

Biomonitoring research on dioxins (PCDD/F and DL-PCB), PFAS and PAH

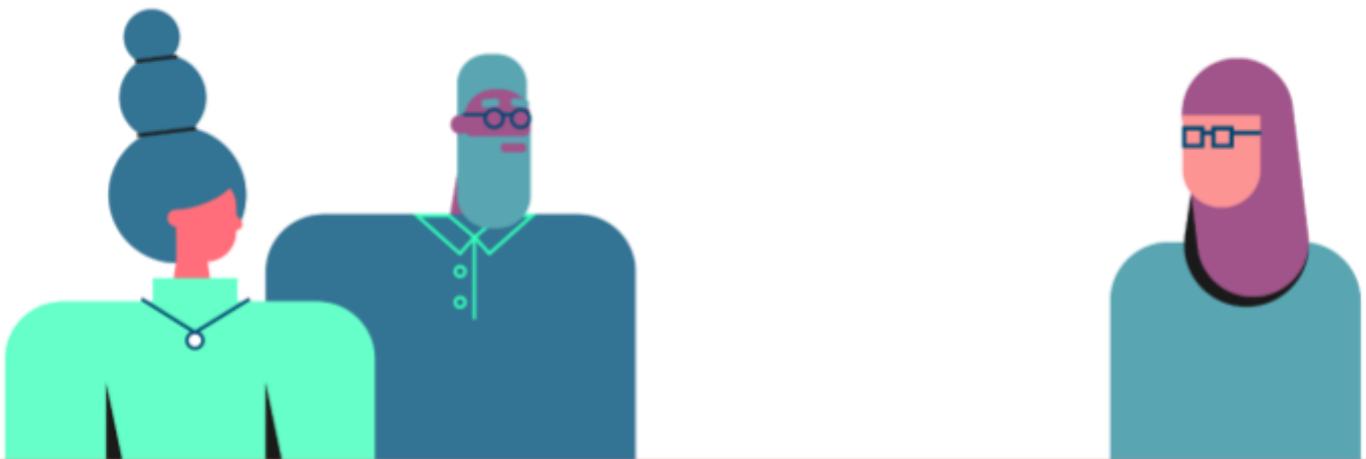
Kaunas, Madrid and Pilsen | 2022



Indicators

Chicken eggs | Moss | Pine needles





Acknowledgements

and credits

Thanks to Zero Waste Europe for making this research on persistent organic pollutants (POPs) possible.

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HARLINGEN, THE NETHERLANDS, TOXICOWATCH FOUNDATION, December 2022
PUBLICATION NUMBER: 2022-BIOM-3Cc
CLIENT: Zero Waste Europe

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Zero Waste Europe gratefully acknowledges financial assistance from the European Union and The Robert H.N. Ho Family Foundation Limited. The sole responsibility for the content of this event materials lies with Zero Waste Europe. It does not necessarily reflect the opinion of the funders mentioned above. The funders cannot be held responsible for any use that may be made of the information contained therein.

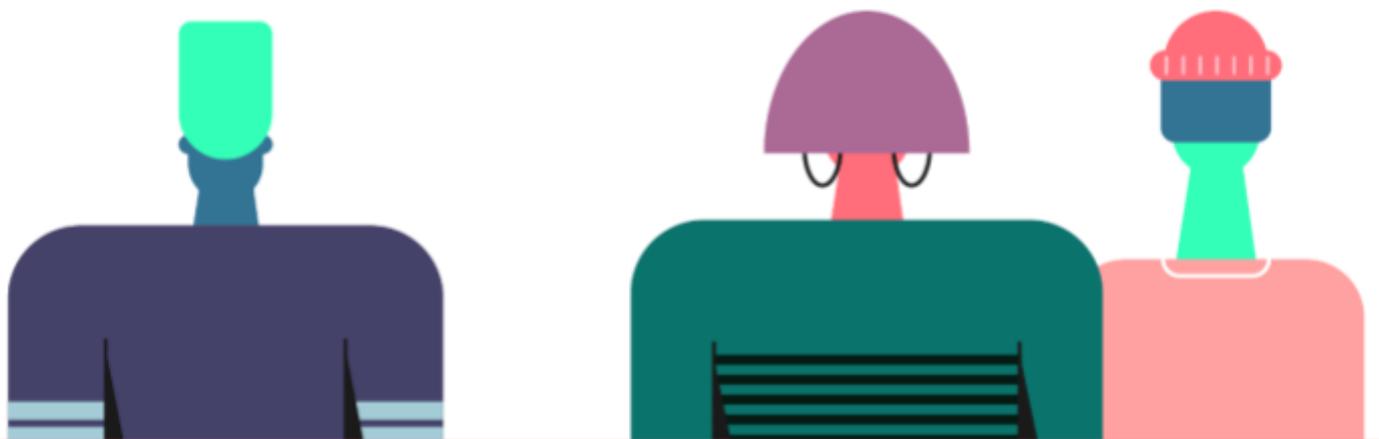


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and abbreviations

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ABBREVIATION	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 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- <i>p</i> -dioxin
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)

Abbreviation	Dioxins, furans (PCDD/F) and dioxin-like PCBs	Toxic equivalency factor
	Congeners	TEF
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

II | The True Toxic Toll - Biomonitoring research on dioxins (PCDD/F and DL-PCB), PFAS and PAH in relation to waste incineration in Kaunas, Madrid and Pilsen

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.zerowasteurope.eu/library/the-true-toxic-toll-biomonitoring-of-incineration-emissions



Executive summary

on biomonitoring, 2022

Kaunas (Lithuania), Madrid (Spain), Pilsen (Czech Rep.)

ToxicoWatch biomonitoring research on backyard chicken eggs and vegetation, such as pine needles and moss, 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. The 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), such as dioxins (PCDD/F and DL-PCB), Per- and PolyFluoroAlkyl Substances (PFAS) and Polycyclic Aromatic Hydrocarbons (PAH). The focus of this biomonitoring research is to analyse the deposition of POPs near 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 just affect backyard chicken eggs. 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 backyard chicken eggs in the vicinity of the three (3) incinerators exceeded EU limits for the DR CALUX bioassay and the GC-MS chemical analysis as regulated in EU Regulation 2017/644.
2. Analysis of the vegetation, pine needles and moss shows high concentrations of dioxins in the areas around the waste incinerators in all three (3) countries according to the DR CALUX bioassay.
3. High quantities of PFAS were found in moss, pine needles and backyard chicken eggs in all three (3) areas around the waste incinerators according to FITC-T4 bioassay measurements.
4. High levels of PAH were found in moss and pine needles around all three (3) waste incinerators according to the PAH CALUX bioassay.

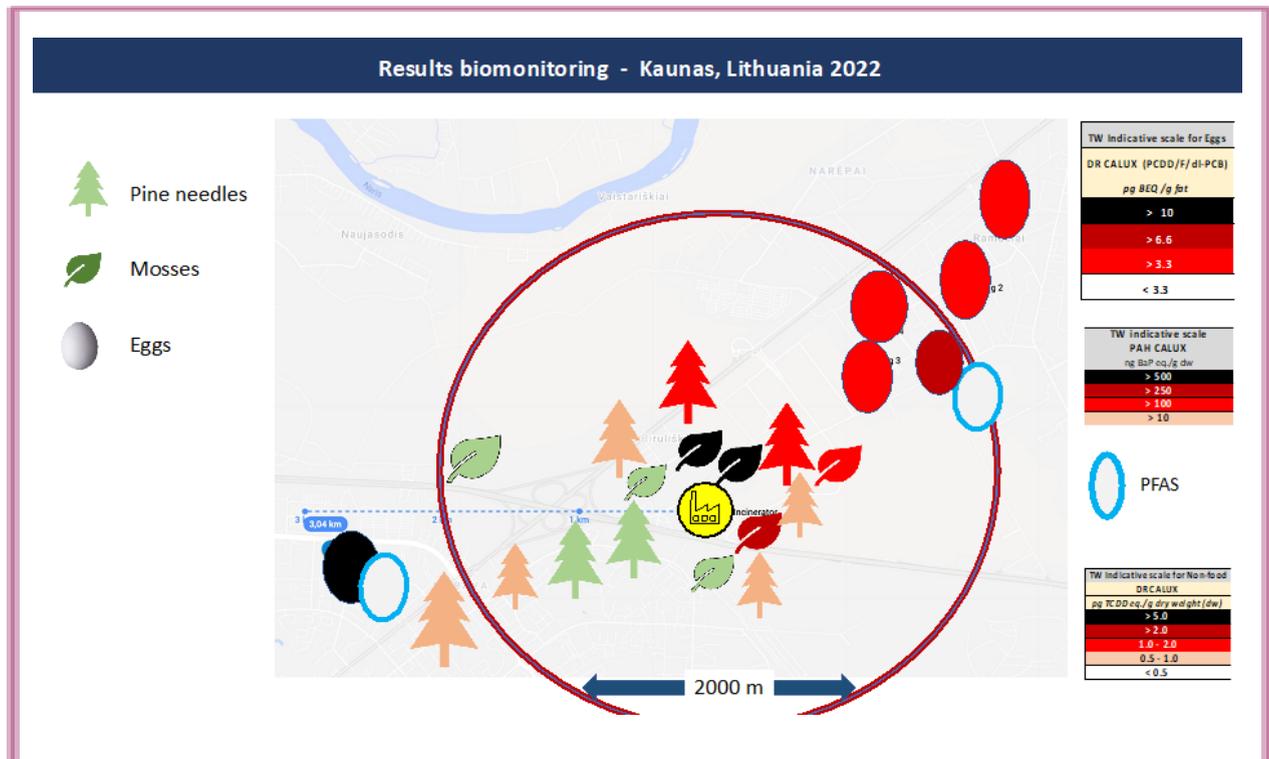
An additional consideration is the limitations of chemical analysis (GC-MS) for POPs. It can determine individual congeners but does not, however, measure the total toxicity (of thousands of toxic substances) of dioxins and dioxin-like substances (PCDD/F and DL-PCB), PAH and PFAS. The DR CALUX bioassay does measure the total toxicity of dioxins and dioxin-like substances, while the chemical analysis (GC-MS) is limited to only 17 congeners, with the exclusion of 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 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.

Biomonitoring results for Kaunas, Lithuania, 2022

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



Eggs

All of the 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.

Moss

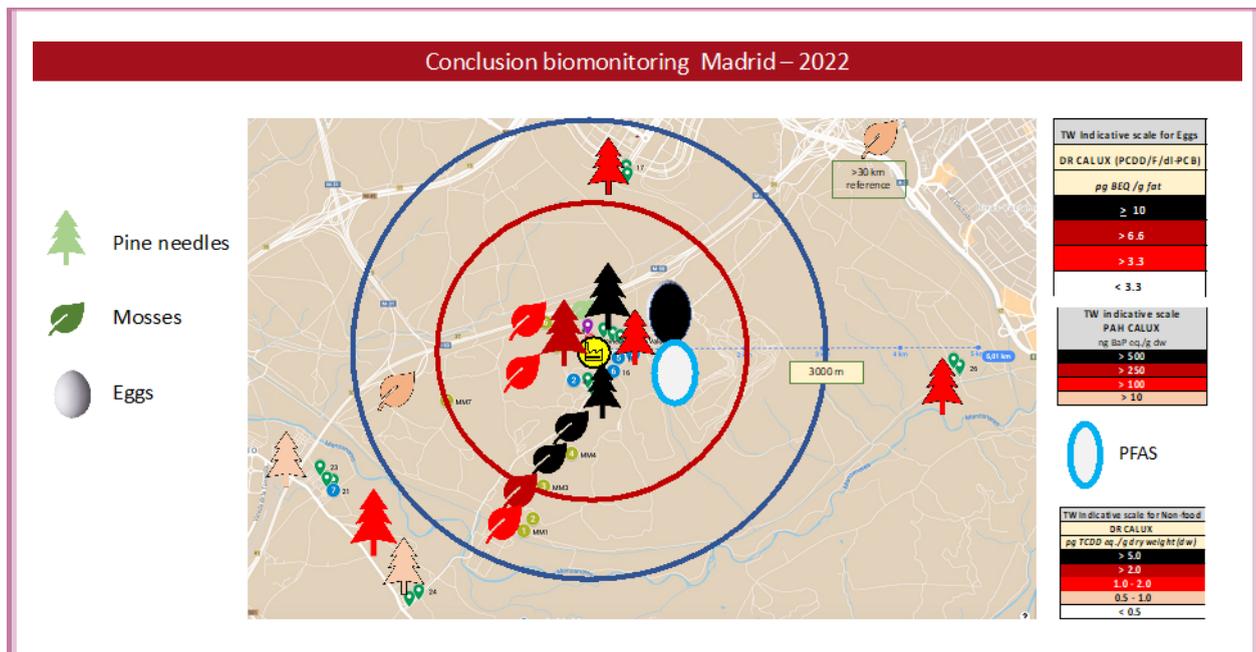
High levels of dioxins and furans (PCDD/F) were measured in moss in the area directly around the incinerator. The difference between the results of the bioassay and the chemical analysis indicates the contribution of substances not covered by the limited GC-MS analyses, such as brominated dioxins or other mixed halogenated substances. Dioxins in moss 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 moss 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 and 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.

