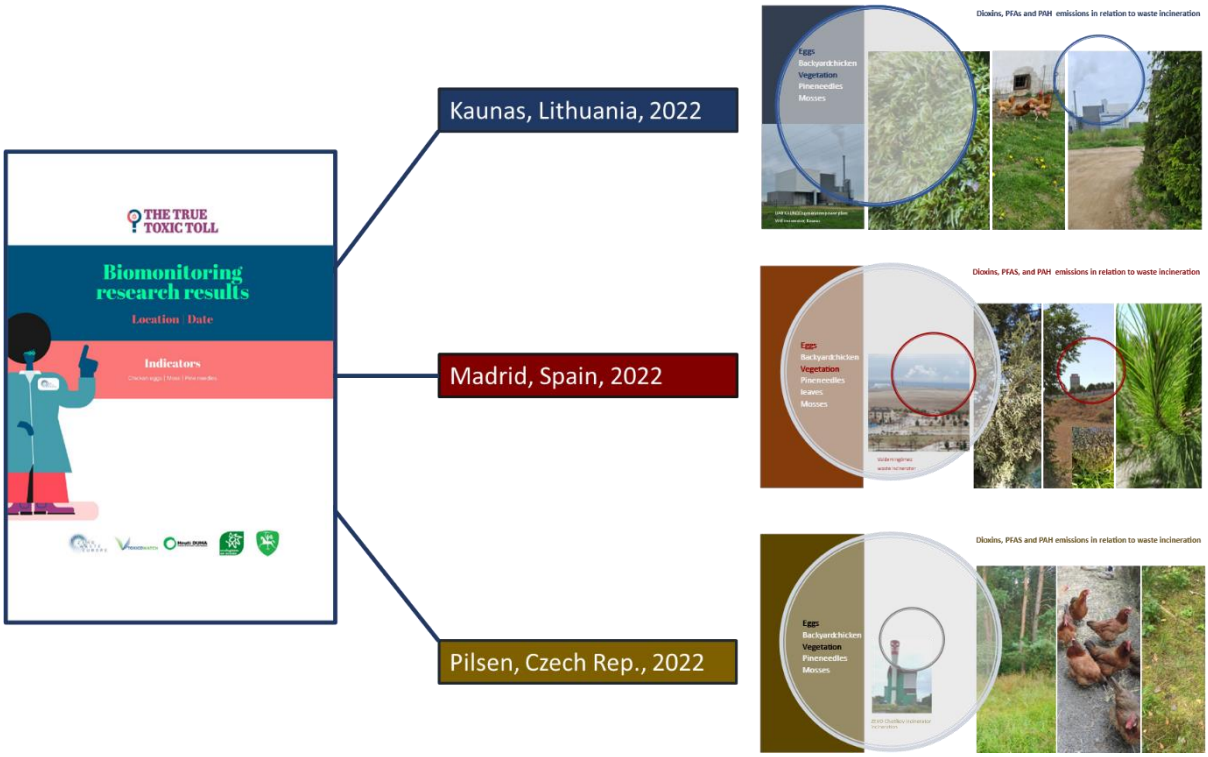
TOXICO WATCH

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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Acknowledgements

Thanks to Zero Waste Europe for enabling research on persistent organic pollutants (POPs).

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

All photographs, graphs and tables are designed by ToxicoWatch, unless stated otherwise. Location photo's Lithuania, Kaunas by D. Tracevičius (*Žiedinė Ekonomika*), Czech Rep. Pilsen by Ivo Kropacek (*Hnutí DUHA)* and Madrid, Spain by Beatriz Martinez (*Ecologistas en Acción*).

www.toxicowatch.org

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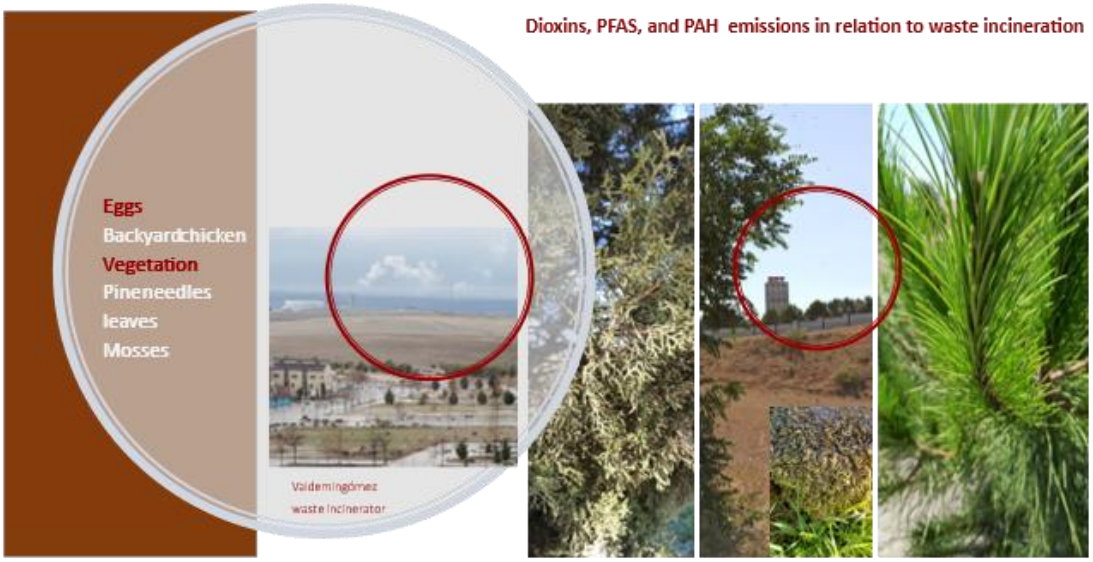
Abbreviation	Meaning
APCD	Air Pollution Control Devices
BAT	Best Available Techniques
BEP	Best Environmental Practice
BEQ	Biological Equivalents
BMI	Body Mass Index
dl-PCB	Dioxin-Like Polychlorinated Biphenyls
DR CALUX®	Dioxin Responsive Chemical-Activated LUciferase gene eXpression
dw	Dry Weight
EFSA	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 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)

Abbreviation	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 TW Biomonitoring reports 2021 on
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 biomonitoring 2022
Kaunas (Lithuania), Madrid (Spain), Pilsen (Czech Rep.)

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

The biomarkers were analysed on 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. Also, in vegetation increased amounts of hazardous persistent organic pollutants are found in the vicinity of the waste incinerators. Summarising the results of 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 exceed 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.

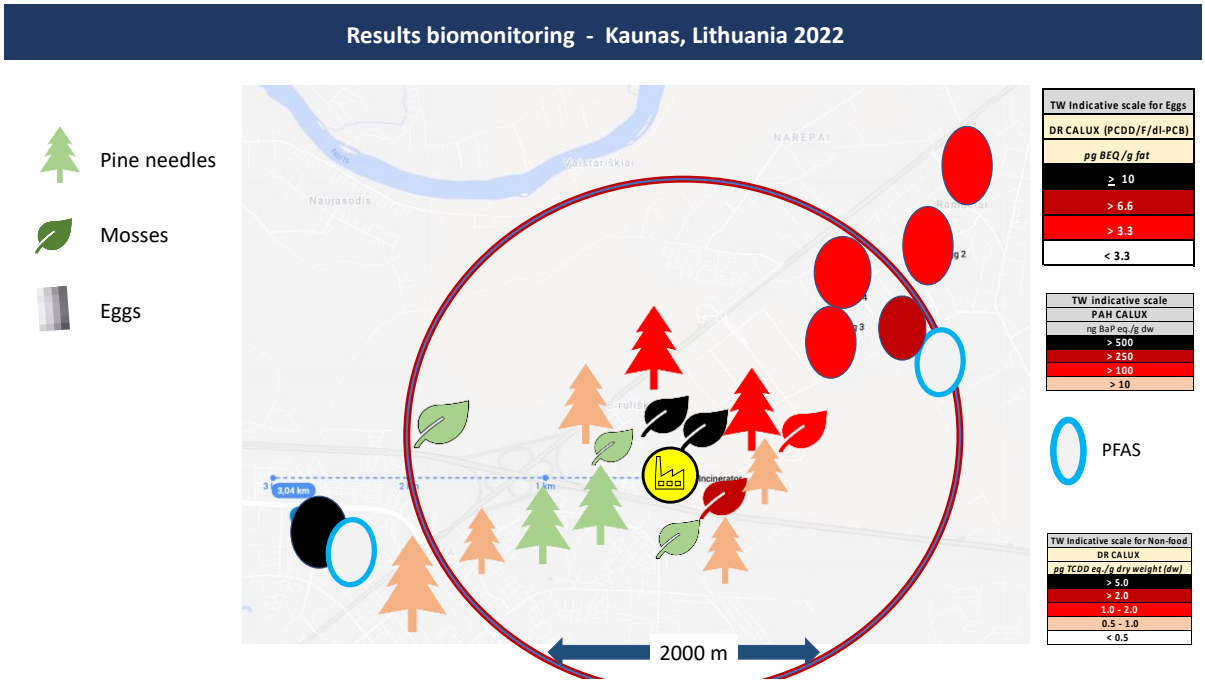
An additional consideration is the limitations of chemical analysis (GC-MS) for POPs, which can however determine individual congeners but does not measure the total toxicity (of thousands of toxic substances) like dioxins and dioxin-like substances (PCDD/F/dl-PCB), PAH and PFAS. The DR CALUX bioassay 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 4 regulated PFAS compounds versus the total toxicity of thousands of other PFAS compounds, which could be present. Therefore, it is highly recommendable that bioassays should be included as standard as well in the regulated (EU) monitoring of POPs from emission sources, such as waste incinerators.

The most polluted area in this biomonitoring research, is found to be in Madrid near Valdemingómez, with one of the oldest waste incinerators in Europe. Recalling the Stockholm Convention on Persistent Organic Pollutants 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.*

Kaunas, Lithuania biomonitoring results 2022

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



Eggs

All backyard chicken eggs exceed the EU limit on the bioassay for dioxins (PCDD/F) with notably increasing levels of dioxins (PCDD/F) in locations nearby the incinerator. One location with an exceptionable high level of dl-PCB is found. Biomarkers of eggs, northeast and west, show elevated levels of dioxins and dioxin-like PCB. PFAS is found in eggs with the bioassay and with the limited chemical analysis, PFOS and Gen-X could be identified.

Mosses

High levels of dioxins and furans (PCDD/F) are measured in mosses in the direct environment of the incinerator. The difference between the results of the bioassay and the chemical analysisindicates 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 are elevated in the direct environment of the incinerator, northwest and west oriented. In 2022 PAH is 20 times increased in mosses northwest from the incinerator

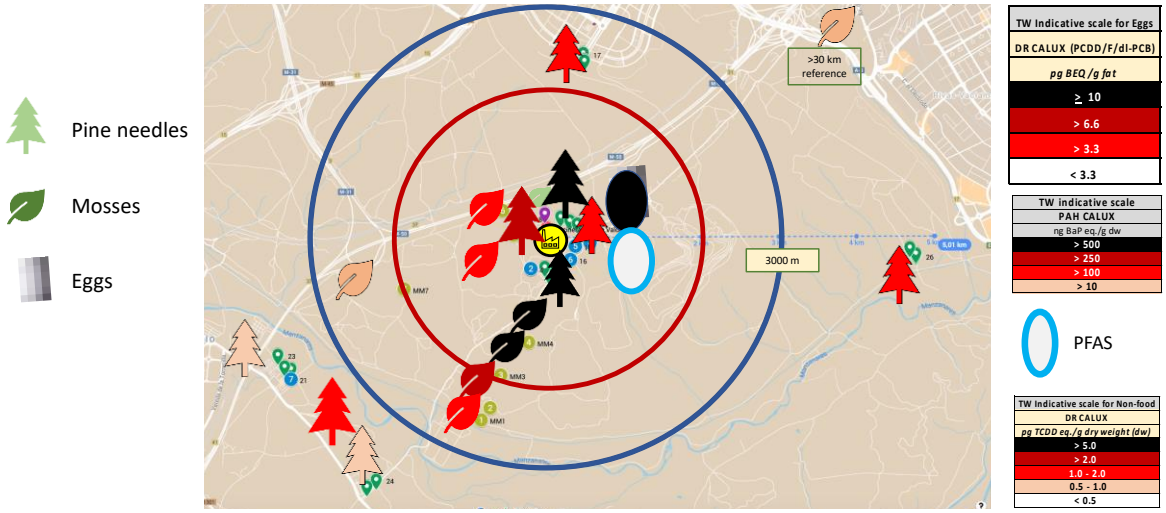
Pine needles

The bioassay DR CALUX analyses demonstrate a predominantly increase in dioxins (PCDD/F) inthe vicinity of the waste incinerator. In pine needles located east also PAH is detected.

