THE TRUE TOXIC TOLL

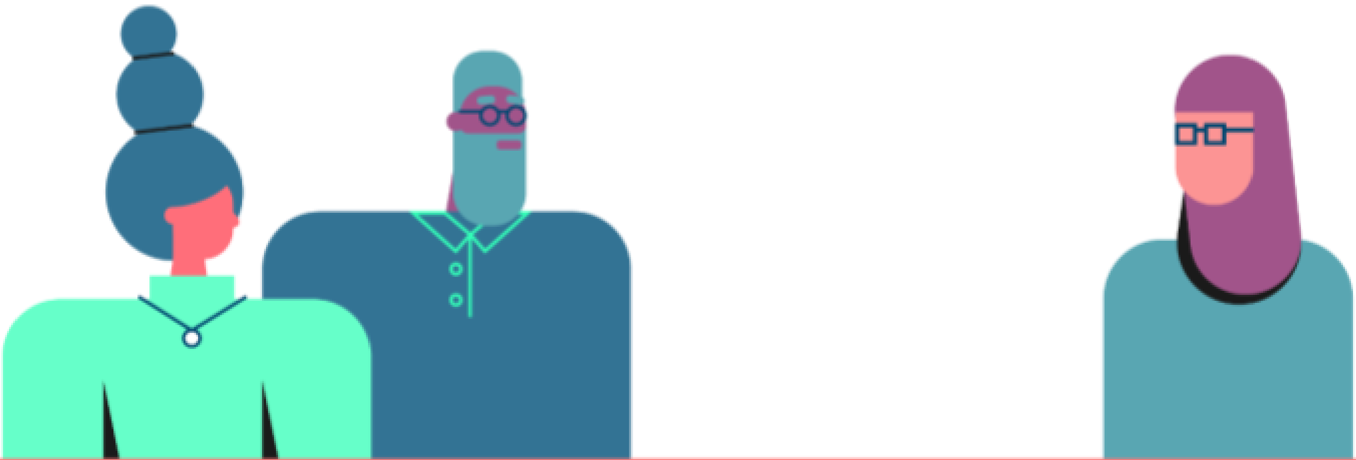
Biomonitoring research results

Pilsen, Czech Republic | 2021

Indicators

Chicken eggs | Moss | Pine needles

ZERO WASTE EUROPE | TOXICOWATCH | Hnutí DUHA | ecologistas en acción



Acknowledgments

And credits

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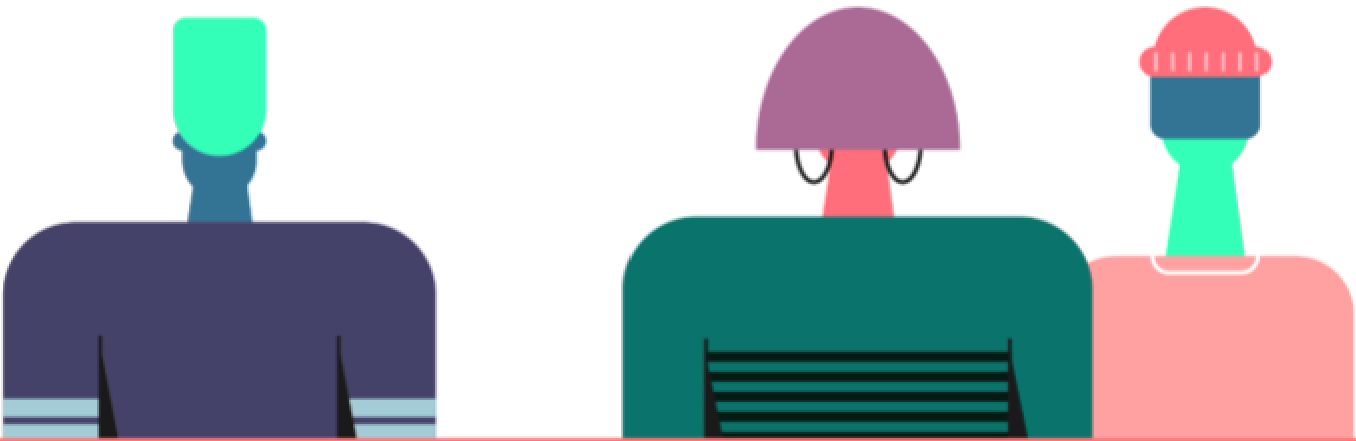

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and abbreviations

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	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
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

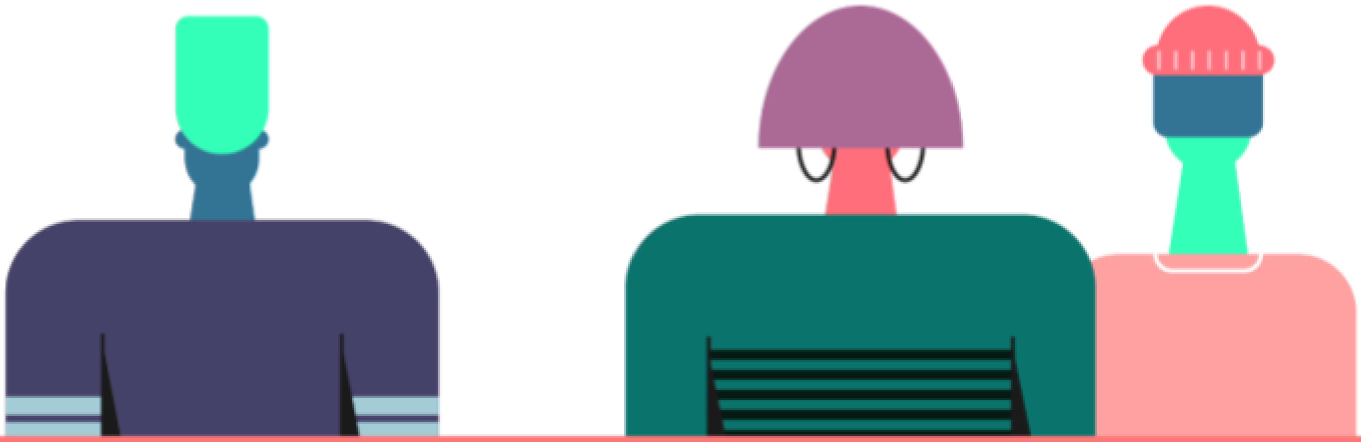
Introduction

The complexity of the chemical content of today's household and industrial waste presents a challenge for turning modern waste into energy in (WtE) waste incinerators. Even with the application of the most developed air pollution control devices (APCD), it is still a huge challenge to eliminate the multitude of persistent organic pollutants (POPs) in waste incinerator residues and flue gases. The dynamics of combustion processes and the inevitable emissions of toxic substances of very high concern (SVHC) into the environment is the main topic of ongoing research worldwide. Even in the most remote areas of the world, such as the Arctic (marine environment), toxic chemicals are found, which have been transported huge distances from industry in other parts of the world. Because of the transboundary behaviour of persistent organic pollutants, international treaties are required to regulate, mitigate or even eliminate toxic chemical emissions. Loopholes still exist in national and international regulations, resulting in an underestimated registration of persistent organic pollutants. Mandatory measurements for waste incineration relating to toxic pollutants like dioxins are sampled in a very short time frame of 6-12 hours a year in optimal conditions and pre-announced, all according to the EU regulations. These regulations are based on chemical analyses of only a few chlorinated dioxins and furans, while many other POPs remain outside the scope, such as brominated dioxins and PFAS. The limitations of the chemical GC-MS analyses could be overcome with the application of bioassays for measuring POPs even in the flue gases of an incinerator. Continuous monitoring of dioxins and other substances of very high concern in the chimney gives a far more accurate picture of the emission from combustion, especially when it is measured in the event of incomplete combustion as in exceptional operating conditions such as shutdown or start-up.

All over the world, there is growing public awareness and concern over the potentially toxic effects of persistent organic pollutants on human health and the environment. In particular, people living near waste incinerators need to be reassured about their health risks, (short- and long-term exposure to incineration emissions), the safety of such combustion facilities, and compliance with regulations – not only under normal conditions, but also in other than normal operating conditions (OTNOC), such as shut-downs, start-ups, and failures.

ToxicoWatch (TW) aims to function as a bridge between people, science, and government when it comes to dioxins, POPs, and waste incineration. TW performs research on dioxins with a focus on possible sources like waste incineration emissions by carefully selecting biomarker samples in an area. A sampling with focused matrices like distance, sample location and collecting information about the research area needs to be performed according to the theory of sampling (TOS) with references in the interest of the research. The biomatrices for this study are primarily backyard chicken eggs, pine needles, and mosses. The chemical analyses are expanded with innovative bioassays to investigate a broader spectrum of POPs such as dioxin-like PCBs, other (mixed) halogenated dioxins, PAHs, and PFAS.

This study is part of a Europe-wide biomonitoring research project on POP emissions in possible relation to waste (WtE) incineration. The project is running simultaneously for 2021 and 2022 in three countries: Lithuania, Spain, and the Czech Republic. ToxicoWatch Foundation, based in the Netherlands, is participating as a scientific partner together with three environmental organisations, Ecologists in Action in Spain, Hnutí DUHA in Czech Republic, and Žiedinė Ekonomika in Lithuania, all coordinated by Zero Waste Europe.



The incinerator

and surronding conditions

Chotíkov, near Pilsen (Czech: Plzeň) is the location of the fourth municipal WtE waste incinerator in the Czech Republic: ZEVO Chotíkov or ZEVO Plzeň. The owner is Plzeňská teplárenská, a.s. The WtE incinerator receives about 12,369 tons of waste per hour, with an annual load of 95,000 tons of waste. The input is mixed municipal waste (about 60% of the total) and non-hazardous industrial waste (about 40%) (various types of packaging, furniture boards, building materials, etc., from factories), as well as sewage sludge (granulated, dried sludge). The environment of Chotíkov and Pilsen is flat with pine trees, like Pinus sylvestris all around. In the early days, mines were operating in this region. The waste incinerator in Pilsen officially began operations on 21 March 2019. The 80-metre-high chimney is a typical landmark in the environment.

Figure 1: ZEVO Plzeň of Plzeňská teplárenská

Sewage sludge is co-incinerated in Pilsen. In the Netherlands, the WtE REC incinerator in Harlingen has a lot of problems with combusting sewage sludge. The moisture content in the waste input creates problems with efficient processing and combusting of the waste. The higher the moisture content of the waste, the more difficult it is to maintain the right combustion temperature to prevent dioxin emissions. Another concern is PFAS in the sewage sludge. The excessive amounts of PFAS can be a serious problem if contaminated sewage sludge is applied to the soil as fertilizer, because of the PFAS content.[1] If PFAS contaminated sludge is incinerated the outcome can result in the re-emission of these fluorinated forever chemicals into the air with depositions on soil and water. Research is still going on and pilot studies show the challenge of destroying persistent organic pollutants like PFAS and dioxins in the Post Combustion Zone at relatively low temperatures of 8500 in WtE incinerators. At the time of preparation of this report, no data were available (yet) on the chemical composition of the sludge, nor on emissions during start-up and shutdown, insofar as these were measured with the appropriate equipment and analysis methods in the exhaust gases from the waste incinerator in Pilsen.

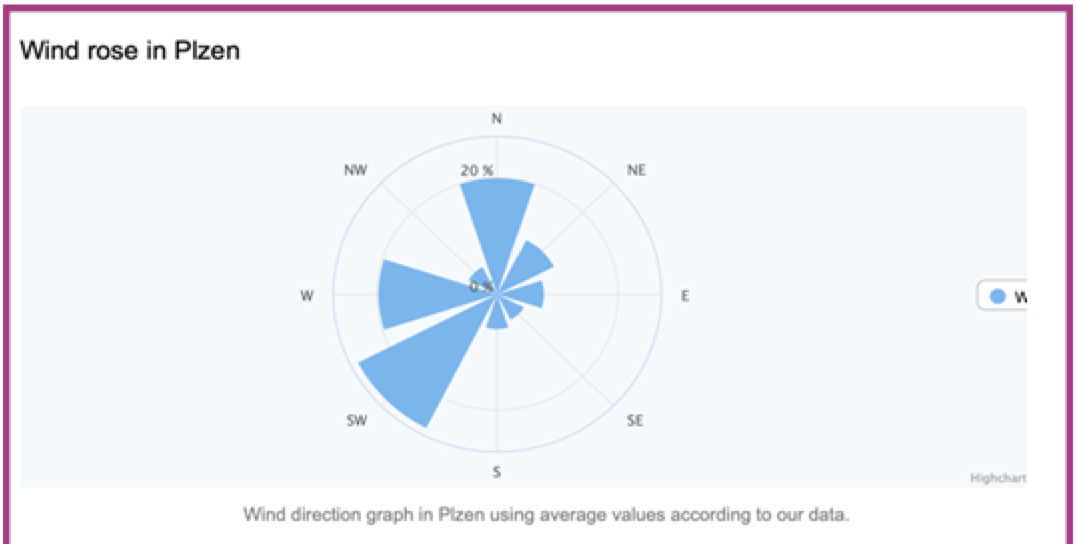
[1] *Semerad J. et al. (2020) Screening for 32 per- and polyfluoroalkyl substances (PFAS) including GenX in sludges from 43 WWTPs located in the Czech Republic - Evaluation of potential accumulation in vegetables after application of biosolids Chemosphere 261,128018*

Wind direction

and depositions

The annual average wind direction in Pilsen is shown in Figure 2. This can be used as a model to predict possible depositions by the incinerator. The dominant wind direction is mainly from the South-west and North. The use of a wind rose for modelling deposition emissions from incineration processes is limited. Figure 3 displays on the left the dominant wind direction in Harlingen, the Netherlands (NL), South-West wind from the North Sea On 1 October 2015, a major malfunction occurred at the WtE waste incineration plant, which was accompanied by prolonged emissions of black clouds that blew in the direction of the UNESCO Wadden Sea on that particular day. The city and region of Harlingen (NL) escaped, during this calamity, of being hit by an enormous toxic cloud of dioxins. This example of a calamity in a waste incineration process illustrates the limitations of using annual average wind direction “safety-models” to determine the load of emission depositions. Dense clouds of emission-loaded dust can and will occur during OTNOC situations like failures, shutdowns, and start-ups. TW studies have learned that in just a few hours emissions of dioxins can emit far more than the annual load of a dioxin model calculated by the regulatory 12 hours (2x 6 hours/year, preannounced) measurement during normal operating conditions. Assuming the emission of dioxins is a discontinued process, calculation with average wind direction and speed is of little importance as large emissions can occur in a very short time frame. Figure 3c shows a TW research of dioxin-contaminated eggs of backyard chicken around the WtE waste incinerator Harlingen (NL).

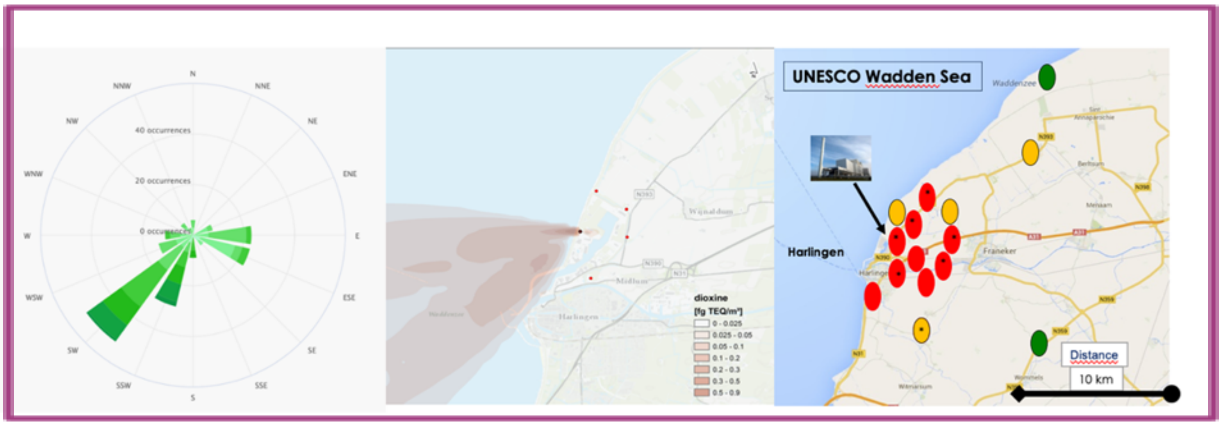
Figure 2: Annual wind rose for Pilsen, Czech Republic

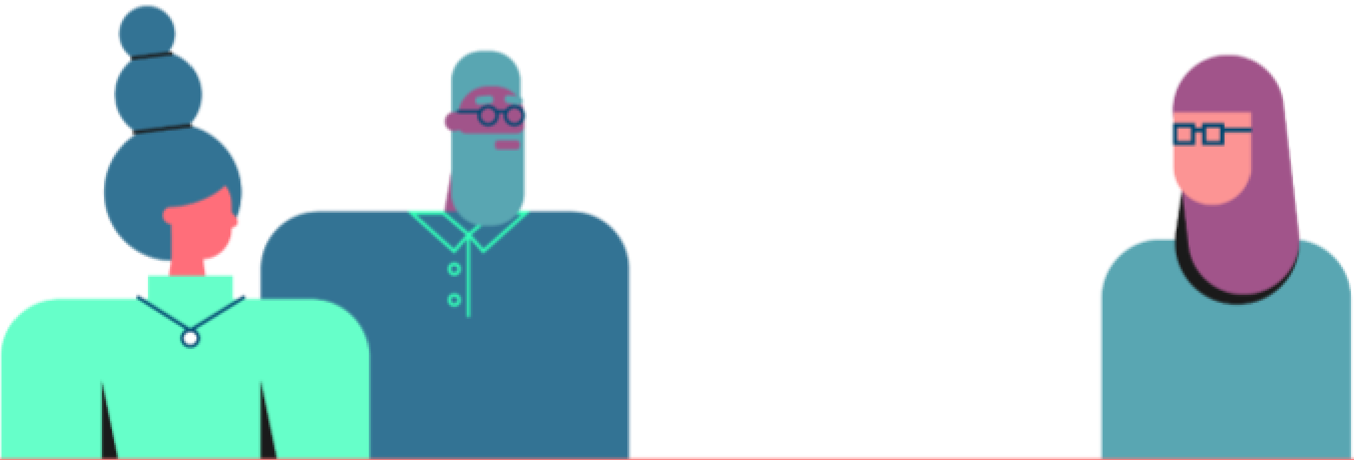


N	NE	E	SE	S	SW	W	NW
Northern	Northeastern	Eeastern	Southeastern	Southern	Southwestern	Western	Northwes
20%	10.5%	7.7%	4.9%	6%	25.9%	19.7%	5.3%

Wind direction is an indication, but the deposition of emissions can differ completely when OTNOC and other parameters like coastline fumigation, along seashores are included, as they should be. In a very short time, in hours or even minutes, extremely polluted POP clouds of loaded dust can be emitted in whichever wind direction is dominant at that moment. This relativizes the use of average dominant wind directions in calculation models for POP emissions.

Figure 3: Wind rose for Harlingen (a), dioxin cloud during calamity, 2015 (b), contaminated eggs, Harlingen ©





Dioxins

Dioxins and furans are classified as highly toxic chemicals that have a serious effect on human health, causing cancer, diabetes, neurotoxicity, immunotoxicity, and chloracne. The emission of dioxins by incinerators was discovered in 1977 in the Netherlands.[2] Although dioxins also can be formed by volcanic eruptions, forest fires, or other natural events, the anthropogenic origin of dioxin is far more than the natural source. Major sources of atmospheric dioxins (PCDD/Fs) include stationary emissions, especially from various types of incinerators, including secondary aluminium smelters, sinter plants, small-scale municipal solid waste incinerators (MSWI), medical waste incinerators (MWI), electric-arc furnaces, industrial waste incinerators, cement kilns, and crematoria. At the Stockholm Convention in 2004, 184 nations agreed to do their utmost to reduce the emissions of dioxins and other unintentionally produced organic **pollutants**. To achieve the goal of the Convention, Parties are required to implement the Best Available Techniques (BAT) and apply the Best Environmental Practices (BEP).[3]

The term 'dioxin' refers to three groups of substances: polychlorinated dibenzo-p-dioxins, (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs). Figure 4 provides a schematic view where the black balls represent carbon atoms, the red oxygen, and the orange chlorine atoms (these can be substituted by other halogenated elements, like bromine, fluorine and iodine to form dioxins). The possible combinations with chlorine atoms (congeners) are 75 for dioxins (PCDDs), 135 for furans (PCDFs), and 217 PCBs congeners. Of these chlorinated congeners, 29 are found to be toxic and therefore regulated in the EU; 7 PCDDs, 10 PCDFs, and 12 dl-PCBs.

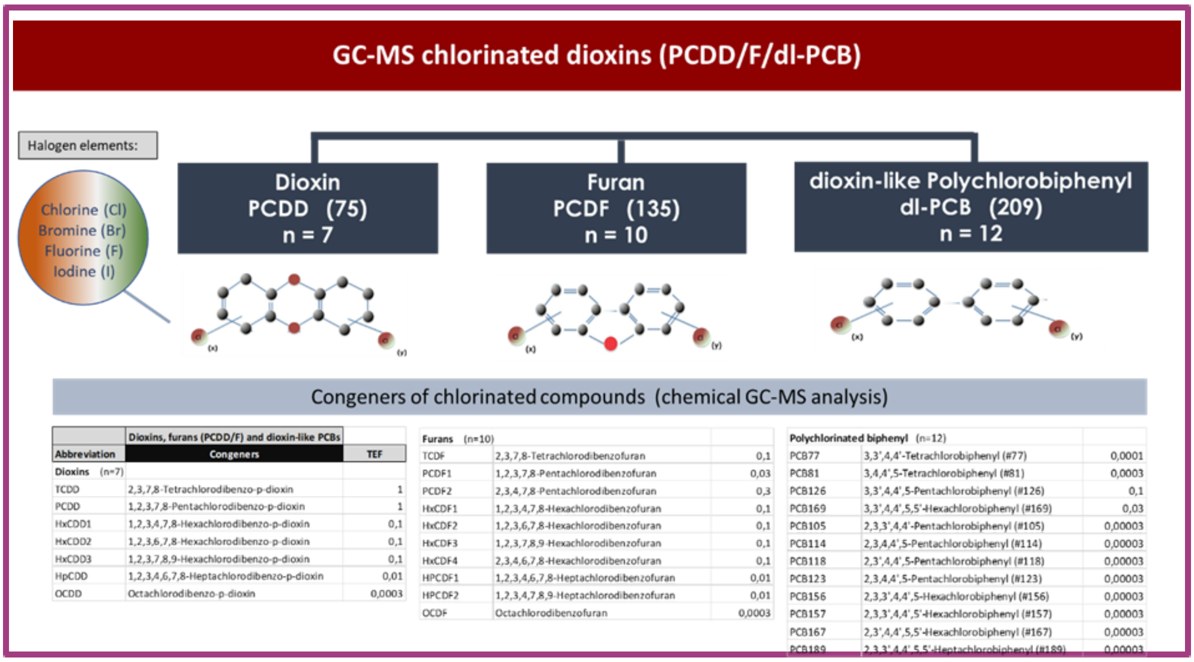
Only chlorinated dioxins and furans (PCDD/F) are regulated by the EU for emissions of persistent organic pollutants (POPs) from waste incinerators. Dioxin-like polychlorinated biphenyls, brominated and mixed halogenated dioxins, all substances with dioxin-like properties, are (still) not regulated in the EU.[4]

[2] *Olie K. , Vermeulen P.L., Hutzinqer O. (1977). Chemosphere No. 8, po 455 - 459, 1977.*

[3] *Guidelines on Best Available Techniques and Provisional Guidance on Best Environmental Practices relevant to Article 5 and Annex C of the Stockholm Convention on Persistent Organic Pollutants (2008). Stockholm Convention on Persistent Organic Pollutants.*

[4] *C. Budin et al. (2020). Chemosphere 251, 126579*

Figure 4: Schematic overview of dioxins (PCDD/F/dl-CBP), © ToxicoWatch



The EU sets limits of 2.5 pg TEQ/g fat for PCDD/F and of 5.0 pg TEQ/g fat for the sum of dioxin (PCDD/F/dl-PCB) for eggs. An EU action limit is set on 1.75 pg TEQ/g fat for PCDD/F and dl-PCB in eggs - see figure 6. For bioassay DR CALUX the EU limits are 1.7 pg BEQ/g fat (eggs) and 3.3. pg BEQ/g fat (eggs) for the sum of dioxins (PCDD/F/dl-PCB), see figure 7.

