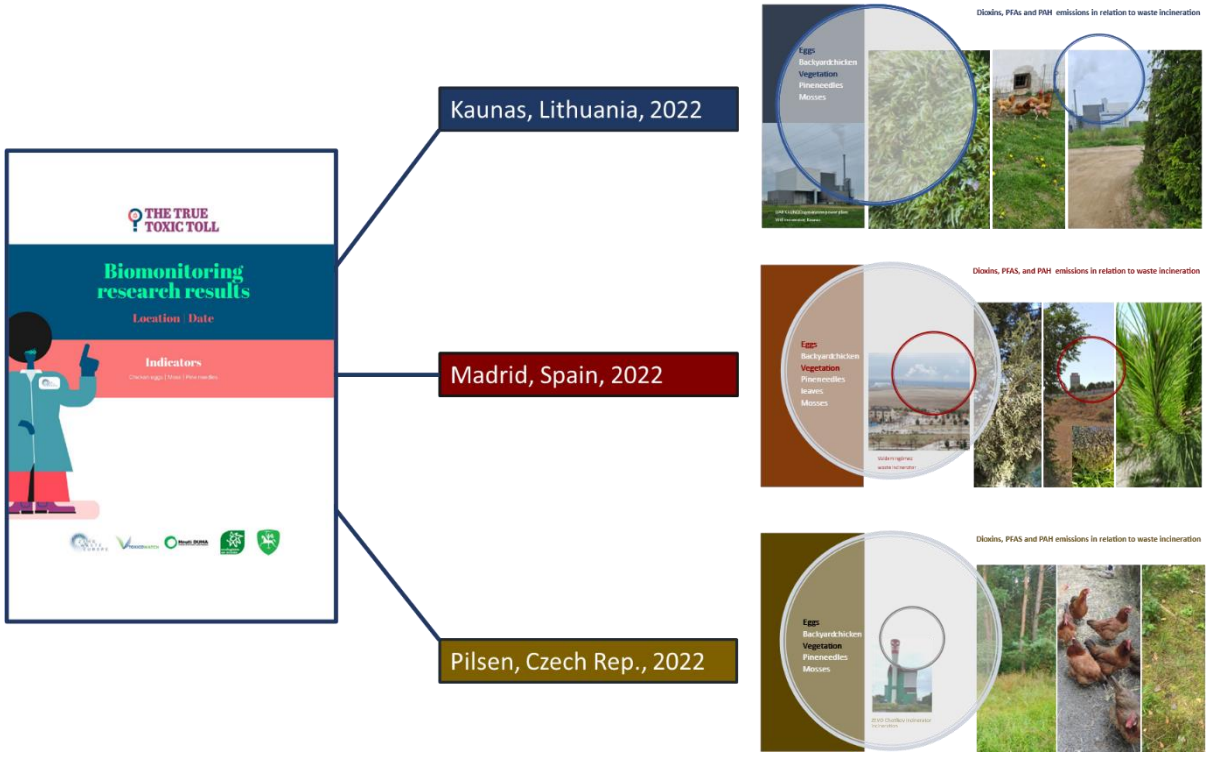
TOXICO WATCH

Biomonitoring research dioxins (PCDD/F/dl-PCB), PFAS and PAH in relation to waste incineration in Kaunas, Madrid, and Pilsen



December 2022

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Kaunas, Pilsen, and Madrid

Acknowledgements

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AUTHORS: A. ARKENBOUT Head of research ToxicoWatch Foundation
K.J.A.M. BOUMAN Research ToxicoWatch Foundation

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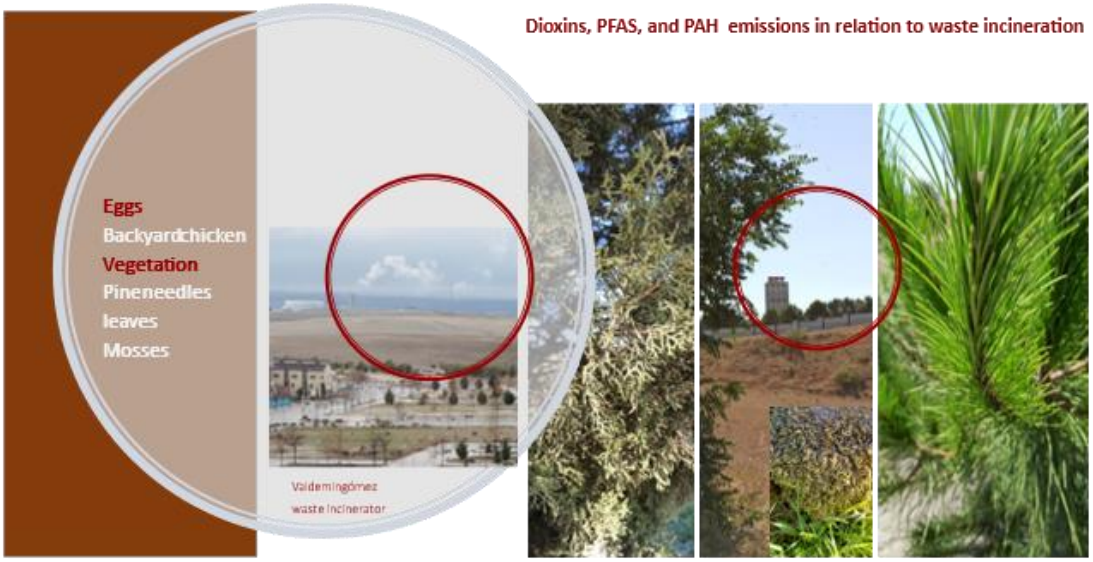
Abbreviations	Meaning
APCD	Air Pollution Control Devices
BAT	Best Available Techniques
BEP	Best Environmental Practice
BEQ	Biological Equivalents
BMI	Body Mass Index
dl-PCB	dioxin-like Polychlorinated Biphenyls
DR CALUX®	Dioxin Responsive Chemical-Activated LUciferase gene eXpression
dw	Dry Weight
EFSA	European Food and Safety Authority
FITC-T4	Fluorescein IsoThioCyanate L-Thyroxine (T4)
GC-MS	Gas Chromatography Mass Spectrometry GC-MS
GenX	Group of fluorochemicals related to of hexafluoropropylene oxide dimer acid (HFPO-DA)
i-PCB	Indicator Polychlorinated Biphenyl
LB	Lower Bound; results under detection limit are set to zero
LOD	Limit of Detection
LOQ	Limit of Quantification
MB	Middle Bound; values are set as half the detection limit values
MWI	Municipal Waste Incineration
ndl-PCB	Non-Dioxin-Like Polychlorinated Biphenyl (Non-Dioxin-Like PCB)
ng	Nanogram; 10^{-9} gram
OTNOC	Other Than Normal Operating Conditions
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
PFAS	Per- and PolyFluoroAlkyl Substances
pg	Picogram; 10^{-12} gram
POP	Persistent Organic Pollutants
RPF	Relative Potency Factors
RvA	Dutch Accreditation Council
SVHC	Substances of Very High Concern
SWI	Solid Waste Incineration
TCDD	2,3,7,8-tetrachloordibenzo-*p*-dioxine
TDI	Tolerable Daily Intake
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TOF	Total Organic Fluorine
TW	ToxicoWatch
TWI	Tolerable Weekly Intake
UB	Upper Bound (ub), results under detection limit are set as detection limit values.
µg	Microgram 10^{-3} gram
WtE	Waste to Energy (waste incinerator)

Abbreviations	Dioxins, furans (PCDD/F) and dioxin-like PCBs	Toxic equivalency factor
	Congeners	
Dioxins (n=7)		
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1
PCDD	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	1
HxCDD1	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.1
HxCDD2	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.1
HxCDD3	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.1
HpCDD	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.01
OCDD	Octachlorodibenzo-p-dioxin	0.0003
Furans (n=10)		
TCDF	2,3,7,8-Tetrachlorodibenzofuran	0.1
PCDF1	1,2,3,7,8-Pentachlorodibenzofuran	0.03
PCDF2	2,3,4,7,8-Pentachlorodibenzofuran	0.3
HxCDF1	1,2,3,4,7,8-Hexachlorodibenzofuran	0.1
HxCDF2	1,2,3,6,7,8-Hexachlorodibenzofuran	0.1
HxCDF3	1,2,3,7,8,9-Hexachlorodibenzofuran	0.1
HxCDF4	2,3,4,6,7,8-Hexachlorodibenzofuran	0.1
HPCDF1	1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.01
HPCDF2	1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.01
OCDF	Octachlorodibenzofuran	0.0003
Polychlorinated biphenyl (n=12)		
PCB77	3,3',4,4'-Tetrachlorobiphenyl (#77)	0.0001
PCB81	3,4,4',5-Tetrachlorobiphenyl (#81)	0.0003
PCB126	3,3',4,4',5-Pentachlorobiphenyl (#126)	0.1
PCB169	3,3',4,4',5,5'-Hexachlorobiphenyl (#169)	0.03
PCB105	2,3,3',4,4'-Pentachlorobiphenyl (#105)	0.00003
PCB114	2,3,4,4',5-Pentachlorobiphenyl (#114)	0.00003
PCB118	2,3',4,4',5-Pentachlorobiphenyl (#118)	0.00003
PCB123	2,3,4,4',5-Pentachlorobiphenyl (#123)	0.00003
PCB156	2,3,3',4,4',5-Hexachlorobiphenyl (#156)	0.00003
PCB157	2,3,3',4,4',5'-Hexachlorobiphenyl (#157)	0.00003
PCB167	2,3',4,4',5,5'-Hexachlorobiphenyl (#167)	0.00003
PCB189	2,3,3',4,4',5,5'-Heptachlorobiphenyl (#189)	0.00003

Background information can be found in the 2021 TW Biomonitoring reports at
www.toxicowatch.org
www.zerowasteeurope.eu/library/the-true-toxic-toll-biomonitoring-of-incineration-emissions/


Dioxins, PFAs and PAH emissions in relation to waste incineration
Eggs
Backyardchicken
Vegetation
Pineneedles
Mosses


Dioxins, PFAS, and PAH emissions in relation to waste incineration
Eggs
Backyardchicken
Vegetation
Pineneedles
leaves
Mosses
Valdemingómez
waste incinerator


Dioxins, PFAS and PAH emissions in relation to waste incineration
Eggs
Backyardchicken
Vegetation
Pineneedles
Mosses
ZEVO Chotíkov incinerator
incineration

Executive summary on biomonitoring, 2022
Kaunas (Lithuania), Madrid (Spain), Pilsen (Czech Rep.)

ToxicoWatch biomonitoring research on eggs of backyard chickens, and vegetation such as pine needles and mosses as biomarkers is a European project, coordinated by Zero Waste Europe. The project was run simultaneously in Lithuania, Spain, and the Czech Republic in 2021 and 2022. ToxicoWatch (TW) Foundation, based in the Netherlands, participated as a scientific partner together with three environmental organisations: for Spain, *Ecologistas en Acción* in Madrid, for Lithuania, *Žiedinė Ekonomika,* and for the Czech Republic, *Hnutí DUHA*.

The biomarkers were analysed for persistent organic pollutants (POPs), like dioxins (PCDD/F/dl-PCB), Per- and PolyFluoroAlkyl Substances (PFAS), and Polycyclic Aromatic Hydrocarbons (PAH).[1] The focus of this biomonitoring research is to analyse the deposition of POPs nearby three (3) WtE incinerators*: UAB Kauno Cogeneration Power Plant* (Kaunas, Lithuania), *Valdemingómez waste-to-energy (WtE) incinerator* (Madrid, Spain) and *ZEVO Chotíkov* (Pilsen, Czech Rep.).

The contamination does not only concern the eggs of backyard chickens. Increased amounts of hazardous persistent organic pollutants were also found in vegetation in the vicinity of the waste incinerators. To summarise the results for 2022, the second year of biomonitoring, in Kaunas, Pilsen and Madrid:

1) Most eggs of backyard chickens in the vicinity of the three (3) incinerators exceeded EU limits for the bioassay DR CALUX and the chemical GC-MS analysis as regulated in the EU Regulation 2017/644.
2) Analysis of the vegetation, pine needles and mosses, shows dioxins in elevated concentrations in the areas around the waste incinerators in all three (3) countries by bioassay DR CALUX.
3) High quantities of PFAS are found in mosses, pine needles and eggs of backyard chickens in all three (3) areas around the waste incinerators by bioassay FITC-T4 measurements.
4) High levels of PAH are found in mosses, and pine needles around all three (3) waste incinerators by bioassay PAH CALUX.

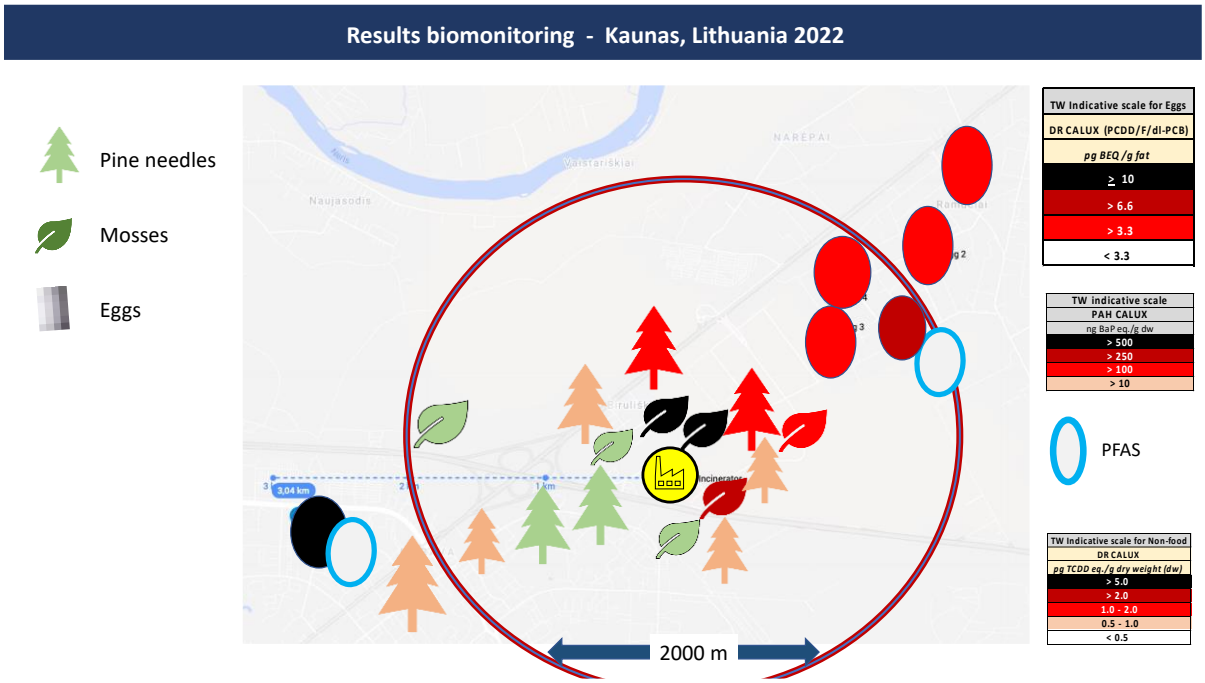
An additional consideration is the limitations of chemical analysis (GC-MS) for POPs, which can however determine individual congeners but does not, however, measure the total toxicity (of thousands of toxic substances) of dioxins and dioxin-like substances (PCDD/F/dl-PCB), PAH and PFAS. The DR CALUX bioassay does measure the total toxicity of dioxins and dioxins-like substances, while the chemical analysis (GC-MS) is limited to only 17 congeners with the exclusion of i.e., brominated dioxins (PBDD/F). The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four (4) regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should also be included as standard in the (EU) regulated monitoring of POPs from emission sources, such as waste incinerators.

The most polluted area in this biomonitoring research was found to be near Valdemingómez in Madrid, which has one of the oldest waste incinerators in Europe. The Stockholm Convention on Persistent Organic Pollutants aims to protect human health and the environment through measures which will reduce and/or eliminate emissions and discharges of persistent organic pollutants.

[1] The term POP is used to refer to toxic chemicals that are resistant to degradation processes, travel over long distances, and bioaccumulate in the human body and ecosystems.

Biomonitoring results for Kaunas, Lithuania, 2022

The infographic shows dioxin (PCDD/F/dl-PCB), PFAS and PAH contamination in the area around the *UAB Kauno Cogeneration Power Plant* waste incinerator (WtE).



Eggs

All backyard chicken eggs exceeded the EU limit in the bioassay for dioxins (PCDD/F) with dioxin levels increasing considerably in locations near the incinerator. One location was found to have an exceptionally high level of dl-PCB. Egg biomarkers in the northeast and west show elevated levels of dioxins and dioxin-like PCB. PFAS were found in eggs in the bioassay and the limited chemical analysis identified PFOS and GenX.

Mosses

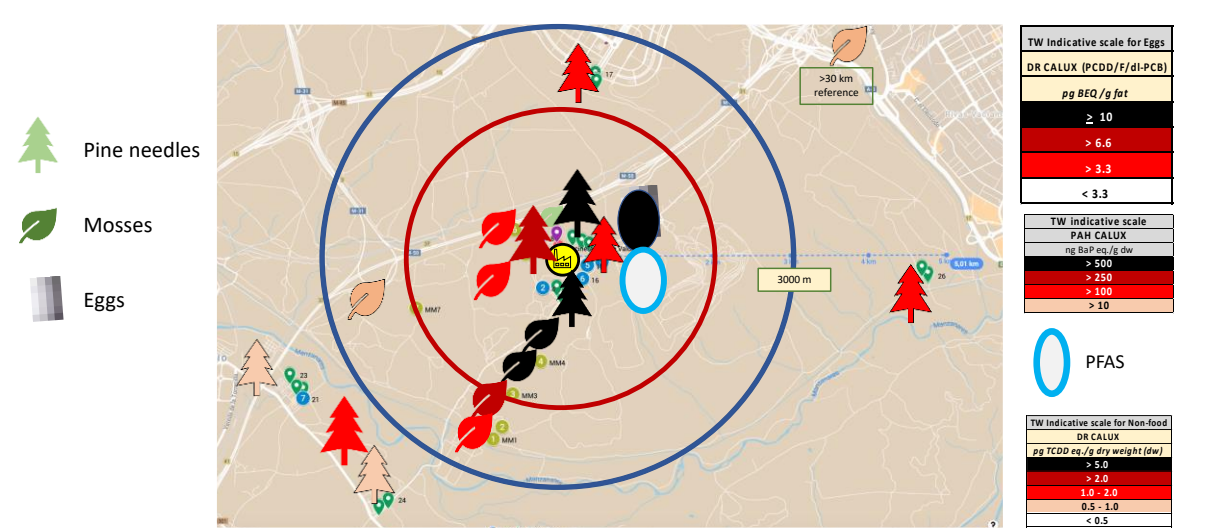
High levels of dioxins and furans (PCDD/F) were measured in mosses in the area directly around the incinerator. The difference between the results of the bioassay and the chemical analysis indicates the contribution of substances not covered by the limited GC-MS analyses, such as brominated dioxins or other mixed halogenated substances. Dioxins in mosses and pine needles were elevated in the area directly around the incinerator, in the northwest and west. In 2022, PAH were 20 times higher in mosses northwest of the incinerator

Pine needles

The DR CALUX bioassay analyses predominantly demonstrated an increase in dioxins (PCDD/F) in the vicinity of the waste incinerator. PAH were also detected in pine needles located in the east.

Biomonitoring results for Madrid, Spain, 2022

The infographic shows dioxins (PCDD/F/dl-PCB), PFAS and PAH contamination in the area around the Valdemingómez waste incinerator.



