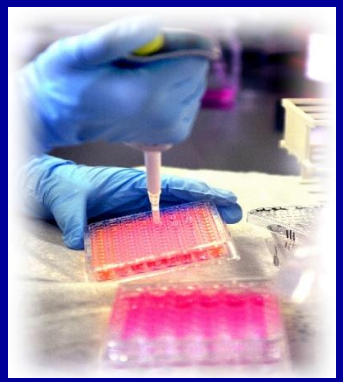
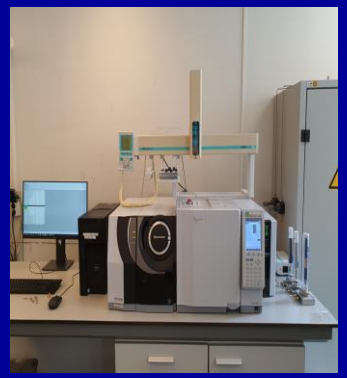
BioDetection Systems

Shimadzu-Büchi Symposium 2022

“Quō vādis POPs testing?”

Dr. Peter A. Behnisch,
BioDetection Systems,
Amsterdam





In the focus….

- State of the art POPs analysis
- Combi screening & confirmative testing
- Dioxin International Food Crisis & their Costs
- Total PFAS by biological & chemical analysis
- POPs in consumer products
- Human Biomonitoring
- Quo Vadis POPs testing ?

BioDetection Systems B.V. (BDS)

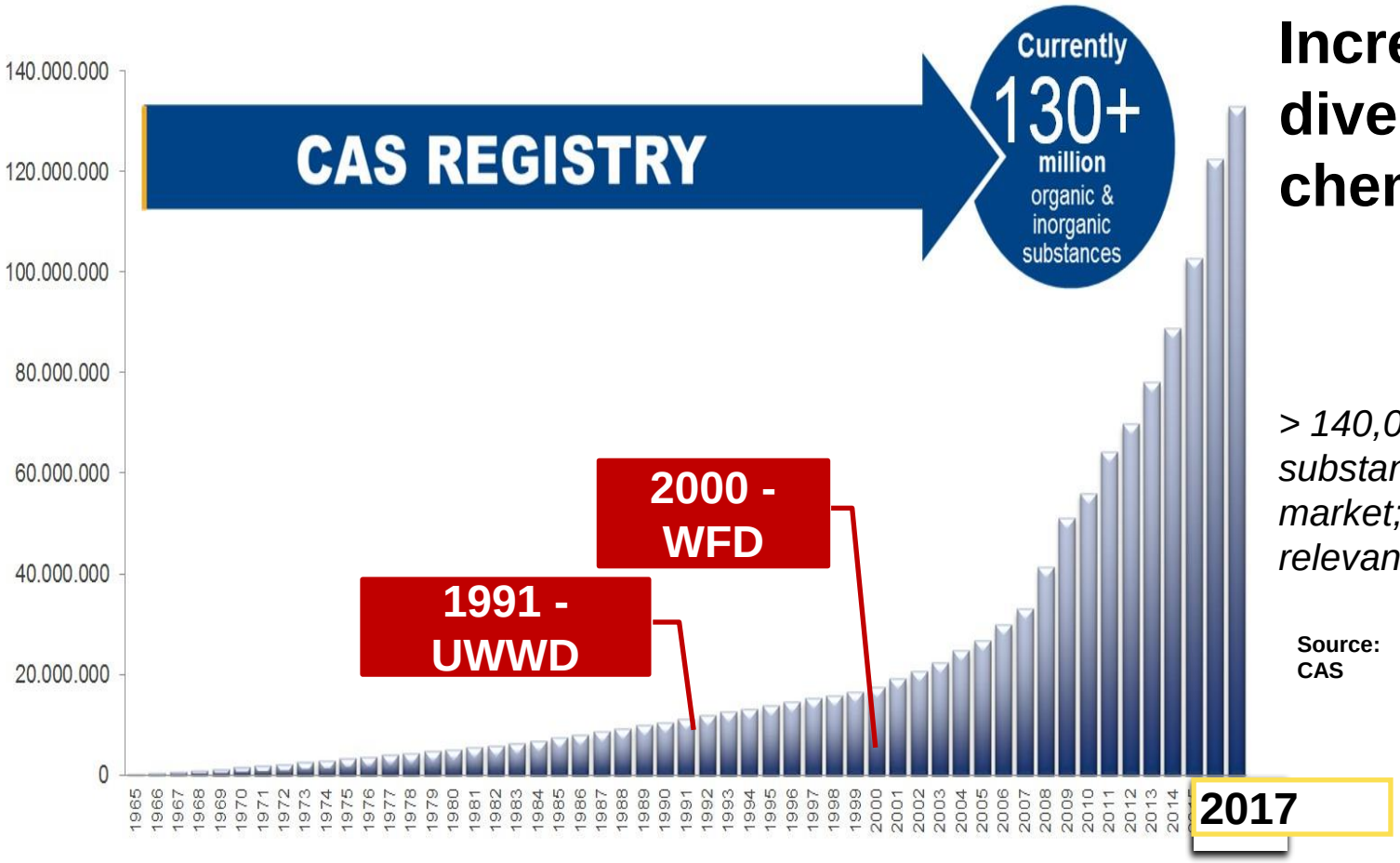
BioDetection Systems B.V. (“BDS”) is a

- Amsterdam based ISO 17025 accredited service laboratory

- International approved for dioxins/PCBs analysis in environmental, food/feed and human biomonitoring
- Bioanalysis for Hormones & Endocrine Disrupting Chemicals (e.g., plastic additives, pesticides, PFAS)

- Non-animal alternatives testing laboratory (OECD TG 455, 456 and 458 approved)
- CRO for safe & healthy food

MISSION
“Know more about the Unknown”



Increasing diversity of chemicals

> 140,000,000 substances on global market; 30,000 water relevant

Source: CAS

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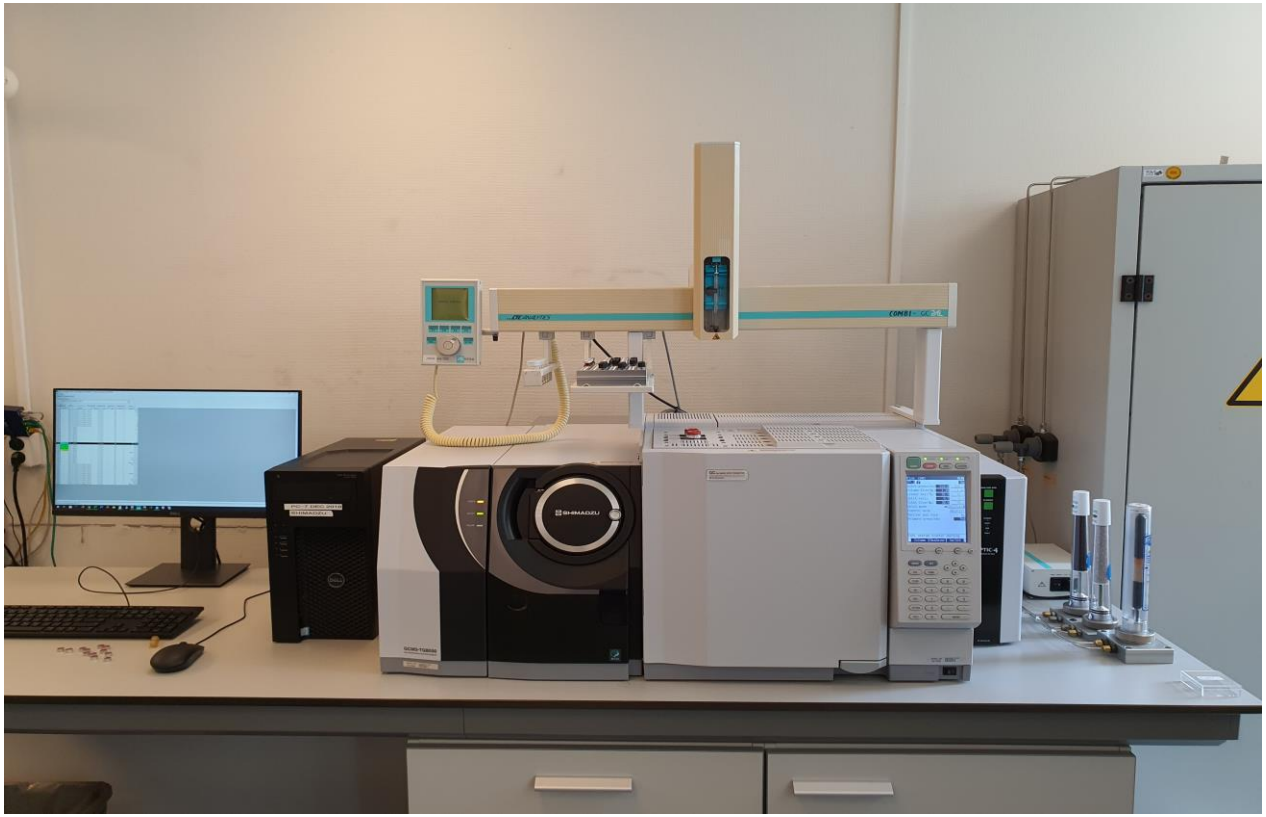
Automated extraction and clean-up of dioxins and PCBs in envi/feed/food