Figure 5: EU regulations for dioxins (PCDD/F/dl-PCB), © ToxicoWatch

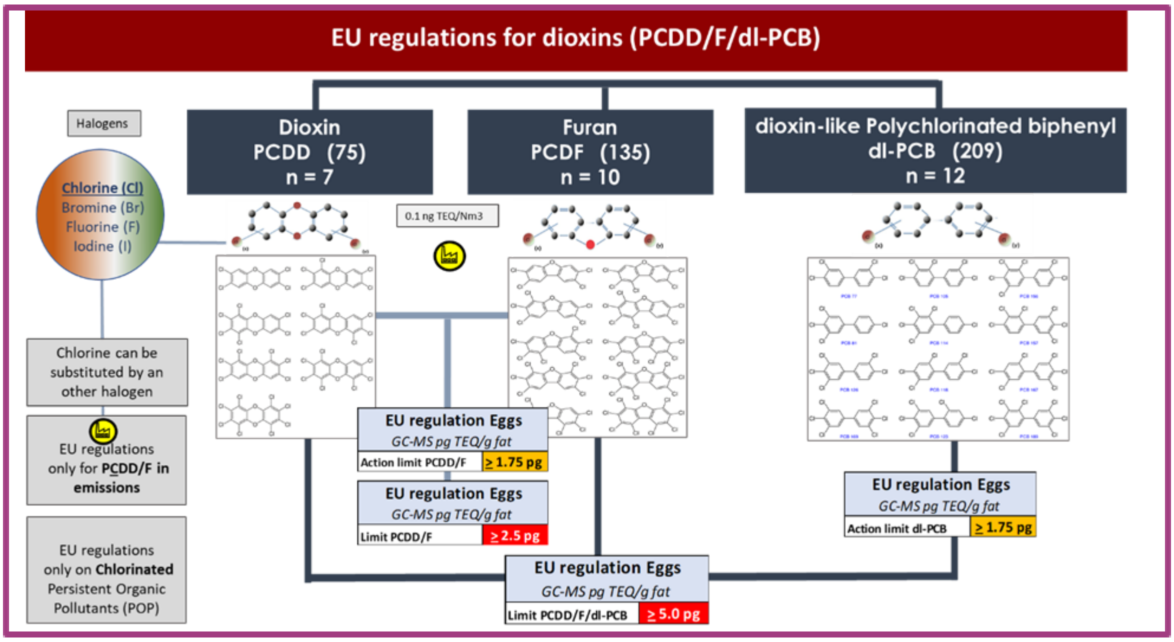
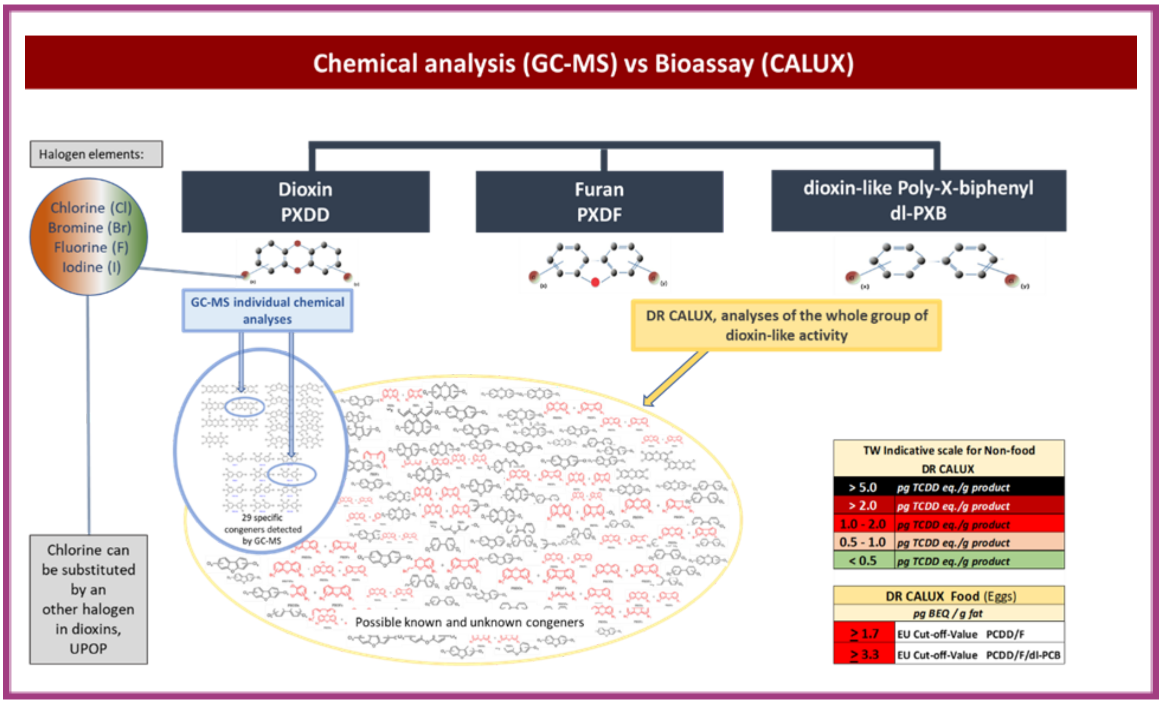
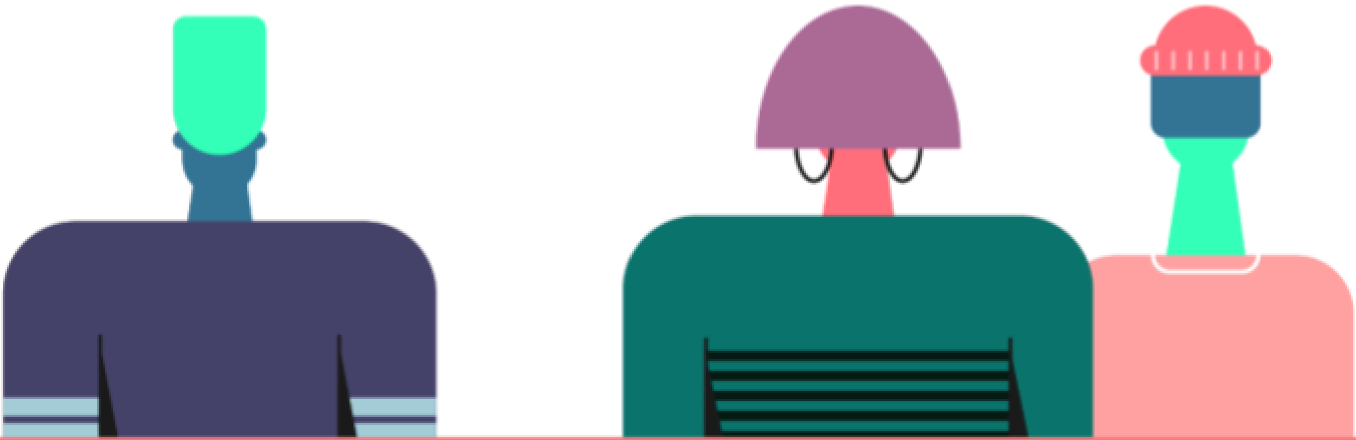


Figure 6 displays the difference between the chemical analysis with GC-MS and the bioassay DR CALUX. GC-MS analysed specific compounds, while DR CALUX measures the total toxic effect of a mixture of dioxin-like activity.

Figure 6: Chemical GC-MS analysis of dioxins (PCDD/F/dl-PCB) vs bioassay DR CALUX analysis, © ToxicoWatch





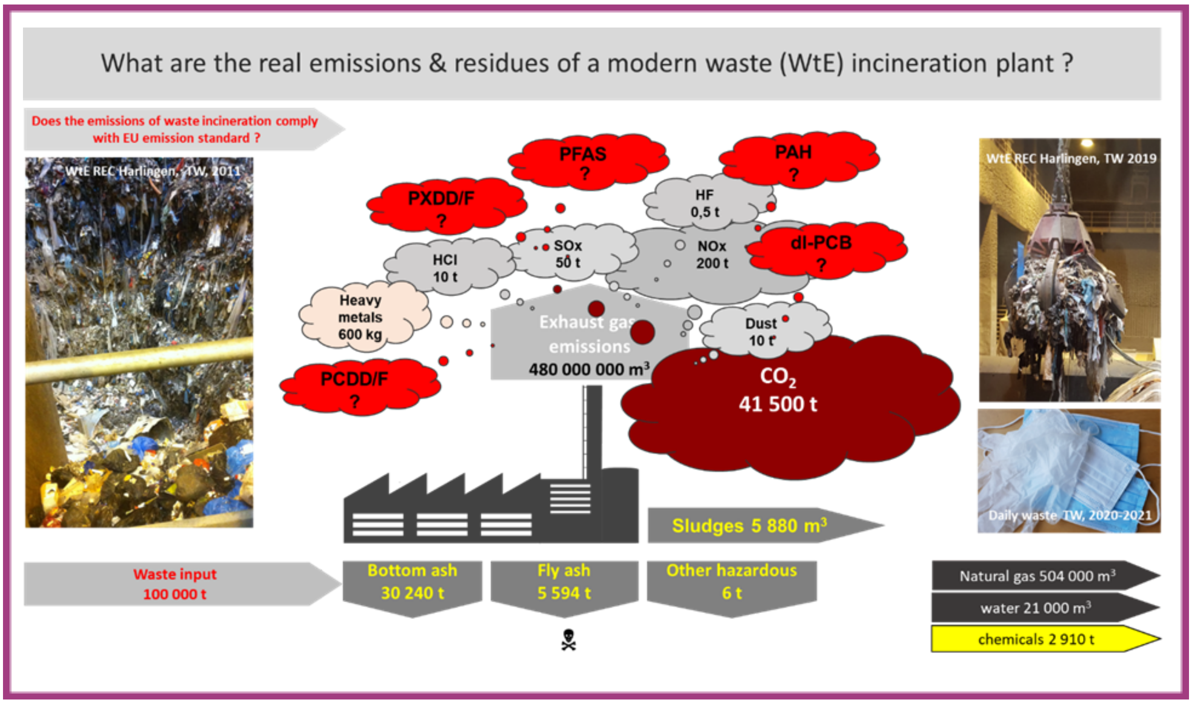
Emissions

of waste incineration

In this biomonitoring research the focus will be on persistent organic pollutants (POPs) like PCDD/F, PXDD/F, PAH and PFAS. See red clouds in Figure 7. A central question in this research is whether waste incineration is a solution for waste disposal and energy production, when there is an unintentional production and emissions of POPs, such as dioxins (PCDD/F/dl-PCB). Figure 8 shows the quantities of emissions per 100,000 tonnes of waste. This figure shows the configuration of the WtE waste incinerator REC in Harlingen, the Netherlands with the specific configuration of Air Pollution Control Devices (APCD) and specific waste input. A big difference in volume of mega-tonnage CO_2 and the relative tiny amount of the extreme toxicity of dioxins, expressed in milligrams.

Although this research is mainly focused on the emissions of substances by air, which is only a small amount of the toxic substances, the main output are the incinerator residues, like fly and bottom ash. The processing, storage and sustainable application of toxic incineration residues is an environmental risk.[5] For more sustainability and a healthy environment the focus needs to be on more recycling of waste materials. Important in this context, the production of non-toxic material in order to prevent (unknown) toxic recycling and with that to prevent a possible toxic greenwashed recycling waste tsunami in the future.

Figure 7: What are the real emissions of WTE incineration?, © ToxicoWatch



[5] *ToxicoWatch (2020). The hidden impacts of incineration residues, Zero Waste Europe*

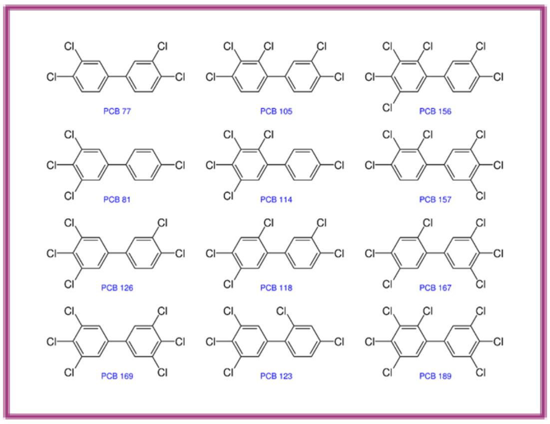
Polychlorinated biphenyl

(PCB)

Polychlorinated biphenyls (PCBs) are chemicals that were widely used in industrial processes from the 1930s until the late 1970s. PCBs were used extensively in many industrial applications, including fire-resistant transformers and insulating condensers. The substances were used as heat exchanger fluids, and in aluminium, copper, iron, and steel manufacturing processing. PCBs were also used as plasticizers, in natural and synthetic rubber products, as adhesives, insulating materials, flame retardant, lubricants in the treatment of wood, clothes, paper, and asbestos, chemical stabilizers in paints, pigments, and as dispersing agents in formulations of aluminium oxide. PCBs were added in small quantities to inks, plastics, paints, sealants, adhesives, and dye solvents for carbonless paper. Although their production ended in 1979, huge amounts of PCBs are still in the environment.[6]

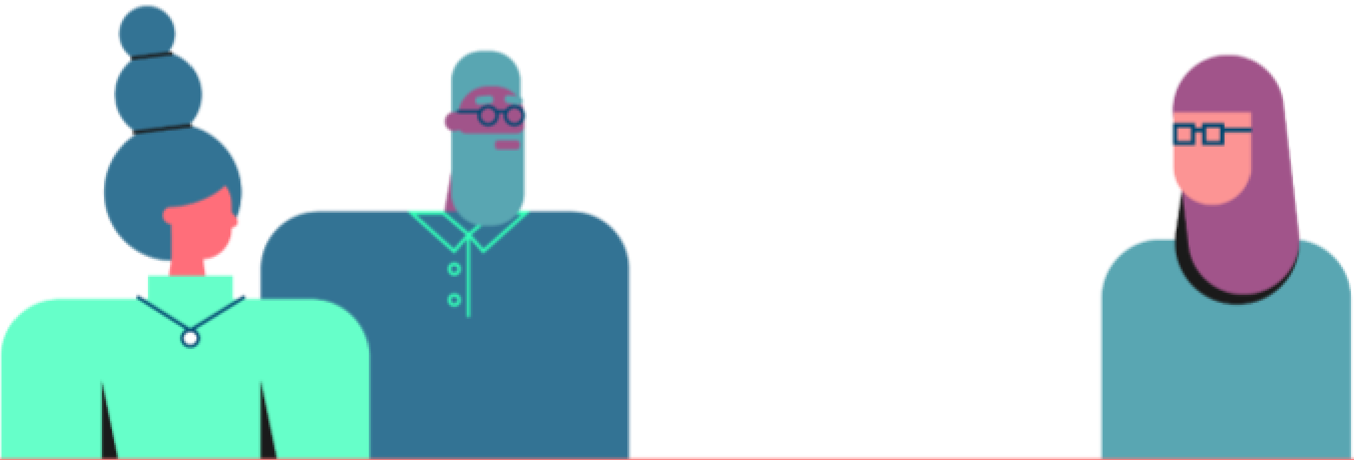
From a toxicological point of view, there is a significant difference between dioxin-like PCBs and non-dioxin-like PCBs. Polychlorinated biphenyl congeners without chlorines in the ortho positions are called “coplanar” because the two phenyl rings can assume a planar state. This subgroup of 12 PCB congeners (non-ortho or mono-ortho chlorine substituted) with at least four chlorine substituents easily adopt a coplanar structure with toxicological properties similar to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), see Figure 8. This subgroup is termed dioxin-like PCBs (dl-PCBs) and are referred to as the 12 dioxin-like PCBs, see also Figure 4, 5. Due to their lipophilic properties and poor degradation, PCDD/Fs and dl-PCBs accumulate in the food chain and are persistent in the environment. Prevention or reduction of human exposure is best performed by source-directed measures, i.e., strict control of industrial processes to reduce the formation of dioxins. The greatest uncertainty with PCB and incinerator emissions lies in the composition of waste content and the distribution of PCB between air and waste. A TW study revealed that 10% of the emissions in the flue gases of an incinerator chimney were dioxin-like PCBs (dl-PCBs).[7] However, in biomatrices around the incinerator, including eggs, milk and vegetation, the contribution of the TEQ dl-PCB is often more than 50%. More research is needed to confirm a direct relation to the emissions from a waste incinerator. PCB 126 was particularly dominant in all biomatrix samples.

Figure 8: Dioxin-like pCB (dl-PCB) congeners



[6] Petrlík J., Arkenbout A. (2019) Dioxins – The old dirty (dozen) guys are still with us www.researchgate.net/publication/332877688

[7] Toxicowatch (November 2018). Hidden Emissions: A story from the Netherlands, a case study, Zero Waste Europe, https://zerowasteeurope.eu/wp-content/uploads/2018/11/NetherlandsCS-FNL.pdf

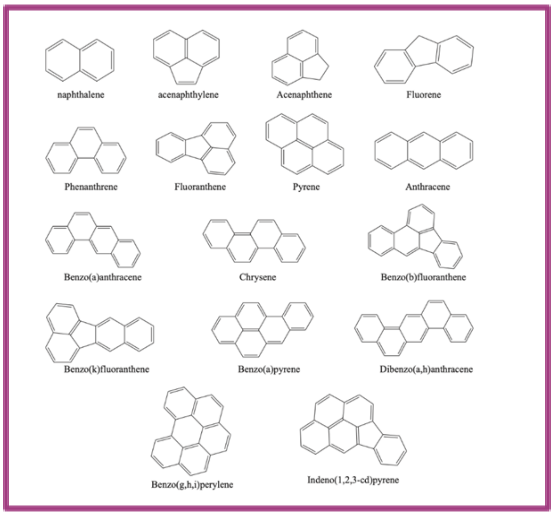


Polycyclic aromatic hydrocarbon

(PAH)

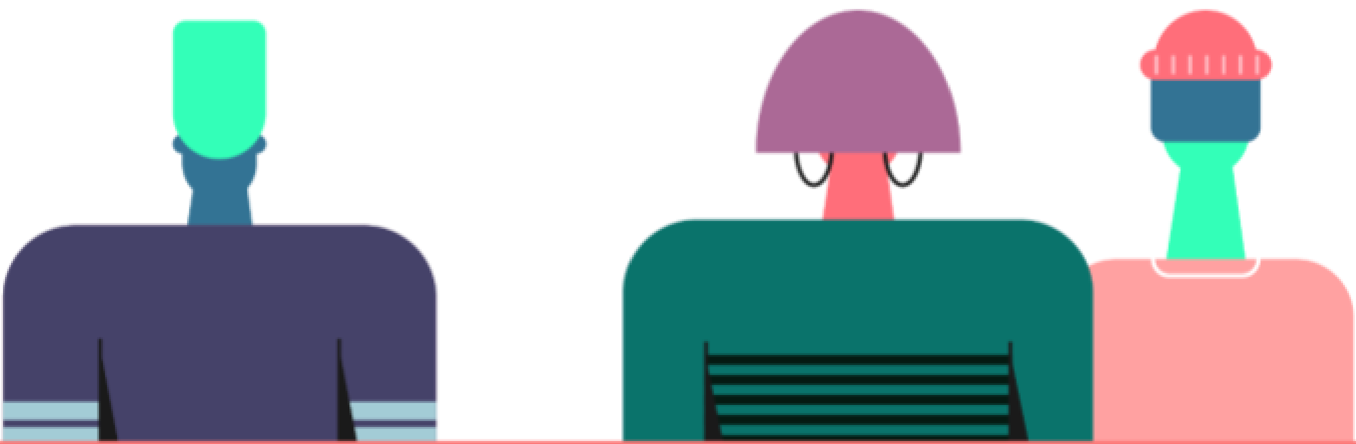
Polycyclic aromatic hydrocarbon (PAH) represents a class of ubiquitously occurring environmental compounds that are implicated in a wide range of toxicological effects. This class of compounds is known by their carcinogenic, mutagenic, and teratogenic properties. PAH leads to the development of a variety of disorders affecting all body systems as well as causing skin cancer and other skin diseases in animals and humans.The PAHs with more than four (4) benzene rings have the most carcinogenic activity. PAH is able to reduce the effectiveness of measles vaccination through immunotoxicity to innate and adaptive immune cells.[8] Routine measurement of PAH contamination generally involves chemical analytical analysis of a selected group of representatives. The United States Environmental Protection Agency (EPA) and the European Commission (EU) classify 16 PAHs as priority pollutants (EPA-16):naphthalene,acenaphthylene,acenaphthene,fluorene,anthracene,phenanthrene,fluoranthene,pyrene,chrysene,benz[a]anthracene,benzo[b]fluoranthene,benzo[k]fluoranthene,benzo[a]pyrene(B[a]P),indeno[1,2,3-c,d]pyrene,benzo[g,h,i]perylene,and dibenz[a,h]anthracene, see Figure 9 . However, this will result in an underestimation of the PAH in a sample.[9] PAHs form a very large group of several tens of thousands (>10.000) of compounds when taking into account the attaching with halogens, hydroxyl or when a nitrogen atom can be in the place of a carbon atom in the ring. In this research a bioassay (PAH CALUX) analysis method is used to measure the total toxic effect of all toxic PAH in a sample. When measuring with a chemical (GC-MS) analysis on a pure sample with known PAH individual congeners, like benzo[a]pyrene, the results with a bioassay (PAH CALUX) analysis, are the same in measured values if the Relative Potency Factor (RPF) are taken into account. In environmental samples, like in this research, high levels of PAH are found, because the bioassay measures the total toxic effect of all present PAH in the sample. The results of a PAH CALUX analysis will be expressed in equivalent benzo[a]pyrene, a class 1B carcinogen.

Figure 9: Molecular structures of the most common PAHS (Hussain 2018)



[8] *Ruri Vivian Nilamsari et al. 2020. Polycyclic Aromatic Hydrocarbons (PAH) Reduces the Effectiveness of Measles Vaccination Through Immunotoxicity to Innate and Adaptive Immune Cells. Research J. Pharm. and Tech. 2020; 13(12):6128-6131.*

[9] *Andersson J.T., Achten C. (2015). Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes - Polycyclic Aromatic Compounds, 35:330–354*

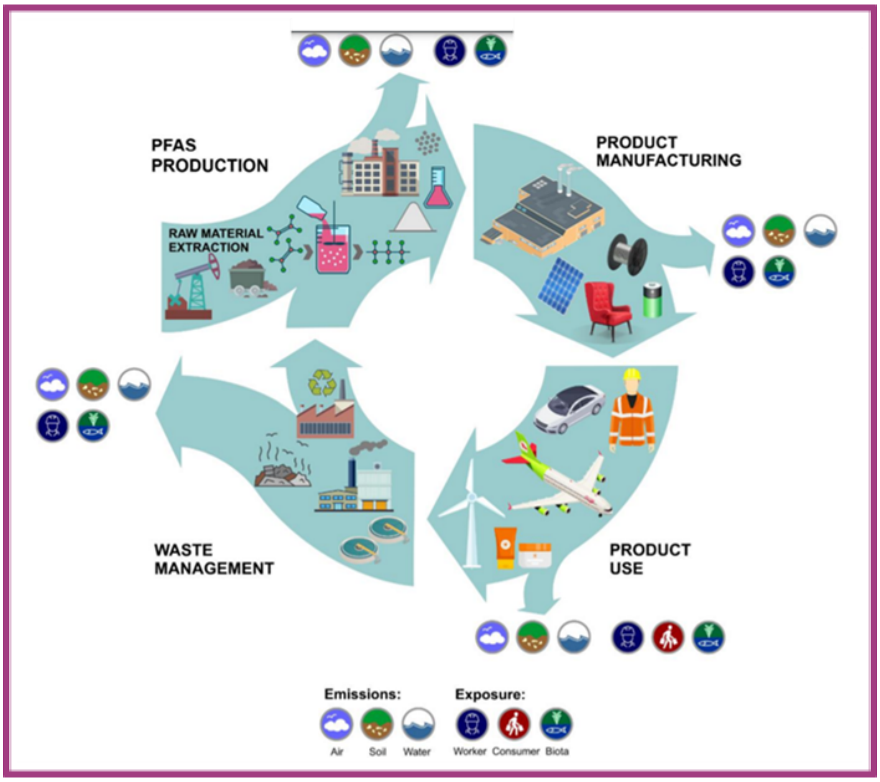


Per- and PolyFluoroAlkyl Substances

(PFAS)

Per- and PolyFluoroAlkyl Substances (PFAS) are a class of man-made chemicals with a wide range of industrial and commercial applications, which has resulted in their ubiquitous presence in the environment. The consolidated PFAS list of EPA contains 6330 PFAS CAS-name substances, of which 5264 are represented with a defined chemical structure resulting in increasingly complex mixtures entering the environment. PFAS possess thermal, chemical, and biological stability, non-flammability, and surface-active properties. Their high applicability combined with chemical stability has led to an inevitable accumulation of PFASs in the environment and as a result to their detection in environmental matrices (air, sewage, rivers, and dust) in food products and food packaging, in drinking water, and also in human samples (breast milk, blood) PFAS are associated with adverse human health effects on thyroid function, metabolism (including overweight/obesity, diabetes, insulin resistance, and high cholesterol, foetal development, and the immune system.[10] The risk of immunotoxicity for humans and wildlife cannot be discounted.[11]

Figure 10: Overview figure of EU Commission staff working document on PFAS, October 2020



According to the EU Commission Staff Working Document on Poly- and perfluoroalkyl substances (PFAS), October 2020, SWD(2020) 249 final, see Figure 10, "A recent opinion from the European Food Safety Agency (EFSA) concluded that both PFOS and PFOA are associated with reduced antibody response to vaccination. PFOS also causes a reduced

[10] *Young, A.S. et al.,(2021). Env. Health Perspect. 129 (4), 047010-1 to 047010-13.*

[11] *Corsini, E., et al., Perfluorinated compounds: Emerging POPs with potential immunotoxicity. Toxicol. Lett. (2014).*

resistance to infection”. EFSA concluded that parts of the European population exceed the tolerable weekly intake (TWI) from food of four PFAS.[12]

However, analysis techniques for PFAS are only available for a limited number of PFAS substances. Chemical (GC-MS) analyses are not capable of detecting the currently known > 8000 PFAS congeners. Some substances are known to be present, these are called known unknowns, the substances that are not known to be present are called the unknown unknowns. It is a struggle for quality for laboratories to produce consistent data in PFAS analysis. Laboratories may suffer from multiple difficulties, which hinder clear identification of the error sources. The lack of analytical standards, the distinctive physical-chemical properties of the PFCs, and matrix effects, at every step of the analysis from sampling to detection is a common problem.[13] Therefore, in this biomonitoring study, a different analysis methodology is chosen to measure the PFAS in the biomarkers around a waste incinerator.

The used analysis method in this research is based on the competition between thyroid hormone (T4) and PFAS for T4-binding site on the blood-protein transthyretin (TTR). The analysis methods are the FITC-T4 assay and the bioassay PFAS CALUX. The Relative Potency Factor (RPF) for 12 different PFAS congeners are expressed in PFOA equivalency (Table 1, Zeilmaker 2018)[14] - see Table 1.