Eggs

In the second year of this biomonitoring research, levels of dioxins (PCDD/F and DL-PCB) were elevated, especially for dioxin-like PCB (DL-PCB) activity. PFAS exceeded the EFSA safety limit. The bioassay for PFAS (FITC-T4) found 500x PFAS activity in these eggs.

Mosses

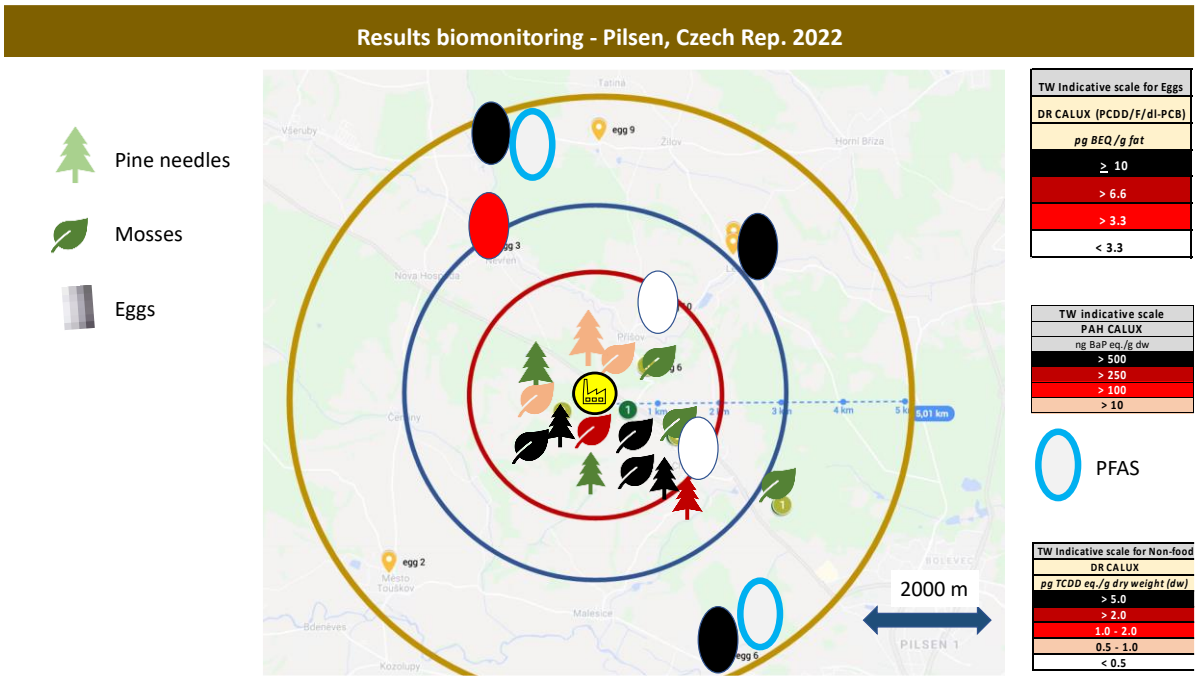
The pattern of elevated dioxins (PCDD/F and DL-PCB) in mosses, most elevated 1500-2000 metres southwest, was found again in this research. The highest values for dioxins ever measured in a TW European biomonitoring survey were measured in the mosses around Valdemingómez.

Pine needles

Pine needles and foliage from evergreen trees surrounding the fence around the waste incinerator had the highest levels of dioxins (PCDD/F and DL-PCB) ever measured in TW biomonitoring research, mostly due to the dioxins and furans (PCDD/F), a typical combustion-related emission value. A slight decrease was observed near the incinerator, as well as a considerable increase in dioxins (PCDD/F) in pine needles at a distance of 3000-5000 m. The dioxins and dioxin-like PCB (PCDD/F and DL-PCB) again showed the highest level found in pine needles in thisTW biomonitoring research, as was also the case in 2021, the first year of this biomonitoring study.

Biomonitoring results for Pilsen, Czech Rep., 2022

The infographic shows dioxin (PCDD/F and DL-PCB), PFAS and PAH contamination in the area around the ZEVO Chotíkov waste incinerator.



Eggs

Analyses on locations within 3000-5000 m show high levels of dioxins (PCDD/F), more than three times the EU limit values for bioassays and chemical analyses. Dioxin congener patterns are related to incomplete combustion. There was a significant increase in dioxin-like PCB (DL-PCB) in the eggs, although these substances have been banned for almost 40 years. With FITC-T4, high concentrations of PFAS were found and PFOS and GenX were identified in the chemical analysis.

Mosses

Dioxins (PCDD/F and DL-PCB) were found in elevated concentrations northwest of the waste incinerator. Elevated levels of PFAS and PAH were found in mosses 2000 m southwest, near the city of Pilsen.

Pine needles

Elevated levels of dioxins (PCDD/F and DL-PCB) were found in pine needles northeast of the incinerator. PFAS were found near the incinerator and in pine needles 2000 m east, near the city of Pilsen.

Analysis methods

DR CALUX bioassay®

The DR CALUX bioassay® **(Dioxin Responsive Chemical Activated Luciferase gene eXpression)** is a bioanalytical screening method[2] used for quantification of dioxins/furans (PCDD/F) and dioxin-like PCBs (DL-PCBs). The results in this research, using DR CALUX® for analyses on dioxins (PCDD/F and DL-PCBs), on eggs are given in **Bioassay Equivalent, BEQ (pg BEQ/g fat)**. The term "**BEQ**" is used for food elements to distinguish between the **TEQ** (Toxic Equivalence) derived from chemical analyses (Gas Chromatography-Mass Spectrometry GC-MS, pg TEQ/g fat). For non-food biomatrices like mosses or pine needles, the results with DR CALUX are given in **TCDD eq./g product** or abbreviated as **pg TEQ/g product**. TCDD stands for 2,3,7,8-Tetrachlorodibenzo-p-dioxin, the most toxic dioxin congener.

The relevant EU legislation is:
- Commission Regulation (EU) **2017/644 of 5 April 2017**[3], which lays down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs.
- Commission Regulation (EU) **1881/2006**[4], which sets maximum levels for certain contaminants e.g. dioxins (PCDD/F and DL-PCB) in foodstuffs.
- Commission Recommendation (EU) **2013/711/EU**[5] on the reduction of the presence of dioxins, furans and PCBs in feed and food sets out action levels[6] for dioxins, furans and dioxin-like PCBs. If the bioassay analysis exceeds 1.75 pg BEQ/g fat for PCDD/F and/or 3.3 pg BEQ/g fat for the sum of PCDD/F and DL-PCB, a confirmatory GC-MS analysis of the hen egg sample is recommended for establishing the results.

Chemical analyses

The maximum permitted levels of contaminants found in hen eggs through GC-MS chemical analyses are given in pg TEQ/g. The analysis was done on 7 dioxins (PCDDs), 10 furans (PCDFs) and 12 dioxin-like polychlorinated biphenyls (DL-PCBs). The concentration results of the chemical analyses of dioxins (PCDD/F and DL-PCBs) were calculated with a specific Toxic Equivalency Factor (TEF) to give a TEQ value (see page 4 Abbreviations and TEF for dioxins and DL-PCBs). The **maximum limit value** for dioxins in eggs is 2.5 pg TEQ/g fat for PCDD/F and the limit is set at 5 pg TEQ/gram fat for the sum of dioxins and dioxin-like PCBs (PCDD/F and DL-PCBs). When these GC-MS limit values are exceeded, hen eggs are not allowed to be placed on the commercial market (see Figures 5 and 6).

2013/711/EU[7] includes the **GC-MS** analysis **action levels** both for dioxins (PCDD/F) and dioxin-like PCBs (DL-PCBs) in hen eggs, which are set at 1.75 pg TEQ/g fat, see Figure 5. These action levels are a tool for competent authorities and operators to highlight cases where it is appropriate to identify the source of contamination and to take measures for its reduction or elimination.

PFAS chemical analyses were performed on 24 PFAS by Normed, Rotterdam NL, using LC-LC-MS.

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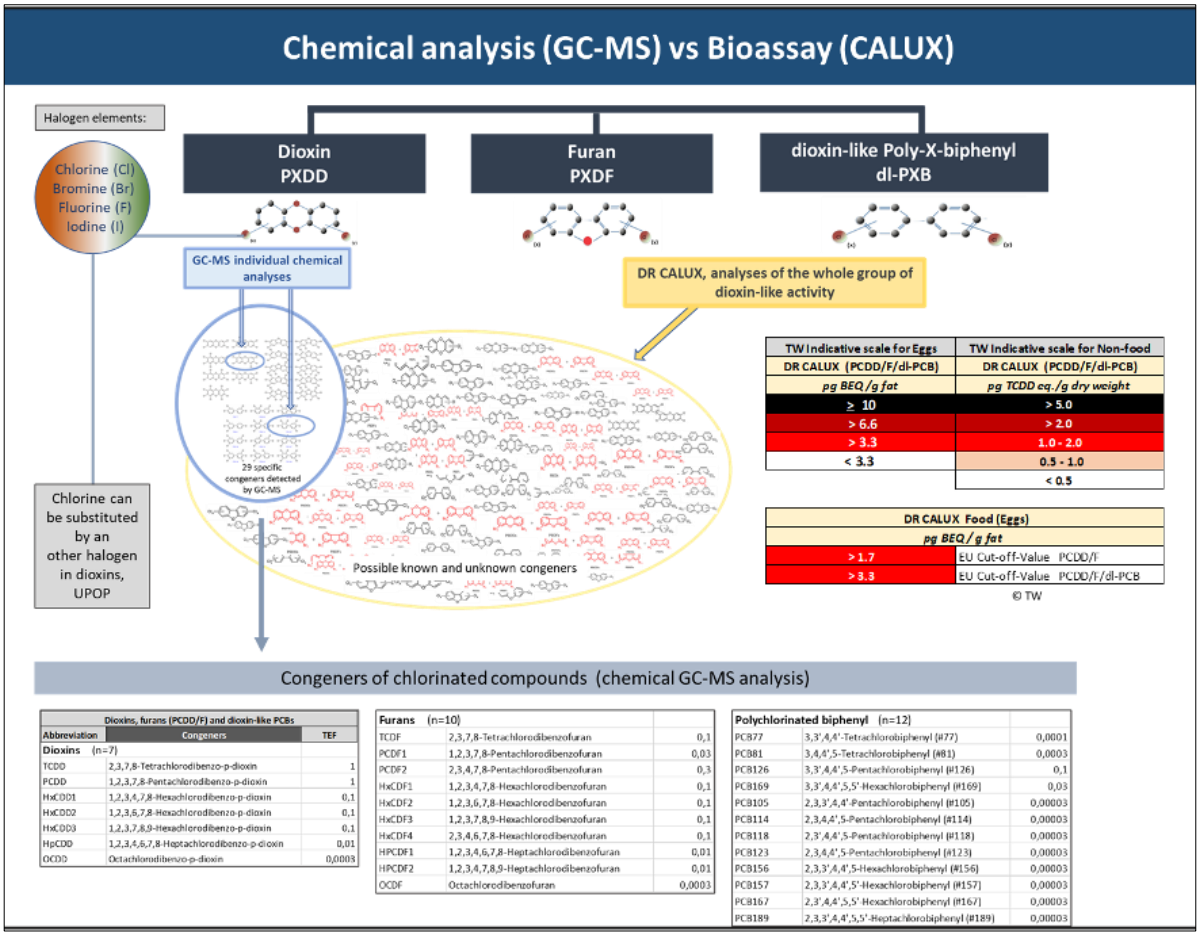
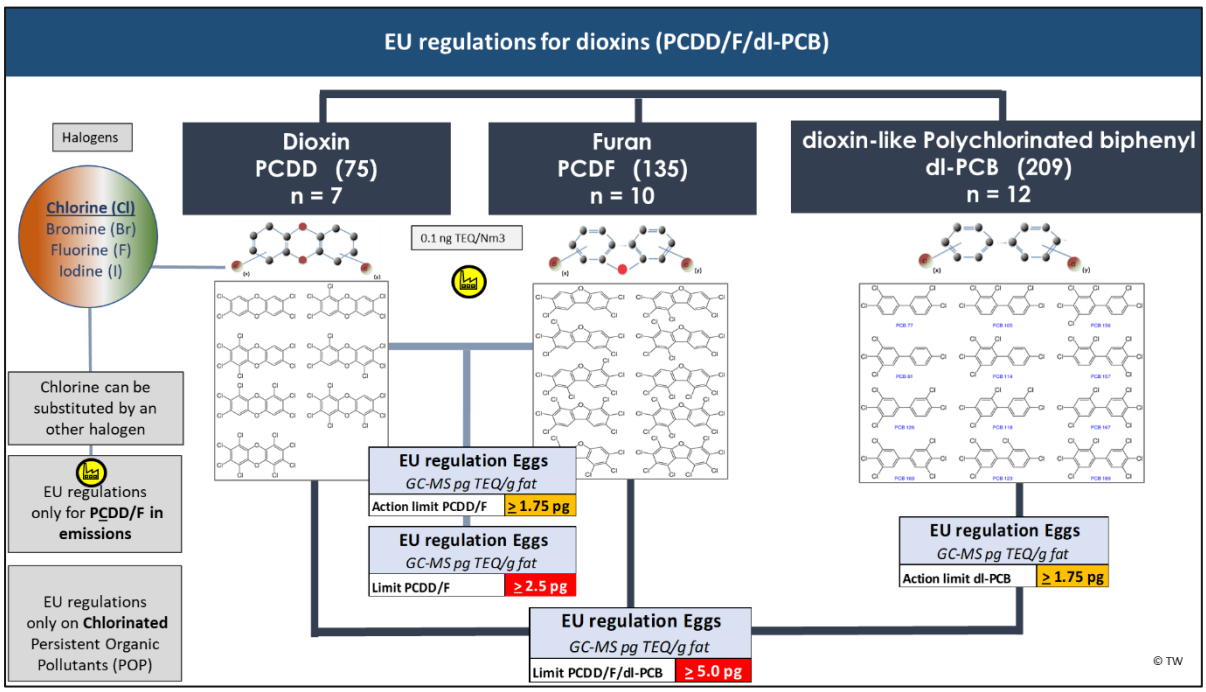
[2] 'Bioanalytical methods' are methods based on the use of biological principles such as cell-based assays, receptor assays and immunoassays.
[3] https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32017R0644
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20210919&from=EN
[5] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN
[6] 'Action level' means the level of a given substance, as laid down in the Annex to Recommendation 2013/711/EU, which triggers investigations to identify the source of that substance in cases where increased levels of the substance are detected
[7] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013H0711&from=EN

EU regulation of dioxins on eggs

The figures below are an explanation of the bioassay (DR CALUX)) and the chemical GC-MS analyses on dioxins (PCDD/F/dl-PCB) related to the EU regulation of dioxins in eggs.





PAH CALUX®

High molecular weight PAHs are known ligands of the aryl hydrocarbon (AhR) receptor, a nuclear receptor that mediates toxic effects related to these compounds. The PAH CALUX assay uses a mammalian, H4IIE- cell-based reporter assay for the hazard identification of total PAH mixtures. The PAH CALUX reporter cell line allows for specific, rapid (4-hour exposure time) and reliable quantification of AhR-induced luciferase induction relative to benzo[a]pyrene (BaP). BaP is a compound with five benzene rings and a class 1B carcinogen and is used here as a toxicity indicator of PAH exposure[8,9].

FITC-T4 bioassay

In the FITC-T4 binding bioassay, sample extracts, suspected to be contaminated with PFAS, were tested for the potency of binding with the thyroid hormone thyroxine (T4) and the plasma transport protein Transthyretin (TTR). The fluorescent-labelled thyroxine (FITC-T4), consisting of fluorescein isothiocyanate (FITC) and L-thyroxine (T4), was used in this assay (Smith, 1977, Hamers 2020)[10,11].Thyroid hormone homeostasis can be disrupted by environmental chemicals at different points of interaction in the thyroid pathway, including during transport of the hormone through the blood. Some chemicals are known to bind to the transport protein TTR, thereby replacing the endogenous ligand T4. PFAS are such chemicals, known for their capability to bind TTR and thus replacing T4. The measurement is based on the difference in fluorescence between bound and non-bound FITC-T4 and the TTR-binding site. Bound FITC-T4 will result in higher fluorescence than non-bound. The FITC-T4 analysis results are given in µg PFOA equivalent/g product.

The DR CALUX®, PFAS CALUX®, FITC-T4 and GC-MS analyses were performed by BioDetection Systems, Amsterdam, the Netherlands. BDS is accredited under RvA L401.

[8] Category 1B carcinogen according to Annex VI to the CLP Regulation (EC) No 1272/2008 of the European Parliament and is classified as a Substance of Very High Concern by the POP Regulation EC No 850/2004.
[9] Pieterse, B., et al. (2013). PAH-CALUX, an optimized bioassay for AhR-mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs). Environ Sci Technol. 2013 Oct 15;47(20):11651-9.
10 Smith, D.S., (1977). FEBS Lett. 77, 25-27.
[11] Hamers T. (2020). Transthyretin-Binding Activity of Complex Mixtures Representing the Composition of Thyroid-Hormone Disrupting Contaminants in House Dust and Human Serum, Environmental Health Perspectives 017015-1 128(1)

Kaunas, Lithuania, 2022

Biomonitoring results for Kaunas, Lithuania, 2022

All locations were visited for sampling in person by D. Tracevičius, *Žiedinė Ekonomika*. The sampling of eggs, vegetation and mosses took place on 12th and 13th June 2022 and the third round took place on 3rd October 2022.

Analysis of eggs of backyard chickens

Two (2) methods were used for the analysis of the eggs. First, the eggs were analysed using the DR CALUX bioassay. This method detects the total toxicity of dioxins, not only regulated chlorinated dioxins (PCDD/F and DL-PCB), but also brominated (PBDD/F) and other (mixed) halogenated dioxins, see figures below. Then, chemical analyses were performed using GC-MS, which is also mandated when the results of DR CALUX analyses exceed the cut-off/maximum limit.

Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	3.8	2.5	-35%
	Egg-02	3.2	1.8	-45%
	Egg-03	1.5	1.6	5%
	Egg-04	3.0	1.9	-36%
	Egg-05	4.3	12.1	181%
	Egg-06	20.0	4.9	-76%
Cut-off	GC-MS limit	5.0		

Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	5.9	4.8	-19%
	Egg-02	6.7	3.3	-51%
	Egg-03	2.1	3.8	81%
	Egg-04	3.0	3.2	7%
	Egg-05	7.0	15.0	114%
	Egg-06	9.3	8.6	-8%
Cut-off	DR CALUX	3.30		

Kaunas, Lithuania 2021/2022				
PCDD/F - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	2.4	1.6	-35%
	Egg-02	2.3	1.2	-49%
	Egg-03	0.8	0.9	18%
	Egg-04	1.9	1.2	-39%
	Egg-05	2.2	1.5	-30%
	Egg-06	1.7	1.4	-15%
Cut-off	GC-MS limit	2.5		
Cut-off	GC-MS action	1.75		

Kaunas, Lithuania 2021/2022				
PCDD/F - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	4.5	3.3	-27%
	Egg-02	5.0	2.6	-48%
	Egg-03	1.2	1.7	42%
	Egg-04	2.1	2.3	10%
	Egg-05	5.0	3.0	-40%
	Egg-06	2.8	3.8	36%
Cut-off	DR CALUX	1.70		

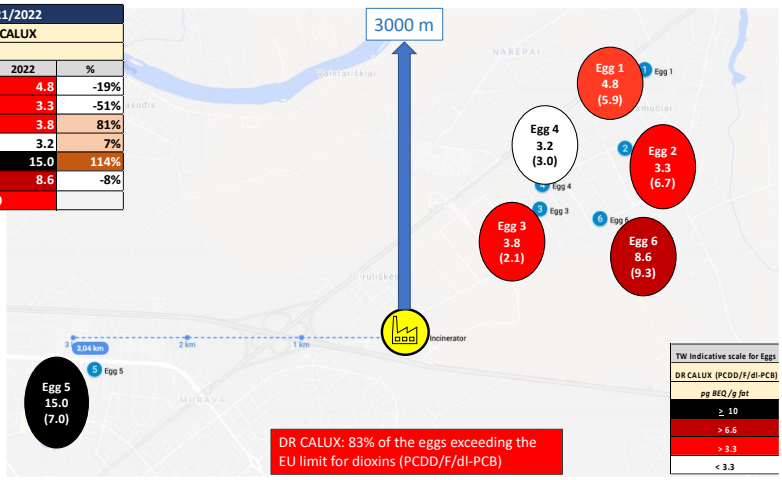
Kaunas, Lithuania 2021/2022				
dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	0.9	-35%
	Egg-02	0.9	0.6	-38%
	Egg-03	0.8	0.7	-13%
	Egg-04	1.1	0.7	-32%
	Egg-05	2.1	10.5	401%
	Egg-06	18.0	3.4	-81%
Cut-off	GC-MS action	1.75		

Kaunas, Lithuania 2021/2022				
dl-PCB - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	1.5	7%
	Egg-02	1.7	0.7	-59%
	Egg-03	0.9	2.1	133%
	Egg-04	0.9	0.9	0%
	Egg-05	2.0	12.0	500%
	Egg-06	6.5	4.8	-26%
Cut-off	DR CALUX			

Dioxin results in backyard chicken eggs using the DR CALUX bioassay

Dioxins (PCDD/F/dl-PCB) in eggs - Kaunas, 2022 (2021) DR CALUX

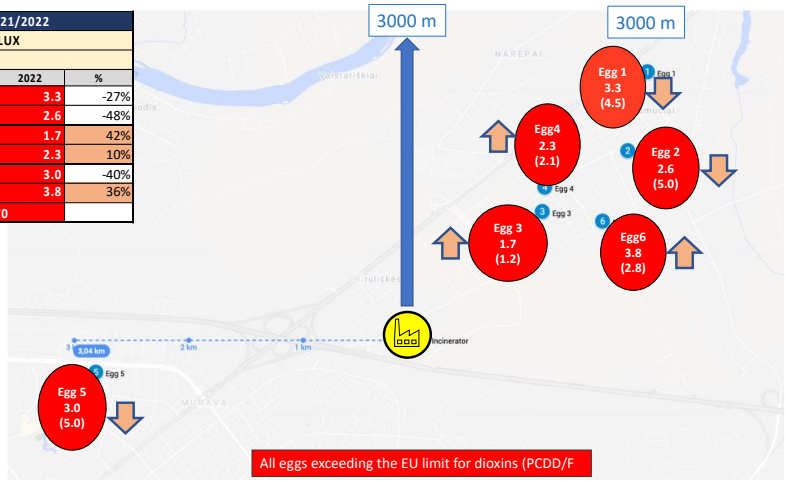
Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	5.9	4.8	-19%
	Egg-02	6.7	3.3	-51%
	Egg-03	2.1	3.8	81%
	Egg-04	3.0	3.2	7%
	Egg-05	7.0	15.0	114%
	Egg-06	9.3	8.6	-8%
Cut-off	DR CALUX	3.30		



DR CALUX: 83% of the eggs exceeded the EU limit for dioxins (PCDD/F/dl-PCB).

Results DR CALUX dioxins (PCDD/F) in eggs - Kaunas, 2022

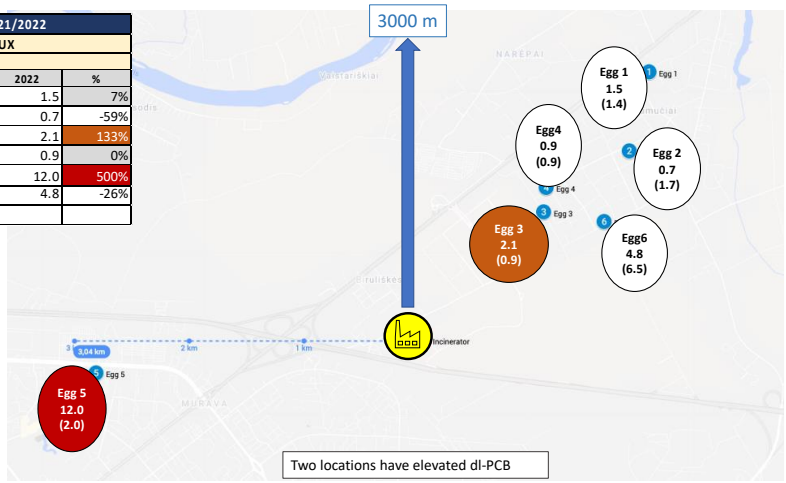
Kaunas, Lithuania 2021/2022				
PCDD/F - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	4.5	3.3	-27%
	Egg-02	5.0	2.6	-48%
	Egg-03	1.2	1.7	42%
	Egg-04	2.1	2.3	10%
	Egg-05	5.0	3.0	-40%
	Egg-06	2.8	3.8	36%
Cut-off	DR CALUX	1.70		



All eggs exceeded the EU limit for dioxins (PCDD/F) when the DR CALUX bioassay was used, with notable increases in dioxin (PCDD/F) levels in locations near the incinerator.

Results DR CALUX dioxin-like PCB (dl-PCB) in eggs - Kaunas, 2022

Kaunas, Lithuania 2021/2022				
dl-PCB - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	1.5	7%
	Egg-02	1.7	0.7	-59%
	Egg-03	0.9	2.1	133%
	Egg-04	0.9	0.9	0%
	Egg-05	2.0	12.0	500%
	Egg-06	6.5	4.8	-26%
Cut-off	DR CALUX			

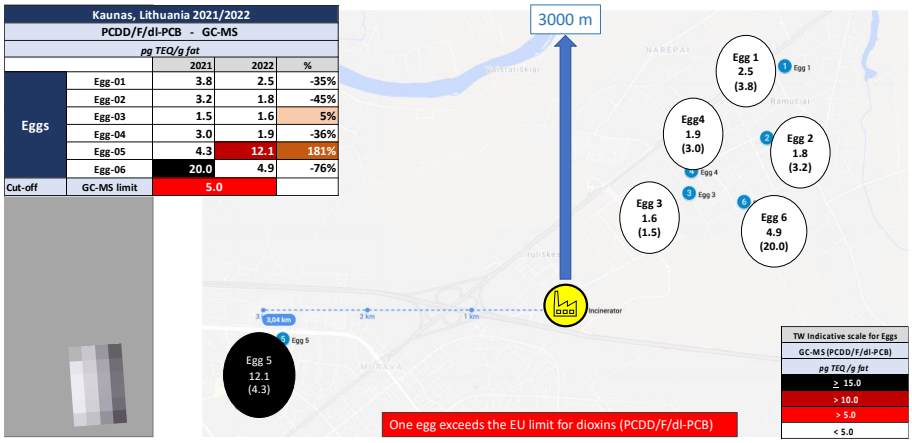


Two locations showed elevated DL-PCB levels of 133% and 500%.

GC-MS results for dioxins in eggs of backyard chicken

Results GC-MS dioxins (PCDD/F/dl-PCB) in eggs - Kaunas, 2022 (2021)

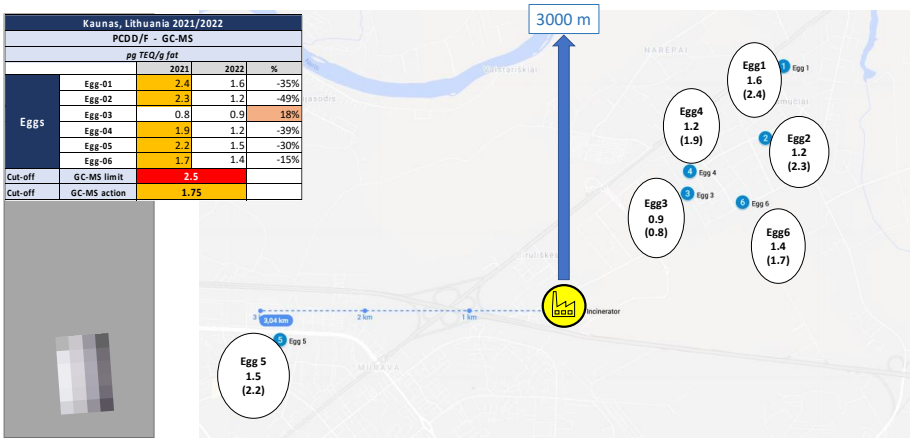
Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	3.8	2.5	-35%
	Egg-02	3.2	1.8	-45%
	Egg-03	1.5	1.6	5%
	Egg-04	3.0	1.9	-36%
	Egg-05	4.3	12.1	181%
	Egg-06	20.0	4.9	-76%
Cut-off	GC-MS limit	5.0		



Four (4) locations showed a slight decrease in dioxins. One (1) location exceeded the GC-MS safety limits for eggs by a factor of nearly 2, at 5 pg TEQ/g fat

Results GC-MS dioxins (PCDD/F) in eggs - Kaunas, 2022 (2021)

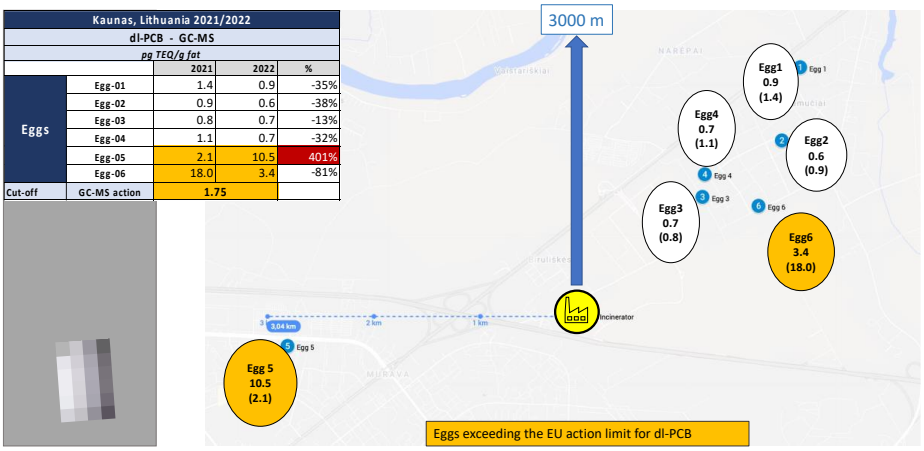
Kaunas, Lithuania 2021/2022				
PCDD/F - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	2.4	1.6	-35%
	Egg-02	2.3	1.2	-49%
	Egg-03	0.8	0.9	18%
	Egg-04	1.9	1.2	-39%
	Egg-05	2.2	1.5	-30%
	Egg-06	1.7	1.4	-15%
Cut-off	GC-MS limit	2.5		
Cut-off	GC-MS action	1.75		



Five (5) locations showed slight decreases in PCDD/F levels compared to 2021. The start-up of the incinerator could have been a reason for the elevated PCDD/F levels. The difference from the DR CALUX bioassay can be explained by the presence of brominated dioxins, not included in regular measurements and not included in this research.

Results GC-MS dioxin-like PCB (dl-PCB) in eggs - Kaunas, 2022 (2021)

Kaunas, Lithuania 2021/2022				
dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	0.9	-35%
	Egg-02	0.9	0.6	-38%
	Egg-03	0.8	0.7	-13%
	Egg-04	1.1	0.7	-32%
	Egg-05	2.1	10.5	401%
	Egg-06	18.0	3.4	-81%
Cut-off	GC-MS action	1.75		

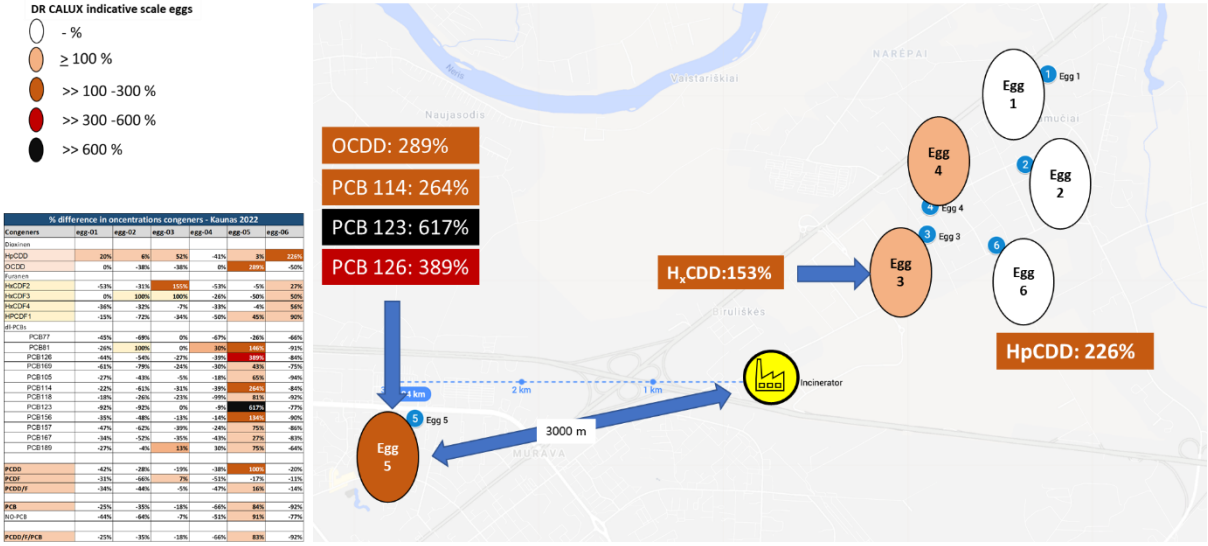


Two locations exceeded the DL-PCB action limit of 1.75 pg TEQ/g fat. There was a significant decrease in DL-PCB levels in location 5 and an increase in location 6.

Results - eggs congener patterns in eggs of backyard chicken

The results of the analysis done in 2021 indicate that the Kaunas incinerator emitted more dioxins during the testing phase. By focusing on the specific congeners of dioxins in eggs using GC-MS analyses, typical increases in congeners due to waste incineration can be seen, such as OCDD, PCB 126, HxCDD and HpCDD. More detailed data from the emission patterns of the incinerator could provide more conclusive evidence of this.

GC-MS congeners : Results dioxins in eggs - Kaunas, 2022

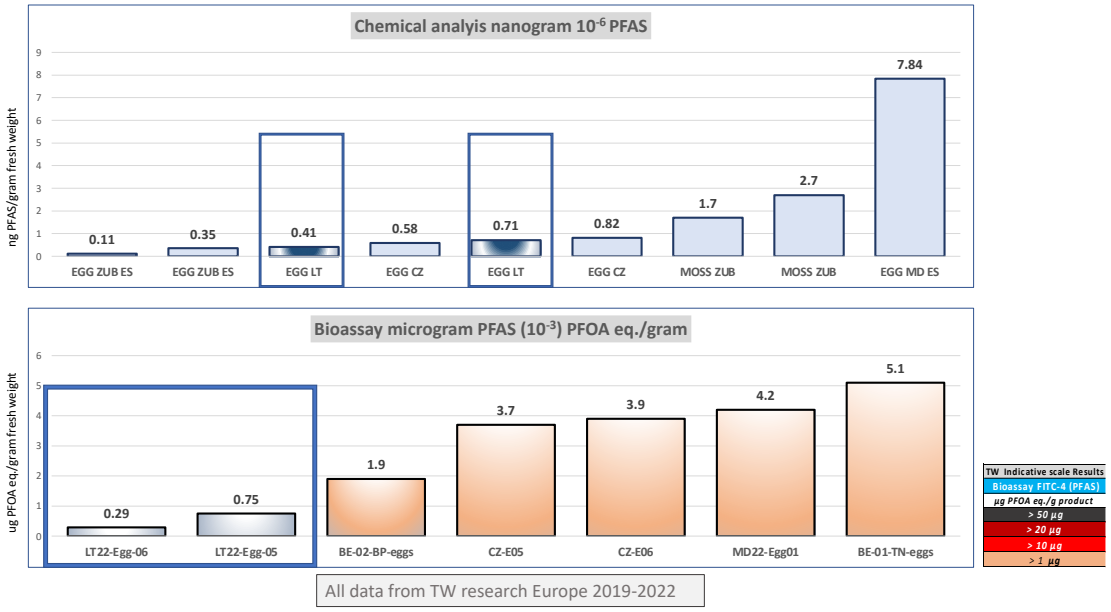


Significantly, egg location 5 shows high values of DL-PCBs. The figure below shows the specific congeners found. Most significant are the increased levels of PCB 123 and PCB 126.

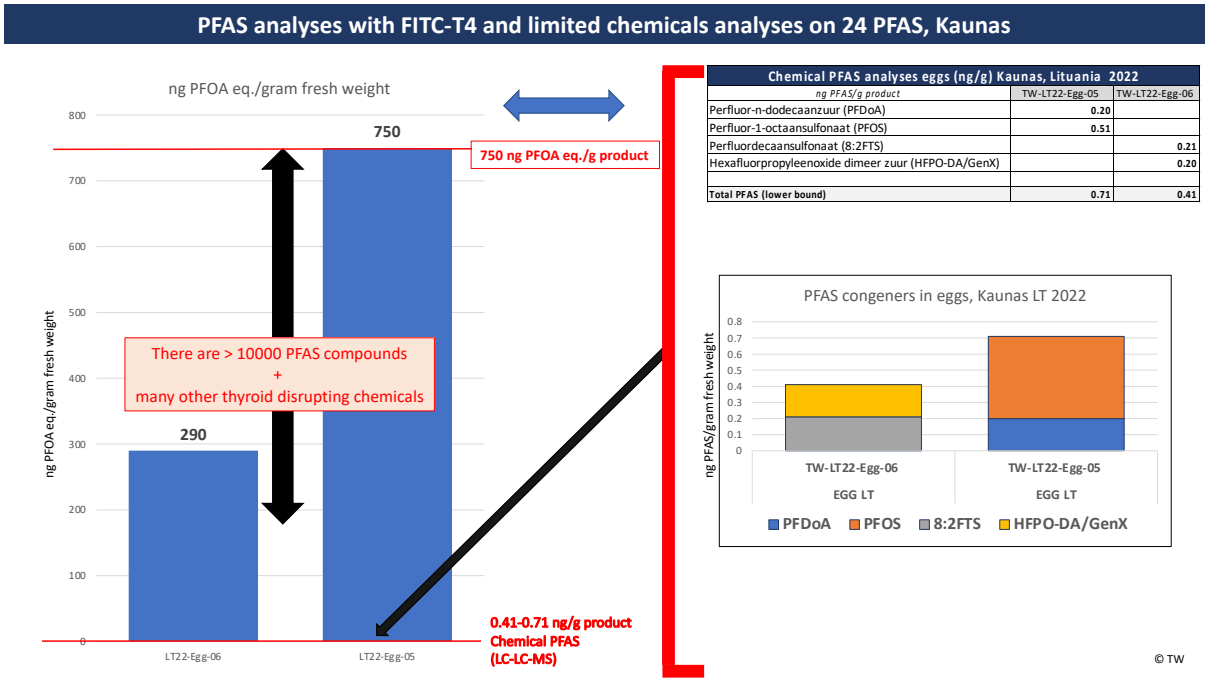
% difference in oncentrations congeners - Kaunas 2022						
Congeners	**egg-01**	**egg-02**	**egg-03**	**egg-04**	**egg-05**	**egg-06**
Dioxinen						
HpCDD	20%	6%	52%	-41%	3%	226%
OCDD	0%	-38%	-38%	0%	289%	-50%
Furanen						
HxCDF2	-53%	-31%	155%	-53%	-5%	27%
HxCDF3	0%	100%	100%	-26%	-50%	50%
HxCDF4	-36%	-32%	-7%	-33%	-4%	56%
HPCDF1	-15%	-72%	-34%	-50%	45%	90%
dl-PCBs						
PCB77	-45%	-69%	0%	-67%	-26%	-66%
PCB81	-26%	100%	0%	30%	146%	-91%
PCB126	-44%	-54%	-27%	-39%	389%	-84%
PCB169	-61%	-79%	-24%	-30%	43%	-75%
PCB105	-27%	-43%	-5%	-18%	65%	-94%
PCB114	-22%	-61%	-31%	-39%	264%	-84%
PCB118	-18%	-26%	-23%	-99%	81%	-92%
PCB123	-92%	-92%	0%	-9%	617%	-77%
PCB156	-35%	-48%	-13%	-14%	134%	-90%
PCB157	-47%	-62%	-39%	-24%	75%	-86%
PCB167	-34%	-52%	-35%	-43%	27%	-83%
PCB189	-27%	-4%	13%	30%	75%	-64%
PCDD	-42%	-28%	-19%	-38%	100%	-20%
PCDF	-31%	-66%	7%	-51%	-17%	-11%
PCDD/F	-34%	-44%	-5%	-47%	16%	-14%
PCB	-25%	-35%	-18%	-66%	84%	-92%
NO-PCB	-44%	-64%	-7%	-51%	91%	-77%
PCDD/F/PCB	-25%	-35%	-18%	-66%	83%	-92%

Results - PFAS in eggs of backyard chicken

Chemical analysis (LC-LC-MS) of the eggs showed PFAS. PFOS and PFDoA were found in the location west of the incinerator, while GenX and 8:2 FTS were found in the egg location in the southwest. Chemical analysis (LC-LC-MS) of two eggs showed different PFAS congeners. PFOS and PFDoA were found in the location west of the incinerator, while GenX and 8:2 FTS were found in the egg location in the southwest.