Speed extraction (Büchi)

Automated Clean-ups

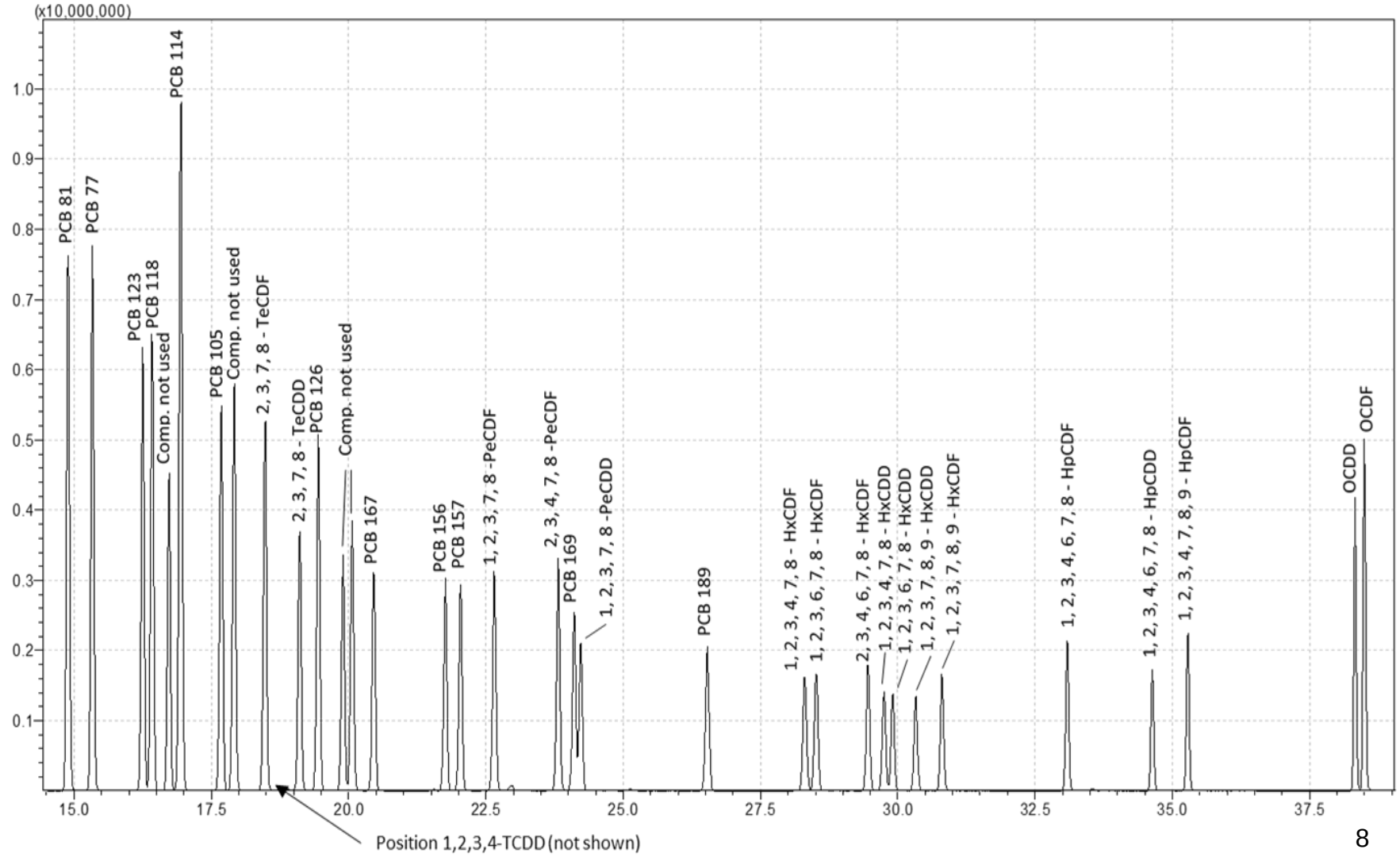
Our GC/MS/MS analysis by Shimadzu for dioxins and PCBs in feed/food

