BioDetection Systems

8th BioDetectors Conference, Torino

Dioxins/PCBs in Feed/Food crisis

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Why using effect-based bioanalysis for POP cocktails ?

- **Many dioxin-like compounds (DLC s) such as PXDD/Fs, PXBs and N-Dioxins are not in the regulatory frameworks = due to the high costs of chemical analysis**
- **Effect-based analysis can cover these & more DLCs, without any additional costs ►► safer risk assment of DLCs!**
- **Many relevant mode-of actions of POPs are not yet included in the risk assessments and TEF validation**
- **Automated sample preparation & robotic analysis make it possible to screen cost-efficient all those DLCs**
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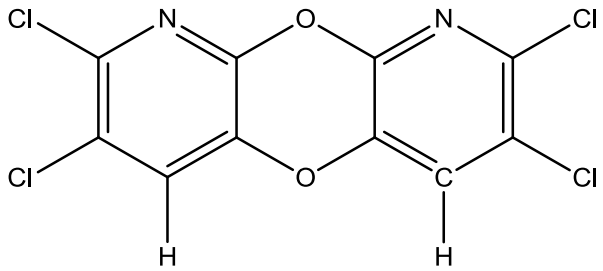
Which toxic DLC compounds can be excluded in case of low DR CALUX results are obtained?

Compounds	WHO-TEF	DR CALUX-REP
2,3,7,8-T4ClDD	1	1
2,3,7,8-T4BrDD	1	0.8
2,3,7,8-TClDF	0.1	
2,3,7,8-TBrDF	0.1	0.60
2,3,7,8-N-Polyhalogentaed Dioxins	?	0.01-0.1
PClB 126	0.1	0.07
PBrB-126	0.1	0.16
8-Methyl-2,3,7-Tri CDD	?	0.01
8-Fluor-2,3,4-TriCDF	?	0.0002
8-Iod-2,3,4-TriCDF	?	0.0011
PBrDE 126	?	0.00013
2,3,6,7-TCN	?	0.0004

1) Van den Berg et al 2013, Tox Sci. 133(2), 197–208 2013:

"use of similar interim TEF values for BR and CL-congeners for human risk assessment is recommended by the WHO and UNEP"

2) 2,3,7,8-N-T4CDD by fire with chlorpyrifos: REP 0.1 to 0.01



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Which mode-of actions can be covered by bioanalysis CALUX tools?

Bioactive Food Pollutants	HTPS CALUX	Pathway
C- and N-Dioxins PXDD/Fs, dl-PXBs (X= Cl, Br, F, methyl)	DR CALUX	Dioxin receptor
Carcinogenic PAHs (such as Benzo(a)pyrene)	PAH CALUX	Dioxin receptor
Estrogens, EDCs, Bisphenol A, Phthalates, Pesticides, Pharmaceuticals, cosmetics	ER CALUX	Estrogen receptor mix
Androgens, EDCs, Bisphenol A, Pesticides, Pharmaceuticals	AR CALUX	Androgen receptor
Progestins, EDCs, Anti-babypill, Pesticides, Pharmaceuticals	PR CALUX	Progesterone receptor
Glucocorticoids, EDCs, Asthma spray, Immune-suppressive agents	GR CALUX	Glucocorticoid receptor
Thyroid hormones, EDCs, Brominated flame retardants	TR CALUX	Thyroid receptor
Retinoids, Pesticides, Pharmaceuticals	RAR CALUX	Retinoic acid receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARgamma CALUX	Peroxisome proliferatorγ1 receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARalpha CALUX	Peroxisome proliferatorα receptor
Pro-inflammatory cytokines	NFkappaB CALUX	NFkappaB activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p21 CALUX	p21 activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p53 CALUX	p53 transcriptional activity
Electrophiles, oxidative stress, heavy metals	Nrf2 CALUX	Nrf2 transcriptional activity
β-Catenin/ involved in development and carcinogenesis	TCF	TCF transcriptional activity
Carcinogens, UV	AP1 CALUX	AP1 transcriptional activity
Hypoxia-mediated angiogenesis	HIF1alpha CALUX	HIF1α transcriptional activ-ity
Endoplasmatic reticulum stressors	ER stress CALUX	XBP1 transcriptional activity
Cytotoxic agents, Non-specific luciferase modulators	Cytox CALUX	Constitutive transcriptional activity