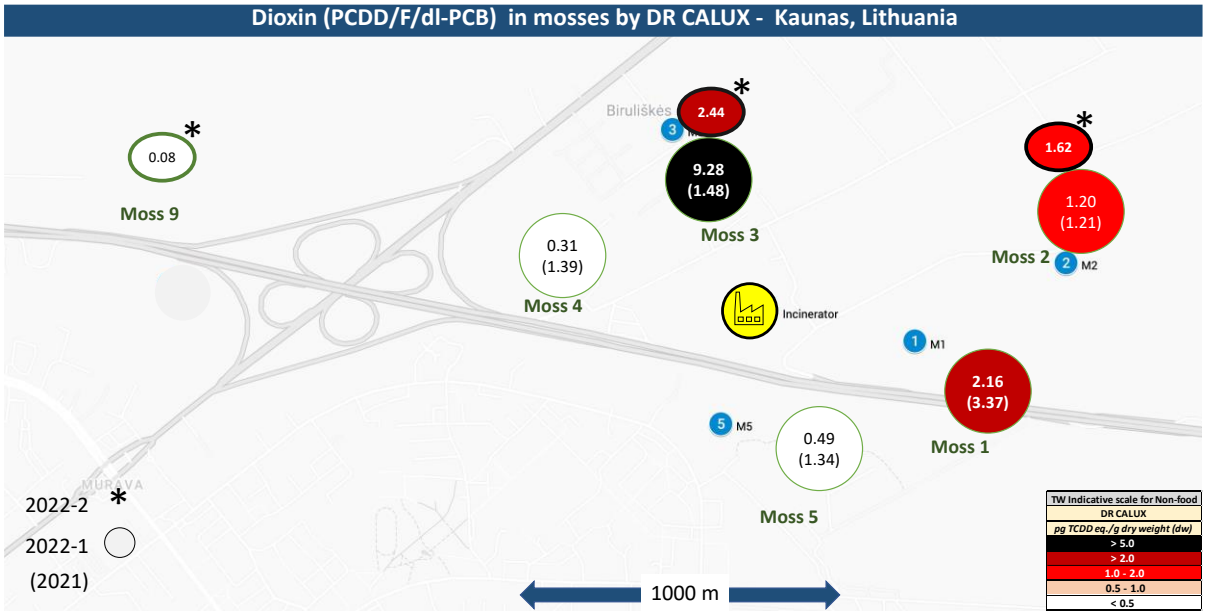
The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should also be included as standard in the (EU) regulated monitoring of POPs from emission sources, such as waste incinerators.



Dioxins results in mosses using the DR CALUX bioassay

The mosses in the six (6) locations was mainly *Hylocomium splendens*, sampled around the incinerator, see the figure below. Mosses was sampled in open fields, avoiding proximity to roads and away from dense tree canopies to avoid fallen leaves and uptake emissions by air. The mosses was sampled in July and October 2022.

The results for the mosses in July and October 2022 were between **0.08 - 9.28 pg TCDD eq./g dw**. The results of sampling in October 2022 are shown with an asterisk. In 2021, location 1 had the highest level of dioxins in mosses. In 2022, an extremely high dioxin level of **9.28 pg TCDD eq./g dw** was measured at location 3, west of the incinerator. The results for 2021 are shown in brackets. Although lower in level, the results of the October sampling confirmed heavily contaminated mosses north of the incinerator, with a dioxin level of **2.44 pg TCDD eq./g dw**.



The measurements in 2021 and July and October 2022 are shown in the table below. There was a significant increase in DL-PCB levels in the samples taken in October 2022.

Mosses Kaunas, 2021-2022									
	2021			Jul-22			Oct-22		
	DR CALUX *pg TCDD eq./g dw*								
	PCDD/F	**dl-PCB**	**PCDD/F/dl-PCB**	**PCDD/F**	**dl-PCB**	**PCDD/F/dl-PCB**	**PCDD/F**	**dl-PCB**	**PCDD/F/dl-PCB**
LT-Mos-01	2.80	0.57	**3.37**	2.12	0.04	2.16			
LT-Mos-02	1.10	0.11	**1.21**	1.17	0.04	1.20	0.83	0.79	1.62
LT-Mos-03	1.30	0.18	**1.48**	9.24	0.04	9.28	1.90	0.54	2.44
LT-Mos-04	1.30	0.09	**1.39**	0.27	0.04	0.31			
LT-Mos-05	1.10	0.24	**1.34**	0.41	0.08	0.49			
LT-Mos-09							0.06	0.01	0.08

Bioassay vs. chemical analyses of mosses

Chemical analyses were performed due to the high levels of dioxins found by the bioassay, 9.28 pg TCDD eq./g dw. The high TEQ values of dioxins using the DR CALUX bioassay could not be found in the GC-MS results. This is probably because of the contribution of other halogenated dioxins, which are not included in the regulated dioxin analyses. The result of 9.28 pg TCDD eq./g dw is unusually high and can only be found at very contaminated sites. The difference in chemical analyses shows 433% more $HpCDF_2$ (1,2,3,4,6,7,8-Heptachlorodibenzofuran) at location M3 northwest of the incinerator. In addition, significantly, almost twice as many PCB were found at mosses location 3, 355.29 versus 191.94 pg DL-PCB.

Bioassay vs. chemical analysis in mosses - Kaunas, Lithuania 2022

Results dioxins in mosses (TEQ) Kaunas

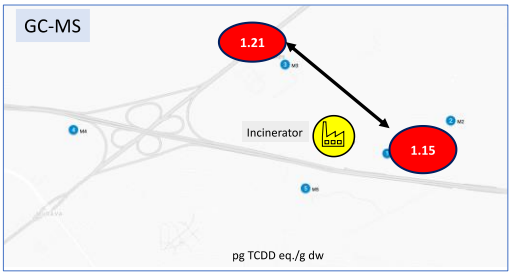
	GC-MS *pg TEQ/g dw*		
	PCDD/F	dl-PCB	PCDD/F/dl-PCB
M1	0.94	0.22	1.15
M3	0.97	0.25	1.21

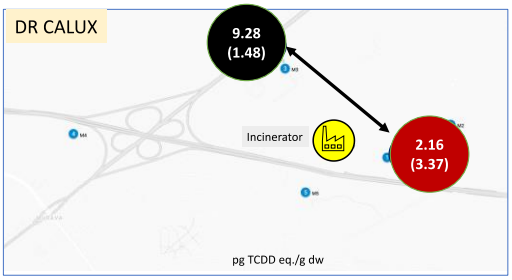
Results dl-PCB in mosses concentration Kaunas

	GC-MS *pg/g dw*		
	PCDD/F	dl-PCB	PCDD/F/dl-PCB
M1	52.25	191.94	204.96
M3	26.71	355.29	373.43

Results Mosses Kaunas, 2021-2022

	DR CALUX pg TCDD eq./g PCDD/F/dl-PCB		
	2021	2022-1	2022-2
LT-Mos-01	3.37	2.16	
LT-Mos-02	1.21	1.20	1.62
LT-Mos-03	1.48	9.28	2.44
LT-Mos-04	1.39	0.31	
LT-Mos-05	1.34	0.49	
LT-Mos-06			0.08





TW Indicative scale for Non-food
DR CALUX pg TCDD eq./g
GC-MS pg TEQ eq./g
> 5.0
> 2.0
1.0 - 2.0
0.5 - 1.0
< 0.5

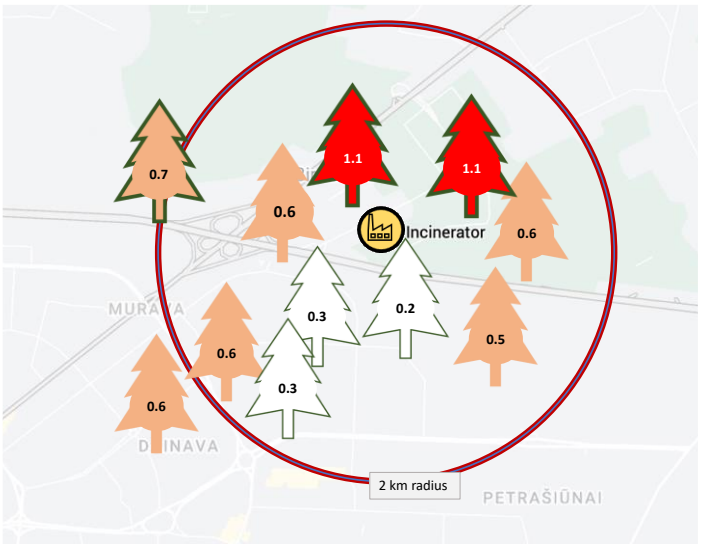
As explained above, the DR CALUX bioassay also reacts to other halogenated compounds, such as brominated and mixed halogenated dioxins. A very large group of brominated and mixed chloro/bromo dioxin and furan analogues is not included in the EU regulations for chemical analyses (GC-MS) nor integrated into measurements for waste incineration emissions. Brominated dioxins are a product of incomplete combustion of material containing brominated flame retardants, such as in electronics. Existing literature suggests that brominated dioxins and furans (PBDDs/Fs) have similar occurrence profiles to their chlorinated analogues, but the data is extremely limited, showing a major gap in estimating the potential risk of these chemicals.[12] More research is needed to explain the exceptionally high results in the DR CALUX bioassay. The inclusion of brominated dioxins in standard measurements of emissions from waste incinerators is strongly recommended.

[12] *Piskorska-Pliszczyńska J., S. Maszewski S. (2014). Bull Vet Inst Pulawy/58 327-335*

Results for dioxins (PCDD/F/dl-PCB) in pine needles

Dioxin (PCDD/F and DL-PCB) analyses on pine needles showed levels of 0.60 – 1.08 pg TCDD eq./g dw (medium bound) northwest and northeast of the waste incinerator. In the south of the incinerator, the dioxin levels were 0.22-0.49 pg TCDD eq./g dw. In the southwest (2000 m from the incinerator), the levels were 0.64 pg and in the west (2000 m), dioxin levels were 0.60 pg TCDD eq./g dw. The samples taken in October 2022 were nearly a factor of 2 higher in dioxin levels compared to analyses in July 2022. The elevations were due to the increase in dioxin-like PCB (DL-PCB). PCBs have been banned since 1985 but are still found in waste and in emissions from waste incinerators.

Results dioxins (PCDD/F/dl-PCB) in pine needles - Kaunas, 2022



Pine needles 2022 Kaunas (MB)				
S-Nr	TW-REF-NR	DR CALUX pg TCDD eq./gr product		
		PCDD/F/dl-PCB		
Pine needles/Picea abies		2021	Jul-22	Oct-22
1	Veg18-PA-SW01	0.38	0.28	
	Veg17-PA-SW02			
2	Veg1a-PA-03	0.73	0.22	
3	Veg16-PA-SW04	0.91	0.27	
	Veg15-SW06			
4	Veg19-PA-SE05	0.97	0.49	
5	PA-07/LT22-V2B	0.52	0.64	1.06
6	Veg14-PA-SW08	0.85	0.64	
	Veg13-PA-SW09			
7	PS-10/LT22-3B	0.93	0.60	1.08
8	Veg08-PA-SW11	0.47	0.64	
9	LT-V4			0.70

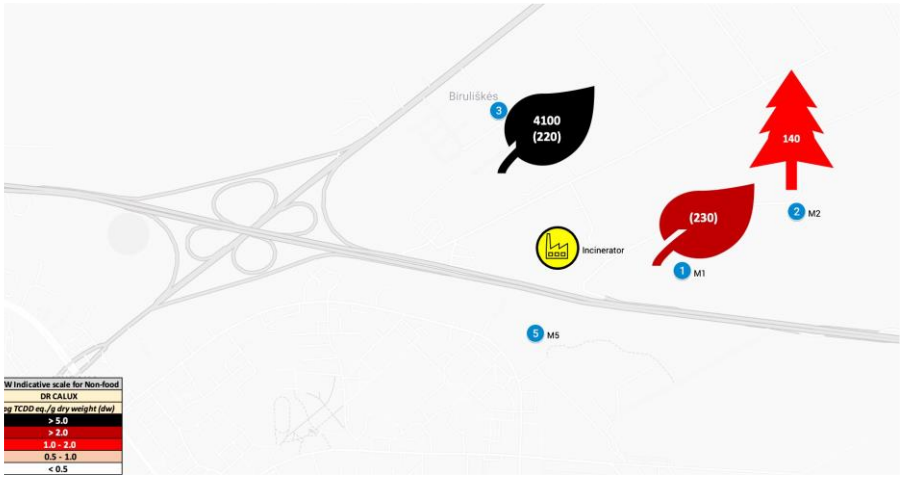
Jul-22 Oct-22

TW Indicative scale for Non-food
DR CALUX
pg TCDD eq./g dry weight (dw)
> 5.0
> 2.0
1.0 - 2.0
0.5 - 1.0
< 0.5

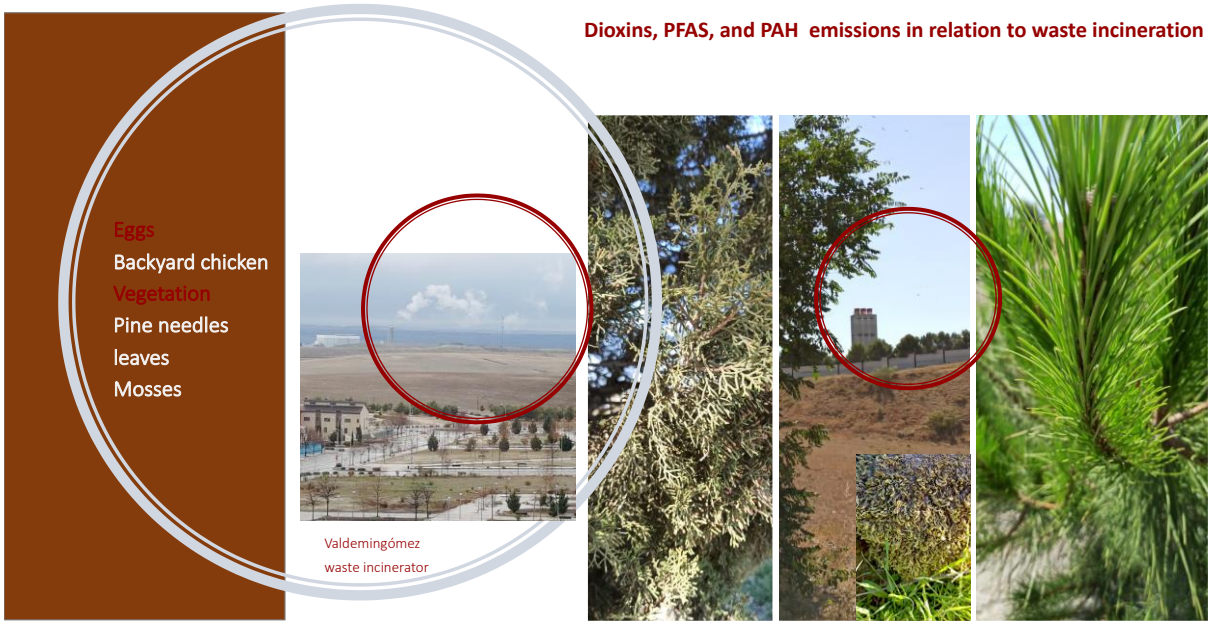
Results - PAHs in mosses and pine needles

The group of polycyclic aromatic hydrocarbons, PAHs, are markers for detecting toxic chemical emissions of thermal confounders. Compounds from the PAH group have carcinogenic, mutagenic, teratogenic and immunosuppressive effects on living organisms. In 2021, PAHs were measured in two mosses locations at 220 and 230 ng BaP eq./g. In mosses location 3, 4100 ng BaP eq./g dw was measured. In the pine needles east of the incinerator, 140 ng BaP eq./g dw was measured.