GC/MS-MS-TQ8050 (Shimadzu) since 2018



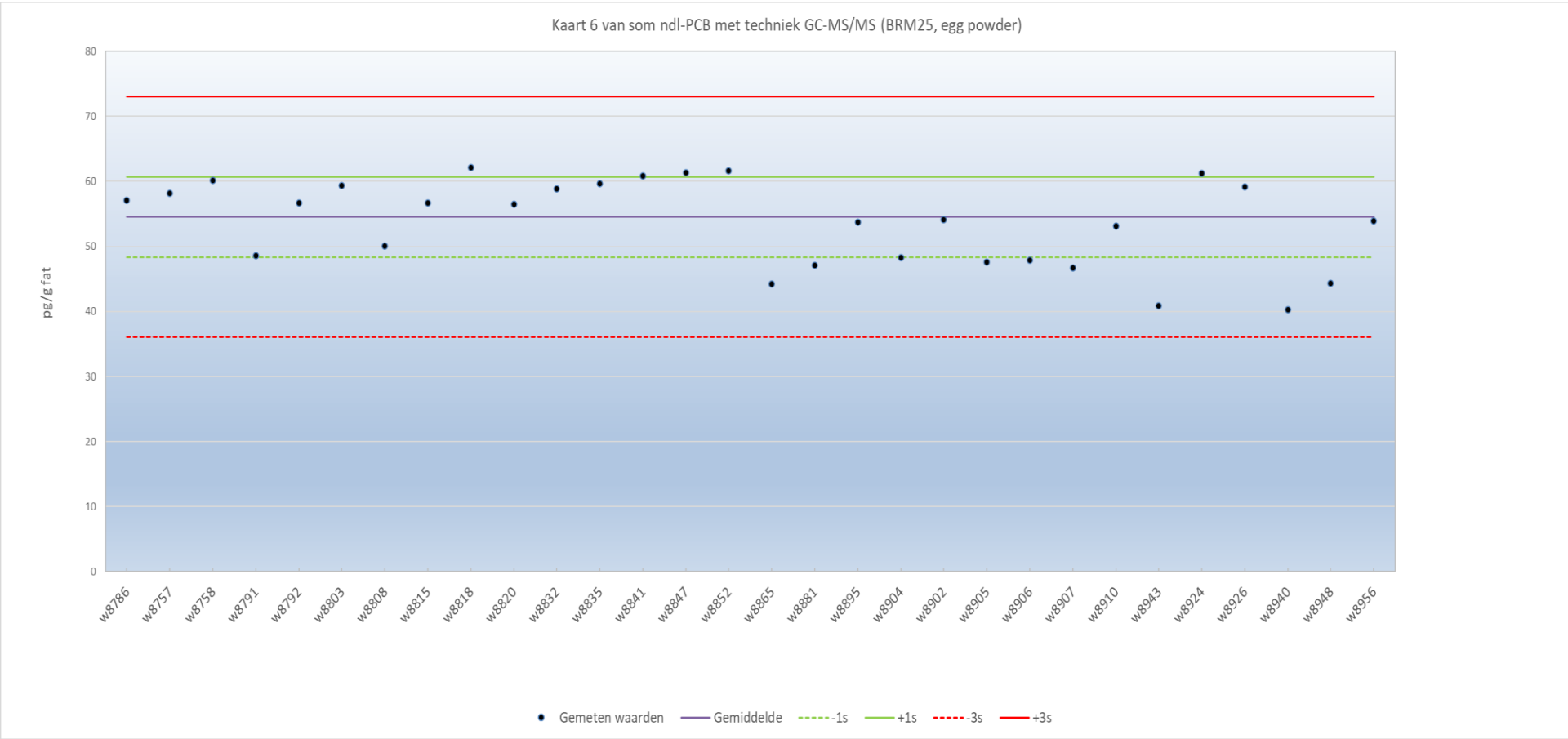
Our GC/MS/MS chromatogram

for all required dioxins and PCB congeners



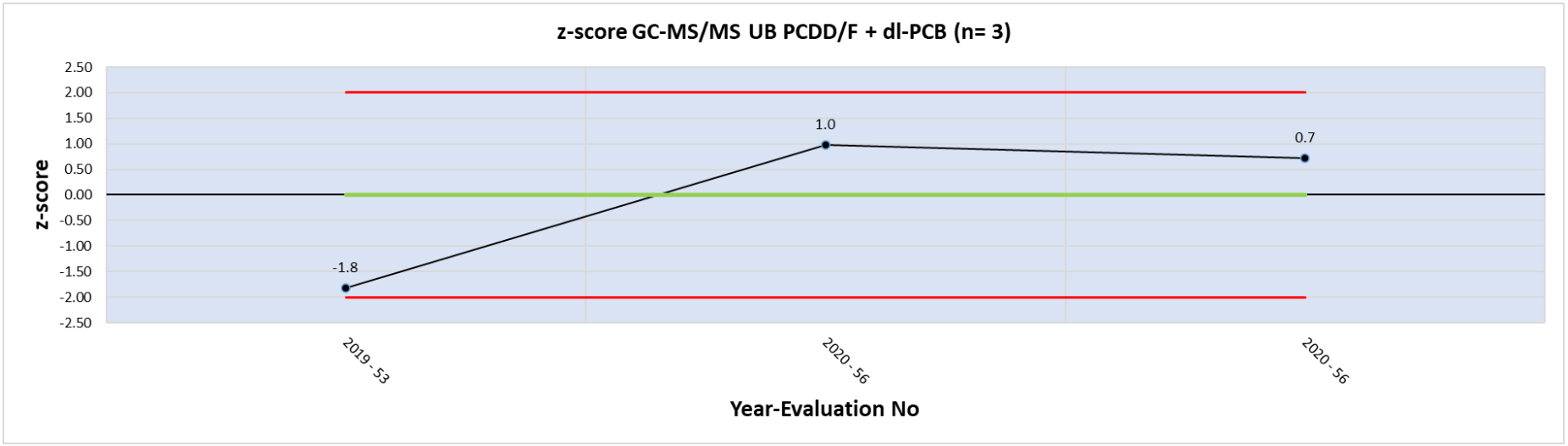
QA/QC GC/MS/MS analysis for dioxins and PCBs

1) Q-Chart for egg powder for NDL-PCBs



QA/QC GC/MS/MS analysis for dioxins and PCBs

3) Z-scores first 3 interlaboratory proficiency tests



z-score GC-MS/MS UB PCDD/F + dl-PCB (n= 3)

No	Organiser	Name Proficiency Test	Year - Evalution number	Sample	Matrix	Z-score	Uppe	Lower	Middl
64	EU-RL	EURL-PT-POP_2001-FI	2019 - 53	Fish fillet	Food, fish (product)	-1.82	2	-2	0
74	EU-RL	EURL-PT-DPB-2003-FF	2020 - 56	PFAD	Feed, plant orgin	0.98	2	-2	0
75	EU-RL	EURL-PT-DPB-2003-FF	2020 - 56	Rapeseed Oil	Feed, plant orgin	0.72	2	-2	0

ISO 17025 scope of BDS

Annex to declaration of accreditation (scope of accreditation)
Normative document: EN ISO/IEC 17025:2017
Registration number: **L 401**

RAAD VOOR ACCREDITATIE

of **Bio Detection Systems B.V.**
BDS
Service Laboratory

This annex is valid from: **23-09-2020** to **01-11-2022** Replaces annex dated: **23-04-2020**

No.	Material or product	Type of activity[1]	Internal reference number	Location
2	Feeding stuffs, raw material for animal feeding stuffs, fats, oils, meat products, dairy products, eggs and fish products	Determination of the content of indicator PCB's; GC-MS/MS PCB 28, PCB 52, PCB 101, PCB 153, PCB 138, PCB 180 and the sum of these six PCB's	P-bds-161 Fats, oils, eggs, meat- , dairy - and fish products: Commission Regulation (EU) No 2017/644) Feeding stuffs and raw material for animal feeding stuffs: Commission Regulation (EU) No 2017/771)	A
3	Feeding stuffs, raw material for animal feeding stuffs, fats, oils, meat products, dairy products, eggs and fish products	Determination of the content of dioxines, furans and dioxine-like PCB; GC-MS/MS 2,3,7,8-TCDD 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD OCDD 2,3,7,8-TCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDF PCB 81 PCB 77 PCB 126 PCB 169 PCB 123 PCB 118 PCB 114 PCB 105 PCB 167 PCB 156 PCB 157 PCB 189 Sum TEQ Dioxine, furanen and dioxine-like PCB Sum TEQ Dioxine and furanes	p-bds-170 in-house methode Fats, oils, eggs, meat- , dairy - and fish products: Commission Regulation (EU) No 2017/644 Feeding stuffs and raw material for animal feeding stuffs: Commission Regulation (EU) No 2017/771	A

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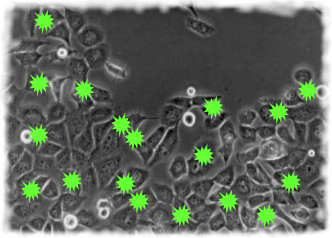
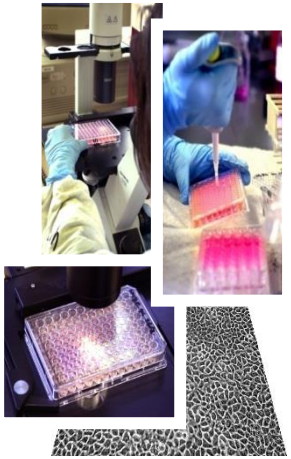
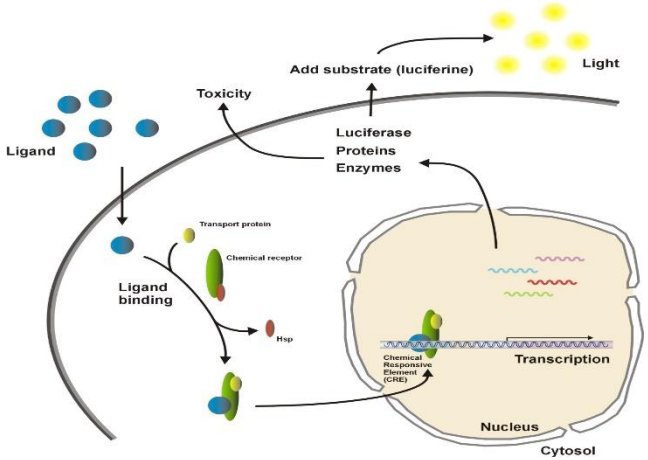
Robotic in vitro CALUX Screening







SEEDING

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

EXPOSURE

Dilution series (TCDD and sample)

DETECTION

In vitro toxicity profiling of POPs

	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.

