	32 (56.14%)	57 (50%)
	Abnormal	32 (56.14%)	25 (43.86%)	57 (50%)
	Total	57 (100%)	57 (100%)	114 (100%)
		OR=1.638, CI: 0.782-3.434, χ^2=0.4298, p=0.1898		

Conclusions: Good correlation between T3 & dioxin levels

Australia:

Endocrine activity in wastewater extracts during treatment

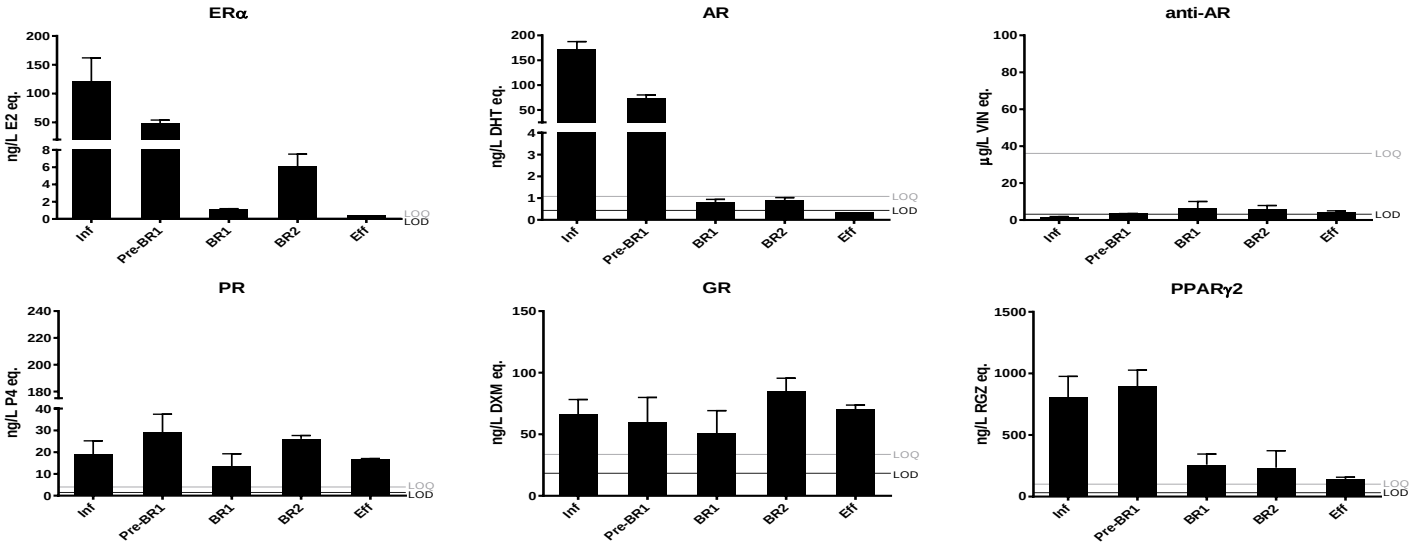


1. (A–C) Schematic diagrams showing the main treatment processes at each wastewater treatment plant (WWTP). Processing of solids has been omitted for