Behnisch (2012). "Hidden cocktails uncovered". Food Lab Intern.2/12, p. 29ff

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HTPS DR CALUX® Method

I, II) Sample treatment

I Extraction:

30 samples/person/3 hrs

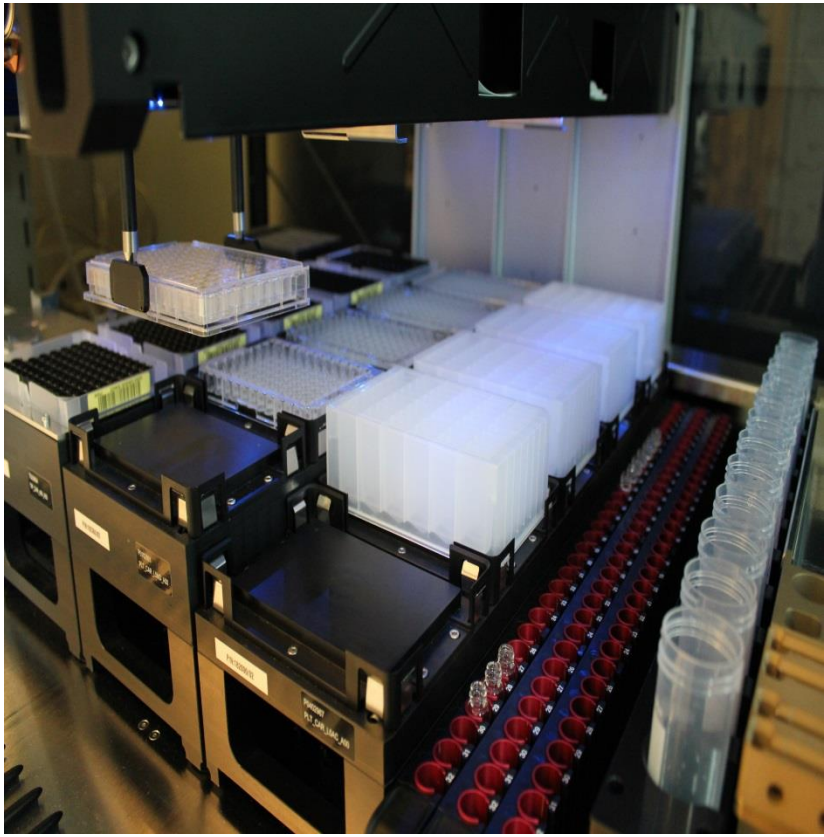
II Clean-up:

15 Proben/hrs

Low costs: ca. <15 Euro/sample

HTPS DR CALUX® Method
III) Robot for Seeding cells and Dosing standards/samples

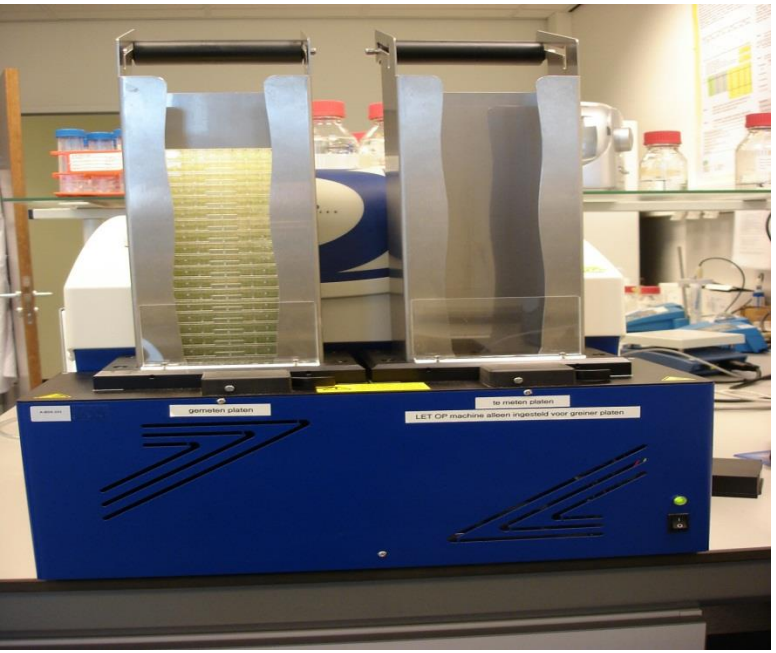
40 samples/hr



Hamilton Microlab Starlet

HTPS DR CALUX® Method

IV) Robotic Luminometer



DR <u>CALUX:</u>

40 samples/hr for the quantitative analysis of the PCDD/F/dl-PCB-TEQ/BEQ in the sample

Critical questions for remediation cases

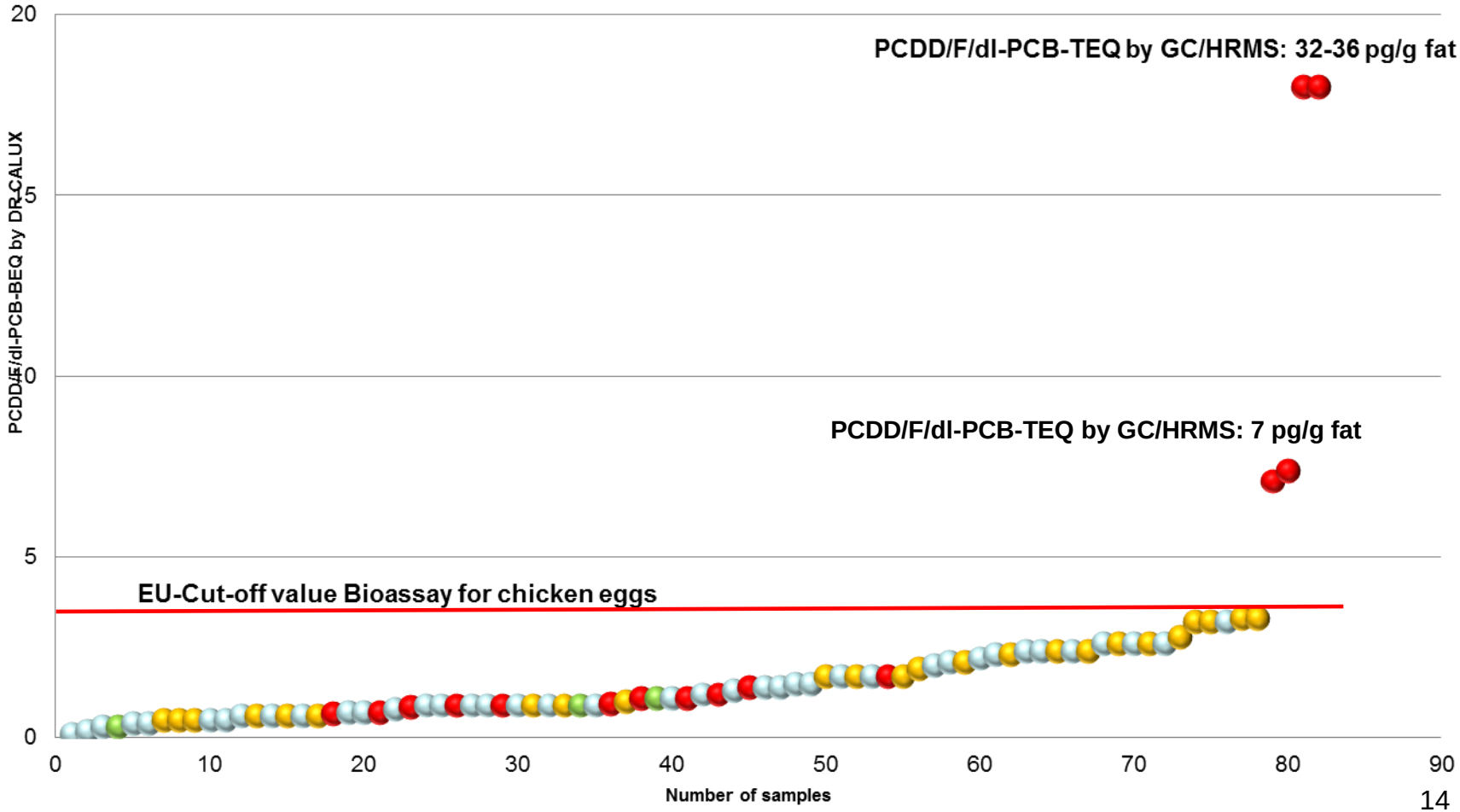
- **Where are the contaminated areas ? ►►need of cost-efficient, fast and high capacity screening**
- **Do we cover the more than 1000 DLCs or only 17 PCDD/Fs? Do we cover many mode of actions or only AhR dependant toxicity?**
- **Can we destroy/remediate most of DLCs or only 17 PCDD/Fs?**
- **How do we asure the destruction of all DLCs in case of a remediation?**