PAH in mosses and pine needles- 2021-2022, Kaunas, Lithuania



Madrid, Spain, 2022



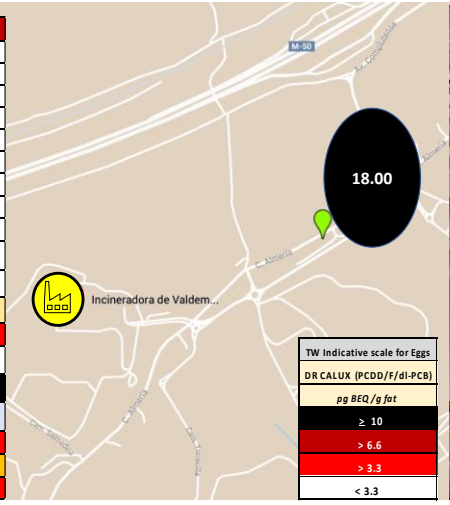
Biomonitoring Research - Madrid, Spain, 2022

Analysis of eggs of backyard chickens

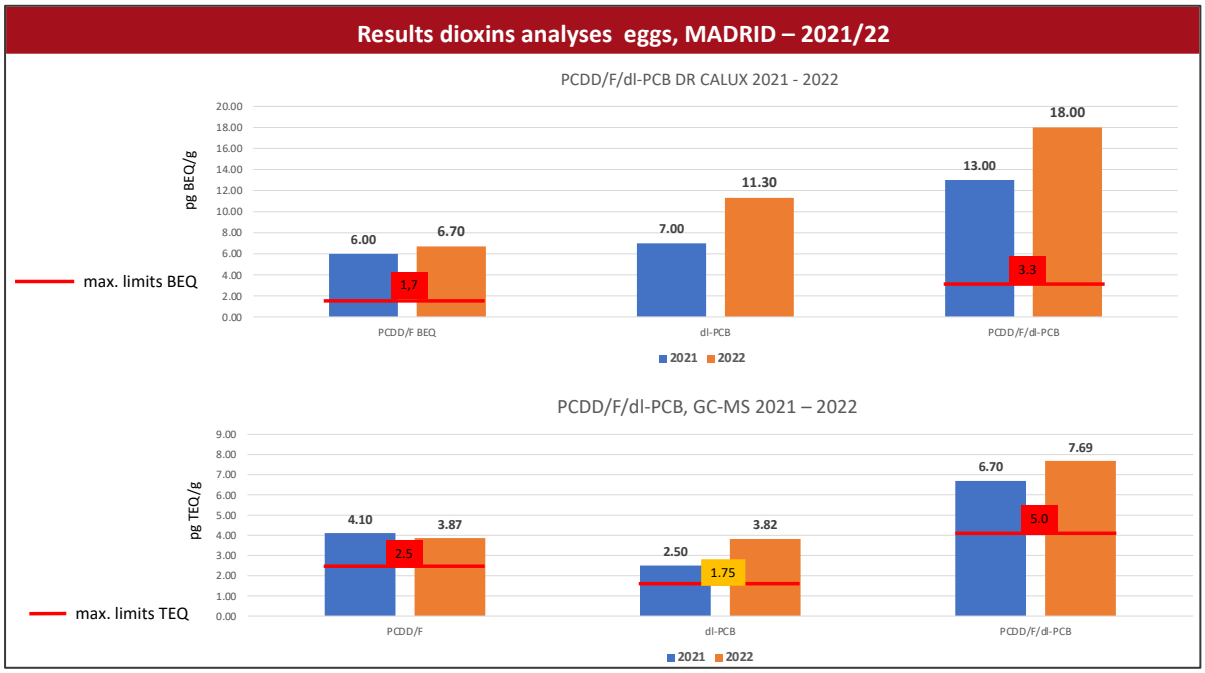
Two (2) methods were used for the analysis of the eggs. First, the eggs were analysed using the DR CALUX bioassay. This method detects the total toxicity of dioxins, not only the regulated chlorinated dioxins (PCDD/F and DL-PCB,) but also brominated (PBDD/F) and other (mixed) halogenated dioxins. Extra GC-MS analysis is mandated in the EU regulation for **commercial** eggs when the results of the DR CALUX analyses are above the cut-off limit of 1.75 pg BEQ/g fat for dioxins (PCDD/F) and 3.3 pg BEQ/g fat for total dioxins, including dioxin-like PCBs (PCDD/F and DL-PCB). In this study, the eggs also underwent a GC-MS analysis. The analyses of the eggs at a location 1000 m from the incinerator showed high levels of PCDD/F and DL-PCB in the DR CALUX bioassay. The GC-MS analysis confirmed these results, partially due to the limited scope of chemical analysis (only 29 congeners were analysed) while the DR CALUX bioassay measured total toxicity of all dioxins).

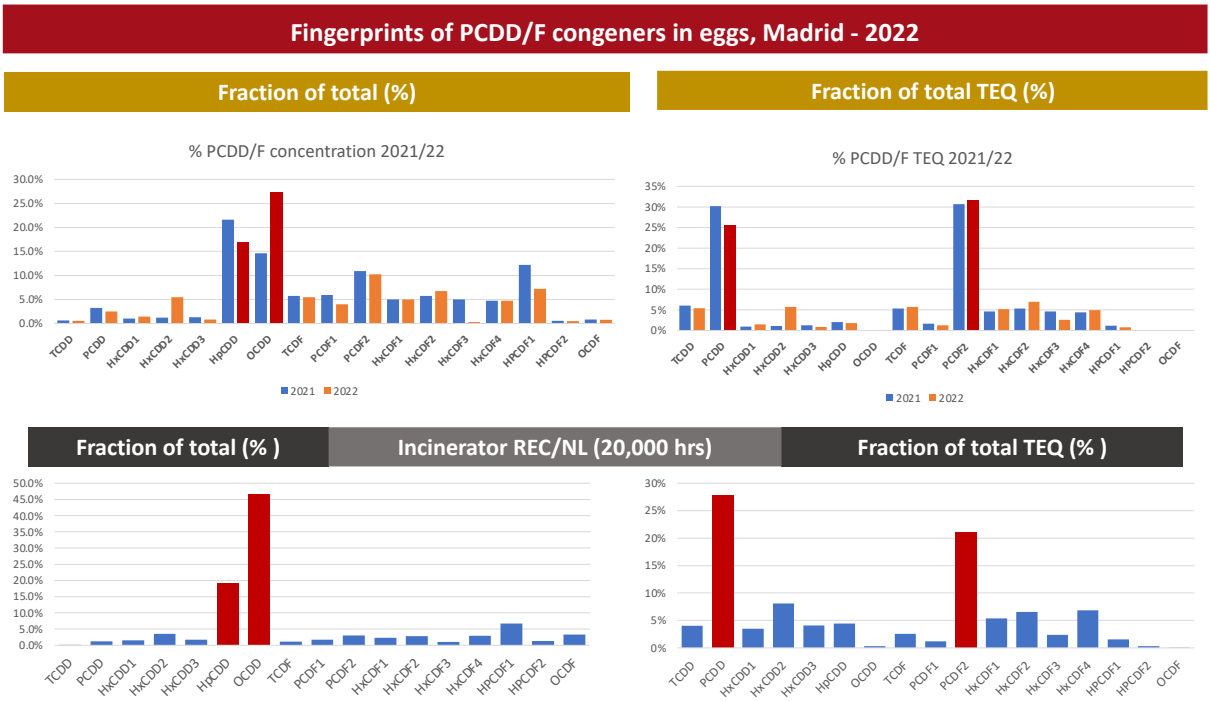
Results eggs sum of dioxins (PCDD/F/dl-PCB) DR CALUX, MADRID – 2021/22

MD-Egg 01		
	2021	2022
Distance	1000	1000
N hens	3	
N rooster		
Age	6-12 mnd	
Eggs/month	*30*	
Foraging area		
Outdoor fireplace		
All purpose burner		
Pesticides use		
DR CALUX *pg BEQ/g fat*		
PCDD/F BEQ	**6.00**	**6.70**
dl-PCB	7.00	11.30
PCDD/F/dl-PCB	**13.00**	**18.00**
GC-MS *pg TEQ/ g fat*		
PCDD/F	4.10	3.87
dl-PCB	2.50	3.82
PCDD/F/dl-PCB	**6.70**	**7.69**



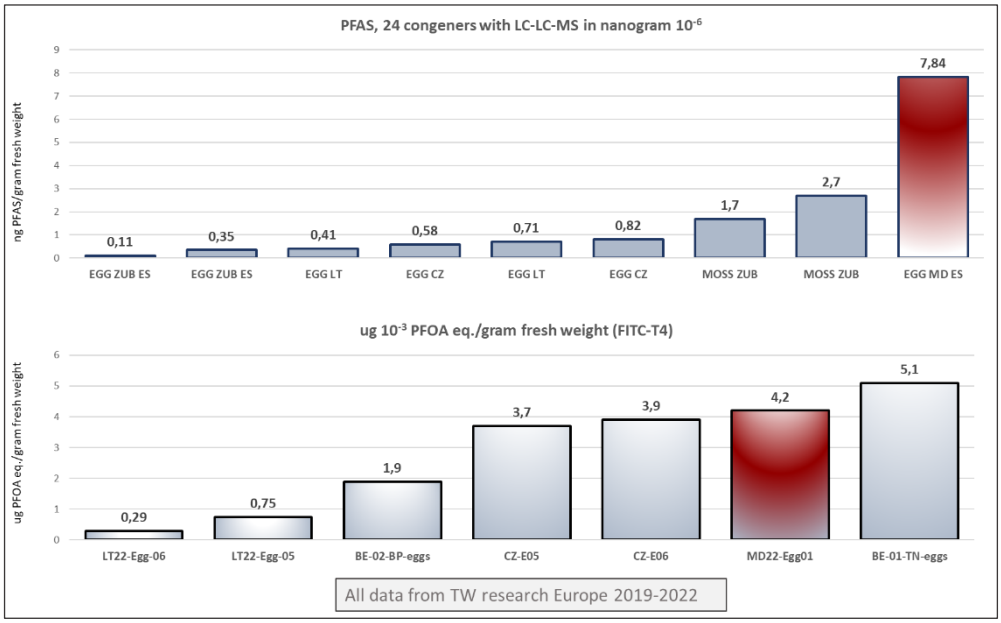
The graphs below show that high levels of dioxins (PCDD/F and DL-PCB) were observed in 2022. The bioassay showed higher levels for dioxins, with brominated dioxins most likely being involved.





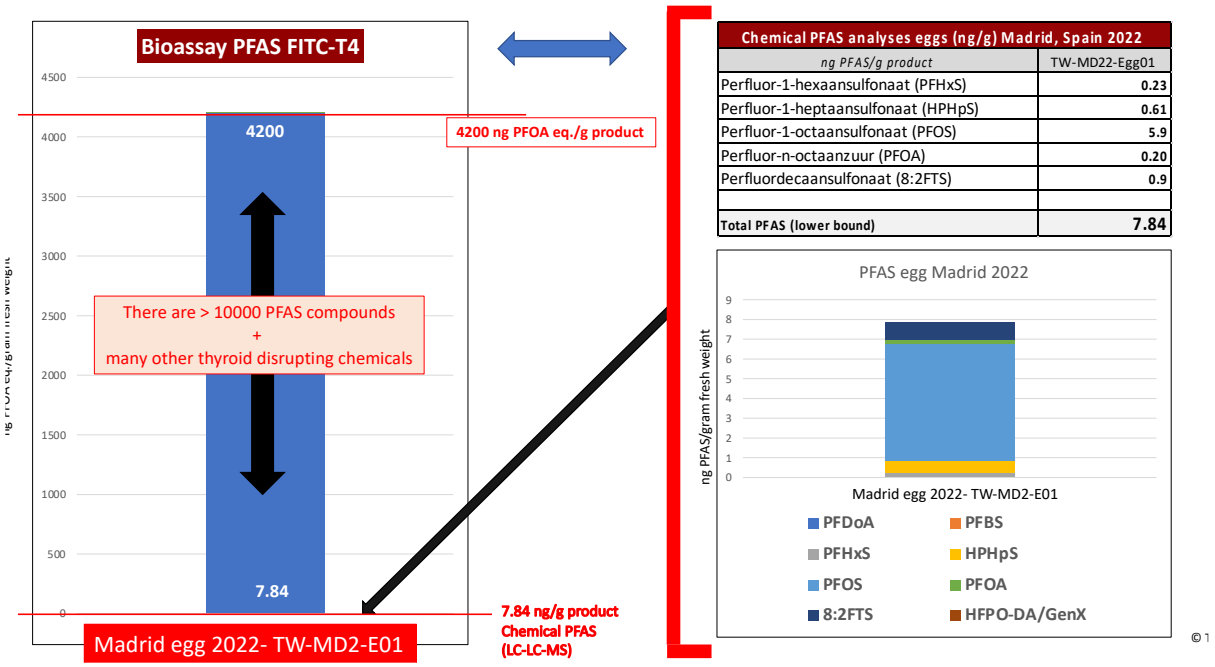
Analysis of PFAS in eggs of backyard chicken

In this biomonitoring research, TW analysed PFAS in eggs. PFAS can also be a product of the waste incinerator (see the explanation in the first TW report in 2021). The chemical PFAS analysis (LC-LC-MS) showed different PFAS congeners in two eggs. PFOS and PFDoA were analysed in the location west of the incinerator, while in the egg location in the southwest, GenX and 8:2 FTS were found.



The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should also be included as standard in the (EU) regulated monitoring of POPs from emission sources, such as waste incinerators.

PFAS analyses with FITC-T4 and limited chemicals analyses on 24 PFAS



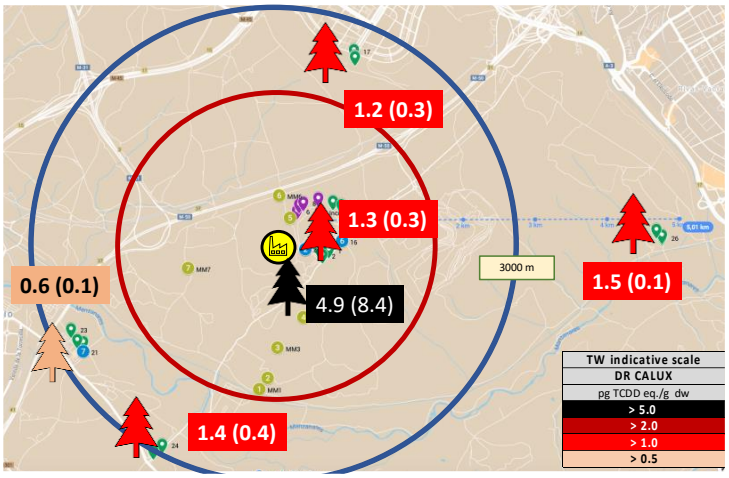
Analysis of pine needles and evergreen trees

The results of the first round of biomonitoring of mosses and pine needles in 2022 were complemented with extra sampling in October 2022. The figure below shows the results for dioxins in pine needles decreasing near the incinerator, but an overall increase in dioxins at all other locations.

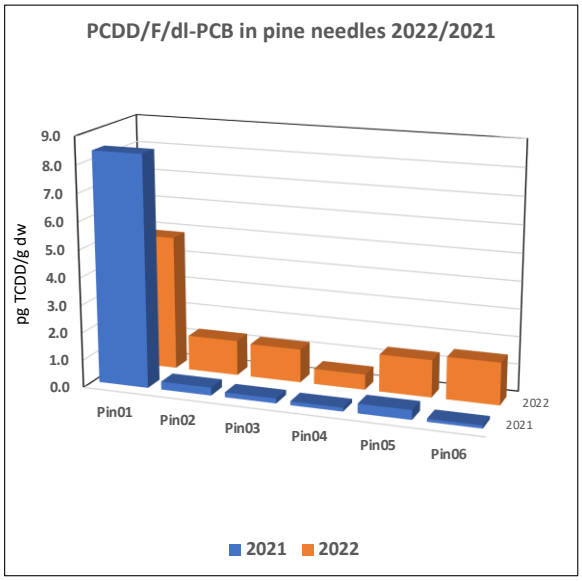
Although commercial use of polychlorinated biphenyls (PCBs) was banned almost half a century ago, contamination of the environment and organisms by PCBs is still observed. A serious increase was seen in several locations in 2022, see the figure on the left.
several locations, see figure left.

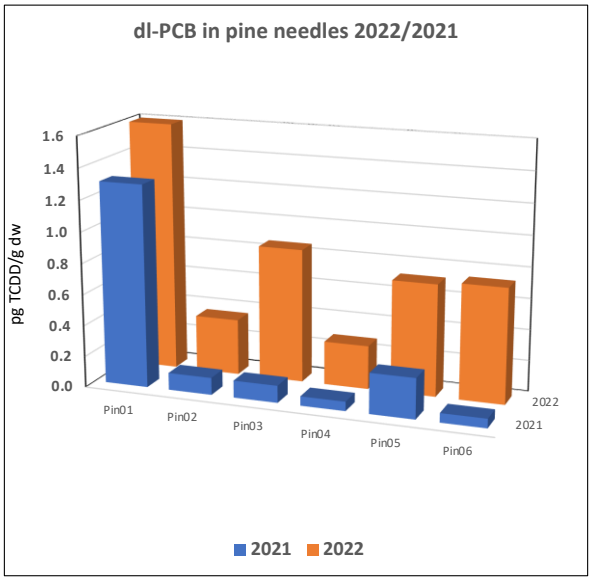
Dioxins in pine needles Madrid, Spain 2022 (2021)

Madrid pine needles 2022	DR CALUX pg TCDD eq./g					
	PCDD/F/dl-PCB		PCDD/F		dl-PCB	
	2021	2022	2021	2022	2021	2022
Pin01	8.4	4.9	7.1	3.2	1.3	1.6
Pin02	0.3	1.3	0.2	0.9	0.1	0.4
Pin03	0.2	1.2	0.1	0.4	0.1	0.9
Pin04	0.1	0.6	0.1	0.3	0.1	0.3
Pin05	0.4	1.4	0.1	0.6	0.3	0.7
Pin06	0.1	1.5	0.1	0.8	0.1	0.7



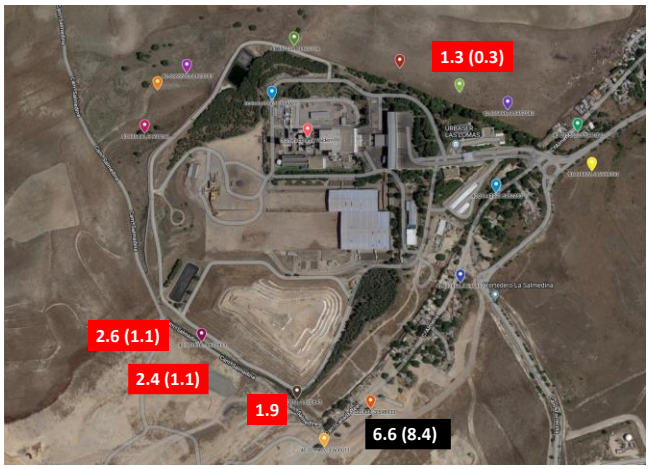
Dioxins in opine needles 2022





When focusing on the area closer to the incinerator, high levels of dioxins (PCDD/F and DL-PCB) were found in the vegetation in 2022 (and in the dioxin values analysed in 2021). Incinerator residue stored in large piles of big bags near the fence (see figure) could be responsible for the POP contamination in the pine needles and evergreen foliage. [13]

Dioxins in pine needles near the incinerator, Madrid, Spain 2022 (2021)



TW indicative scale
DR CALUX
pg TCDD eq./g dw
> 5.0
> 2.0
> 1.0
> 0.5

Dioxins (PCDD/F/dl-PCB) in vegetation around incinerator, Madrid – 2022 (2021)

[13] *https://efeverde.com/sacas-ceniza-toxica-aire-libre-valdemingomez-madrid/*

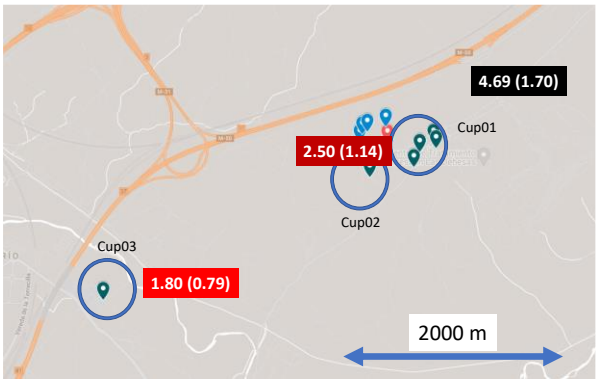
Analysis of needles of evergreen trees - *Cupressus arizonica*

The results for the Cupressus *arizonica* biomarker using the DR CALUX bioassay showed elevated levels of dioxins (PCDD/F and DL-PCB) in all places in 2022. As a comparison, the average level in vegetables according to the 2018 EFSA report is 0.36 pg TEQ/g product, with a lower share of dioxins (PCDD/F), 0.08, and a higher share of dioxin-like PCBs (DL-PCB), 0.28 pg TEQ . High values of PCDD/F were detected in the foliage in these locations, typically combustion-related. More research is needed.