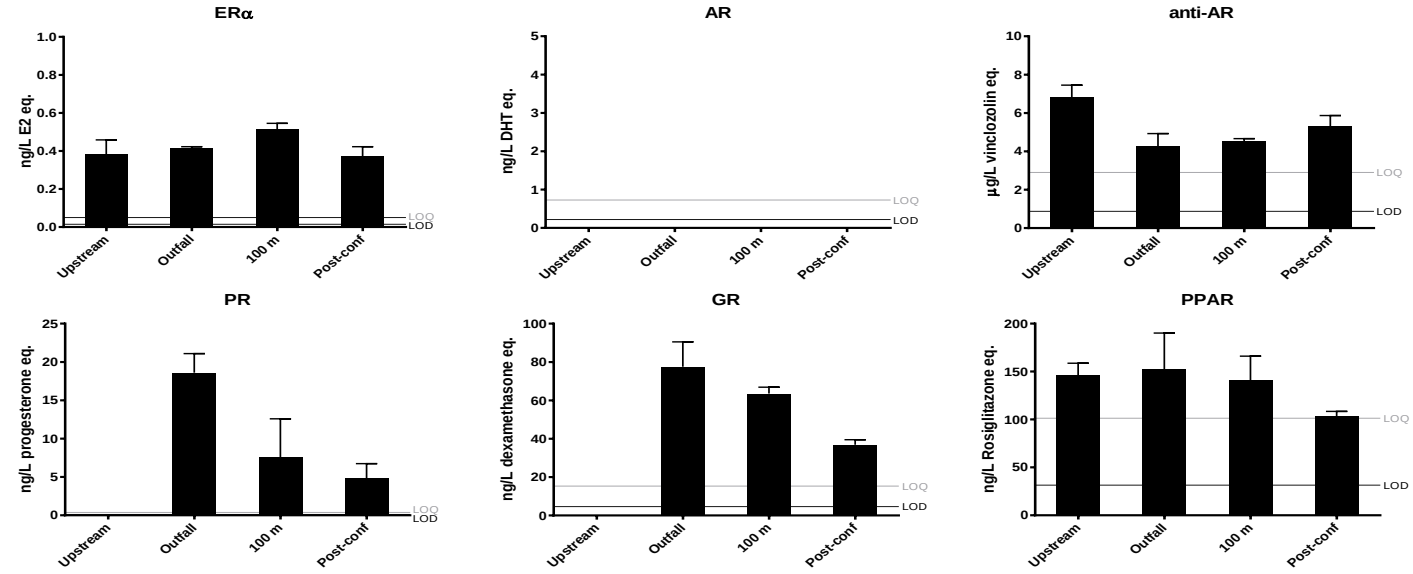
Peter Bain et al. Environmental Toxicology and Chemistry, Vol. 33, No. 10, pp. 2297–2307, 2014

Endocrine activity in wastewater extracts during treatment (CALUX bioassays)

WWTP C



WWTP B



Canada:

Monitoring of water treatment plants (Prof. Yargeau's team)

YARGEAU LABORATORY
Controlling Contaminants of Concern

Potential toxicity of influent and effluent (*i.e.*, untreated and treated water) to be determined using the cell-based bioassays

Approach applied to samples collected at three wastewater treatment plants.

	Treatment Type	Sample Type	Plant Capacity (m^3/day)
WWTP 1	Secondary treatment, conventional activated sludge, non-nitrifying, continuous chlorination	effluent, influent	409,000
WWTP 2	Tertiary treatment, nitrifying, seasonal chlorination	effluent, influent	18,180
WWTP 3	Lagoon (secondary treatment equivalent) with seasonal discharge	effluent (pre- and post-addition of antifoaming agent), influent, surface water downstream of wastewater discharge	1,364

Results: *ERα CALUX*

Estrogenic activity of wastewater samples collected at WWTP 1 and WWTP 2, expressed in terms of ng 17β-estradiol (E2) equivalents/L.

Wastewater Treatment Plant 1		
SAMPLING DATE	SAMPLE TYPE	ng 17β-ESTRADIOL EQUIVALENTS ± %SD
April, 2014	Influent	28.4 ± 6
	Effluent	1.3 ± 9
May, 2014	Influent	37.8 ± 15
	Effluent	9.7 ± 10
June, 2014	Influent	71.6 ± 6
	Effluent	3.8 ± 5
July, 2014	Influent	26.6 ± 13
	Effluent	2.5 ± 4
August, 2014	Influent	46.7 ± 12
	Effluent	1.6 ± 10

Wastewater Treatment Plant 2		
SAMPLING DATE	SAMPLE TYPE	ng 17β-ESTRADIOL EQUIVALENTS ± %SD
April, 2014	Influent	34.1 ± 14
	Effluent	13.7 ± 11
May, 2014	Influent	52.2 ± 2
	Effluent	3.1 ± 15
June, 2014	Influent	50.2 ± 4
	Effluent	2.6 ± 12
July, 2014	Influent	36.4 ± 8
	Effluent	1.5 ± 6
August, 2014	Influent	58.6 ± 4
	Effluent	12.8 ± 10

Effluent samples exhibit significantly lower estrogenic activity than the influent samples.

