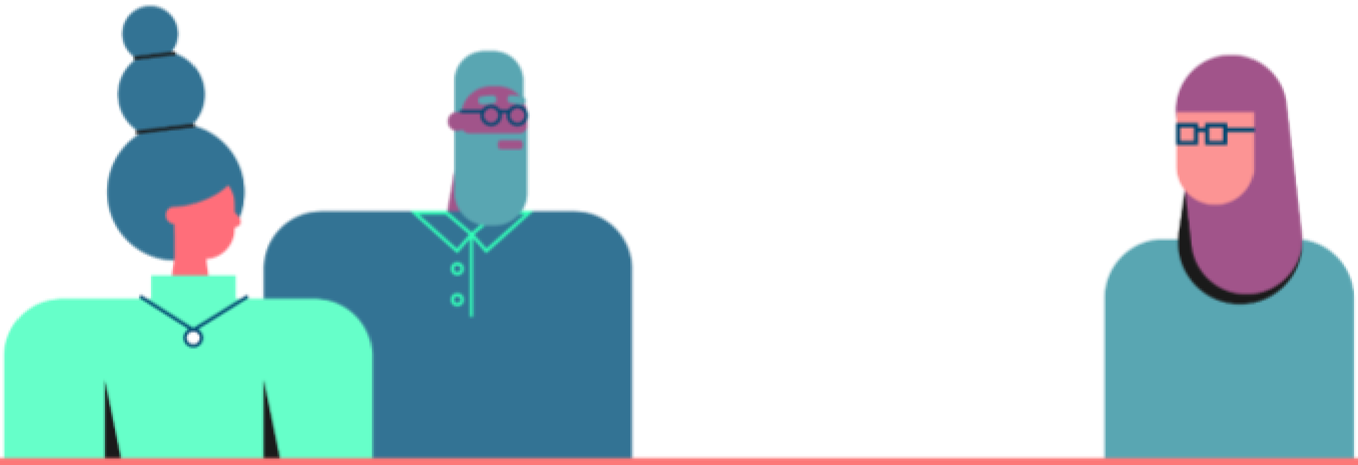
THE TRUE TOXIC TOLL

# Biomonitoring research results

## Kaunas, Lithuania | 2021

## Indicators

Chicken eggs | Moss | Pine needles



# Acknowledgements

## and credits

Thanks to Zero Waste Europe for making it possible to perform this toxicology research on persistent organic pollutants (POPs) in the environment of Kaunas, Lithuania.

## AUTHORS

A. ARKENBOUT - Head of research at ToxicoWatch foundation
K. BOUMAN - Research assistant at ToxicoWatch foundation

**HARLINGEN, THE NETHERLANDS, TOXICOWATCH FOUNDATION, December 2021**
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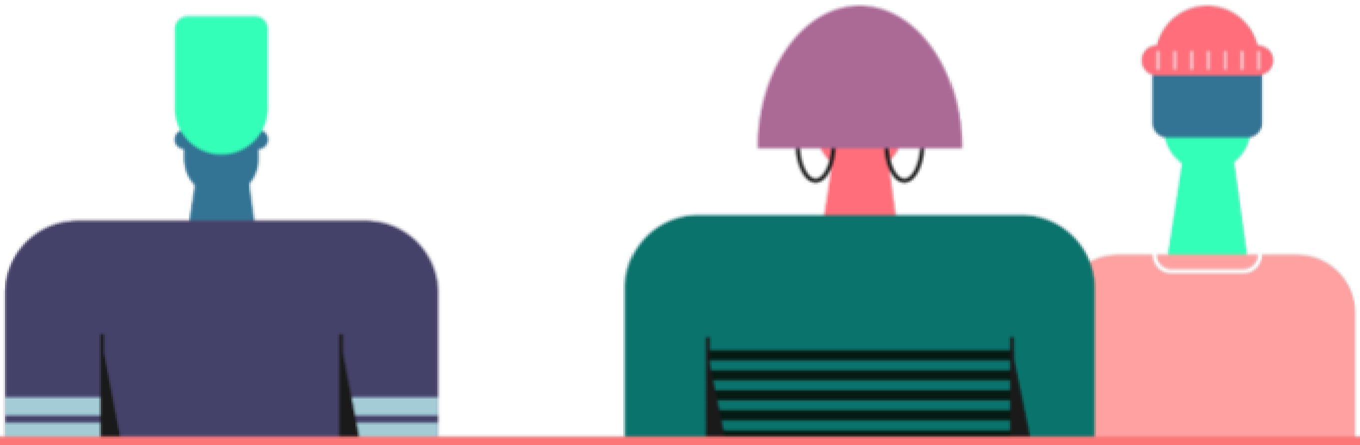

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## and abbreviations

| 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 the Czech Republic, and Žiedinė ekonomika in Lithuania - all coordinated by Zero Waste Europe.

# The incinerator

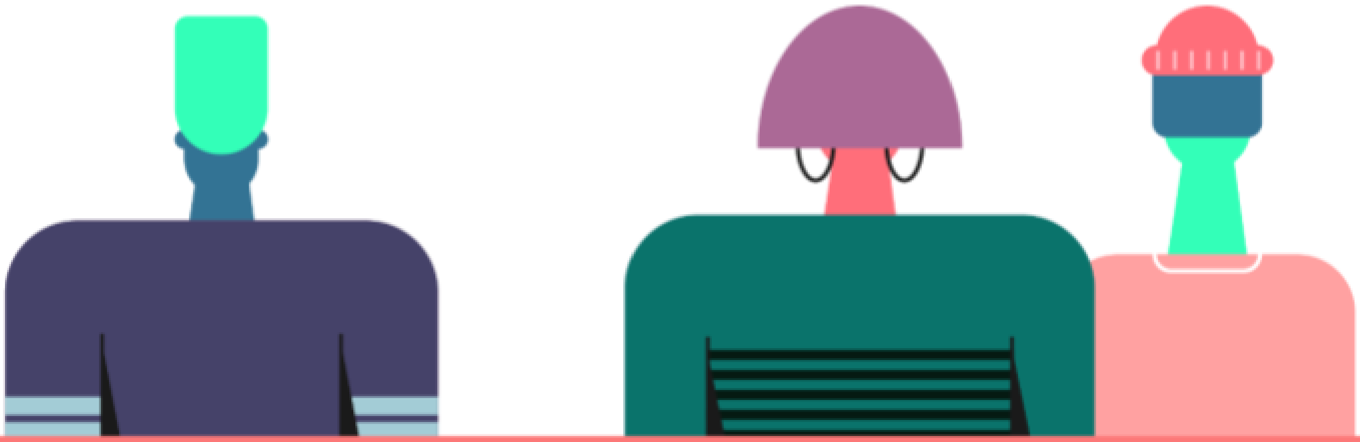
## and surrounding conditions

In Lithuania, most municipal solid waste has generally been disposed of in landfills. However, in recent years, there has been a growing interest in waste-to-energy (WtE) incineration to use the waste heat generated when waste is incinerated. A new high-efficiency waste-fired cogeneration power plant in Kaunas with an electric capacity of about 24 MW and heat production capacity of about 70 MW entered full-scale operation on 27 November 2020.

**Figure 1: UAB Kauno Cogeneration Power Plant, (WtE) waste incineration 2021**

The Waste-To-Energy (WTE) incinerator has a maximum capacity of combustion of 800 tons waste/day for the supply of electric power to the national 110 kV grid and distributes heat to the city of Kaunas. About 200,000 tonnes of municipal waste is generated in the region, after sorting out the waste input, producing around 500 GWh of heat and 170 GWh of electricity per year. The WtE incinerator produces about 40% of the heat requirements of Kaunas city. This waste incinerator is the UAB Kauno Cogeneration Power Plant (Lithuanian: UAB Kauno Kogeneracinė Jėgainė) or the UAB Kauno CHP plant or Kaunas Combined Heat and Power Plant. In this report, it is referred to as the Kaunas WTE (waste) incinerator.

It's unclear how long the testing phase of the newly built Kaunas WtE incinerator will last. The height of the chimney is 43 metres in an open area with the dominant average wind direction being from the southwest.

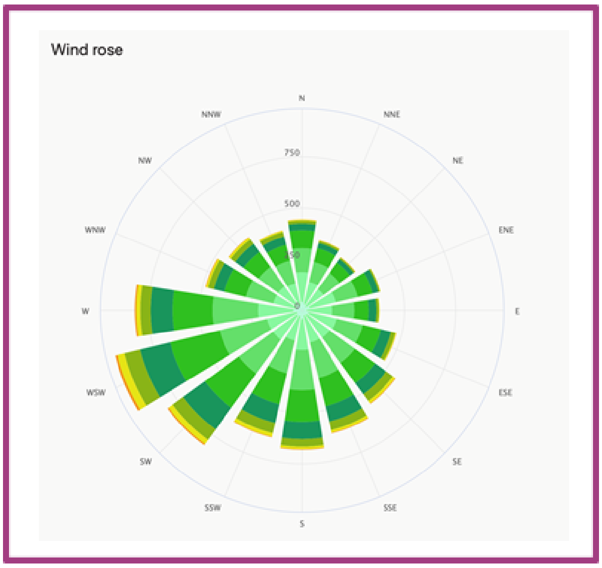


# Wind direction

## and depositions

The annual average wind direction in Kaunas is shown in Figure 2. This can be used as a model to predict possible depositions of emissions by the Kaunas waste incinerator. The dominant wind direction is mainly southwest.

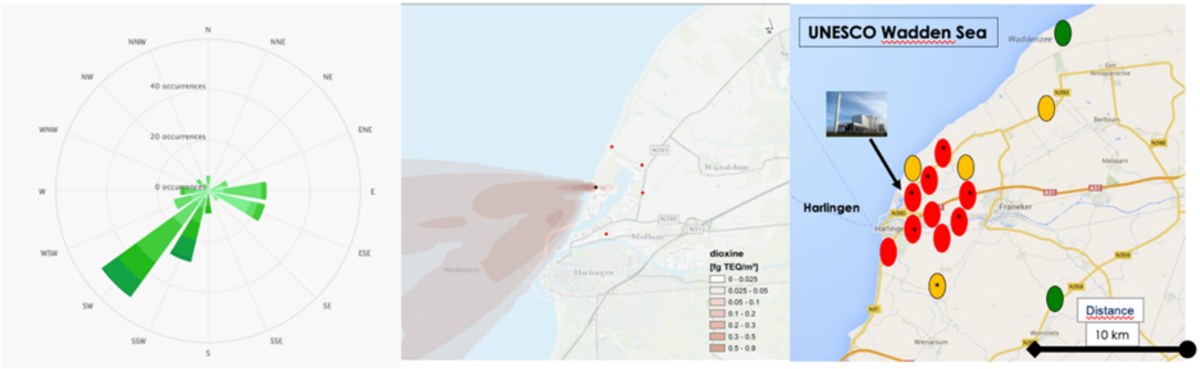
**Figure 2: Annual and 2-week wind rose for Kaunas, Lithuania**



The use of a wind rose for modelling deposition emissions from incineration processes is limited. Figure 3 shows 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 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 timeframe. Figure 3c shows dioxin-contaminated eggs around the WtE waste incinerator in Harlingen (NL).

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 relativises the use of average dominant wind directions in calculation models for POP emissions.

**Figure 3: Wind rose for Harlingen, NL, with dominant average wind direction from SW (a), dioxin cloud during calamity, 2015 (b), contaminated backyard chicken eggs, Harlingen (c)**

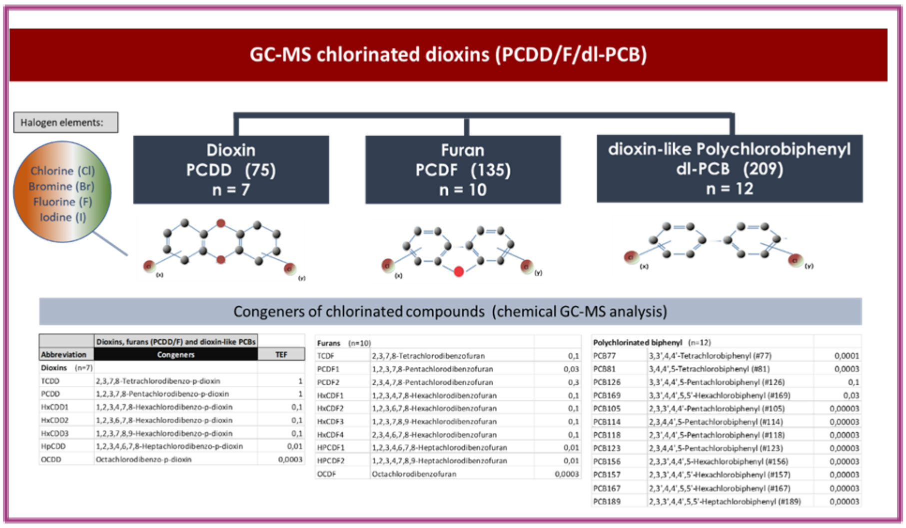


# Dioxins

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.[1] 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).[2]

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 5 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.[3]

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



| | Dioxins, furans (PCDD/F) and dioxin-like PCBs | |
|---|---|---|
| **Abbreviation** | **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 |

