DR-CALUX
Remediation worker monitoring in Switzerland

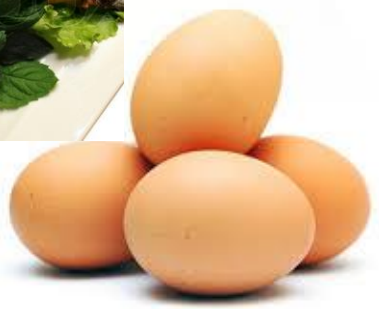
9th Biodetection Conference

Lausanne

14-15 April 2016

Institut de Chimie Clinique

Toxic sources



Acute *vs* chronic

only the dose makes the poison

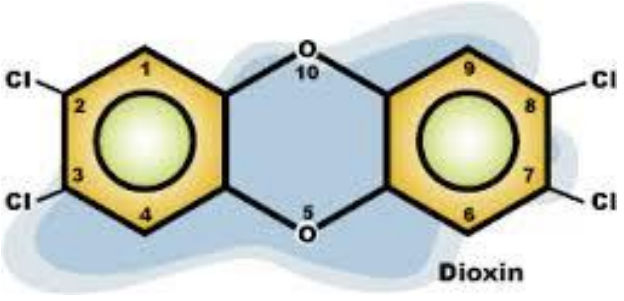
Paracelse

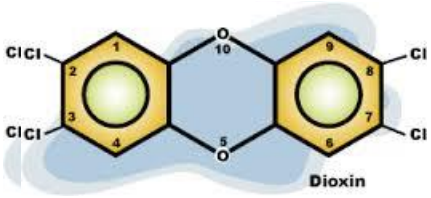
Acute

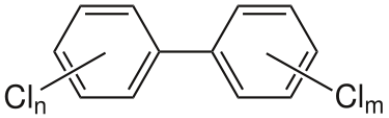
- Short acting toxic effect
- Doses can be measured
- Risk is « visible »

Chronic

- Long time toxicity
- Low doses
- Difficult to measure the risk !







209 PCBs

Where is the risk?

Acute toxicity

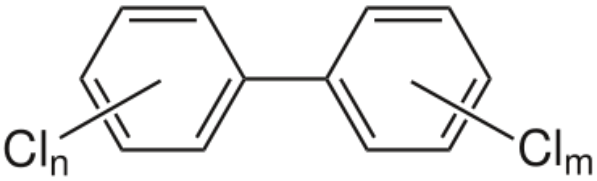
Rare

poisoning

Chronic toxicity

PCB *vs* dioxines…

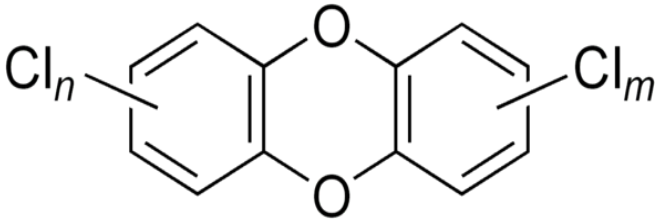
- Liquides visqueux
- Stables à la chaleur, inertes chimiquement
- Isolant électrique
- Très liposoluble et s'accumulent dans le long de la chaîne alimentaire
- Perturbateurs endocriniens
- Cancérogènes probables (IARC 2a)



Polychlorobiphényles
(PCB)

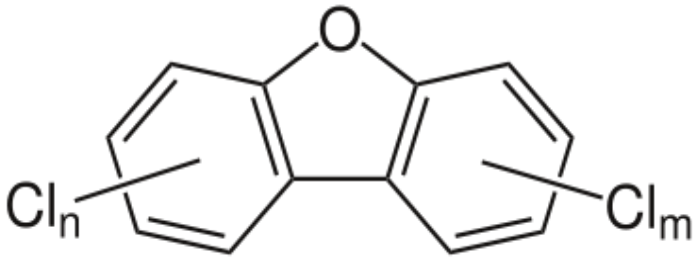
209 congénères

12 congénères planaires



Polychlorodibenzodioxines
(PCDD)

75 congénères



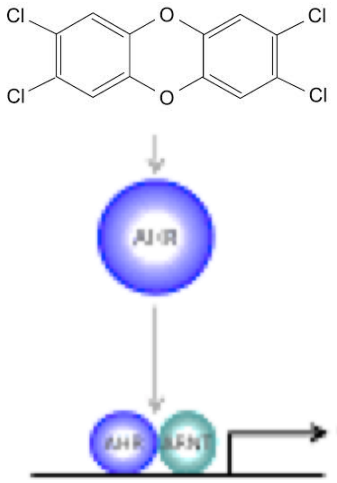
Polychlorodibenzofuranes
(PCDF)

135 congénères

When does human biomonitoring make sense ?