Moss

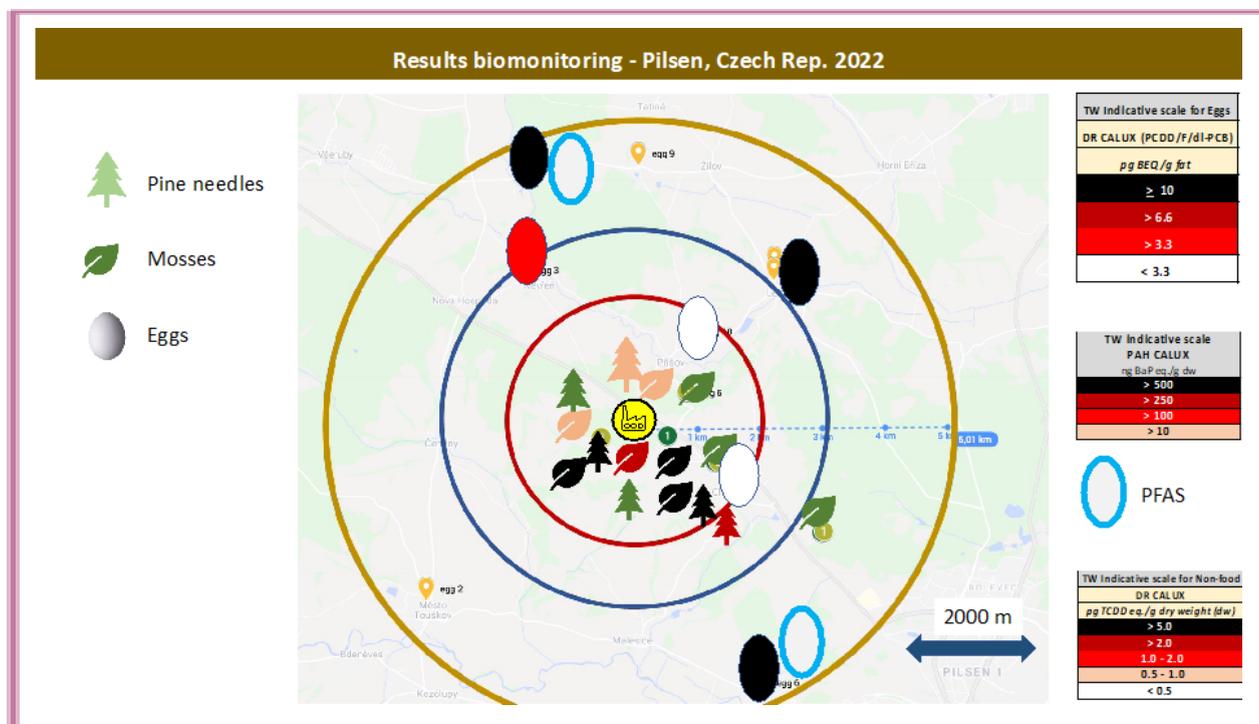
The pattern of elevated dioxins (PCDD/F and DL-PCB) in moss, most elevated 1500-2000 metres southwest, was found again in this research. The highest values for dioxins ever measured in a TW European biomonitoring survey were measured in the moss around Valdemingómez.

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 this TW 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

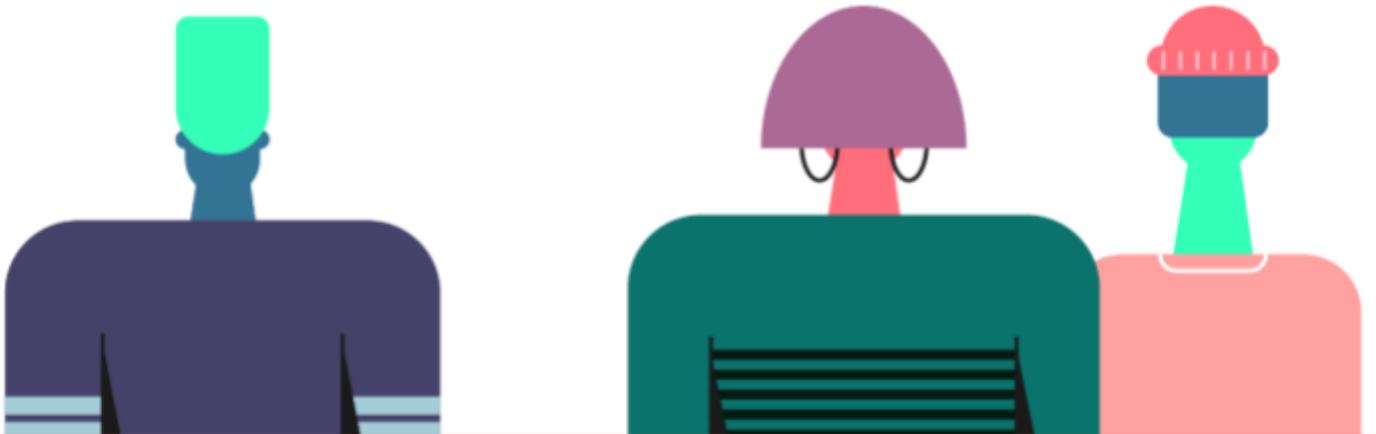
Analyses on locations within 3000-5000 m show high levels of dioxins (PCDD/F), more than three times the EU limit values for bioassays and chemical analyses. Dioxin congener patterns are related to incomplete combustion. There was a significant increase in dioxin-like PCB (DL-PCB) in the eggs, although 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.

Moss

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 moss 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 method¹ 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 moss 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**,² 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**,³ which sets maximum levels for certain contaminants e.g. dioxins (PCDD/F and DL-PCB) in foodstuffs.
- Commission Recommendation (EU) **2013/711/EU**⁴ on the reduction of the presence of dioxins, furans and PCBs in feed and food sets out action levels⁵ 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/EU⁶ 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.

¹ 'Bioanalytical methods' are methods based on the use of biological principles such as cell-based assays, receptor assays and immunoassays.

² eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32017R0644

³ eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20210919&from=EN

⁴ eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN

⁵ '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.

⁶ eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013H0711&from=EN

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.

EU regulation of dioxins in eggs

The figures below are an explanation of the bioassay (DR CALUX)) and the GC-MS chemical analyses on dioxins (PCDD/F and 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.^{7 8}

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)^{9 10}[9][10]. 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**.

⁷ Category 1B carcinogen according to Annex VI to CLP Regulation (EC) No 1272/2008 of the European Parliament and classified as a Substance of Very High Concern by POP Regulation (EC) No 850/2004.

⁸ 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.

⁹ Smith, D.S., (1977). FEBS Lett. 77, 25-27.

¹⁰ 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)

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.



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 moss took place on 12th and 13th June 2022 and the third round took place on 3rd October 2022.

Analysis of backyard chicken eggs

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		

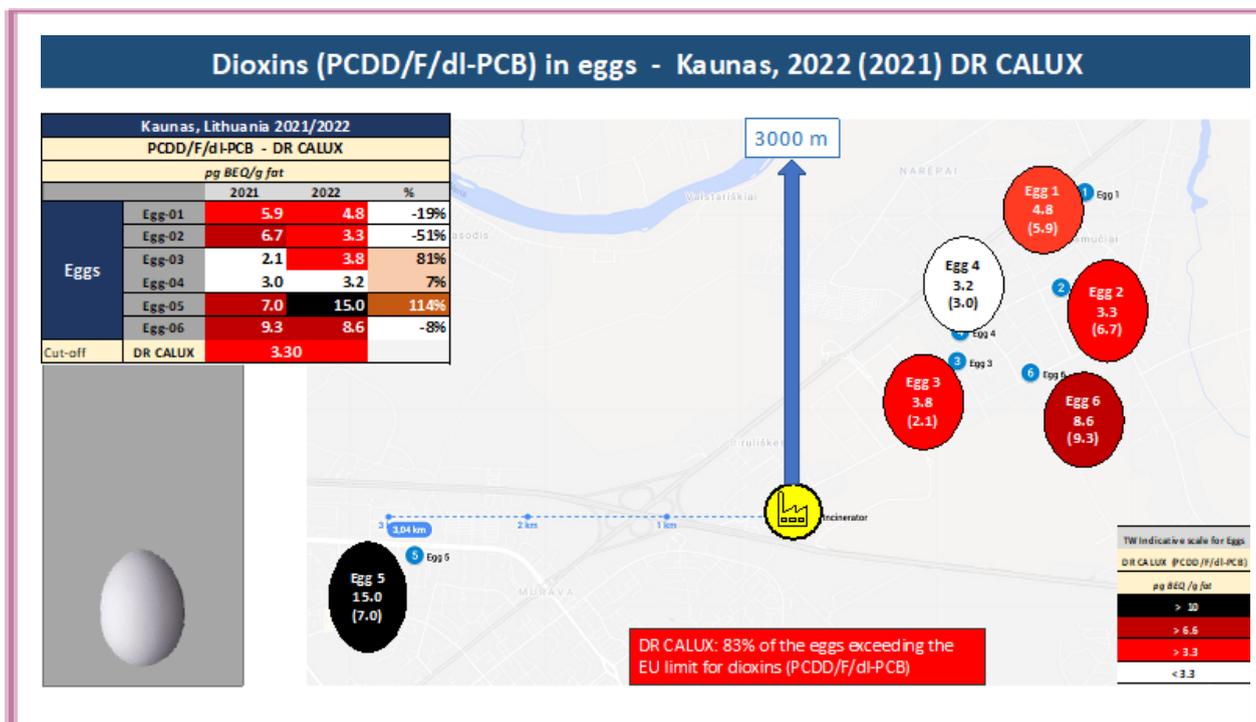
Kaunas, Lithuania 2021/2022				
PCDD/F - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	2.4	1.6	-35%
	Egg-02	2.3	1.2	-49%
	Egg-03	0.8	0.9	18%
	Egg-04	1.9	1.2	-39%
	Egg-05	2.2	1.5	-30%
	Egg-06	1.7	1.4	-15%
Cut-off	GC-MS limit	2.5		
Cut-off	GC-MS action	1.75		

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		

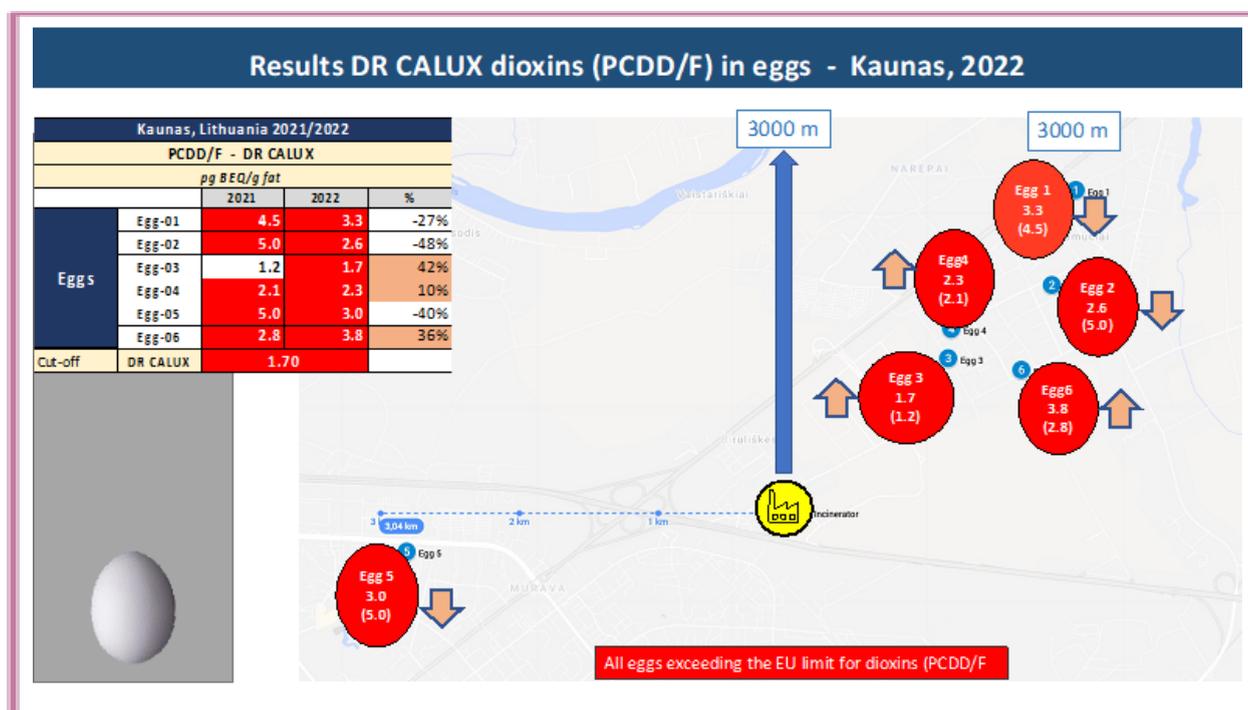
Kaunas, Lithuania 2021/2022				
dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	0.9	-35%
	Egg-02	0.9	0.6	-38%
	Egg-03	0.8	0.7	-13%
	Egg-04	1.1	0.7	-32%
	Egg-05	2.1	10.5	401%
	Egg-06	18.0	3.4	-81%
Cut-off	GC-MS action	1.75		

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

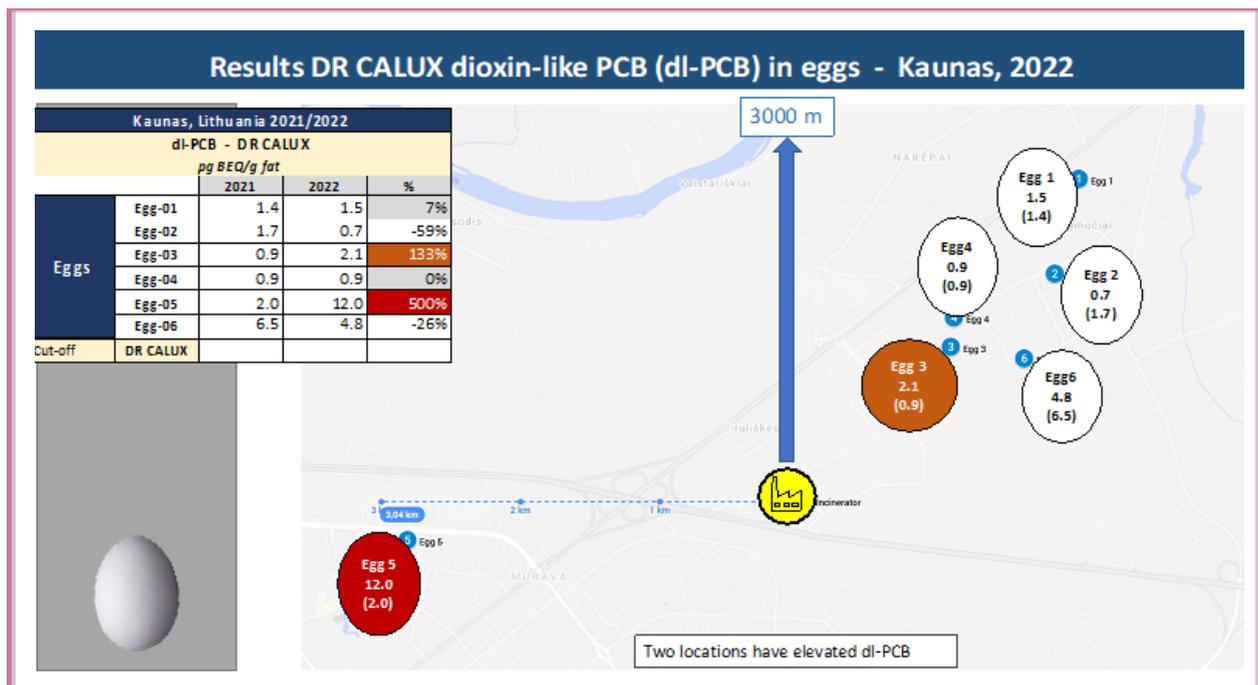


DR CALUX: 83% of the eggs exceeded the EU limit for dioxins (PCDD/F and DL-PCB).