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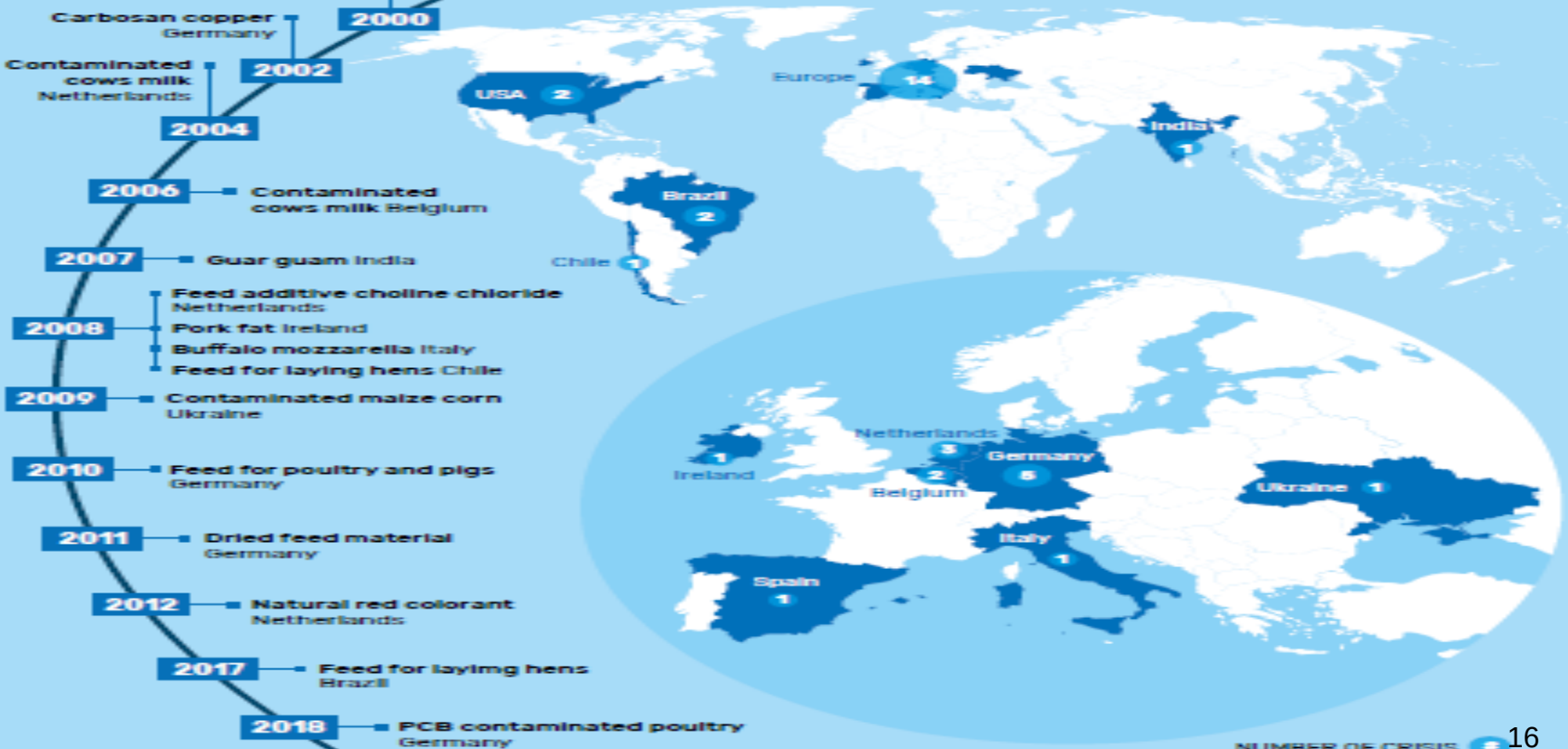
International DIOXIN food crisis

Behnisch, Brouwer in Affidia 01/2020

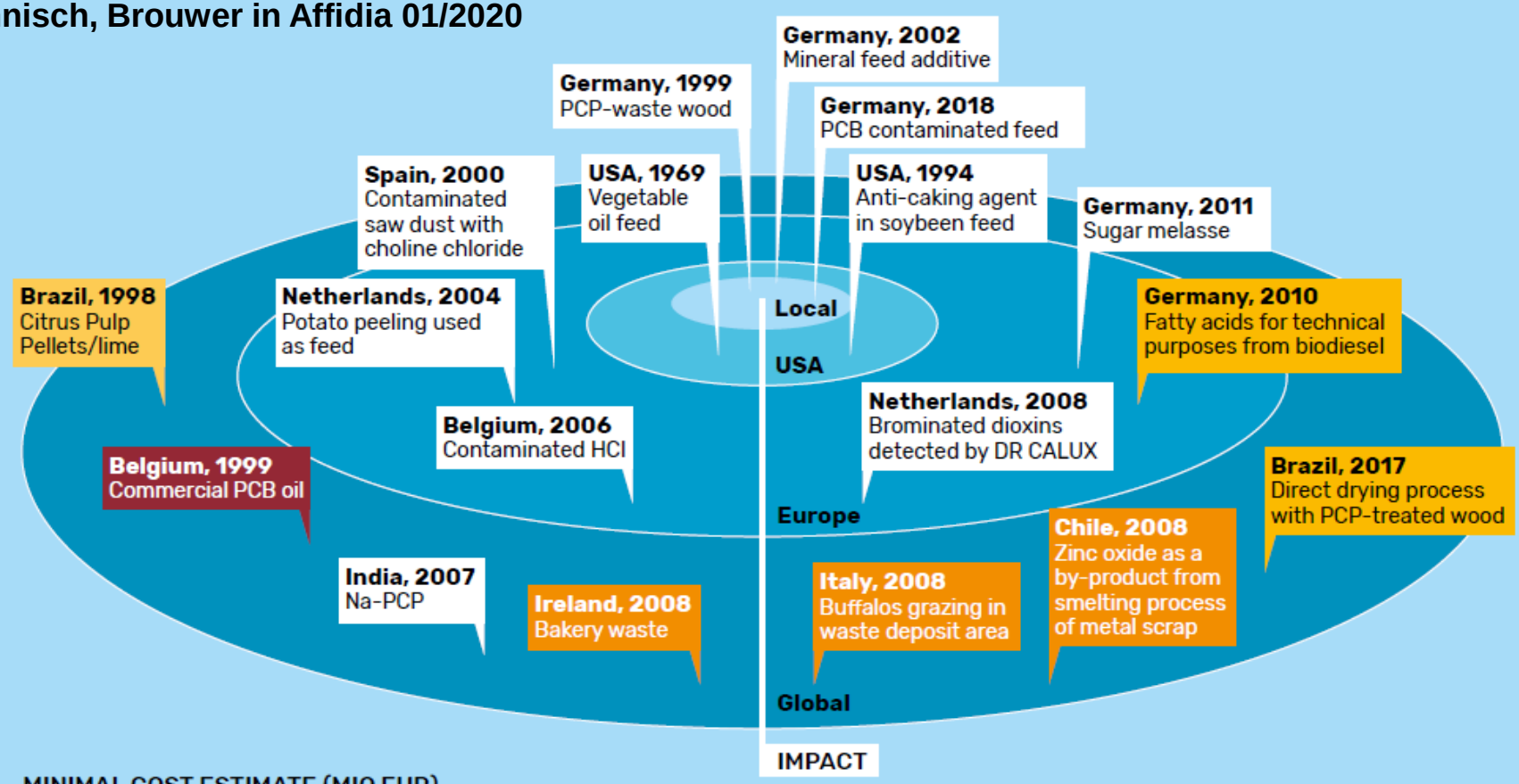
NON EXHAUSTIVE LIST OF GLOBAL DIOXIN CRISIS

- **1969** — Chicken death USA
- **1994** — Contaminated poultry and catfish USA
- **1998** — Contaminated cows milk Brazil
- **1999** — PCB contaminated feed additive Belgium; Contaminated grass meal Germany
- **2000** — Feed Spain
- **2002** — Carbosan copper Germany
- **2004** — Contaminated cows milk Netherlands
- **2006** — Contaminated cows milk Belgium
- **2007** — Guar guam India
- **2008** — Feed additive choline chloride Netherlands; Pork fat Ireland; Buffalo mozzarella Italy; Feed for laying hens Chile
- **2009** — Contaminated maize corn Ukraine
- **2010** — Feed for poultry and pigs Germany
- **2011** — Dried feed material Germany
- **2012** — Natural red colorant Netherlands
- **2017** — Feed for laying hens Brazil
- **2018** — PCB contaminated poultry Germany