Madrid, Spain biomonitoring results 2022

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

Conclusion biomonitoring Madrid – 2022



Eggs

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

Mosses

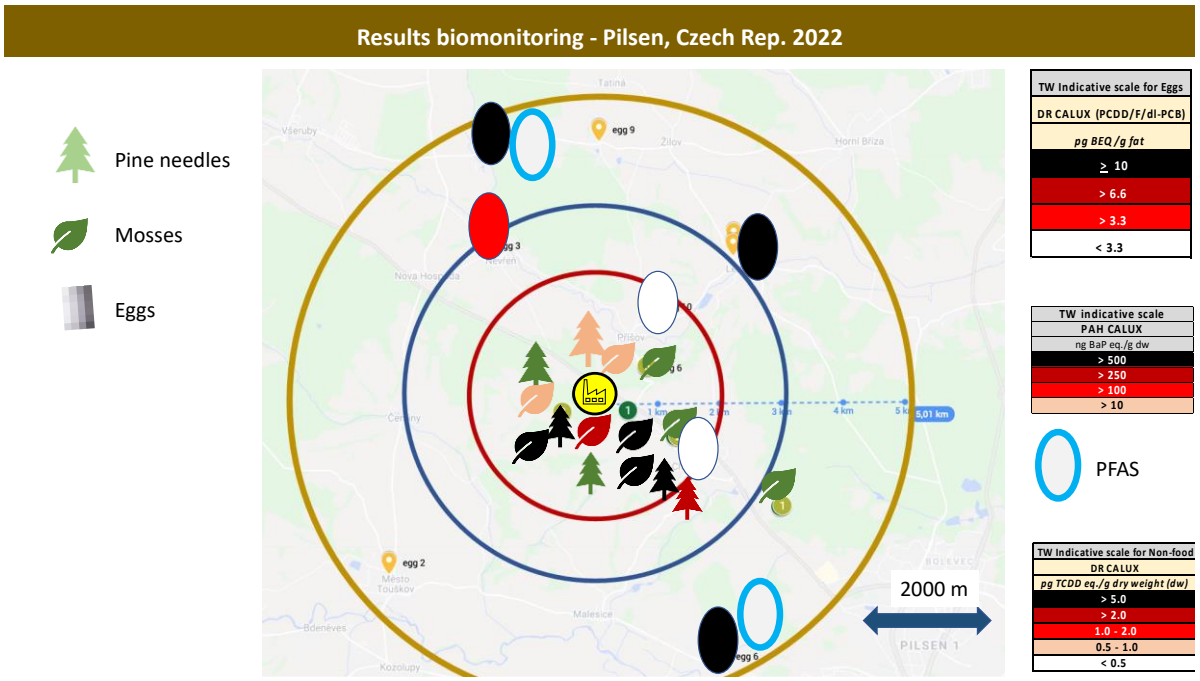
The pattern of elevated dioxins (PCDD/F/dl-PCB) in mosses, most elevated 1500 -2000 meter southwest, has been found again in this research. The highest values for dioxins ever measured in TW European biomonitoring survey were measured in the mosses around Valdemingómez.

Pine needles

Pine needles and foliage of evergreen trees surrounding the fence of the site of the waste incinerator are loaded with the highest levels of dioxins (PCDD/F/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 is observed near the incinerator and a considerable increase of dioxins (PCDD/F) in pine needles at a distance of 3000- 5000 m. The dioxins and dioxin-like PCB (PCDD/F/dl-PCB) belong again to the highest level found in pine needles of thisTW biomonitoring research, as was also the case in the first year in 2021 of the start of this biomonitoring study.

Pilsen, Czech Rep., biomonitoring results 2022

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



Eggs

Analyses on locations within 3000-5000 m show high levels of dioxins (PCDD/F), more than three times the EU limit values for bioassay and chemical analyses. Dioxin congener patterns are related to incomplete combustion. A remarkable increase of dioxin-like PCB (dl-PCB) in eggs, while these substances have been banned for 40 years. With FITC-T4, high concentrations of PFAS are found, with the chemical analysis PFOS and Gen-X being identified.

Mosses

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

Pine needles

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

Analyses methods

Bioassay DR CALUX®

The bioassay **DR CALUX® (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 with DR CALUX® for analyses on dioxins (PCDD/F/dl-PCBs) on eggs are expressed 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 the DR CALUX will be expressed 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 are:
- Commission Regulation (EU) **2017/644 of 5 April 2017**[3] 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] sets maximum levels for certain contaminants e.g., dioxins (PCDD/F/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/dl-PCB, a confirmatory GC-MS analysis of the hen egg sample is recommended to establish the results...

Chemical analyses

The maximum permitted levels of contaminants in hen eggs using chemical analyses of GC-MS are expressed as pg TEQ/g. The analysis is 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/dl-PCBs) will be calculated with a specific Toxic Equivalency Factor (TEF) towards a TEQ value (see page 4 Abbreviation 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 for the sum of dioxins and dioxin-like PCBs (PCDD/F/dl-PCBs), the limit is set at 5 pg TEQ/gram fat. When exceeding these GC-MS limit values, hen eggs are not allowed to be placed on the commercial market (see Figures 5 and 6).

2013/711/EU[7] includes the **action levels GC-MS** for both dioxins (PCDD/F) and dioxin-like PCBs (dl-PCBs) in chicken eggs 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.

Chemical analyses on PFAS are performed with LC-LC-MS on 24 PFAS substances by Normed, Rotterdam NL.

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2 *'Bioanalytical methods' means methods based on the use of biological principles such as cell-based assays, receptor-assays or 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*

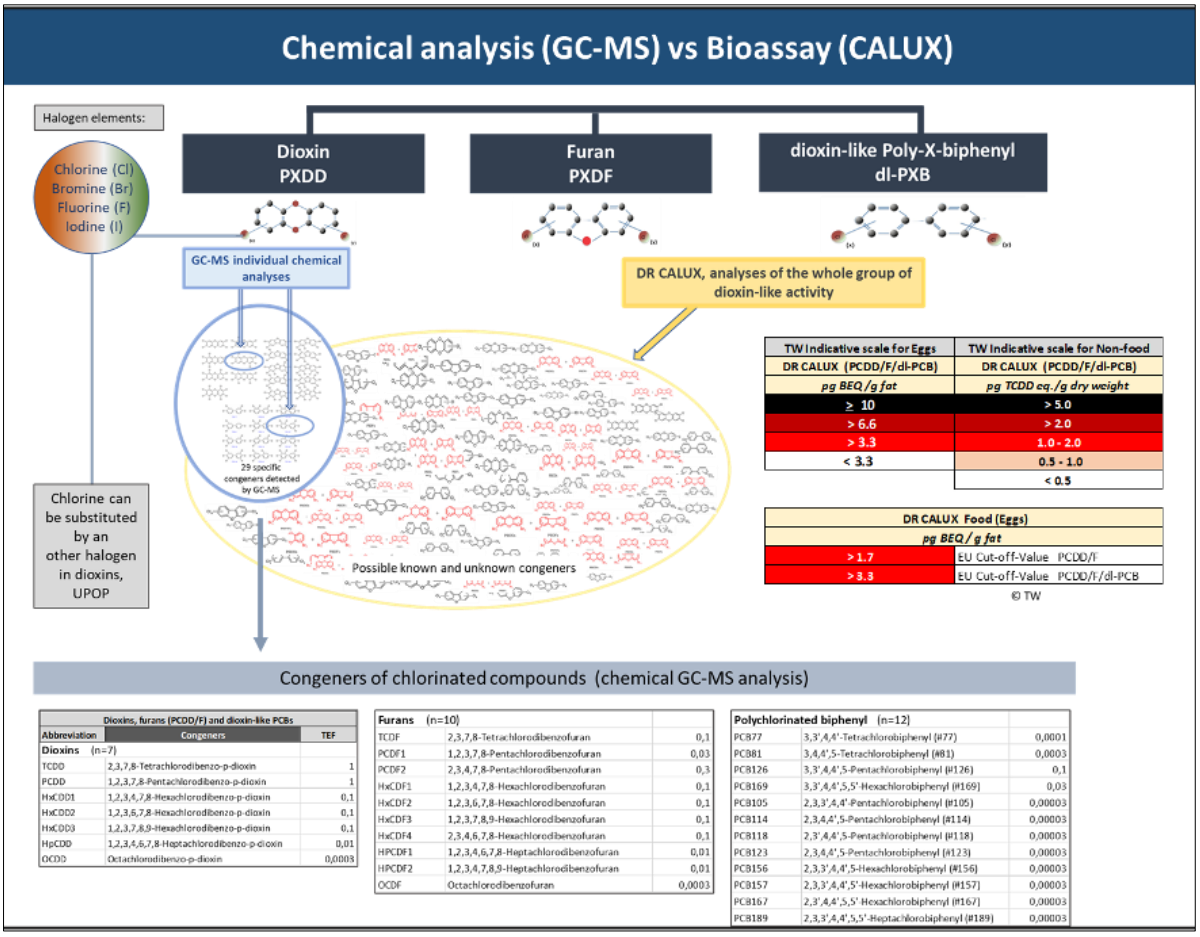
5 *https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN*

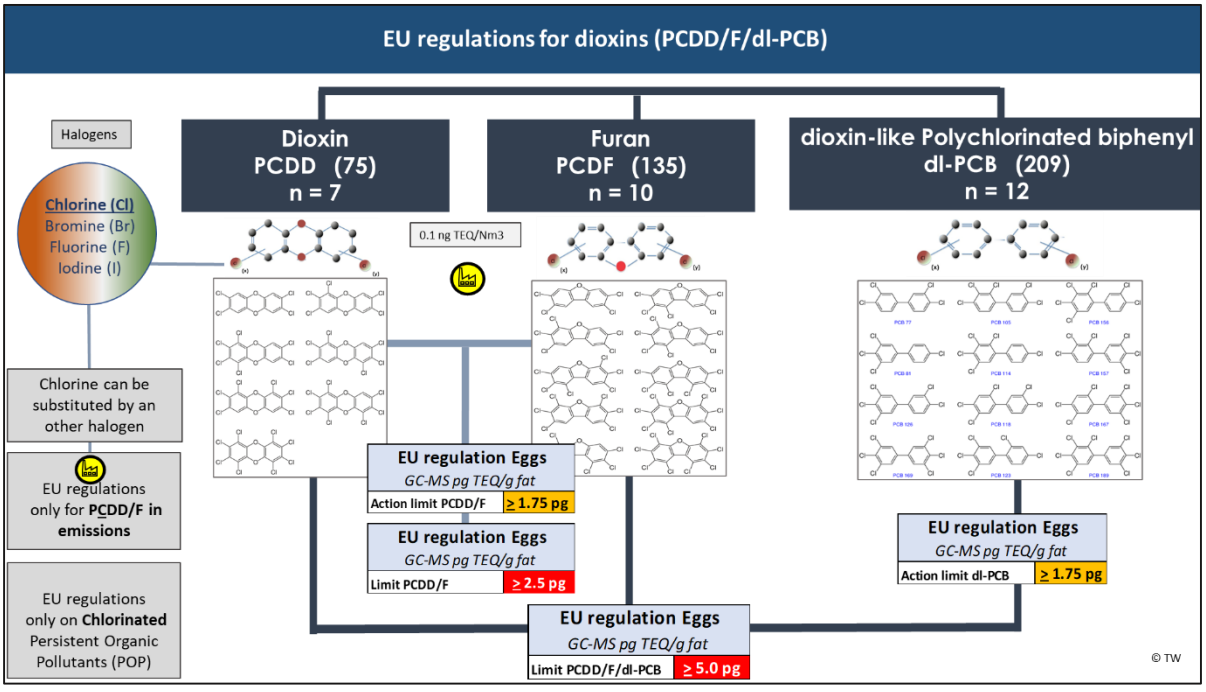
6 *'Action level' means the level of a given substance, as laid down in the Annex to Recommendation 2013/711/EU, which triggers investigations to identify the source of that substance in cases where increased levels of the substance are detected*

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 on eggs.





PAH CALUX®

High molecular weight PAHs have known ligands of the aryl hydrocarbon receptor (AhR), 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, are tested for the potency of binding with the thyroid hormone thyroxine (T4) to the plasma transport protein Transthyretin (TTR). The fluorescent-labelled thyroxine (FITC-T4) consisting of Fluorescein isothiocyanate (FITC) and L-thyroxine (T4) are used in this assay (Smith, 1977, Hamers 2020)[10,11].The 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 thereby replacing T4. The measurement is based on the difference in fluorescence between bound and non-bound FITC-T4 to the TTR-binding site. Bound FITC-T4 will result in a higher fluorescence than non-bound. The analysis results of the FITC-T4 will be expressed in µg PFOA equivalent/g product.

The DR CALUX®, PFAS CALUX®, FITC-T4, and GC-MS-analysis 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

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

Analysis of eggs of backyard chickens

For the analysis of eggs, two (2) methods are used. First, eggs are analysed with the bioassay DR CALUX. This method detects the total toxicity of dioxins, and not only the regulated chlorinated dioxins (PCDD/F/dl-PCB) but also brominated (PBDD/F) and other (mixed) halogenated dioxins, see figures below. Secondly, the eggs are analysed with the chemical analyses GC-MS, this 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		

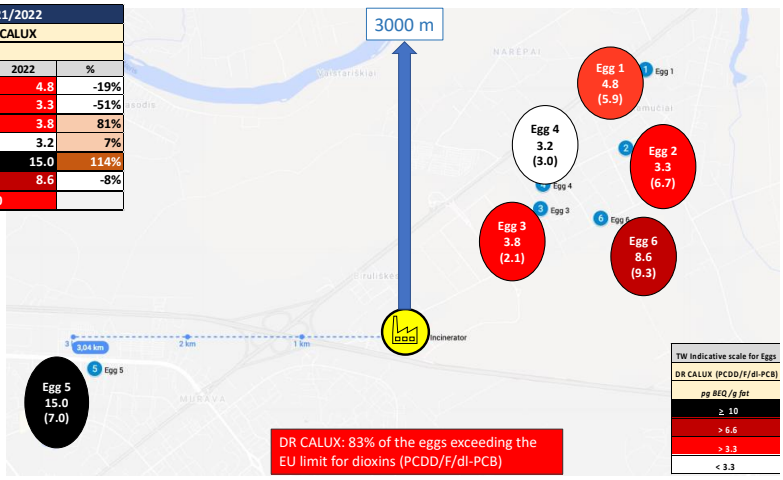
Kaunas, Lithuania 2021/2022				
dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	0.9	-35%
	Egg-02	0.9	0.6	-38%
	Egg-03	0.8	0.7	-13%
	Egg-04	1.1	0.7	-32%
	Egg-05	2.1	10.5	401%
	Egg-06	18.0	3.4	-81%
Cut-off	GC-MS action	1.75		