Overview of global biomonitoring cases for Dioxins & other POPs

Landfill/Dumpside Cases/Industrial Production side

Typical distribution of PCDD/F/dl-PCB analysed by DR CALUX in different kinds of rearing of chicken eggs

Typical DR CALUX results for separated PCDD/Fs and dl-PCBs for eggs from organic (0-Country, orange), free-range (1-; red), floor (2-; blue) and cage (3-; green) rearing: > 95% compliant = lower than cut-off 3.3 pg PCDD/F/dl-PCB/g fat (n=80)



Landfill Eyller Mountain: Germany

Egg biomonitoring by DR CALUX



Egg samples (pg/g)	PCDD/F/dl-PCB-BEQ DR CALUX	PCDD/F-BEQ DR CALUX	PCDD/F/dl-PCB-TEQ	PCDD/F-TEQ
Place 1	7.1	5.3	10	6.3
Place 2	6.5	4.4	8.7	3.6

See for more info: www.bund-nrw.de/.../pcb-eier-am-fuss-des-eyller-berg

Former PCB condenser production side: Germany

Egg biomonitoring by DR CALUX

Egg Samples (pg/g)	PCDD/F/ dl-PCB- & others -BEQ DR CALUX	PCDD/F-BEQ DR CALUX	PCDD/F/ dl-PCB-TEQ	PCDD/F/ -TEQ
Place 1	18	5,1	32	6,4
Place 2	18	6,9	36	11

See for more info at: www.sauberes-grundwasser.de

Pesticide Burial Site, Armenia

Soil/egg monitoring by DR CALUX

- Soils around the landfill Nubarashen are heavily contaminated by DDT, HCHs and therefore also PCDD/Fs measured by DR CALUX and GC/HRMS

- Chicken Eggs 1-2 km from the burial place also contaminated with PCDD/Fs measured by DR CALUX

Sample (pg/g)	**PCDD/F/ dl-PCB- & others -TEQ DR CALUX**	**PCDD/F-TEQ**	
Soil	6600	2680	
Egg 1	25		
Egg 2	21		

See for further info at: "Toxic Hotspots in Armenia" by Petrlik et al (ARNIKA); EU Development project for Armenia

Burning of industrial waste, Naples, Italy

human cancer tissue biomonitoring by DR CALUX

Place	Sex	Age	Cancer	PCDD/F-BEQ	Dl-PCB BEQ	SUM (pg BEQ/g fat)
Caserta	M	65	sarcomasG1	10	2.7	13
Casoria	F	44	sarcomasG3	LOQ (3.3)	LOQ (1.6)	LOQ (4.9)
Baronissi	F	70	sarcomasG3	18	4.1	22
Briatico	F	60	sarcomasG3	3.7	LOQ (1.9)	5.6
Ischia	F	34	Condro-sarcomasG3	4.5	3.2	7.7

Conclusions:

Results with GC/HRMS in this area with other cancer patients have been similar to the here presented data by DR CALUX.