- If exposure is repeated
- To compare levels over time
- A screening method to measure the effect in the sample
- Not only presence/abundance of congeners

DR-CALUX = toxicity of dioxins, furans and dl-PCBs



Use of PCBs

Use of PCBs	repartition
Condensateurs	50.3%
Transformateurs	26.7%
Plastifiants (joints)	9.2%
Huiles hydrauliques	6.4%
Papier carbone	3.6%
Fluides caloporteurs	1.6%
Additifs pétrolier	0.1%
Autres	2.2%

Usage industriel des PCB (1929-1975) – EPA 97

Production mondiale : 1.5 x 10^6 tonnes (UNEP 98).

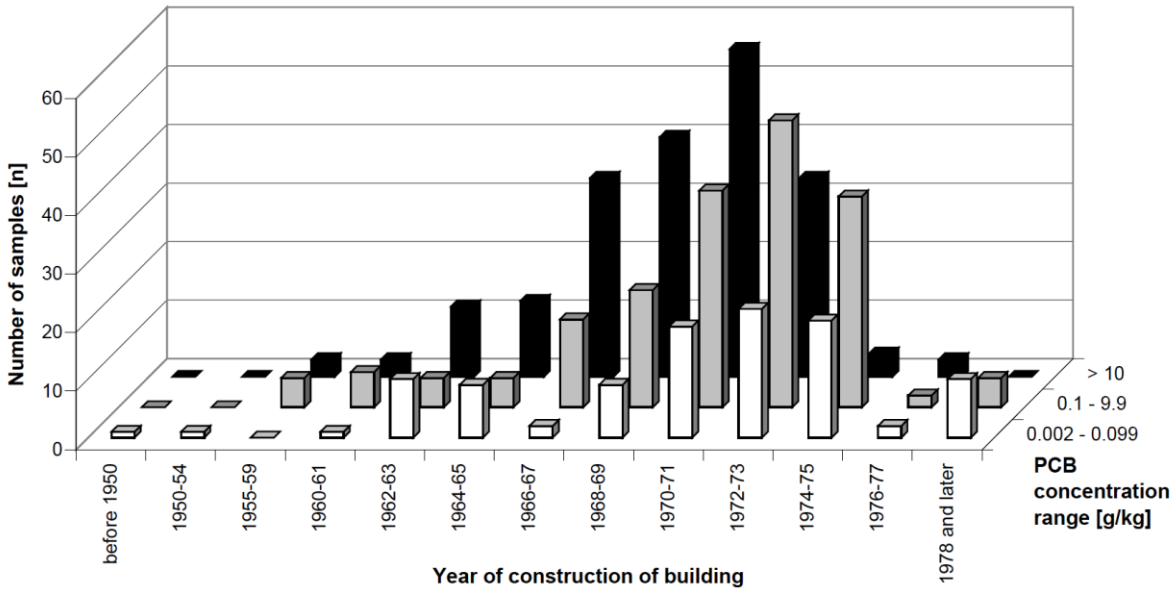
2004

Joint sealants, an overlooked diffuse source of polychlorinated biphenyls (PCB) - results of a nationwide study in Switzerland

ORGANOHALOGEN COMPOUNDS – Volume 66 (2004)

Markus Zennegg[1], Martin Kohler [1], Josef Tremp[2], Cornelia Seiler[1], Salome Minder-Kohler[3], Marcel Beck[3], Peter Lienemann[4], Lukas Wegmann[2], Peter Schmid[1]

PCB in joint sealants – occurrence and inventory in Switzerland: Figure 1 represents the PCB contents determined in 647 of 1348 samples collected from public buildings in Switzerland. Sampling focused on concrete buildings erected between 1950 (estimated start of use of joint sealants containing PCB as a plasticizer) and 1980 (eight years after the use of PCB in open applications has been banned in Switzerland).



The historical record of PCB and PCDD/F deposition at Greifensee, a lake of the Swiss plateau, between 1848 and 1999

Markus Zennegg [a,*], Martin Kohler [a], Paul C. Hartmann [b], Michael Sturm [b], Erika Gujer [a], Peter Schmid [a], Andreas C. Gerecke [a], Norbert V. Heeb [a], Hans-Peter E. Kohler [b], Walter Giger [b]

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[b] *Eawag, Swiss Federal Institute for Environmental Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland*