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			

Results dioxins by DR CALUX in eggs backyard chicken

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

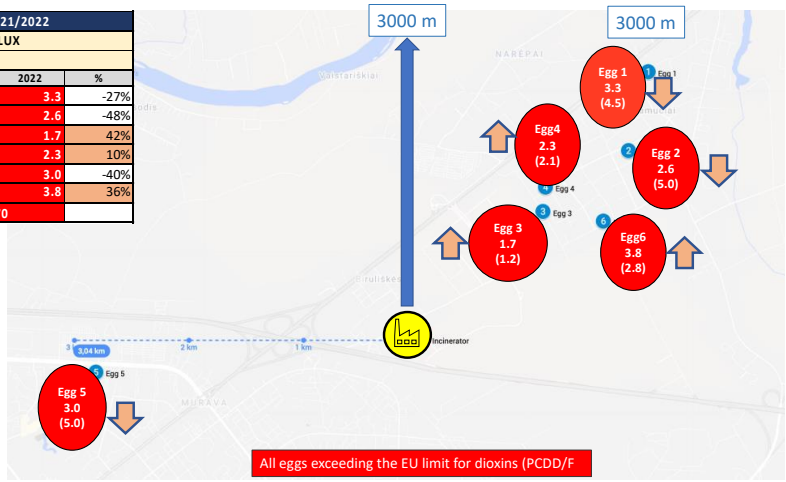
Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	5.9	4.8	-19%
	Egg-02	6.7	3.3	-51%
	Egg-03	2.1	3.8	81%
	Egg-04	3.0	3.2	7%
	Egg-05	7.0	15.0	114%
	Egg-06	9.3	8.6	-8%
Cut-off	DR CALUX	3.30		



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

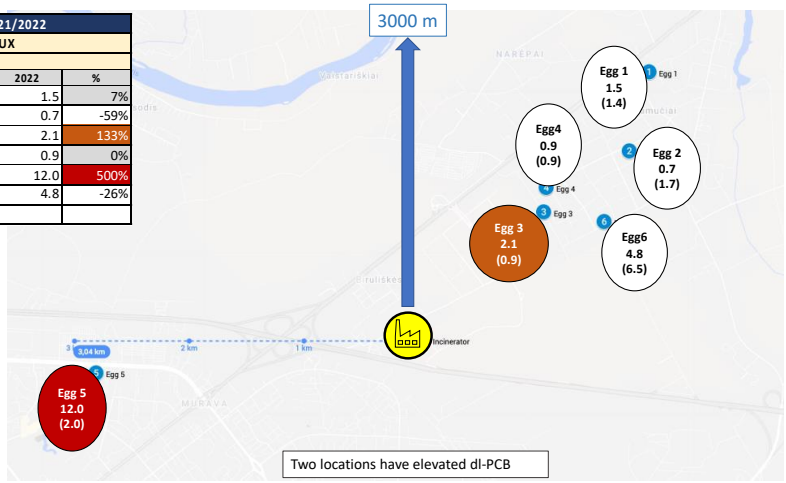
Kaunas, Lithuania 2021/2022				
PCDD/F - DR CALUX				
pg BEQ/g fat				
		2021	2022	%
Eggs	Egg-01	4.5	3.3	-27%
	Egg-02	5.0	2.6	-48%
	Egg-03	1.2	1.7	42%
	Egg-04	2.1	2.3	10%
	Egg-05	5.0	3.0	-40%
	Egg-06	2.8	3.8	36%
Cut-off	DR CALUX	1.70		



All eggs exceed the EU limit for dioxins (PCDD/F) with the bioassay of DR CALUX, notably increasing levels of dioxins (PCDD/F) in locations nearby 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			

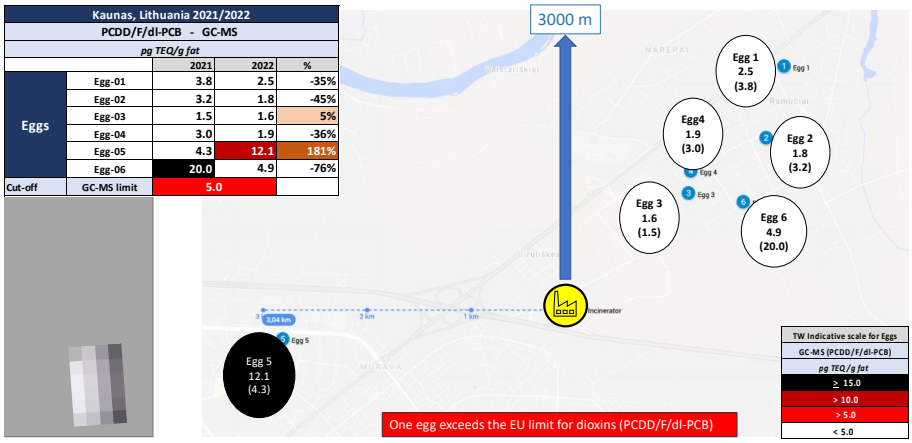


Two locations show an elevation of dl-PCB of 133 and 500%.

Results of dioxins by GC-MS in eggs of backyard chicken

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

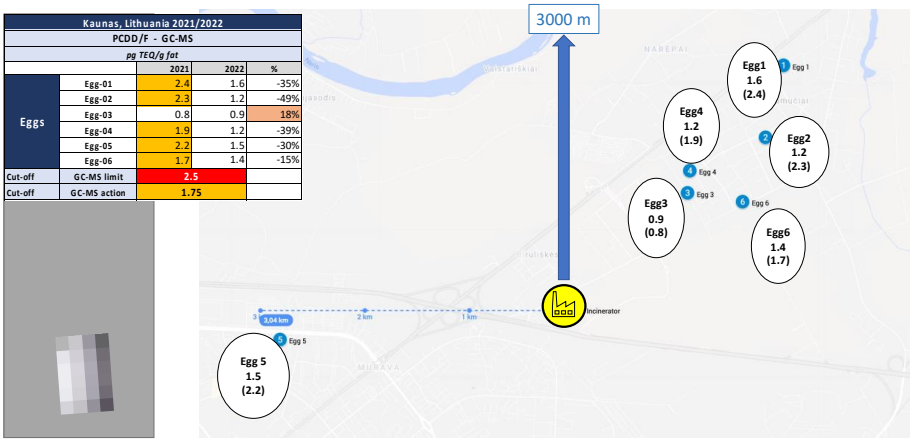
Kaunas, Lithuania 2021/2022				
PCDD/F/dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	3.8	2.5	-35%
	Egg-02	3.2	1.8	-45%
	Egg-03	1.5	1.6	5%
	Egg-04	3.0	1.9	-36%
	Egg-05	4.3	12.1	181%
	Egg-06	20.0	4.9	-76%
Cut-off	GC-MS limit	5.0		



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

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

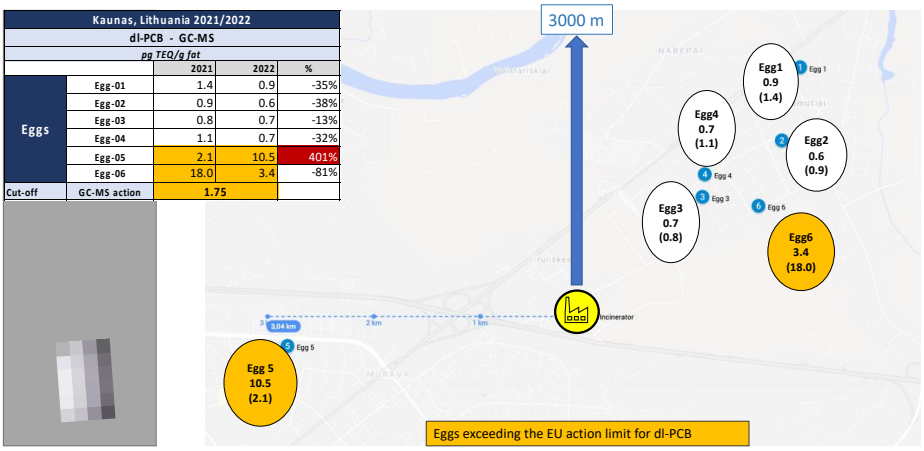
Kaunas, Lithuania 2021/2022				
PCDD/F - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	2.4	1.6	-35%
	Egg-02	2.3	1.2	-49%
	Egg-03	0.8	0.9	18%
	Egg-04	1.9	1.2	-39%
	Egg-05	2.2	1.5	-30%
	Egg-06	1.7	1.4	-15%
Cut-off	GC-MS limit	2.5		
Cut-off	GC-MS action	1.75		



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

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

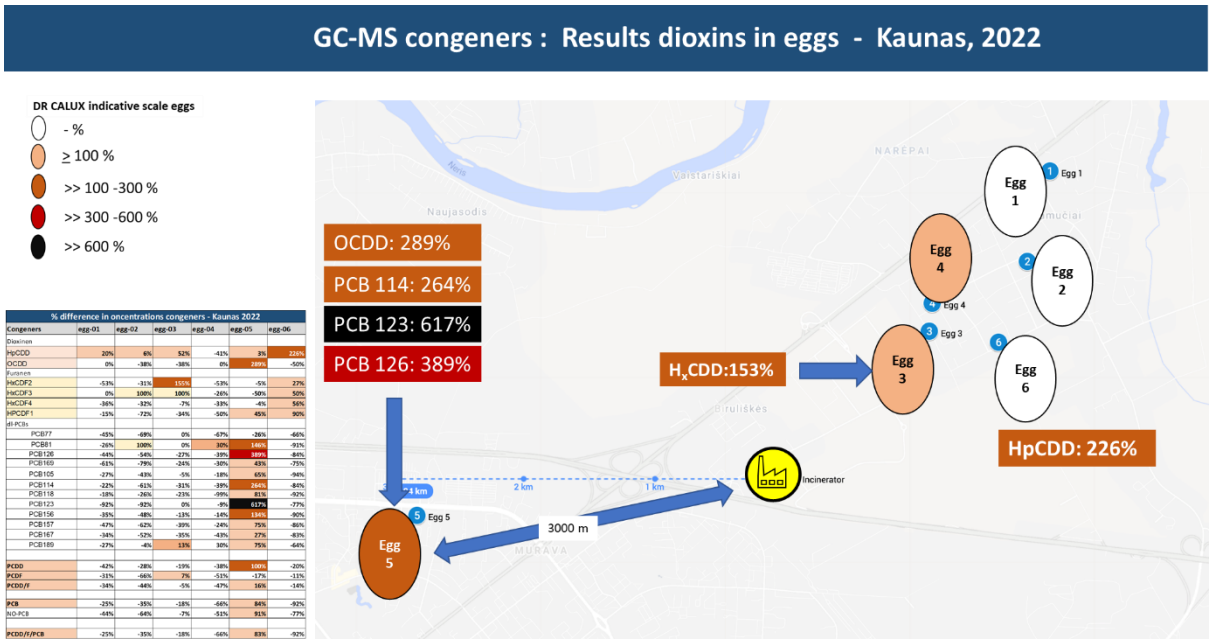
Kaunas, Lithuania 2021/2022				
dl-PCB - GC-MS				
pg TEQ/g fat				
		2021	2022	%
Eggs	Egg-01	1.4	0.9	-35%
	Egg-02	0.9	0.6	-38%
	Egg-03	0.8	0.7	-13%
	Egg-04	1.1	0.7	-32%
	Egg-05	2.1	10.5	401%
	Egg-06	18.0	3.4	-81%
Cut-off	GC-MS action	1.75		



Two locations exceed the action limit for dl-PCB of 1.75 pg TEQ/g fat. Remarkable is the decrease of dl-PCB in location 5 and the increase in location 6.

Results - eggs congener patterns

The results of the analysis done in 2021, indicate that the Kaunas incinerator emitted more dioxins during the testing phase. By zooming in on the specific congeners of dioxins in eggs by using the GC-MS analyses, typical congener elevations of waste incineration can be noticed as OCDD, PCB 126, HxCDD and HpCDD. More detailed data from the emission patterns of the incinerator might be more conclusive about this.

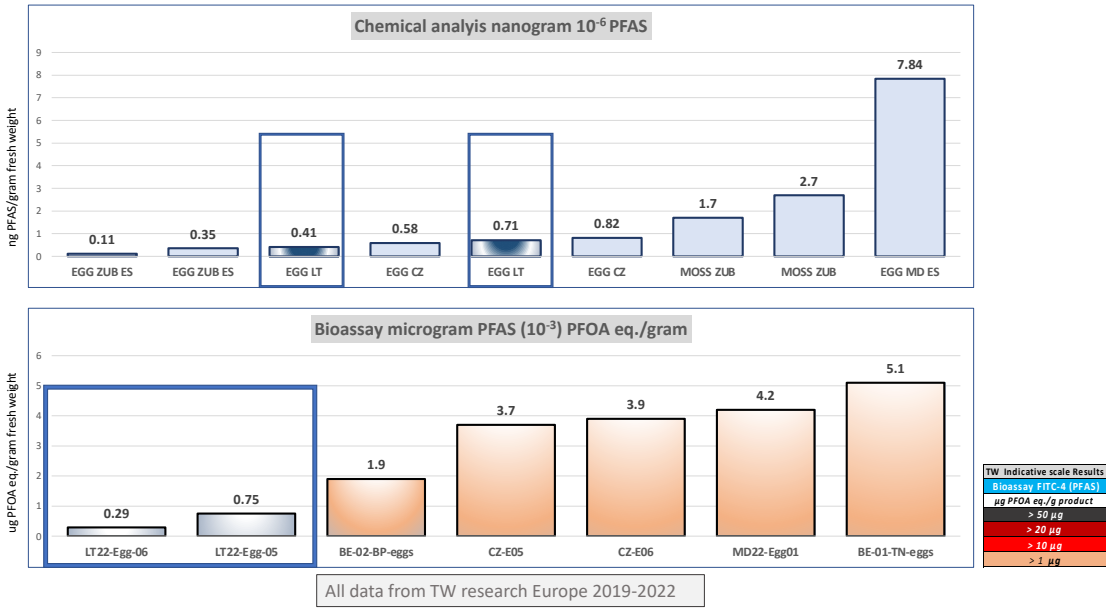


Remarkably egg location 5 shows high values of dl-PCBs. In the figure below are the specific congeners to be found. Most remarkable is the elevation of PCB 123 and PCB 126.