Metal-Recycling Cases

Metal-Recycling Case Hardenberg: the Netherlands

Wipe sample monitoring of waste materials by DR CALUX

- **Dioxins were found in wipe dust samples up to 500 meters in the surroundings of the aluminum recycling plant, as a result of the emissions from the stored filtering material.**

- **Here we report DR CALUX analysis from recycling materials such as wood, iron and concrete from the remediation and demolition of this former aluminum recycling plant.**

- **In the cleanup of the contaminated site, DR CALUX was used to separate clean materials from polluted materials, and to determine the cleaning level of the buildings. For this purpose, the surface was sampled with cotton wipes and analyzed.**

- **Therefore wipe samples from several storage rooms, recycling materials (iron and wood) from demolition of the building structures, as well as from water and soil samples from this former aluminum recycling plant have been analyzed by DR CALUX.**

- **Not many results of wipe samples of background locations are published and we would refer to a typical background level of between 5 to 25 pg WHO-TEQ/m^2. It is recommended to use a dioxin norm of 25 pg TEQ/m^2 as trigger value.**

- **In Germany, in the Regulation for Fire case remediation is using a value of 10 ng I-TEQ/m^2 for such wipe samples in indoor contaminations is used. Lindert and Fiedler reviewed in 1999 several fire cases with a wide range of PCDD/F results (80 to 15.000 ng PCDD/F-TEQ/m^2) in wipe sample.**

DR CALUX analysis from wipe, waste water and soil

- **Wipe samples from different locations (e.g. wood storage, iron storage, concrete floor) showed Total-TEQ levels by DR CALUX® ranging from 14 to 400.000 pg TEQ/m²**
- **All tested soil samples have been below the here applied norm of 55 ng TEQ/kg d.w.**
- **One waste water sample did also show elevated levels of 17 ng TEQ/l water**

Samples	Sampled surface (m^2)	DR CALUX-TEQ (pg TEQ/m^2)
Wipe samples		
Wipe sample M3, wood	0.002	570
Wipe sample M4, concrete floor	0.09	540
Wipe Sample M5, Iron from hall	0.09	2100
Wipe Sample M6, Iron from hall	0.09	580
Wipe Sample M7, cleaned area	0.09	100
Wipe Sample M8, floor cleaned area	0.09	47
Wipe Sample M9, cleaned concrete floor hall 1	0.09	15
Wipe Sample M10, Iron from hall 1	0.09	2000
Wipe Sample G6, cleaned with high pressure	0.09	100
Wipe Sample M11, concrete floor, cleaned with high pressure	0.09	230
Wipe Sample H2, Wood, cleaned with water	0.01	22000
Wipe Sample H3, Wood, cleaned with high pressure	0.0025	400.000
Wipe Sample M12, floor cleaned with soap	0.09	110
Wipe Sample Y7 to Y23, Steel storage	0.09	1 to 50
Sample H4, Wood from roof loc. C	0.01	20.000
Soil samples		
Soil Samples G1 to G2	Soil samples	All below norm of 55 ng TEQ/kg
Water samples		
Waste Water Sample WW1		17 ng TEQ/l water