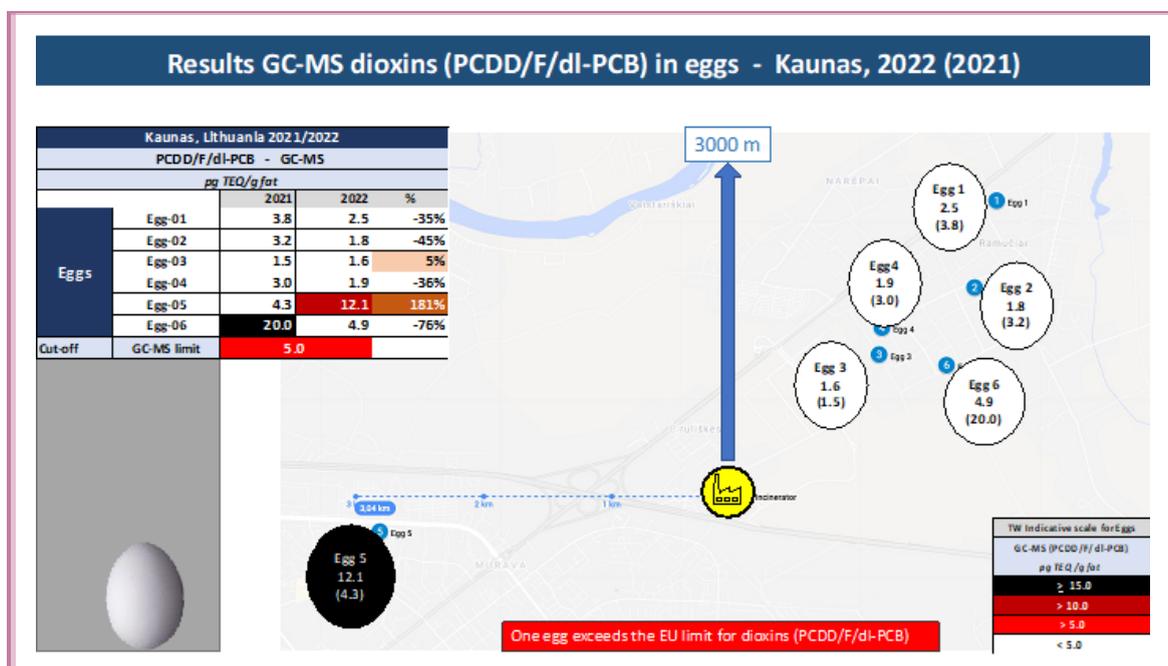


All eggs exceeded the EU limit for dioxins (PCDD/F) when the DR CALUX bioassay was used, with notable increases in dioxin (PCDD/F) levels in locations near the incinerator.

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



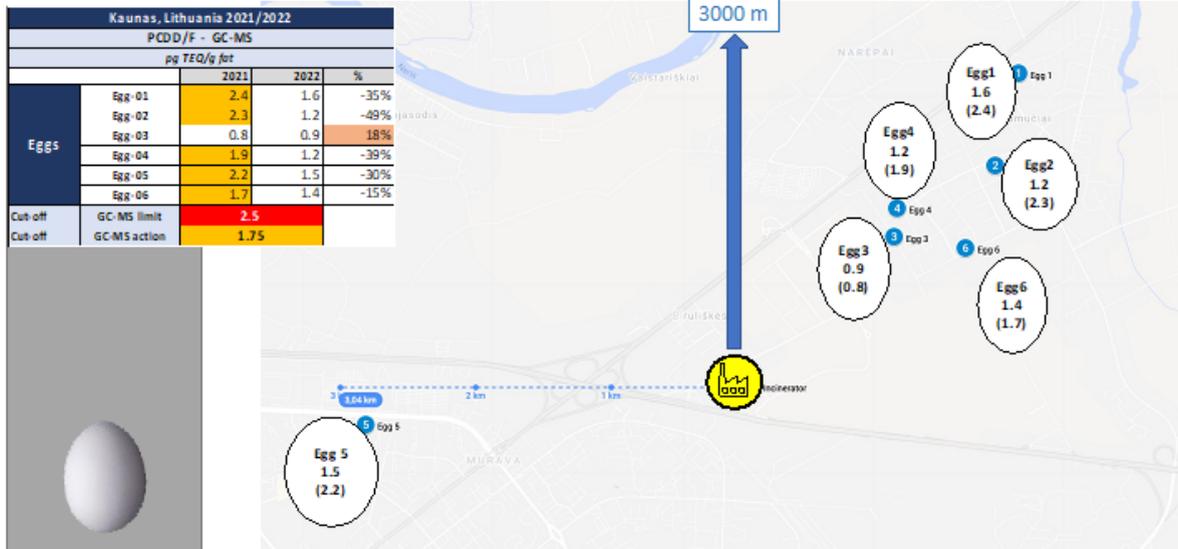
GC-MS results for dioxins in backyard chicken eggs



Four (4) locations showed a slight decrease in dioxins.

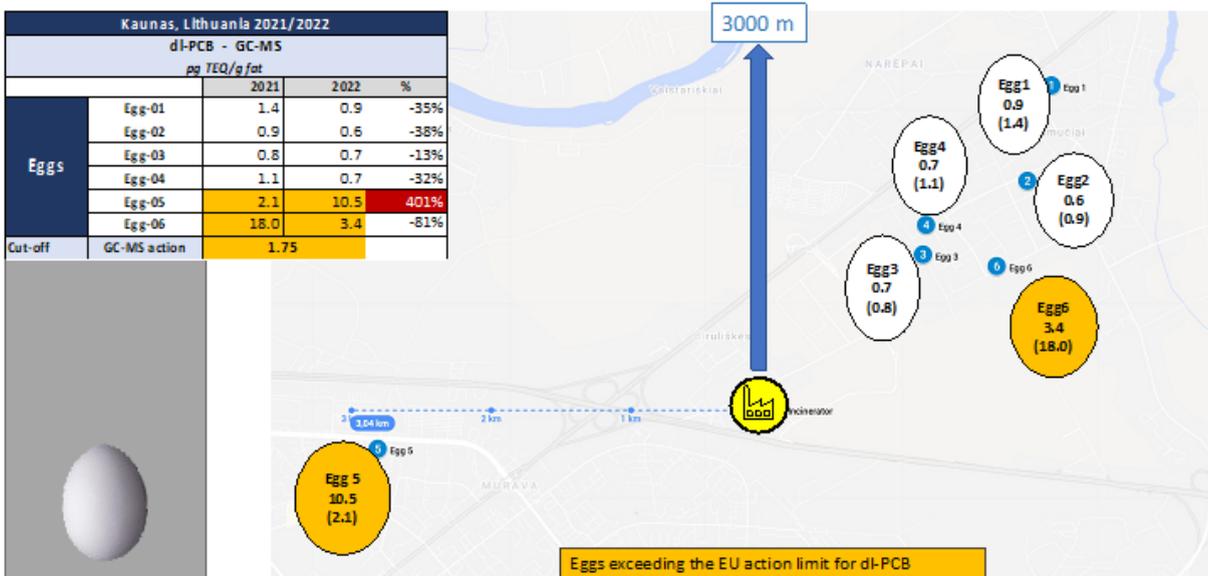
One (1) location exceeded the GC-MS safety limits for eggs by a factor of nearly 2, at 5 pg TEQ/g fat.

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



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.

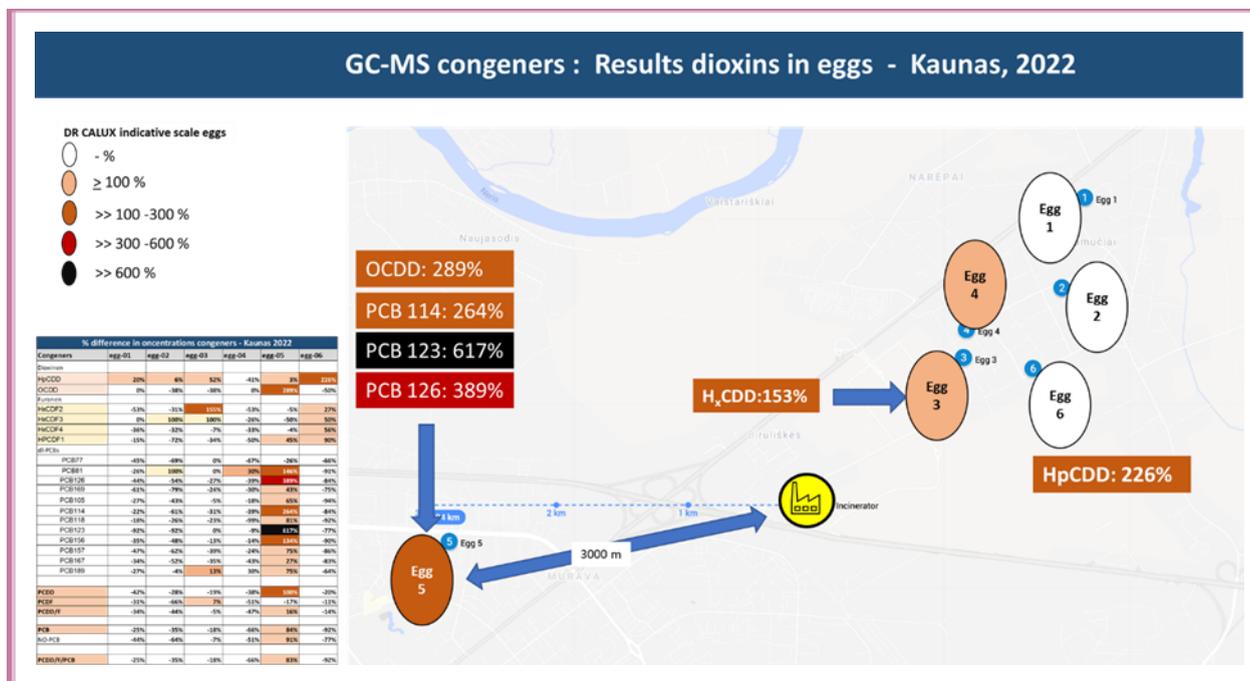
Results GC-MS dioxin-like PCB (dl-PCB) in eggs - Kaunas, 2022 (2021)



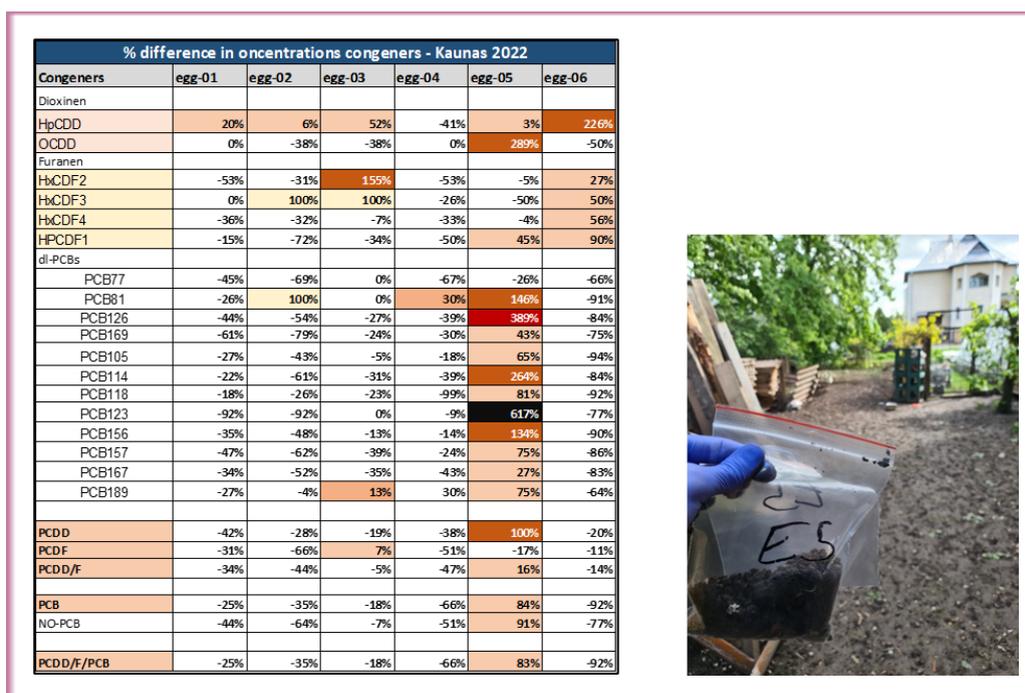
Two locations exceeded the DL-PCB action limit of 1.75 pg TEQ/g fat. There was a significant decrease in DL-PCB levels in location 5 and an increase in location 6.

Results - congener patterns in eggs

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.

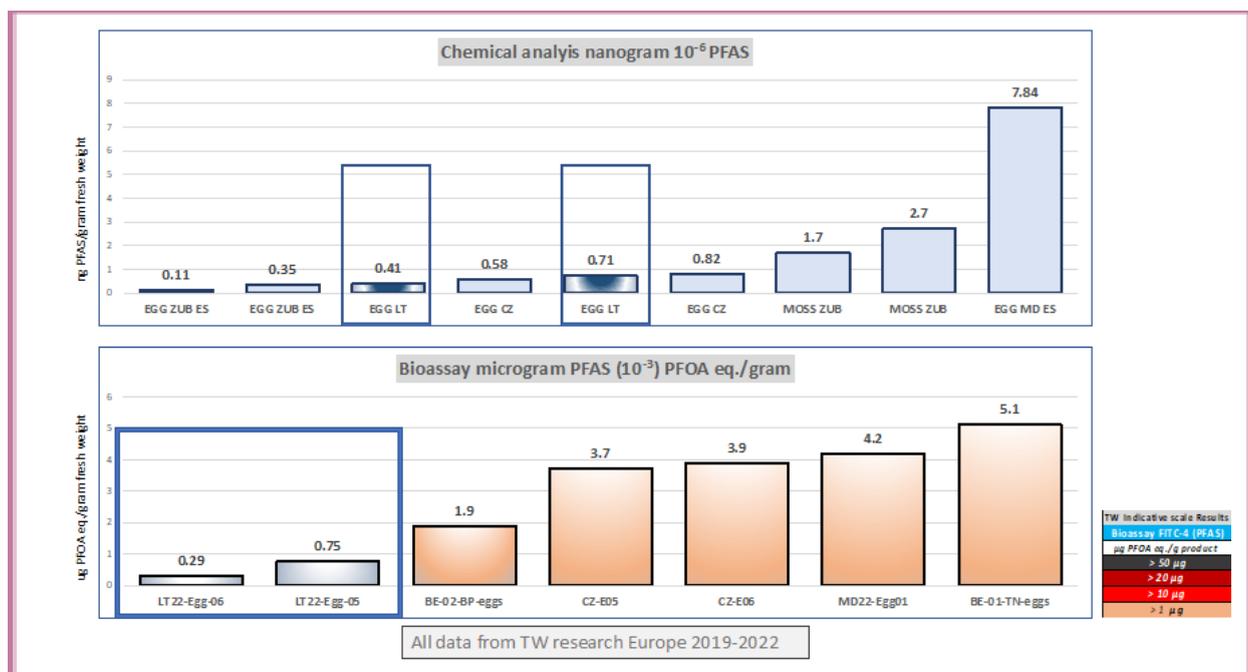


Significantly, egg location 5 shows high values of DL-PCBs. The figure below shows the specific congeners found. Most significant are the increased levels of PCB 123 and PCB 126.