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

[2] *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.*

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

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**

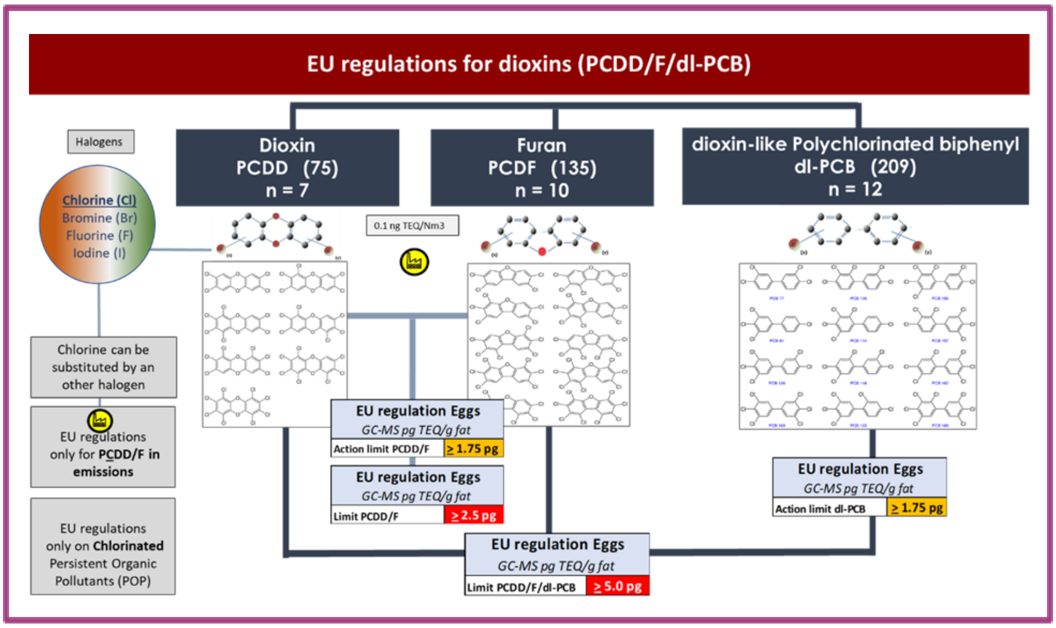
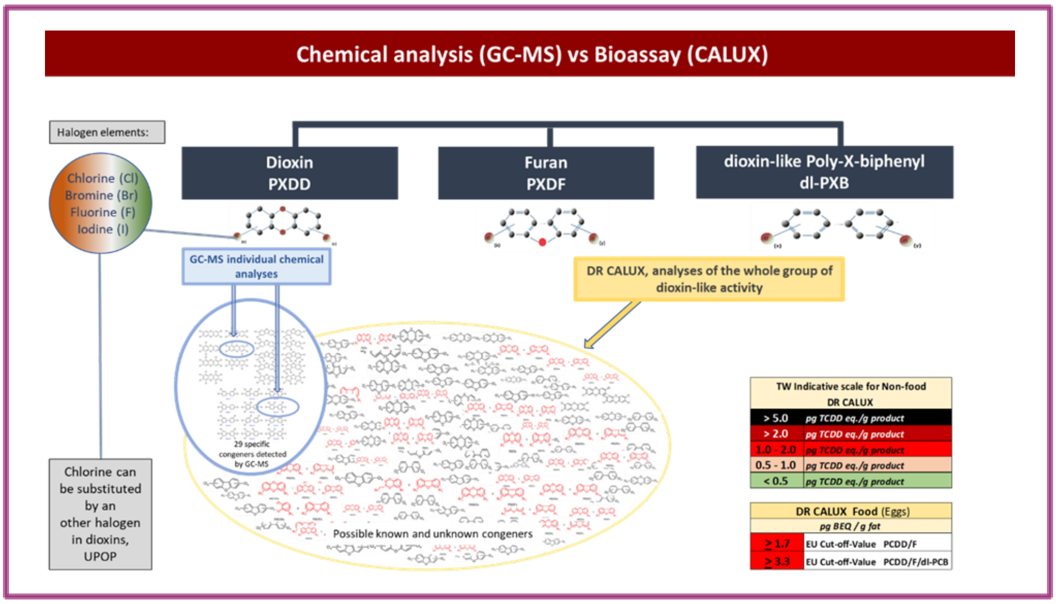
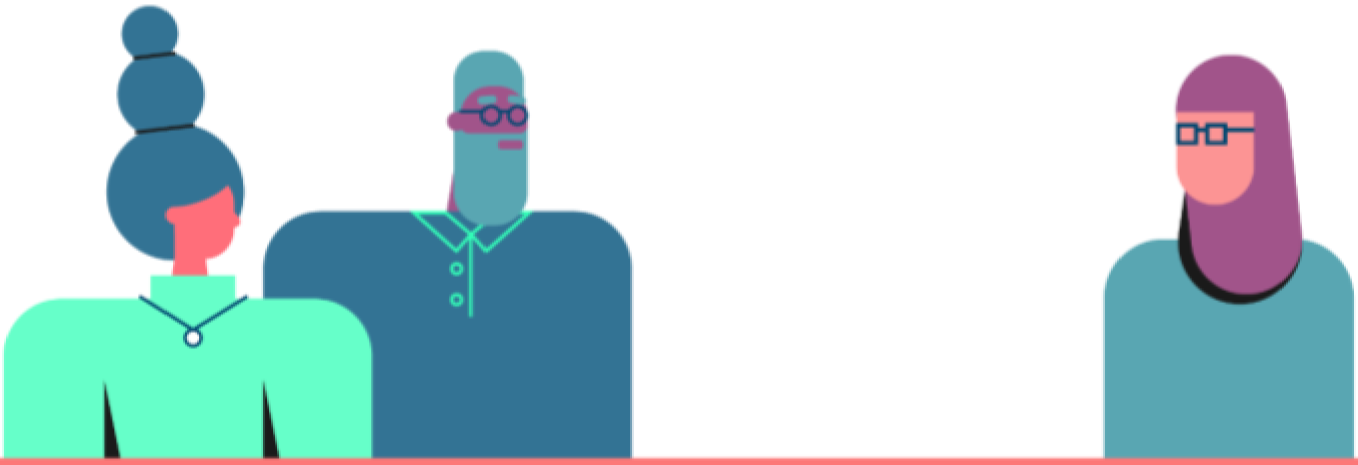


Figure 6 displays the difference between the chemical analysis with GC-MS and the bioassay DR CALUX. GC-MS analysed specific compounds, while DR CALUX measures the total toxic effect of a mixture of dioxin-like activity.

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





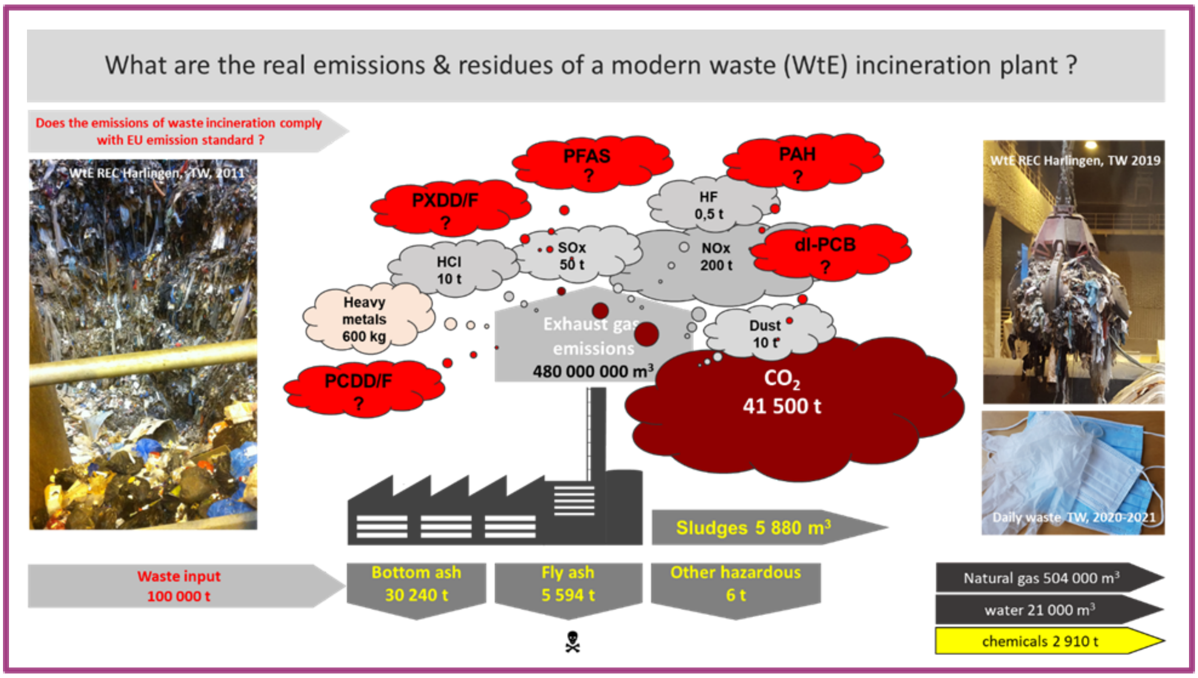
# Emissions

## 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 8. 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.[4] 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**



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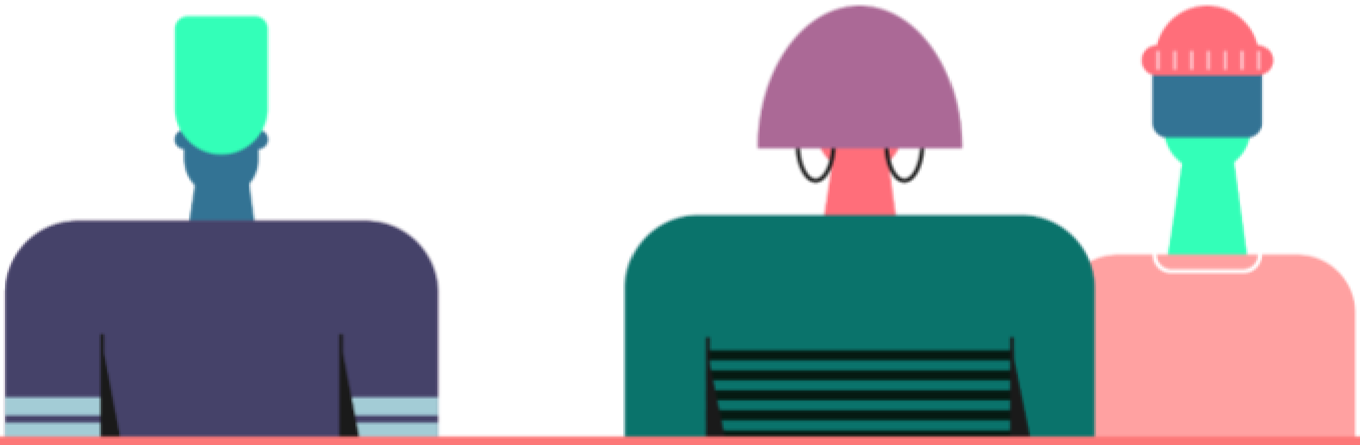
[4] *ToxicoWatch (2020). The hidden impacts of incineration residues, Zero Waste Europe*



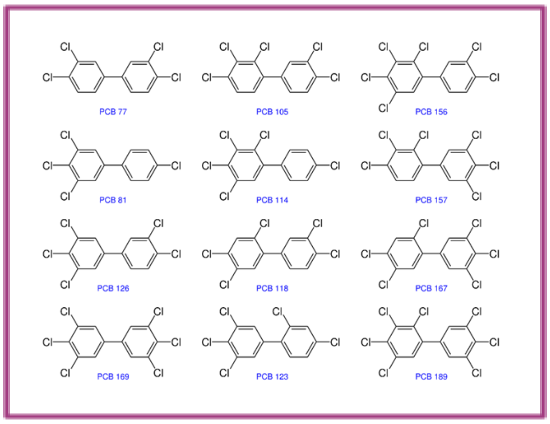
# Polychlorinated biphenyl

## (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.[5]

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 9. This subgroup is termed dioxin-like PCBs (dl-PCBs) and are referred to as the 12 dioxin-like PCBs, see also Figure 5, 6. 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).[6] 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**



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

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

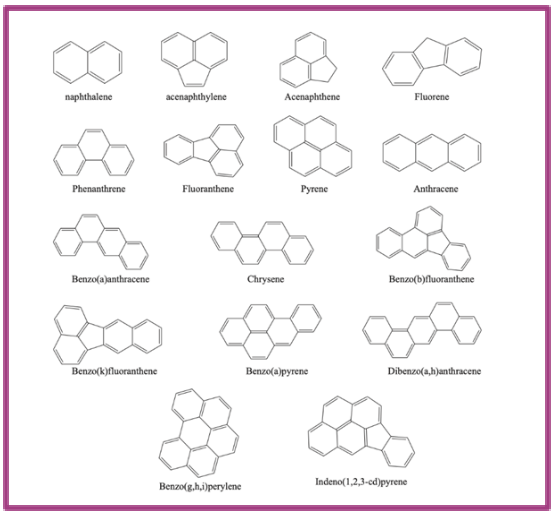
# Polycyclic aromatic hydrocarbon

## (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.[7] 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 10.

However, this will result in an underestimation of the PAH in a sample.[8] 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)**