% 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

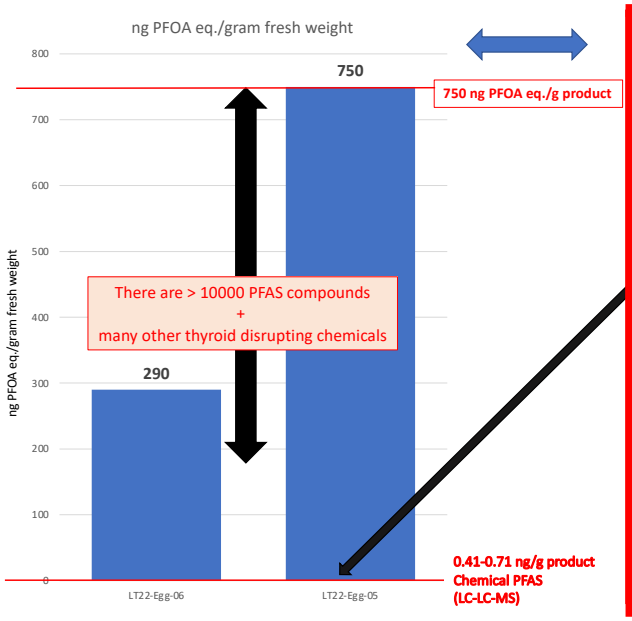
Chemical analysis (LC-LC-MS) of eggs shows PFAS. In the location West of the incinerator, PFOS and PFDoA were found, while in the egg location Southwest Gen-X and 8:2 FTS were found. Chemical analysis (LC-LC-MS) in two eggs shows different congeners of PFAS. In the location West of the incinerator, PFOS and PFDoA were found, while in the egg location Southwest Gen-X and 8:2 FTS were found.



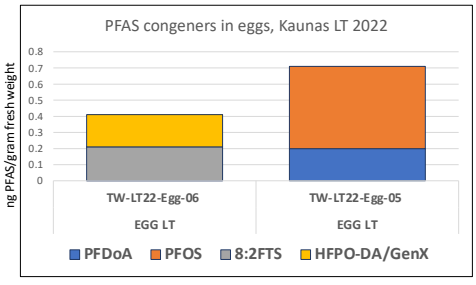
All data from TW research Europe 2019-2022

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

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



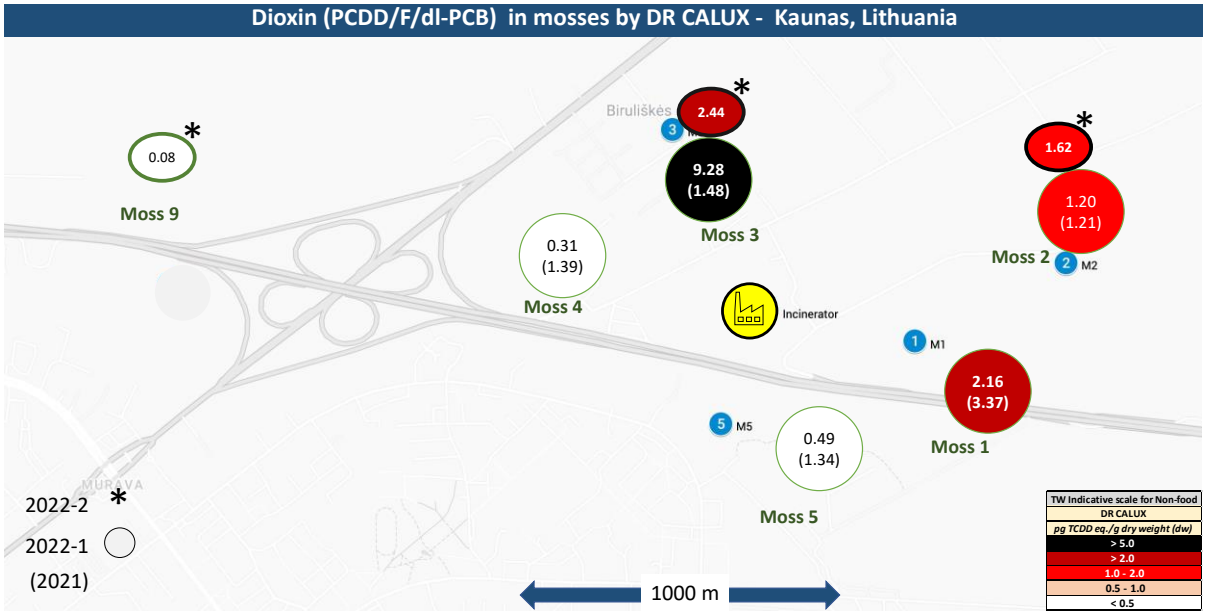
Chemical PFAS analyses eggs (ng/g) Kaunas, Lituania 2022		
ng PFAS/g product	TW-LT22-Egg-05	TW-LT22-Egg-06
Perfluor-n-dodecaanzuur (PFDoA)	0.20	
Perfluor-1-octaansulfonaat (PFOS)	0.51	
Perfluordecaansulfonaat (8:2FTS)		0.21
Hexafluorpropyleenoxide dimeer zuur (HFPO-DA/GenX)		0.20
Total PFAS (lower bound)	0.71	0.41



Results dioxins in mosses by bioassay DR CALUX

The mosses of the six (6) locations are mainly *Hylocomium splendens*, sampled around the incinerator, see the figure below. Mosses are sampled in the open field, avoiding the proximity of roads and not under the dense tree canopies to avoid the shedding of leaves for uptake emissions by air. Mosses are sampled in July and October 2022.

The results of the mosses of July and October 2022 are between **0.08 - 9.28 pg TCDD eq./g dw**. The results of sampling in October 2022 are given 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** is measured at location 3, west of the incinerator. In brackets, the results are given for 2021. Although lower in level, the result of the October sampling confirms the heavily contaminated mosses north from the incinerator, with a dioxin level of **2.44 pg TCDD eq./g dw**.



The measurements in 2021, July and October 2022 are shown in the table below. Remarkable is the elevation of the dl-PCB levels in the sampling of 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 on mosses

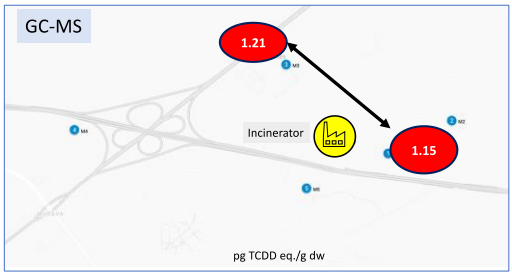
Due to the analyse results of high levels of dioxins by the bioassay of 9.28 pg TCDD eq./g dw, chemical analyses were performed. The high TEQ values of dioxins by the DR CALUX could not be found in the GC-MS results. Probably because of the contribution of other halogenated dioxins, which are not included 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, it is remarkable, twice as much PCB is found at moss location 3, 355.29 versus 191.94 pg dl-PCB.

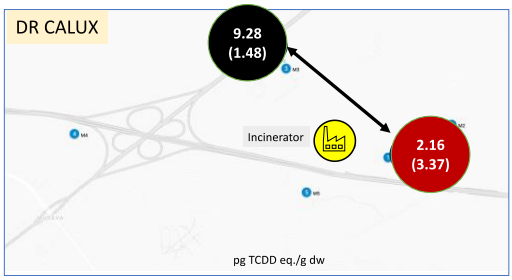
Bioassay vs. chemical analysis in mosses - Kaunas, Lithuania 2022

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

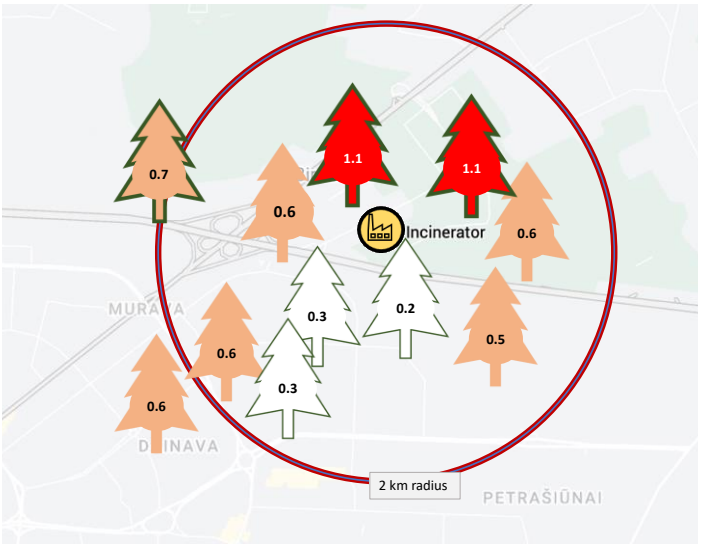
The DR CALUX bioassay, as explained above, also reacts to other halogenated compounds such as brominated and mixed halogenated dioxins. A very large group of brominated and mixed chloro/bromo dioxin and furan analogues is not included in the EU regulations for chemical analyses (GC-MS) nor integrated 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. A strong recommendation is to include brominated dioxins in standard measurements of emissions from waste incinerators.

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

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

Dioxin (PCDD/F/dl-PCB) analyses on pine needles show levels 0.60 – 1.08 pg TCDD eq./g dw (medium bound) Northwest, Northeast from the waste incinerator. In the South of the incinerator, the dioxin levels are 0.22-0.49 pg TCDD eq./g dw. In the Southwest (2000 m from the incinerators) the levels are 0.64 pg, also West (2000 m) levels of dioxins are 0.60 pg TCDD eq./g dw. The samples taken in October 2022 are nearly a factor 2 higher in dioxins levels compared to analyses in July 2022. The elevations were due to the increase of dioxin-like PCB (dl-PCB). PCBs are banned since 1985 but are still found in waste and in emissions of 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

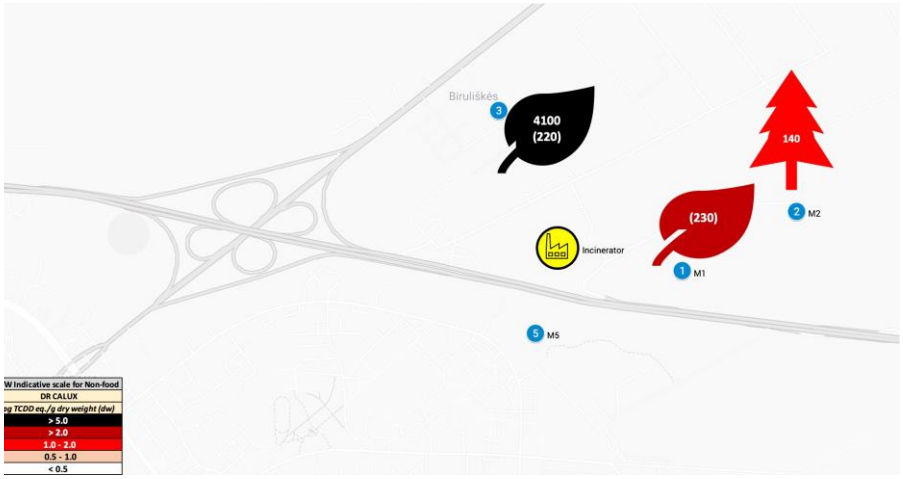
Jul-22 Oct-22

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

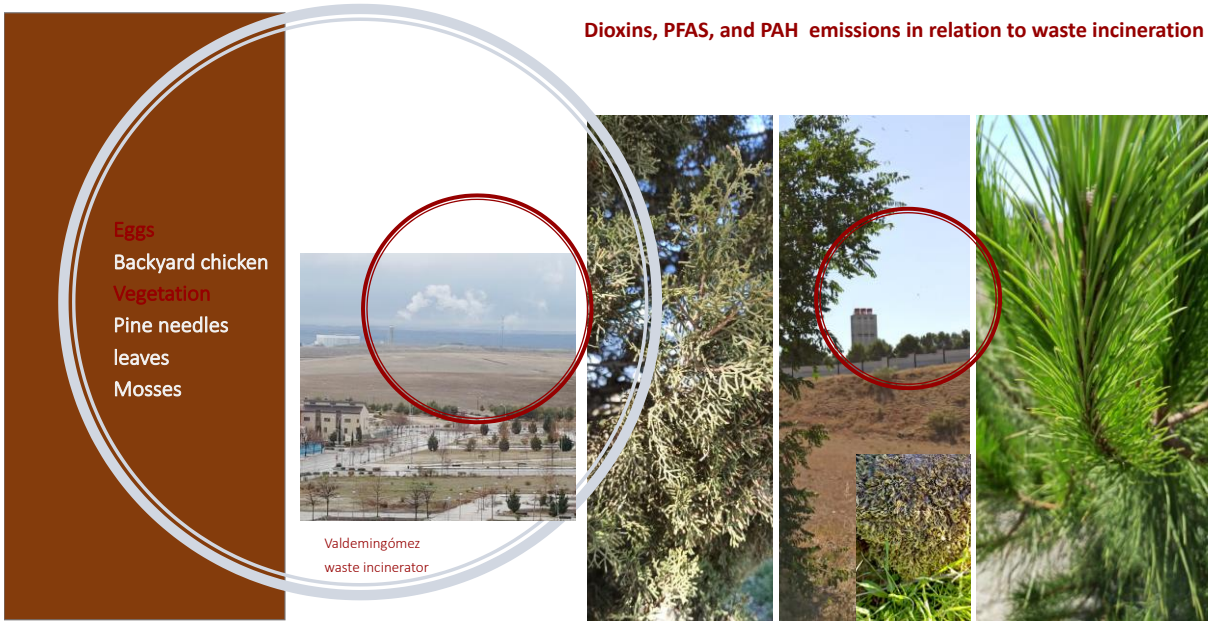
Results - PAHs in mosses and pine needles

The group of polycyclic aromatic hydrocarbons, PAHs, are markers for detecting toxic chemical emissions of thermo-confounders. Compounds from the PAH group have carcinogenic, mutagenic, teratogenic, and immunosuppressive effects on living organisms. In 2021 PAH was measured on two moss locations 220 and 230 ng BaP eq./g. On 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.