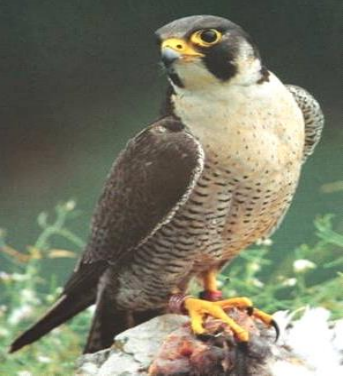
Results: *ERα CALUX*

Wastewater Treatment Plant 3		
SAMPLING DATE	SAMPLE TYPE	ng 17β-ESTRADIOL EQUIVALENTS ± %SD
May, 2014	Influent	172.9 ± 7
	Effluent Pre-Antifoam	2.7 ± 5
	Effluent Post-Antifoam	4.5 ± 3
	Surface Water	4.9 ± 12
June, 2014	Influent	56.3 ± 8
	Effluent Pre-Antifoam	1.1 ± 21[*,†]
	Effluent Post-Antifoam	TBD
	Surface Water	15.4 ± 6
September, 2014[‡]	Influent	214.7 ± 8
	Effluent Pre-Antifoam	7.1 ± 15
	Effluent Post-Antifoam	13.4 ± 20[†]
	Surface Water	17.1 ± 10
September, 2014[‡]	Influent	137.5 ± 13
	Effluent Pre-Antifoam	TBD
	Effluent Post-Antifoam	NOT PROVIDED
	Surface Water	4.9 ± 15

Estrogenic activity of the wastewater samples collected at WWTP 3, expressed in terms of ng 17β-estradiol (E2) equivalents/L.

Once more, effluent samples exhibit significantly lower estrogenic activity than influent samples.

While influent estrogenic activity at WWTP 3 is higher than at WWTPs 1 and 2, effluent levels are comparable to other plants.



Germany: Falcon egg biomonitoring by CALUX panel

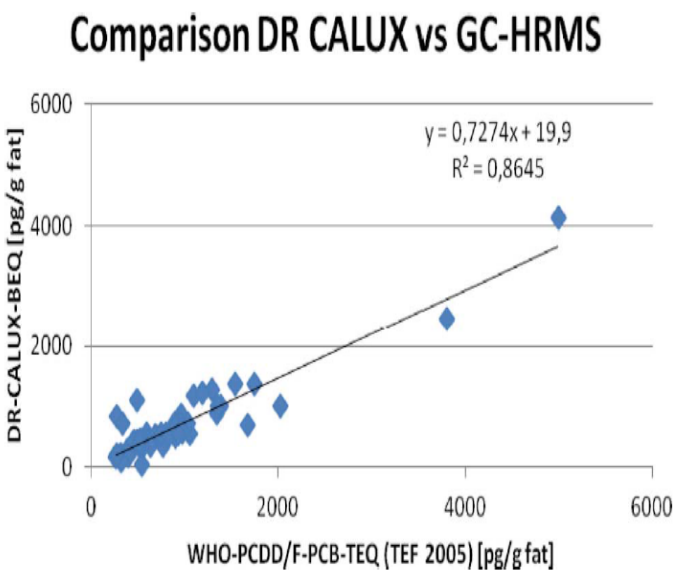


Figure 7. Sum of PCDD/F and dl-PCB levels in peregrine falcon eggs from BW measured by the semi-quantitative (DR CALUX) and quantitative (GC/HRMS) methods.
Scale: pg (PCDD/F + dl-PCB-TEQ) or (BEQ)/g fat. Samples from 2004 to 2011 ($n = 59$).

Further hormone and obesity analysis results:

ER CALUX: 12-766 pg EEQ/g

AR CALUX: 8-491 mg FEQ/g

GR, TR or PPARg CALUX: < LOQ

Schwarz et al (2016). Peregrine falcon egg pollutants.TOXICOLOGICAL & ENVIRONMENTAL CHEMISTRY, 2016. http://dx.doi.org/10.1080/02772248.2015.1126717

Germany:

Flame retardants PBDEs in Fish x cooking = PBDFs!

Each minute of heating 1 g salmon fillet spiked with 200 ng BDE-209 generated about 0.5 ‰ PBDFs confirmed by DR CALUX and GC/MS.