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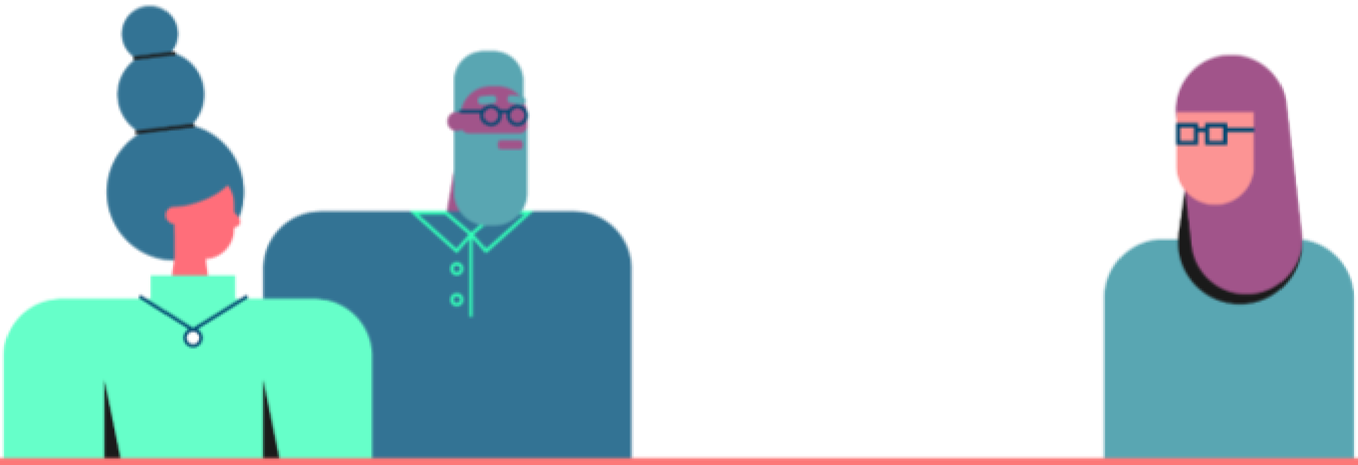
[7] *Ruri Vivian Nilamsari et al. 2020. Polycyclic Aromatic Hydrocarbons (PAH) Reduces the Effectiveness of Measles Vaccination Through Immunotoxicity to Innate and Adaptive Immune Cells. Research J. Pharm. and Tech. 2020; 13(12):6128-6131.*

[8] *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*

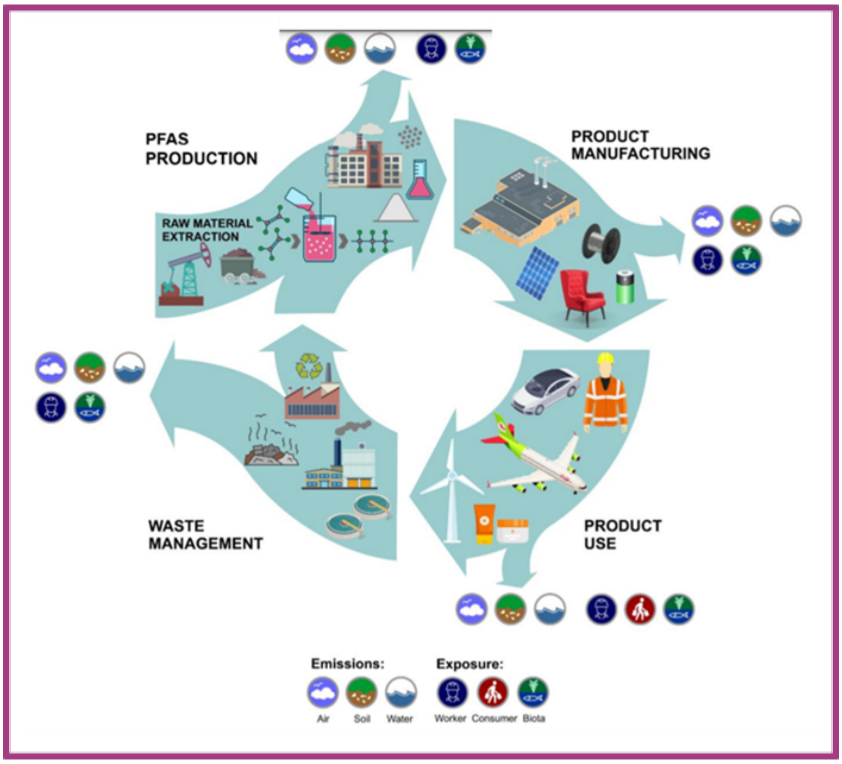


# Per- and PolyFluoroAlkyl Substances

## (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.[9] The risk of immunotoxicity for humans and wildlife cannot be discounted.[10]

**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 11, “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 resistance to infection”. EFSA concluded that parts of the European population exceed the tolerable weekly intake (TWI) from food of four PFAS.[11]

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

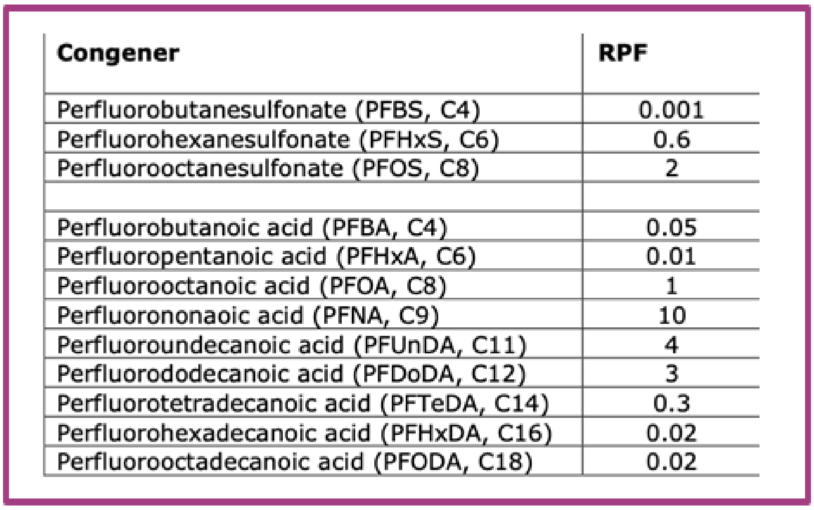
[10] *Corsini, E., et al., Perfluorinated compounds: Emerging POPs with potential immunotoxicity. Toxicol. Lett. (2014).*

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

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.[12] 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)[13] - see Table 1.

Overview of PFAS exposure pathways to the human population and the environment - see Figure 12, (Sunderland et al. 2019).[14] “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.”[15]

**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 |

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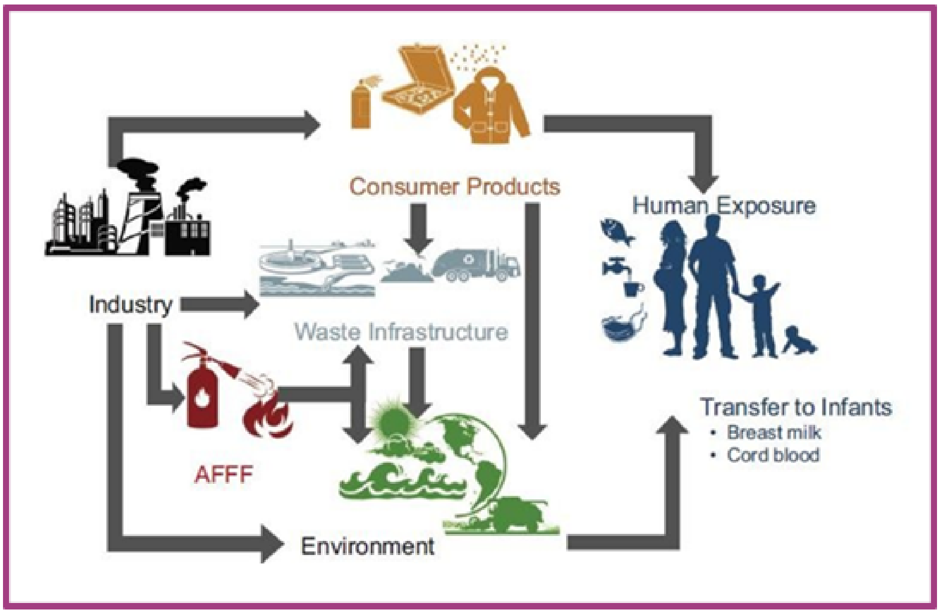
[12] *Van Leeuwen SPJ, Kärrman A, Van Bavel B, De Boer J and Lindstrom G, 2006. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples. Environmental Science and Technology, 40, 7854–7860.*

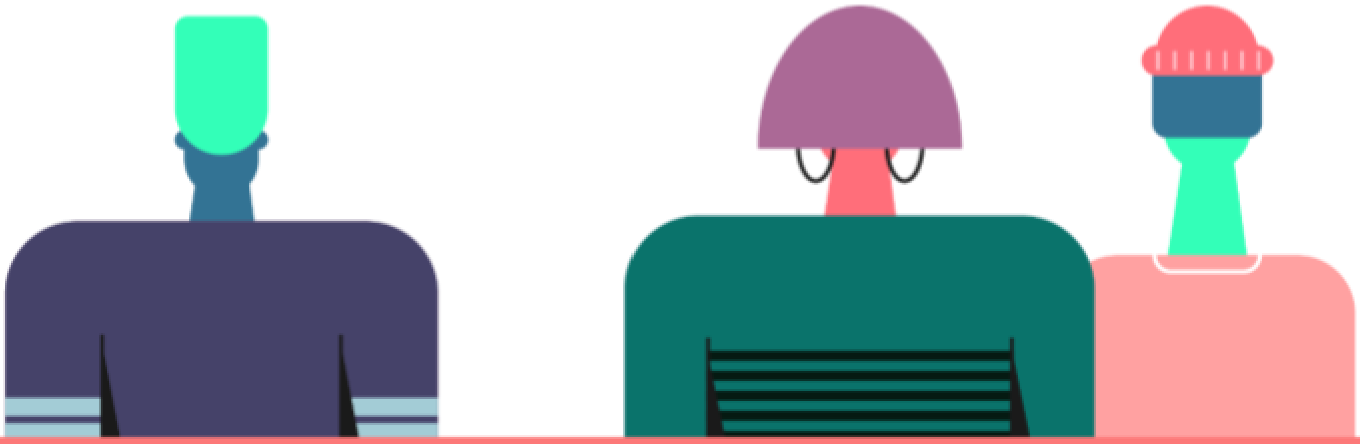
[13] *M.J. Zeilmaker et al 2018. Mixture exposure to PFAS: A Relative Potency Factor approach, National Institute for Public Health and the Environment, RIVM Report 2018-0070.*

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

[15] *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**[16] 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 6 and 7).

**Directive 2013/711/EU**[17] 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[18] 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 6. 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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[16] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20210919&from=EN
[17] eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0709&from=EN
[18] 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).[19,20]

## 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).[21] 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.[22] 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).[23,24] The thyroid hormone homeostasis can be disrupted by environmental chemicals at different points of interaction in the thyroid pathway, including

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[19] *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.*

[20] *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.*

[21] *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.*

[22] *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*

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

[24] *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.

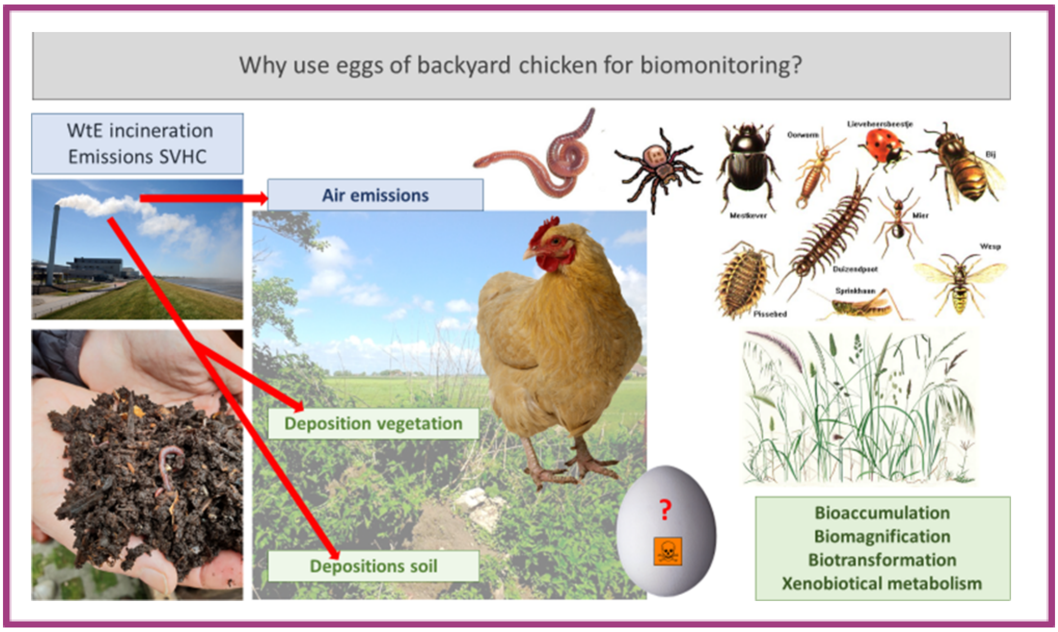
# Backyard chicken

## 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).[25,26]

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 13). 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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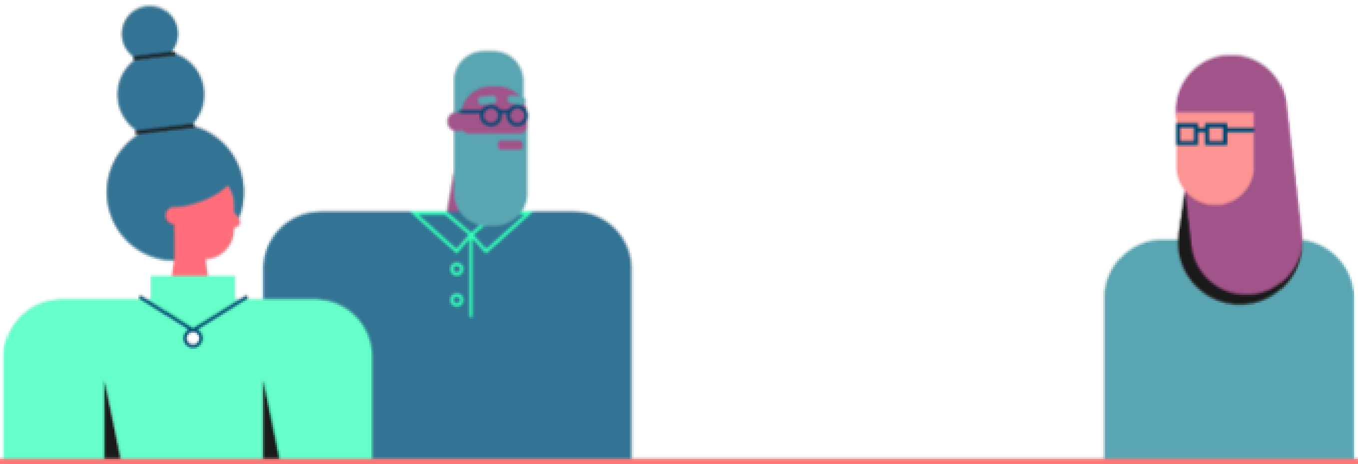
[25] *Arkenbout A, Esbensen K H. (2017) Sampling, monitoring and source tracking of Dioxins in the environment of an incinerator in the Netherlands, Proceedings Eighth World Conference On Sampling and Blending / Perth*