Industrial Cases

Crisis related monitoring: Dioxin Feed and Food crises

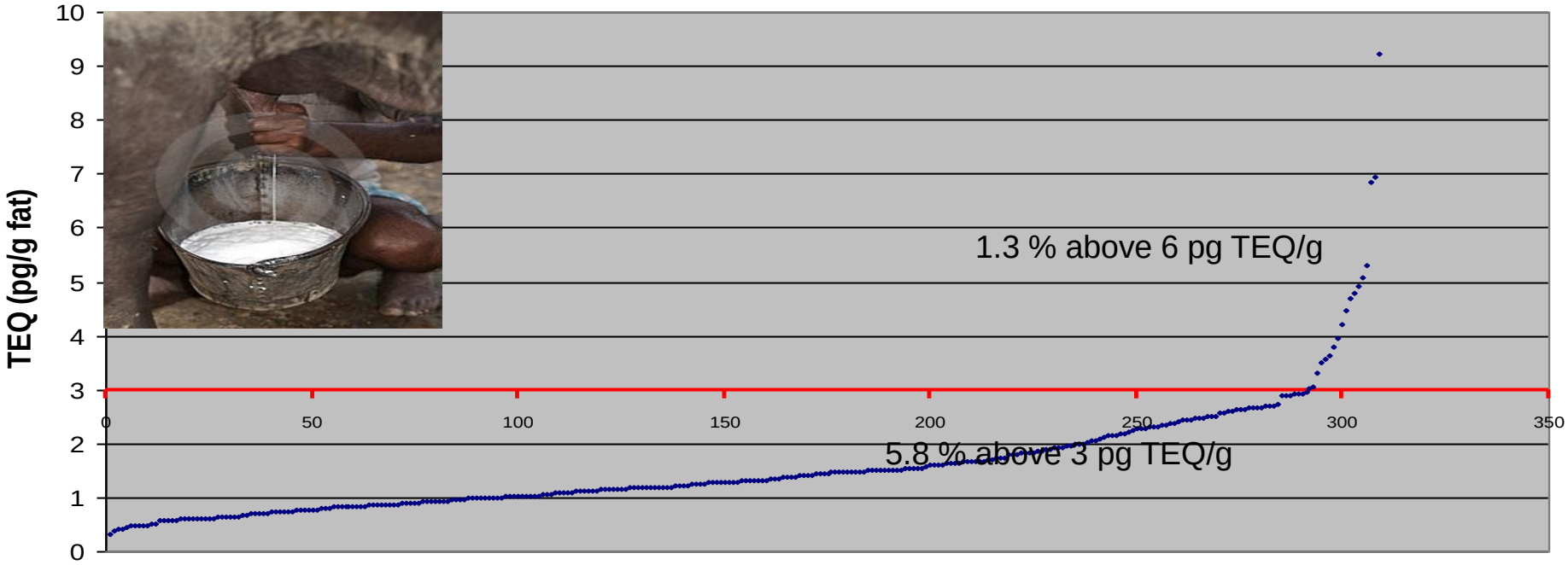
Brazilian	**Citrus pulp 1998**
Belgian	**Chicken PCB fat 1999**
German	**Kaolinic clay 1999**
Belgian	**Cholin chloride 2002**
German	**Bakery waste 2003**
Netherlands	**Potato peels/kaolinic clay 2004**
Belgium	**Gelatin/Hydrochloric acid 2006**
Australia	**Fish/Home bush bay-2,4,5-T 2006**
India	**Guar Gum/PCP 2007**
Italy	**Mozzarella/waste disposal 2008**
Germany	**Chicken, pig and egg pollutions through food fat (2011)**

Dutch Dioxin crisis 2004: 300 milk sample in just 4 weeks

Clay effecting potato peelings used in animal feed

No of samples analyzed during crisis (4 months) several thousands

total DR CALUX TEQ distribution for milk samples, 2004 Dutch clay crisis



60 % below 1.5 pg TEQ/g

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Toxic Trade

The players in the dioxin scandal

Petrotech AG (Emden, Germany) manufactures biofuel from spent cooking fats. The process also produces mixed fats.

Olivet (Rotterdam, the Netherlands) buys the fats and sells them on.