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



Madrid, Spain, 2022



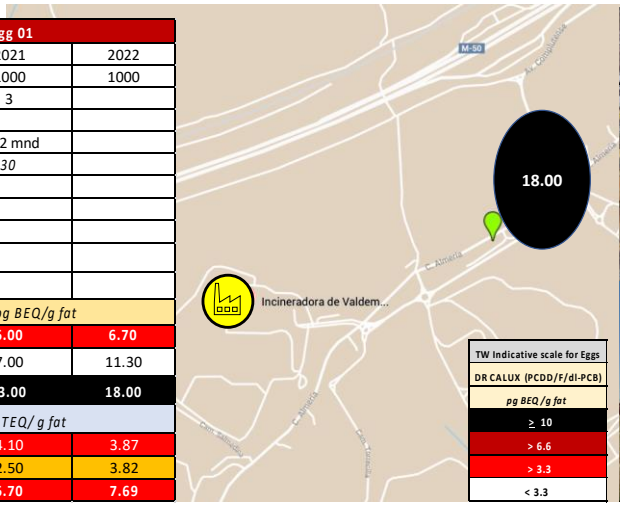
Biomonitoring Research Madrid, Spain, 2022

Analysis of eggs of backyard chickens

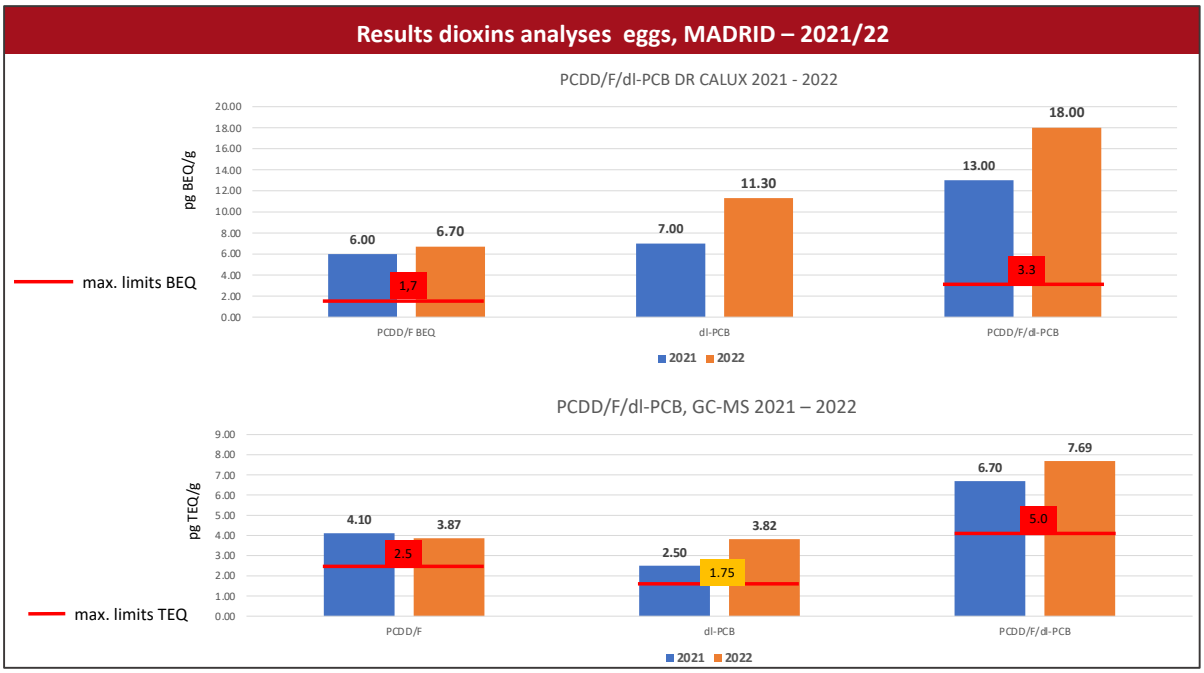
For the analysis of the eggs, two (2) methods are used. First, the eggs are analysed with the bioassay DR CALUX. This method detects the total toxicity of dioxins, and not only the regulated chlorinated dioxins (PCDD/F/dl-PCB) but also brominated (PBDD/F) and other (mixed) halogenated dioxins.
Extra GC-MS analysis is mandated in the EU regulation for **commercial** eggs when the results of 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 the total of dioxins including dioxin-like PCBs (PCDD/F/dl-PCB). In this research also the eggs are analysed by GC-MS. In the analyses of the eggs at a location 1000 m from the incinerator, a strong elevation of PCDD/F/dl-PCB was found by the DR CALUX. The GC-MS verified these results partially due to the limited scope of chemical analysis (only 29 congeners are analysed) while the bioassay of DR CALUX measured the total toxicity of all dioxins).

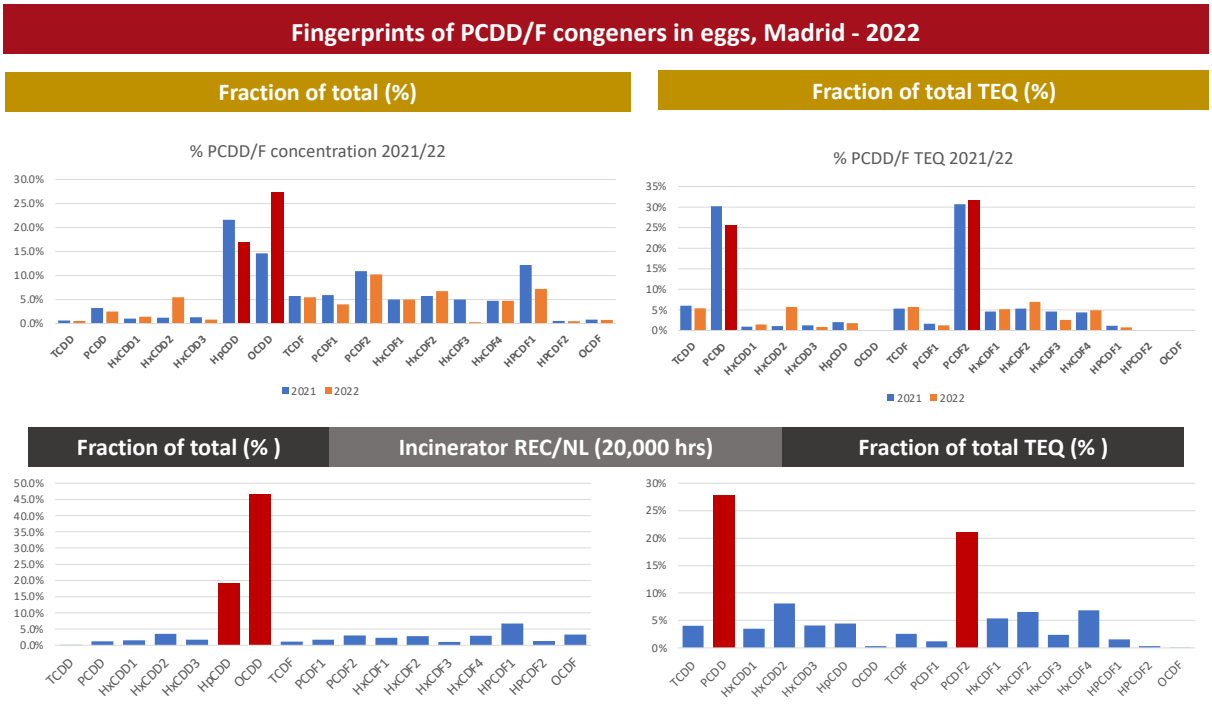
Results eggs sum of dioxins (PCDD/F/dl-PCB) DR CALUX, MADRID – 2021/22

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**



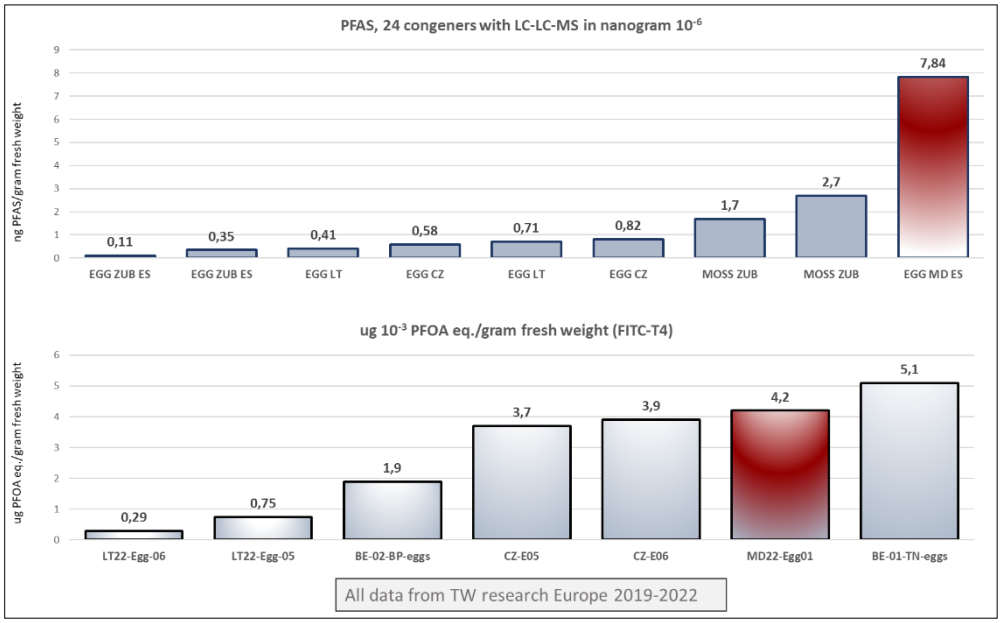
In the graphs below an elevation of dioxins (PCDD/F/dl-PCB) is observed in 2022. The bioassay showed for dioxins higher levels, most likely brominated dioxins are involved.





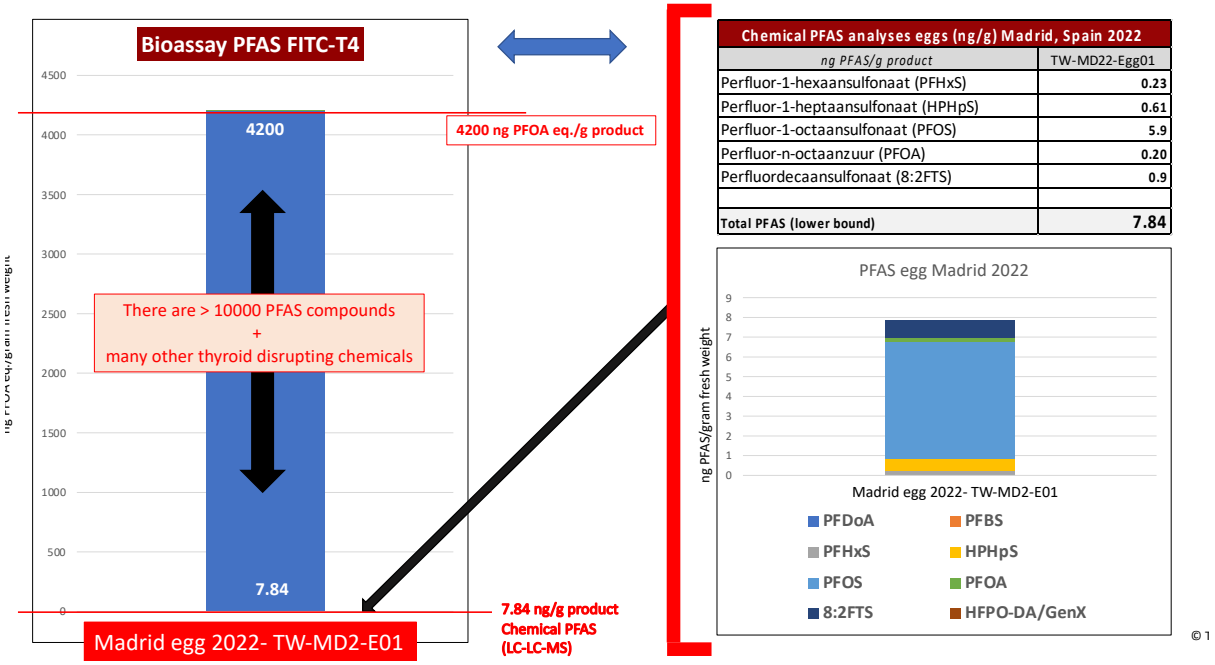
Analysis of PFAS in eggs of backyard chicken

In this biomonitoring research, TW has analysed PFAS in eggs. PFAS can also be a product from the waste incinerator (see explanation in the first TW report in 2021). The chemical PFAS analysis (LC-LC-MS) shows in the two eggs different congeners of PFAS. In the location West of the incinerator, PFOS and PFDoA are analysed, while in the egg location Southwest Gen-X and 8:2 FTS is found.



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

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



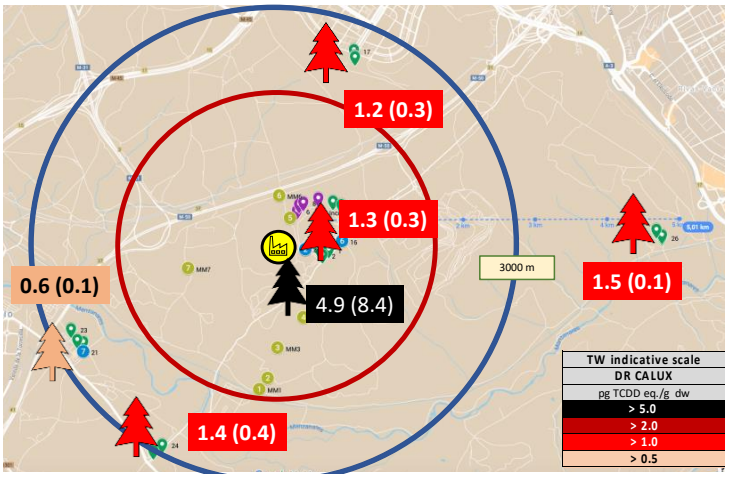
Analysis of pine needles and evergreen trees

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

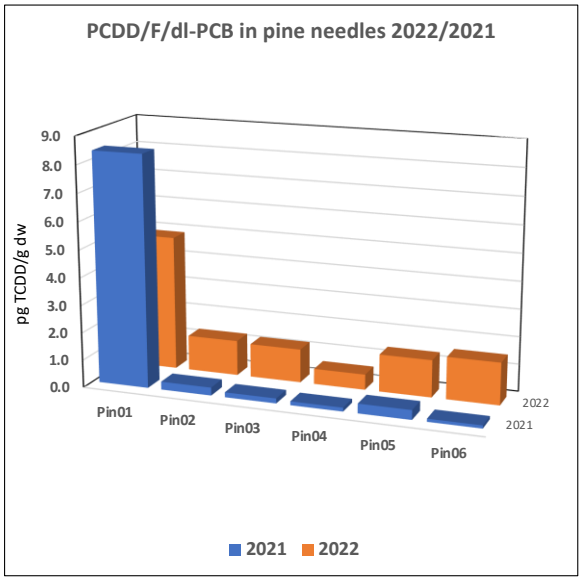
Although polychlorinated biphenyls (PCBs) were commercially banned half a century ago, contamination of the environment and organisms by PCBs is still observed. In 2022 a serious increase is seen in several locations, see figure left.