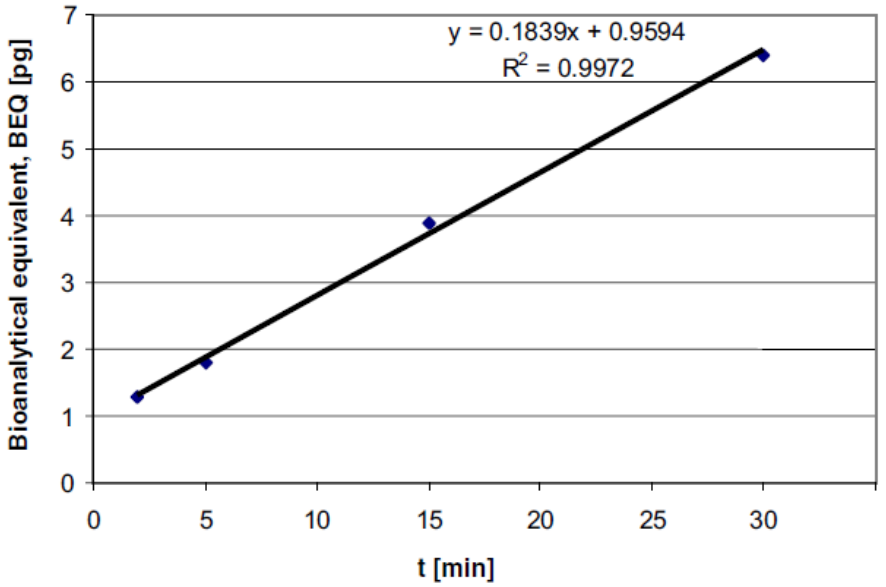


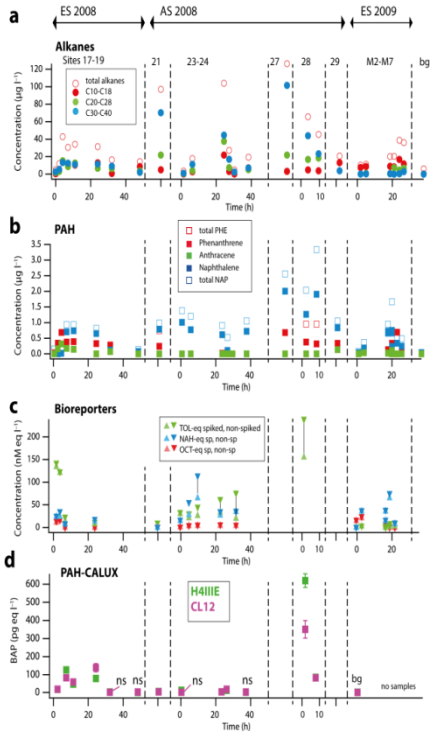
Fig. 2 Toxic response expressed in toxic equivalents per gram of fish in the DR CALUX bio-assay generated upon the heating of 1 g of fish fillet fortified with 200 ng of the PBDEs for different periods of time

Vetter et al. Formation of polybrominated dibenzofurans (PBDFs) after heating of a salmon sample spiked with decabromodiphenyl ether (BDE-209). Environ Sci Pollut Res 2014.

Switzerland:
PAH CALUX to measure effects of oil spills

Immediate Ecotoxicological Effects of Short-Lived Oil Spill on Marine Biota

- **Assess impact of small oil spills using bioassays (PAH CALUX, bacterial)**
- **Experimental spills at sea: high bioavailability and toxicity of dissolved oil within 24 h after the spills up to 8 m below the slicks.**
- **Selective decline of marine plankton is observed**
- **> Contrary to common thinking, even small spills have immediate adverse biological effects and their recurrent nature is likely to affect marine ecosystem functioning.**



Brussard et al. Nature Communications, 2016, Apr 4;7:11206.

Global environment and human safety – knowing more about the unknown

- **Bioassays can easily cover complex questions & cocktails from all over the world….**
- **They offer an easy and care taking answer to many daily questions**
- **POPs & EDCs…but also many other relevant types of toxicity**
- **Thanks for YOUR interest…**
- **YOUR questions are welcome..!!**