Overview of PFAS exposure pathways to the human population and the environment - see Figure 11, (Sunderland et al. 2019).[15] “PFAS are man-made substances that do not naturally occur in the environment. Examples of PFAS are GenX, PFOA perfluoro octanoic acid and PFOS perfluorooctane sulfonates. PFASs are used in many products. As a result, and due to emissions and incidents, these substances have ended up in the environment and are now found in, among other things, soil, dredging spoils and surface water.”[16]

Table 1: Relative Potency Factor (RPF) for 12 PFAS expressed in PFOA equivalency (RIVM, Zeilmaker 2018)

Congener	RPF
Perfluorobutanesulfonate (PFBS, C4)	0.001
Perfluorohexanesulfonate (PFHxS, C6)	0.6
Perfluorooctanesulfonate (PFOS, C8)	2
Perfluorobutanoic acid (PFBA, C4)	0.05
Perfluoropentanoic acid (PFHxA, C6)	0.01
Perfluorooctanoic acid (PFOA, C8)	1
Perfluorononaoic acid (PFNA, C9)	10
Perfluoroundecanoic acid (PFUnDA, C11)	4
Perfluorododecanoic acid (PFDoDA, C12)	3
Perfluorotetradecanoic acid (PFTeDA, C14)	0.3
Perfluorohexadecanoic acid (PFHxDA, C16)	0.02
Perfluorooctadecanoic acid (PFODA, C18)	0.02

[12] ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_PFAS.pdf

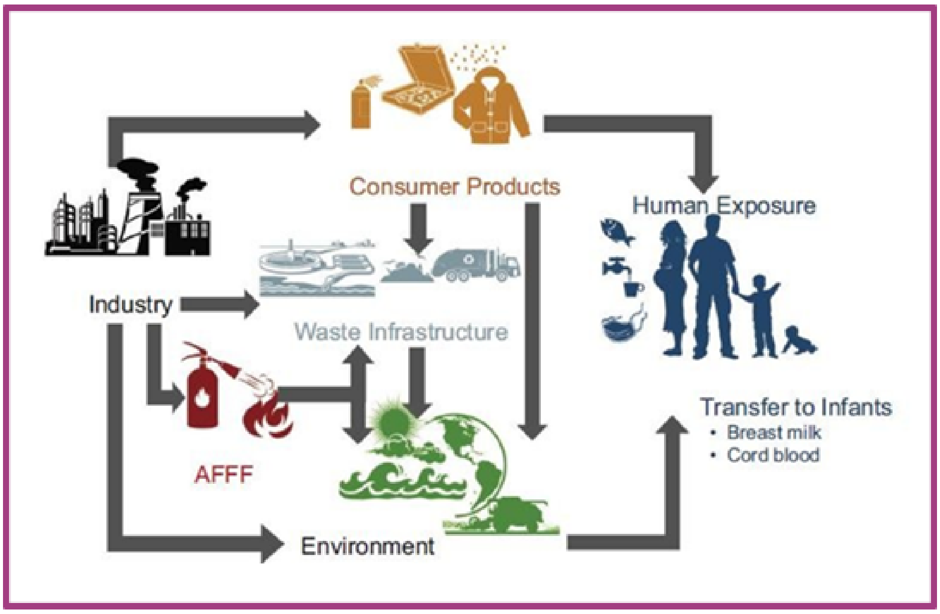
[13] *Van Leeuwen SPJ, Kärrman A, Van Bavel B, De Boer J and Lindstrom G, 2006. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples. Environmental Science and Technology, 40, 7854–7860.*

[14] *M.J. Zeilmaker et al 2018. Mixture exposure to PFAS: A Relative Potency Factor approach, National Institute for Public Health and the Environment, RIVM Report 2018-0070.*

[15] *Sunderland EM. (2019). Journal of Exposure Science & Environmental Epidemiology (2019) 29:131–147*

[16] www.rivm.nl/en/pfas

Figure 11: Overview of PFAS exposure pathways to the human populations and the environment (Sunderland et al. 2019)



Bioassays

for quantification of chemicals

DR CALUX®

The bioassay **DR CALUX® (Dioxin Responsive Chemical Activated LUciferase gene eXpression)** is 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.

Like all EU regulations, **Regulation EU 1881/2006**[17] is immediately enforceable as law in all member states. This regulation sets maximum levels for certain contaminants in food products. The food products which are listed should not be placed on the commercial market if a contaminant exceeds the maximum level set out in the Annex of the EU documents.

The limits set in legislation are expressed in pg TEQ/g, based on GC-MS measurements. The GC-MS analysis concerns 7 dioxins (PCDDs), 10 furans (PCDFs), 12 dioxin-like polychlorinated biphenyls (dl-PCBs), and 6 indicator polychlorinated biphenyls (i-PCB).

The results of the chemical analyses with GC-MS of dioxins (PCDD/F/dl-PCBs) will be calculated with a specific Toxic Equivalency Factor (TEF) towards a TEQ value (see page 5 Abbreviation and TEF for dioxins, and dl-PCBs). The sum of the TEQ will be measured with upper bound values, meaning calculation with the value of the limit of detection (LOD) of a specific congener. These GC-MS **limit values** for chicken eggs are 2.5 pg TEQ/g fat for dioxins (PCDD/F) and for the sum of dioxins (PCDD/F) and dioxin-like PCBs (dl-PCBs), the GC-MS limit value is set at 5 pg TEQ/gram fat. When exceeding these GC-MS limit values, chicken eggs are not allowed to be on the commercial market, (see Figure 4 and 5).

Directive 2013/711/EU[18] sets out the cut-off values of the DR CALUX analysis determined. If the analysis exceeds the 70% value of PCDD/F, i.e. 1.7 pg BEQ/g and/or 70% of the limit of the sum of dioxins (PCDD/F/dl-PCB) i.e. 3.3 pg BEQ/g a GC-MS analysis of the egg sample is recommended to establish the results with the GC-MS chemical analysis, where **EU 1881/2006** can be applied.

2013/711/EU [19] 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 a source of contamination and to take measures for its reduction or elimination.

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[17] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20210919&from=EN
[18] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN
[19] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013H0711&from=EN

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 benz[a]pyrene, a compound with five benzene rings and a class 1B carcinogen, is used as an indicator of PAH exposure (see Annex V for relative potency factors PAH).[20,21]

PFAS CALUX®

The chemical analyses on individual PFAS congeners are very limited, depending on the lab, only 8 - 55 substances can be analysed. Practically, this means that only 0.1- 1% can be determined with the chemical analyses, compared with the value of the Total Organic Fluorine (TOF).[22] The bioassay of PFAS CALUX® comprises human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Thyroid Responsive Elements (TREs) as a reporter gene for the presence of thyroid-like inhibiting compounds. It is based on the TTR-binding of PFAS in combination with the TRβ CALUX detection. The presence of increasing concentrations of PFAS capable of competing with T4 for TTR-binding sites will result in a decreased amount of TTR-bound T4. Disruption of T4-TTR binding is benchmarked against the reference compound Perfluorooctanoic acid (PFOA), whose value is set to one (1), just like TCDD in the TEQ calculation.[23] See table 1 for relative potency factors of other PFAS. The analysis results of the PFAS CALUX are expressed in: µg PFOA equivalent/g product.

FITC-T4 assay

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).[24,25] The thyroid hormone homeostasis can be disrupted by environmental chemicals at different points of interaction in the thyroid pathway, including

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[20] *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.*

[21] *Pieterse B, Felzel E, Winter R, van der Burg B, Brouwer A. PAH-CALUX, an optimized bioassay for AhR-mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. Environ Sci Technol. 2013 Oct 15;47(20):11651-9. doi: 10.1021/es403810w. Epub 2013 Sep 25. PMID: 23987121.*

[22] *Straková, J., Schneider, J., Cingotti, N. et al., 2021. Throwaway Packaging, Forever Chemicals: European wide survey of PFAS in disposable food packaging and tableware. 54 p.*

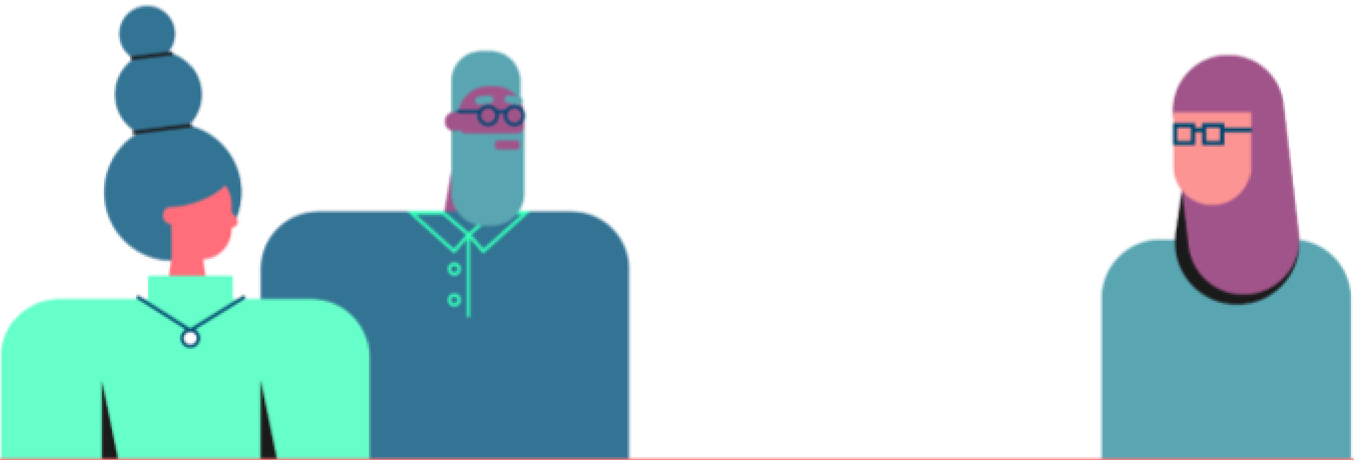
[23] *P.A. Behnisch et al. Developing potency factors for thyroid hormone disruption by PFASs using TTR-TRβ CALUX® bioassay and assessment of PFASs mixtures in technical products, Environment International 157 (2021) 106791*

[24] *Smith, D.S., (1977). FEBS Lett. 77, 25-27.*

[25] *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)*

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.



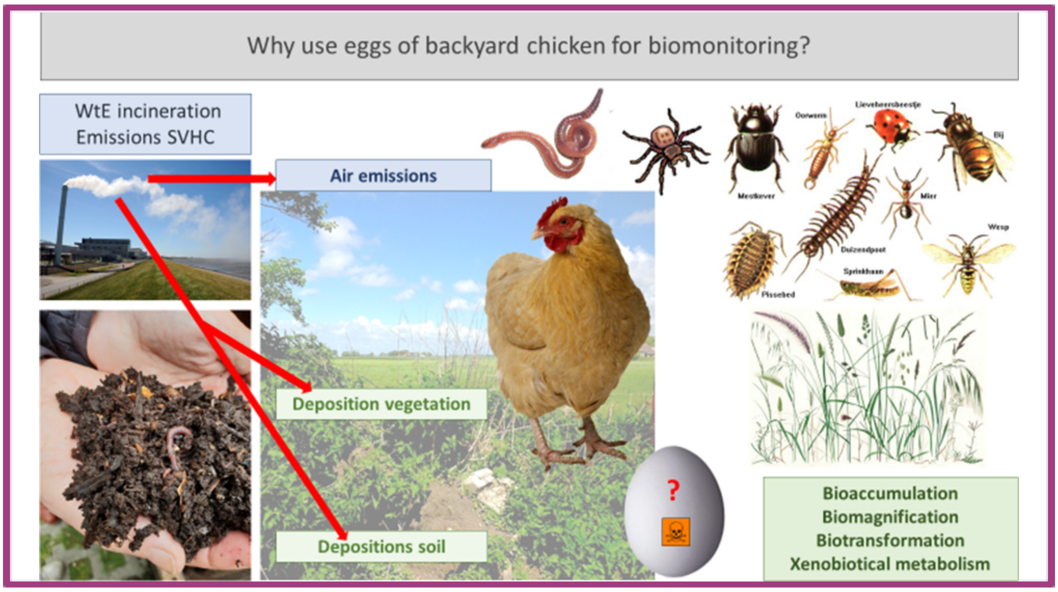
Backyard chicken

eggs

Backyard chicken eggs are used for biomonitoring levels of contamination by POPs in various studies. Eggs are sensitive indicators of POP contamination in soil and dust and are a significant exposure pathway from soil pollution to humans. Eggs from contaminated areas can readily lead to exposures that exceed thresholds for the protection of human health. Chickens and their eggs might, therefore, be ideal “active samplers”: an indicator species for the evaluation of contamination levels of sampled areas by POPs, particularly by dioxins (PCDD/Fs) and dioxin-like-PCBs (dl-PCBs).[26,27]

When chickens are free to forage on natural uncovered soil in the open air without roofing, they are in optimal contact with the environment. Eggs can reflect the chemical situation of soil biota related to the atmospheric deposition of hazardous chemical particles from industrial emissions, such as car shredding, metallurgy, coal-fired power plants, foundries, the PVC industry, cement kilns, the paper industry, and waste incineration. Chickens forage on and in the soil, eating insects, invertebrates, vegetation, and even grass (Figure 12). As a result, persistent organic pollutants (POPs) like dioxins (PCDD/F/dl-PCB) can be found in the fatty egg yolk and act as a biomarker for the environment. The chicken excretes the toxic compounds like dioxins into the fatty yolk when producing the eggs (dioxins are fat related). The older the chicken is, the more toxic compounds can be collected in the body, a process called bioaccumulation. Biotransformation refers to the capability of an organism to break down certain substances. Xenobiotic metabolism refers to the metabolism or breakdown of foreign substances not belonging to the substances of an organism of an ecological system.

Figure 12: Overview of backyard chicken eggs in natural environment



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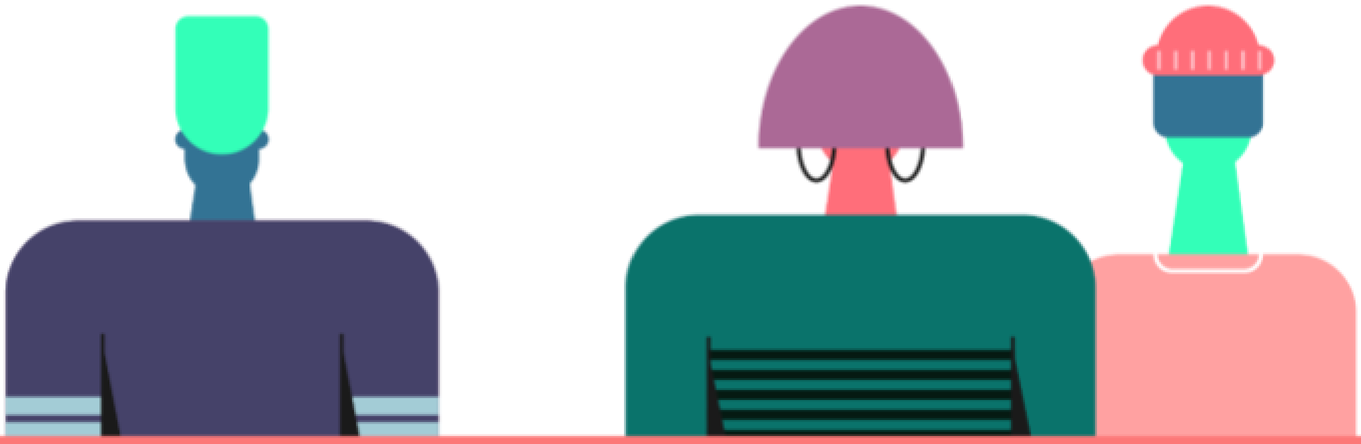
[26] *Arkenbout A, Esbensen K H. (2017) Sampling, monitoring and source tracking of Dioxins in the environment of an incinerator in the Netherlands, Proceedings Eighth World Conference On Sampling and Blending / Perth*
[27] *Petrlík J. (2015). Persistent Organic Pollutants (POPs) in Chicken Eggs from Hot Spots in China.Beijing-Gothenburg-Prague, Arnika - Toxics and Waste Programme.*

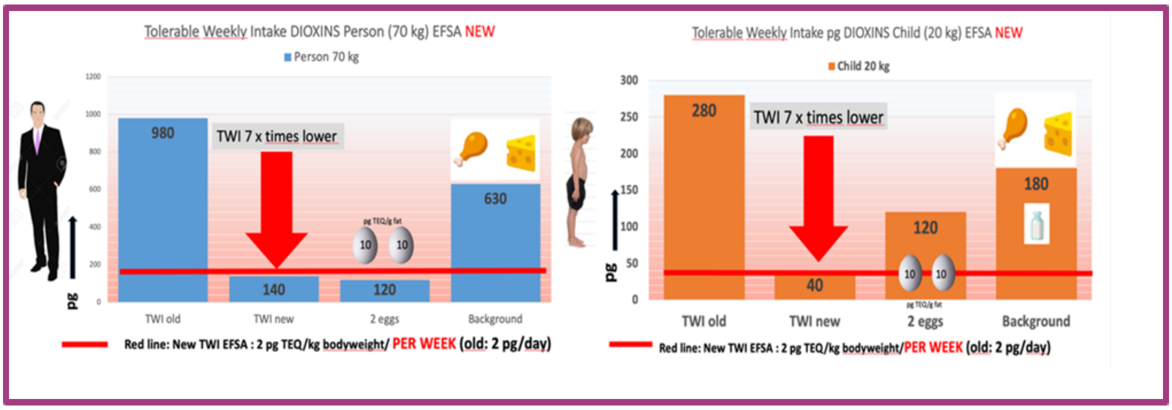


European Food Safety Authority

(EFSA)

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are important contaminants in the food chain. In 2018, the European Food Safety Authority (EFSA) reduced the tolerable weekly intake (TWI) from 14 to 2 pg TEQ (Toxic Equivalents)/kg body weight per week, based on extended scientific reviews conducted on humans and animals (EFSA, 2018)[28] - see Figure 13. It demonstrates the present exposure to dioxins for most consumers in the EU exceeds the TWI. The maximum levels for PCDD/Fs and dl-PCBs in food and feed have to be reduced according to the EFSA advice, however the EU has taken, so far, no action. The actual dioxin limit value for eggs is 2.5 pg TEQ PCDD/g fat and 5.0 pg TEQ/fat PCDD/F/dl-PCB. A reduction of these limit values with a factor of 7 will have enormous implications - see Figure 14. The actual EU limits (Figure 6 and 7), based on pre EFSA advice, before 2018, can be seen as more the result of political and economic arguments, rather than preliminary ones on behalf of human health.

Figure 13: Tolerable Weekly Intake of dioxins revision for adults and children (EFSA 2018), graphics by © ToxicoWatch.



Public concern about ongoing contamination of POPs in human bodies has increased since several of these substances of very high concern have been identified as hormone disruptors and immune depressors. There are many risks and effects of having these chemicals in our environment and, as far as dioxins are concerned, they are of no benefit. Pollutants like dioxins contaminate the environment, persist for decades, and cause problems such as cancer, birth defects, learning disabilities, immunological deficiency, behavioural, neurological, and reproductive discrepancies in human and other animal species. **For PFOS and PFOA** the EFSA established **a tolerable weekly intake (TWI) of 13 ng/kg body weight per week (PFOS) and 6 ng/kg body weight per week (PFOA) respectively**.[29] For both compounds, the exposure of a considerable proportion of the population exceeds the proposed TWI. A safe daily dose of **GenX or HFPO-DA is 3 ng/kg of body weight,** according to the EPA.

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

[29] *EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK et al, 2018. Scientific Opinion on the risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 2018;16(12):5194, 284 pp.*

Sampling

of backyard chicken eggs

This project was carried out in cooperation with Ing. Ivo Kropáček, waste expert Hnutí DUHA – Friends of the Earth Czech Republic. For the preparation, a manual was applied to explain the sample steps. Annex I provides an outline of this 9-step Sampling Plan by TW. First, an inventory was undertaken for the possibilities of biomonitoring in the region around the waste incinerator on backyard chicken eggs and vegetation.

In February to June 2021, several actions were undertaken to find the locations of backyard chicken owners who were willing to participate in this biomonitoring study, along with locations for monitoring vegetation and mosses. Seven egg locations were found within a 3- and 5-km radius of the waste incinerator and two in the inner 2-km circle. See Table 2 for an overview of the questionnaire responses given by each chicken coop owner.

TW conducted this research together with the sampling team at locations in Pilsen, Czech Republic. Working with the Czech sampling team was essential because of the need to communicate with the participants in the Czech language. TW underlines the importance of this kind of participation with the local community when conducting biomonitoring research. This is needed to gain local information about the region and for optimum sampling of biomarkers. The local team provided TW with photo material of the sampling, handling, and locations of the backyard chicken coops as well as about the vegetation locations of pine trees and mosses. The reason for taking samples of evergreen gymnosperm trees (in this research needles of the family of Pinaceae and particularly of the species Pinus sylvestris) is that the deposition or uptake of dioxins can take place continuously throughout the year. Pine needles remain on pine trees for more than 2-5 years, depending on the species. The pine needles are good biomarkers, thanks to the fatty cuticle, the protective layer on the outside of the pine needles that prevents too much water evaporating. From each vegetation sample (pine needles and mosses) per location 100-150 grams were collected in plastic HDPE bags and stored dry and cool until they could be shipped in boxes to the Netherlands.

Between July 28th and August 2nd 2021, sampling was conducted on backyard chicken eggs, feed, soil, and vegetation of pine needles and mosses according to the 9-step TW Sampling Plan, see Annex I.

For the egg sampling: 10 eggs were collected by the Czech team at each location, together with samples of the chicken feed and soil in the foraging area. The eggs of all locations were collected in 24 hours and sent the following day by courier service to the Netherlands. The eggs were split and the fatty yolk of all 10 eggs from one location mixed together for a homogeneous sample per location. The mixed egg yolk of 10 eggs/location was divided into two parts, one used for the DR CALUX and the other kept in reserve or stored in the freezer (-170C) for other analyses such as PFAS or PAH.

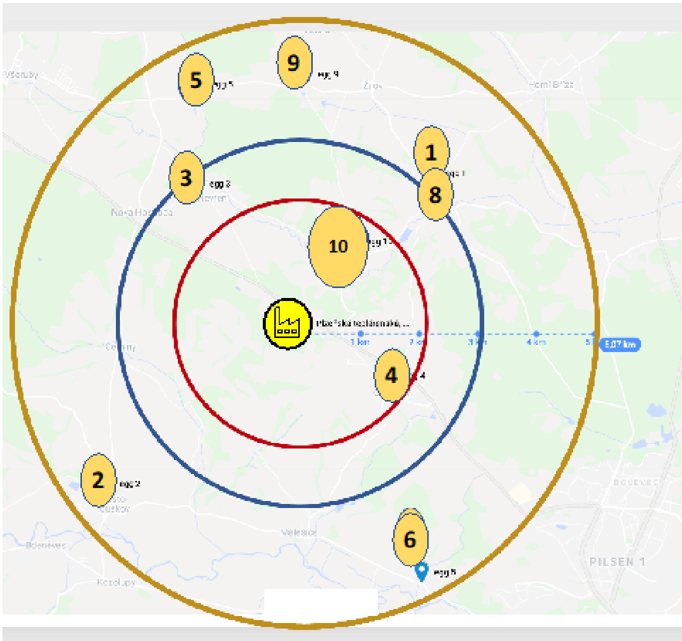
Biomonitoring dioxins (PCDD/F/dl-PCB) and PFAS in eggs

Biomonitoring of backyard chicken eggs as biomarkers started with chicken coop owners who want to participate in this study. In total. Nine (9) chicken coop owners were willing to cooperate. In this research TW used also an ordinary box of 12 eggs from a supermarket for reference, so in total ten (10) egg locations with 10-12 eggs/location for a pooled sample were used for this research in the region of Pilsen, 2021, see Figure 14, with 2 locations in the inner circle of <2 km from the waste incinerator.

Figure 14: Locations of backyard chicken eggs, Pilsen, Czech Rep. 2021.

Locations of backyard chicken, Pilsen Czech 2021

Eggs Czech Rep.		
Location	TW-REF-NR	Distance (m)
EGG01	TW-CZ21-Egg01	3250
EGG02	TW-CZ21-Egg02	4290
EGG03	TW-CZ21-Egg03	3000
EGG04	TW-CZ21-Egg04	1780
EGG05	TW-CZ21-Egg05	4400
EGG06	TW-CZ21-Egg06	4700
EGG07	TW-CZ21-Egg07	reference
EGG08	TW-CZ21-Egg08	3170
EGG09	TW-CZ21-Egg09	4240
EGG10	TW-CZ21-Egg10	1630



Questionnaire

All the chicken coop owners in the nine (9) participating egg locations were asked to take part in a questionnaire provided by TW. Their answers to questions about keeping chickens, such as numbers of hens and roosters, breed, foraging area, and possible confounders are summarized in Table 2. Eight (8) chicken coop owners gave their permission for photos to be used in this biomonitoring report. One sample of eggs was taken from the local supermarket for reference purposes.

Table 2: Summary of questionnaire involving chicken coop owners, Pilsen, 2021

Sample egg location questionnairy Pilsen, Czech 2021									
TW-REF-NR	CZ21-Egg01	CZ21-Egg02	CZ21-Egg03	CZ21-Egg04	CZ21-Egg05	CZ21-Egg06	CZ21-Egg08	CZ21-Egg09	CZ21-Egg10
Distance (m)	3250	4290	3000	1780	4400	4700	3170	4240	1630
Pics permissions	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NO	Yes
Breed	Hens of red	Black sumen	Mix	Mix	Mix	Mix	Mix	Mix	Susex
									Isa brown
N hens	20	12	20	14	22	22	35	20	6
N rooster	-	-	-	-	5	1	3	-	-
Age	-	12 mnd	-	12 mnd	36 mnd	48 mnd	24 mnd	12-36 mnd	6 mnd
Eggs/day	8	8	10	5	8	13	20	15	4
Eggs/week									
Eggs/month	*240*	*240*	*300*	*150*	*240*	*390*	*600*	*450*	*120*
Foraging area	360	40	160	150	30	84	30	300	17,5
Housing	15	6	16	25	12	6	50	6	1,3
Terrain	soil	soil	soil	soil	soil	soil	concrete	soil	soil
	grass		grass	grass	concrete	trees	manure	grass	grass
	vegetation		vegetation	vegetation	trees	stones		vegetation	plastic
			wood	rock					
			concrete	concrete	asbestos				stones
Feed	corn	old pastry	corn	corn	corn	corn	corn	potatoes	corn
	waste	shells		grass	kitchen	grain	kitchen		grain
		ground grain				poll			garden
Outdoor fireplace	moderate	no	moderate	noderatly (wood	no	no	no	no	no
Housing material	earth/stone	saw dust	concrete	wood	saw dust	straw	concrete	wood	chipboard
		earth		concrete					
All purpose burner	many times	not	often	many times	many times	regular	regular	not	regular
Pesticides use	no	not	not	not	not	not	not	no	no
Industry nearby	no	no		no	no	not	no	no	no
Highway nearby	no	no		no		no	no	no	no

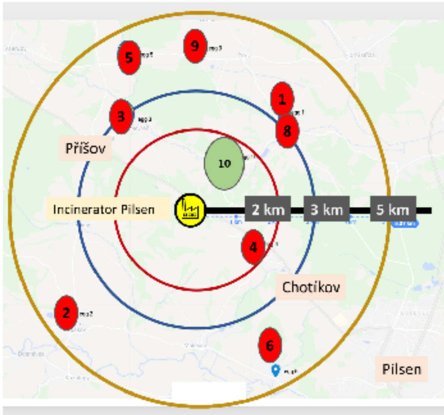
Results DR CALUX analysis on eggs of backyard chicken

In this study nine (9) egg locations (with 10 eggs/per location) were used for the biomonitoring and one (1) egg sample, TW-CZ21-Egg07, is used for reference (supermarket). Figure 15 displays the results of the DR CALUX analyses. The results on the left of the table are marked in red if they exceed the cut-off or limit value of the EU regulation on food safety. With the exception of TW-CZ21-Egg10 and the eggs from the supermarket, all the egg locations exceeded the limit value for the sum of dioxins (PCDD/F/dl-PCB). The map demonstrates the various locations of contaminated eggs.