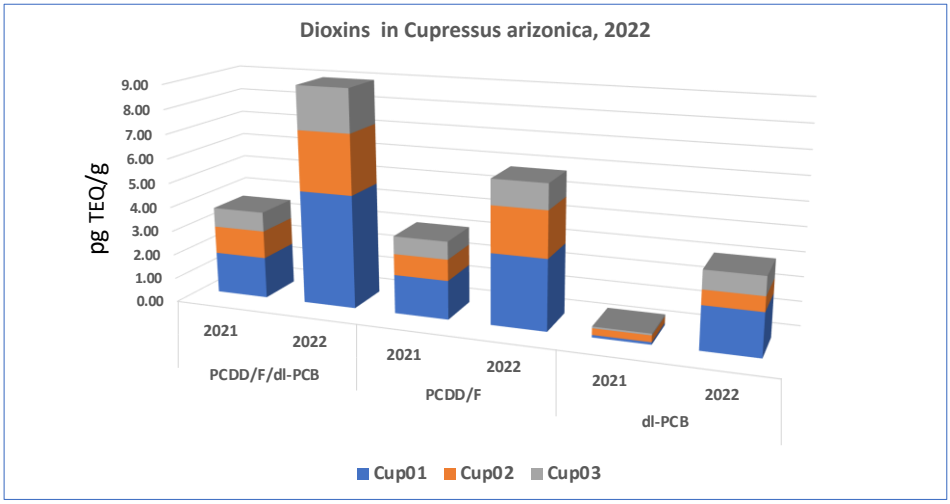
Dioxins in *Cupressus arizonica* 2022 (2021)

Dioxins in vegetation July and October, 2022						
	2022			2021		
	DR CALUX			DR CALUX		
	PCDD/F/dl-PCB	PCDD/F	dl-PCB	PCDD/F/dl-PCB	PCDD/F	dl-PCB
	pg TCDD eq./g dw			*pg TCDD eq./g product*		
Cup01	4.69	2.92	1.77	1.70	1.60	0.10
Cup02	2.50	1.90	0.60	1.14	0.87	0.27
Cup03	1.80	1.04	0.76	0.80	0.73	0.07

TW indicative scale
DR CALUX
pg TCDD eq./g dw
> 5.0
> 2.0
> 1.0
> 0.5



Dioxins in *Cupressus arizonica* 2022 (2021)



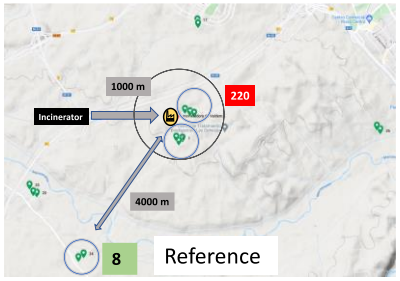
Dioxins in Cupressus arizonica, 2022						
DR CALUX						
	PCDD/F/dl-PCB		PCDD/F		dl-PCB	
	2021	*2022*	*2021*	*2022*	*2021*	*2022*
Cup01	1.70	4.69	1.60	2.92	0.10	1.77
Cup02	1.14	2.50	0.87	1.90	0.27	0.60
Cup03	0.80	1.80	0.73	1.04	0.07	0.76

TW indicative scale
DR CALUX
pg TCDD eq./g dw
> 5.0
> 2.0
> 1.0
> 0.5

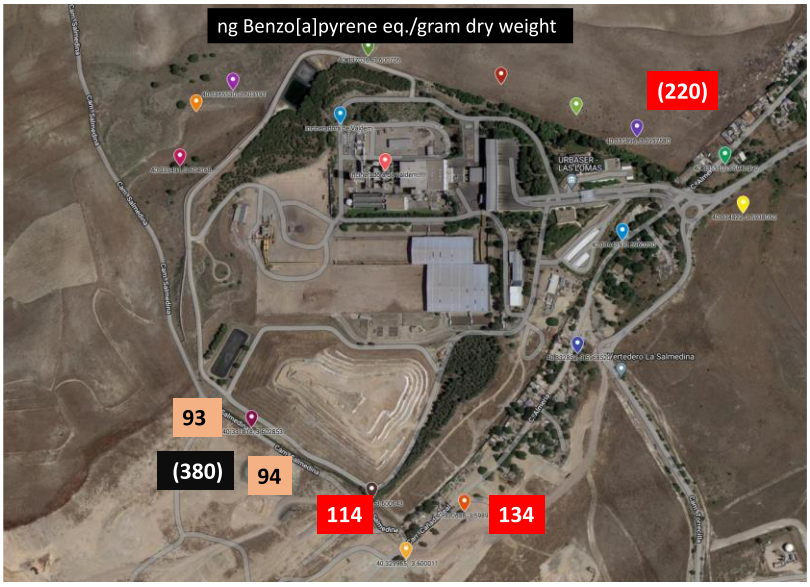
Analysis of PAH in pine needles and evergreen trees

In 2021, biomonitoring research found high levels of PAH northeast of the waste incinerator. In 2022, TW analysed four sites for PAH and found high levels of PAH in pine needles and evergreen trees directly around the perimeter of the incinerator. The figure below on the left shows the 2021 results and the figure on the right shows the results from four (4) PAH analyses around the incinerator in 2022. The results of the PAH analyses on the pine needles showed high levels, 93, 94, 114 and 134 nanogram benzo(a)pyrene per gram dry weight.

PAH in pine needles and evergreen trees, Madrid – 2022 (2021)



TW indicative scale
PAH CALUX
ng BaP eq./g dw
> 500
> 250
> 100
> 10



Valdemingómez waste-to- energy incinerator (WtE)

Analysis of dioxins (PCDD/F/dl-PCB) in mosses

The first sample round in 2022 took place in May. However, temperatures were high and not favourable for sampling mosses. The samples consisted mainly of soil. See the photos below and the soil quantity estimates. The analysis results were highly anomalous, which is why we decided to carry out a second round of sampling in October.

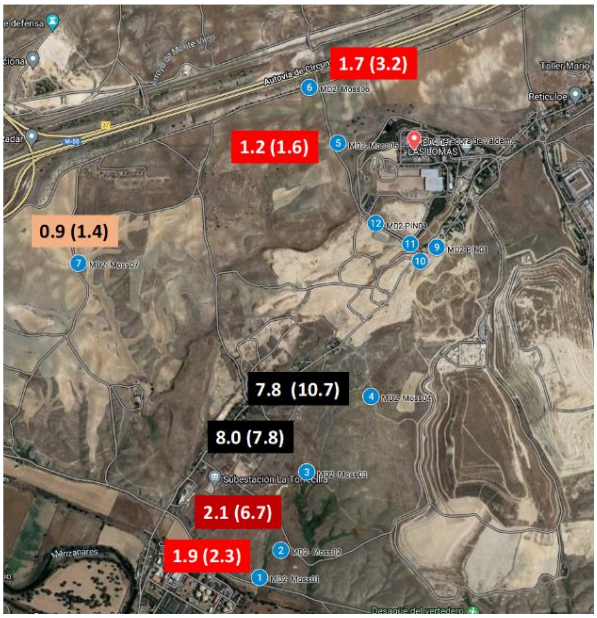
Moss-Soil 3, sample date, Madrid 03-07-2022

Moss-Soil 4, sample date, Madrid 03-07-2022

The results of the analysis on the mosses around the Valdemingómez incinerator are similar to those for 2021. The difference is that an increase is seen both in the references nearby and in the reference location 35 km away, in Castello de Villa. The elevated level of dioxins (PCDD/F and DL-PCB) is mostly observed around 2 km southwest of the waste incinerator, the same increase in dioxins as found in the research on pine needles in 2022. A reference sample for the mosses, located 20 km north of Madrid, in Castillo de Viñuelas, was taken from a natural environment, see the figure below.

Results sum of Dioxins (PCDD/F/dl-PCB) in *Mosses* Madrid – 2022 (2021)

Results Mosses, Madrid 2022 (Extra SET-B)				
		Dioxin - DR CALUX 2022 (A)		
Sample date	TW-REF-NR	PCDD/F/dl-PCB	PCDD/F	dl-PCB
Madrid		*(pg TCDD eq./g product)*		
01/09/2022	MD2-M01A	1.9	1.3	0.6
01/09/2022	MD2-M02A	2.1	1.5	0.6
01/09/2022	MD2-M03A	8.0	6.3	1.7
01/09/2022	MD2-M04A	7.8	6.5	1.3
01/09/2022	MD2-M05A	1.2	1.0	0.2
01/09/2022	MD2-M06A	1.7	1.4	0.3
01/09/2022	MD2-M07A	0.9	0.7	0.3
01/09/2022	MD2-M08A	0.9	0.6	0.3



Pilsen, Czech Republic, 2022

Dioxins, PFAS and PAH emissions in relation to waste incineration

Biomonitoring Research Pilsen - Czech Republic, 2022

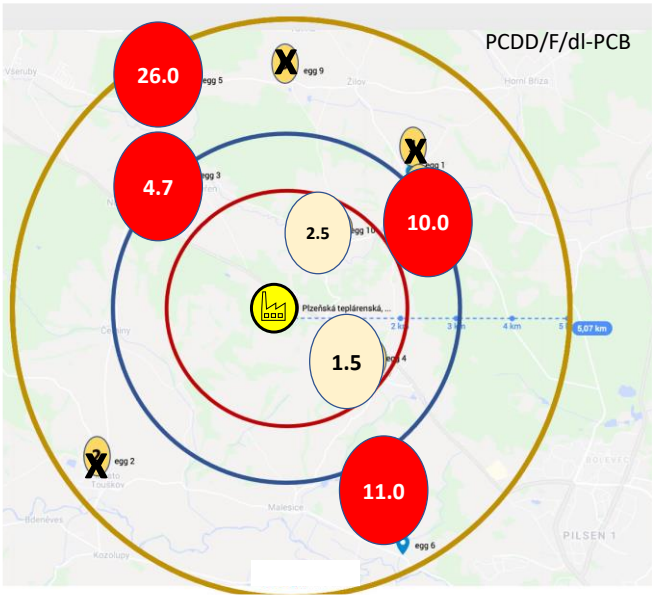
Bioassay analysis of dioxins in eggs of backyard chickens

The DR CALUX bioassay found four locations exceeding the EU cut-off/limit for eggs, which needed to be analysed using GC-MS chemical analyses. Two locations near the incinerator (< 2 km) comply with the limits. Three participants discontinued the biomonitoring research, partly due to having found contaminated eggs in 2021. The overall results are shown in the figure below.

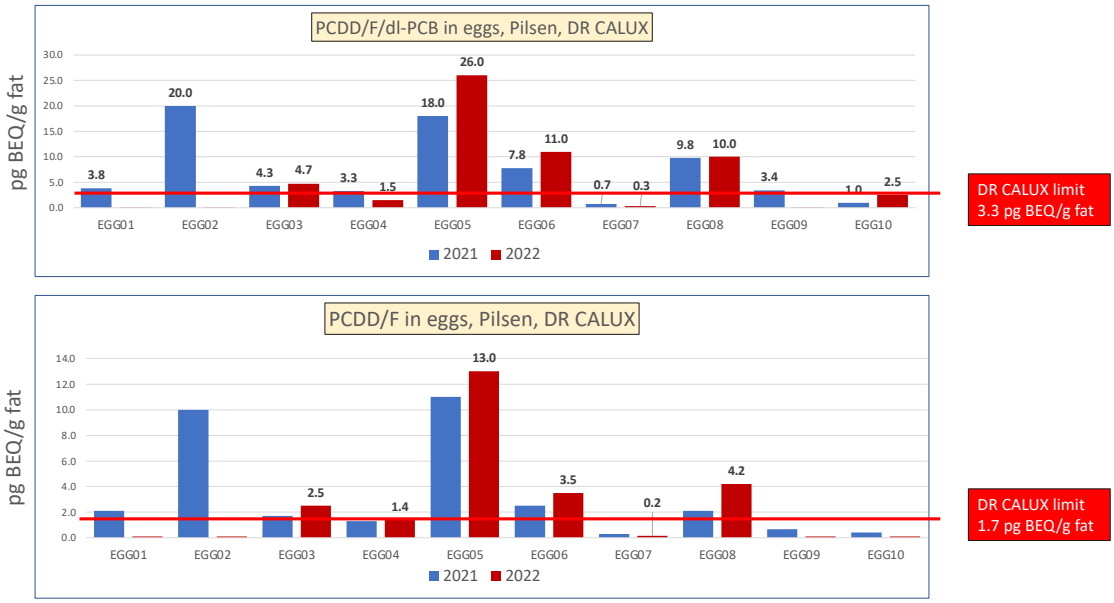
Results DR CALUX dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2022

Results Eggs Pilsen, Czech Republic 2022							
		DR CALUX *pg BEQ/g fat Medium bound (MB)*					
Location	Distance (m)	2021	2022	2021	2022	2021	2022
		PCDD/F/dl-PCB		PCDD/F		dl-PCB	
EGG01	3250	3.8		2.1		1.7	
EGG02	4290	20.0		10.0		10.0	
EGG03	3000	4.3	4.7	1.7	2.5	2.6	2.2
EGG04	1780	3.3	1.5	1.3	1.4	2.0	0.1
EGG05	4400	18.0	26.0	11.0	13.0	7.0	13.0
EGG06	4700	7.8	11.0	2.5	3.5	5.3	7.5
EGG07	reference	0.7	0.3	0.3	0.2	0.4	0.2
EGG08	3170	9.8	10.0	2.1	4.2	7.7	5.8
EGG09	4240	3.4		0.7		2.7	
EGG10	1630	1.0	2.5	0.4	0.2	0.5	2.4
DR CALUX	EU limit	3.3		1.7			
EGG	quit						

TW Indicative scale for Eggs
DR CALUX (PCDD/F/dl-PCB)
pg BEQ/g fat
≥ 10
> 6.6
> 3.3
< 3.3



DR CALUX results sum of dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2021 -2022



Chemical analysis of dioxins in eggs

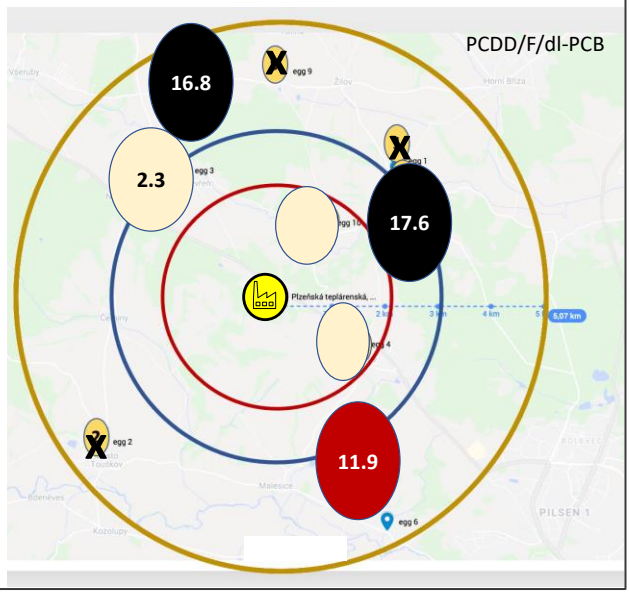
The GC-MS showed that two egg locations exceeded the maximum limit for dioxins by a factor of 3 and one location by a factor 2. The levels are 11.9, 16.8 and 17.6 pg TEQ/g fat. Three egg locations exceeded the DL-PCB action level of 1.75 pg TEQ/g.

Results GC-MS analyse dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2022

Results Eggs Pilsen, Czech Republic 2021

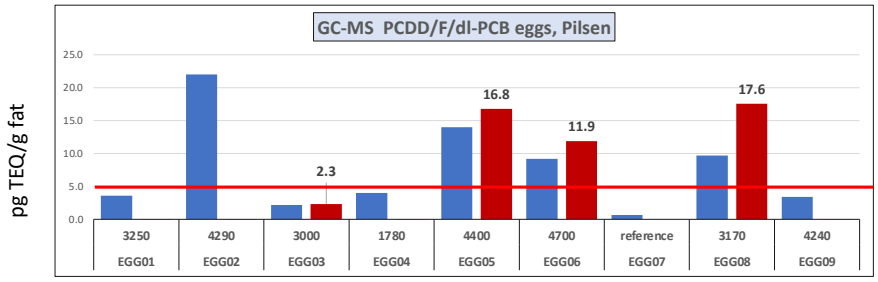
		GC-MS		pg TEQ/g fat		Medium bound (MB)	
Location	Distance (m)	2021	2022	2021	2022	2021	2022
		PCDD/F/dl-PCB		PCDD/F		dl-PCB	
EGG01	3250	3.6		2.2		1.4	
EGG02	4290	22.0		6.7		16.0	
EGG03	3000	2.2	2.3	1.0	1.3	1.2	1.0
EGG04	1780	4.0		3.0		1.0	
EGG05	4400	14.0	16.8	8.9	11.1	5.0	5.7
EGG06	4700	9.2	11.9	1.7	3.2	7.4	8.7
EGG07	reference	0.7		0.2		0.6	
EGG08	3170	9.7	17.6	1.3	3.0	8.4	14.5
EGG09	4240	3.4		0.7		2.7	
EGG10	1630						
EU Limit	GC-MS	5,0		2,5			
Action limit	GC-MS			1.75		1.75	
EGG	quit						

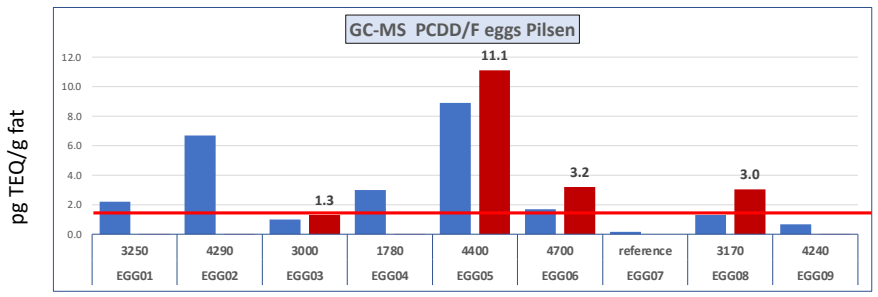
TW Indicative scale for Eggs
GC-MS (PCDD/F/dl-PCB)
pg TEQ/g fat
≥ 15.0
> 10.0
> 5.0
< 5.0



All three egg locations have increasing levels of PCDD/F and dl-PCB compared to 2021. On the next page, the three contaminated locations are given with some additional data.