Harles and Jentzsch (Uetersen and Bösel, Germany) uses the fats in the production of feed fats. It is still not clear at what point the dioxin got into the fats.

Feed producers mix the feed fats in with their animal feed.

Farmers buy the animal feed and use it to feed their hens, turkeys and pigs.

German Dioxin crisis 2011: Egg testing by BDS = all negative

Test results for eggs

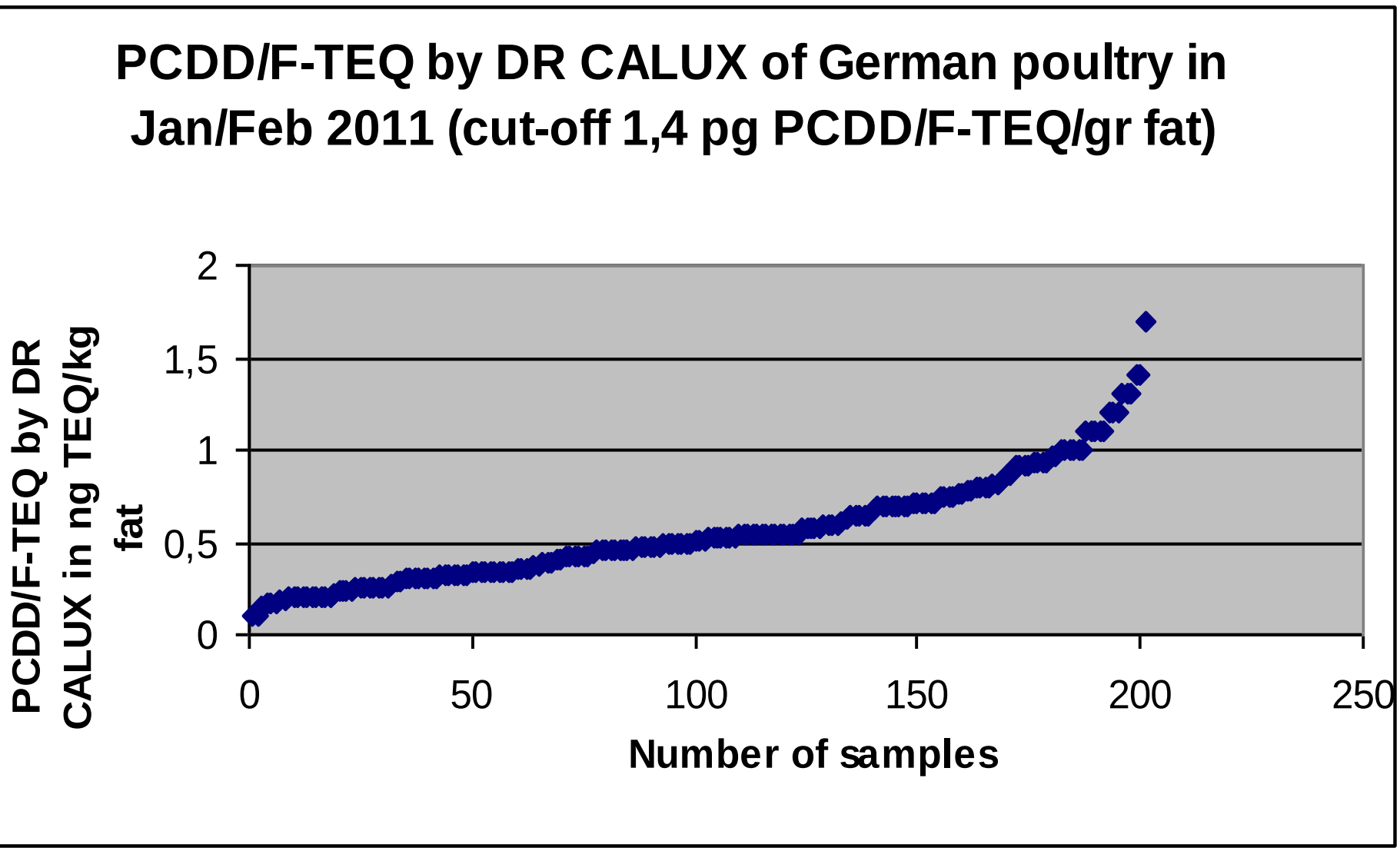
Land	Number of samples	Samples above MRL
Bayern	19	7
Baden-Württemberg	1	0
Brandenburg	3	0
Hessen	11	0
Niedersachsen	81	16
Nordrhein-Westfalen	32	8
Schleswig-Holstein	9	0
Mecklenburg-Vorpommern	7	0
Sachsen-Anhalt	1	0
Total	164	31*