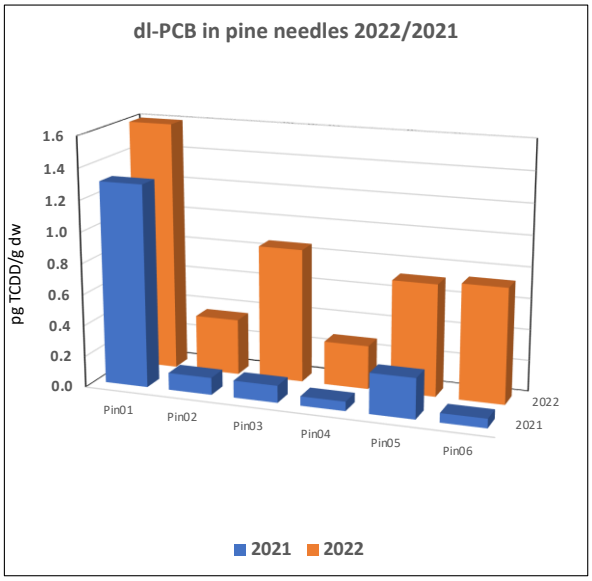
Dioxins in pine needles Madrid, Spain 2022 (2021)

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



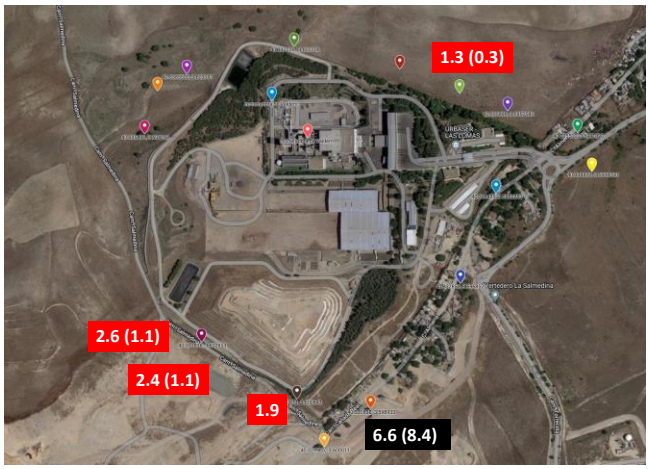
Dioxins in opine needles 2022





By zooming in more nearby the incinerator, high results of dioxins (PCDD/F/dl-PCB) are found in the vegetation in 2022 (and dioxin values analysed in 2021). Incinerator residues stored in the huge piles of big bags near the fence (see figure) could be responsible for the contamination of toxic POPs 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/*

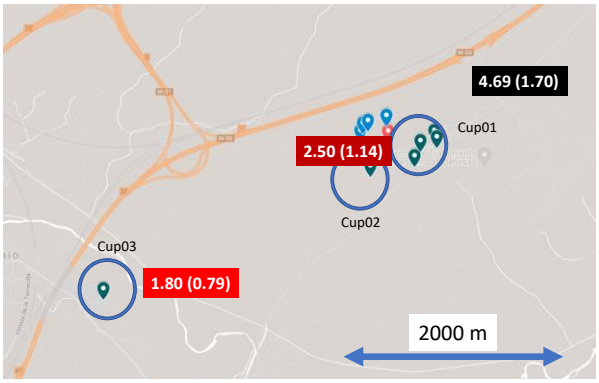
Analysis of needles of evergreen trees - *Cupressus arizonica*

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

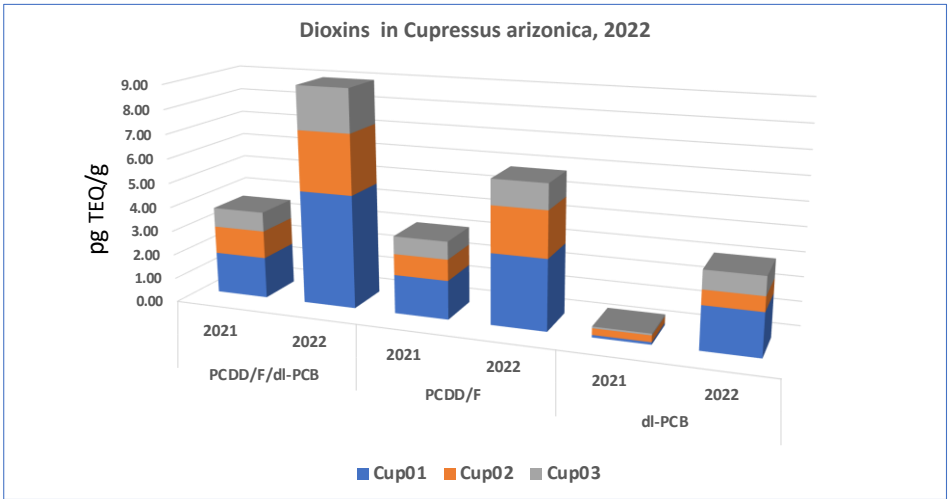
Dioxins in *Cupressus arizonica* 2022 (2021)

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)



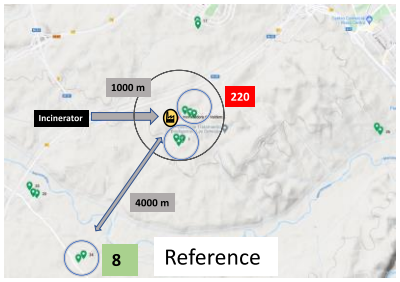
Dioxins in Cupressus arizonica, 2022						
DR CALUX						
	PCDD/F/dl-PCB		PCDD/F		dl-PCB	
	2021	*2022*	*2021*	*2022*	*2021*	*2022*
Cup01	1.70	4.69	1.60	2.92	0.10	1.77
Cup02	1.14	2.50	0.87	1.90	0.27	0.60
Cup03	0.80	1.80	0.73	1.04	0.07	0.76

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

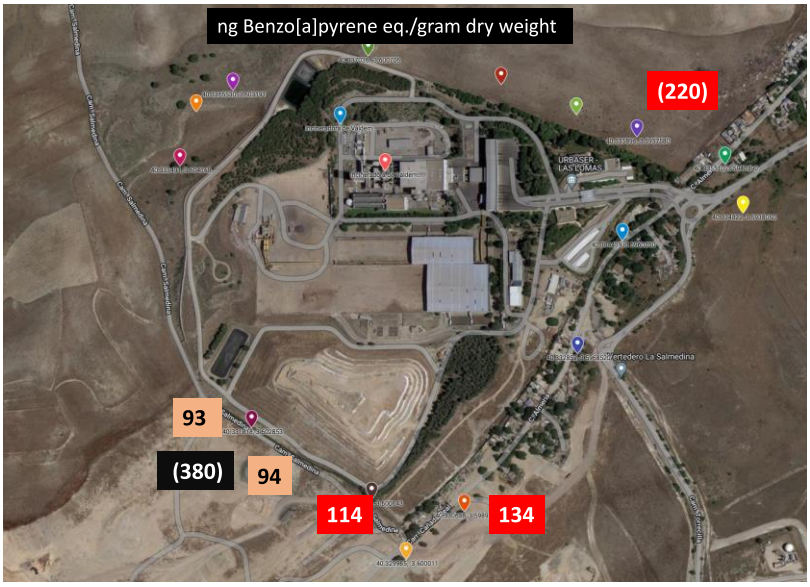
Analysis of PAH in pine needles and evergreen trees

In 2021, biomonitoring research found high levels of PAH on the Northeast side of the waste incinerator. This year (2022) TW has analysed 4 sites on PAH and verified the high levels of PAH in pine needles and evergreen trees directly around the borders of the incinerator. In the figure below on the left are shown the results of 2021 and on the right side are shown the results from four (4) PAH analyses around the incinerator in 2022. The results of the PAH analyses on the pine needles show high levels of 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. Temperatures were, however, high, and not favourable to sample 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 do a second round of sampling in October.

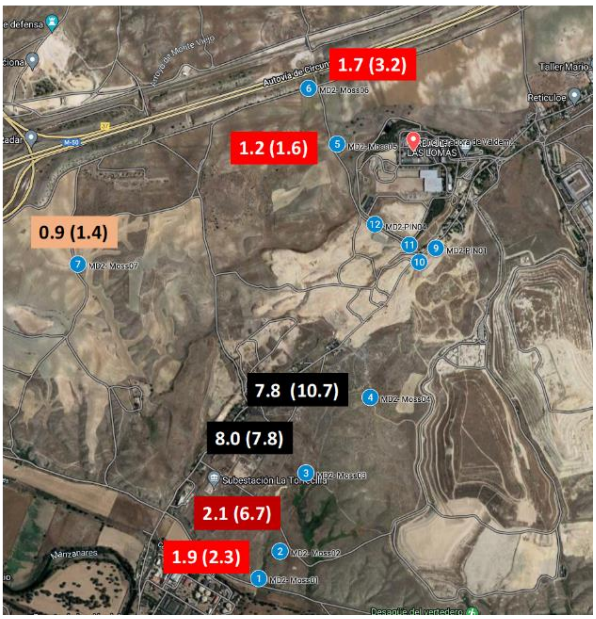
Moss-Soil 3, sample date, Madrid 03-07-2022

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

The analysis results in the mosses around the incinerator Valdemingómez are similar to those of 2021. The difference is that also an elevation is seen in the references nearby and the reference location 35 km away, in Castello de Villa. The elevation of dioxins (PCDD/F/dl-PCB) is most observed around 2 km southwest of the waste incinerator, the same dioxin elevation as for the research on pine needles this year has shown. 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

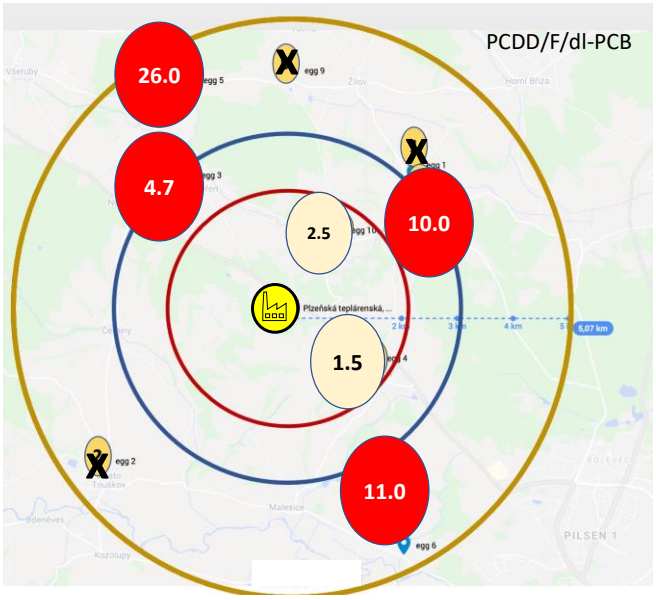
Bioassay analysis of dioxins in eggs of backyard chickens

The bioassay DR CALUX found four locations exceeding the cut-off/limit of the EU limit for eggs that needed to be analysed with the chemical analyses GC-MS. Two locations nearby 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.

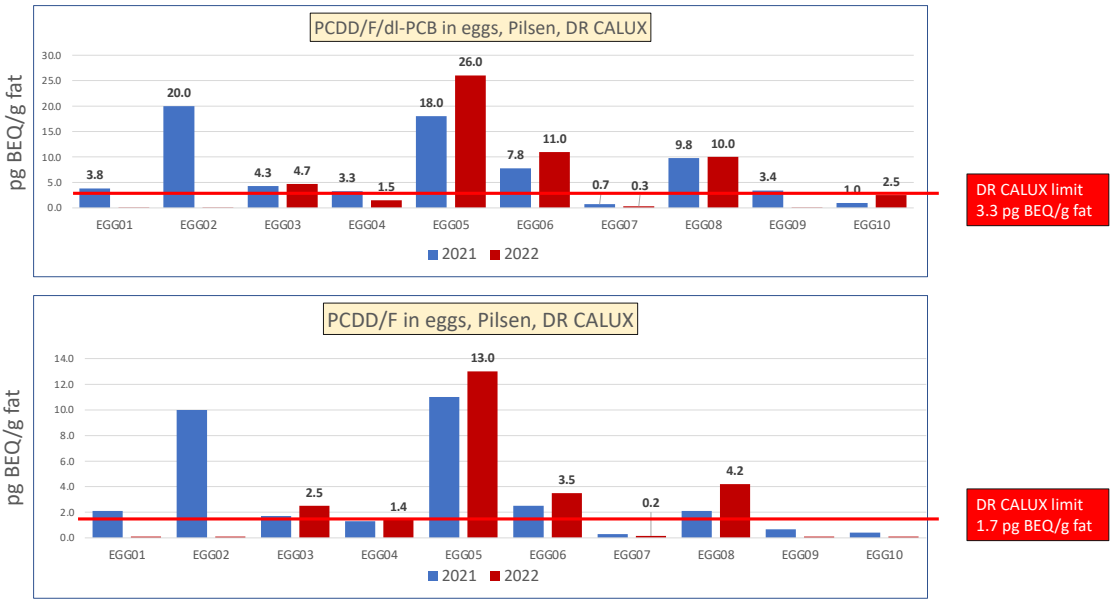
Results DR CALUX dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2022

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

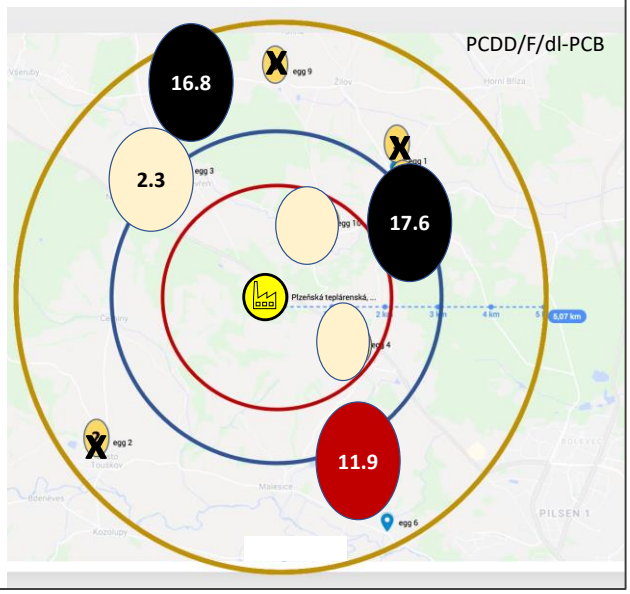
The GC-MS shows 2 egg locations exceeded the maximum limit for dioxins with a factor 3 and one location with a factor 2. The levels are 11.9, 16.8 and 17.6 pg TEQ/g fat. Three egg locations exceed the action limit for dl-PCB of 1.75 pg TEQ/g.