GC-MS results dioxins eggs Pilsen - 2021 -2022





High dioxin-contaminated egg locations

Egg location 5 Pilsen Czech Rep. - 2022

TW-CZ-Egg05		
	2021	2022
Distance	4510	4510
N hens	22	
N rooster	5	
Age	36 mnd	
Eggs/month	*240*	
Area m2	30	30
Outdoor fireplace	no	no
Wood burning stove	many times	
Pesticides use	not	not
Industry nearby	no	no
Highway nearby	no	no
DR CALUX BEQ		
PCDD/F BEQ	11.00	13.00
dl-PCB	7.00	13.00
PCDD/F/dl-PCB	18.00	26.00
GC-MS TEQ		
PCDD/F BEQ	8.90	11.10
dl-PCB	5.00	5.70
PCDD/F/dl-PCB	14.00	16.80

TW Indicative scale for eggs
DR CALUX (PCDD/F/dl-PCB)
pg BEQ /g fat
≥ 10
> 6.6
> 3.3
< 3.3

TW Indicative scale for eggs
GC-MS (PCDD/F/dl-PCB)
pg TEQ /g fat
≥ 15.0
> 10.0
> 5.0
< 5.0

Egg location 6 Pilsen Czech Rep. - 2021

TW-CZ-Egg06		
	2021	2022
Distance	4670	4670
N hens	22	
N rooster	1	
Age	48 mnd	
Eggs/month	*390*	
Area m2	84	84
Outdoor fireplace	no	no
Wood burning stove	regular	regular
Pesticides use	not	not
Industry nearby	not	not
Highway nearby	no	no
DR CALUX *pg BEQ/g fat*		
PCDD/F	2.5	7.50
dl-PCB	5.3	3.50
PCDD/F/dl-PCB	7.8	11.00
GC-MS *pg TEQ/ g fat*		
PCDD/F	1.7	8.67
dl-PCB	7.4	3.20
PCDD/F/dl-PCB	9.2	11.87

TW Indicative scale for eggs
DR CALUX (PCDD/F/dl-PCB)
pg BEQ /g fat
≥ 10
> 6.6
> 3.3
< 3.3

TW Indicative scale for eggs
GC-MS (PCDD/F/dl-PCB)
pg TEQ /g fat
≥ 15.0
> 10.0
> 5.0
< 5.0

Egg location 8 Pilsen Czech Rep. - 2021

TW-CZ-Egg08		
	2021	2022
Distance	3290	4670
N hens	35	
N rooster	3	
Age	24 mnd	
Eggs/month	*600*	
Area m2	30	84
Outdoor fireplace	no	no
Wood burning stove	regular	regular
Pesticides use	not	not
Industry nearby	no	not
Highway nearby	no	no
DR CALUX *pg BEQ/g fat*		
PCDD/F	2.1	4.20
dl-PCB	7.7	5.80
PCDD/F/dl-PCB	9.8	10.00
GC-MS *pg TEQ/ g fat*		
PCDD/F	1.7	8.67
dl-PCB	7.4	3.20
PCDD/F/dl-PCB	9.2	17.57

TW Indicative scale for eggs
DR CALUX (PCDD/F/dl-PCB)
pg BEQ /g fat
≥ 10
> 6.6
> 3.3
< 3.3

TW Indicative scale for eggs
GC-MS (PCDD/F/dl-PCB)
pg TEQ /g fat
≥ 15.0
> 10.0
> 5.0
< 5.0

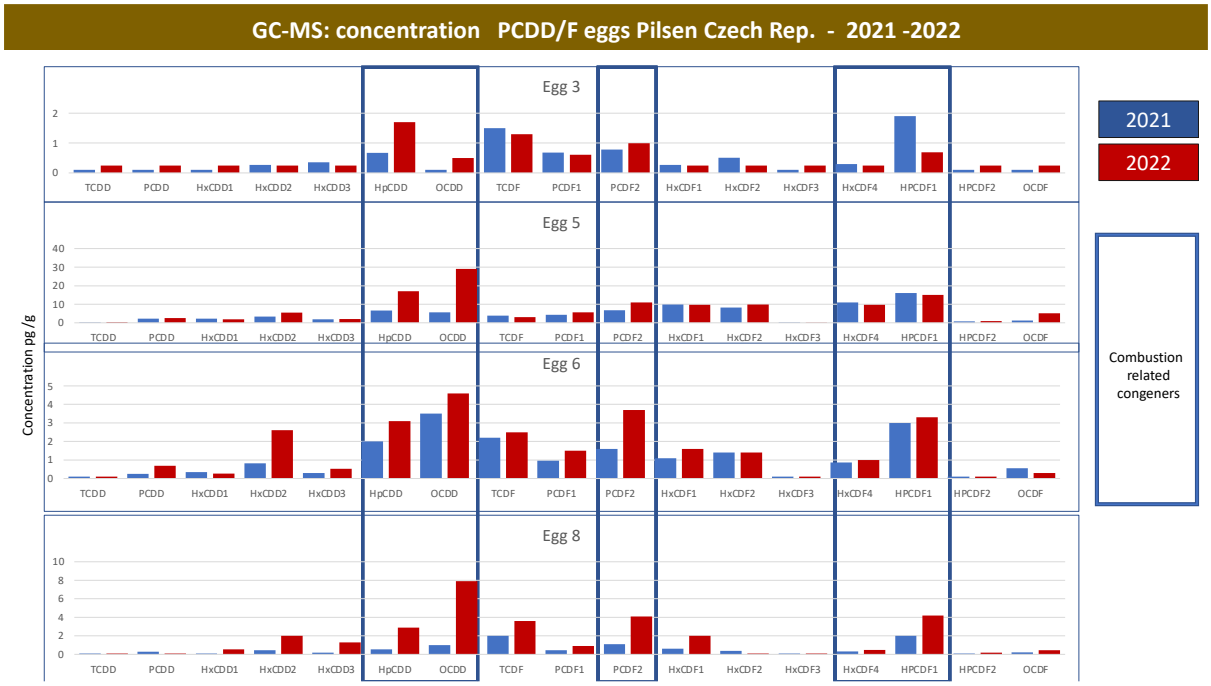
Analysis of feed and soil

Feed and soil were analysed using DR CALUX for additional research concerning dioxin contamination in egg location 5. The soil measured 2.1 pg BEQ/g PCDD/F and DL-PCB, mostly caused by a PCDD/F level of 1.49 pg TCDD eq./g. No dioxins could be detected in feed above the limit of detection (LOD=0.1 TCDD eq./g).

Analyse Date	Location	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB
				pg BEQ/g fat		
27/10/2022	Feed mais	TW22-CZ-F05M		0.2	0.1	0.1
27/10/2022	Feed grain	TW22-CZ-F05G		0.2	0.1	0.1
27/10/2022	Soil Extra	TW22-CZ-F05S		2.1	1.49	0.61

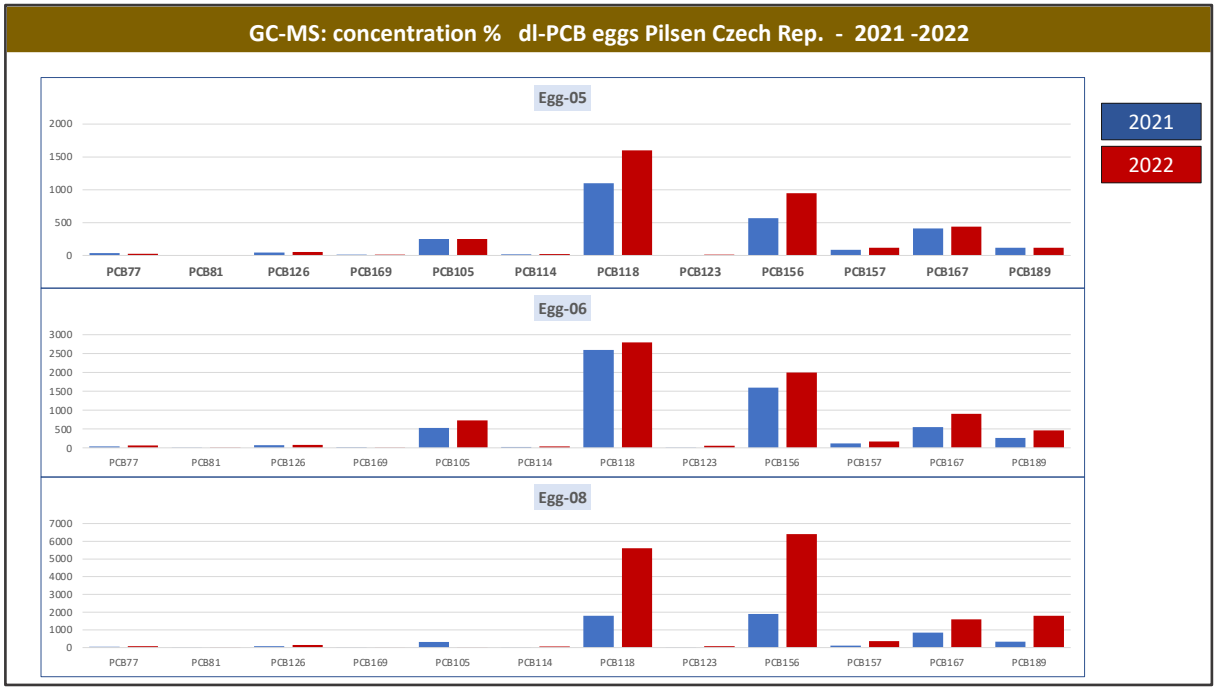
Analysis of dioxin congeners

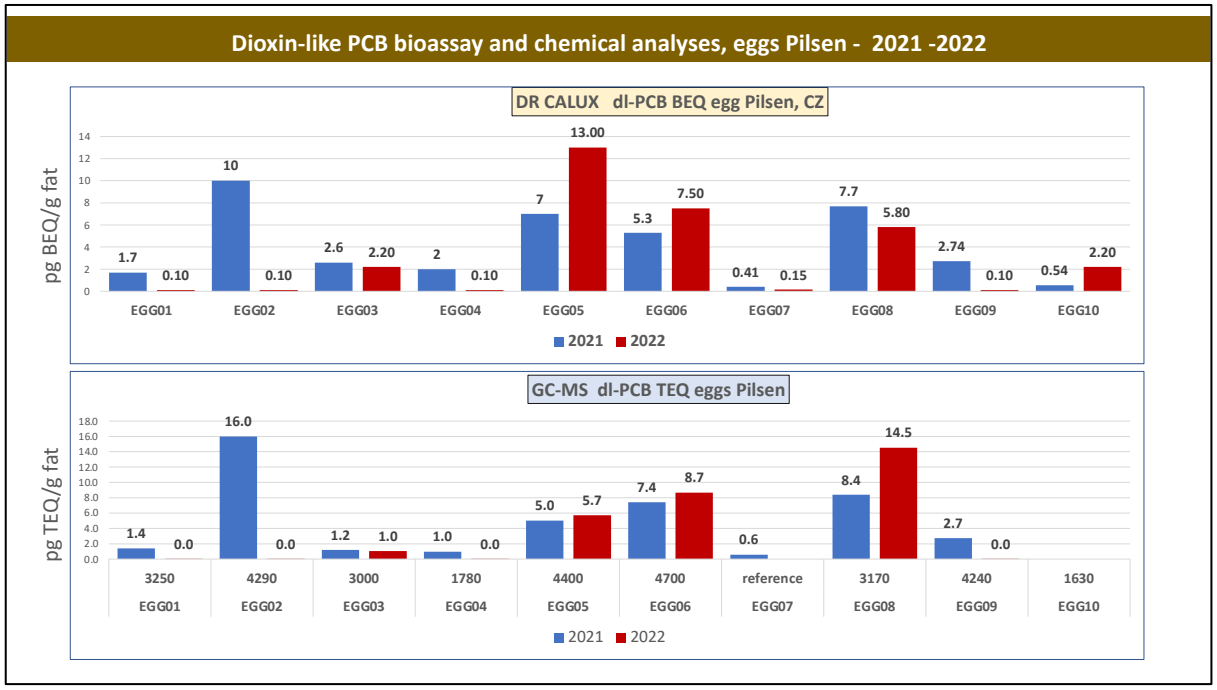
The figure shows the congeners found in the chemical analysis of the eggs. A pattern of elevation of those specific congeners related to (incomplete) combustion is present.



Analysis of dioxin-like PCB (dl-PCB)

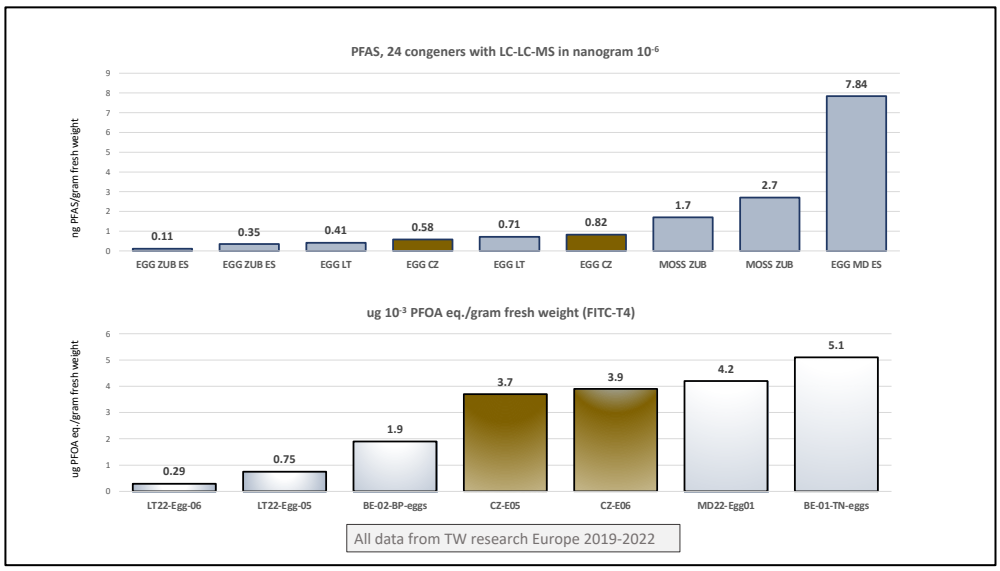
In 2022, levels of DL-PCB were elevated at locations 5 and 6. Although DL-PCB have long been banned, elevated levels can be still found in eggs. Soil measurements (see page 14) show 0.61 pg TCDD eq./g.



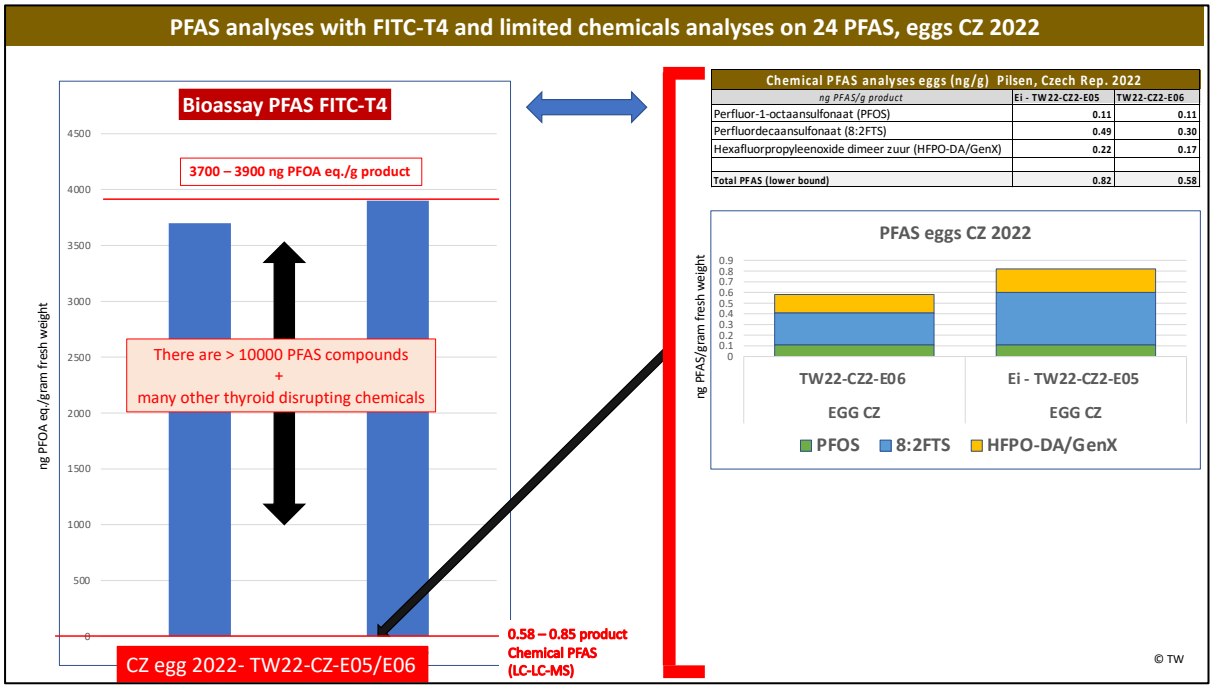


Analysis of PFAS in eggs

In this biomonitoring research, TW analysed PFAS in eggs. PFAS can also be a product of the waste incinerator (see the explanation in the first TW report in 2021[14]). The PFAS chemical analysis (LC-LC-MS) showed different PFAS congeners in two eggs. PFOS and PFDoA were analysed in the location west of the incinerator, while GenX and 8:2 FTS were found in the egg location in the southwest.



The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four (4) regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should also be included as standard in the (EU) regulated monitoring of POPs from emission sources, such as waste incinerators.



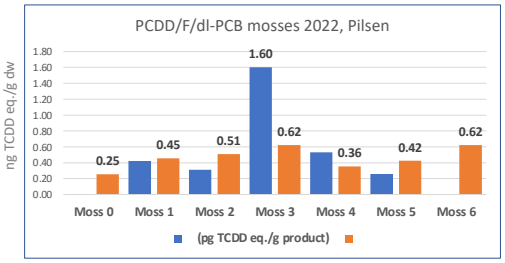
[14] https://zerowasteeurope.eu/library/the-true-toxic-toll-biomonitoring-of-incineration-emissions/

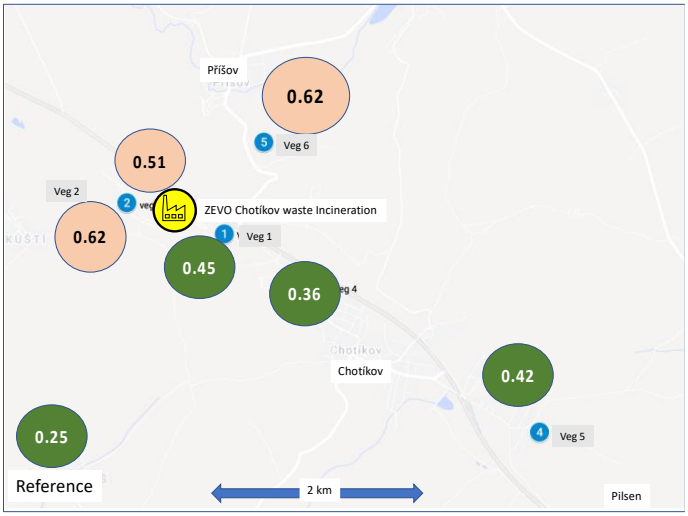
Analysis of mosses

Dioxins are slightly elevated at sites 2, 3 and 6 in the northwest, with values above 0.5 pg TCDD. eq/g dw. This mainly concerns the PCDD/F fraction, 0.13-0.41 TCDD.eq/g dw. The large outlier of 1.6 pg TCDD eq./g in 2021 decreased to 0.62 pg TCDD eq./g. If values like these were detected in vegetables, farms or vegetable gardens, they would be deemed too high for consumption. Normally, dioxin values in vegetables are very low, around 0.05 pg TEQ/g.[15]

Dioxins in mosses Pilsen, Czech Rep. 2022 (2021)

Results Mosses Pilsen, Czech Rep. 2022	DR CALUX (UB)					
	PCDD/F/dl-PCB		PCDD/F		dl-PCB	
	(pg TCDD eq./g product)					
	2021	2022	2021	2022	2021	2022
Moss 0		0.25		0.13		0.12
Moss 1	0.42	0.45	0.29	0.36	0.13	0.09
Moss 2	0.31	0.51	0.17	0.41	0.14	0.09
Moss 3	1.60	0.62	1.20	0.41	0.40	0.21
Moss 4	0.53	0.36	0.31	0.28	0.22	0.07
Moss 5	0.26	0.42	0.18	0.17	0.08	0.25
Moss 6		0.62		0.36		0.27





[15] *Knutsen HK et al. (2018) Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331, p. 189*

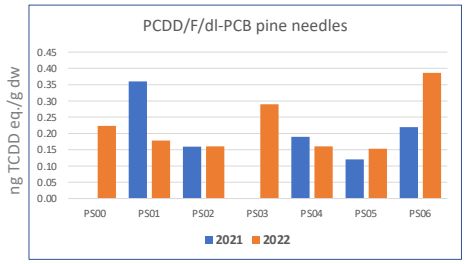
Analysis of pine needles

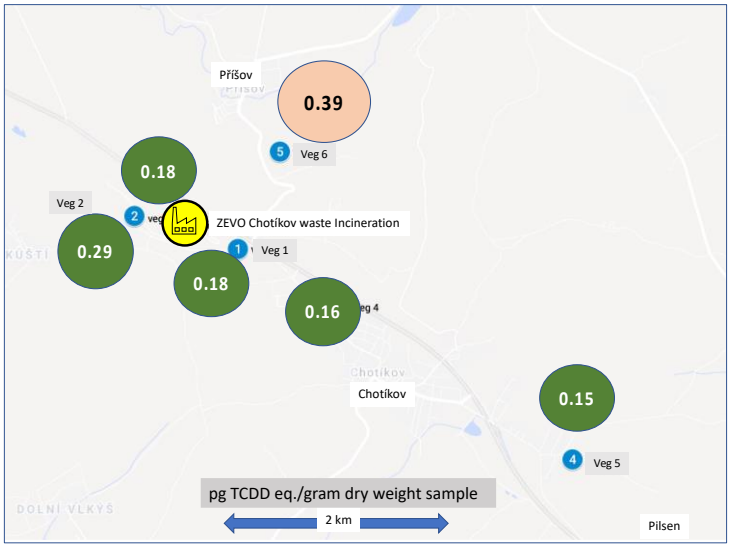
Dioxins were slightly elevated at sites 3 and 6, mainly due to PCDD/F. Dioxin-like PCBs were slightly elevated in 2021 but are now lower.