Figure 15: Results of DR CALUX PCDD/F/dl-PCB, Pilsen 2021

Results sum of dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2021

Results eggs Pilsen, Czech Rep. - 2021					
Location	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB
			DR CALUX *pg BEQ/g fat*		
EGG01	TW-CZ21-Egg-01	3250	3.80	2.10	1.70
EGG02	TW-CZ21-Egg-02	4290	20.00	10.00	10.00
EGG03	TW-CZ21-Egg-03	3000	4.30	1.70	2.60
EGG04	TW-CZ21-Egg-04	1780	3.30	1.30	2.00
EGG05	TW-CZ21-Egg-05	4400	18.00	11.00	7.00
EGG06	TW-CZ21-Egg-06	4700	7.80	2.50	5.30
EGG07	TW-CZ21-Egg-07	reference	0.71	0.30	0.41
EGG08	TW-CZ21-Egg-08	3170	9.80	2.10	7.70
EGG09	TW-CZ21-Egg-09	4240	3.40	0.66	2.74
EGG10	TW-CZ21-Egg-10	1630	0.95	0.41	0.54
	Cut-off	DR CALUX	3.30	1.70	



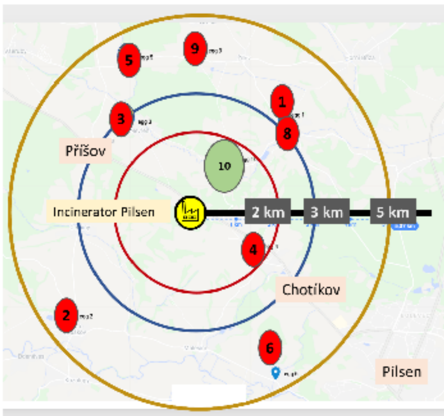
Only one location meets the requirements for safe eggs and this location is closest to the waste incinerator. In this particular location with the Sussex and Isa Brown breeds of hens, these hens are probably too young to actually take up pollutants from the environment. Hens usually start laying eggs from the 20th week, these hens are 24 weeks old. So bioaccumulation in the chicken body is almost 0 with this young age and therefore this egg location is not a particularly good indicator, biomarker, for dioxins in the environment. Next year's follow-up results at this location will be interesting, because by then the hens will have had time to bioaccumulate one year of possible depositions from the air. For this year this location can be marked as a zero measurement.

In figure 16 the results are given of DR CALUX PCDD/F separately. Six (6) egg locations exceeded the action limit set for the DR CALUX on dioxins (PCDD/F). All the values in figures are given in bioassay equivalency or abbreviated as BEQ.

Figure 16: Results of DR CALUX PCDD/F eggs, Pilsen 2021

Results sum of dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2021

Results eggs Pilsen, Czech Rep. - 2021					
Location	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB
			DR CALUX *pg BEQ/g fat*		
EGG01	TW-CZ21-Egg-01	3250	3.80	2.10	1.70
EGG02	TW-CZ21-Egg-02	4290	20.00	10.00	10.00
EGG03	TW-CZ21-Egg-03	3000	4.30	1.70	2.60
EGG04	TW-CZ21-Egg-04	1780	3.30	1.30	2.00
EGG05	TW-CZ21-Egg-05	4400	18.00	11.00	7.00
EGG06	TW-CZ21-Egg-06	4700	7.80	2.50	5.30
EGG07	TW-CZ21-Egg-07	reference	0.71	0.30	0.41
EGG08	TW-CZ21-Egg-08	3170	9.80	2.10	7.70
EGG09	TW-CZ21-Egg-09	4240	3.40	0.66	2.74
EGG10	TW-CZ21-Egg-10	1630	0.95	0.41	0.54
	Cut-off	DR CALUX	3.30	1.70	



Results GC-MS analysis on eggs of backyard chicken

A chemical GC-MS analysis has needed to be performed for the verification of the DR CALUX results and to have recommendations and obligations in the framework of the European food safety regulation, when bioassay results are above the action values of the DR CALUX screening test. That is for dioxins PCDD/F 1.7 pg BEQ/gr fat and for the sum of dioxins and dioxin-like PCBs (PDD/F/dl-PCBs) 3.3 pg BEQ/gr fat. The regulation is in principle meant for the commercial egg sector, the production of eggs by hobby hens at these sampling sites in Pilsen is between 150 to 600 eggs per month, which is a considerable amount.

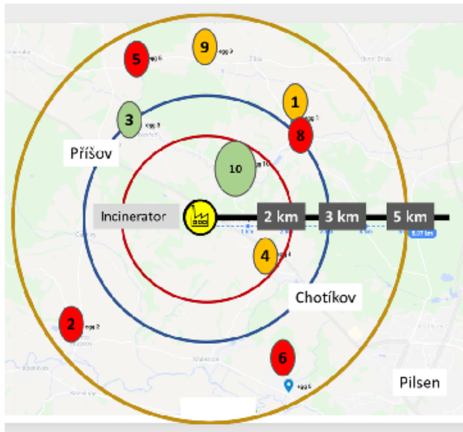
Figure 17: Results of the GC-MS analyses of eggs in the region of Pilsen 2021

Results GC-MS sum of dioxins (PCDD/F/dl-PCB) eggs Pilsen - 2021

Eggs Pilsen, Czech Republic - 2021

Location	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB
			GC-MS *pg TEQ/g fat*		
EGG01	TW-CZ21-Egg01	3250	3.60	2.20	1.40
EGG02	TW-CZ21-Egg02	4290	22.00	6.70	16.00
EGG03	TW-CZ21-Egg03	3000	2.20	1.00	1.20
EGG04	TW-CZ21-Egg04	1780	4.00	0.95	3.00
EGG05	TW-CZ21-Egg05	4400	14.00	8.90	5.00
EGG06	TW-CZ21-Egg06	4700	9.20	1.70	7.40
EGG07	TW-CZ21-Egg07	*reference*	0.69	0.63	0.06
EGG08	TW-CZ21-Egg08	3170	9.70	1.30	8.40
EGG09	TW-CZ21-Egg09	4240	3.50	0.74	2.80
EGG10	TW-CZ21-Egg10	1630	*NA*	*NA*	*NA*
	Cut-off	GC-MS	5.00	2.50	
	Action limit	GC-MS		1.75	1.75

Legenda GC-MS	
pg TEQ/g fat	
2.5	> EU-limit PCDD/F
5.0	> EU-limit PCDD/F/dl-PCB
1.75	> Action level PCDD/F
1.75	> Action level dl-PCB



The results of the chemical analysis with the GC-MS shows four (4) egg locations, numbers 2, 5, 6 and 8, above the limit of 5 pg TEQ/g fat for the sum of dioxins and dl-PCB. Six (6) egg locations, numbers 2, 4, 5, 6, 8 and 9, exceed the limit of 1.75 pg TEQ/g fat for dl-PCBs. Two (2) egg locations, 2 and 5, exceed the EU limit with a factor 3 and 4 with TEQ values of 22.00 and respectively 14.00 pg TEQ/g fat. The highest dioxin (PCDD/F) value is found in egg location 5 with 8.90 pg TEQ/g fat, see figure 17.

Congeners

In Table 3 the 17 dioxin and furan congeners are presented as a percentage of the total TEQ PCDD/F at the different egg locations. Analyses of congener patterns can be helpful to determine the source of the contamination[30]. The dominant congeners (brown) are TCDD, PCDD, PCDF2 and to some extent TCDF. The highest value is given in a box marked black, the second and third in grey with white and black letters respectively. In the individual egg location profile, the congener fraction in concentrations will be mentioned, see Annex II. Locations 2, 5, and 8 seem to have another pattern than the less contaminated sites of 1, 3, 4 and 9. Location 7 concerns the supermarket eggs, where the percentage TEQ is mainly based on upper bound numbers, because all 17 dioxin and furan congeners are below the detection limit. Due to the low dioxins results of Egg location 10 in the DR CALUX bioassay no GC-MS analysis is needed to be performed.

Table 3: Fraction of total TEQ (%) on dioxins (PCDD/F) in eggs, Pilsen, Czech Rep., 2021

% TEQ individual congeners PCDD/F eggs, Pilsen Czech Rep. - 2021										
TW-CZ21	Egg01	Egg02	Egg03	Egg04	Egg05	Egg06	Egg07	Egg08	Egg09	Egg10
TCDD	13%	5%	19%	21%	4%	12%	32%	15%	27%	
PCDD	35%	22%	19%	21%	24%	14%	32%	22%	27%	
HxCDD1	3%	2%	2%	2%	2%	2%	3%	2%	3%	
HxCDD2	6%	6%	3%	3%	4%	5%	3%	3%	3%	
HxCDD3	4%	3%	3%	2%	2%	2%	3%	2%	3%	
HpCDD	2%	2%	1%	1%	1%	1%	0%	0%	1%	
OCDD	0%	0%	0%	0%	0%	0%	0%	0%	0%	
TCDF	6%	9%	14%	16%	4%	13%	3%	15%	11%	
PCDF1	1%	1%	2%	1%	1%	2%	1%	1%	1%	
PCDF2	11%	22%	23%	22%	23%	28%	10%	25%	9%	
HxCDF1	4%	5%	3%	4%	11%	6%	3%	5%	3%	
HxCDF2	4%	8%	5%	2%	9%	8%	3%	3%	4%	
HxCDF3	4%	1%	2%	2%	0%	1%	3%	2%	3%	
HxCDF4	2%	7%	3%	2%	12%	5%	3%	2%	3%	
HPCDF1	4%	6%	2%	1%	2%	2%	0%	2%	3%	
HPCDF2	0%	0%	0%	0%	0%	0%	0%	0%	0%	
OCDF	0%	0%	0%	0%	0%	0%	0%	0%	0%	
DR CALUX										
PCDD/F	2,10	10,00	1,70	1,30	11,00	2,50	0,30	2,10	0,66	0,41
dl-PCB	1,70	10,00	2,60	2,00	7,00	5,30	0,41	7,70	2,74	0,54
PCDD/F/dl-PCB	3,80	20,00	4,30	3,30	18,00	7,80	0,71	9,80	3,40	0,95
GC-MS										
PCDD/F	2,20	6,70	1,00	0,95	8,90	1,70	0,63	1,30	0,74	
dl-PCB	1,40	16,00	1,20	3,00	5,00	7,40	0,06	8,40	2,80	
PCDD/F/dl-PCB	3,60	22,00	2,20	4,00	14,00	9,20	0,69	9,70	3,50	

In Table 4 the fraction of total (%) of congeners in concentration is given. The concentrations of fractions from egg locations 5 and 8 have deviating patterns of Octachlorodibenzo-p-dioxin (octa) and 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (hepta) dioxins. These congeners, which are characteristic of incomplete combustion, are found at locations 1, 2 and 4. The ratio of octa and hepta dioxin concentration with waste incineration is more or less 2 to 1. See in the next chapters more in detail the congener patterns of dioxins in TEQ and concentrations. Because of the absence of detailed information of the

[30] *Hoogenboom R. LAP et al (2020). Congener patterns of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls as a useful aid to source identification during a contamination incident in the food chain, Science of the Total Environment 746 (2020) 141098*

emission patterns incinerator of Pilsen, the emissions patterns of the WtE incinerator REC in the Netherlands is given as reference (based on a TW study of > 20,000 hours measurements of the flue gas inside the chimney of the WtE waste incinerator REC).[31] In the study of Chen [32] also the HpCDF1 congener is an indicator of incineration. Perhaps a different configuration or a different waste input is to be held responsible for this particular emission. Also in Pilsen HpCDF1 is dominantly present.

Table 4: Fraction of total (%) concentration dioxins (PCDD/F) in eggs, Pilsen 2021

% concentrations individual congeners PCDD/F eggs, Pilsen Czech Rep. - 2021										
TW-CZ21	Egg01	Egg02	Egg03	Egg04	Egg05	Egg06	Egg07	Egg08	Egg09	Egg10
TCDD	1%	0%	2%	2%	0%	1%	4%	2%	2%	
PCDD	2%	1%	2%	2%	3%	1%	4%	3%	2%	
HxCDD1	2%	1%	2%	2%	3%	2%	4%	2%	2%	
HxCDD2	4%	4%	3%	3%	4%	4%	4%	4%	2%	
HxCDD3	2%	2%	3%	2%	2%	2%	4%	2%	2%	
HpCDD	12%	12%	6%	9%	8%	10%	4%	5%	8%	
OCDD	20%	17%	19%	23%	7%	18%	38%	18%	24%	
TCDF	4%	6%	14%	17%	4%	11%	4%	18%	10%	
PCDF1	2%	3%	7%	5%	5%	5%	4%	4%	3%	
PCDF2	3%	4%	8%	8%	8%	8%	4%	10%	3%	
HxCDF1	3%	3%	3%	4%	12%	6%	4%	5%	3%	
HxCDF2	3%	5%	5%	3%	10%	7%	4%	3%	4%	
HxCDF3	3%	0%	2%	2%	0%	1%	4%	2%	2%	
HxCDF4	2%	4%	3%	2%	13%	4%	4%	3%	2%	
HPCDF1	29%	36%	18%	8%	19%	15%	4%	18%	23%	
HPCDF2	2%	0%	2%	2%	1%	1%	4%	2%	2%	
OCDF	6%	2%	2%	2%	2%	3%	4%	2%	2%	
DR CALUX										
PCDD/F	2,10	10,00	1,70	1,30	11,00	2,50	0,30	2,10	0,66	0,41
dl-PCB	1,70	10,00	2,60	2,00	7,00	5,30	0,41	7,70	2,74	0,54
PCDD/F/dl-PCB	3,80	20,00	4,30	3,30	18,00	7,80	0,71	9,80	3,40	0,95
GC-MS										
PCDD/F	2,20	6,70	1,00	0,95	8,90	1,70	0,63	1,30	0,74	
dl-PCB	1,40	16,00	1,20	3,00	5,00	7,40	0,06	8,40	2,80	
PCDD/F/dl-PCB	3,60	22,00	2,20	4,00	14,00	9,20	0,69	9,70	3,50	

Egg locations of backyard chicken region Pilsen

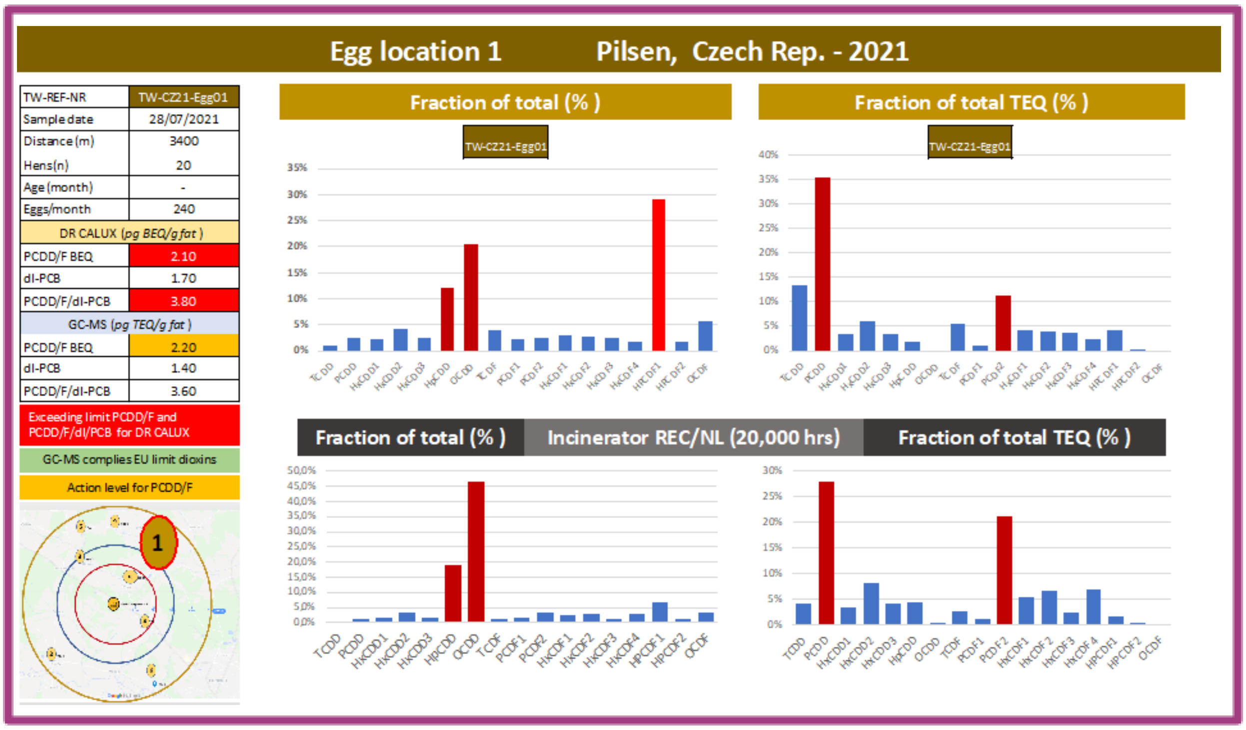
Location 1 is located 3,400 metres North-West of the incinerator. The eggs exceed the cut-off values for dioxins (PCDD/F) and the sum of dioxins (PCDD/F/dl-PCB) with the DR CALUX analyse method. The GC-MS give a compliance for the sum of dioxins (PCDD/F/dl-PCB), but the dioxins (PCDD/F) exceed the action level for PCDD/F. In this overview the congener patterns of the fraction of concentrations and TEQ are compared with the incineration patterns of the WtE incinerator (REC) in Harlingen, the Netherlands. The reason is to provide some interpretation of the patterns, although the waste input and

[31] *Arkenbout A, Esbensen K H, Sampling, monitoring and source tracking of Dioxins in the environment of an incinerator in the Netherlands, Proceedings Eighth World Conference On Sampling And Blending / Perth, May 2017, 117 – 124*

[32] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*

therefore the emission output may differ. The patterns of the REC waste incinerator are the results of more than 20,000 hours of (semi-) continuous measurements of the flue gases. With the colour dark red, the typical incinerator patterns are marked, like Octachlorodibenzo-p-dioxin (OCDD) and 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD) in the concentrations, and the low chlorinated 1,2,3,7,8-Pentachlorodibenzo-p-dioxin (PCDD) and 2,3,4,7,8-Pentachlorodibenzofuran (PCDF2) in the TEQ profiles. Remarkable is the high contribution of the furan congener 1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF) to the concentration of dioxins (PCDD/F) congeners at the eggs locations in Pilsen (except location 4). In the study of Chen (2017).[33] HpCDF is designated as typical for waste incineration. The configuration of the waste incinerator may be altered or another input of waste may be the cause. The results with the bioassay exceed the limits, the GC-MS results complies with the EU regulations for dioxins (PCDD/F) in eggs, except an exceeding of the action limit for dl-PCB, see figure 18.

Figure 18: Results of dioxin analysis in egg location 1.



Location two (2) is 4,300 metres South-West of the incinerator in Pilsen. The eggs do not comply with the EU limit of dioxins and dl-PCBs. The results of 22.00 pg TEQ/g fat for PCDD/F/dl-PCB exceed the EU limit with a factor of 4.5. The dl-PCB content is 16.00 pg TEQ/g fat, exceeding the action limit with a factor 9, (Figure 19). The dioxin (PCDD/F) content is nearly 4 times higher than the EU action level. 1,2,3,4,6,7,8-Heptachlorodibenzofuran has the highest contribution to the concentration, 1,2,3,7,8-Pentachlorodibenzo-p-dioxin and 2,3,4,7,8-Pentachlorodibenzofuran the highest contribution in TEQ, as typical representants of incineration.

[33] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*

Figure 19: Results of dioxin analysis of egg location 2.

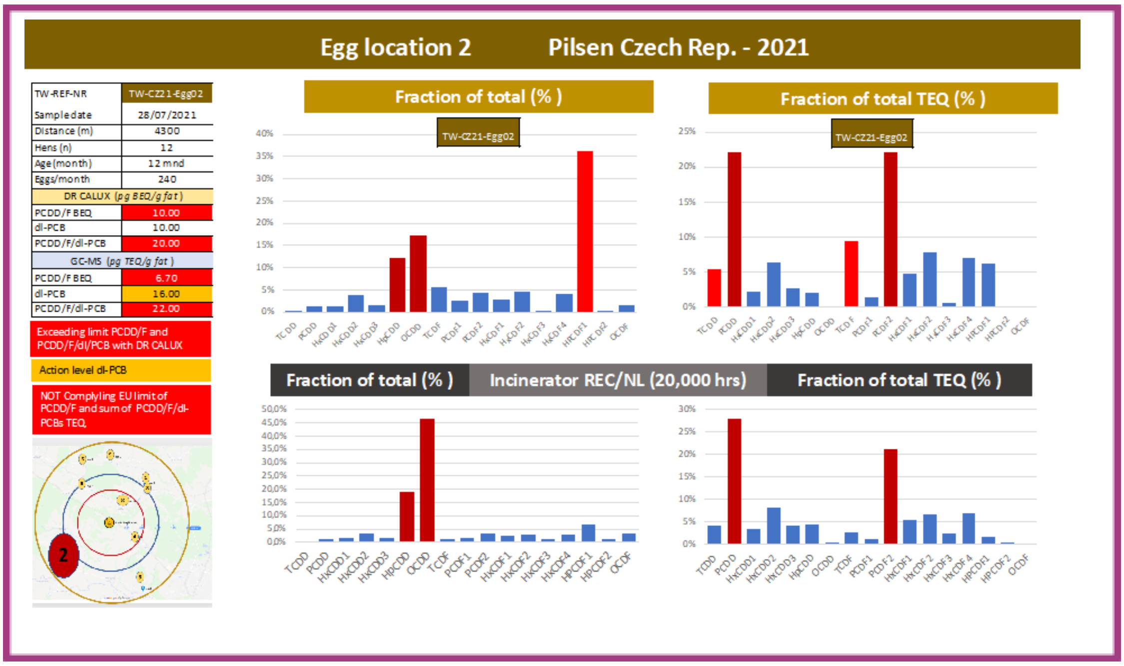
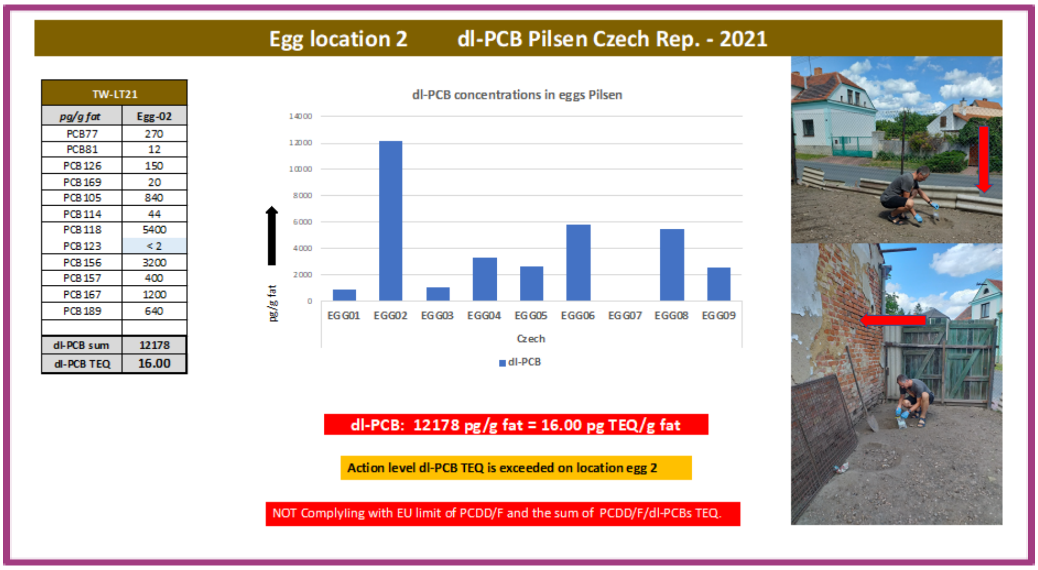


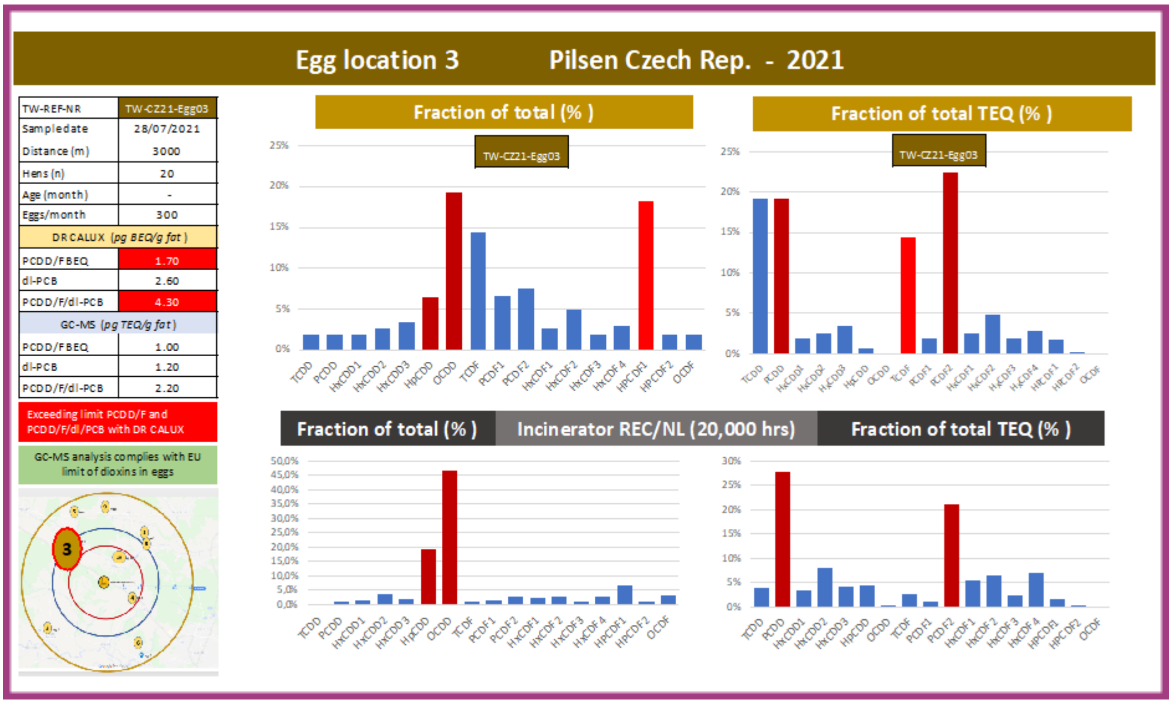
Figure 20 for egg location two (2) shows the highest level of dioxin-like PCBs found in this biomonitoring research. It is strongly recommended to find the source(s) to reduce or eliminate the toxic substances at this location. Possible PCB-source are given with red arrows in Figure 19, like possible PCB -containing paint or PCB coated asbestos plates.

Figure 20: PCB contamination at egg location 2 with possible sources (red arrows).