[26] *Petrlík J. (2015). Persistent Organic Pollutants (POPs) in Chicken Eggs from Hot Spots in China.Beijing-Gothenburg-Prague, Arnika - Toxics and Waste Programme.*

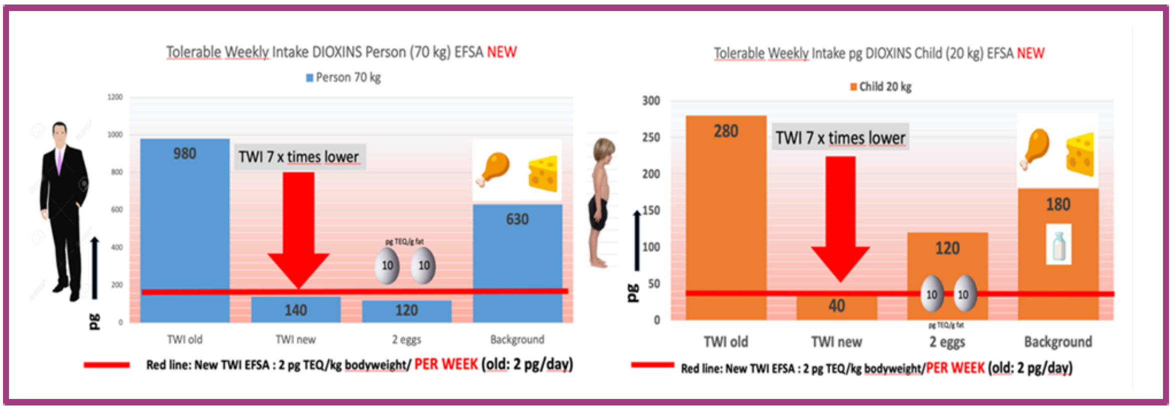


# European Food Safety Authority

## (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)[27] - see Figure 14. 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.**[28] 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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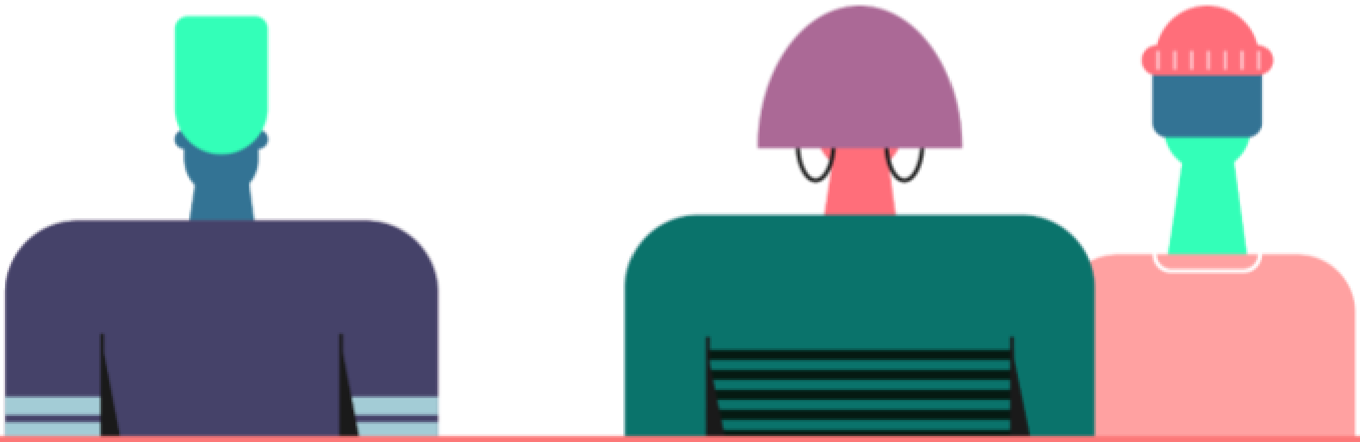
[27] *EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK et al. 2018. Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331 pp.*

[28] *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

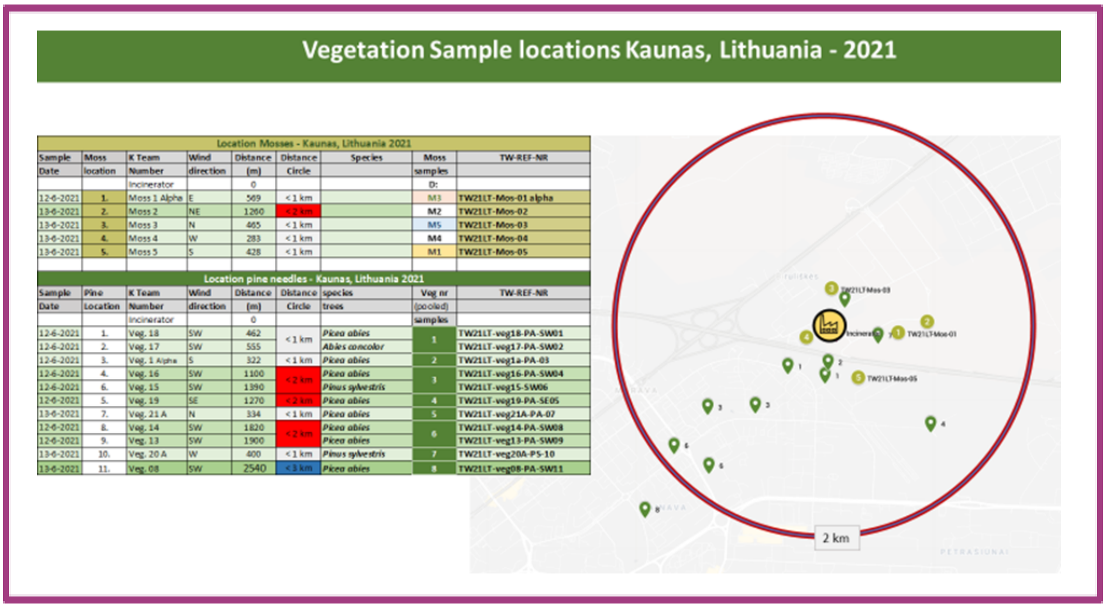
This biomonitoring project is performed on sample locations by D. Tracevičius of Zero Waste Lithuania in cooperation with TW. Participation of a local sample team is important and needed for communication about this biomonitoring research with the local community and the actual sampling. A TW manual is applied to explain the needed sample steps for preparation and handling in this study, see Annex I, Sampling Plan. For the initial sampling plan Vegetation (pine needles of *Picea abies* and *Pinus sylvestris* besides Mosses) – see Figure 14.

At first, an exploration was undertaken on 14 April to find the locations of chicken coop owners of backyard chicken in the surrounding of Kaunas, who were willing to participate in this biomonitoring research and to make an inventory of locations for monitoring vegetation and mosses. Six backyard chicken locations were found within a 3-km radius, five (5) towards the North-East and one on the West side of the waste incinerator. All the chicken coop owners completed the questionnaire provided by TW (table 2). Photos were taken of the sample backyard chicken egg locations and of the vegetation for collecting pine needles and mosses.

Samples of evergreen (gymnosperm) trees were taken because the deposition or uptake of dioxins can take place continuously throughout the year. Pine needles can stay on pine trees for 2-5 years, depending on the species. Pine needles have a fatty cuticula, the protective wax layer on the outside of the pine needle, to prevent too much water from evaporating, as well as for the protection of UV radiation and pathogens attacks. Since pine needles are lipophilic, these are a good biomarker for analysing persistent organic pollutants (POPs) like dioxins. Several years of deposition into the wax layer of the pine needle can be analysed with a chemical and bioassay method.

On 12 June 2021, sampling was conducted on the vegetation of pine needles (11 locations) and mosses (5 locations) – see Figure 13; as well on six (6) locations of backyard chicken eggs, feed, soil (see Figure 14) for biomonitoring in Kaunas, 2021.

**Figure 14: Vegetation sampling locations – Kaunas 2021**



Vegetation Sample locations Kaunas, Lithuania - 2021

Location Mosses - Kaunas, Lithuania 2021

| Sample Date | Moss location | K Team Number | Wind direction | Distance (m) | Distance Circle | Species | Moss samples | TW-REF-NR |
|---|---|---|---|---|---|---|---|---|
| | | Incinerator | | 0 | | | D: | |
| 12-6-2021 | 1. | Moss 1 Alpha | E | 569 | <1 km | | M3 | TW21LT-Mos-01 alpha |
| 13-6-2021 | 2. | Moss 2 | NE | 1260 | <2 km | | M2 | TW21LT-Mos-02 |
| 13-6-2021 | 3. | Moss 3 | N | 465 | <1 km | | M5 | TW21LT-Mos-03 |
| 13-6-2021 | 4. | Moss 4 | W | 283 | <1 km | | M4 | TW21LT-Mos-04 |
| 13-6-2021 | 5. | Moss 5 | S | 428 | <1 km | | M1 | TW21LT-Mos-05 |

Location pine needles - Kaunas, Lithuania 2021

| Sample Date | Pine Location | K Team Number | Wind direction | Distance (m) | Distance Circle | species trees | Veg nr (pooled) samples | TW-REF-NR |
|---|---|---|---|---|---|---|---|---|
| | | Incinerator | | 0 | | | | |
| 12-6-2021 | 1. | Veg. 18 | SW | 462 | <1 km | *Picea abies* | 1 | TW21LT-veg18-PA-SW01 |
| 12-6-2021 | 2. | Veg. 17 | SW | 555 | | *Abies concolor* | | TW21LT-veg17-PA-SW02 |
| 12-6-2021 | 3. | Veg. 1 Alpha | S | 322 | <1 km | *Picea abies* | 2 | TW21LT-veg1a-PA-03 |
| 12-6-2021 | 4. | Veg. 16 | SW | 1100 | <2 km | *Picea abies* | 3 | TW21LT-veg16-PA-SW04 |
| 12-6-2021 | 6. | Veg. 15 | SW | 1390 | | *Pinus sylvestris* | | TW21LT-veg15-SW06 |
| 12-6-2021 | 5. | Veg. 19 | SE | 1270 | <2 km | *Picea abies* | 4 | TW21LT-veg19-PA-SE05 |
| 13-6-2021 | 7. | Veg. 21 A | N | 334 | <1 km | *Picea abies* | 5 | TW21LT-veg21A-PA-07 |
| 12-6-2021 | 8. | Veg. 14 | SW | 1820 | <2 km | *Picea abies* | 6 | TW21LT-veg14-PA-SW08 |
| 12-6-2021 | 9. | Veg. 13 | SW | 1900 | | *Picea abies* | | TW21LT-veg13-PA-SW09 |
| 13-6-2021 | 10. | Veg. 20 A | W | 400 | <1 km | *Pinus sylvestris* | 7 | TW21LT-veg20A-PS-10 |
| 13-6-2021 | 11. | Veg. 08 | SW | 2540 | <3 km | *Picea abies* | 8 | TW21LT-veg08-PA-SW11 |