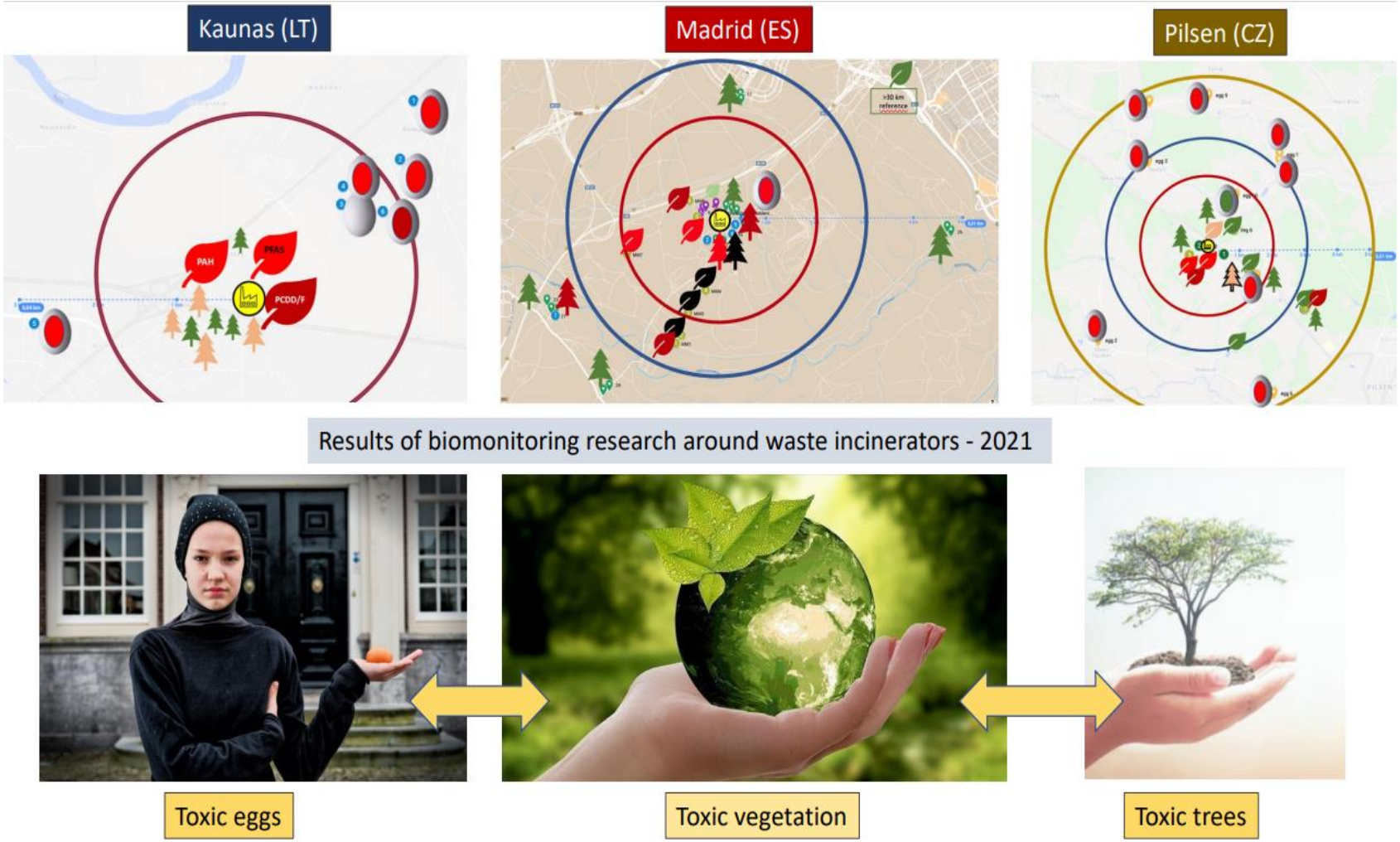
International DIOXIN food crisis & costs

Behnisch, Brouwer in Affidia 01/2020



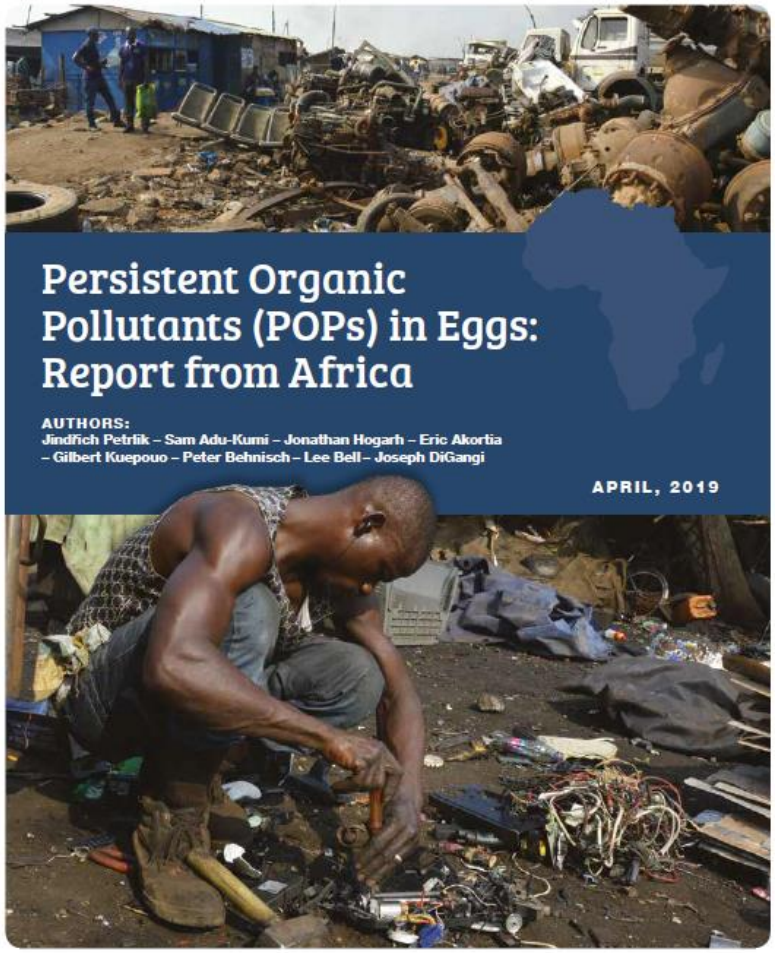
SOURCES AND IMPACT IN DIOXIN CRISIS

Dioxin Egg monitoring surrounding incinerators: Zero Waste Europe report (2022): The True Toxic Toll



in courtesy to Abel Arkenbout, Toxicowatch

Dioxins & other POPs in chicken eggs at E-waste recycling sides in Africa (Arnika report 2019)



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	

Free range chicken eggs in Indonesia & World (Arnika 2019)

PLASTIC WASTE FLOODING INDONESIA LEADS TO TOXIC CHEMICAL CONTAMINATION OF THE FOOD CHAIN

December 2019

NEXUS3 ARNIKA ecoton IPEN for a toxics-free future

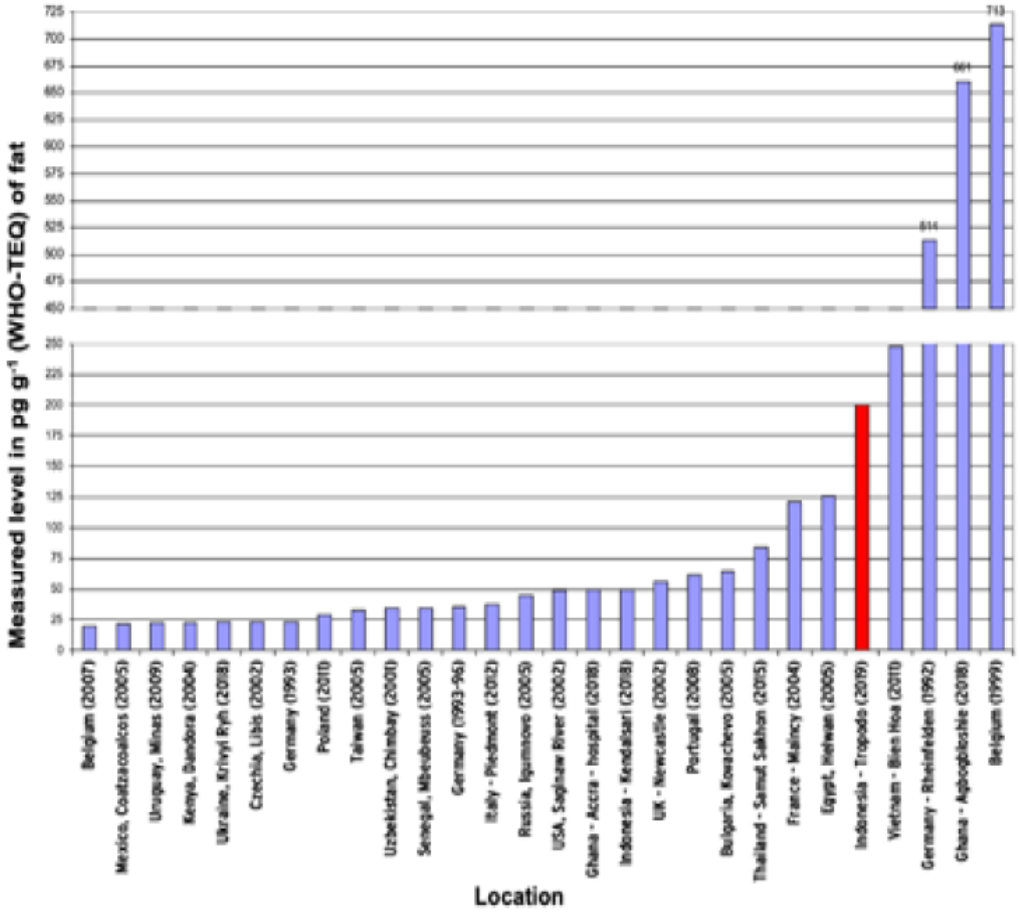
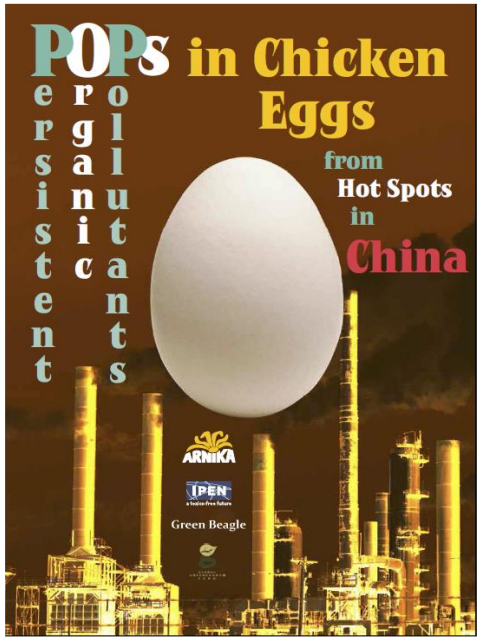