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

Results Eggs Pilsen, Czech Republic 2021

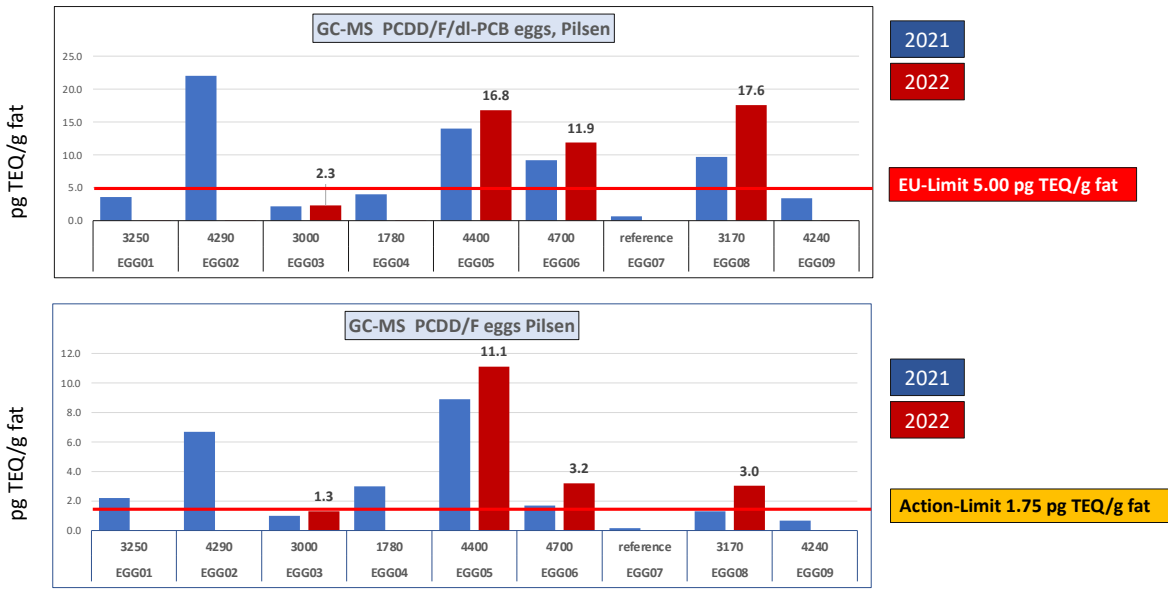
		GC-MS		pg TEQ/g fat		Medium bound (MB)	
Location	Distance (m)	2021	2022	2021	2022	2021	2022
		PCDD/F/dl-PCB		PCDD/F		dl-PCB	
EGG01	3250	3.6		2.2		1.4	
EGG02	4290	22.0		6.7		16.0	
EGG03	3000	2.2	2.3	1.0	1.3	1.2	1.0
EGG04	1780	4.0		3.0		1.0	
EGG05	4400	14.0	16.8	8.9	11.1	5.0	5.7
EGG06	4700	9.2	11.9	1.7	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. At 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

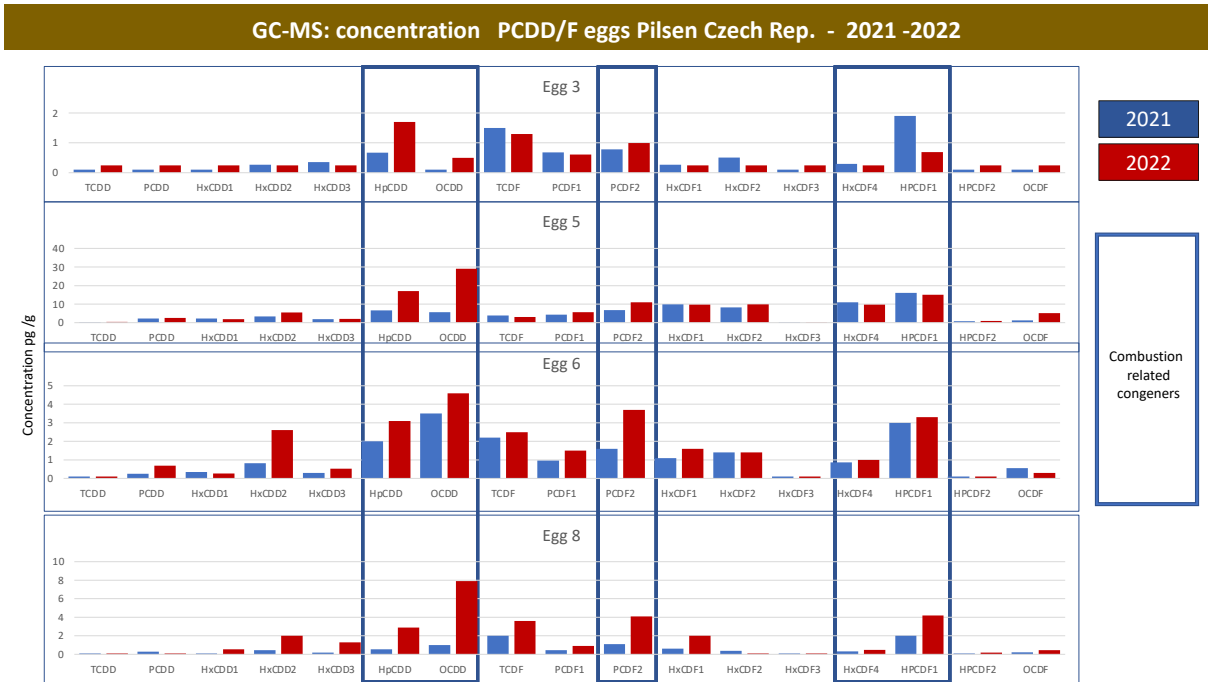
Analysis of feed and soil

Feed and soil were analysed by DR CALUX for additional research concerning dioxin contamination on egg location 5. The soil measured 2.1 pg BEQ/g PCDD/F/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

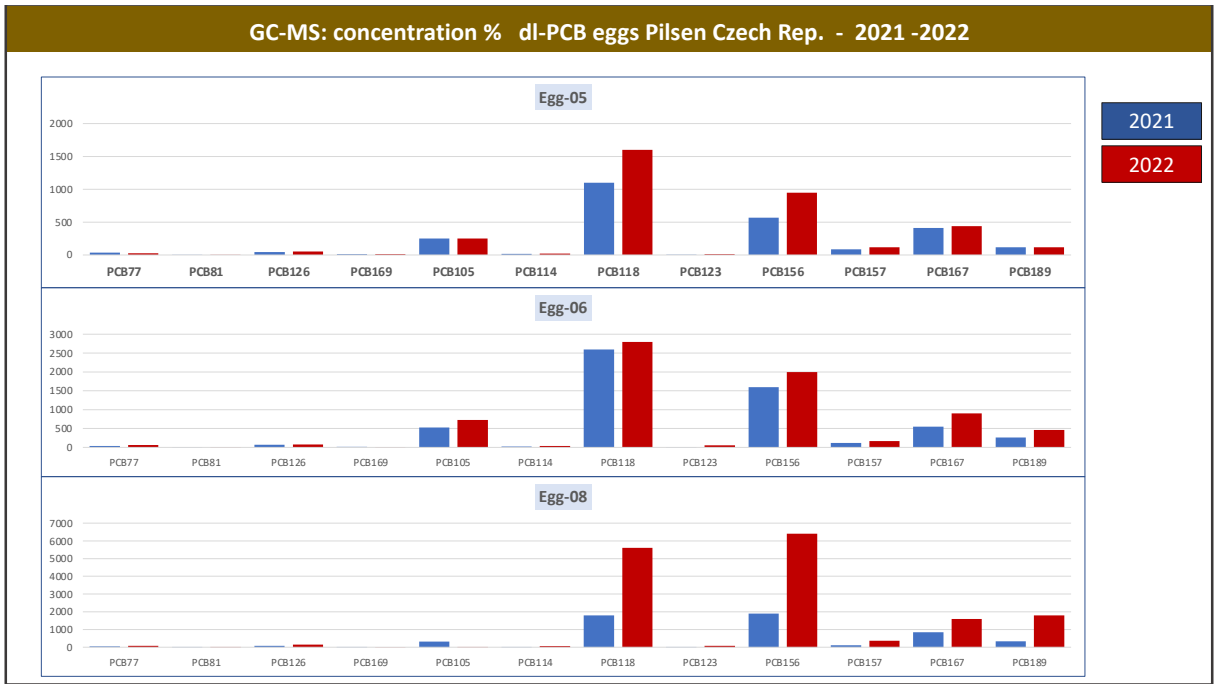
Analysis of dioxin congeners

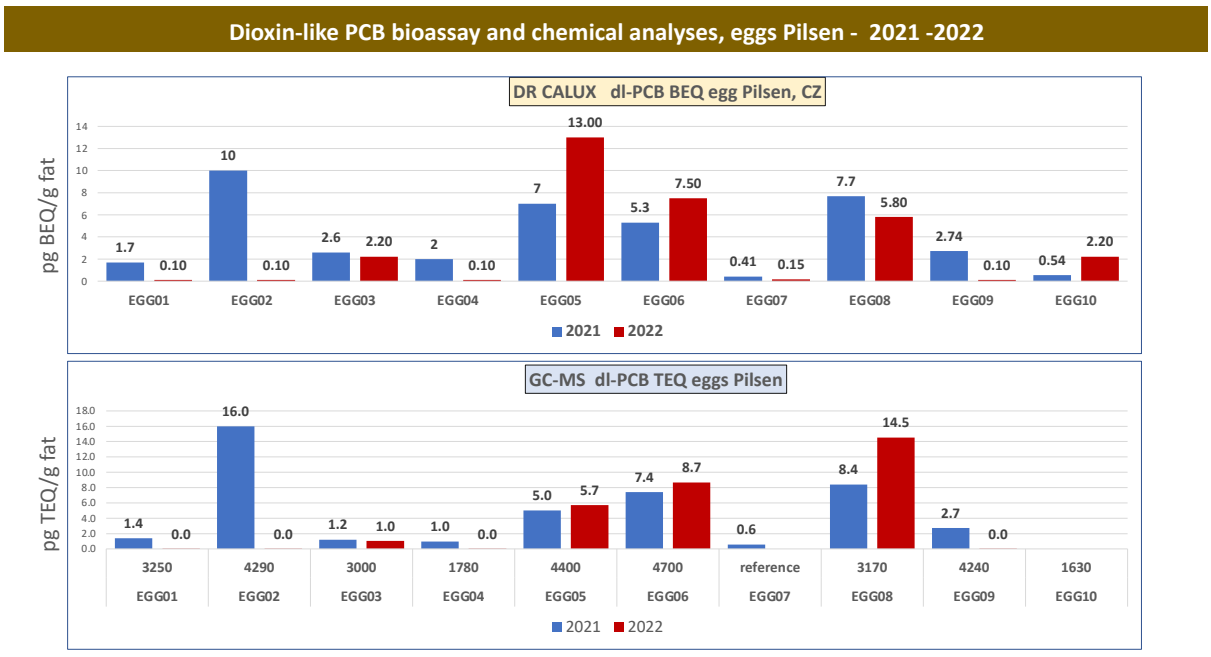
The figure shows the congeners found with the chemical analysis of the eggs. A pattern of elevation of those specific congeners related to (incomplete) combustion is present.



Analysis of dioxin-like PCB (dl-PCB)

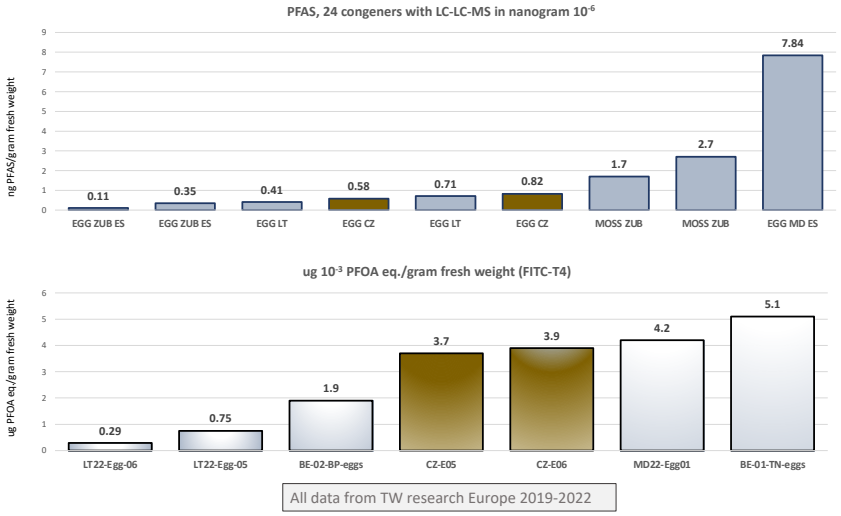
In 2022 levels of dl-PCB are elevated at locations 5 and 6. Although dl-PCB are for a long time banned, elevation can be still found in eggs. Measurement in soil (see page 14) shows 0.61 pg TCDD eq./g.