Dioxins in pine needles Pilsen, Czech Rep. 2022 (2021)

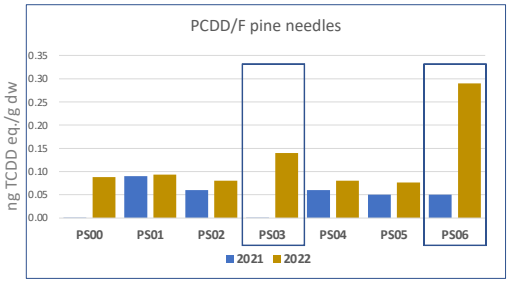
Pinus sylvestris - Czech Republic 2022	DR CALUX (UB)					
	PCDD/F/dl-PCB		PCDD/F		dl-PCB	
	(pg TCDD eq./g product)					
	2021	2022	2021	2022	2021	2022
PS00		0.22		0.09		0.14
PS01	0.36	0.18	0.09	0.09	0.27	0.08
PS02	0.16	0.16	0.06	0.08	0.10	0.08
PS03		0.29		0.14		0.15
PS04	0.19	0.16	0.06	0.08	0.13	0.08
PS05	0.12	0.15	0.05	0.08	0.07	0.08
PS06	0.22	0.39	0.05	0.29	0.17	0.10

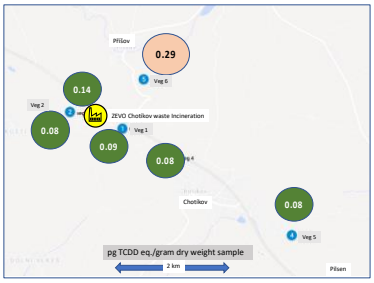
Elevated levels
Below level of detection

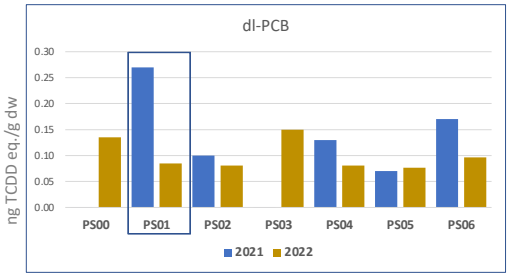


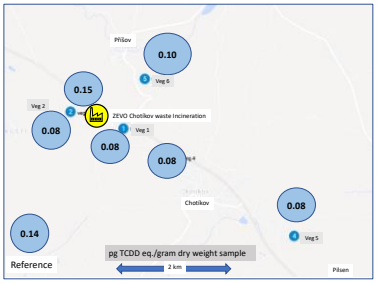


PCDD/F and dl-PCB in pine needles Pilsen, Czech Rep. 2022 (2021)



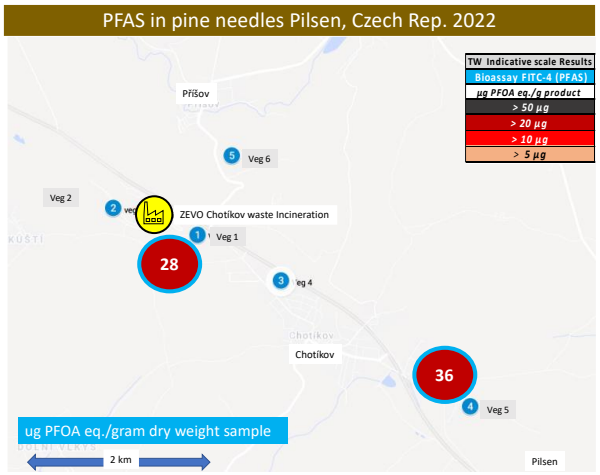


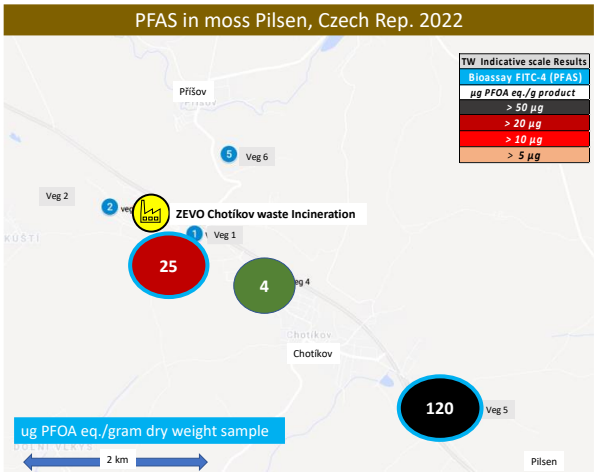




Analysis of PFAS in mosses and pine needles

Analyses were performed on PFAS using the FITC-T4 bioassay. It should be noted that the highest concentrations of PFOA equivalent were found both in pine needles and mosses at a location (5) 2000 m southwest of the waste incinerator, near the city of Pilsen.

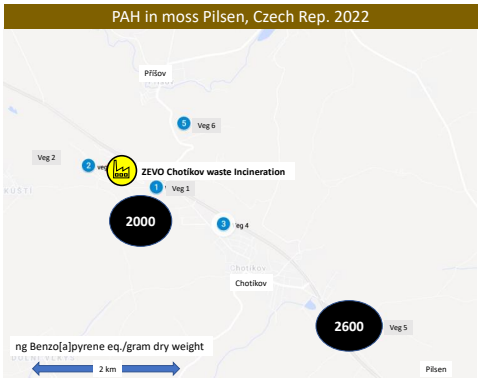




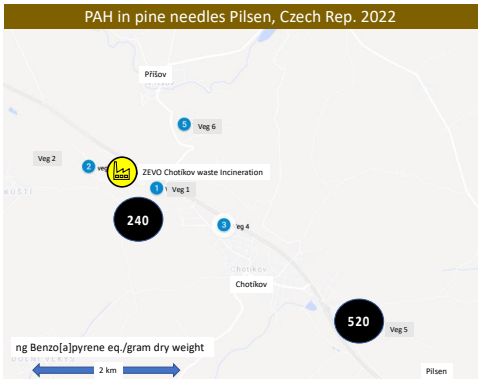
Analysis of PAH in mosses and pine needles

Analyses were performed on PAH using the PAH CALUX bioassay. The highest concentrations of benzo(a)pyrene equivalent were found both in pine needles and mosses at a location (5) 2000 m southwest of the waste incinerator. PAH were found to be 2600 ng benzo(a)pyrene eq./g dw in the mosses and 520 ng benzo(a)pyrene eq./g dw in the pine needles at this location.

PAH in moss and pine needles Pilsen, Czech Rep. 2022 (2021)



TW indicative scale
PAH CALUX
ng BaP eq./g dw
> 500
> 250
> 100
> 10



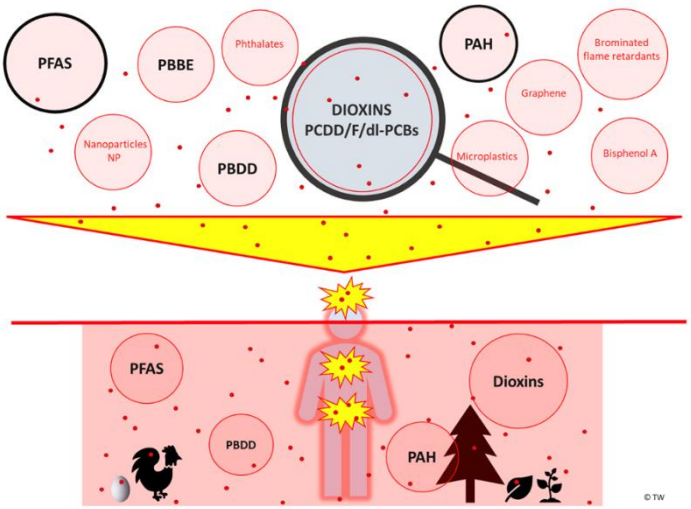
Mosses Pilsen, Czech Rep. 2022					
		DR CALUX 2022			PAH
TW-REF-NR	Distance (m)	CDD/F/dl-PCB	PCDD/F	dl-PCB	(07-11-2022)
		pg TCDD/g dw (18-08-2022)			g BAP eq./g pr.
TW-CZ22_MOSS-00	150000	0.25	0.13	0.12	
TW-CZ22-MOS-01	420	0.45	0.36	0.09	240
TW-CZ21-MOS-02	1400	0.51	0.41	0.09	
TW-CZ21-MOS-03	420	0.62	0.41	0.21	
TW-CZ21-MOS-04	980	0.36	0.28	0.07	
TW-CZ21-MOS-05	3440	0.42	0.17	0.25	520
TW-CZ21-MOS-06	953	0.62	0.36	0.27	

Results Pine needles - *Pilsen* Czech Republic 2022 (UB)					
		DR CALUX 2022 (18-08-2022)			PAH
TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB	(07-11-2022)
		(pg TCDD eq./g product)			ng BAP eq./g pr.
TW-CZ22-PS-Veg-00	150000	0.22	0.09	0.14	
TW-CZ22-PS-Veg-01	520	0.18	0.09	0.08	240.00
TW-CZ22-PS-Veg-02	274	0.16	0.08	0.08	
TW-CZ22-PS-Veg-03	1380	0.29	0.14	0.15	
TW-CZ22-PS-Veg-04	3410	0.16	0.08	0.08	
TW-CZ22-PS-Veg-05	1000	0.15	0.08	0.08	520
TW-CZ22-PS-Veg-06	953	0.39	0.29	0.10	

“It is not an egg problem”

One must realise that **no safe level of exposure to dioxin exists for human health and the environment**. Every level of dioxin and dioxin-like chemicals is associated with adverse human health effects. All opportunities should be undertaken to eliminate persistent organic pollutants such as dioxins. Incinerating waste always results in dioxin production. Measurements have been taken in general in this industry to reduce dioxin emissions; however total elimination is not possible for these Substances of Very High Concern (SVHC). The industry claimed to have diminished dioxin emissions, instead, emissions and deposition of dioxins are increasingly found in the environment, as reported in TW biomonitoring research in Europe.

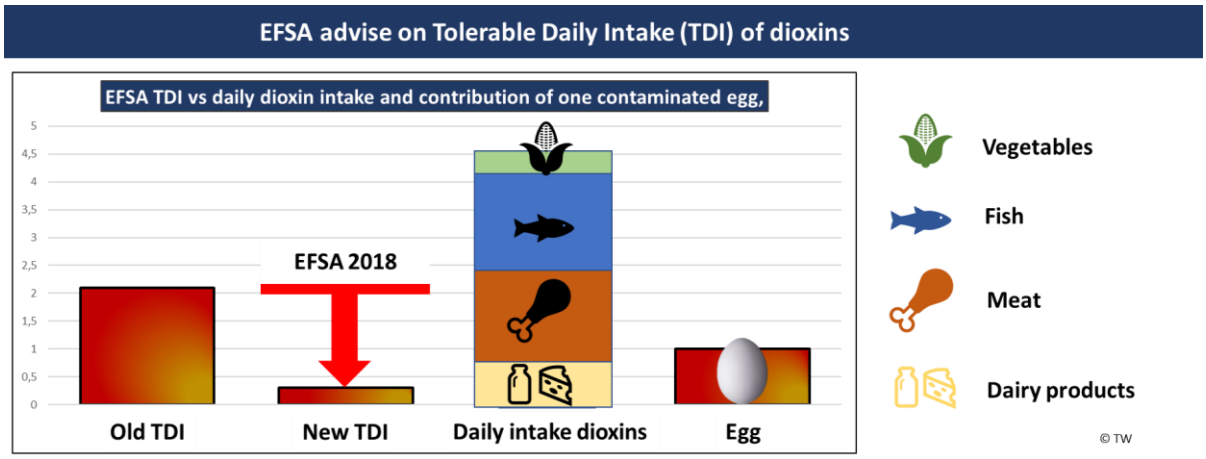
For TW biomonitoring research on emissions related to waste incineration, eggs of backyard chickens are used as biomarkers to obtain an indication of the environmental contamination of dioxins (PCDD/F/dl-PCB), PFAS and PAH of a specific area. TW biomonitoring results in 2021 and 2022 indicates an environment under stress, presented by high analyse values of dioxins in - besides eggs of backyard chicken - other biomatrices, like vegetation such as pine needles and mosses. To summarise these high results of dioxins as “*only an egg problem*”, is just a one-sided focus that distracts from what the conclusion is in the overall perspective of a contaminated environment by persistent organic pollutants (POPs). The TW biomonitoring analyse results of 2021 and 2022 should have bells ringing for serious local dioxin pollution. Banning the consumption of backyard chicken eggs, as an answer to the biomonitoring studies on environmental dioxin contamination, is not addressing the real problem at the source of dioxin pollution. An EFSA study[16], on page 189, shows clearly that the sharing part in a total of dioxins in meat, fish, milk, butter, and cheese all is a much bigger threat to our health than focusing only on dioxins in eggs. A **backyard chicken egg is a sensitive tool for measuring dioxin pollution in the environment**. Backyard chickens feed most of the time on seeds, insects, worms, snails, vegetation, natural soil and breaching the outdoor air. In ideal circumstances, the eggs of backyard chickens should be healthier than chicken eggs from the semi-indoor bioindustry. Just because their feed contains natural biodiversity, without the toxic compounds of agro-industry farming and/or frequent veterinary pharmacy. If toxic compounds are found in backyard chicken eggs, the real cause of the presence of dioxins should be addressed and measurements should be undertaken to achieve a reduction/elimination of the level of dioxins in the environment.



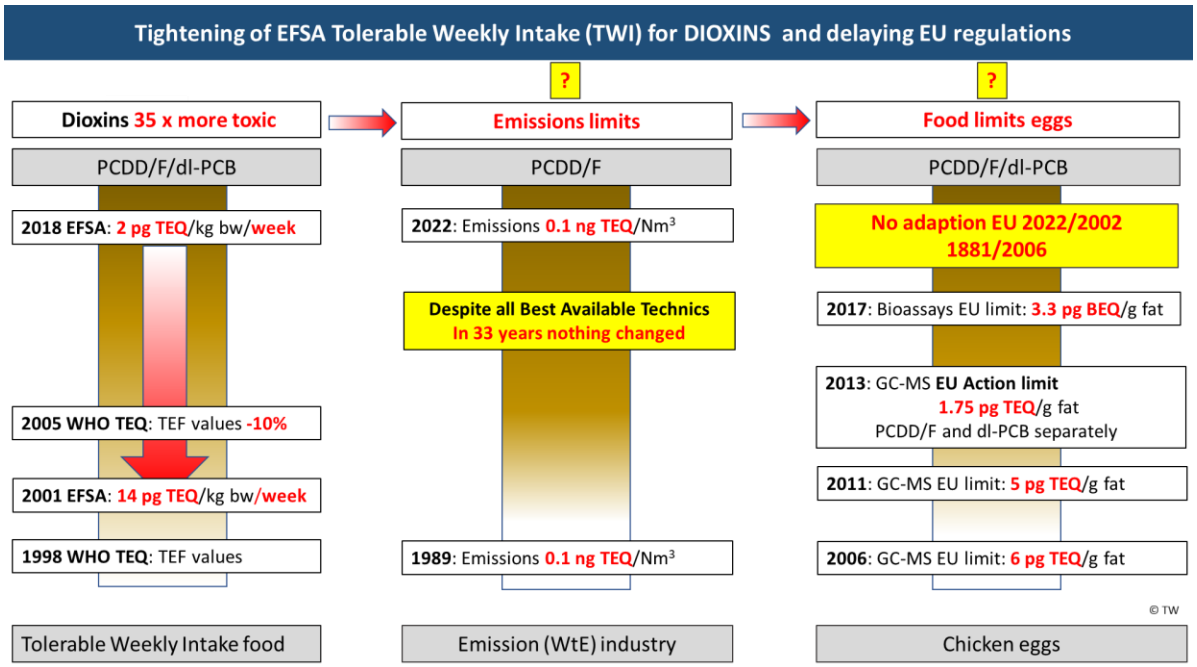
[16] *Knutsen HK et al. (2018) Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331, p. 189*

EU regulations and EFSA

In the biomonitoring report Kaunas of the last year 2021, the EU regulation on eggs was referenced. TW mentioned the update of the European Food Safety Authority (EFSA) advice in 2018 on the toxicity of dioxins by lowering the safe levels of dioxins with a factor of seven (7), or 7 x more toxic advice than the EFSA set up in 2001 for specific eggs.[17] This has not yet been implemented in the EU regulation of food. Until now the official EU limits on food (eggs) are based on the old TWI levels for dioxins (2001). If the new EFSA advice 2018 would be implemented, all the eggs would be passing this adapted limit for 'safer' dioxin levels in eggs.



For example, see the figure below for the GC-MS analyse results for the sum of dioxins (PCDD/F/dl-PCB) in eggs Kaunas 2022, if the updated dioxins safety levels, according to the EFSA advice 2018, were applied to the result of the chemical analyses on eggs.



[17] *EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK et al. (2018). Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331 pp.*

Reference list

- *Chen P. et al. (2017). Characteristic accumulation of PCDD/Fs in pine needles near an MSWI and emission levels of the MSWI in Pearl River Delta: A case study. Chemosphere 181 (2017) 360 – 367*
- *EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK et al. (2018). Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331 pp.*
- *EU 2017/644: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32017R0644*
- *EU No 1881/2006: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20210919&from=EN*
- *EU No 709/2014: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN*
- *EU: 2013/711/EU: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013H0711&from=EN*
- *Hamers T. (2020). Transthyretin-Binding Activity of Complex Mixtures Representing the Composition of Thyroid-Hormone Disrupting Contaminants in House Dust and Human Serum, Environmental Health Perspectives 017015-1 128(1)*
- *Knutsen HK et al. (2018) Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331, p. 189*
- *Photo from https://efeverde.com/sacas-ceniza-toxica-aire-libre-valdemingomez-madrid/*
- *Piskorska-Pliszczyńska J., S. Maszewski S. (2014). Bull Vet Inst Pulawy/58 327-335*
- *Pieterse, B., et al. (2013). PAH-CALUX, an optimized bioassay for AhR-mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs). Environ Sci Technol. 2013 Oct 15;47(20):11651-9.*
- *Smith, D.S., (1977). FEBS Lett. 77, 25-27.*
- *ToxicoWatch: Biomonitoring Kaunas, Madrid, Pilsen 2021, www.toxicowatch.org*
- *Zero Waste Europe: Biomonitoring Kaunas, Madrid, Pilsen 2021, www.zerowasteeurope.eu/library/the-true-toxic-toll-biomonitoring-of-incineration-emissions/*