Figure 19: Dioxins measured in free-range chicken eggs from different locations in the world.

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



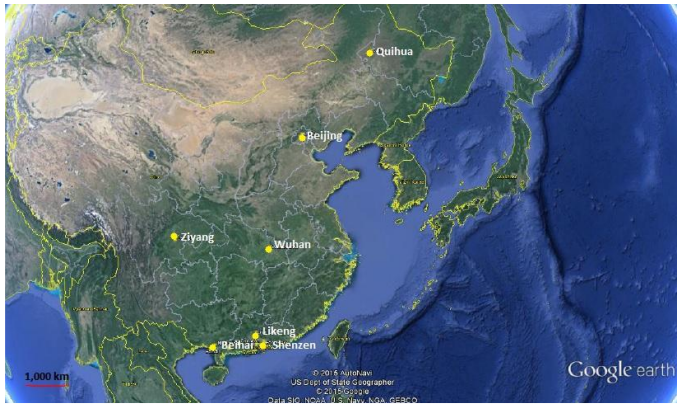
Beihei –metallurgic plant: 5-7 x EU Max

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

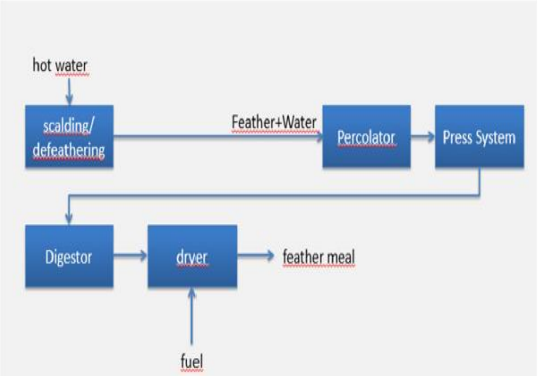


"This publication is part of Strengthening the capacity of pollution victims and civil society organisations to increase chemical safety in China"

China bans meat from Brazil (May 2015): intensive food screening by DR CALUX

Reason:	China Physicochemical Notification AQSIQ SIF 1001.
AQSIQ Notification:	DIOXIN
Product:	Chicken meat from the Industry Plant under SIF 1001 control.
Production dates involved:	**17, 18 and 29 th March 2015:** Health Certificates (IHCs) nº 01054/1001/15, shipped on 31/03/2015, nº 00896/1001/15, shipped on 20/03/2015, **16, 25 and 27 th April 2015 and 4, 5, 6, 7, 8 and 09 th May 2015:** Health Certificates nº 02101/1001/15, shipped on 02/06/2015, **8, 9, 13, 15, 16, 18, 19, 20 and 21 th May 2015:** Health Certificates nº 02415/1001/15, shipped on 19/06/2015.

Fuel used in unsuitable process (contaminated).
Burning of fuel with the potential to generate dioxins.
Burning of used pallets disposed of treated wood, used for drying feathers.
Method of drying feathers by direct heat.



BDS DR CALUX analysis:

- **2000 samples for Brazilian feed/food industry**
- **Less than 1% suspect in DR CALUX**
- **Caused by burning PCP contaminated pallets for drying chicken feathers, which are used as feed additive**

Free range chicken eggs in Western Balkan Countries (Arnika report 2014)

Table 4: Summarized results of analyses for POPs and mercury for thirteen pooled free range chicken eggs samples and one cheese sample. There are also EU limit values for comparison. (Table continues on page 13.)

Locality	Podbrežje (Zenica)	Gračanica (Zenica)	Tetovo (Zenica)	Donja Gračanica (Zenica)	Donja Vraca (Zenica)	Divkovići I (Tuzla)	Divkovići II (Tuzla)	Plužine – Orah	Plužine – Seoce
Sample	ZEN 1	ZEN 15/1	ZEN 15/2 + ZEN 15/4	ZEN 15/3	ZEN 15/5	BiH-E-01	BiH-E-02	PLZ-E1+E2+E3	PLZ-E4+E5+E1
Fat content	11.15	15.7	14.1	11.5	15.6	12.3	15.6	12.5	10.6
PCDD/Fs (pg WHO TEQ g^{-1} fat)	1.93	2.40	5.57	4.73	3.86	2.51	NA	NA	NA
DL PCBs (pg WHO TEQ g^{-1} fat)	5.15	1.55	3.09	3.56	3.75	1.56	NA	NA	NA
Total PCDD/F + DL PCBs (pg WHO TEQ g^{-1} fat)	7.08	3.95	8.66	8.29	7.61	4.07	NA	NA	NA
PCDD/Fs and DL PCB (DR CALUX); (pg BEQ g^{-1} fat)	NA	NA	12	NA	NA	NA	6.5	0.98	NA
PCDD/Fs (DR CALUX); (pg BEQ g^{-1} fat)	NA	NA	8.8	NA	NA	NA	4.3	0.34	NA

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Interlaboratory study of various reference materials

- **<u>Interlaboratory study with 4 food samples</u> (see for more info at Uhlig, Simon, Frost, QuoData, Germany, 2015):**
  - **Calf liver spiked**
  - **Wild pig unspiked, but from a contaminated area**
  - **Fish muscle**
- **Several feed samples from LHL:**
  - **Calf liver un-spiked**
  - **Potato**
  - **Gras**
  - **Feed**
  - **Compost**

PFOA-EQ (CHEM) and PFOA-BEQ (PFAS CALUX) values of a wild pig sample

PFAS	Conc. ng/g	REP-factors PFAS CALUX	PFOA-EQ	% Kongener/ PFAS-EQ
PFPeA	2,5	0,0093	0,02	0,0
PFHxA	0	0,041	0,0	0,0
PFHpA	4,2	0,24	1,0	1,0
PFOA	13,2	1	13	13,5
PFNA	24,6	0,1	2,5	2,5
PFDA	18,1	0,06	1,1	0,5
PFBS	2,3	0,025	0,1	0,1
PFHxS	2,5	3,2	8	8,2
PFOS	358	0,2	72	73
SUM ng PFOA-EQ/g (CHEM)			98	
SUM ng PFOA-BEQ/g (PFAS CALUX)			1600	

Screening & chemical analysis of total PFAS in food
PFOA-EQ vs PFOA-BEQ [in ng/g]

Sample	ng PFOA-EQ/g (CHEM)	ng PFOA-BEQ/g (PFAS CALUX)	Ratio BIO/CHEM	% PFAS/PFOA -EQ (CHEM)
Wild pig	**98**	**1600**	**16**	PFOS 73%
Calf liver spiked	**120**	**130**	**same range**	PFHxS 53%
Calf liver	**3,5**	**580**	**160**	PFOS 60%
Fish	**11**	**11000**	**1000**	PFOS 61%, PFOA 34%
Potato	**110**	**500**	**4,5**	PFOS 30%, PFOA 70%
Gras	**640**	**140**	**0,22**	PFHxS 80%
Feed	**320**	**120**	**0,38**	PFHxS 85%
Compost	**21**	**370**	**18**	PFOA 87%