Analysis of PFAS in eggs

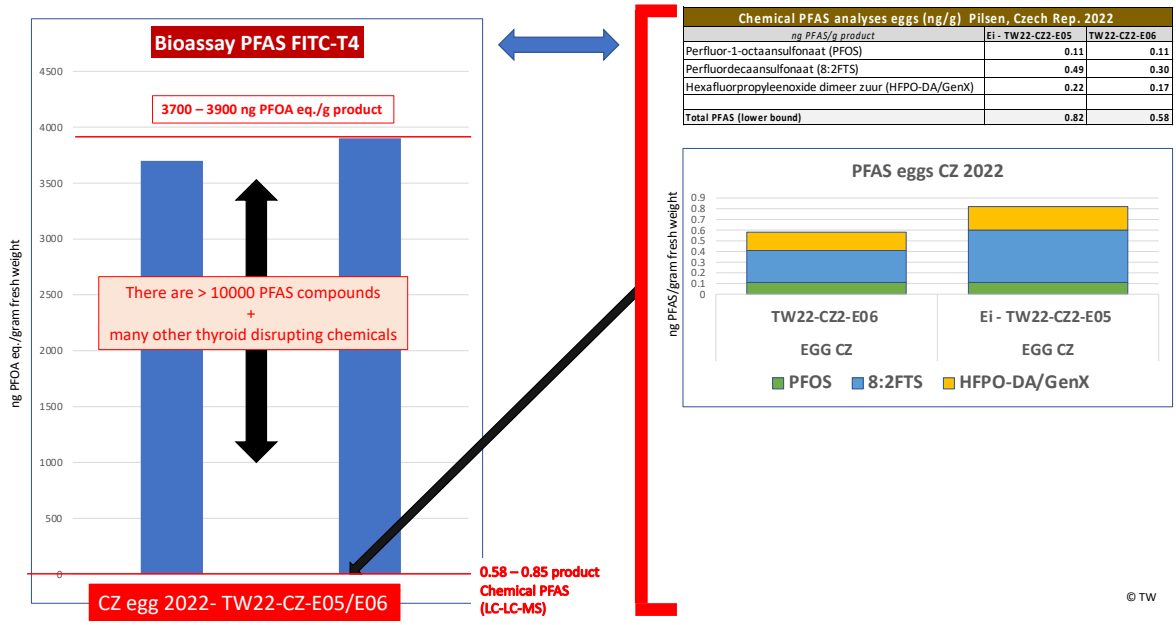
In this biomonitoring research, TW has analysed PFAS in eggs. PFAS can also be a product from the waste incinerator (see explanation in the first TW report in 2021[14]). The chemical PFAS analysis (LC-LC-MS) shows in the two eggs different congeners of PFAS. In the location West of the incinerator, PFOS and PFDoA are analysed, while in the egg location Southwest Gen-X and 8:2 FTS is found.



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

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

PFAS analyses with FITC-T4 and limited chemicals analyses on 24 PFAS, eggs CZ 2022



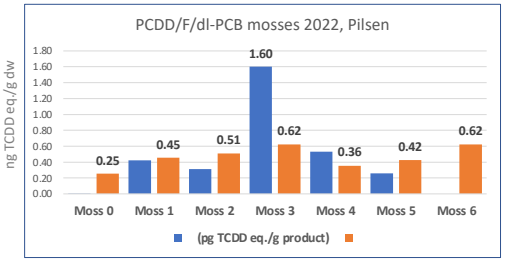
Chemical PFAS analyses eggs (ng/g) Pilsen, Czech Rep. 2022		
ng PFAS/g product	Ei - TW22-CZ2-E05	TW22-CZ2-E06
Perfluor-1-octaansulfonaat (PFOS)	0.11	0.11
Perfluordecaansulfonaat (8:2FTS)	0.49	0.30
Hexafluorpropyleenoxide dimeer zuur (HFPO-DA/GenX)	0.22	0.17
Total PFAS (lower bound)	0.82	0.58

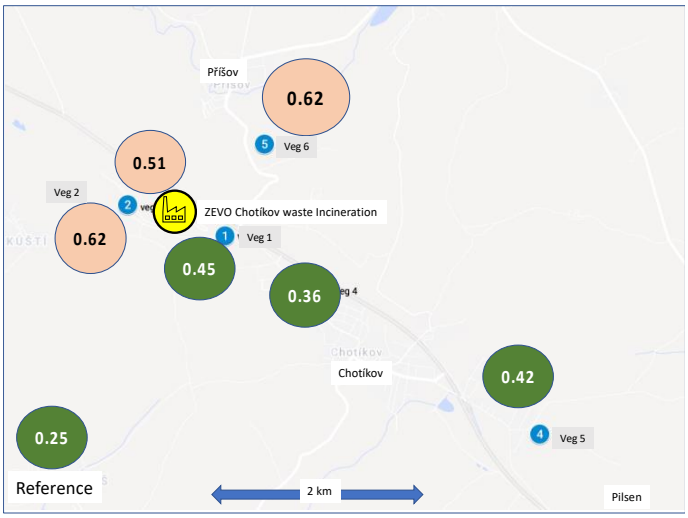
Analysis of mosses

Dioxins are slightly elevated at sites 2, 3 and 6, 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, has decreased to 0.62 pg TCDD eq./g. If this value was detected on vegetables, agricultural or vegetable gardens, these values would be too high for consumption. Normally, dioxins 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

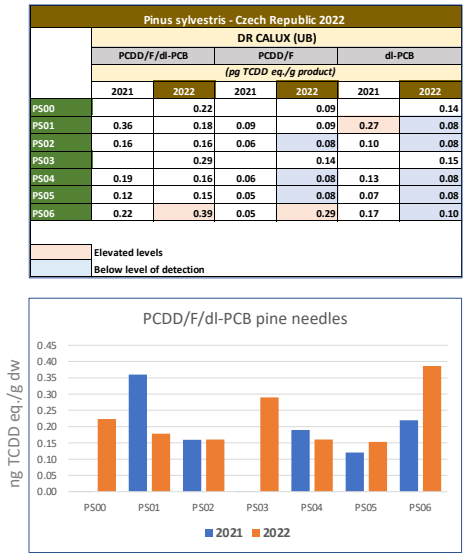
Dioxins are slightly elevated at sites 3 and 6, mainly due PCDD/F. The dioxin-like PCBs were slightly elevated in 2021 but are now lower.

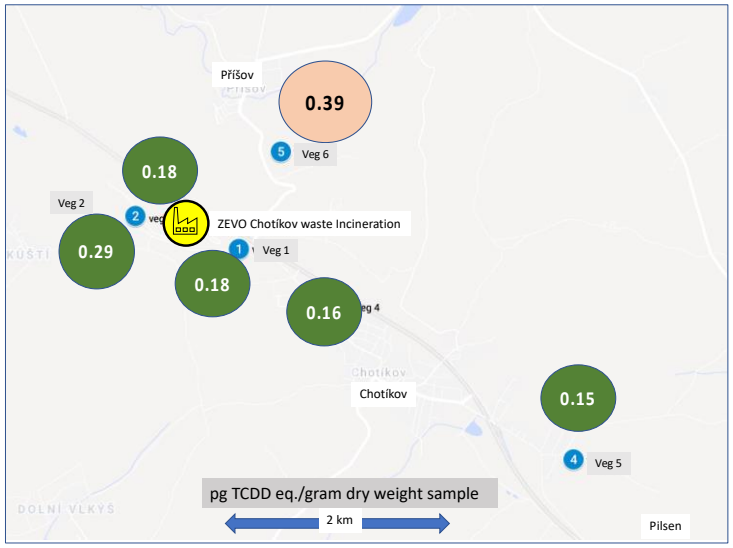
Dioxins in pine needles Pilsen, Czech Rep. 2022 (2021)

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

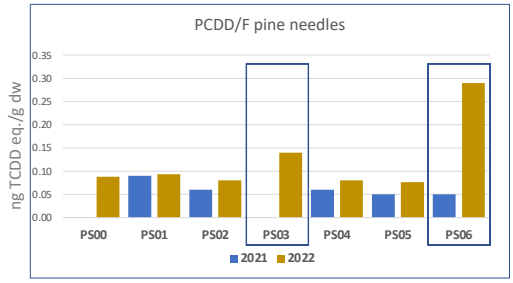
Elevated levels

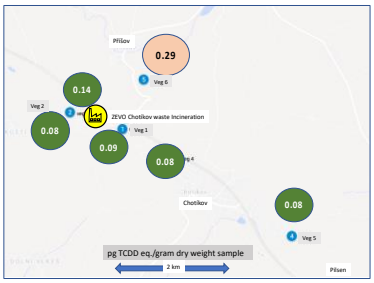
Below level of detection

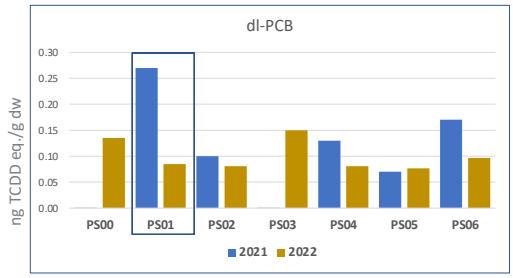


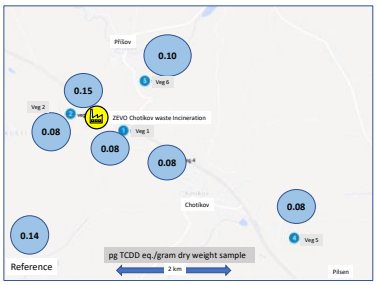


PCDD/F and dl-PCB in pine needles Pilsen, Czech Rep. 2022 (2021)





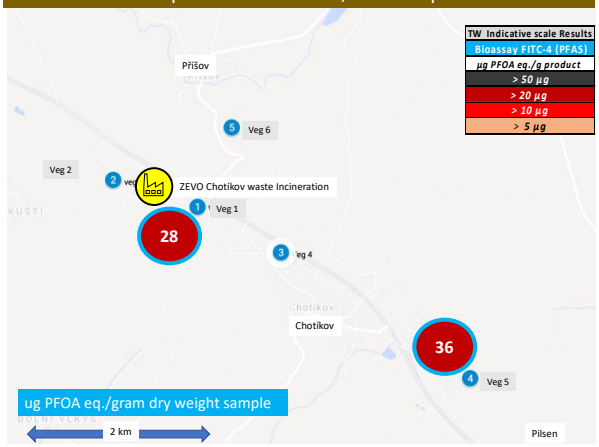




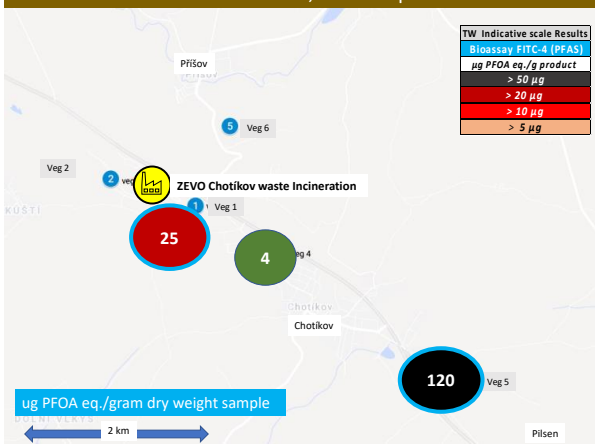
Analysis of PFAS in moss and pine needles

Analyses were performed on PFAS with the bioassay of FITC-T4. It is noteworthy that the highest concentrations of PFOA equivalent are found both in pine needles and mosses at the location (5) 2000 m Southwest of the waste incinerator, near the city of Pilsen.

PFAS in pine needles Pilsen, Czech Rep. 2022



PFAS in moss Pilsen, Czech Rep. 2022

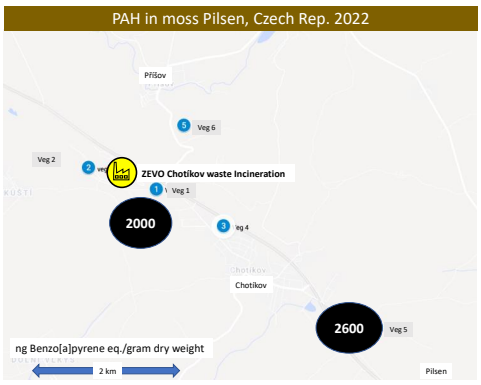


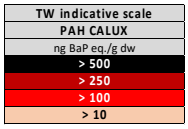
Analysis of PAH in moss and pine needles

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

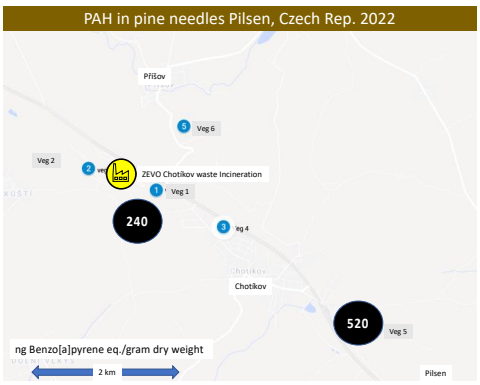
PAH in moss and pine needles Pilsen, Czech Rep. 2022 (2021)

PAH in moss Pilsen, Czech Rep. 2022





PAH in pine needles Pilsen, Czech Rep. 2022



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	

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