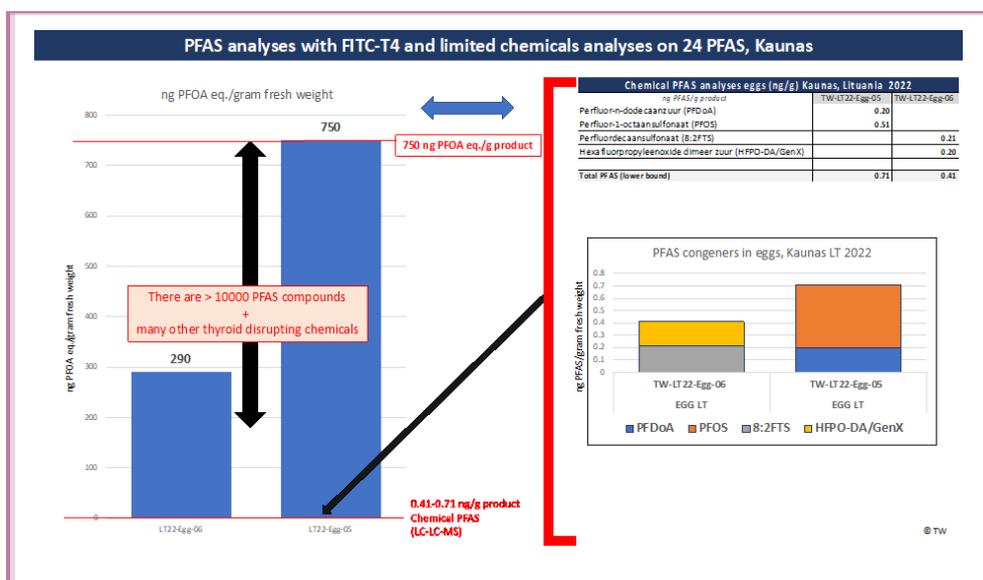


Results - PFAS in backyard chicken eggs

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



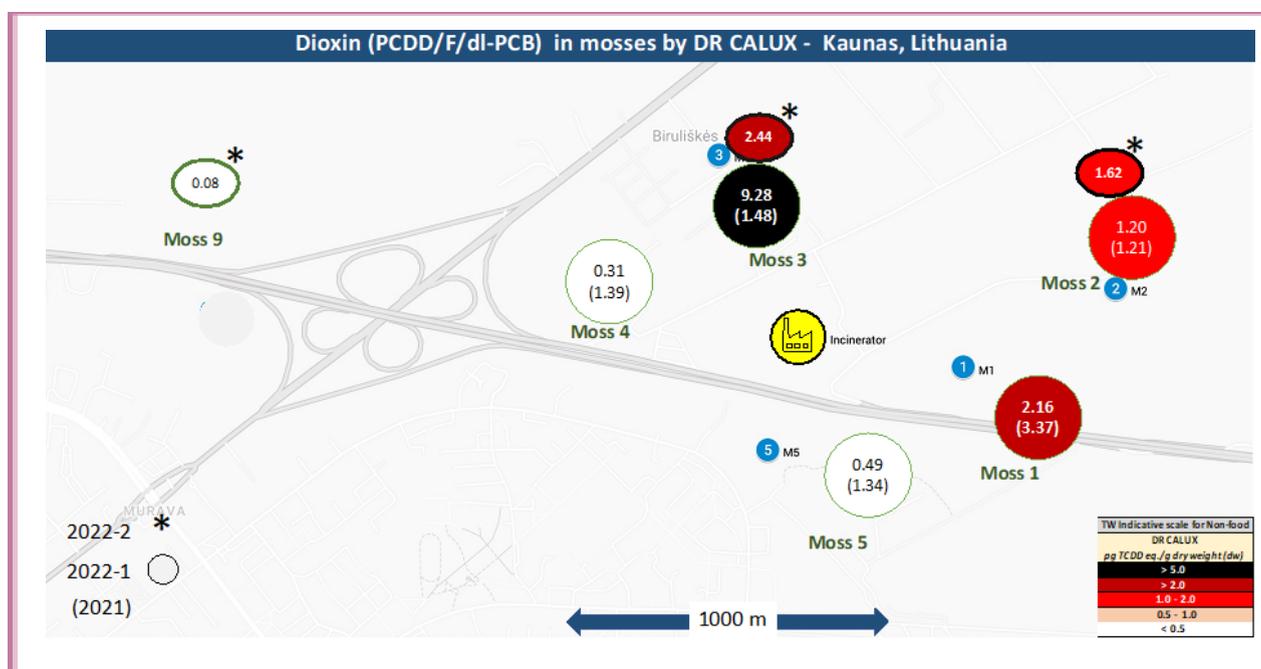
The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should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



Dioxins results in moss using the DR CALUX bioassay

The moss in the six (6) locations was mainly *Hylocomium splendens*, sampled around the incinerator, see the figure below. Moss 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 moss was sampled in July and October 2022.

The results for the moss 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 moss. 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 moss north of the incinerator, with a dioxin level of **2.44 pg TCDD eq./g dw**.



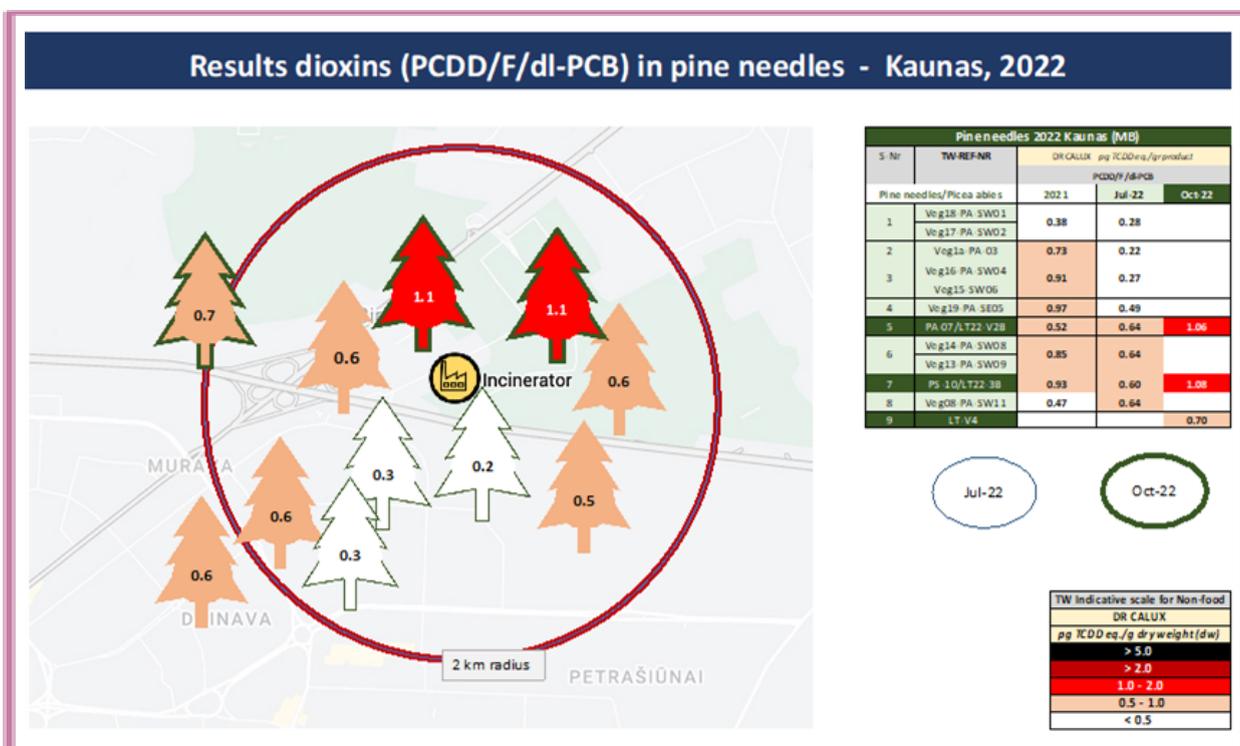
The measurements in 2021 and July and October 2022 are shown in the table below. There was a significant increase in DL-PCB levels in the samples taken in October 2022.

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



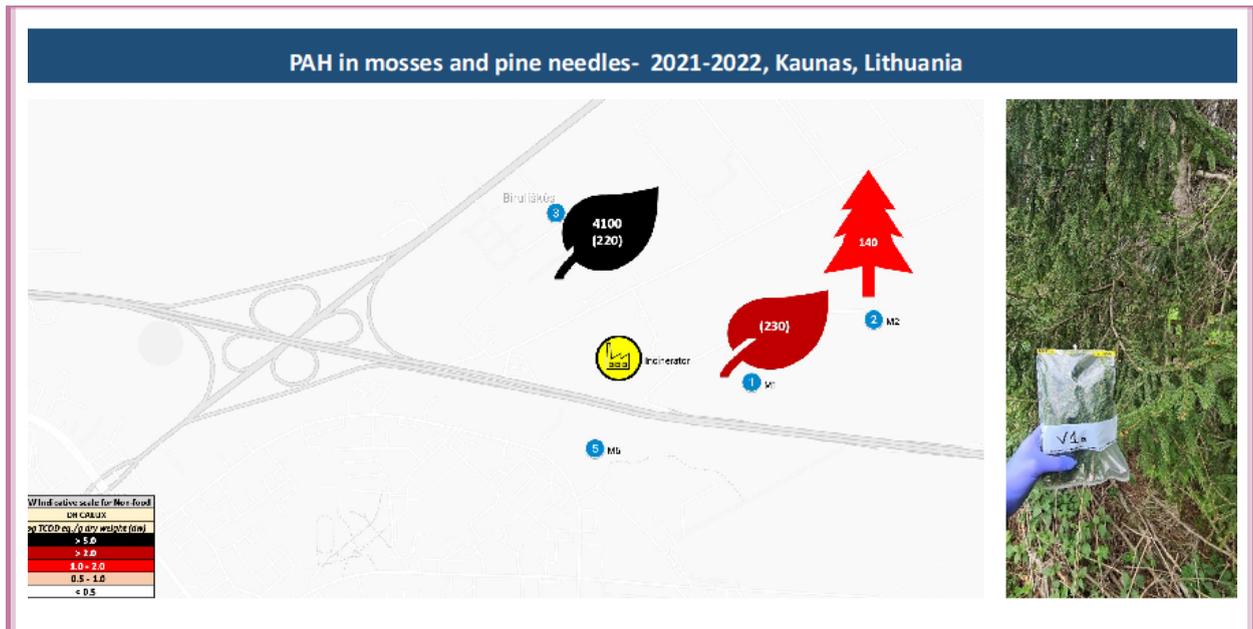
Results for dioxins (PCDD/F and DL-PCB) in pine needles

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 - PAHs in moss 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 moss locations at 220 and 230 ng BaP eq./g. In moss 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.

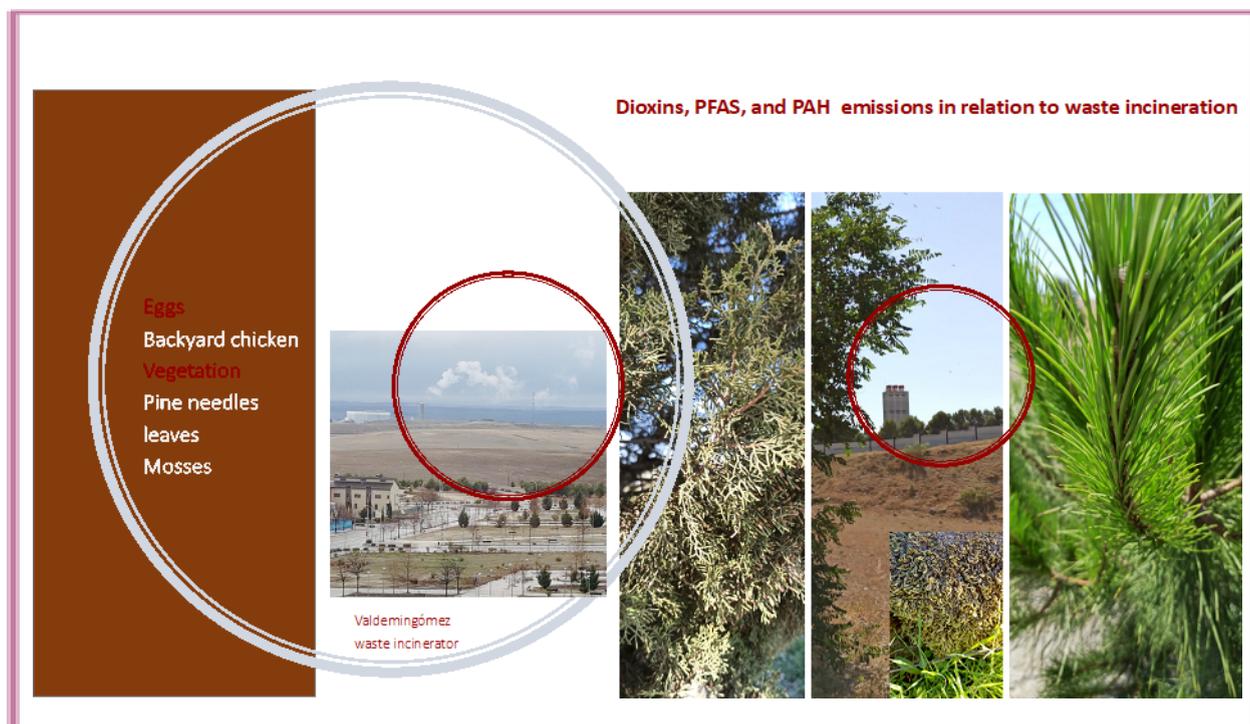




Madrid

Spain, 2022

Biomonitoring Research – Madrid, Spain, 2022



Analysis of backyard chicken eggs

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.