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Toys & consumer products with high levels of brominated dioxins (Arnika report 2018)

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

PFAS in food packaging by chemical and biological analysis (Arnika report 2021)

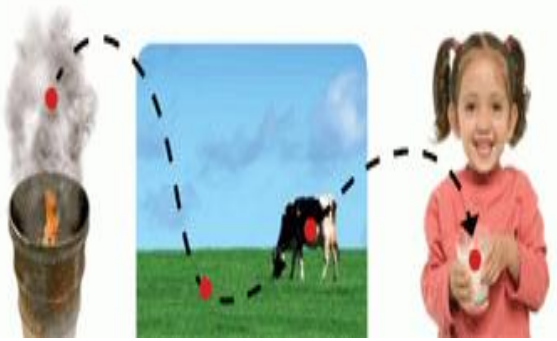
OIL-BEADING TAKEAWAY PAPER

SAMPLE ID	TOF (mg/kg dw)	TOF (µg/dm2 dw)	6:2 FTOH (ng/g)	6:2 FTS (ng/g)	10:2 FTS (ng/g)	% identified fluorine	TTR - FITC-T4 activity (µg PFOA/g)	TTR - FITC-T4 LOQ (µg PFOA/g)
DE-PAP-KFC-17a	**770**	247	**528**	<LOQ	<LOQ	0.047	**341**	26
FastF-FR-5	**700**	215	**706**	<LOQ	<LOQ	0.068	**220**	29
FastF-FR-3	**670**	224	**192**	**39.5**	**104**	0.033	NA	-
DE-PAP-NRDS-19a	**640**	291	**234**	<LOQ	<LOQ	0.025	NA	-
FastF-FR-2	**530**	351	**219**	<LOQ	<LOQ	0.028	NA	-
DE-PAP-DDNT-20a	**510**	270	**194**	<LOQ	<LOQ	0.026	NA	-
FasF-UK-5a	**480**	157	**16.9**	<LOQ	<LOQ	0.0024	39	19
CZ-FCM-KFC-06	**480**	134	**634**	<LOQ	<LOQ	0.090	69	33
CZ-FCM-MCD-01b	**470**	176	**335**	<LOQ	<LOQ	0.048	**52**	16
FastF-UK-2	**440**	177	<LOQ	<LOQ	**34.4**	0.0050	60	30
CZ-FCM-BB-01b	**400**	400	**345**	<LOQ	<LOQ	0.059	NA	-
FastF-UK-4	**390**	125	**248**	<LOQ	<LOQ	0.043	NA	-
DE-PAP-MCD-26	**370**	159	**132**	<LOQ	<LOQ	0.024	**180**	26

In the focus….

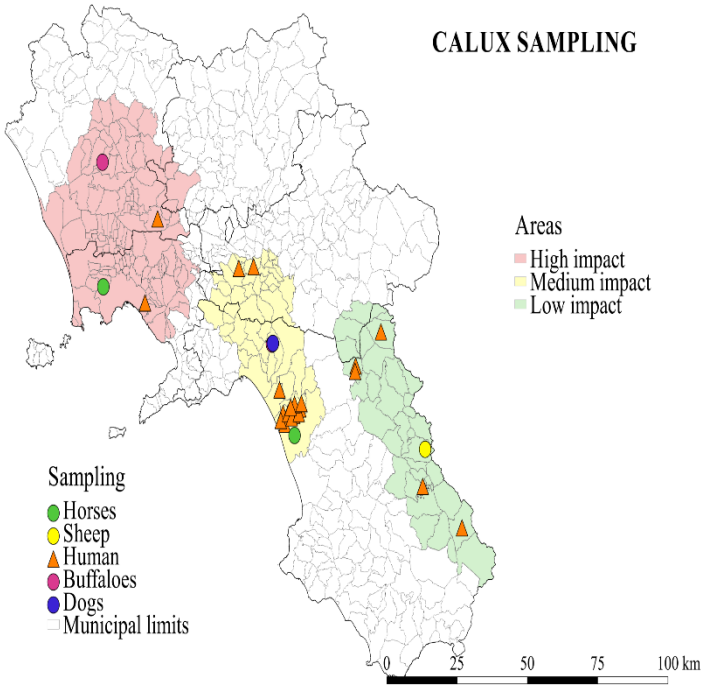
- State of the art POPs analysis
- Combinatorial screening & confirmative testing
- Dioxin International Food Crisis & their Costs
- Total PFAS by biological & chemical analysis
- POPs in consumer products
- **Human Biomonitoring**
- Quo Vadis POPs testing ?

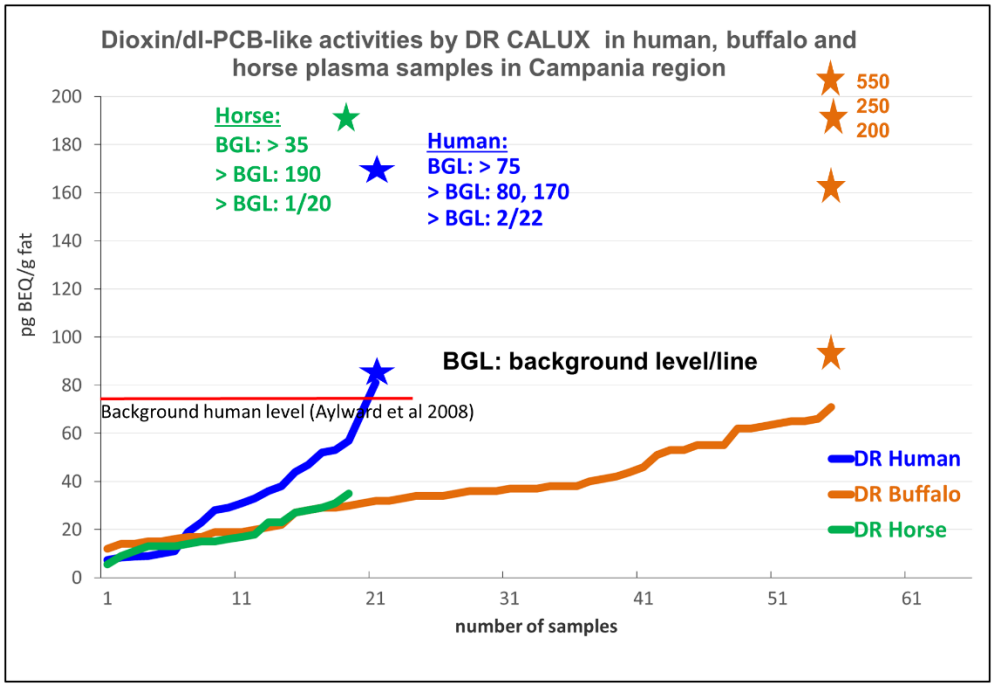
Blood testing by screening CALUX and chemical analysis