Revised 14 February 2006; accepted 26 May 2006

2007

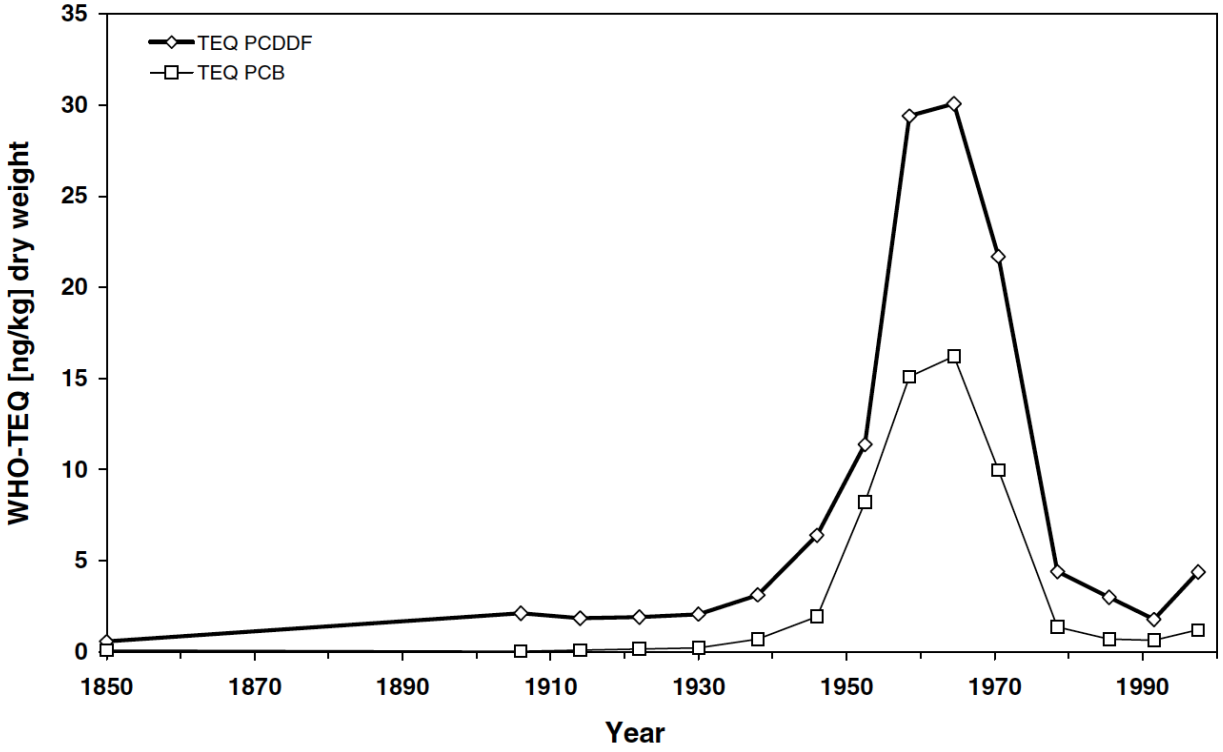


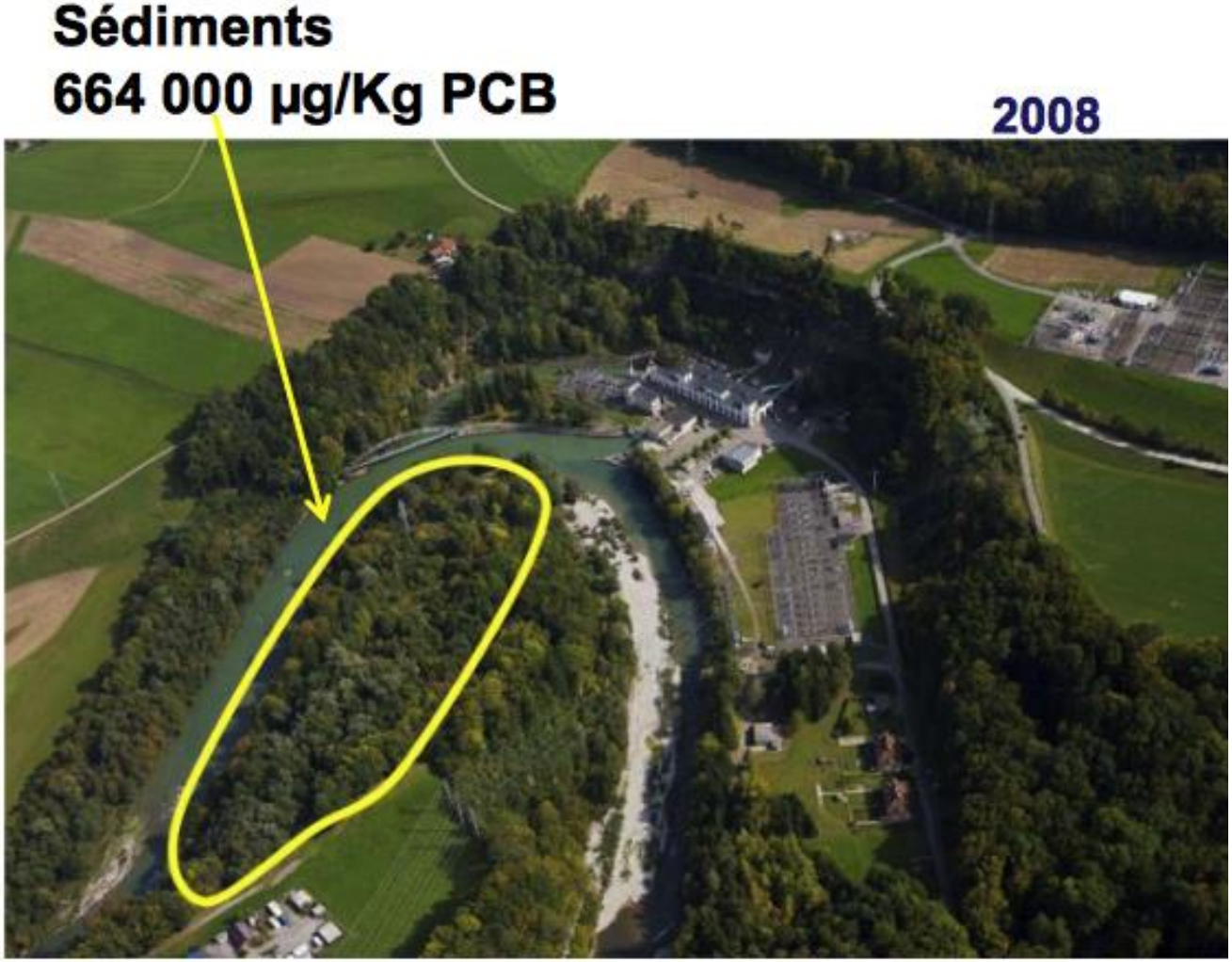
Fig. 2. Historical record of PCB and PCDD/F concentrations in Greifensee sediment, calculated as WHO-TEQ.