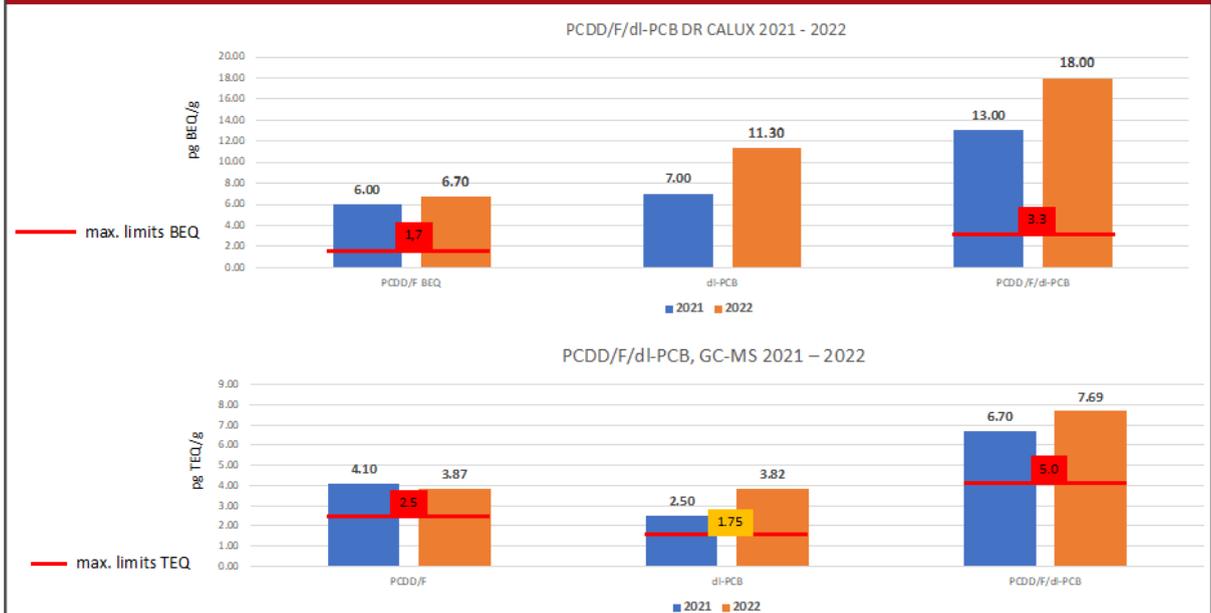
Extra GC-MS analysis is mandated in the EU regulation for commercial eggs when the results of the DR CALUX analyses are above the cut-off limit of 1.75 pg BEQ/g fat for dioxins (PCDD/F) and 3.3 pg BEQ/g fat for total dioxins, including dioxin-like PCBs (PCDD/F and DL-PCB). In this study, the eggs also 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).

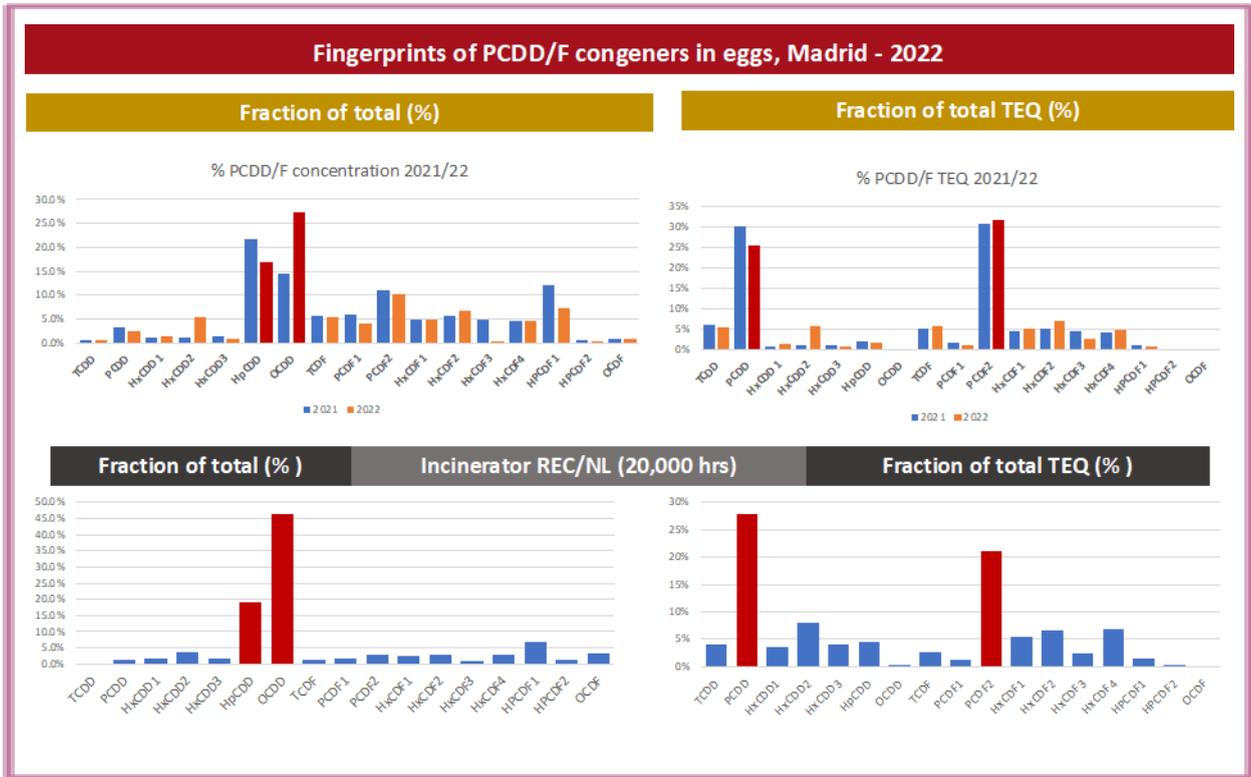
Results eggs sum of dioxins (PCDD/F/dl-PCB) DR CALUX, MADRID – 2021/22



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.

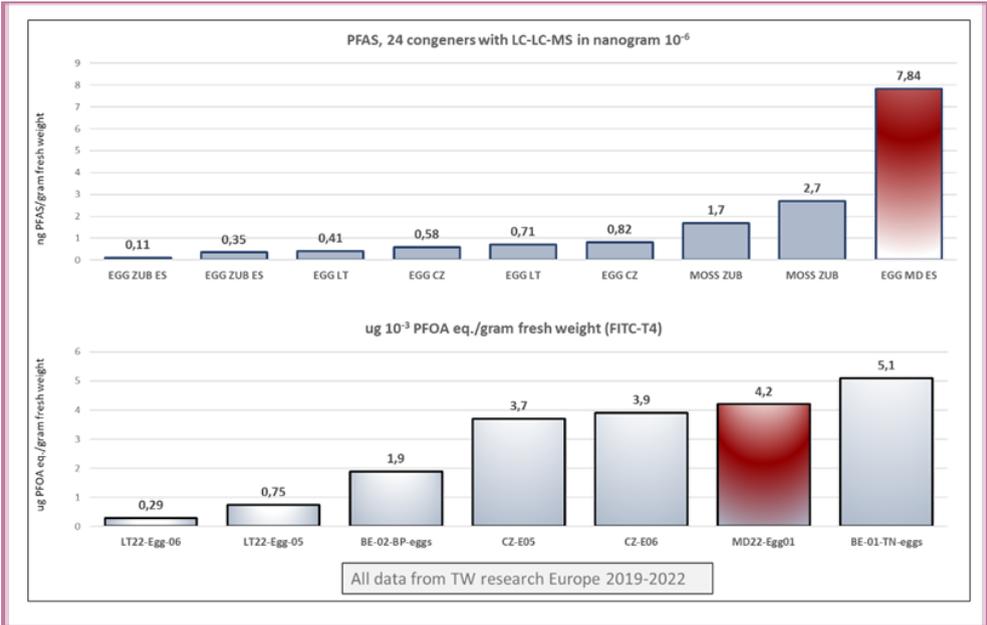
Results dioxins analyses eggs, MADRID – 2021/22



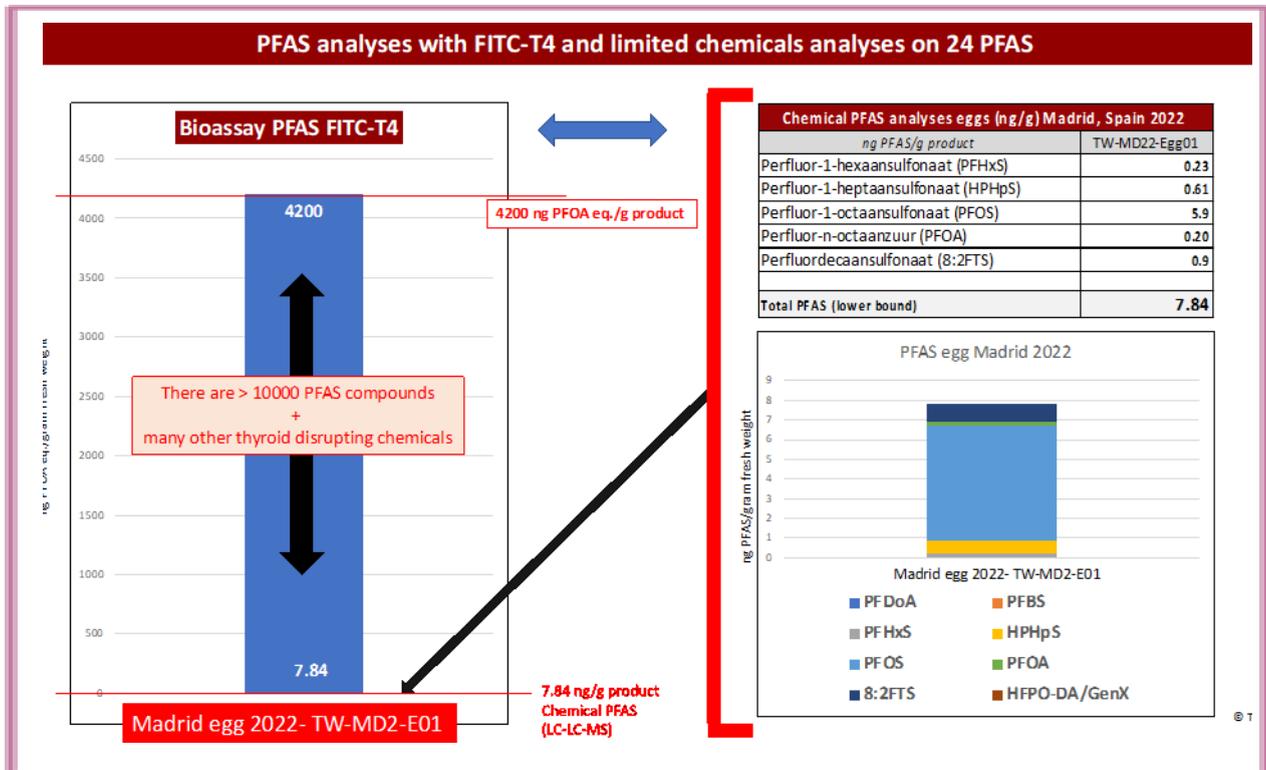


Analysis of PFAS in backyard chicken eggs

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). The chemical PFAS analysis (LC-LC-MS) showed different PFAS congeners in two eggs. PFOS and PFDaA 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.



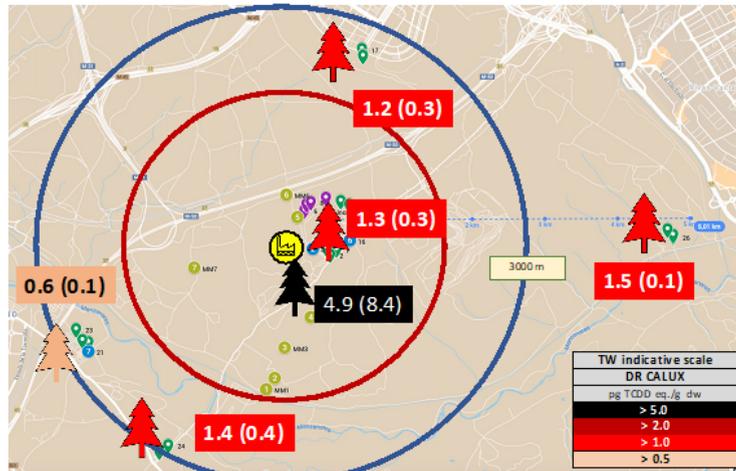
Analysis of pine needles and evergreen trees

The results of the first round of biomonitoring of moss 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.

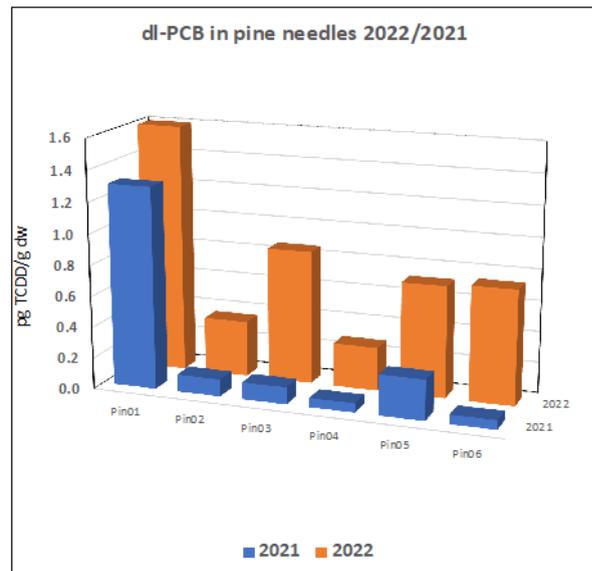
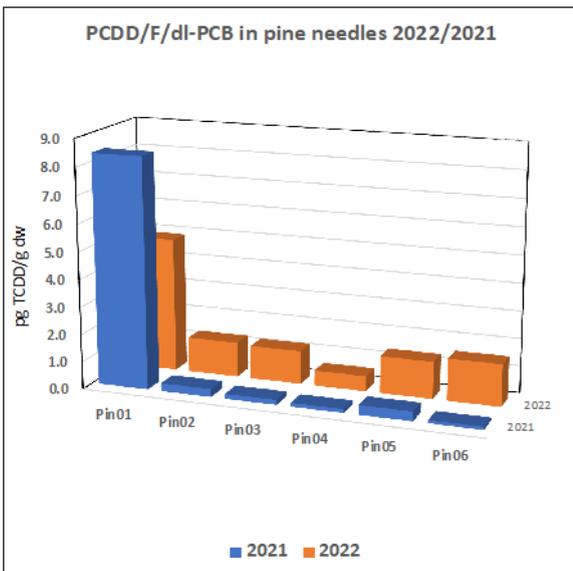
Although commercial use of polychlorinated biphenyls (PCBs) was banned almost half a century ago, contamination of the environment and organisms by PCBs is still observed. A serious increase was seen in several locations in 2022, see the figure on the left.