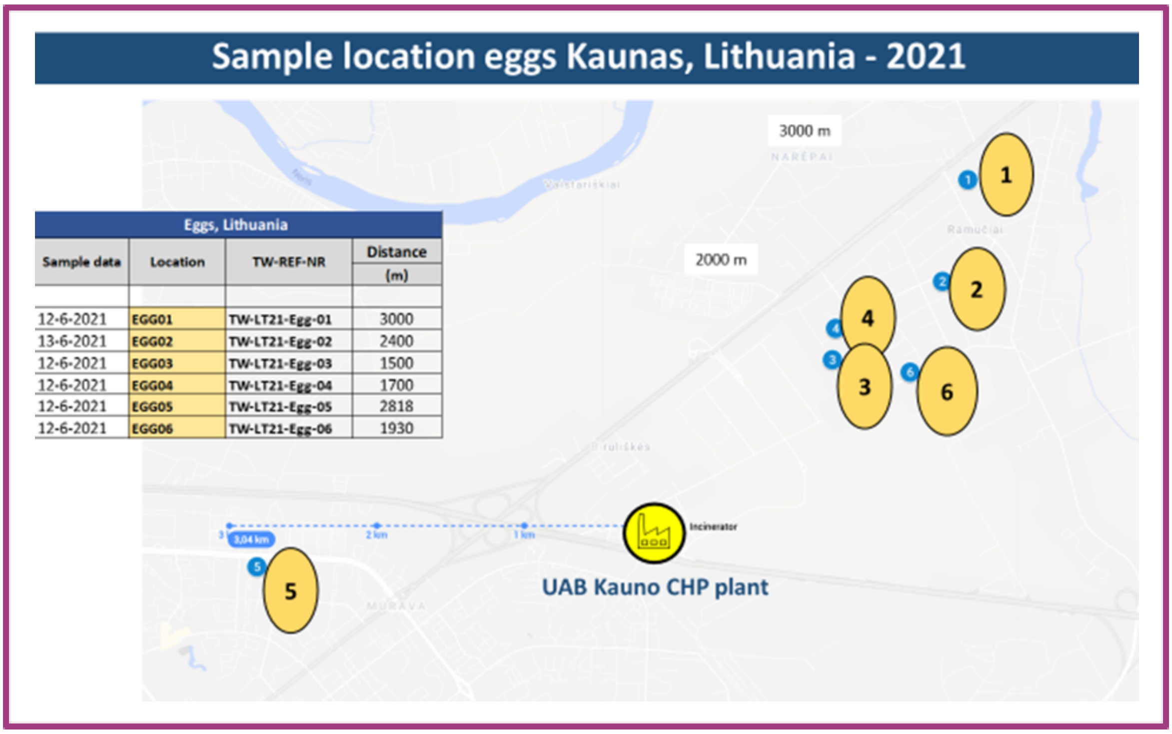
# Biomonitoring dioxins

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

## Egg location for sampling

All egg locations were visited in person by the Zero Waste Lithuania team, by D. Tracevičius of Zero Waste Lithuania. The sampling of eggs, vegetation and moss took place on 12 and 13 June 2021, 6 months after the official starting of operations of the Kaunas WtE incinerator.

**Figure 15: Six (6) egg locations for sampling and biomonitoring – Kaunas 2021**



Sample location eggs Kaunas, Lithuania - 2021

Eggs, Lithuania

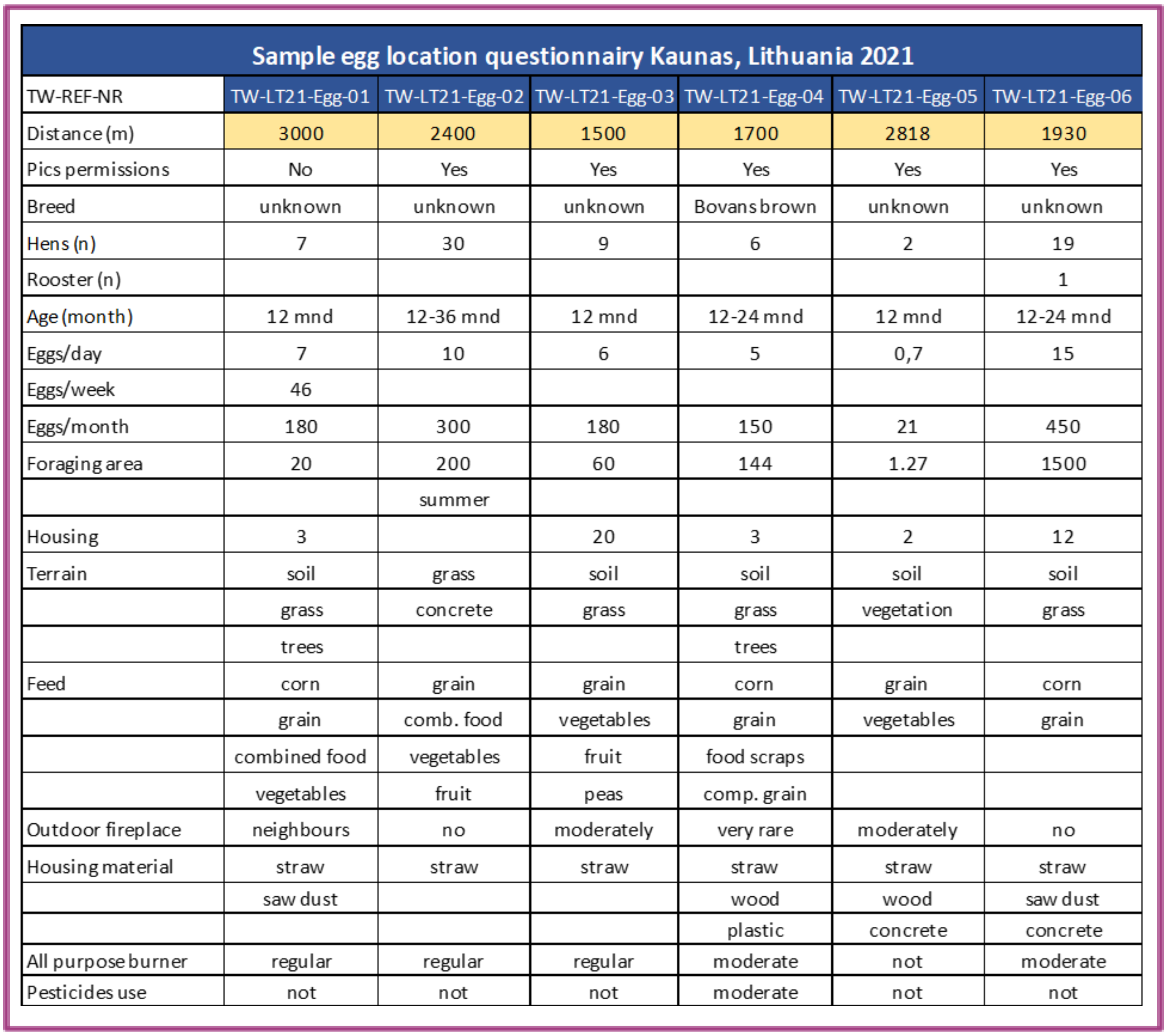
| Sample data | Location | TW-REF-NR | Distance (m) |
|---|---|---|---|
| | | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 |

It would be more preferable to show a map with egg locations for biomonitoring emissions of dioxins, in possible relation to the waste incinerator, in every wind direction around the waste incinerator with a distance < 1-3 km, according to the initial TW Sample Plan. Actually, for this research, these six (6) egg locations could be found and used for biomonitoring Kaunas 2021.

## Questionnaire

All the chicken coop owners of the six (6) 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. Five chicken coop owners gave their permission for photos to be used in this biomonitoring report.

**Table 2: Summary of questionnaire involving chicken coop owners – Kaunas 2021, provided by TW**



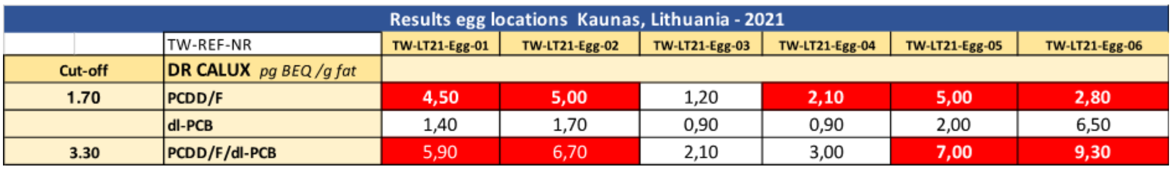
Sample egg location questionnairy Kaunas, Lithuania 2021

| TW-REF-NR | TW-LT21-Egg-01 | TW-LT21-Egg-02 | TW-LT21-Egg-03 | TW-LT21-Egg-04 | TW-LT21-Egg-05 | TW-LT21-Egg-06 |
|---|---|---|---|---|---|---|
| Distance (m) | 3000 | 2400 | 1500 | 1700 | 2818 | 1930 |
| Pics permissions | No | Yes | Yes | Yes | Yes | Yes |
| Breed | unknown | unknown | unknown | Bovans brown | unknown | unknown |
| Hens (n) | 7 | 30 | 9 | 6 | 2 | 19 |
| Rooster (n) |  |  |  |  |  | 1 |
| Age (month) | 12 mnd | 12-36 mnd | 12 mnd | 12-24 mnd | 12 mnd | 12-24 mnd |
| Eggs/day | 7 | 10 | 6 | 5 | 0,7 | 15 |
| Eggs/week | 46 |  |  |  |  |  |
| Eggs/month | 180 | 300 | 180 | 150 | 21 | 450 |
| Foraging area | 20 | 200 | 60 | 144 | 1.27 | 1500 |
|  |  | summer |  |  |  |  |
| Housing | 3 |  | 20 | 3 | 2 | 12 |
| Terrain | soil | grass | soil | soil | soil | soil |
|  | grass | concrete | grass | grass | vegetation | grass |
|  | trees |  |  | trees |  |  |
| Feed | corn | grain | grain | corn | grain | corn |
|  | grain | comb. food | vegetables | grain | vegetables | grain |
|  | combined food | vegetables | fruit | food scraps |  |  |
|  | vegetables | fruit | peas | comp. grain |  |  |
| Outdoor fireplace | neighbours | no | moderately | very rare | moderately | no |
| Housing material | straw | straw | straw | straw | straw | straw |
|  | saw dust |  |  | wood | wood | saw dust |
|  |  |  |  | plastic | concrete | concrete |
| All purpose burner | regular | regular | regular | moderate | not | moderate |
| Pesticides use | not | not | not | moderate | not | not |

## Results of DR CALUX analysis on eggs of backyard chicken

The backyard chicken eggs sampled in the marked locations (Figure 14) were analysed using a DR CALUX bioassay. Concerning the result values of these DR CALUX analyses of the six (6) egg locations, (Table 3), a strong recommendation is given by the EU regulation for food (in the case of commercial eggs) for chemical GC-MS analysis to verify the results of DR CALUX, if the DR CALUX exceeds the action levels. The action levels for DR CALUX are 1.7 pg BEQ/g fat for dioxins (PCDD/F) and 3.3 pg BEQ/g fat for the sum of dioxins and dl-PCBs (PCDD/F/dl-PCB). This cut-off for DR CALUX values, action levels, is set on 70% of the limit values set for GC-MS analysis. The analysis with DR CALUX shows that four (4) out six (6) egg locations exceed the limit of 3.3 pg BEQ/g fat for the sum of dioxins (PCDD/D/dl-PCB) and five (5) out of six (6) locations exceed the limit for dioxins (PCDD/F) values of 1.7 pg BEQ/g fat.

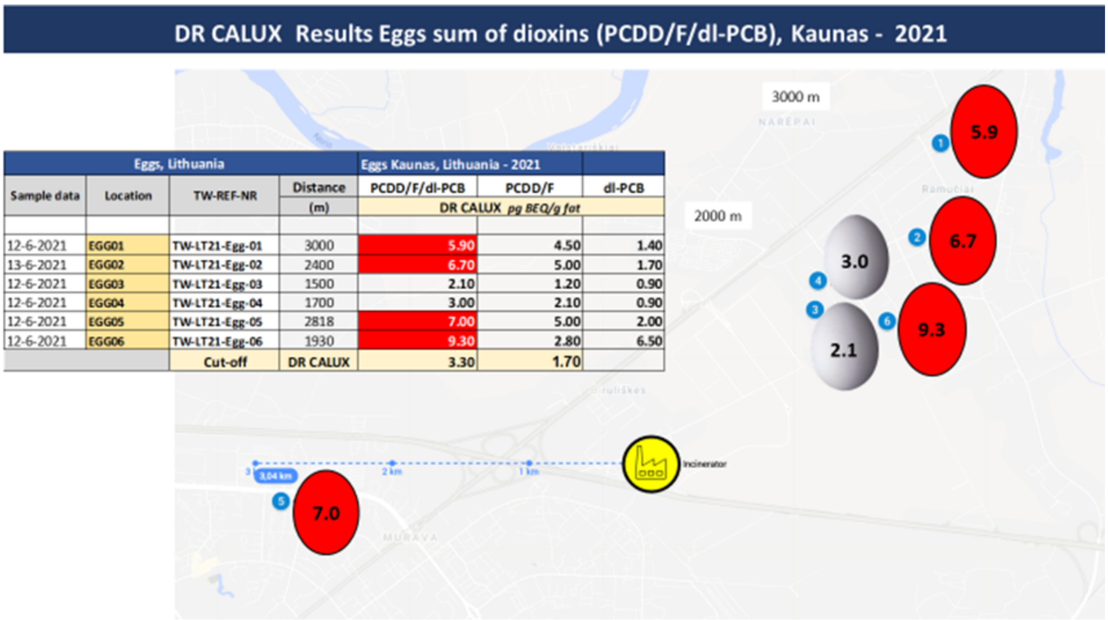
### Table 3: Results in egg locations using DR CALUX analysis – Kaunas 2021



| Results egg locations Kaunas, Lithuania - 2021 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | TW-REF-NR | TW-LT21-Egg-01 | TW-LT21-Egg-02 | TW-LT21-Egg-03 | TW-LT21-Egg-04 | TW-LT21-Egg-05 | TW-LT21-Egg-06 |
| **Cut-off** | **DR CALUX** *pg BEQ /g fat* | | | | | | |
| **1.70** | **PCDD/F** | 4,50 | 5,00 | 1,20 | 2,10 | 5,00 | 2,80 |
| | **dl-PCB** | 1,40 | 1,70 | 0,90 | 0,90 | 2,00 | 6,50 |
| **3.30** | **PCDD/F/dl-PCB** | 5,90 | 6,70 | 2,10 | 3,00 | 7,00 | 9,30 |

Figure 16 shows the results of the sum of dioxins (PCDD/F/dl-PCB) with DR CALUX at the six (6) egg sampling locations shown on the map of the Kaunas region. The egg locations are mainly located in the North-East and one in the West wind direction, between 1,500 and 3,000 metres from the waste incinerator.