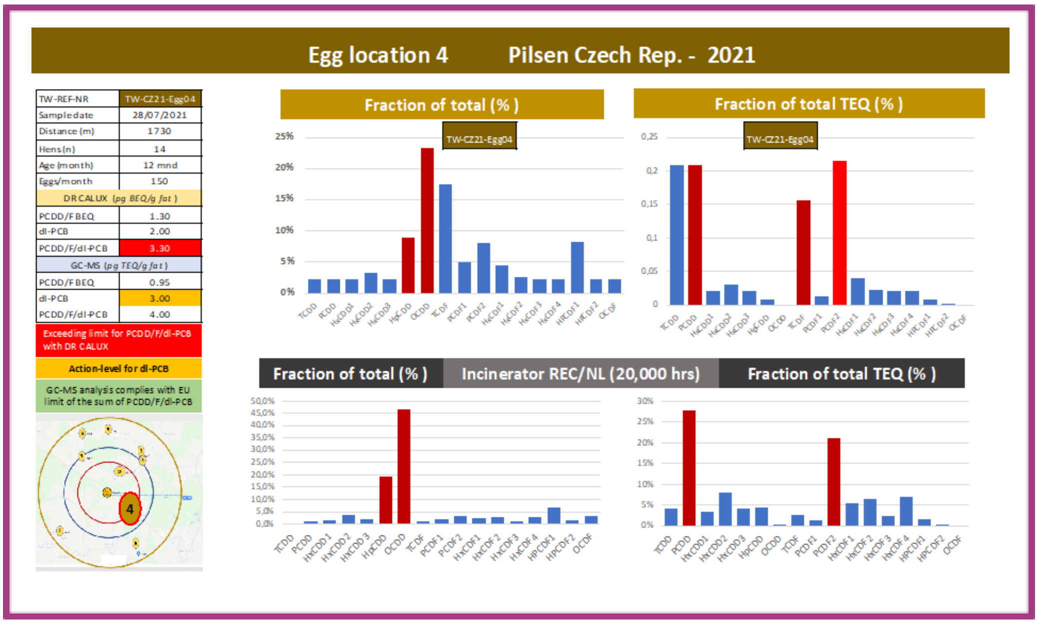
DR CALUX analysis for location 3 is suspect for the PCDD/F and PCDD/F/dl-PCB with 1.7 pg BEQ/g fat and 4.3 pg BEQ/g fat respectively, see Figure 21. The GC-MS measures lower values, indicating the possibility of brominated dioxins not measured by the chemical analysis of GC-MS (see discussion page 34). The result of TCDD is below the limit of detection with < 0,2 pg/g and this value is used in this graph (upper bound).

Figure 21: Results of analysis in egg location 3, Pilsen, Czech Rep., 2021.

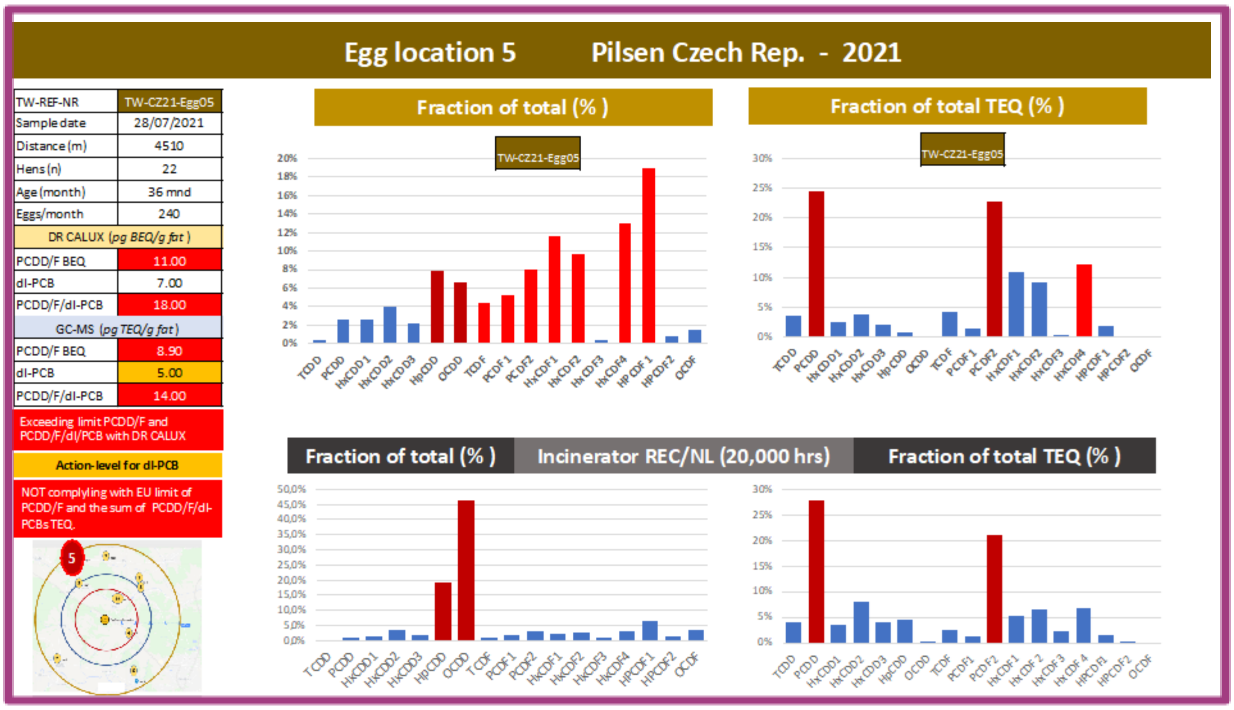


The DR CALUX analysis for location 4 (Figure 22) is suspect for the sum of dioxins (PCDD/F/dl-PCB) with 3.3 pg BEQ/g fat, just on the cut-off value of the action limit. The GC-MS measures a higher exceeding value of the action limit of dl-PCB. Remarkable is presence of TCDF, with 1.5 pg/g. The result of TCDD is below the limit of detection with < 0,2 pg/g and this value is used in this graph (upper bound).

Figure 22: Results of analysis in egg location 4, Pilsen, Czech Rep. , 2021.



A high PCDD/F level of 8.90 pg TEQ/g is measured in at egg location 5 (Figure 23). The difference in the bioassay and chemical analyses could indicate the presence of other dioxins, like brominated dioxins as discussed on page 35. In the research of Chen the high PCDF/PCDD concentration ratio of 2,8 indicates emissions from an incinerator.[34]



[34] Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367

Figure 23: Results of analysis in egg location 5, Pilsen, Czech Rep., 2021.

The action level for dl-PCB of 1.75 pg TEQ/g is exceeded with the measured value of 5.00 pg TEQ/g fat.

The value of dl-PCB is 2,654 pg/g fat. As can be seen in Figure 24, PCB 118, PCB 156 and PCB 167 are dominant. The pattern of PCBs found most resembles with Aroclor 1254, in which these specific PCB ratios occurred. PCBs have been banned since the 1970s and are still found today in the environment, animals, people and, as in Pilsen, in eggs.

Figure 24: Results of dl-PCB analysis in egg location 5, Pilsen, Czech Rep., 2021

Egg location 6, Figure 25, demonstrate a large share of furans in the concentration and TEQ of dioxins PCDD/F, respectively 62 and 64%. This is probably due to the large amount of PCB at this location, 82% of the TEQ is determined by dl-PCB. Remarkable is the presence of TCDF. TCDD value is the Limit of Detection (upper bound). Another explanation of the congener pattern of location 3, 4 and 6 can be deposition of crematoria.[35] There are two crematoria in Pilsen, but at a distance of more than 5 km from the different egg locations.

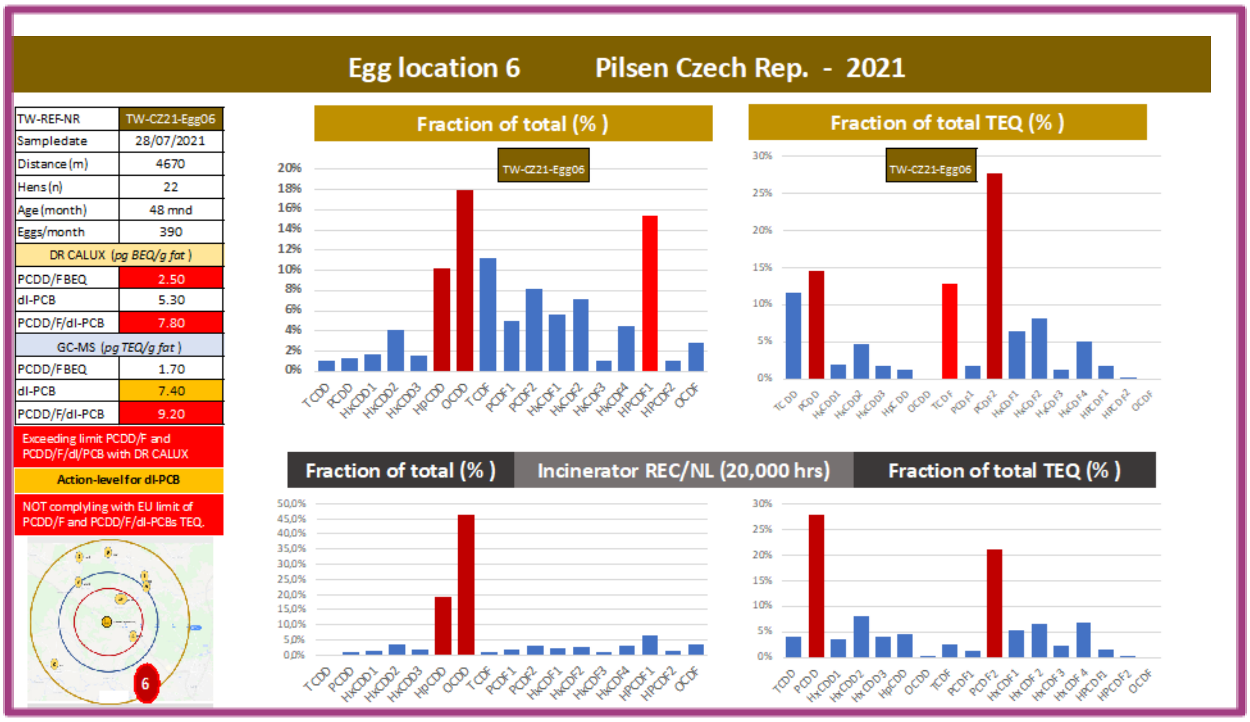
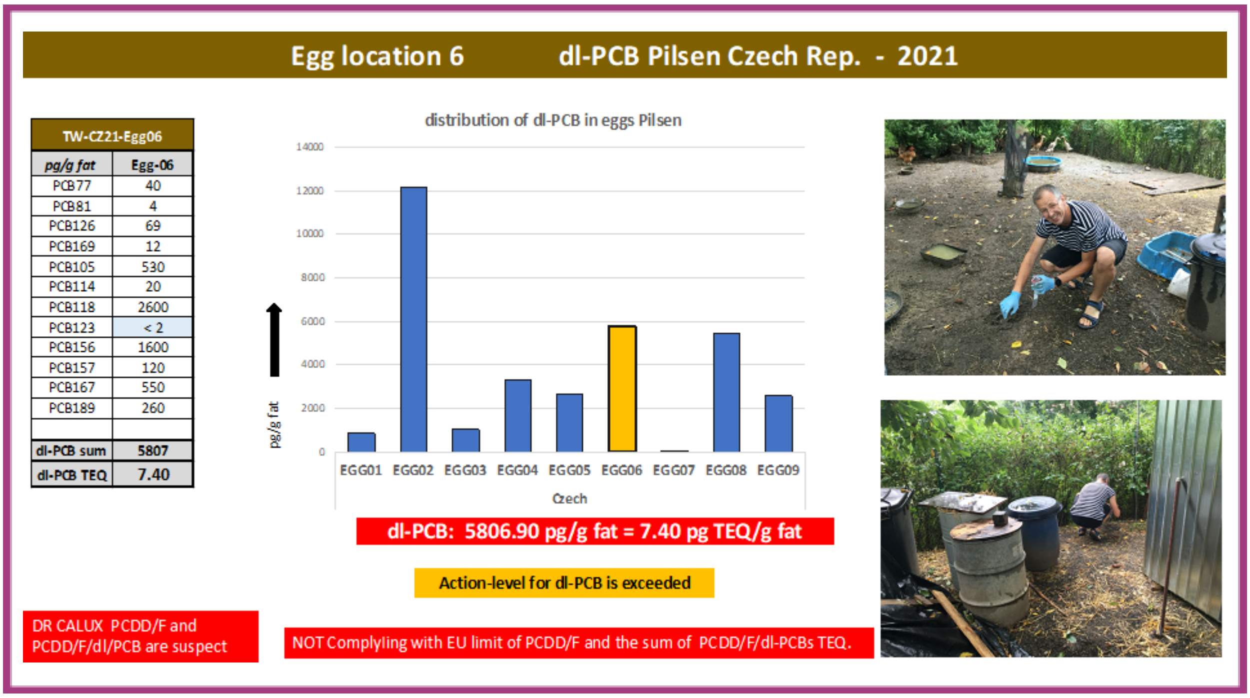


Figure 25: Results of analysis at egg location 6, Pilsen, Czech Rep., 2021.

At location 6, the EU action limit for dl-PCB is exceeded with a factor 4. On the picture is seen some barrels, it could be a spill-over. In the table right of Figure 26 are shown the main contributors of the dl-PCB contamination, PCB 118 and PCB 156.

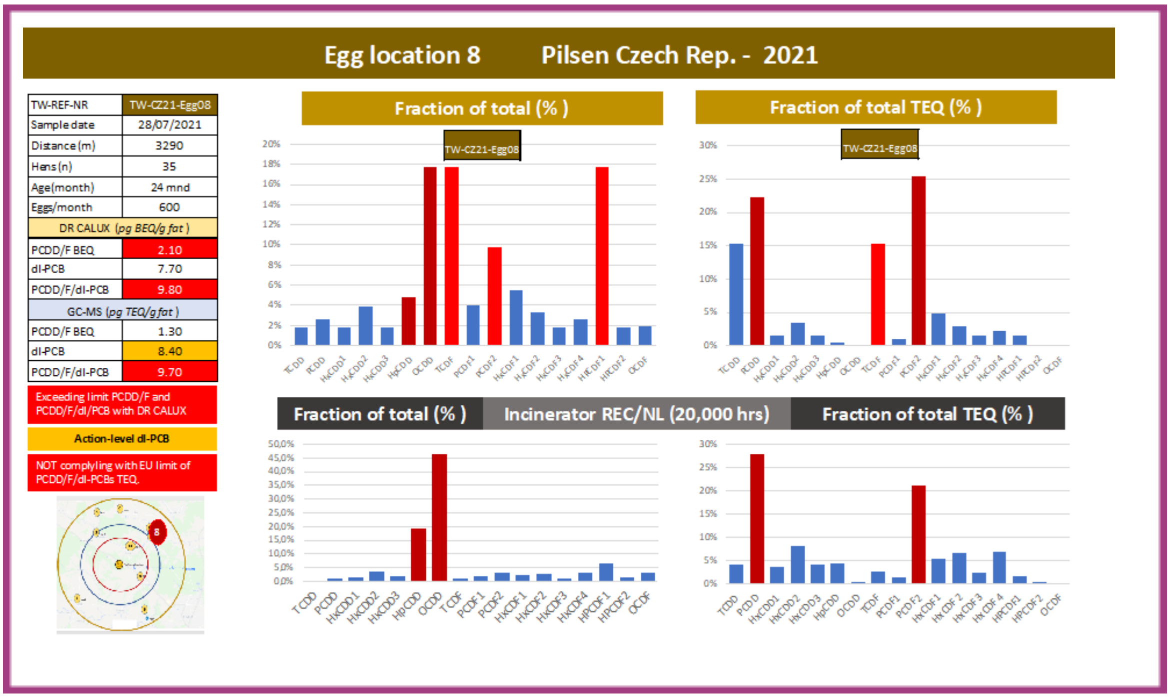
[35] *Kao et al., Aerosol and Air Quality Research, Vol. 6, No. 2, pp. 170-179, 2006*

Figure 26: Results of dl-PCB analysis in egg location 6, Pilsen, Czech Rep., 2021



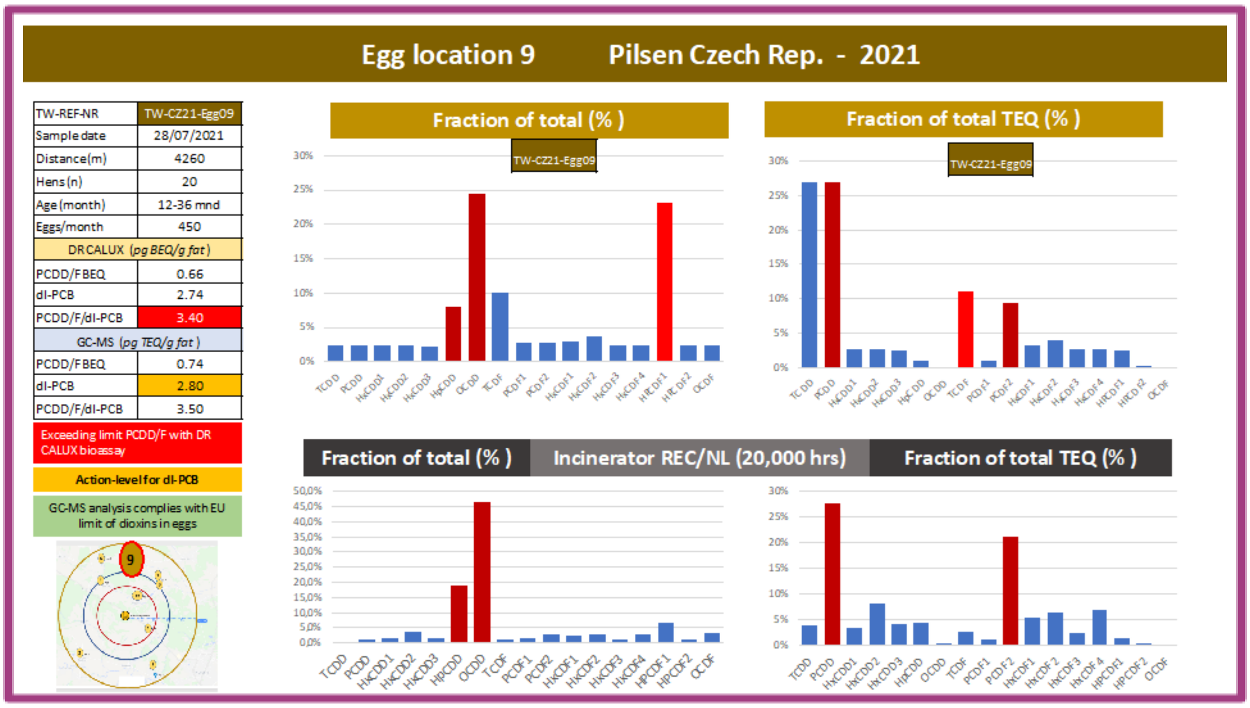
In Figure 27 the profile is given for egg location 8. Typically are the three dominant congeners of TCDF, HpCDF and OCDD in this pattern. Compared with location 1, only 110 meters distance, a big difference in patterns can be observed.

Figure 27: Results of analysis in egg location 8, Pilsen, Czech Rep., 2021



At Location 9, 4260 meters from the incinerator, four congeners OCDD, HpCDF, TCDF and HpCDF are dominating the patterns. The result of TCDD is below the limit of detection, < 0,2 pg/g, but gives a high value in the graph due to the LOD being used in the TEQ calculation (upper bound). The GC-MS analysis gives a warning signal for the presence of dl-PCB with a level of 2.80 pg TEQ/g (Figure 28).

Figure 28: Results of analysis in egg location 9, Pilsen, Czech Rep., 2021

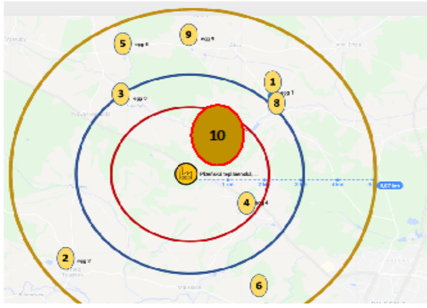


Egg location 10, Figure 29, the sampling location nearest to the incinerator is found to be the less dioxin contaminated place in this study. At this particular location the Sussex and Isa Brown breeds of hens are probably too young to actually up take pollutants from the environment. Hens usually start laying eggs from the 20th week, these hens are 24 weeks old. So bioaccumulation in the chicken body is almost 0 at this young age and therefore this egg location is not a particularly good indicator for biomonitoring dioxin contamination in the environment for this year. Next year's follow-up results at this location will be interesting, because by then the hens will have had time to bioaccumulate one year of possible depositions from the air into the soil. For this year this location can be marked as a zero measurement location.

Figure 29: Results of analysis in egg location 10, Pilsen, Czech Rep., 2021

Egg location 10 — Pilsen Czech Rep. - 2021

TW-REF-NR	TW-CZ21-Egg10
Sampledate	28/07/2021
Distance (m)	1630
Hens (n)	6
Age (month)	6 mnd
Eggs/month	120
DR CALUX *(pg BEQ/g fat)*	
PCDD/F BEQ	0.41
dl-PCB	0.54
PCDD/F/dl-PCB	0.95
GC-MS *(pg TEQ/g fat)*	
PCDD/F BEQ	
dl-PCB	
PCDD/F/dl-PCB	



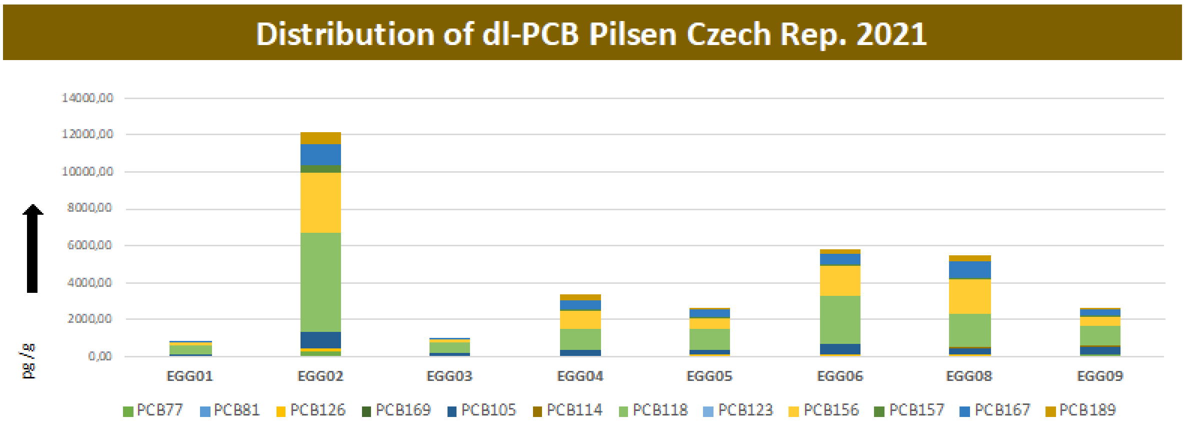
Dl-PCB contamination in eggs of backyard chicken

Although their production ended in 1979, huge amounts of PCBs are still in the environment. Most of the PCBs found today in the environment originate from legacy sources (i.e., releases from transformers or capacitors are still in use, building materials, stored waste, or contaminated soils) or as unintentional by-products of combustion processes (i.e., waste incineration).