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.¹²

¹² efeverde.com/sacas-ceniza-toxica-aire-libre-valdemingomez-madrid

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



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



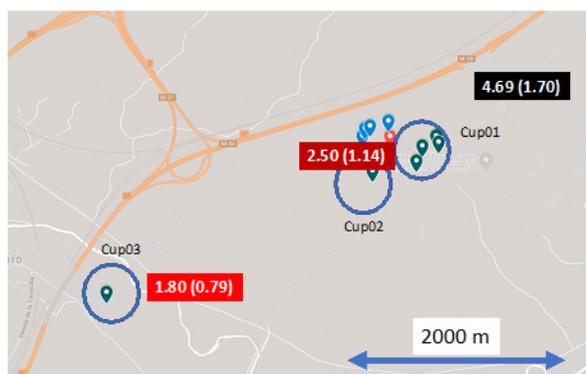
Analysis of needles of evergreen trees - *Cupressus arizonica*

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.

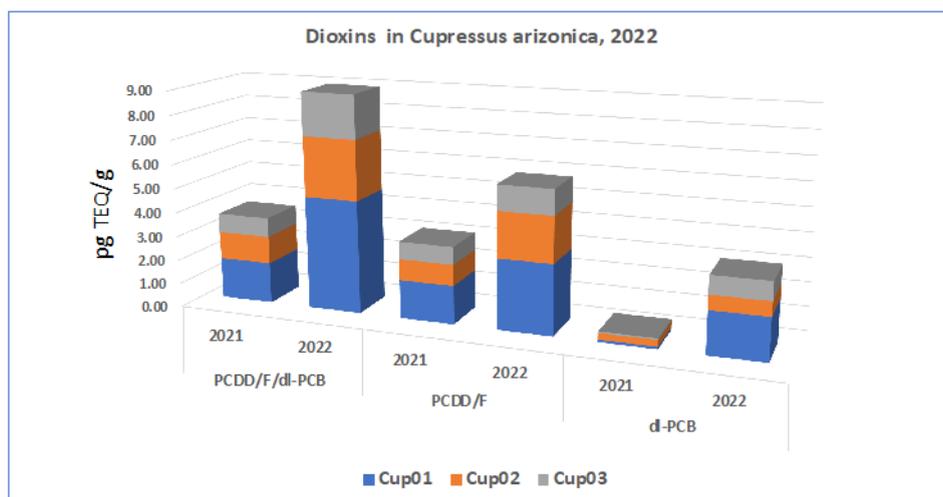
Dioxins in *Cupressus arizonica* 2022 (2021)

	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 <i>Cupressus arizonica</i> , 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.



Analysis of dioxins (PCDD/F and DL-PCB) in moss

The first sample round in 2022 took place in May. However, temperatures were high and not favourable for sampling moss. 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

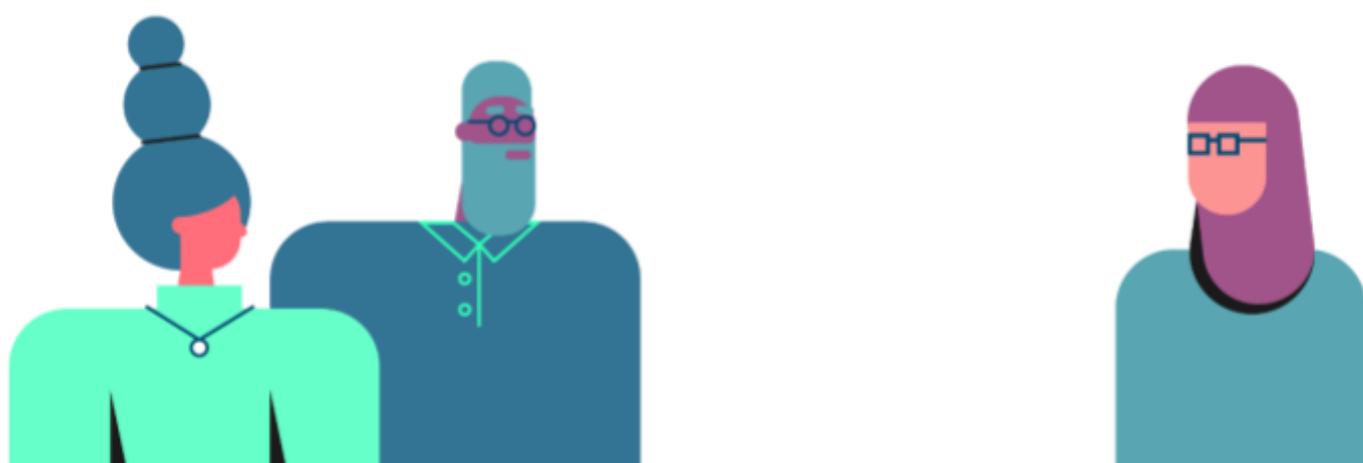


The results of the analysis on the moss around the Valdemingómez incinerator are similar to those for 2021. The difference is that an increase is seen both in the references nearby and in the reference location 35 km away, in Castello de Villa. The elevated level of dioxins (PCDD/F and DL-PCB) is mostly 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 moss, 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)				
Sample date	TW-REF-NR	Dioxin - DR CALUX 2022 (A)		
		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

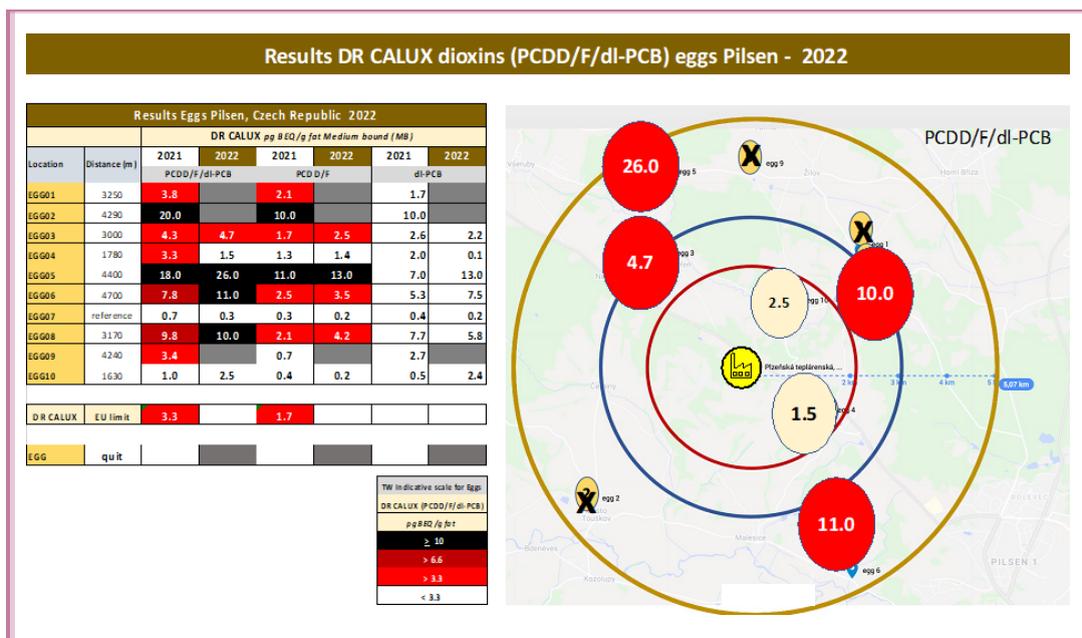
Czech Republic, 2022

Biomonitoring Research – Pilsen, Czech Republic, 2022

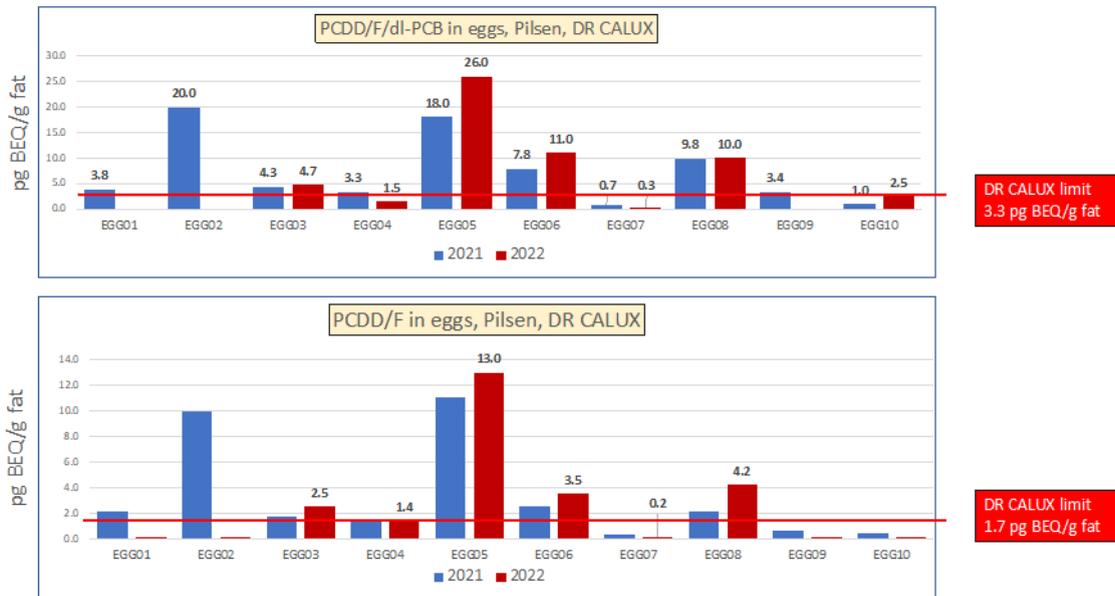


Bioassay analysis of dioxins in backyard chicken eggs

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.



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

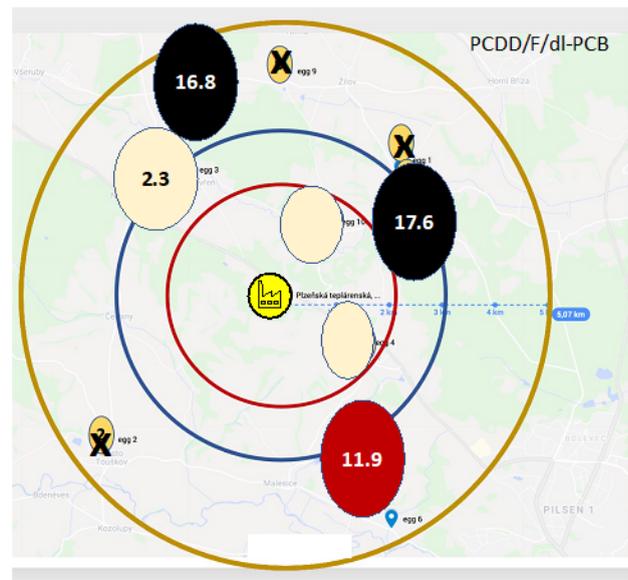


Chemical analysis of dioxins in eggs

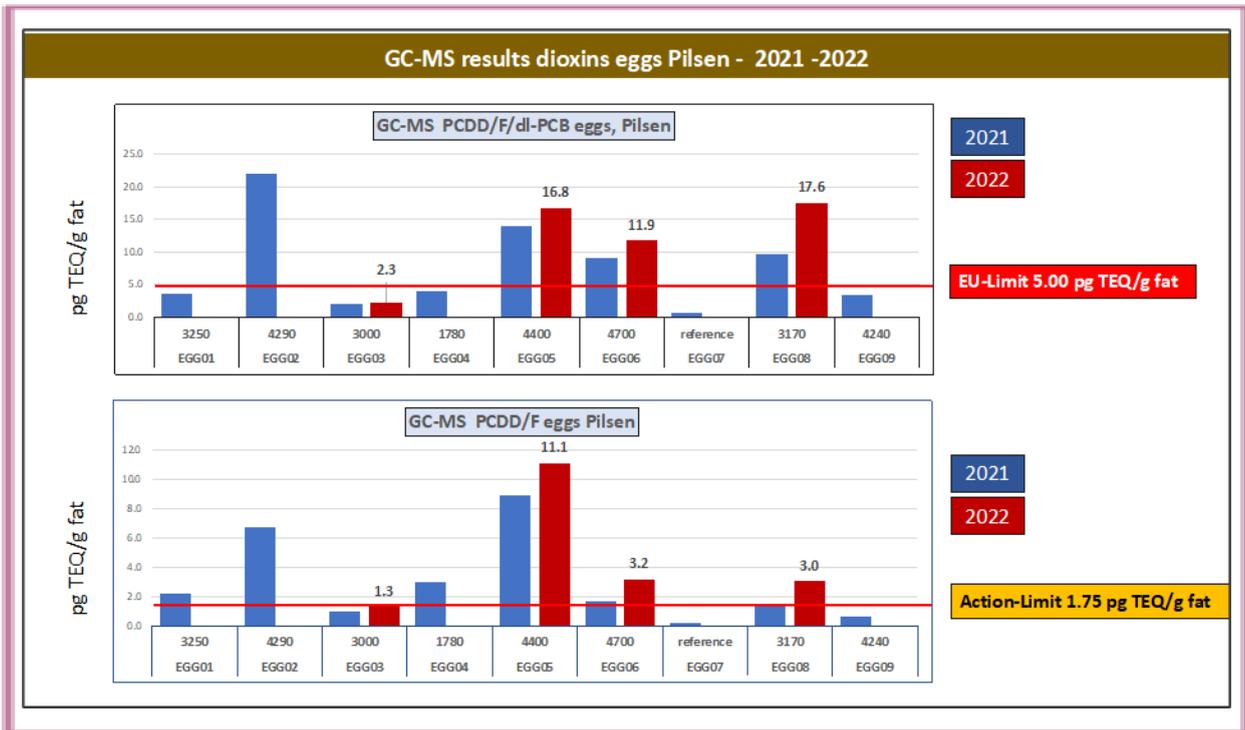
The GC-MS showed that two egg locations exceeded the maximum limit for dioxins by a factor of 3 and one location by a factor 2. The levels are 11.9, 16.8 and 17.6 pg TEQ/g fat. Three egg locations exceeded the DL-PCB action level of 1.75 pg TEQ/g.

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

Results Eggs Pilsen, Czech Republic 2021						
Location	Distance (m)	GC-MS		Medium bound (MB)		
		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	1830					
EU Limit	GC-MS	5,0		2,5		
Action limit	GC-MS			1.75		1.75
EGG	quit					



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.