### Figure 16: Sum of dioxins (PCDD/F/dl-PCB) in eggs using DR CALUX analysis – Kaunas 2021.

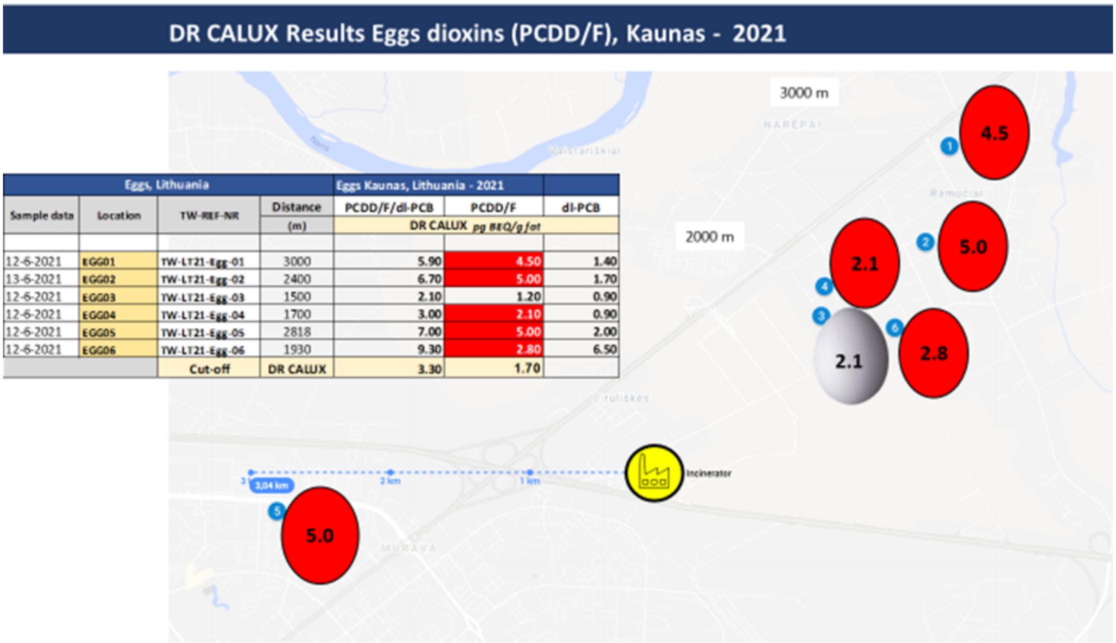


DR CALUX Results Eggs sum of dioxins (PCDD/F/dl-PCB), Kaunas - 2021

| Eggs, Lithuania | | | | Eggs Kaunas, Lithuania - 2021 | | |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance (m) | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | | | | DR CALUX *pg BEQ/g fat* | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 5.90 | 4.50 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 6.70 | 5.00 | 1.70 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 2.10 | 1.20 | 0.90 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 2.10 | 0.90 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 7.00 | 5.00 | 2.00 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 9.30 | 2.80 | 6.50 |
| | | Cut-off | DR CALUX | 3.30 | 1.70 | |

The five (5) egg locations in Figure 16 exceed the limit of dioxins (PCDD/F) of 1.7 pg BEQ/g fat and with that considered to be suspicious by the DR CALUX analysis (Figure 17).

### Figure 17: Dioxins (PCDD/F) in eggs using DR CALUX analysis – Kaunas 2021

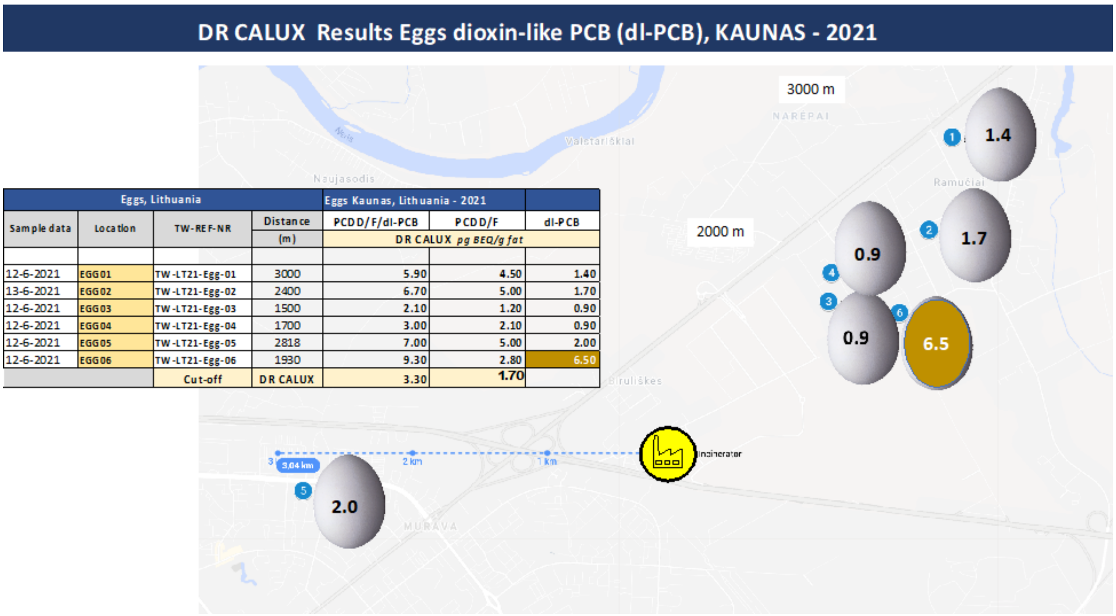


DR CALUX Results Eggs dioxins (PCDD/F), Kaunas - 2021

| Eggs, Lithuania | | | | Eggs Kaunas, Lithuania - 2021 | | |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | | | (m) | DR CALUX *pg BEQ/g fat* | | |
| | | | | | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 5.90 | 4.50 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 6.70 | 5.00 | 1.70 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 2.10 | 1.20 | 0.90 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 2.10 | 0.90 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 7.00 | 5.00 | 2.00 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 9.30 | 2.80 | 6.50 |
| | | Cut-off | DR CALUX | 3.30 | 1.70 | |

The content of dioxin-like PCB (dl-PCB) at egg location 6 has a high value of 6.50 pg BEQ/g fat, as presented in Figure 18.

### Figure 18: Dioxin-like PCBs (dl-PCBs) in eggs by DR CALUX analysis – Kaunas 2021



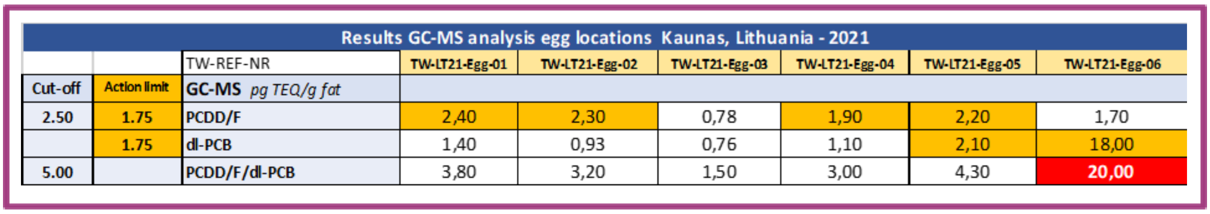
DR CALUX Results Eggs dioxin-like PCB (dl-PCB), KAUNAS - 2021

| Eggs, Lithuania | | | | Eggs Kaunas, Lithuania - 2021 | | |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | | | (m) | DR CALUX *pg BEQ/g fat* | | |
| | | | | | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 5.90 | 4.50 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 6.70 | 5.00 | 1.70 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 2.10 | 1.20 | 0.90 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 2.10 | 0.90 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 7.00 | 5.00 | 2.00 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 9.30 | 2.80 | 6.50 |
| | | Cut-off | DR CALUX | 3.30 | 1.70 | |

## Results GC-MS analysis on eggs of backyard chicken

GC-MS results for the sum of dioxins (PCDD/F/dl-PCB) assess five (5) out of six (6) egg locations to comply with the EU regulations. However, location 6 (sample TW-LT21-EGG-06) exceeds the maximal safety level of 5 pg TEQ/gram fat with a factor of four (4). Egg location number 6 needs to be further investigated if more clarity is required about the cause of the high dioxin-like PCB content of the contamination. More research can give a better understanding of how to reduce and/or eliminate these high dioxin-like PCB values. Based on these analysis results, consumption of eggs at location 6 should be strongly discouraged. Also, egg location 5, (TW-LT21-EGG-05) exceeds the action limit of 1.75 pg TEQ/g for dioxin-like PCBs (dl-PCBs) and dioxins (PCDD/F) – see also Figure 5. At three (3) other egg locations (1,2,4), the dioxin (PCDD/F) activity values have been exceeded. It is advised to identify the source(s) of these dioxin elevations, highly toxic substances, before continuing consuming the eggs at locations 1, 2, 4 and 5. If these are commercial eggs, these results mean that action must be taken at sites 1, 2, 4, 5 and 6 to eliminate or reduce the contamination. For egg location 6 this means that these eggs should be withdrawn from the market immediately.

**Table 4: Results in egg locations using GC-MS analysis – Kaunas 2021**

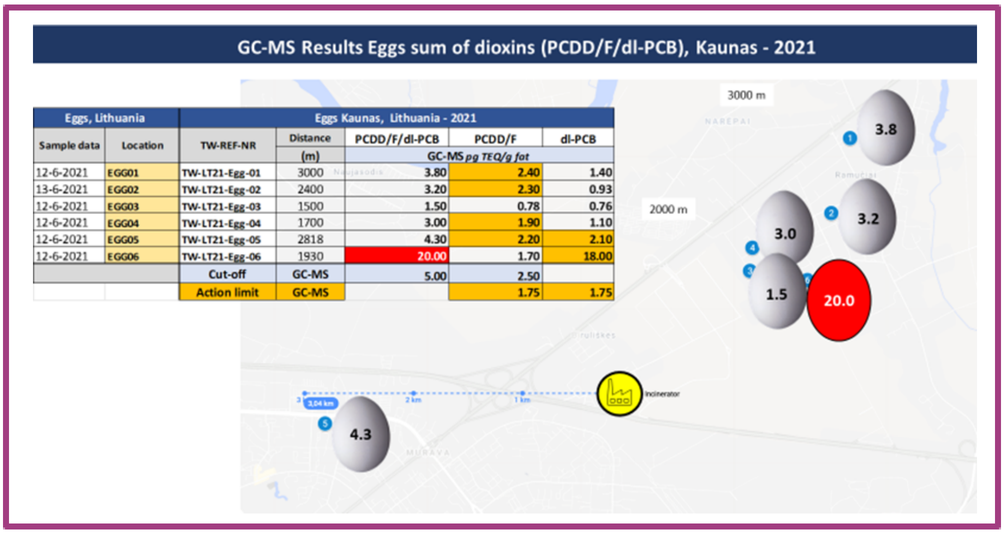


Results GC-MS analysis egg locations Kaunas, Lithuania - 2021

|  |  | TW-REF-NR | TW-LT21-Egg-01 | TW-LT21-Egg-02 | TW-LT21-Egg-03 | TW-LT21-Egg-04 | TW-LT21-Egg-05 | TW-LT21-Egg-06 |
|---|---|---|---|---|---|---|---|---|
| Cut-off | Action limit | **GC-MS** *pg TEQ/g fat* |  |  |  |  |  |  |
| 2.50 | 1.75 | PCDD/F | 2,40 | 2,30 | 0,78 | 1,90 | 2,20 | 1,70 |
|  | 1.75 | dl-PCB | 1,40 | 0,93 | 0,76 | 1,10 | 2,10 | 18,00 |
| 5.00 |  | PCDD/F/dl-PCB | 3,80 | 3,20 | 1,50 | 3,00 | 4,30 | 20,00 |

Figure 19 shows a map of the Kaunas region with the GC-MS analysis results of the sum of dioxins (PCDD/F/dl-PCB) in the eggs per sampling location and shows the distance from the waste incinerator.