The different PCB-congeners in eggs are showed in Figure 30. The contribution of PCB 118 is 33% - 54%, PCB 156 8% -35% and PCB 105 from 7% - 17%. Compared with other TW-biomonitoring researches the average contributions of PCB156 (22%), PCB167 (11%) and PCB189 (5%) are remarkable. In other countries the contribution of these PCBs are resp. 6%, 3% and 1%. There could be a connection with the former operations of Škoda Works, although these are mainly related to sewage sludge and contamination of the (tributaries) of the Elbe.[36]

[36] *The environment of the city of Pilsen, Municipality of Pilsen, https://ozp.plzen.eu/Files/ozp/publikace/ZP_2012_EN.pdf*

Figure 30: Distribution of dl-PCB in eggs, Pilsen 2021



% individual congeners dl-PCB eggs, Pilsen Czech 2021												
TW-CZ21	PCB77	PCB81	PCB126	PCB169	PCB105	PCB114	PCB118	PCB123	PCB156	PCB157	PCB167	PCB189
Egg01	1%	0%	1%	0%	12%	1%	54%	0%	17%	2%	8%	3%
Egg02	2%	0%	1%	0%	7%	0%	44%	0%	26%	3%	10%	5%
Egg03	1%	1%	1%	0%	12%	1%	53%	0%	17%	2%	7%	4%
Egg04	1%	0%	1%	0%	8%	0%	36%	0%	29%	2%	14%	9%
Egg05	1%	0%	2%	1%	9%	1%	41%	0%	21%	3%	15%	5%
Egg06	1%	0%	1%	0%	9%	0%	45%	0%	28%	2%	9%	4%
Egg07	9%	3%	1%	0%	16%	3%	46%	3%	8%	3%	5%	3%
Egg08	1%	0%	1%	0%	6%	0%	33%	0%	35%	2%	16%	6%
Egg09	3%	0%	1%	0%	17%	1%	44%	0%	19%	2%	13%	3%

The EU mandates a reduction in the amount of toxic dioxin-like substances by making serious efforts to find the source of this contamination. And what is the contribution of the incinerator to the PCB contamination? In a continue measurements of the emissions of a WtE incinerator 10% of the TEQ found to be related to dioxin-like PCBs, mainly PCB 126.[37] A remark has to be made, that semi-continuous measurements are by far the best in measuring emissions of dioxins during normal operation.[38] However, measuring emissions during transient phases, such as start-up and shutdown, requires a different methodology of measuring due to changing conditions such as temperature and gas velocity. The amount of data is limited, however a study by Li from 2018 indicates formation of dioxin-like PCBs during transient phases such as start-ups and shutdowns is very likely.[39]

Brominated and mixed halogenated dioxins (PBDD/F and PXDD/F) in eggs of backyard chicken

The higher levels of dioxins (PCDD/F) with the DR CALUX may cause by the fact that the bioassay analysis also reacts to polyhalogenated dioxins like the brominated (PBDD/F) and

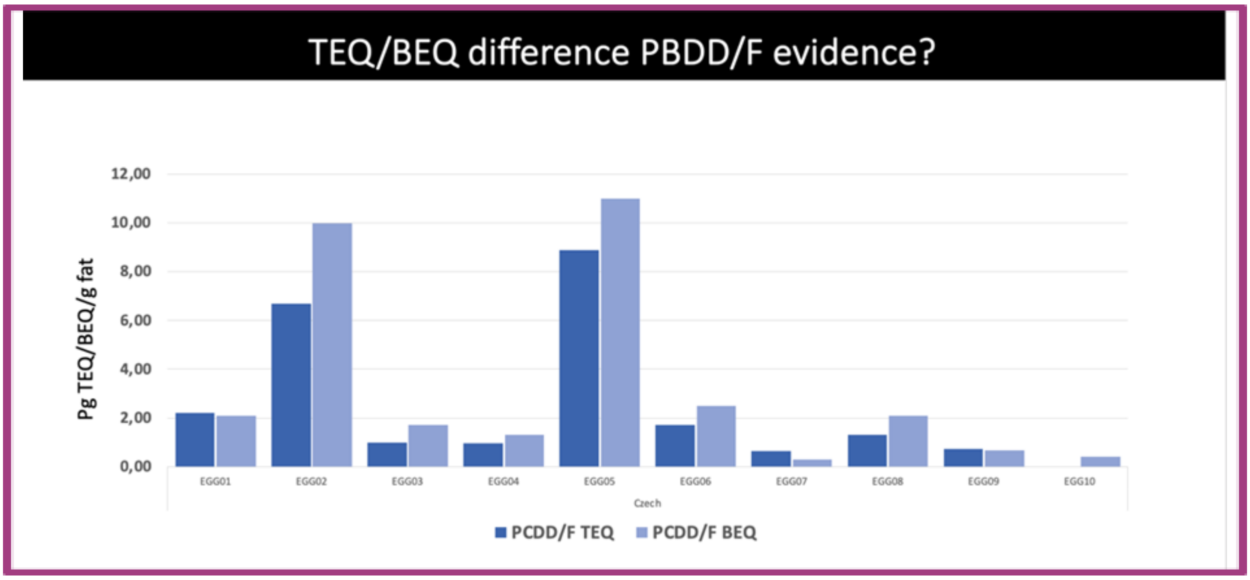
[37] *Hidden Emissions of incinerators, 2017. Toxicowatch Foundation, publication by Zero Waste Europe*

[38] *Arkenbout, A, Olie K, Esbensen, KH, 2018. Emission regimes of POPs of a Dutch incinerator: regulated, measured and hidden issues, Conference paper Dioxin2018*

[39] *Li M, Wang C, Cen K, Ni M, Li X. 2018 Emission characteristics and vapour/particulate phase distributions of PCDD/F in a hazardous waste incinerator under transient conditions. R. Soc. open sci. 5: 171079.*

mixed halogenated chloro/bromo/fluorinated dioxins (PXDD/F). In a study of ToxicoWatch with continuous measurement in the chimney of a WtE incinerator, a broad scale of POPs was found.[40] The EU regulation covers only the chlorinated dioxins (GC-MS: PCDD/F in TEQ and DR CALUX: PCDD/F in BEQ), see Figure 31, 5 and 6. While more and more scientific publications show the proportion of other halogenated dioxins cannot be neglected and should integrated in EU regulation. This is especially true when (municipal) waste with brominated and fluorinated (flame retardant) content are combusted. The problem is the analysis of all these halogenated compounds. There are about 4,600 chlorinated and brominated dioxins, without any international guideline, besides the fluorinated (PFAS) compounds. At the moment only one detection method (bioassay DR CALUX) is suitable for measuring the total toxic effect. Brominated dioxins make up to 15% of the total dioxin in human body (Jogsten et al 2010).[41]

Figure 31: Difference between GC-MS (TEQ) and DR CALUX (BEQ) indicates evidence of brominated dioxins



It is widely recognized that unintentional produced persistent organic pollutants (UPOPs) in emission from thermal processes, especially incineration of e-waste containing PBDEs, is the principal source of PBDD/Fs in the environment. PBDE can primarily found in black electronic devices like TV casings. Waste incineration and metallurgical processes, including secondary metal smelting and arc furnace steelmaking, are important anthropogenic sources of dioxins. Although less data are available on PBDD/Fs formation during waste incineration and metallurgical process than for PCDD/Fs, pilot studies have demonstrated that PBDD/Fs are formed during thermal processes. [42]

[40] *Arkenbout, A., Bouman KJAM, 2018.Emissions of dl-PCB, PBB, PBDD/F, PBDE, PFOS, PFOA and PAH from a waste incinerator, Dioxin2018, see reference list*

[41] *I.E. Jogsten et al. / Food and Chemical Toxicology 47 (2009) 1577–1583*

[42] *L. Yang et al. 2021. Environment International 152 (2021) 106450*

PFAS in eggs of backyard chicken

In 2015 PFAS is detected in eggs of backyard chickens in the Netherlands and Greece. In the study by Zafeiraki (2015), only 11 PFAS are analyzed, while there are more than 7,000 different PFAS substances with a toxicologic activity. Research into PFAS is expanding, with more than 600 publications last year, but most studies are focused on chemical analyses, which have their limitations. A paradigm shift is needed to address the analysis of such substances in a more efficient way. In this TW biomonitoring research, two different analysis PFAS methods are used: the chemical method of FITC-T4 and the bioassay of PFAS CALUX. The FITC-T4 measures the decrease in T4 binding capacity, while the PFAS CALUX measures the substances that cause the decrease in T4 binding capacity. The results of the two different PFAS analysis methods for egg sample numbers 2 and 4 are given in Figure 32. The PFAS CALUX measures 130 and 250 ng PFOA eq./g at egg location 4 respectively egg location 2. The method FITC-T4 gives higher results on the same samples of these egg locations, namely 1200 and 1400 ng PFOA eq./g. This study is probably the first to analyse eggs from hens with the combination of PFAS CALUX and the FITC-T4 method.

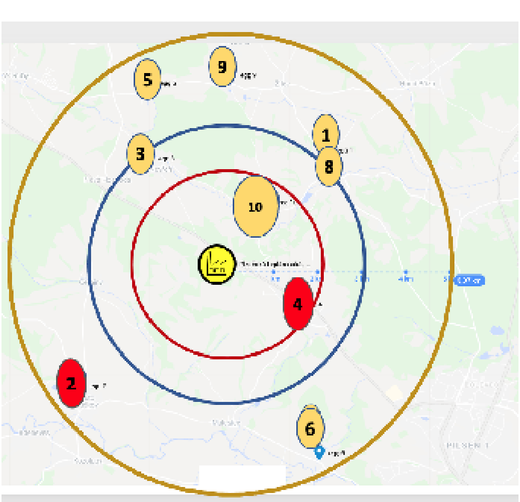
Figure 33 shows a graph of the difference found in PFOA concentration in this study, 130 – 1400 ng PFOA eq./g with the level of 1.5 ng PFOA in the study of Zafeiraki. This is a huge difference, no explanation can be found. In the study of Zafeiraki a linear relation between dioxins (PCDD/F) and PFAS is confirmed. This could be an indication of relation with (in-)complete incineration processes. There are too few studies to confirm that the possible huge amount of PFAS-containing (single use) everyday products, (see Figure 9 and 10, page 13, 14), will be sufficiently degraded by waste incineration.

The EFSA set the Tolerable Weekly Intake (TWI) for PFOA at 6 ng PFOA/kg bodyweight. On the left-hand side of Figure 32, a calculation has been made of the consequences of this high value of PFAS/PFOA equivalents. It means that the TWI set by EFSA is exceeded by a factor of 1400.

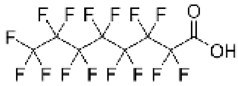
The EU underlines in Regulation (EU) 2019/1381 the need for continuous risk analysis and transparent risk communication. The EU wants to increase trust in government and is therefore open to a participatory and open dialogue between all stakeholders. When it comes to prioritizing the public interest, accuracy, transparency, and accountability are important in the risk analysis process. And this is certainly true in the case of contaminated eggs in the Pilsen area. It is in the public interest that emissions from the waste incinerator should be investigated clearly and transparently in order to protect the health of local residents.

Figure 32: PFAS in eggs Pilsen, Czech Rep. 2021

PFAS eggs Pilsen Czech Rep. 2021



EGG04		
PFAS CALUX	*ng PFOA eq./g product*	130.00
PFAS (FITC-T4)	*ng PFOA eq./g product*	1400.00

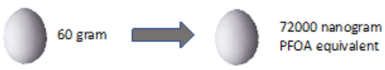


Results eggs Pilsen, Czech Rep. - 2021							
Location	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB	PFAS CALUX	PFAS (FITC-T4)
			DR CALUX *pg BEQ/g fat*			*ng PFOA eq./g product*	*ng PFOA eq./g product*
EGG02	TW-CZ21-Egg-02	4290	20.00	10.00	10.00	250.00	1200.00
EGG04	TW-CZ21-Egg-04	1780	3.30	1.30	2.00	130.00	1400.00
	Cut-off	DR CALUX	3.30	1.70			

Figure 33: PFAS in backyard chicken eggs exceeding the limits, Pilsen, Czech Rep. 2021

PFAS eggs Pilsen Czech Rep. 2021

EGG02		
PFAS CALUX	*ng PFOA eq./g product*	250.00
PFAS (FITC-T4)	*ng PFOA eq./g product*	1200.00



EFSA: Tolerable Weekly Intake (TWI) for PFOA is 6 ng PFOA/kg bodyweight.

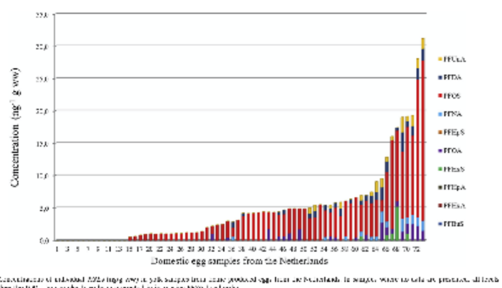
TWI adult 70 kg is 420 ng PFOA/week

one egg a day = 7 x 72000 =

504000 ng PFOA a week

exceeding the EFSA limit PFOA with a factor 1200-1400 !

What about our immune system?

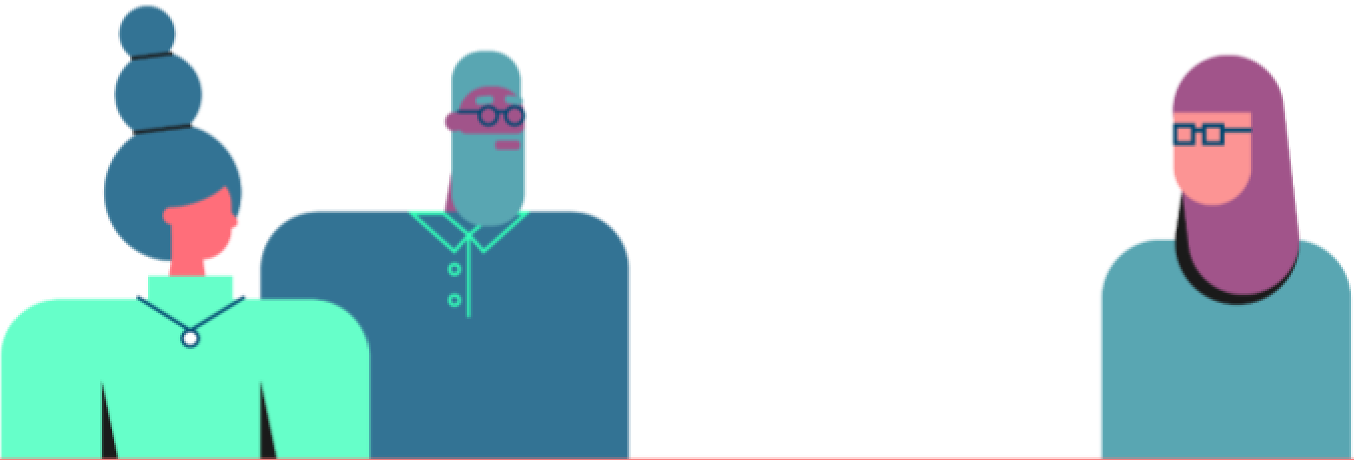


Zafeiraki et al, 2016 Perfluoroalkylated substances (PFASs) in home and commercially produced chicken eggs from the Netherlands and Greece, Chemosphere 144 2106–2112

In the Netherlands with chemical analyses GC-MS less than 2 ng PFOA found in eggs (2016)

The results in the graph are performed by chemical analyses, measured in 1-2 ng.

TW use bioassay PFAS CALUX and FITC-T4 methods for analyses of the eggs in this biomonitoring research with measurements in PFOA equivalents (eq.). The results in this research show a lot of PFAS with PFOA toxicity, presumably because of the PFAS CALUX and FITC-T4 method.



Biomonitoring

of vegetation

Sampling of pine needles

Pines are widespread among evergreen species and are characterized by a high-fat content. Pine needles have been used for many decades to monitor atmospheric POP pollution on a global and regional scale. The uptake of pollutants by vegetation occurs mainly through gas-phase partitioning or deposition of dust particles that adsorb on the surface and then diffuse into the waxy cuticle of the leaves. It has been identified that waste incinerators contribute significantly to the environmental concentrations of PCDD/Fs.[43] Pine needles have an advantage over the use of Polyurethane Foam discs (PUF), which are vulnerable to vandalism.

Pine trees are able to survive long periods of stressful drought conditions due to the special constructions of pine needles, especially the epicuticular waxes and the distribution of tubular waxes which are species specific.[44] Meaning the epicuticular wax layer, which helps protect the leaves from the more toxic form of ultraviolet light called UV-B, as well to prevent water loss of the plant system, risks of pathogen and insects attacks. Dioxins (PCDD/F/dl-PCB) partition in this fatty wax layer because of its lipophilic properties. In fact, lipophilic xenobiotica have been found to have a greater affinity to one of the main components of the cuticle membrane, the cuticular waxes compared to other cuticle membrane components.[45] Persistent organic pollutants are thought to sorb to the cuticular waxes and diffuse into internal leaf (pine needle) compartments.[46] Therefore, plant leaves/ pined needles can be used as a natural sampler for persistent organic pollutants (POPs) in the environment.

In the environment of Pilsen pine needles are sampled of the Pinus sylvestris. As a reference pine needles of Pinus sylvestris were taken from a location at a distance of 3,410 metres from the waste incinerator, as presented in Figure 34.

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[43] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*
[44] *Lamppu J., Huttunen S. (2002). Environmental Pollution 122 (2003) 119–126*
[45] *Moeckel C., 2008. Environ Sci Technol 42:100–105*
[46] *Barber, JL. (2004).. Environ Pollut 128: 99–138*

Figure 34:Vegetation sample locations Pilsen 2021

Pine needles, Pilsen Czech rep. 2021					
CZ team location	TW	Species	sample	TW-REF-NR	Distance
Delivering nr	nr		date		(m)
1 (A)	PS01	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg01	520
2 (B)	PS02	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg02	274
4 (E)	PS03	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg04	1380
5 (F)	PS04	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg05	3410
6 (D)	PS05	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg06	1000

Results DR CALUX pine needles

The results of DR CALUX analyse method for the sum of dioxins (PCDD/F/dl-PCB), and dioxins (PCDD/F) and dioxin-like PCBs (dl-PCB), separately are presented in table 5.

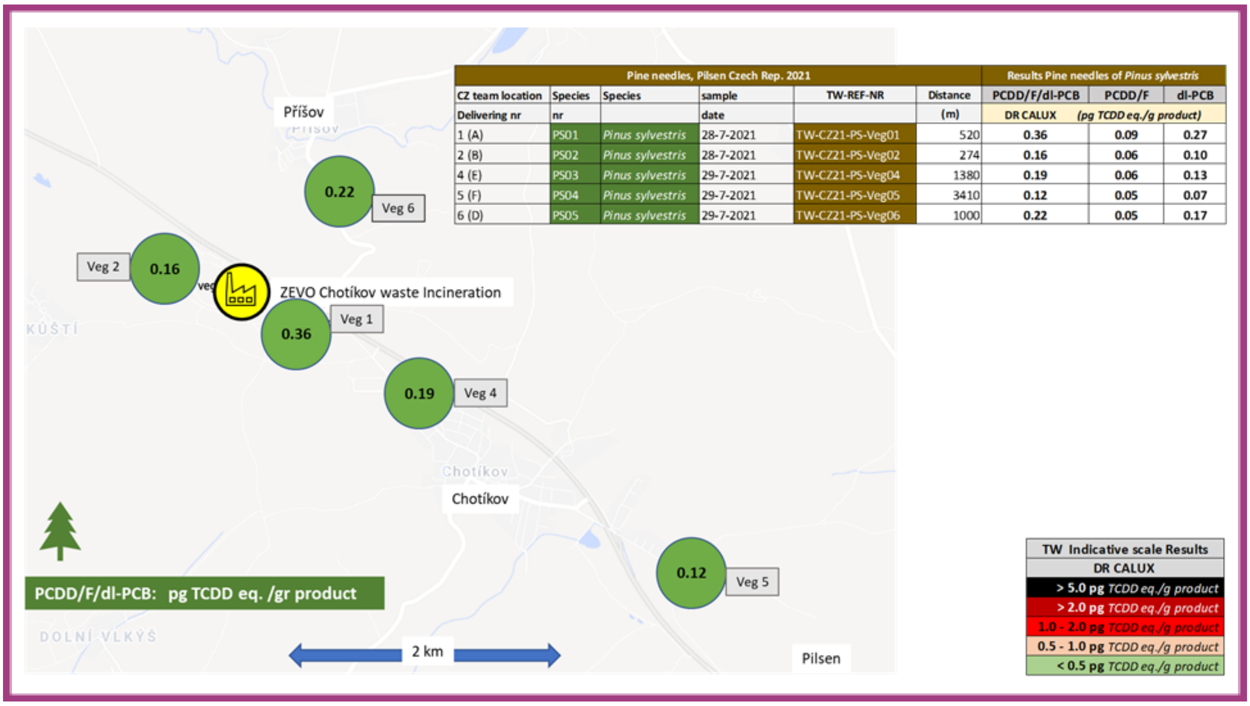
Pine needles, Pilsen Czech Rep. 2021						Results Pine needles of *Pinus sylvestris*		
CZ team location	Species	Species	sample	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	dl-PCB
Delivering nr	nr		date		(m)	DR CALUX	*(pg TCDD eq./g product)*	
1 (A)	PS01	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg01	520	0.36	0.09	0.27
2 (B)	PS02	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg02	274	0.16	0.06	0.10
4 (E)	PS03	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg04	1380	0.19	0.06	0.13
5 (F)	PS04	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg05	3410	0.12	0.05	0.07
6 (D)	PS05	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg06	1000	0.22	0.05	0.17

Loc. 1 (A)

Table 5: Results PCDD/F/dl-PCB in pine needles of Pinus sylvestris, Pilsen, Czech Rep. 2021

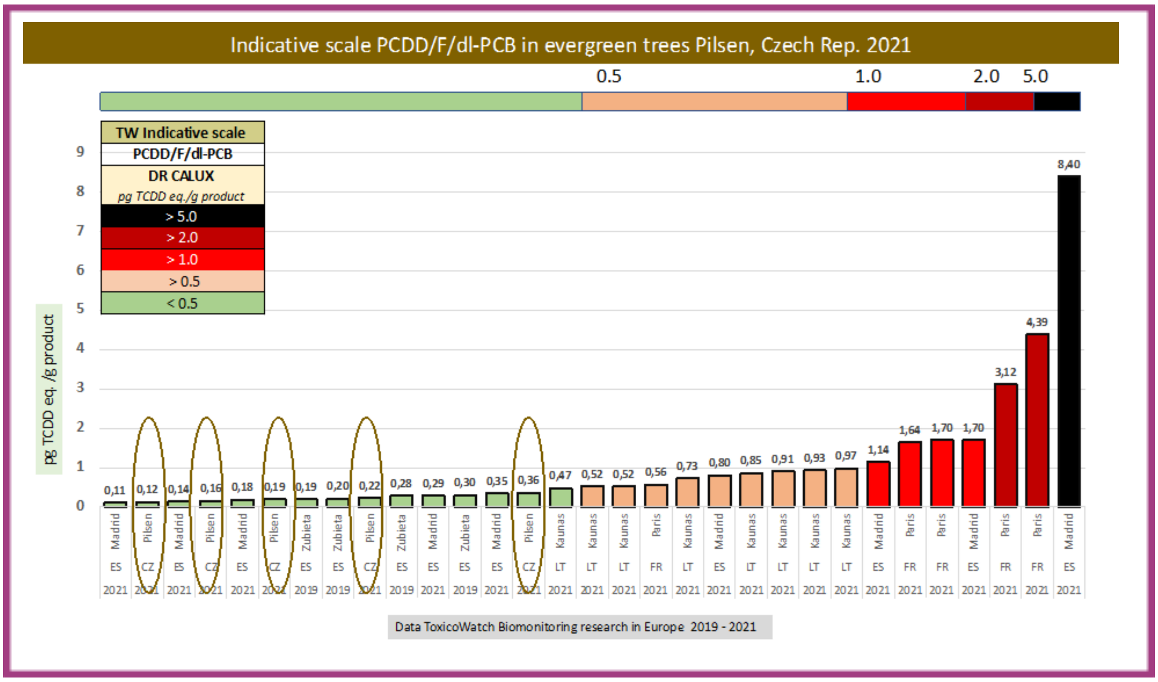
Figure 35 shows the DR CALUX results on the map. Three times more dioxins (PCDD/F/dl-PCB) were found near the incinerator compared to reference location 5.

Figure 35: Dioxins (PCDD/F/dl-PCB) pine needles Pinus sylvestris at 5 locations Pilsen, Czech Rep. 2021



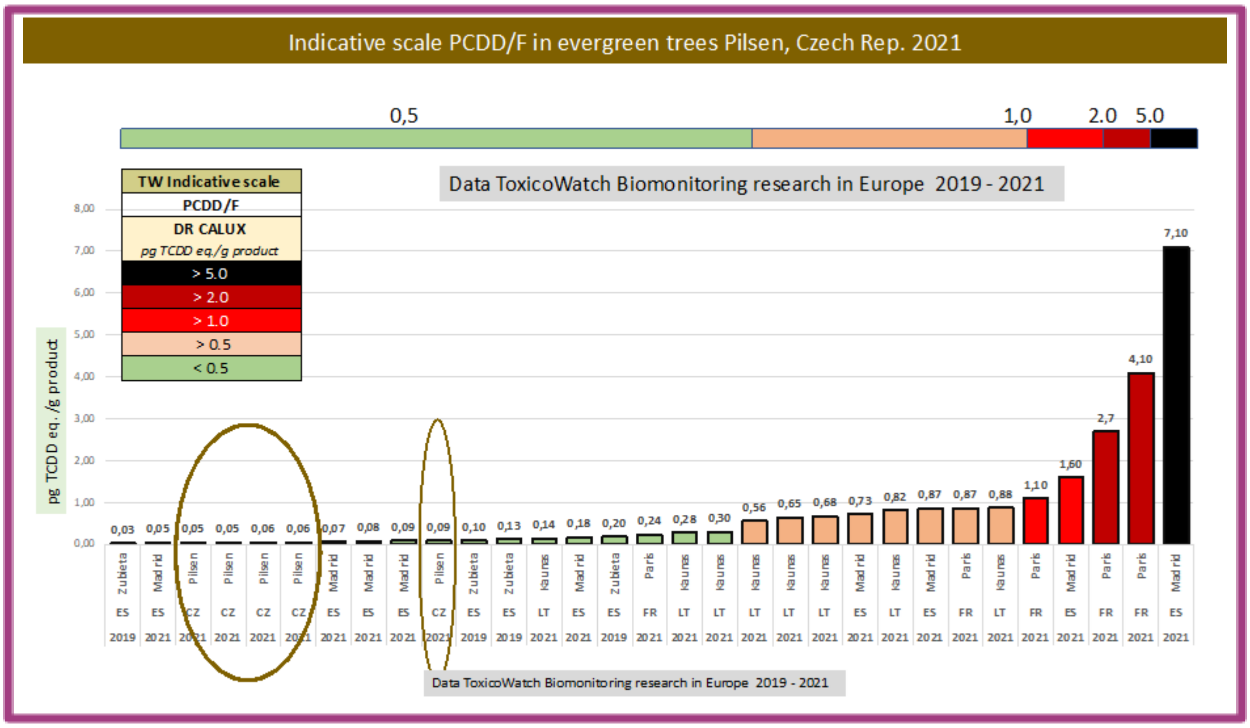
Compared with the results of other TW biomonitoring data analyses of evergreen trees (Europe) the results are relative low compared to other TW researches, Figure 36.

Figure 36: TW Indicative scale for dioxins (PCDD/F/dl-PCB) in evergreen trees Europe 2019-2021



The relatively low values of seperatly dioxin (PCDD/F) measurements become even more apparent when they are included in the TW indicative comparison scale with measurements in evergreen trees in Europe, Figure 37. More detailed maps in the Annex III vegetation.

Figure 37: TW Indicative scale for dioxins (PCDD/F) in evergreen trees, TW research Europe 2019-2021

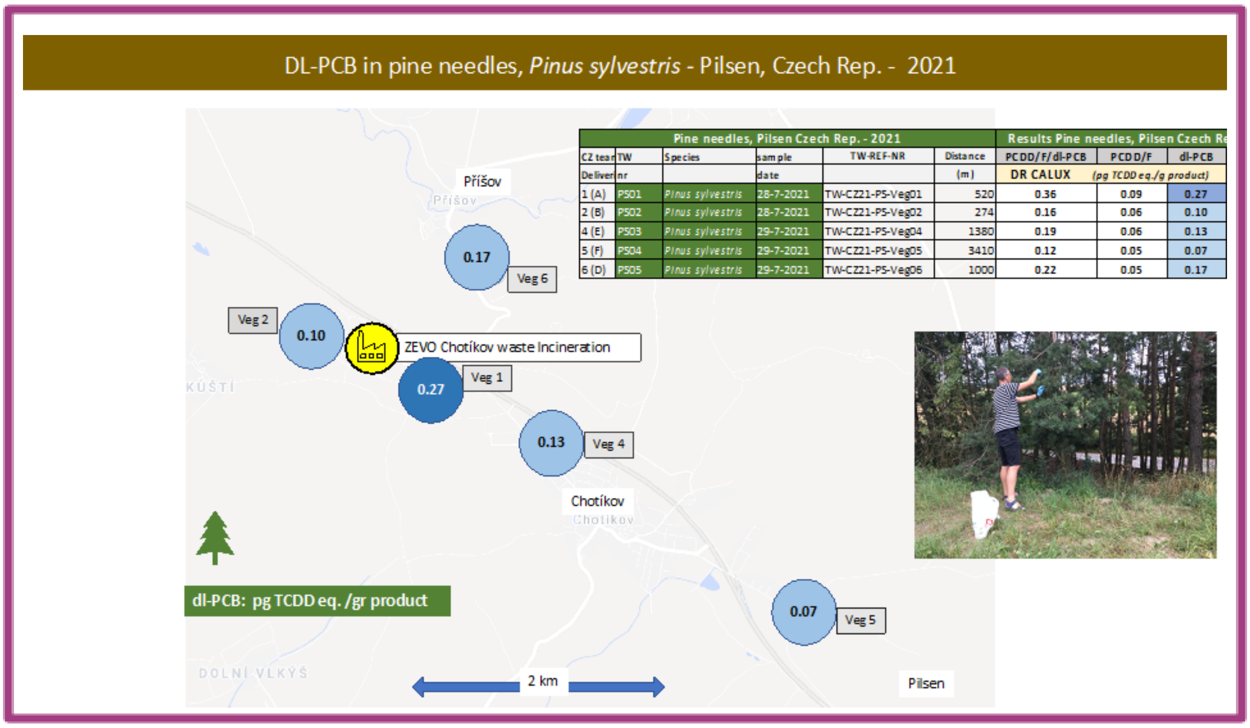


Results dioxin-like PCB (dl-PCB) in pine needles

Dioxin-like polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, and toxic (PBT) compounds that have the potential to move far from original sources. Therefore, routine monitoring of these compounds, in the vicinity of pointed sources is important. Figure 38 shows the results of the dl-PCBs in pine needles of Pinus sylvestris. At location Veg 1 is a level of dl-PCB measured of 0.27 pg TEQ/g product, see Figure 38. In a research of Holt (2016) levels of dl-PCB in industrial sites dioxins in pine needles are measured with the GC-MS and resulted in values between 0.25 – 1.6 pg TEQ/g for industrial sites in Czech Republic.[47] This TW-biomonitoring study on pine needles is performed by DR CALUX analysis method which give lower results of PCB TEQ in general. One of the reason is the use of a relative high toxicity factor for the TEQ calculation for PCB 126 in the GC-MS analysis.