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

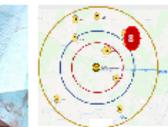
TW-CZ-Egg06		
	2021	2022
Distance	4670	4670
N hens	22	
N rooster	1	
Age	48 mnd	
Eggs/month	390	
Area m2	84	84
Outdoor fireplace	no	no
Wood burning stove	regular	regular
Pesticides use	not	not
Industry nearby	not	not
Highway nearby	no	no
DR CALUX pg BEQ/g fat		
PCDD/F	2.5	7.50
dl-PCB	5.3	3.50
PCDD/F/dl-PCB	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	Yellow
> 6.6	Orange
> 3.3	Red
< 3.3	Green
TW Indicative scale for eggs	
GC-MS (PCDD/F/dl-PCB)	
pg TEQ/g fat	
≥ 15.0	Yellow
> 10.0	Orange
> 5.0	Red
< 5.0	Green

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	Yellow
> 6.6	Orange
> 3.3	Red
< 3.3	Green
TW Indicative scale for eggs	
GC-MS (PCDD/F/dl-PCB)	
pg TEQ/g fat	
≥ 15.0	Yellow
> 10.0	Orange
> 5.0	Red
< 5.0	Green

Analysis of feed and soil

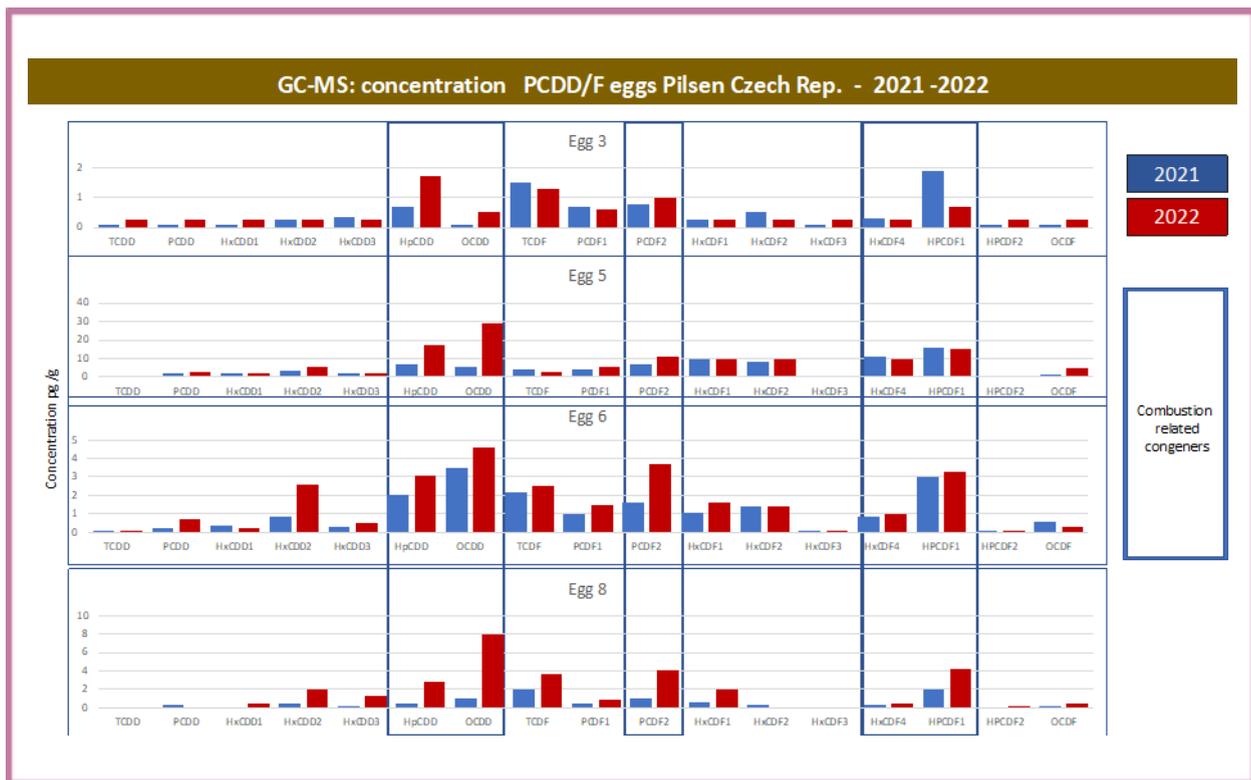
Feed and soil were analysed using DR CALUX for additional research concerning dioxin contamination in egg location 5. The soil measured 2.1 pg BEQ/g PCDD/F and DL-PCB,

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

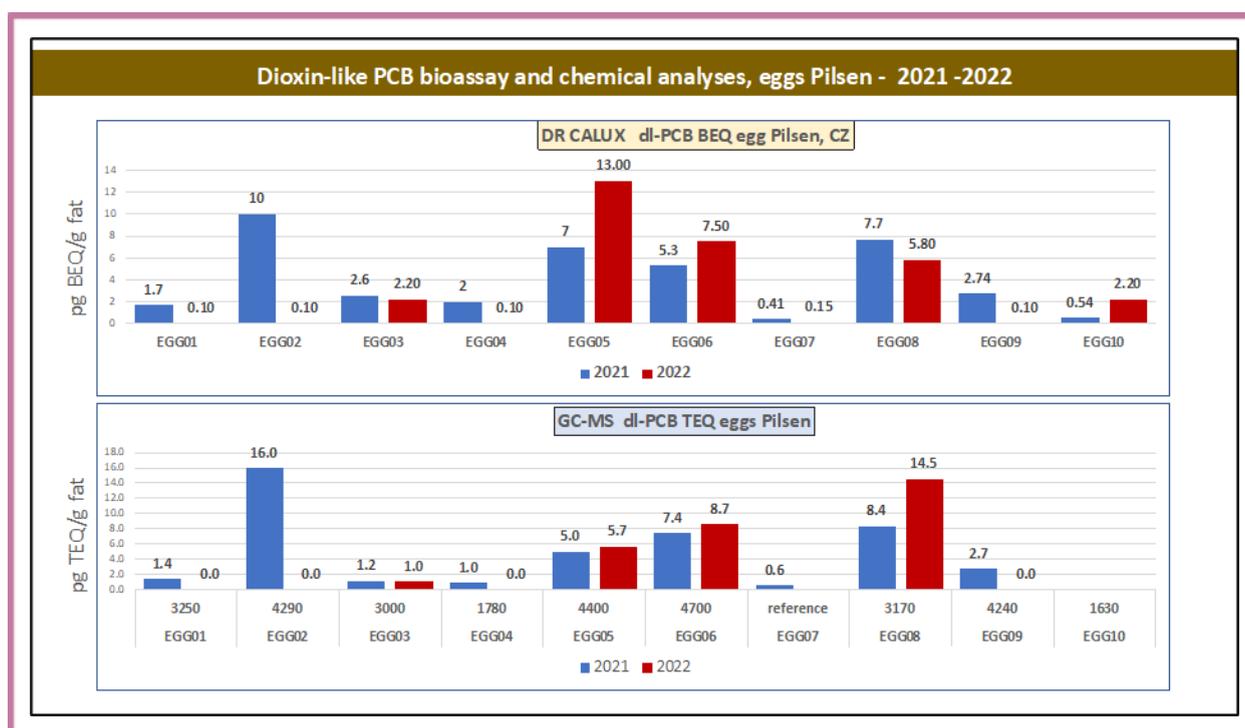
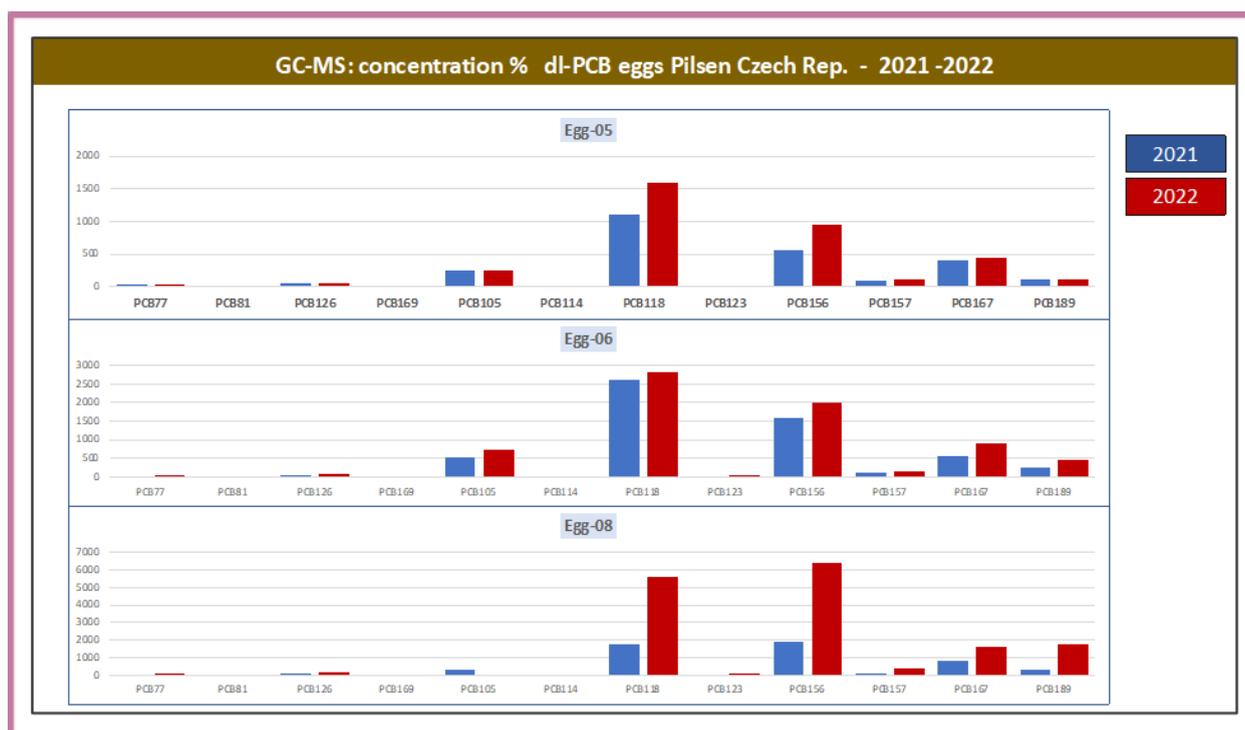
Analysis of dioxin congeners

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.

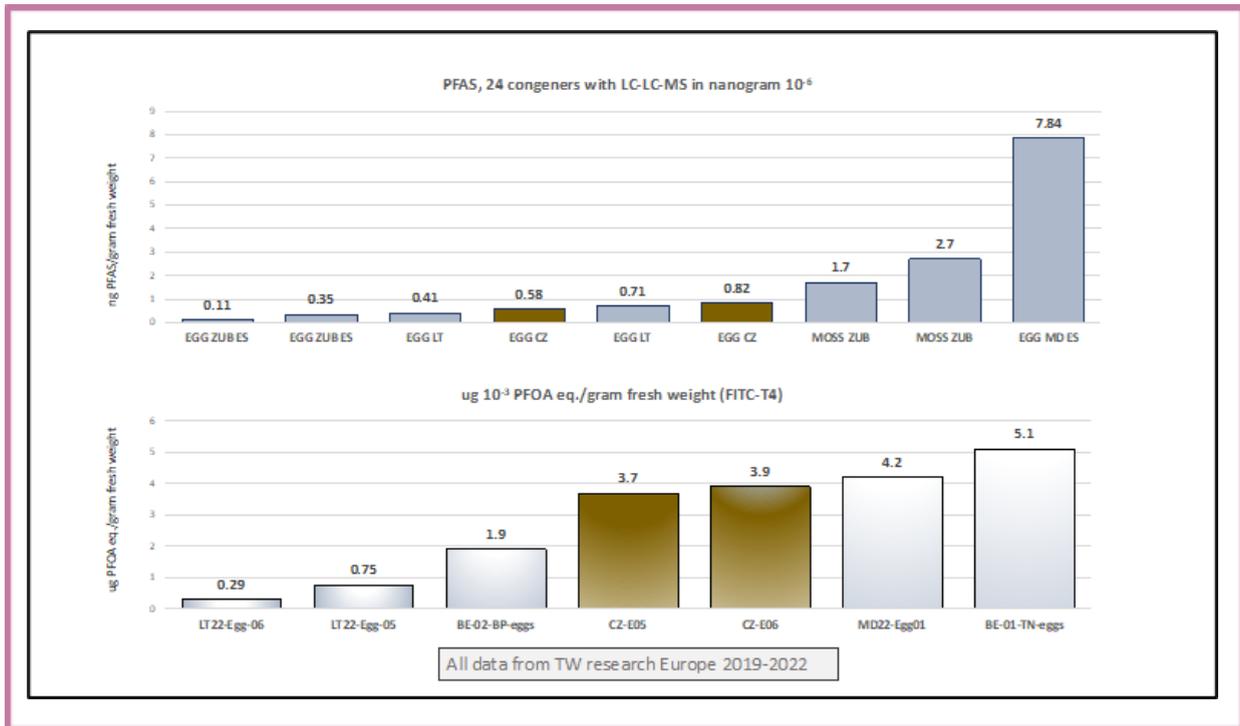


Analysis of PFAS in eggs

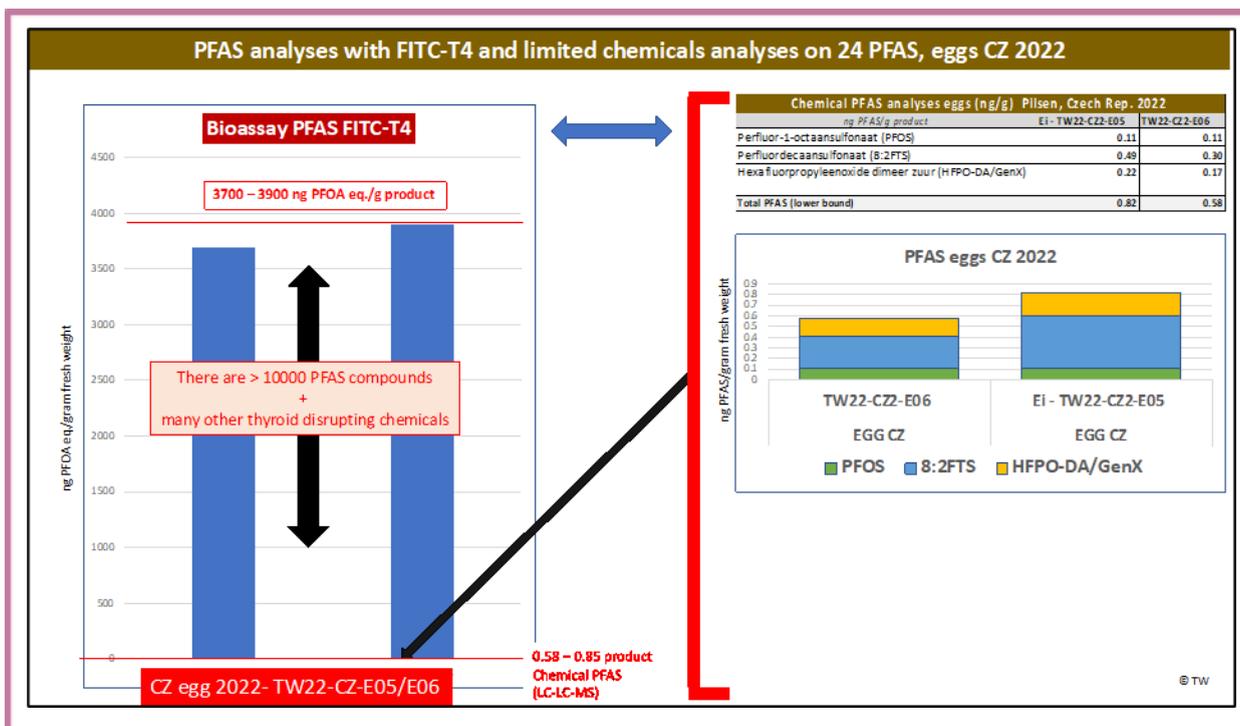
In this biomonitoring research, TW analysed PFAS in eggs. PFAS can also be a product of the waste incinerator (see the explanation in the first TW report in 2021)¹³. The PFAS