29.08.2007

Interdiction de pêche sur le tracé de la Sarine entre les barrages de Rossens et de Schiffenen, ainsi que dans la Gérine inférieure

Suite à des analyses révélant une contamination des poissons dans la zone concernée, le Conseil d'Etat a décidé hier d'une interdiction de la pêche sur le tracé de la Sarine entre les barrages de Rossens et de Schiffenen, ainsi que dans la Gérine inférieure. En effet, les résultats obtenus révèlent des teneurs en PCB de type dioxine (cPCB) trop élevées dans la chair des poissons analysés, les rendant impropres à la consommation. En revanche, la baignade dans les eaux concernées ne pose pas de problème.

The source



1952-1975
Décharge ordures ménagères + déchets industriels

20 tonnes condensateurs + huiles

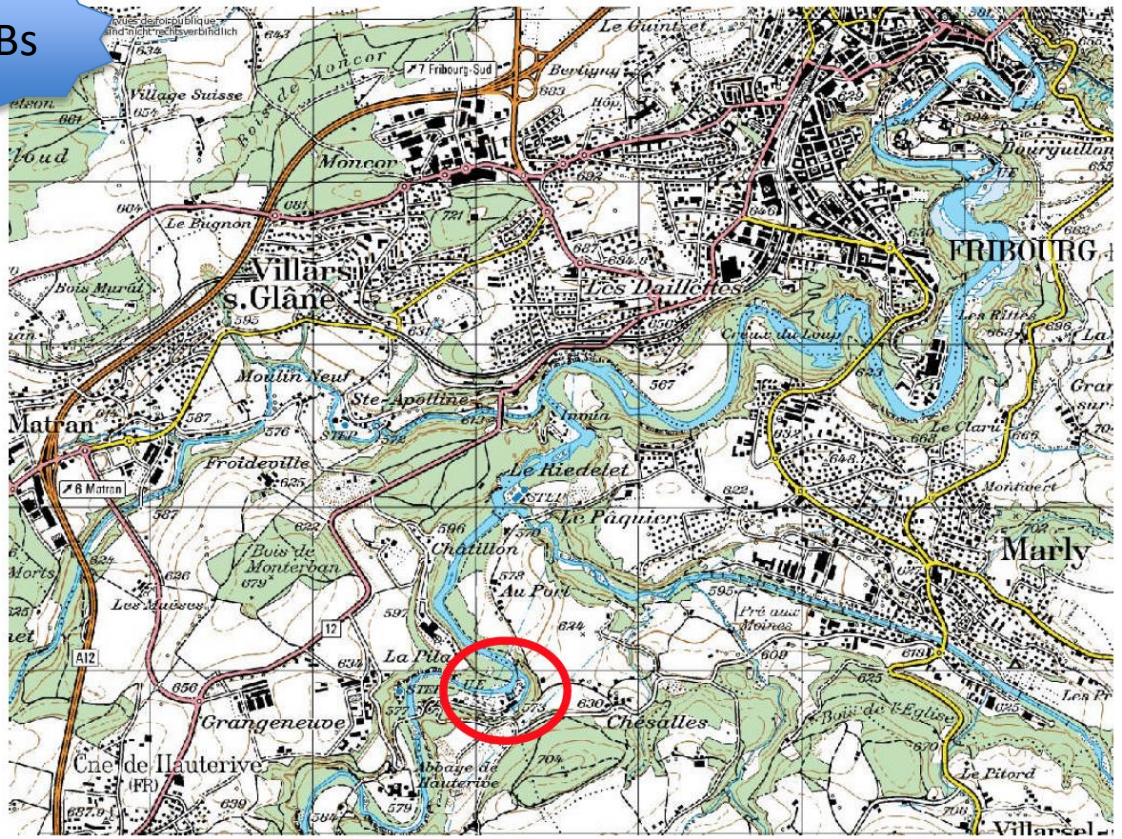
250’000 m^3 de terre, sédiments contaminés