[47] *Holt E. et al. (2016). Spatiotemporal patterns and potential sources of polychlorinated biphenyl (PCB) contamination in Scots pine (Pinus sylvestris) needles from Europe. Environ Sci Pollut Res, DOI 10.1007/s11356-016-7171-6*

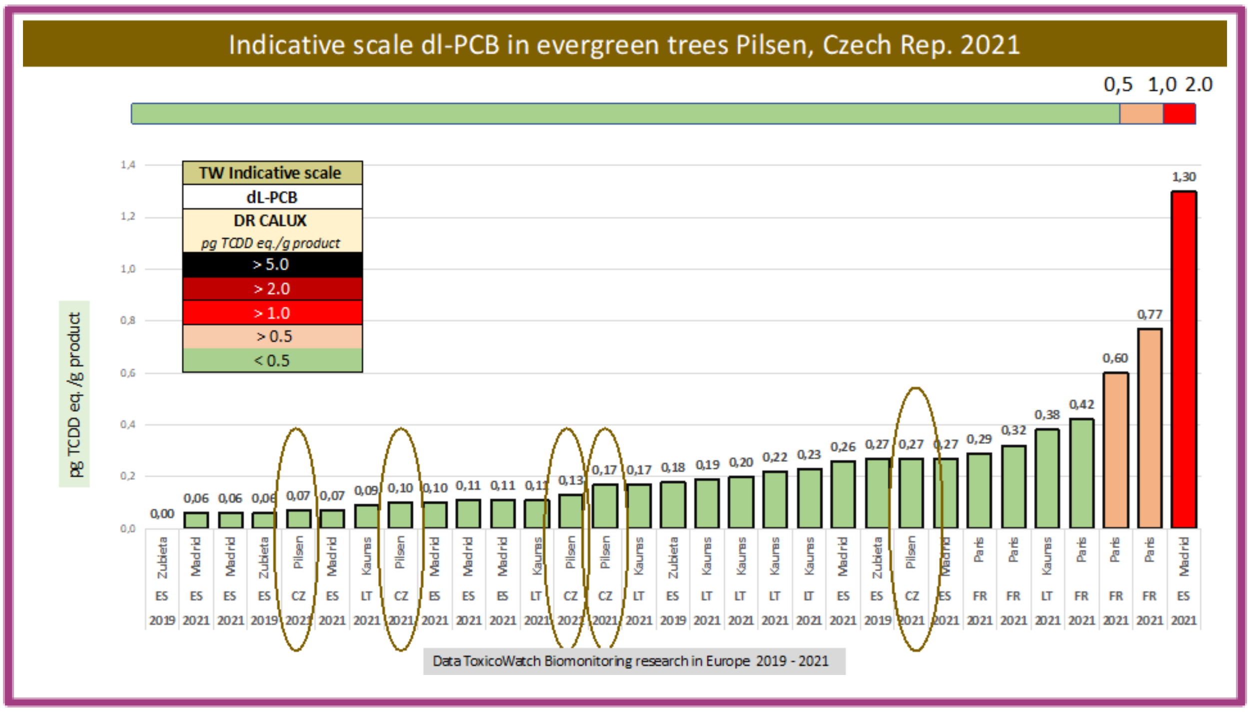
Figure 38: Results of dioxin-like PCB (dl-PCB) in pine needles of Pinus sylvestris, Pilsen, Czech Rep. 2021



Pine needles, Pilsen Czech Rep. - 2021						Results Pine needles, Pilsen Czech Re		
CZ team Deliveri	TW nr	Species	sample date	TW-REF-NR	Distance (m)	PCDD/F/dl-PCB	PCDD/F	dl-PCB
						DR CALUX	(pg TCDD eq./g product)	
1 (A)	PS01	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg01	520	0.36	0.09	0.27
2 (B)	PS02	*Pinus sylvestris*	28-7-2021	TW-CZ21-PS-Veg02	274	0.16	0.06	0.10
4 (E)	PS03	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg04	1380	0.19	0.06	0.13
5 (F)	PS04	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg05	3410	0.12	0.05	0.07
6 (D)	PS05	*Pinus sylvestris*	29-7-2021	TW-CZ21-PS-Veg06	1000	0.22	0.05	0.17

Figure 39 presents a comparative TW indicative scale for dl-PCB in evergreen trees based other TW data of results of DR CALUX measurements of biomonitoring researches on evergreen trees in Europe from 2019-2021.

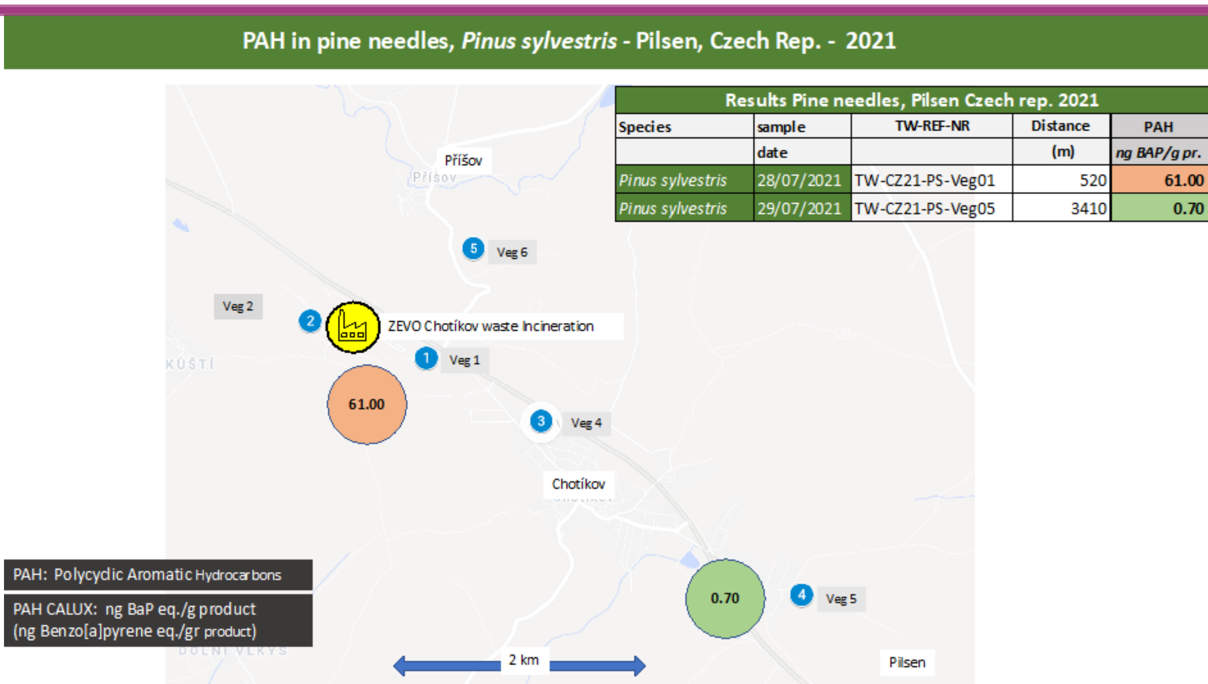
Figure 39: TW Indicative scale dl-PCB in evergreen trees in Europe 2019-2021



PAH in pine needles

Polycyclic Aromatic Hydrocarbons (PAHs) are an excellent tool for capturing certain emissions of thermo-confounders. The concentration of 61 ng B[a]P/g near the incinerator is considerably more than 0.70 ng B[a]P at reference location 5, a factor of 87. The pine needle samples were taken in woodland, from a quiet country road, see Figures 40 and Annex III for more details.

Figure 40: PAH in pine needles, Pilsen, 2021

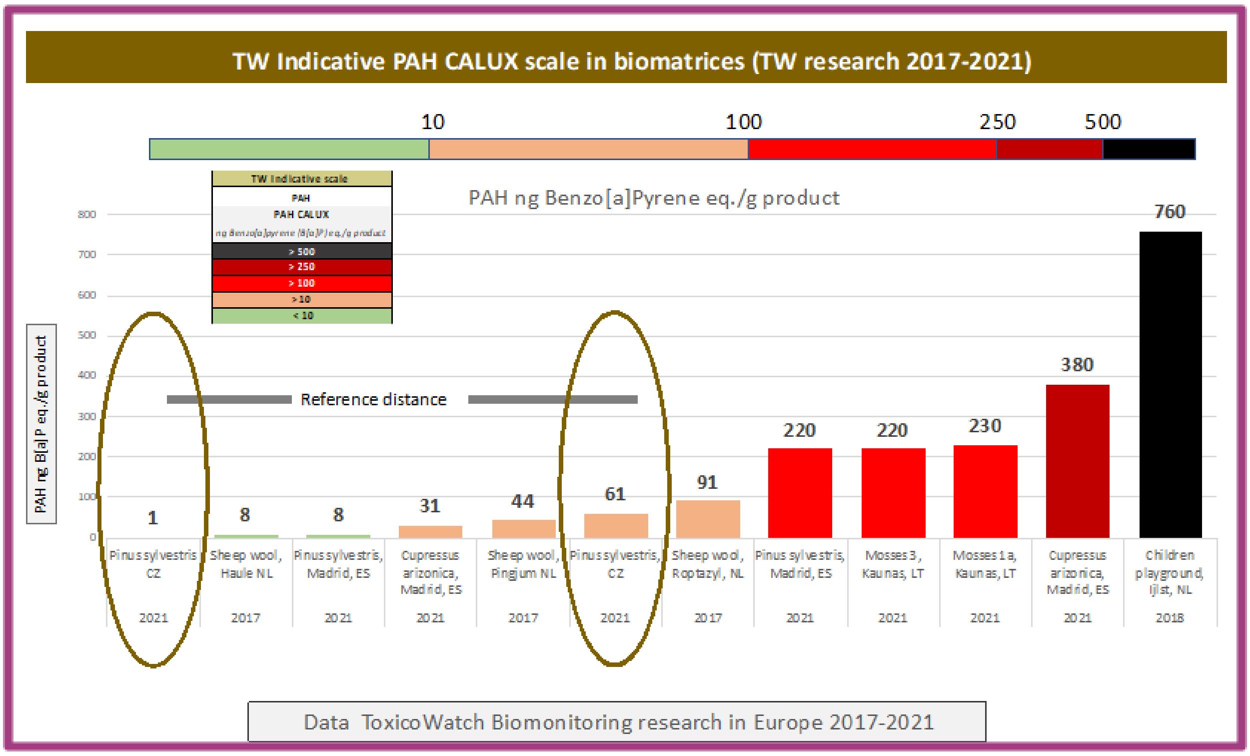


Results Pine needles, Pilsen Czech rep. 2021

Species	sample date	TW-REF-NR	Distance (m)	PAH ng BAP/g pr.
Pinus sylvestris	28/07/2021	TW-CZ21-PS-Veg01	520	61.00
Pinus sylvestris	29/07/2021	TW-CZ21-PS-Veg05	3410	0.70

The concentration of PAH, expressed in Benzo[a]pyrene, found in pine needles near the incinerator can be considered as worrying on this TW indicative scale of PAH in biomatrices (coloured orange)., Figure 41.

Figure 41: Indicative scale for results of PAH CALUX



Mosses

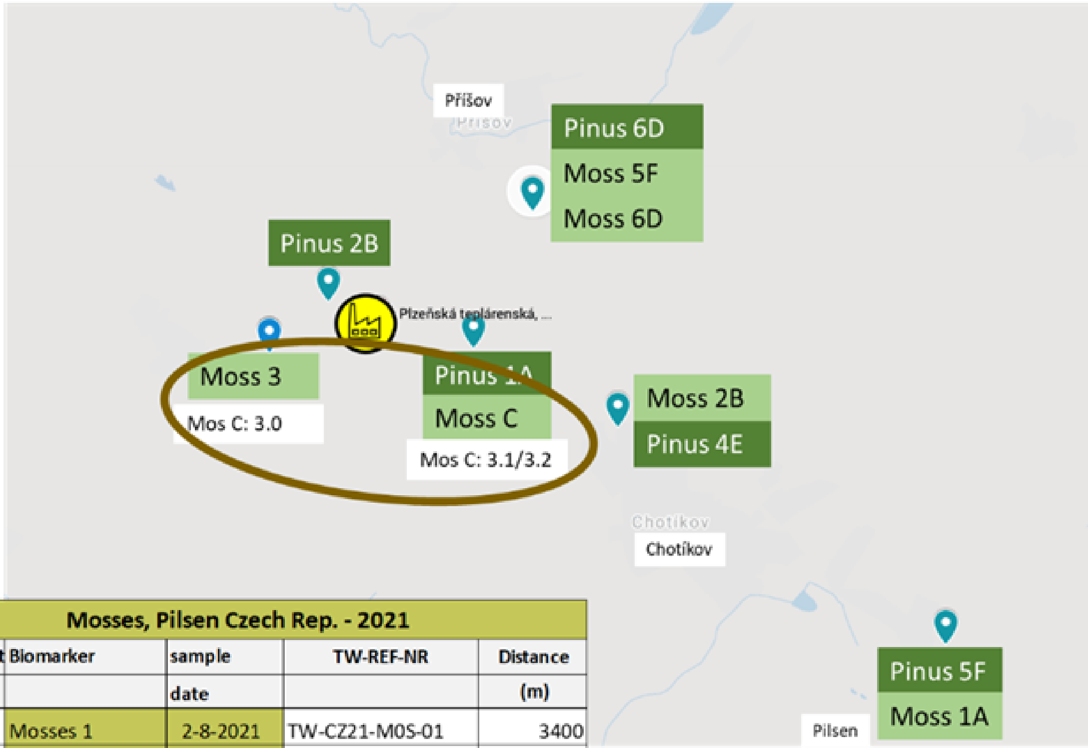
Bryophytes are the non-vascular autotrophic cryptogams with the second-highest conglomeration among land plants after the angiosperms (flowering plants), and nearly 25,000 species were present worldwide.[48] Mosses belong to the kingdom Plantae, and division Bryophyta.Mosses have a crucial ecological role, they represent a largely untapped resource for monitoring and are a useful indicator of the consequences of pollution in the living environment.[49] Carballeira et a (2006)[50] promote mosses as indicators for PCDD/F contamination as they are also for other types of pollutants. In this biomonitoring study, innovative bioassays are used to analyse persistent organic pollutants (POPs). This is done to overcome the limitations of chemical analyses on only a selected group of congeners.

Samples of mosses, mainly Hylocomium splendens, were taken within a 1,500-metre radius of the incinerator in Pilsen, with a reference at 3,400 metres. Mosses were sampled in the open field, avoiding the proximity of roads and not under the dense tree canopies in order to avoid the shedding of leaves. In Figure 42 the moss sample locations in relation with the sample locations of pine needle samples.

[48] *Mishra M, Dash PK, Alam A et al (2016) Plant Sci Today 3:186–194.*
[49] *Mahapatra M. (2018). Environmental Science and Pollution Research 2019 Oct;26(29):29620-29638*
[50] *Carballeira A. et al. (2006). Moss: A powerful tool for dioxin monitoring. Atmospheric Environment 40, 5776–5786*

Figure 42: Sampling location, mosses, Pilsen, Czech Rep., 2021

Mosses Sample locations region Pilsen, Czech Rep. - 2021



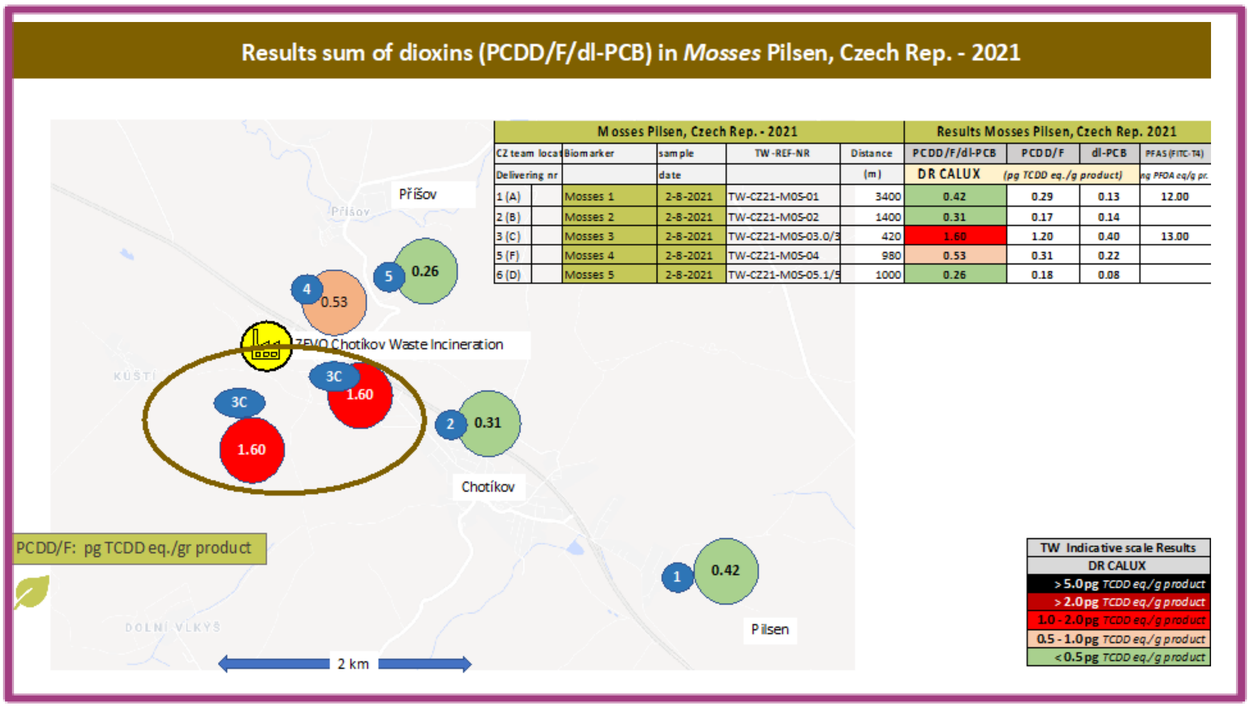
Mosses, Pilsen Czech Rep. - 2021					
CZ team locat		Biomarker	sample	TW-REF-NR	Distance
Delivering nr			date		(m)
1 (A)		Mosses 1	2-8-2021	TW-CZ21-MOS-01	3400
2 (B)		Mosses 2	2-8-2021	TW-CZ21-MOS-02	1400
3 (C)		Mosses 3	2-8-2021	TW-CZ21-MOS-03.0/3	420
5 (F)		Mosses 4	2-8-2021	TW-CZ21-MOS-04	980
6 (D)		Mosses 5	2-8-2021	TW-CZ21-MOS-05.1/5	1000

Loc. 1(A) Loc. 2(B) Loc. 3(C) Loc. 3(C) Loc. 5(F) Loc. 6 (D)

Figure 43 shows that dioxin levels are high at moss location 3, to the south-west and south-east of the incinerator. It is a pooled sample, see Figure 46 for pictures of the moss locations. PCDD/F is 4 times more than the reference mosses at the reference moss location 1 (near Pilsen) and dl-PCB by a factor of 3. Moss locations 4 and 5 in the north measured two different values: 0.26 and 0.53 pg TCDD eq./g product using the DR CALUX method. No explanation could be found for this finding.

In the literature (2018)[51] PCDD/F TEQ concentrations are found between 0.024 pg TEQ to 0.81 pg TEQ (GC-MS analyses). Caraballeira[52] (2006) et al. reported PCDD/F TEQ concentrations of 0.3 pg TEQ/g (in woodlands), 2.5 pg TEQ in relation to an incinerator. Most of the mosses are < 1 pg TEQ/g. Danielsson[53] et al. (2016) observed PCDD/F concentrations in Swedish moss samples (Pleurozium schreberi or Hylocomium splendens) from 0.0001 to 0.57 pg TEQ/g. Generally, the concentrations of the analyzed substances were very low, often close to or below the quantification limits (LOQ) for the dioxin analyses. They found significant correlation between the concentrations of PAHs, dioxin/furans and dioxin-like PCBs in the mosses and the distance to the nearest industry. In the literature most studies of dioxins and pine needles are performed with the chemical analyses of GC-MS. The toxicity will be calculated with toxicity equivalency factors. PCB 126 has a toxicity equivalency factor of 0,1, which needs to be updated to a lower value. The DR CALUX bioassay measures the toxicity of substances on cell culture and measure in general lower dl-PCB values. Probably due to the overestimation of the toxicity of PCB 126.

Figure 43: Sum of dioxins (PCDD/F/dl-PCB) in mosses, Pilsen 2021

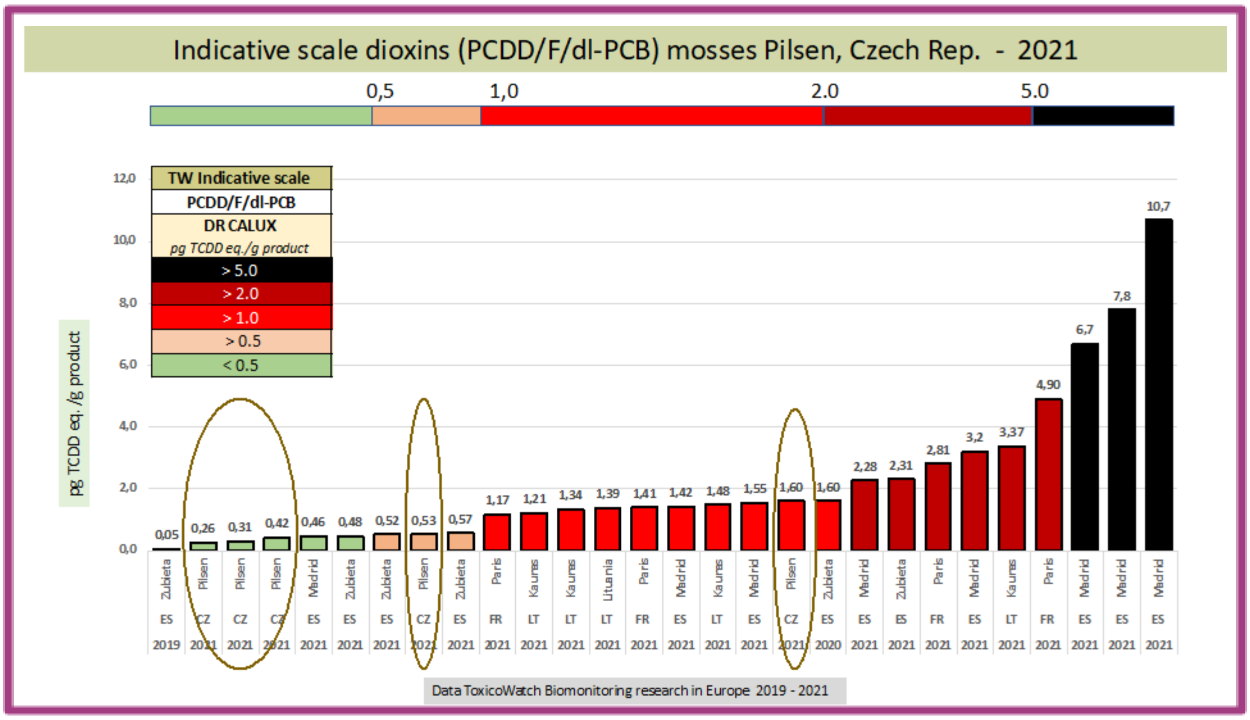


Mosses Pilsen, Czech Rep. - 2021					Results Mosses Pilsen, Czech Rep. 2021			
CZ team locat	Biomarker	sample	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	dl-PCB	PFAS (FITC-T4)
Delivering nr		date		(m)	DR CALUX	(pg TCDD eq./g product)		ng PFOA eq/g pr.
1 (A)	Mosses 1	2-8-2021	TW-CZ21-M05-01	3400	0.42	0.29	0.13	12.00
2 (B)	Mosses 2	2-8-2021	TW-CZ21-M05-02	1400	0.31	0.17	0.14	
3 (C)	Mosses 3	2-8-2021	TW-CZ21-M05-03.0/3	420	1.60	1.20	0.40	13.00
5 (F)	Mosses 4	2-8-2021	TW-CZ21-M05-04	980	0.53	0.31	0.22	
6 (D)	Mosses 5	2-8-2021	TW-CZ21-M05-05.1/5	1000	0.26	0.18	0.08	

51 Dreyer et al. Environ Sci Eur (2018) 30:43 https://doi.org/10.1186/s12302-018-0172-y
52 Carballeira A, Angel Fernandez J, Aboal JR, Real C, Couto JA (2006) Moss: a powerful tool for dioxin monitoring. Atmos Environ 40(30):5776–5786
53 Danielsson et al. (2016). Persistent organic pollutants in Swedish mosses, IVL-report C 188

The value of 1.60 pg TCDD eq./g product in mosses can be considered as high, when this is compared with other results of dioxin biomonitoring in mosses by data of ToxicoWatch researches in Europe. The TW indicative color red is because the value is given between the 1.0 – 2.0 pg TCDD eq./g product. The other analysis results, except moss location 5, can be considered as low. See Figure 44 for an TW indicative scale of dioxin results in mosses.