**Figure 19: Sum of dioxins (PCDD/F/dl-PCB) in eggs using GC-MS analysis – Kaunas 2021**

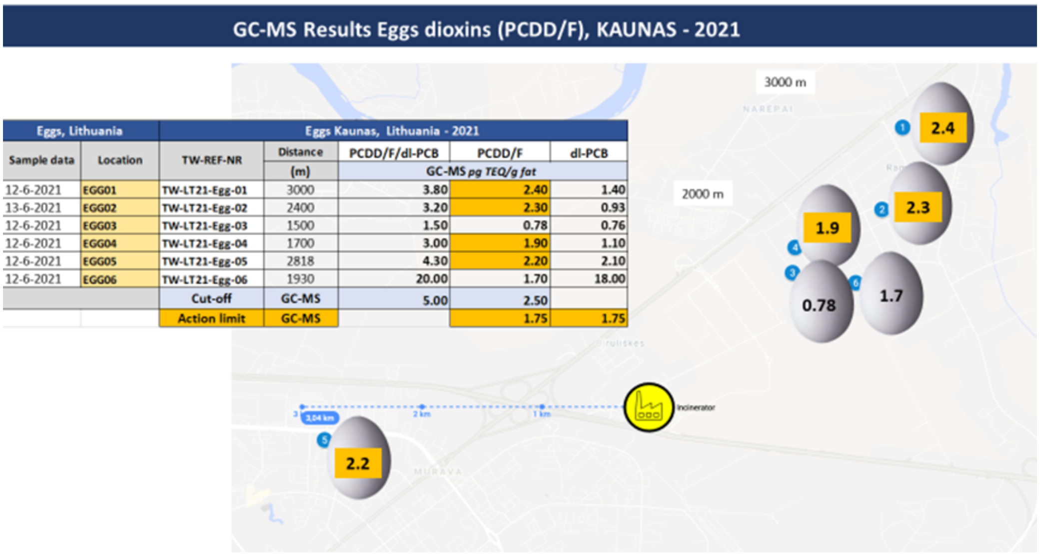


GC-MS Results Eggs sum of dioxins (PCDD/F/dl-PCB), Kaunas - 2021

| Eggs, Lithuania |  | Eggs Kaunas, Lithuania - 2021 |  |  |  |  |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance (m) | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
|  |  |  |  | GC-MS *pg TEQ/g fat* |  |  |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 3.80 | 2.40 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 3.20 | 2.30 | 0.93 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 1.50 | 0.78 | 0.76 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 1.90 | 1.10 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 4.30 | 2.20 | 2.10 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 20.00 | 1.70 | 18.00 |
|  |  | Cut-off | GC-MS | 5.00 | 2.50 |  |
|  |  | Action limit | GC-MS |  | 1.75 | 1.75 |

The GC-MS results show that four (4) out of six (6) egg locations exceed the EU action limit of 1.75 pg TEQ/g fat (Figure 20). In the commercial egg market, responsible authorities are strongly advised to take action to eliminate or reduce the source of dioxin contamination. In this case, certain chicken coop owners of backyard chicken have a production/consumption of more than 5,000 eggs a year. This means there is a serious threat to human health if all these contaminated eggs are consumed.

**Figure 20: Exceeding EU action limit for dioxins (PCDD/F) in eggs by GC-MS analysis – Kaunas 2021**

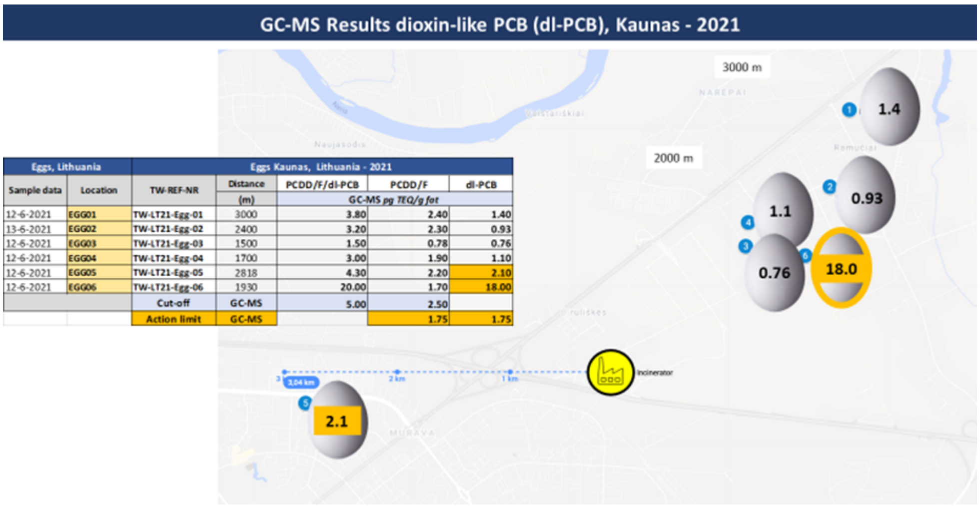


GC-MS Results Eggs dioxins (PCDD/F), KAUNAS - 2021

| Eggs, Lithuania | | Eggs Kaunas, Lithuania - 2021 | | | | |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance (m) | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | | | | GC-MS *pg TEQ/g fat* | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 3.80 | 2.40 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 3.20 | 2.30 | 0.93 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 1.50 | 0.78 | 0.76 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 1.90 | 1.10 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 4.30 | 2.20 | 2.10 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 20.00 | 1.70 | 18.00 |
| | | Cut-off | GC-MS | 5.00 | 2.50 | |
| | | Action limit | GC-MS | | 1.75 | 1.75 |

At egg location 6, the GC-MS analysis shows high results of heavy contamination with dioxin-like PCB (dl-PCBs). This value even exceeds 4.5 times the norm for safe food intake. For health reasons, it is necessary to take action to find the cause of this high dl-PCB contamination (see Figure 21).

**Figure 21: Exceeding dioxin-like PCBs (dl-PCBs) on egg location 6, by GC-MS analysis – Kaunas 2021**



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

| Eggs, Lithuania | | Eggs Kaunas, Lithuania - 2021 | | | | |
|---|---|---|---|---|---|---|
| Sample data | Location | TW-REF-NR | Distance (m) | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | | | | GC-MS *pg TEQ/g fat* | | |
| 12-6-2021 | EGG01 | TW-LT21-Egg-01 | 3000 | 3.80 | 2.40 | 1.40 |
| 13-6-2021 | EGG02 | TW-LT21-Egg-02 | 2400 | 3.20 | 2.30 | 0.93 |
| 12-6-2021 | EGG03 | TW-LT21-Egg-03 | 1500 | 1.50 | 0.78 | 0.76 |
| 12-6-2021 | EGG04 | TW-LT21-Egg-04 | 1700 | 3.00 | 1.90 | 1.10 |
| 12-6-2021 | EGG05 | TW-LT21-Egg-05 | 2818 | 4.30 | 2.20 | 2.10 |
| 12-6-2021 | EGG06 | TW-LT21-Egg-06 | 1930 | 20.00 | 1.70 | 18.00 |
| | | Cut-off | GC-MS | 5.00 | 2.50 | |
| | | Action limit | GC-MS | | 1.75 | 1.75 |

The Estonian Environmental Research Centre (EERC) performed air analyses in May and September 2019 at a sampling point marked in Figure 22, very near the egg sample locations two and six. With a Digitel DHA-80 sampler, 737.76 $m^3$ and 729.94 $m^3$ of air were sampled in 24 hours for measurement of various substances including PAH and dioxins.[29] The dioxin results were below the Limit of Detection (LOD) as well are the results of measurements on polycyclic aromatic hydrocarbons (PAH) in September 2020. In May 2020 0.08 ng B[a]P was measured per $m^3$, far below the EU Directive 2004/107/EC of 1 ng B[a]P/$m^3$. The location of this EERC measuring point is near the three (3) egg locations (Egg 2, 4 and 6) in this biomonitoring project with elevated levels found of dioxins and dioxin-like PCBs in the sampled eggs. The EERC measurements were taken in the pre-testing phase of the incinerator.

The WtE incinerator in Kaunas began full-scale operations on 27 November 2020. Earlier air measurements by the EERC, 24 hours with active air sampling, did not measure dioxins or PAHs above the limit of detection. However, very near this measuring point, in June 2021, heavily contaminated eggs were found with PCDD/F/dl-PCB, and with a dramatic result of four (4) times exceeding the level for safe food. The results of the EERC can be interpreted that the air in this sample region was relatively ‘clean’ in May and September 2019, although this method is limited in sampling time. Perhaps the dioxins were produced after the EERC measurement session had ended, but any conclusions in this direction require more research.

**Figure 22: Sampling point of the EERC and TW egg sampling locations – Kaunas 2021**

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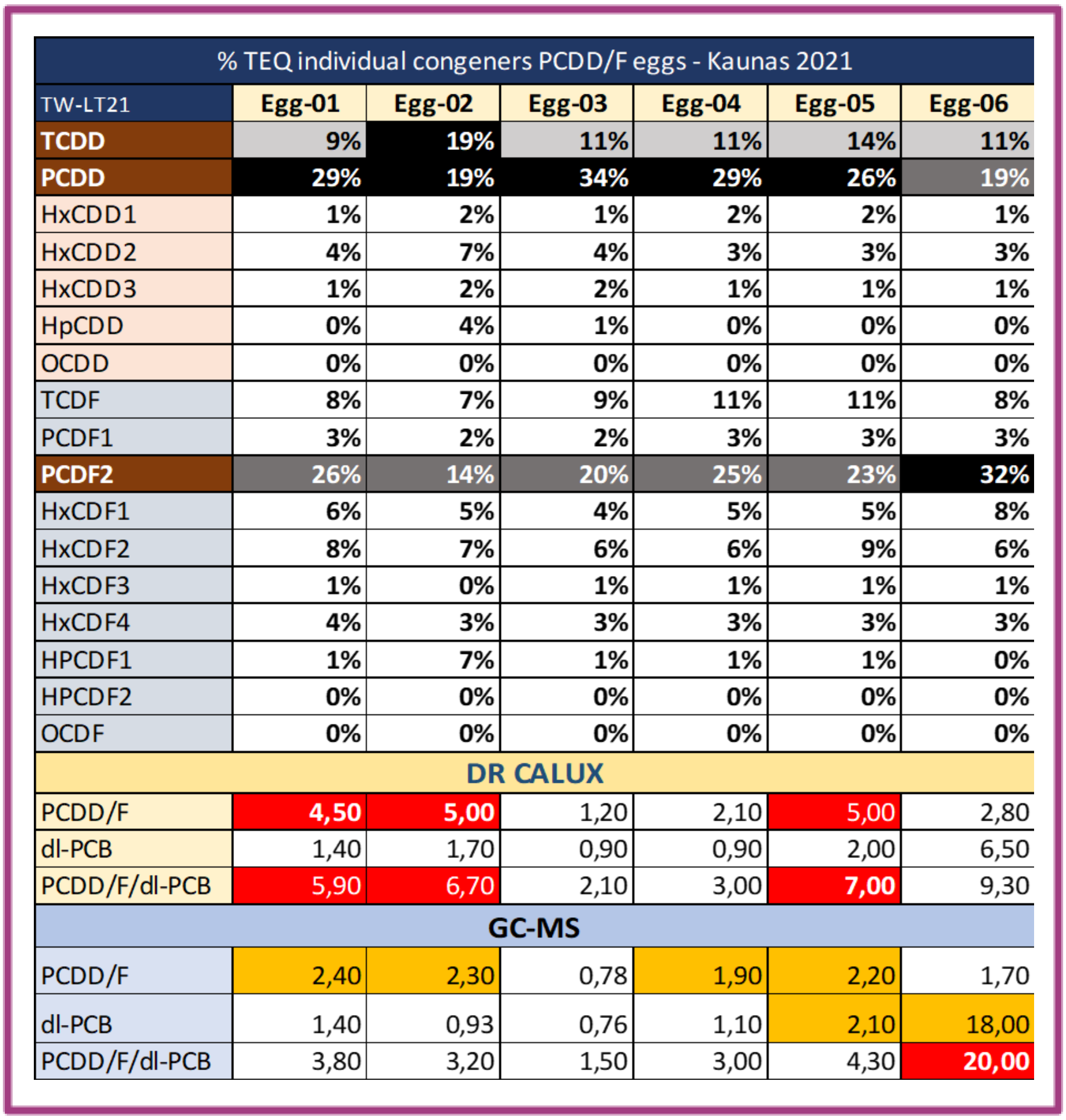
[29] *Aser Sikk, Keio Vainumäe 2020. Ambient Air Quality Monitoring in Ramučiai Township, Kaunas., Eesti Keskkonnauuringute Keskus OÜ / Estonian Environmental Research Centre*

## Congeners

In Table 5 the 17 dioxin and furan congeners (PCDD/F) are shown as a percentage of the total TEQ PCDD/F concentrations at each egg location. The dominant congeners (brown) are TCDD, PCDD and PCDF2. The highest value is shown 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 published, see Annex II. Table 5 shows the toxic load of PCDD/F congeners. Remarkable is the other pattern of location 6 with the dominant PCDF2 and at location 2 the dominant position of 2,3,7,8-Tetrachlorodibenzo-p-dioxin is a reason for further research. Except for locations 2 and 6, the other congener patterns show an unambiguous picture, indicating an unambiguous source or sources of dioxin contamination.