2014: first human biomonitoring in Switzerland

PCBs

A household dump
Contaminated with condensers containing PCBs

→ Remediation needed



The company is worried about the workers protection :

- wear individual protection equipment
- Ask for a biomonitoring of PCBs levels in blood on 10 individuals

Comparison of analytical tools

Exposure analysis Chemical method	Effect analysis, screening Reporter gene assay
Analysis of 209 PCBs congeners by GC/HRMS	Analysis of the induction of AhR by the DR-CALUX bioassay
20 ml of blood needed, expensive (>1000 €)	2 ml of serum needed, 3 to 5 times cheaper
	Validated for food, feed, environmental and blood

Analysis choice

Screening tool
two complementary assays

GC-ECD chemical analysis	DR CALUX bioassay
6 most abundant PCBs congeners (indicator PCBs) Non coplanar PCBs	Dioxin-like PCBs binding to the AhR receptor Coplanar, toxic congeners
2 ml of serum needed cheap	2 ml of serum needed, cheap

Dioxin/PCBs and men

- Lipophilic - Accumulation over time in human (fat)
- Serum fat level is proportional to fat of the body
  - Are levels proportional to weight ?
  - Are levels proportional to age?
- Women can release thought breast feeding
  - Are levels linked to gender?

> Sampling before and at the end of the time spent on the construction site

Data set is small

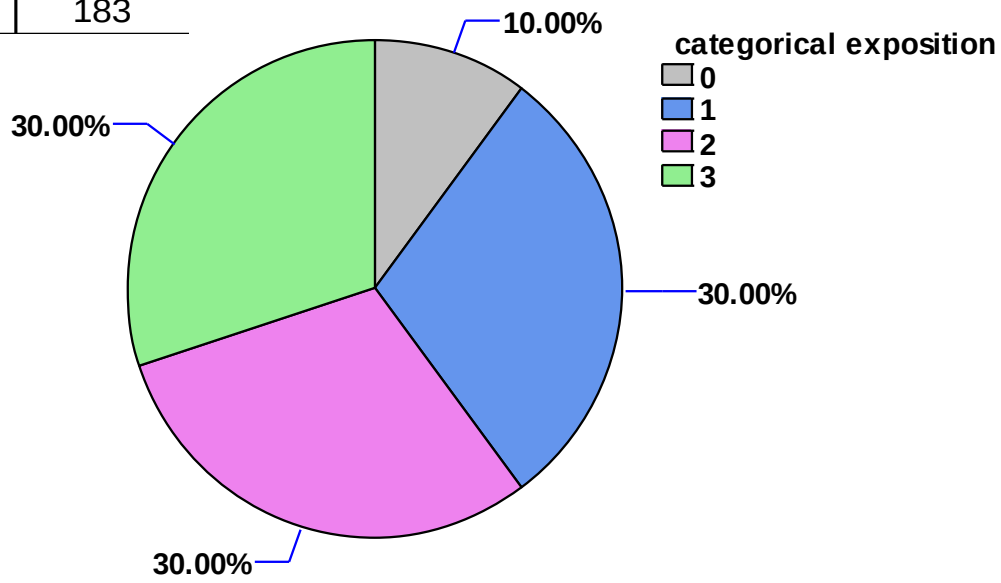
No patient	Exposure	Sexe	Age [yrs]	Weight [kg]	Height [cm]
1	0	M	57	84	182
2	3	M	46	72	165
3	3	M	50	85	172
4	2	M	37	90	174
5	2	M	56	75	165
6	3	M	57	96	184
7	1	M	39	84	179
8	1	F	34	80	175
9	2	M	53	82	175
10	1	M	36	83	183

Bias due to the selection of individuals ?

Average age 46.5 y.o.

Average weight 83.1 kg

Average BMI 26.5



Results DR-CALUX

	before	after
No patient	CALUX A PCDD/PCDF and dl-PCBs (only total TEQ) [pg TEQ/g fat]	CALUX B PCDD/PCDF and dl-PCBs (only total TEQ) [pg TEQ/g fat]
1	19*	29
2	28	25
3	32	18
4	20	16
5	24	16
6	20	13
7	16*	17
8	23	21
9	19	31
10	17	13
Average	21.8	19.9
Median	20	17.5
Standard deviation	5.03	6.42

No significant difference

Values closed to the LOQ for most individuals

Basal serum levels in the general population (Europe):

- non exposed or new born: 23 to 27 pg TCDD TEQ/g fat
- living close to an incinerator: 55 to 76 pg TCDD TEQ/g fat

Responses to individual variables

Variable	rs/ rho	p-value	
Sexe	0.18	0.63	(1)
Age	0.20	0.59	(2)
Weight	-0.36	0.31	(2)
Height	-0.68	0.03	(2)