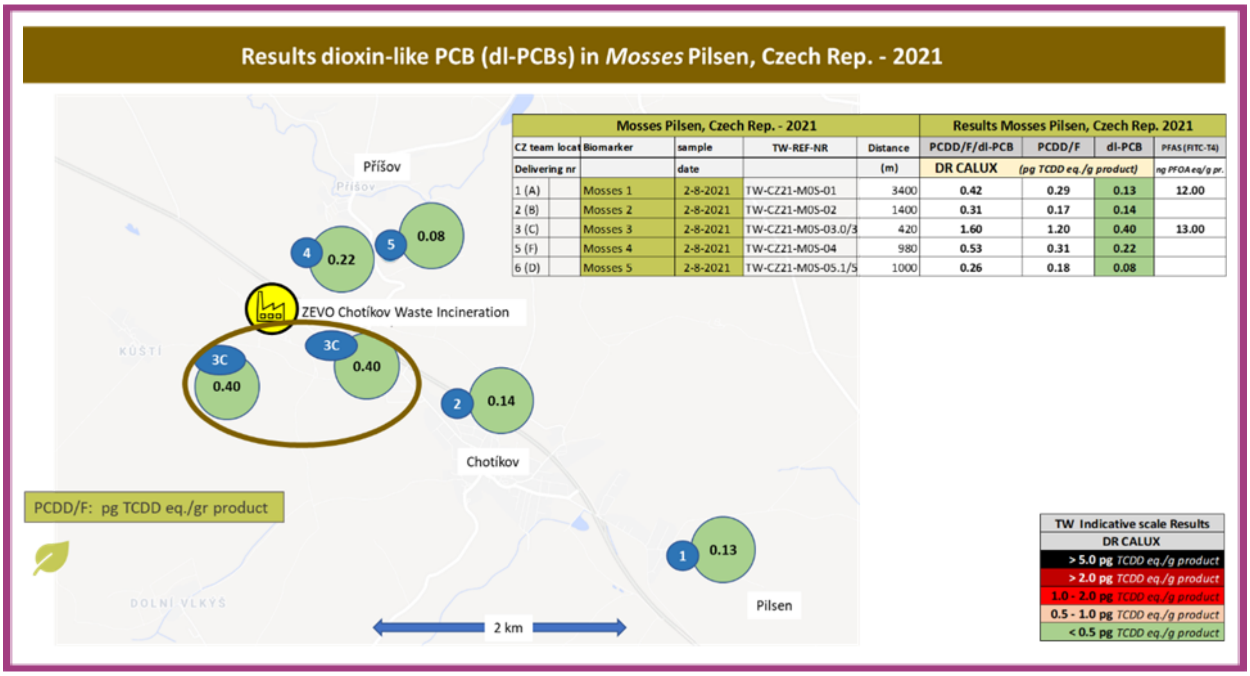
Figure 44: TW Indicative scale for sum of dioxins (PCDD/F/dl-PCB) in mosses, Pilsen 2021



Results dioxin-like PCB (dl-PCB) in mosses

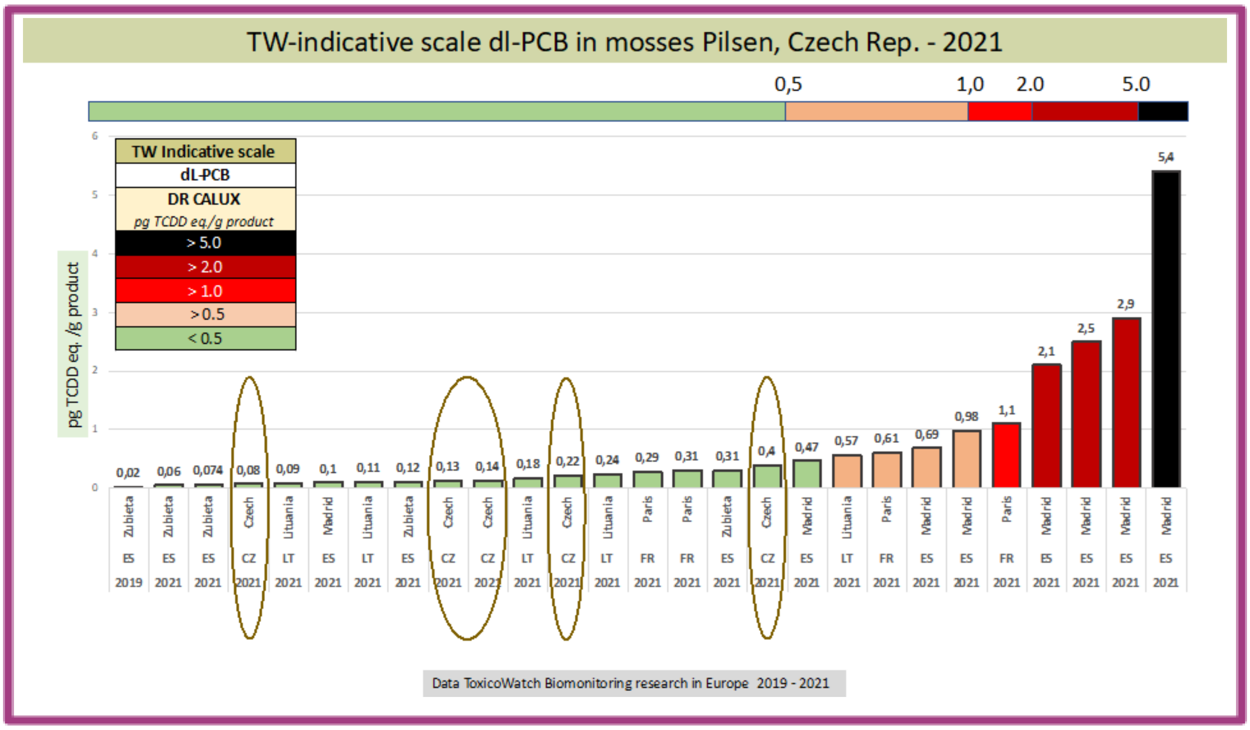
Dioxin-like PCBs (dl-PCBs) are elevated on location 3 with a factor 3. The level of dl-PCB in the environment (Figure 45) can be considered as low when compared with other places around incinerators in Europe (Figure 46).

Figure 45: dl-PCBs in mosses Pilsen, Czech Rep. 2021



Just like in the pine needles relative low values of dl-PCB are measured in mosses. In the literature higher values are calculated with GC-MS, because of a too high TEF value for the TEQ calculation of 0,1.

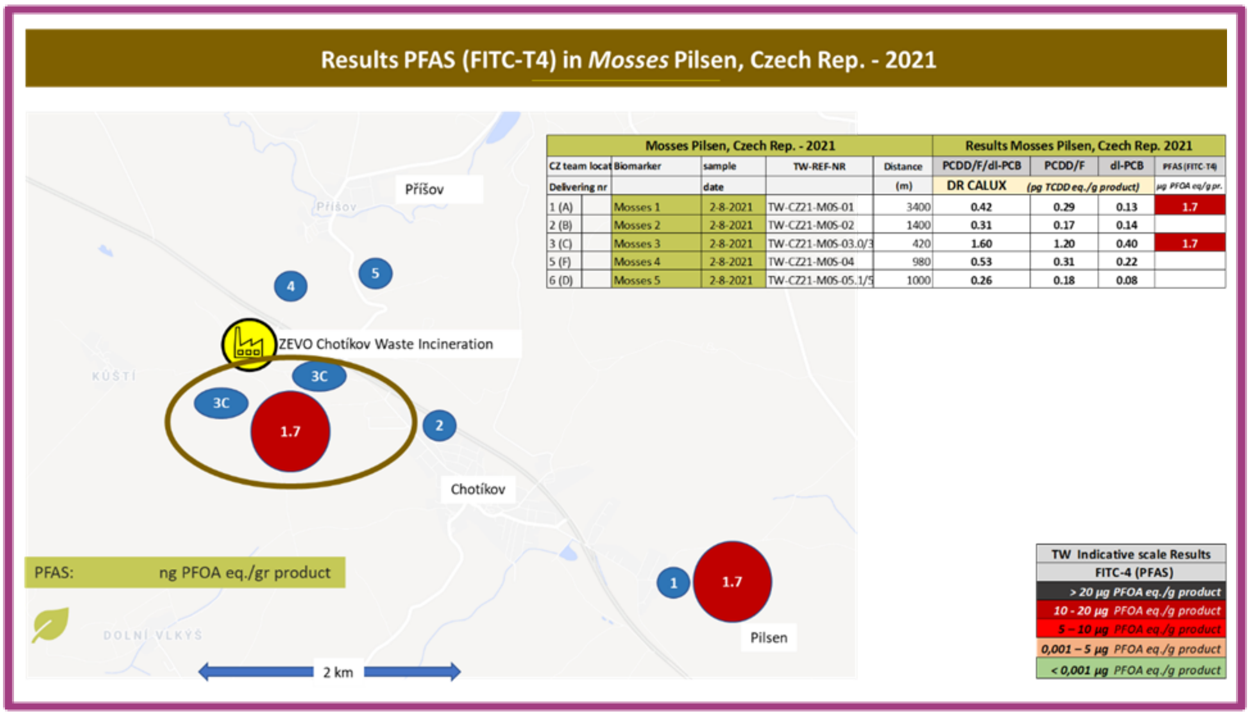
Figure 46: TW Indicative scale dioxin- like PCB (dl-PCB) mosses Pilsen , Czech Rep. 2021



PFAS in mosses

To have an impression of PFAS presence in the environment the FITC-T4 analysis method was used on two samples of mosses. The results for moss location 1 and 3 are 12.0 μg and 13.0 μg PFOA equivalent per gram product (Figure 47), expressed in nanograms 12,000 and 13,000 ng PFOA eq./gram mosses. In a Swedish study[54] the concentrations of 10 perfluorinated alkylated substances (PFAS) were all below the quantification limit (<0.6 ng/g dw) for every compound and moss sample. The results in this biomonitoring research show high levels of PFAS, which is an important reason for more research, particularly now that high quantities of PFAS have also been found in the eggs of backyard chicken in this study. What are the consequences of PFAS on the environment, for vegetation, uptake by domestic animals, dairy and meat products, as well for human health in general? The question arises: what is the cause of this PFAS contamination? And what is the contribution of incineration to the PFAS contamination in the environment? In a study of ToxicoWatch of continuous measurements WtE incineration in the Netherlands, PFOA and PFOS are detected in the flue gases.[55] So the question arise what is the contribution of waste incineration to the PFAS contamination in the environment of Pilsen?

Figure 47: Results of PFAS (FITC-T4) in mosses, Pilsen,, Czech Rep. 2021



Mosses Pilsen, Czech Rep. - 2021					Results Mosses Pilsen, Czech Rep. 2021			
CZ team locat	Biomarker	sample	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	dl-PCB	PFAS (FITC-T4)
Delivering nr		date		(m)	DR CALUX	(pg TCDD eq./g product)		µg PFOA eq/g pr.
1 (A)	Mosses 1	2-8-2021	TW-CZ21-MOS-01	3400	0.42	0.29	0.13	1.7
2 (B)	Mosses 2	2-8-2021	TW-CZ21-MOS-02	1400	0.31	0.17	0.14	
3 (C)	Mosses 3	2-8-2021	TW-CZ21-MOS-03.0/3	420	1.60	1.20	0.40	1.7
5 (F)	Mosses 4	2-8-2021	TW-CZ21-MOS-04	980	0.53	0.31	0.22	
6 (D)	Mosses 5	2-8-2021	TW-CZ21-MOS-05.1/5	1000	0.26	0.18	0.08	

TW Indicative scale Results
FITC-4 (PFAS)
> 20 µg PFOA eq./g product
10 - 20 µg PFOA eq./g product
5 – 10 µg PFOA eq./g product
0,001 – 5 µg PFOA eq./g product
< 0,001 µg PFOA eq./g product

[54] Danielsson H. et al. (2016). *Persistent organic pollutants in Swedish mosses, IVL Swedish Environmental Research Institute 2016, report nr. C 188*

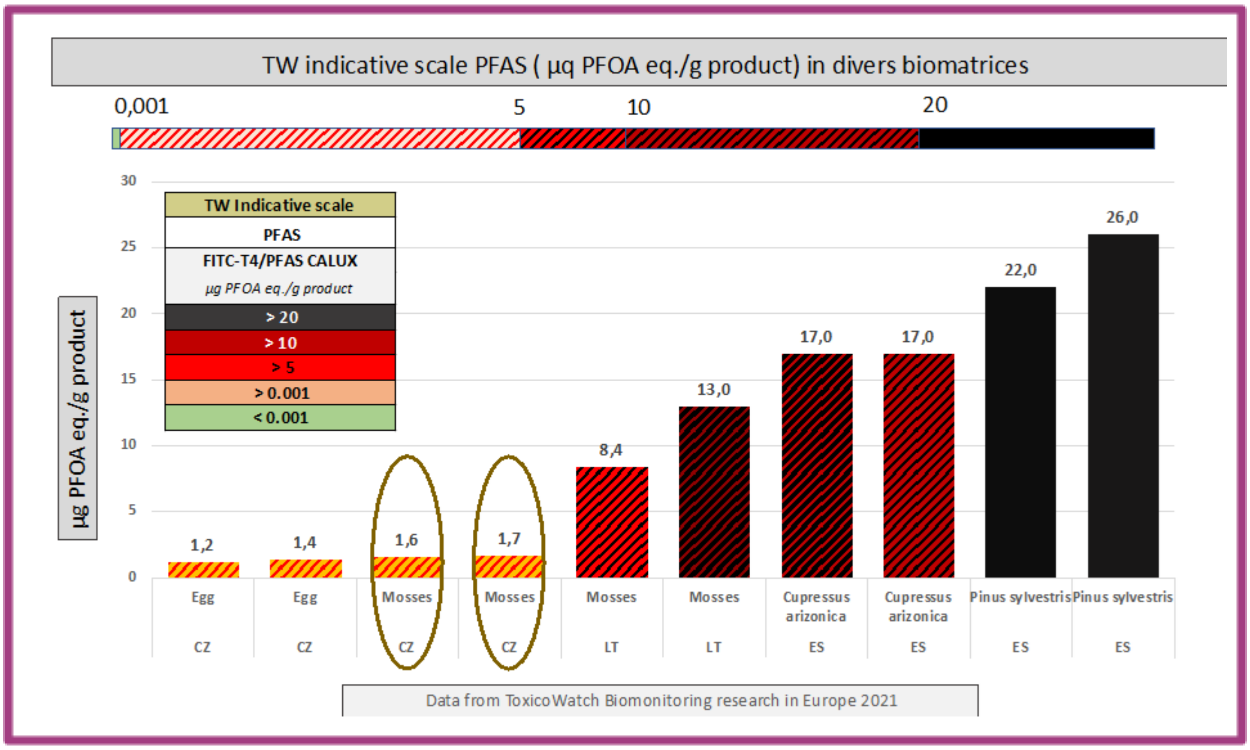
[55] *Arkenbout, A, 2018. Long-term sampling emission of PFOS and PFOA of a Waste-to-Energy incinerator*

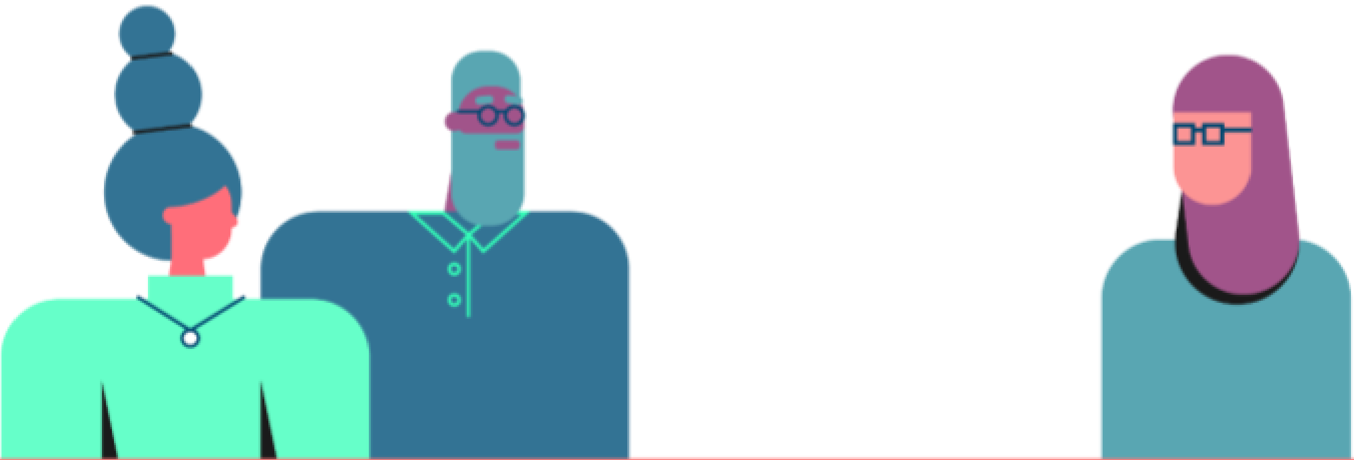
The EFSA advice for a Tolerable Weekly Intake of PFOA is set at 6 nanogram/kg body weight a week. This means the PFAS levels found in the two (2) moss locations are extreme high, raising concerns about the findings on vegetables in food crops in the area, whether for the market or for private use. Additional research on PFAS is needed a for a better understanding of how these results can be interpreted.

In the previous section is explained how the analytical research of PFAS is lagging behind. Only a fraction of the different PFAS components (8-55) can be analysed in a laboratory, whereas it is likely that more than 8,000 different PFAS can be found in the environment. The relative potency factor of only 12 components could have been determined (see page 16). The FITC-4 is an analytical method measuring the total toxicity of a mixture of different PFAS substances. PFAS are associated with adverse human health effects on thyroid function, metabolism (including overweight/obesity, diabetes, insulin resistance, high cholesterol and foetal development, and play an important role in the human immune system. Further research is needed to monitor and analyse the contamination of this "forever" chemical in our environment to establish the consequences of these PFAS for the environment, vegetation, animals, and our human health.

There are no further studies of FITC-T4 on vegetation or mosses. This biomonitoring research, simultaneously performed in the Czech Republic (Pilsen), Lithuania (Kaunas) and Spain (Madrid), see Figure 48, is the first in line with the application of FITC-T4 on biomatrices. There is a great need for data on PFAS distribution in the environment. Chemical analyses (GC-MS) unfortunately fall short of these findings, hence the application of the FITC-T4 methodology. The extent to which the incineration of PFAS-related waste and sewage sludge leads to PFAS contamination in the environment is still unknown. The association with fire-fighting foams (AFFF) is clear, but what is not clear are the combustion products of a fire-fighting event. Pilot studies with PFAS incineration indicate incomplete destruction even at temperatures above 950 ˚C. In a modern waste-to-energy incinerator (WtE) the post-combustion temperature is set at 850 °C, and, as it appears currently, these temperatures are not adequate to destroy persistent organic pollutants like dioxins and PFAS completely.

Figure 48: TW Indicative scale for PFAS in various biomatrices – 2021



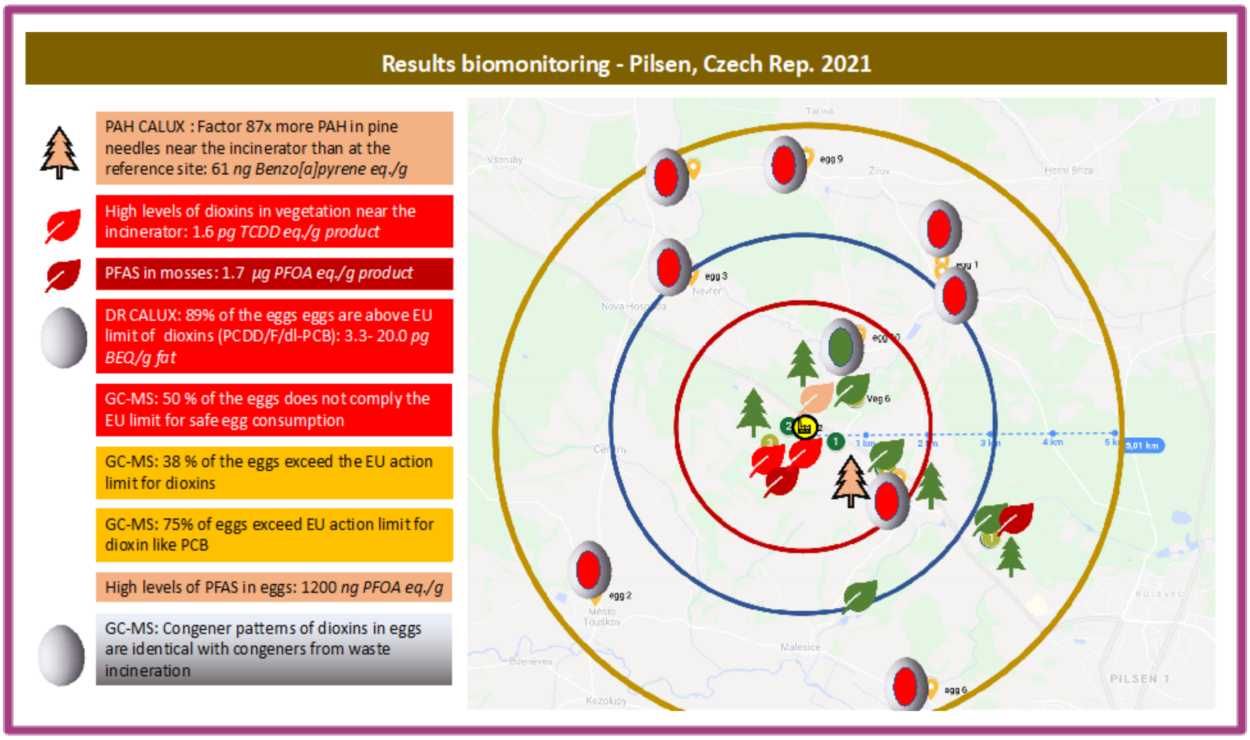


Conclusion

The newly built Pilsen Waste-to-Energy incinerator (WtE), ZEVO Plzeň or ZEVO Chotíkov, has been in operation since March 2019. The first round of a biomonitoring research on biomarkers of backyard chicken eggs, pine needles and mosses in the region around the incinerator has taken place in the year 2021. The second round of biomonitoring in the area of Pilsen around the WtE (waste) incinerator will continue in 2022.

The eggs of backyard chicken, a sensitive biomarker of pollution of substances of very high concern in the environment, show with the bioassay DR CALUX analysis that 89% (8 of the 9 locations) exceed the EU action limits for food safety as regulated in the EU Directive 2013/711/EU. The eggs that met the EU requirements were first-laid eggs from very young hens and supermarket eggs, used as a reference. The chemical GC-MS analyses of the eggs result in six (6) locations exceeding the EU action limit for dioxin-like PCBs (dl-PCB) and four (4) out of nine (9) egg locations exceeding the EU limits for safe egg consumption according to Regulation EU 1881/2006. If these eggs were intended for the commercial market, they should have been withdrawn from the market.

Figure 49: Conclusions biomonitoring Pilsen, Czech Rep. 2021



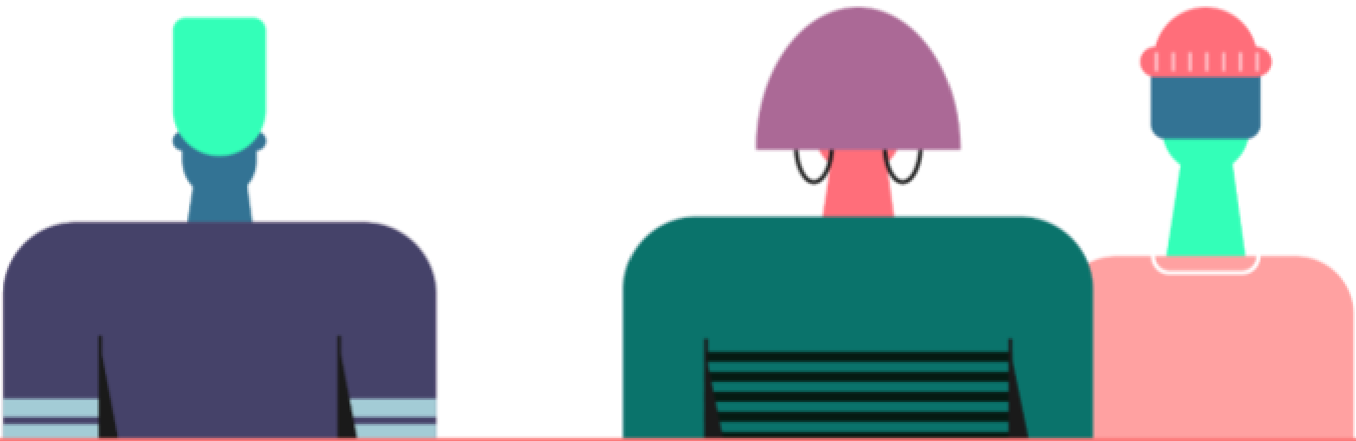
The EU regulations urge for action on these egg locations to find out the source of persistent organic pollutant contamination, in order to eliminate or at least do the utmost to reduce dioxins (PCDD/F) to the minimum level. However, it should be noted that the EU standards are intended for the economic food market and are not primarily based on EFSA's sole health advice. The EU limits for eggs are based on a Tolerable Weekly Intake (TWI) of dioxins. The European Food and Safety Authority (EFSA) has adjusted this TWI by a factor of seven (7) in 2018. This health advice is still not yet implemented by governments in EU. Since private consumption of backyard chicken eggs is as high as 200-600 eggs per month, according to the answers in the questionnaire from the chicken coop owners, this is a serious health risk. The congener patterns of the dioxins (PCDD/F) found in backyard chicken eggs in this research, expressed both in concentrations and TEQ, demonstrate the characteristics fingerprints of incomplete combustion. A long-term study of 20,000 hours in the REC waste incinerator in the Netherlands is used as a reference. Resemblances are found in the concentration and especially in the TEQ profiles for the PCDD/Fs. A contribution from the waste incinerator cannot be completely excluded for the dioxin-like PCBs, although local deposition is more likely. The dominant presence of furans indicates newly formed dioxins from incineration.

The results of the analysis of the vegetation, pine needles and mosses show elevation of dioxin levels in the vicinity of the waste incinerator. The mosses, with a value of 1.60 pg TCDD eq./g product, are a factor of five (5) higher than the reference site. The pine needles are increased by a factor of 3, mainly due dl-PCBs. The results of the dioxin analysis of vegetation in Pilsen demonstrate a clear spatial trend of dioxin. The PAH found in pine needles near the incinerator is 87 times more than at the reference location in Pilsen.

The region of Pilsen shows an environment under threat by contamination of substances of very high concern in eggs of backyard chicken, pine needles, and mosses. This biomonitoring research gives a warning signal for contamination of the environment with serious toxic substances such as dioxins (PCDD/F), dioxin-like PCBs, PAHs and PFAS. The emerging question rises whether waste incineration is a solution for waste disposal and energy production instead of the main focus on recycling.

Measurements of the flue gasses could verify the fingerprints and congener patterns, found in the eggs and most important quantify the emitted POPs during Other Than Normal Operation Conditions (OTNOC). The results of the biomarkers in this biomonitoring research underline that the environment around WtE incinerator ZEVO Plzeň is under threat by contamination of persistent organic pollutants (POPs).

The biomonitoring project will be continued in 2022.



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