SPES

STUDIO DI ESPOSIZIONE NELLA POPOLAZIONE SUSCETTIBILE





Blood testing for dioxins/DL-PCB by chemical analysis

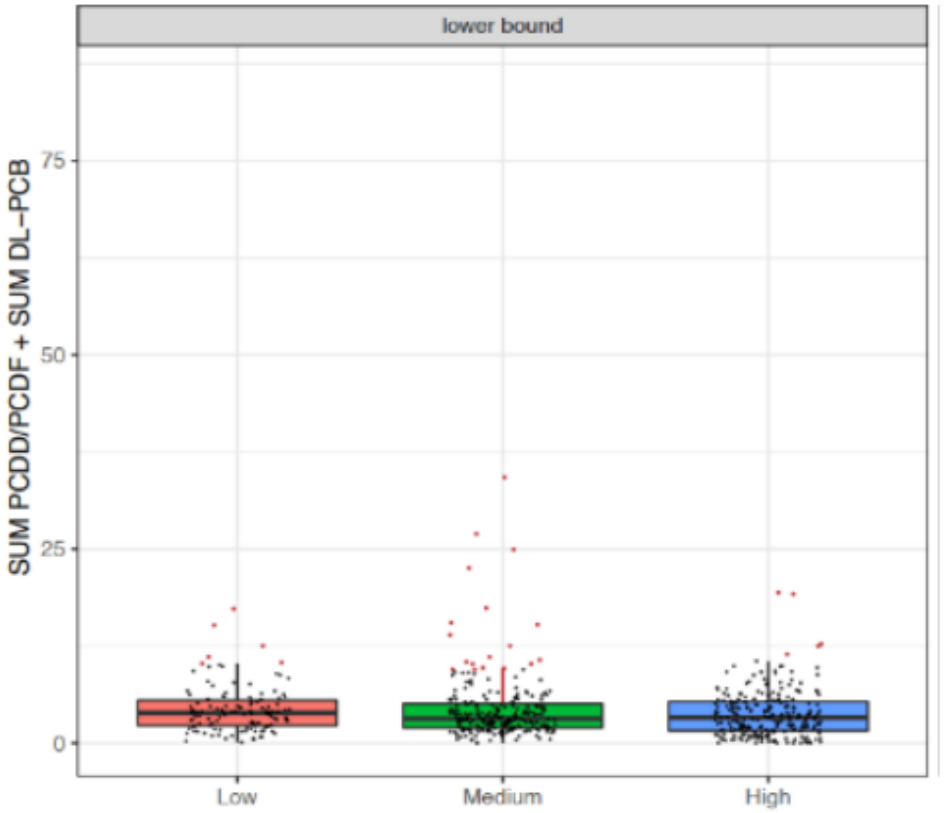


Figure 14. Sum of PCDD/PCDF + sum of DL-PCB expressed as pg WHO-TEQ/g lipids among the three different impact areas: low, medium and high impact area.

Blood testing for dioxins/DL-PCB by DR CALUX

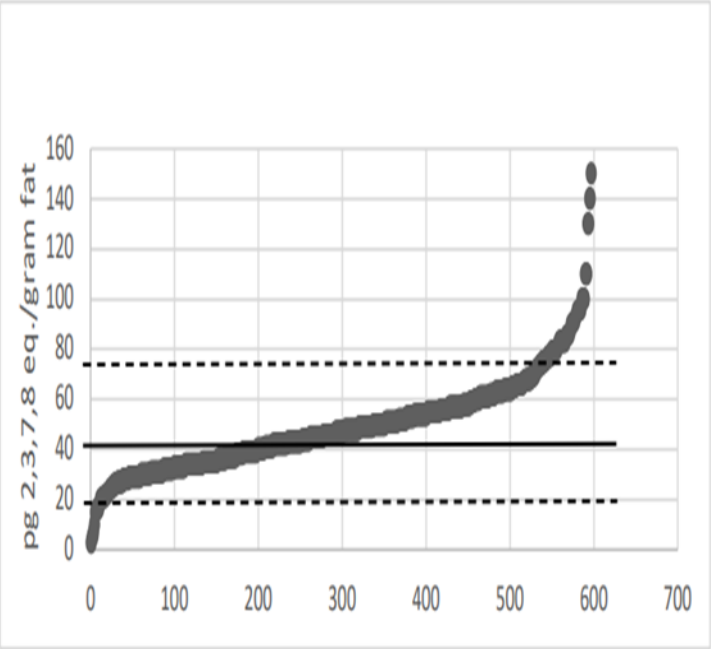


Figure 18. Yardstick-based representation of DR CALUX® results in study sample plasma analyzed. DR CALUX® bioassay results are lined up from lowest to highest obtained result expressed in pg 2,3,7,8-TCDD BEQ/g fat. Back line represents the mean value of 44.89 pg 2,3,7,8-TCDD BEQ/g fat. The top line indicates the

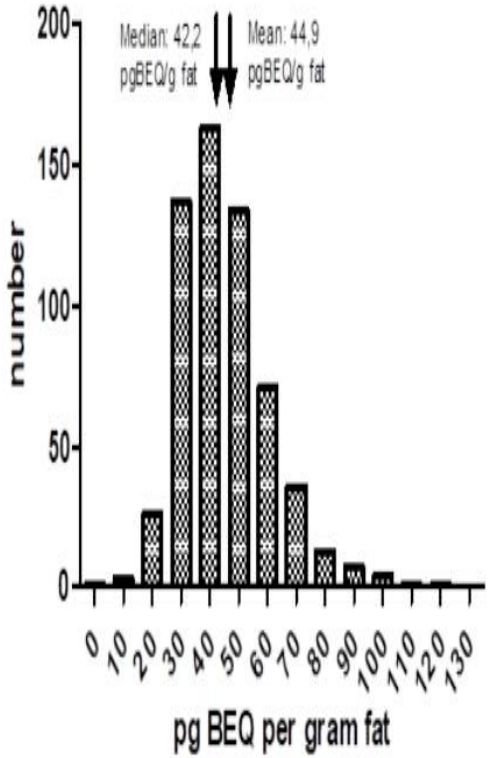


Figure 19. Frequency distribution of DR CALUX® results expressed as pg BEQ/g fat.

DNA marker vs chemical PCDD vs CALUX panel

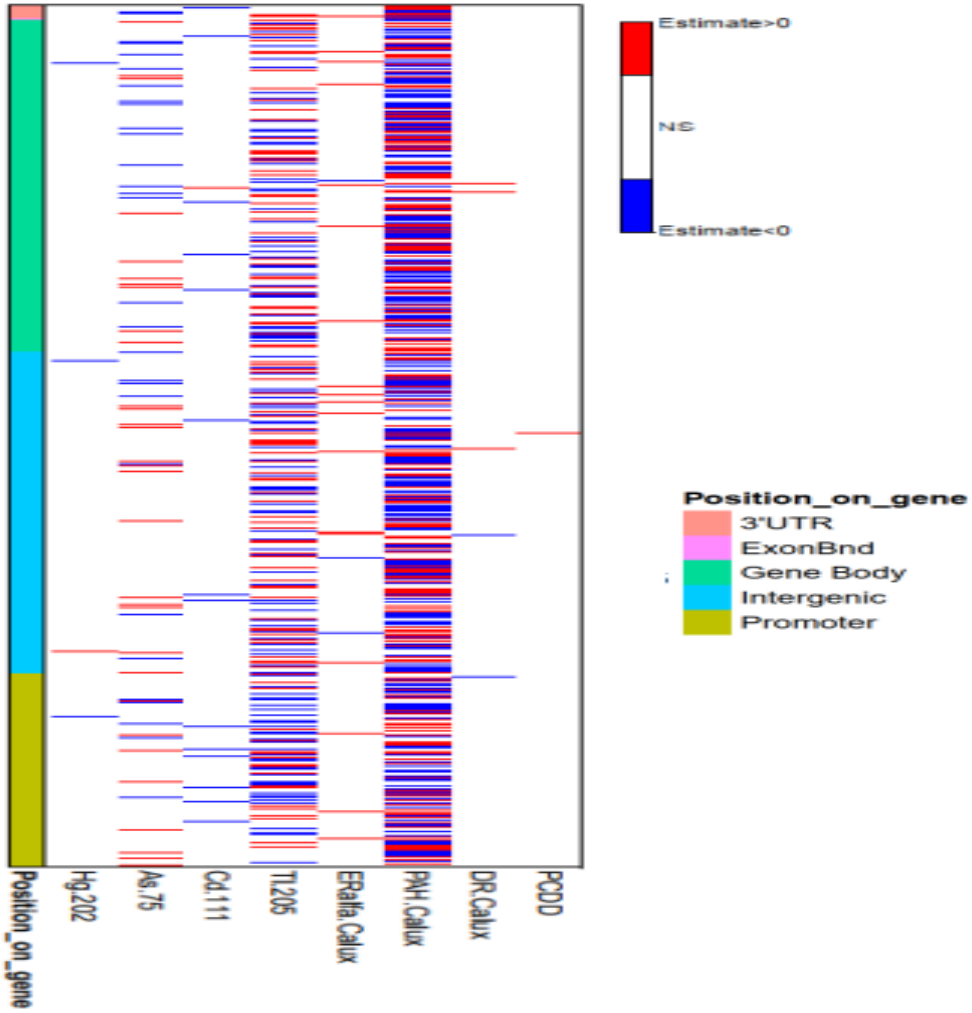


Figure 31. Heatmaps of robust linear multiple regression model

We are ready to go!

QUO VADIS POPs testing ?

- **Monitoring of POPs by combining in vitro CALUX screening and chemical analysis for a safer feed & food!**
- **Providing robust, easy, high capacity and low-cost state-of-the art de-risking solutions**
- **Prevent consumer scares (e.g. plastic) of new POPs through monitoring of sources, food and human uptake**
- **POPs & EDCs AND their toxic pathways as safer umbrella to make the ZERO pollution Green Deal strategy a step further to reality…..**