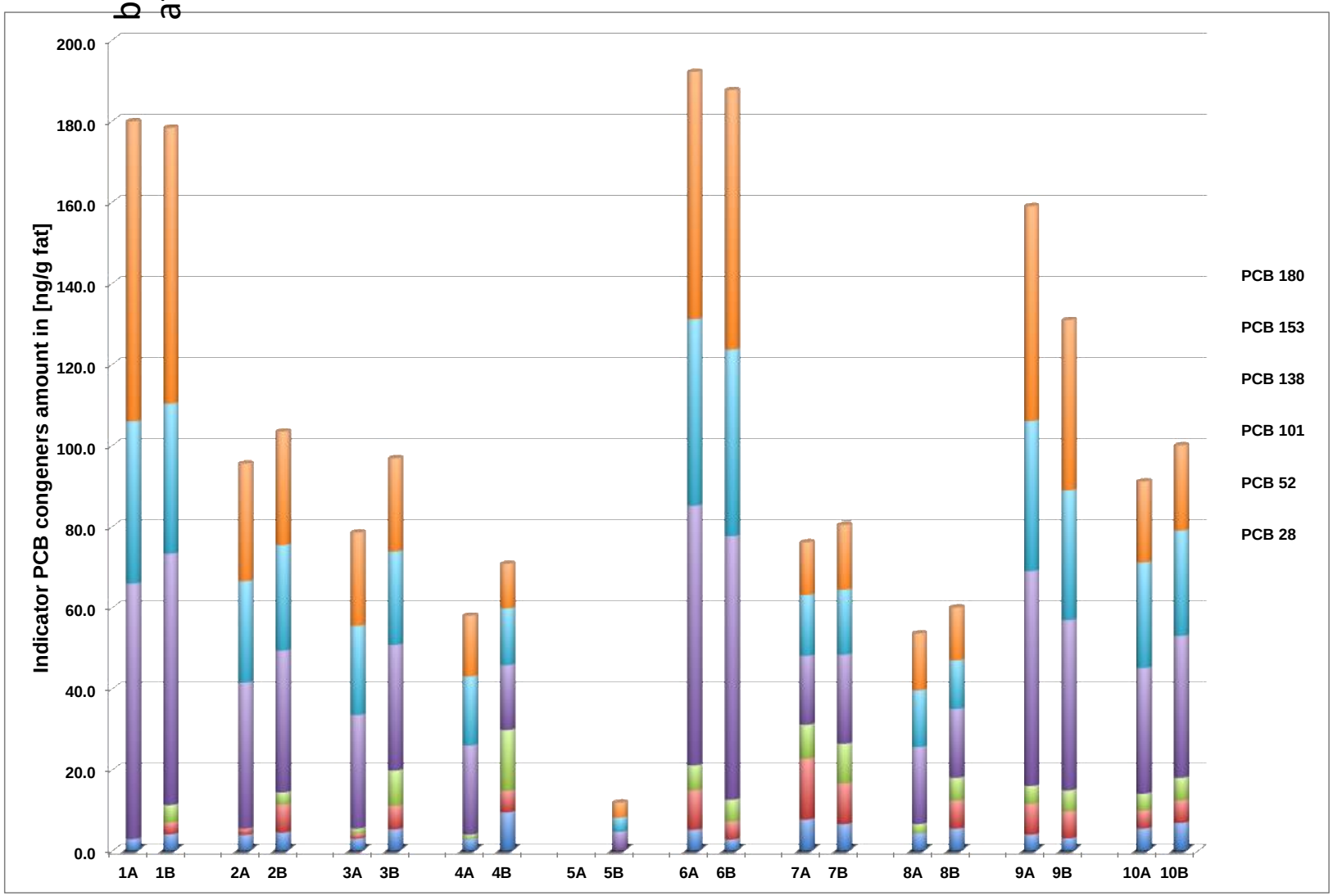
Correlations tests were performed on the CALUX A values to assess for effect of individual variables on PCB loads.
Test. Pearson's correlation for continuous variables (2)

Variable	rs/ rho	p-value to CALUX relative difference	
categorical exposure	-0.60	0.07	(1)

The correlation test was performed on the relative difference between value B and A to assess for exposure effect
Test: Spearman's correlation for categorical variables (1)

- No significant p-value for any variable, but the height.
- Individual sources of variability could not be identified using the correlation tests

Indicator PCBs (GC-ECD)



Neonates (n>1000) (Koppen et al. 2009) :

26.0 ng/g fat

37.7 ng/g fat

21.3 ng/g fat

-

-

-

Higher preponderance of the congeners 138, 153, 180 than 28, 52, 101 (metabolized)

Conclusion of the study

- Screening of a small population of potentially exposed workers
  - Basal values observed for indicator PCBs amounts as well as for TCDD TEQ in the serum of the individuals

- No significant increase found in the levels of PCBs after the exposure period

- The workers were well protected and there is no or negligible effects of the occupational exposure on their health