* No correlation of amount of samples with blocked farms

Chicken testing by BDS
>200 German samples = 99.5% negative

				Sample compliant	Sample compliant	Sample compliant	Sample compliant
	PCB-TEQ	PCDD/F-TEQ	Total-TEQ	PCDD/F-25% below	Total-25% below	PCDD/F-50% below	Total-50% below
1) Pig Meat: N	496	496	502	99,8%	97,6%	94%	89%
1) Pig Meat: Mean	0,23	0,21	0,43				
1) Pig Meat: Range	0,1-1,1	0,1-0,77	0,1-1,47				
2) Pig Liver: N	32	37	34	99%	97,3%	93,2%	90%
2) Pig Liver: Mean	0,80	0,78	1,57				
2) Pig Liver: Range	0,1-2,1	0,1-3,4	0,1-5,2				
3) Eggs: N	9	9	36	100%	100%	44%	100%
3) Eggs: Mean	0,67	1,21	1,48				
3) Eggs: Range	0,1-1,0	0,1-2,3	0,1-2,6				
4) Chicken: N	201	201	207	99,5%	98%	93%	88%
4) Chicken: Mean	0,74	0,56	1,29				
4) Chicken: Range	0,3-3,3	0,3-1,7	0,6-3,8				

Typical distribution dioxins in chicken meat



Valencia, Spain (Prof. Yusà, CSISP, Valencia)

Food Monitoring by DR CALUX

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

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

Harbor City

Harbor City Istanbul, Turkey (Yilmaz et al 2014)

Breast 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).**

Take home message

- Many DLCs (which are in the environment)are routinely not monitored, while bioassays can additionally cover some of them. But than they are called false positive results. Is the truth not that the chemical analysis measures here false negative results?
- Such dioxin/PCB screening tests are widely accepted in the feed/food testing, but rarely used in environmental testing. Only Japan, USA and the Netherlands have set-up national standards to use such cost-efficient screening tests. How can we improve that?
- Here we report results of many polluted sides (e.g. landfills, dumpsites, harbors. Industrial production side) by using a wide range of different monitoring parameters/matrices (such as soil, wipe, eggs or human blood/cancer tissue samples) tested by bioassay
- For most samples analyzed by BDS, the PCB-TEQ and PCDD/F-TEQ have been reported separately to assess both compounds groups separately
- In general, more than 95% of samples tested by DR CALUX method are below existing regulatory levels. Here we only present some positive case, to show the proof of principle

Take home message

- **In case of compliant DR CALUX results, we are also able to exclude relevant effects of PBDD/Fs, PBBs or PCNs = higher environmental, feed/food quality and public health safety!!!**

- **For the price of a typical chemical analysis for dioxins/PCBs you are now able to cover PXDD/PXDF/PXB as well as obesogens (PPAR CALUX), hormones (CALUX panel) and all kinds of endocrine disrupting chemicals (CALUX panel)..please try it..**

- **The results of this presentation shows that the bioassays for screening of PCDD/Fs and dioxin-like PCBs in soils, wipe samples, feed/food and blood/cancer tissue is an important device to identify the few percentage of exceeding samples among the bulk of non-detected samples….!!!**