¹³ zerowasteurope.eu/library/the-true-toxic-toll-biomonitoring-of-incineration-emissions

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

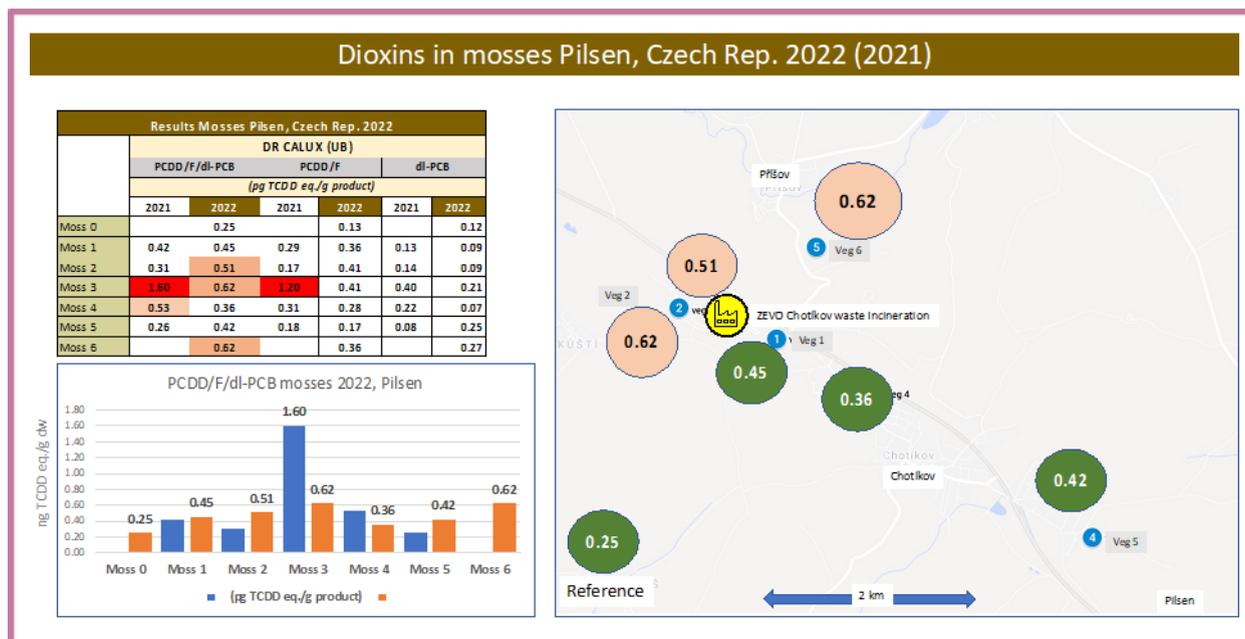


The FITC-T4 bioassay for analysis of total PFAS toxicity demonstrates the huge gap between the current chemical analysis of only four regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is strongly recommended that bioassays should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



Analysis of moss

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.¹⁴



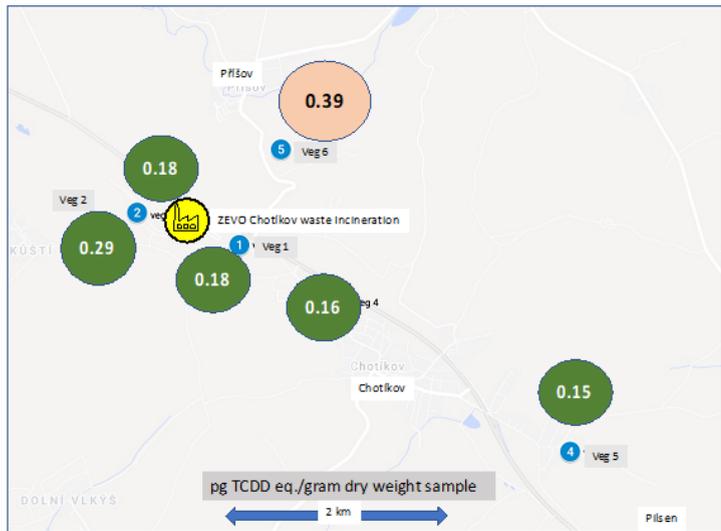
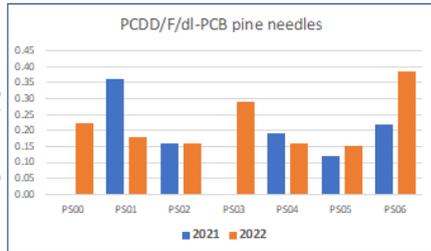
Analysis of pine needles

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.

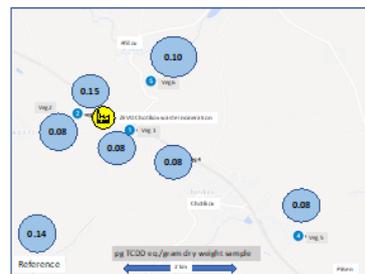
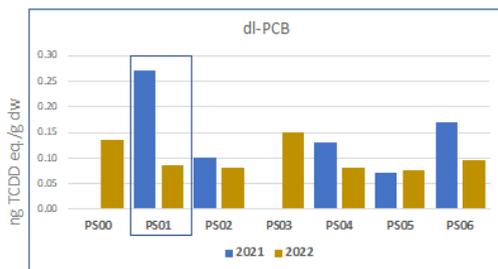
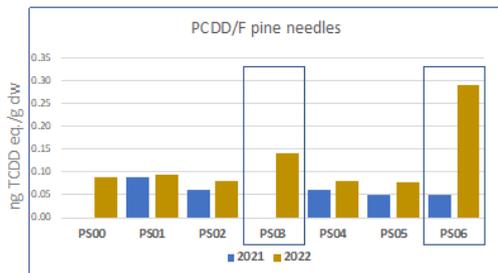
¹⁴ Knutsen HK et al. (2018) *Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food*. EFSA Journal 2018;16(11):5333, 331, p. 189

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

Pinus sylvestris - Czech Republic 2022						
	DR CALUX (UB)					
	PCDD/F/dl-PCB			dl-PCB		
	(pg TCDD eq./g product)					
	2021	2022	2021	2022	2021	2022
PS00		0.22	0.09	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

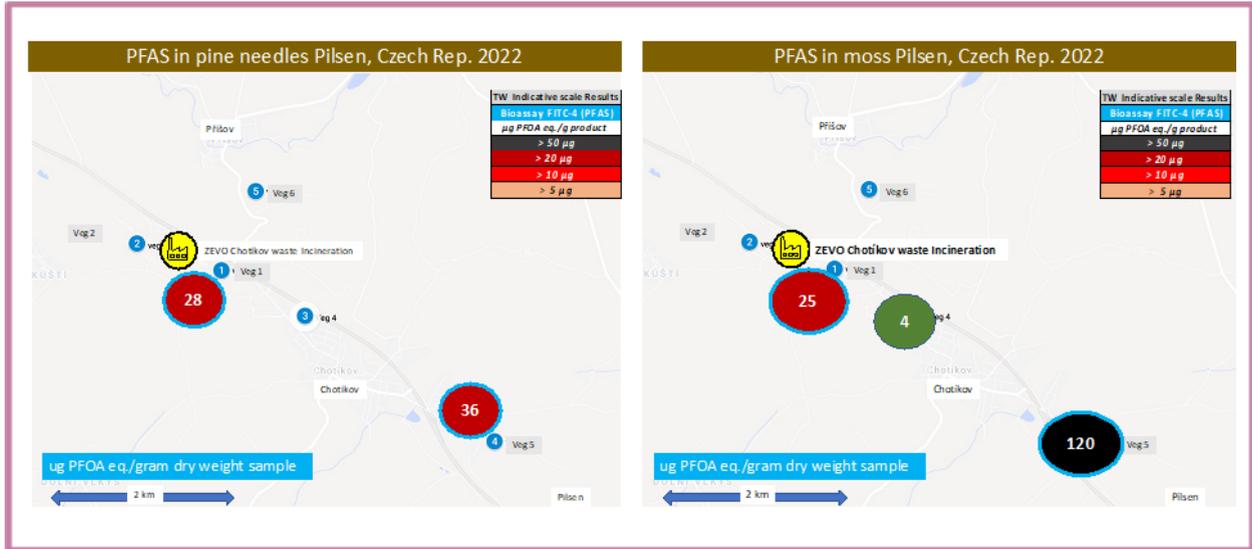


PCDD/F and dl-PCB in pine needles Pilsen, Czech Rep. 2022 (2021)



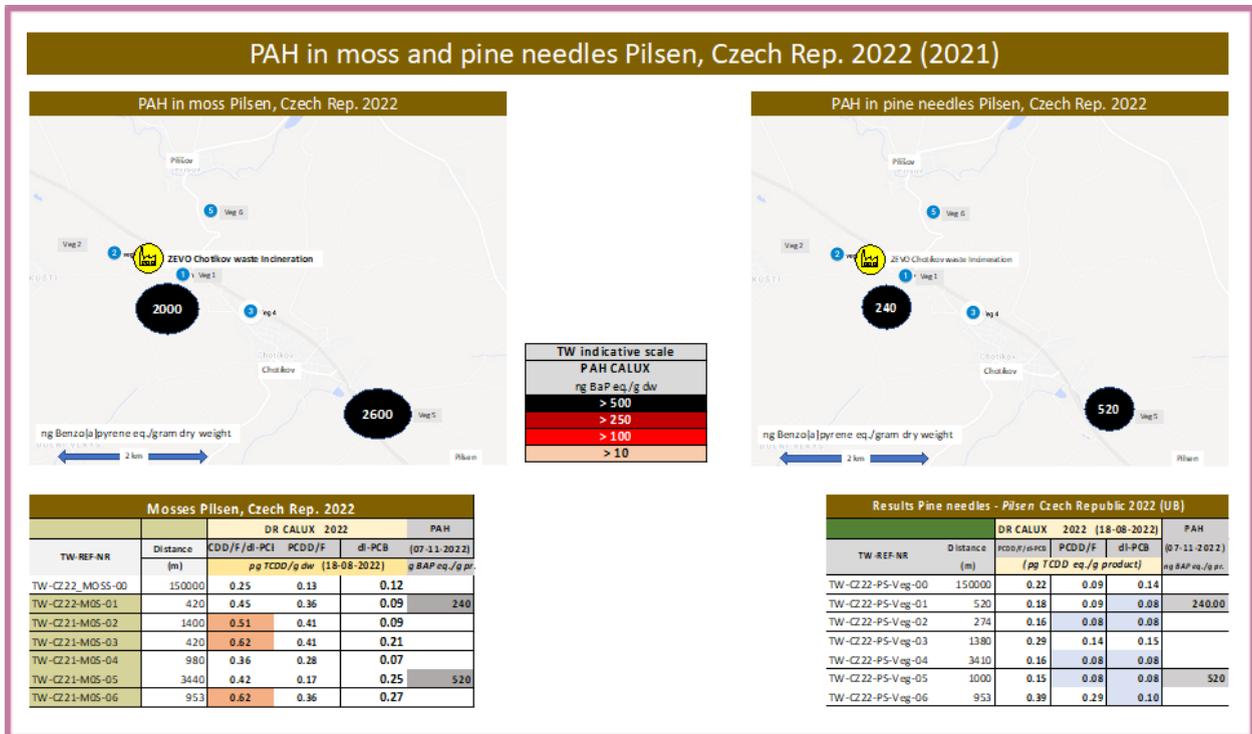
Analysis of PFAS in moss and pine needles

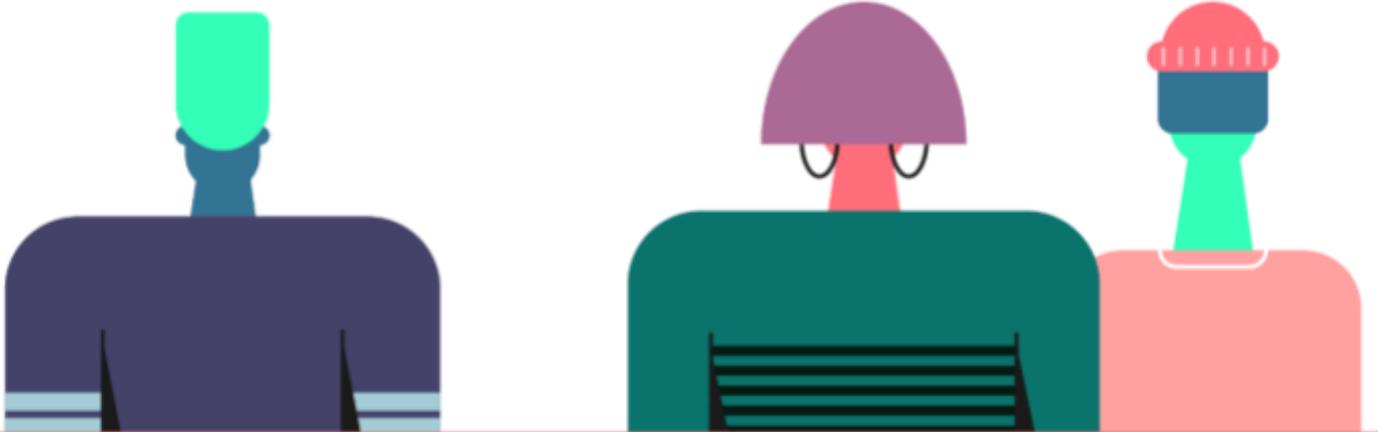
Analyses were performed on PFAS using the FITC-T4 bioassay. It should be noted that the highest concentrations of PFOA equivalent were found both in pine needles and moss at a location (5) 2000 m southwest of the waste incinerator, near the city of Pilsen.



Analysis of PAH in moss 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 moss 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 moss and 520 ng benzo(a)pyrene eq./g dw in the pine needles at this location.





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