- DR CALUX bioassay allows to screen serum for activity of compounds with a dioxin-like activity
- A complementary analysis is required to confirm any positive results

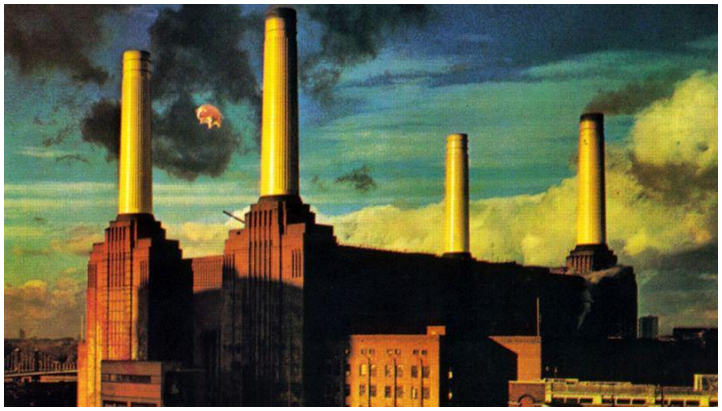
Start to test other workplaces

From 2015 – on

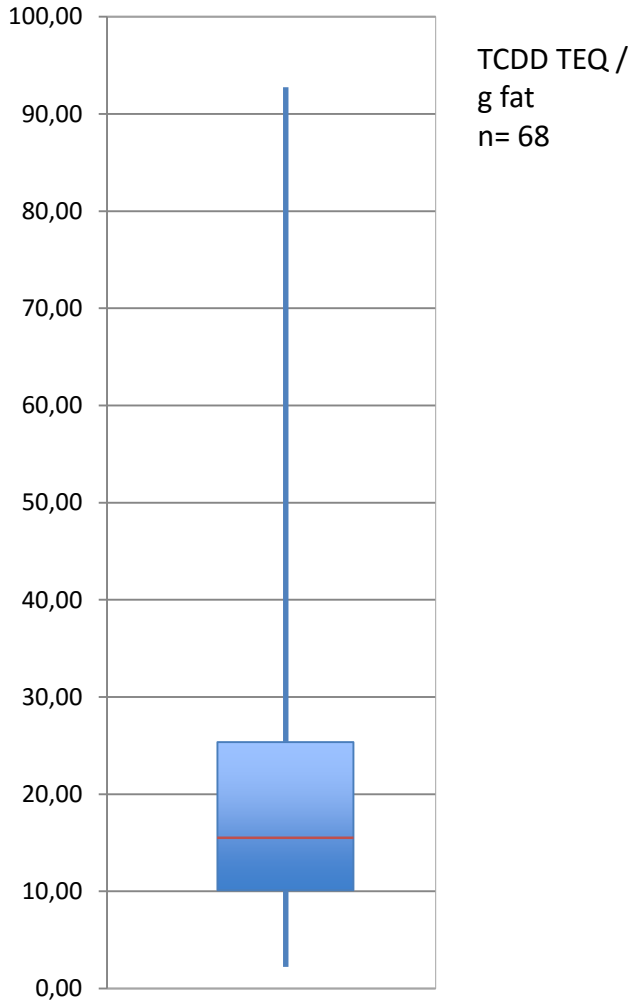
Biomonitoring of 200 individuals working in an incineration plant

Once every second year , check up analyses, under the control of a physican

- Clinical parameters (liver enzymatic activity, thyroid hormones…)
- 1-OH pyrene as biomarker of the exposure to PAH
- Heavy metals
- DR CALUX



Levels of DR-CALUX in worker's serum



Implementation steps of CALUX method

- Think about the room and regulations required in your country
- Acquire equipment

- Qualify equipment
- Train staff

- Start analysing in order to validate your method

ISO /CEI 17025

Validation of the lab with BDS standards

Qualify luminometer

see poster

Results and conclusions

2.1 Phase IA: Data handling

Evaluation of the DR CALUX® excel-sheet. In t
summarised.

Table 1. Data handling

Knows where to find the TEQ outcome in spre
Knows how to fill in the correct weight of sampl
Knows how to fill in the correct product unit
Correct significance presentation of results
Correct filling in of observations
Knows when values for samples are out of ran

Phase IB: cell performance

Figure 1 shows standard calibration curves obt
a normal pattern. In Table 2, the performance c

From the data, it is concluded that the performan
according to BDS' performance criteria.

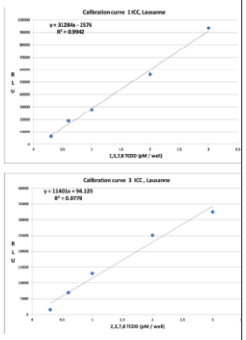


Figure 1. 2,3,7,8-TCDD calibration curves for
concentrations in pM/well are given.

BDS-ICC Lausanne- report cross validation

Calibration curves

2.3 Phase IIA: Undiluted DMSO samples

In Table 4, the reported DR CALUX® analysis results of the undiluted DMS
Lausanne and BDS respectively. The results for both ICC Lausanne and Bl
represented in Figure 3.

From the presented data it can be concluded that a good correlation is ol
results for undiluted DMSO samples obtained by ICC Lausanne and BDS

Table 4. DR CALUX® analysis results of undiluted DMSO samples
and BDS.

Sample code	ICC Lausanne (pM in well)
	Values below LOQ
Cross1	0.08
Cross2	0.53
	Values between LOQ and top of calibration cur
Cross3	1.3
Cross4	1.9
Cross5	2.9
Cross9	1.1
	Values above calibration curve
Cross6	6.62
Cross7	4.85
Cross8	6.09
Cross10	4.13

Note: Values below the LOQ (1.0 pM in the well) and above the highest concentration of the T
well) should be ignored for comparison.

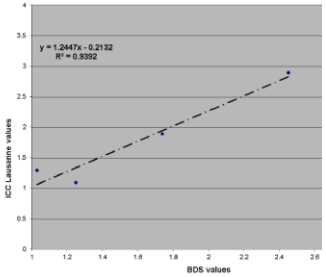


Figure 3. Correlation between ICC Lausanne and BDS for undiluted DM
pM DR CALUX® TEQ in the well

BDS-ICC Lausanne- report cross validation

Measure extracts

2.4 Phase IIB: DMSO samples subject to dilution

In Table 5 the reported DR CALUX® analysis results of the DMSO samples a
and BDS. The results for both ICC Lausanne and BDS are graphically repres

From the presented data it can be concluded that a good correlation betwe
undiluted DMSO samples obtained by ICC Lausanne and BDS is observed

Table 5. DR CALUX® analysis results of DMSO samples, subject to dilution
and BDS.

dilution	Sample code	ICC Lausanne (pM in well)
		Values below LOQ
1	Cross14	0
3	Cross14	0
10	Cross14	0
		Values between LOQ and top of calibration curve
10	Cross11	1.4
10	Cross12	3.5
3	Cross13	3.8
10	Cross13	0.98
		Values above calibration curve
1	Cross11	7.4
3	Cross11	3.4
1	Cross12	9.3
3	Cross12	7.0
1	Cross13	7.3

Note: Values below the LOQ (1.0 pM in the well) and above the highest concentration of the TCD
well) should be ignored for comparison.

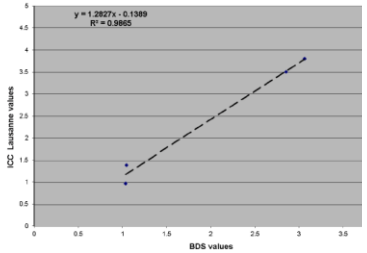


Figure 4. Correlation between ICC Lausanne and BDS for diluted DMSO s
DR CALUX® TEQ in the well

2.5 Phase II: All DMSO samples (diluted and undiluted)

BDS-ICC Lausanne- report cross validation

Extract and measure samples

Table 2. Performance characteristics at ICC Lausanne.

	Induction at 3pM	Status >2	BRM07 Reference	Status >1-<2	R² of fit	Status >0.95
160203_Cross Samples	3.3	OK	1.05	OK	0.9697	OK
160215_Fish Oil_dil 2	4.4	OK	1.40	OK	0.9956	OK
160211_Fish Oil	2.2	OK	1.70	OK	0.9889	OK
160205_SeraOF8255-	2.7	OK	1.44	OK	0.9969	OK

2.2 Phase IC; Luminometer performance

In Table 3, the results of the luminometer performance are given. Figure 2 shows a graphical representation of the obtained data.

From the results, it is concluded that the performance of the luminometer used by ICC Lausanne is according to the requirements.

Table 3. Luminometer results.

pM TCDD	avg RLU
0.15	73311
0.45	95347
0.75	124258
1.05	147584
1.35	160081
1.65	179042
1.95	218655
2.25	243697
2.55	267135
2.85	306340

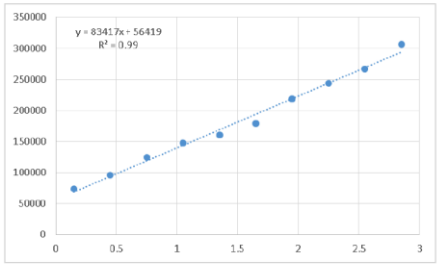


Figure 2. Graphical representation of the linearity of the luminometer used at ICC Lausanne

BDS-ICC Lausanne- report cross validation Page 5 of 12

ISO /CEI 17025

- Last step : find an individual proficiency control organisation /
- Show the results of these controls
- To obtain the ISO/CEI 17025 Accreditation

→ Analyse and report

2016: Food DR-CALUX in CH

- Compare : GCMS screening + GCHRMS confirmation
  to DR-CALUX screening + GCHRMS confirmation

- Collaboration with a cantonal lab to test
  - 24 fish extracts
  - 24 meat extracts

- Validation the method in accordance to EU regulations

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