**Table 5: Fraction of total TEQ (%) on dioxins (PCDD/F) in eggs – Kaunas 2021**

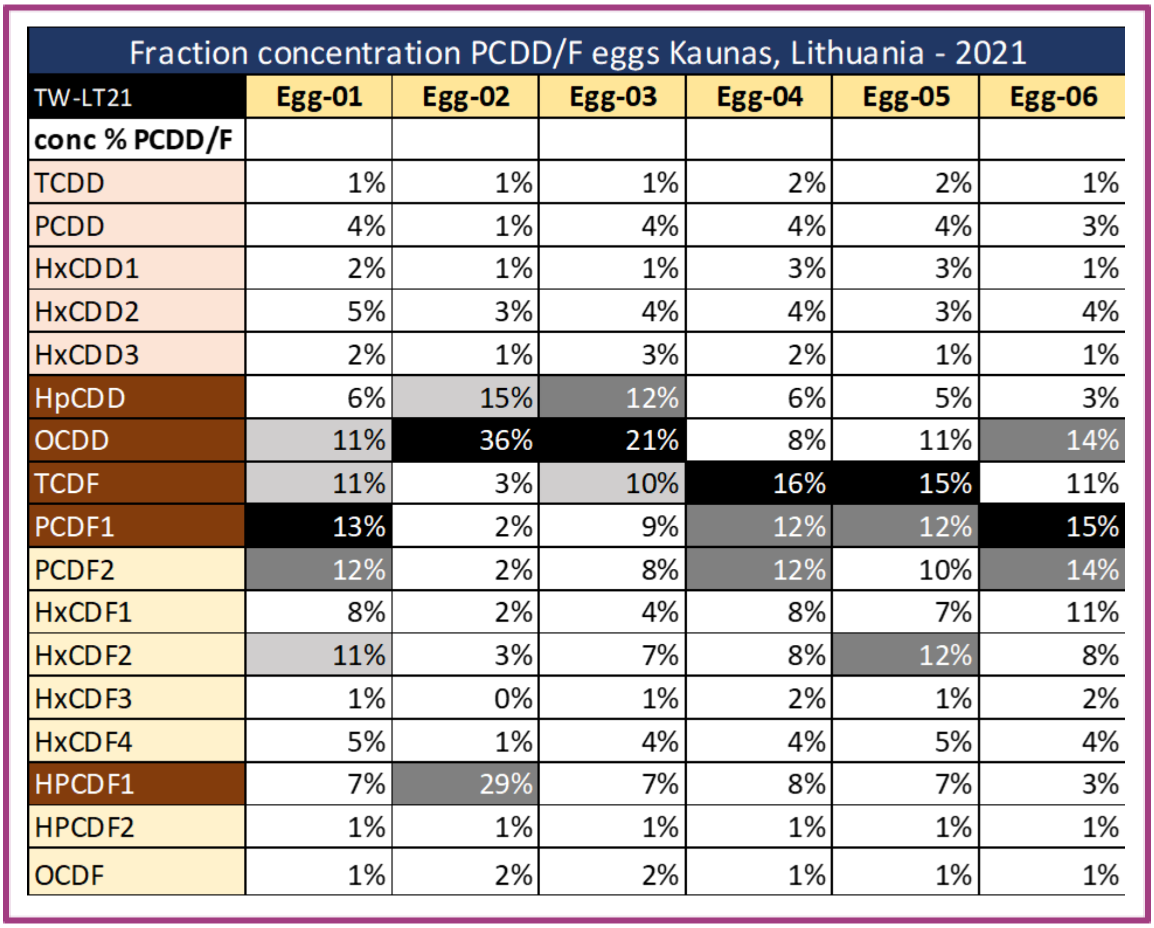


% TEQ individual congeners PCDD/F eggs - Kaunas 2021

| TW-LT21 | Egg-01 | Egg-02 | Egg-03 | Egg-04 | Egg-05 | Egg-06 |
|---|---|---|---|---|---|---|
| **TCDD** | 9% | 19% | 11% | 11% | 14% | 11% |
| **PCDD** | 29% | 19% | 34% | 29% | 26% | 19% |
| HxCDD1 | 1% | 2% | 1% | 2% | 2% | 1% |
| HxCDD2 | 4% | 7% | 4% | 3% | 3% | 3% |
| HxCDD3 | 1% | 2% | 2% | 1% | 1% | 1% |
| HpCDD | 0% | 4% | 1% | 0% | 0% | 0% |
| OCDD | 0% | 0% | 0% | 0% | 0% | 0% |
| TCDF | 8% | 7% | 9% | 11% | 11% | 8% |
| PCDF1 | 3% | 2% | 2% | 3% | 3% | 3% |
| **PCDF2** | 26% | 14% | 20% | 25% | 23% | 32% |
| HxCDF1 | 6% | 5% | 4% | 5% | 5% | 8% |
| HxCDF2 | 8% | 7% | 6% | 6% | 9% | 6% |
| HxCDF3 | 1% | 0% | 1% | 1% | 1% | 1% |
| HxCDF4 | 4% | 3% | 3% | 3% | 3% | 3% |
| HPCDF1 | 1% | 7% | 1% | 1% | 1% | 0% |
| HPCDF2 | 0% | 0% | 0% | 0% | 0% | 0% |
| OCDF | 0% | 0% | 0% | 0% | 0% | 0% |
| **DR CALUX** | | | | | | |
| PCDD/F | **4,50** | **5,00** | 1,20 | 2,10 | 5,00 | 2,80 |
| dl-PCB | 1,40 | 1,70 | 0,90 | 0,90 | 2,00 | 6,50 |
| PCDD/F/dl-PCB | 5,90 | 6,70 | 2,10 | 3,00 | **7,00** | 9,30 |
| **GC-MS** | | | | | | |
| PCDD/F | 2,40 | 2,30 | 0,78 | 1,90 | 2,20 | 1,70 |
| dl-PCB | 1,40 | 0,93 | 0,76 | 1,10 | 2,10 | 18,00 |
| PCDD/F/dl-PCB | 3,80 | 3,20 | 1,50 | 3,00 | 4,30 | **20,00** |

Zooming in on the individual egg locations with a measured problem of dioxins with the DR CALUX analysis method. Dioxins, PCDD/F are known products of incomplete combustion, incineration and could therefore be an indication of emissions from the waste incinerator. Typical congeners Octachlorodibenzo-p-dioxin (OCDD) and 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD) mostly in the concentration ratio of 2 to 1, see table 6. Because of the absence of detailed information on the emission patterns incinerator of Kaunas, 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).[30] In the study of Chen,[31] the 1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF1) congener is an indicator of incineration. Perhaps a different configuration of the incinerator or a different waste input is to be held responsible for this particular emission, different from the patterns of the REC in the Netherlands. Also in Kaunas at location two (2) the furan congener HpCDF1 is dominantly present.

**Table 6: Fraction of total (%) concentration dioxins (PCDD/F) in egg– Kaunas 2021**



Fraction concentration PCDD/F eggs Kaunas, Lithuania - 2021

| TW-LT21 | Egg-01 | Egg-02 | Egg-03 | Egg-04 | Egg-05 | Egg-06 |
|---|---|---|---|---|---|---|
| conc % PCDD/F | | | | | | |
| TCDD | 1% | 1% | 1% | 2% | 2% | 1% |
| PCDD | 4% | 1% | 4% | 4% | 4% | 3% |
| HxCDD1 | 2% | 1% | 1% | 3% | 3% | 1% |
| HxCDD2 | 5% | 3% | 4% | 4% | 3% | 4% |
| HxCDD3 | 2% | 1% | 3% | 2% | 1% | 1% |
| HpCDD | 6% | 15% | 12% | 6% | 5% | 3% |
| OCDD | 11% | 36% | 21% | 8% | 11% | 14% |
| TCDF | 11% | 3% | 10% | 16% | 15% | 11% |
| PCDF1 | 13% | 2% | 9% | 12% | 12% | 15% |
| PCDF2 | 12% | 2% | 8% | 12% | 10% | 14% |
| HxCDF1 | 8% | 2% | 4% | 8% | 7% | 11% |
| HxCDF2 | 11% | 3% | 7% | 8% | 12% | 8% |
| HxCDF3 | 1% | 0% | 1% | 2% | 1% | 2% |
| HxCDF4 | 5% | 1% | 4% | 4% | 5% | 4% |
| HPCDF1 | 7% | 29% | 7% | 8% | 7% | 3% |
| HPCDF2 | 1% | 1% | 1% | 1% | 1% | 1% |
| OCDF | 1% | 2% | 2% | 1% | 1% | 1% |

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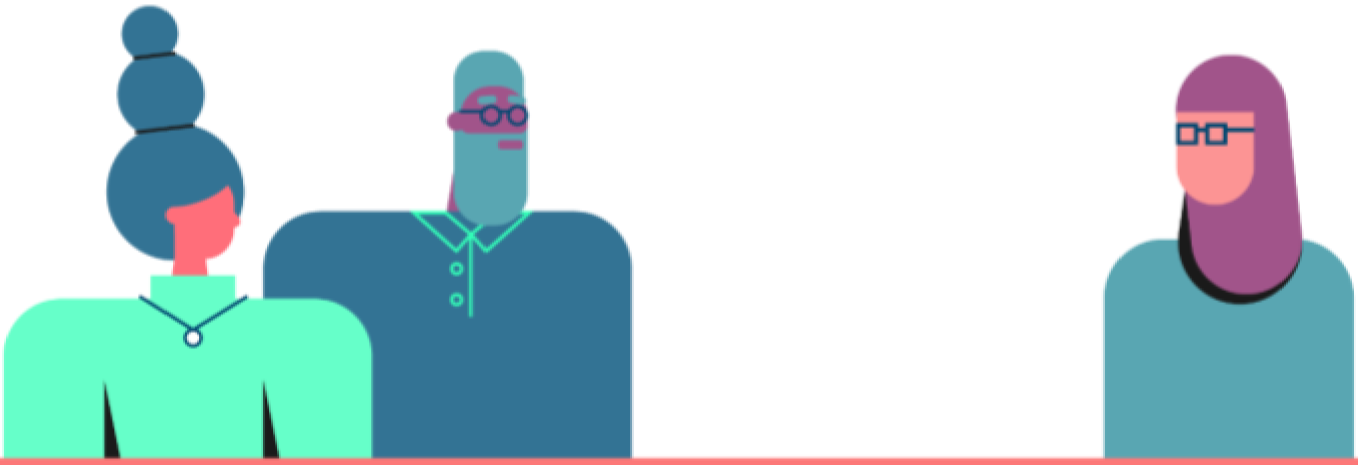
[30] *Arkenbout A, Esbensen K H, Sampling, monitoring and source tracking of Dioxins in the environment of an incinerator in the Netherlands, Proceedings Eighth World Conference On Sampling And Blending / Perth, May 2017, 117 – 124*
[31] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*



# Egg locations

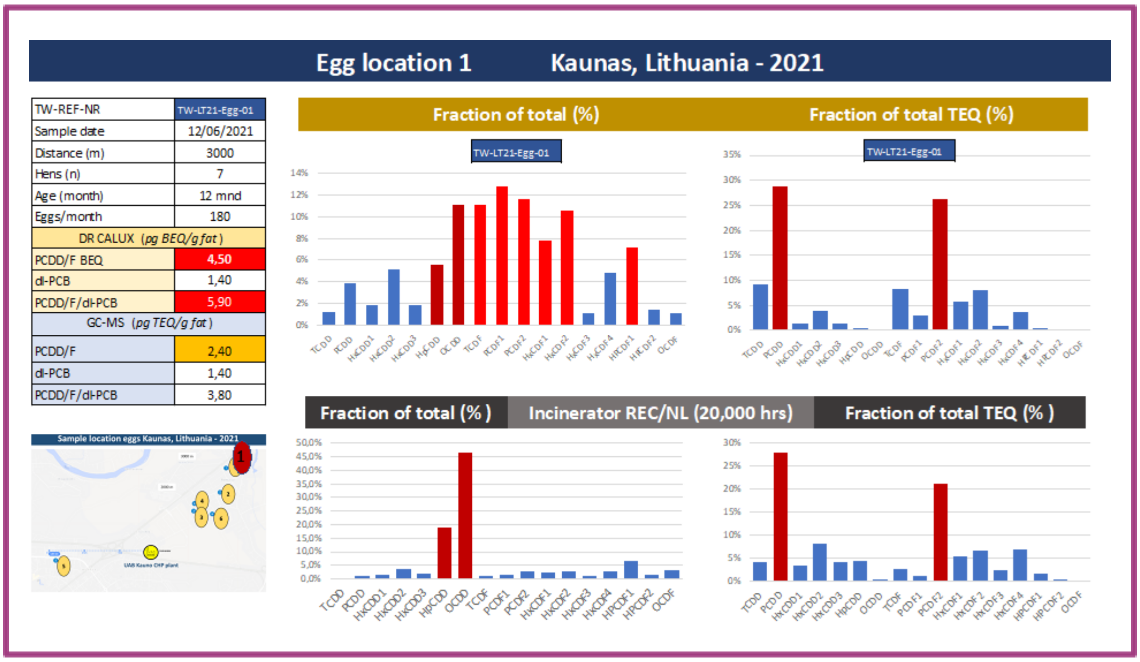
## in the Kaunas regions

## Egg location 1

Egg location one (1) exceeds the values of dioxins (PCDD/F) and the sum of dioxins (PCDD/F/dl-PCB) with the DR CALUX analysis as shown in Figure 23. The GC-MS analysis shows lower values for PCDD/F but exceeds the action limit for PCDD/F. This means additional research is recommended to determine the source of the dioxin contamination in the eggs. 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 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 ($PCDF_2$) in the TEQ profiles.

The higher dioxin (PCDD/F) values, measured with the DR CALUX, can be explained by the presence of brominated dioxins, which are not measured with GC-MS analyses, as explained on page 10. The upper left graph of Figure 22 shows a high contribution of furans (PCDF) to the concentration. The ratio PCDF/PCDD is 2.3 and can be indicative of newly formed emissions from waste incineration.[32]

**Figure 23: Overview of data, egg location 1 Kaunas – 2021, see Annex II Eggs**



Egg location 1 Kaunas, Lithuania - 2021

| | |
|---|---|
| TW-REF-NR | TW-LT21-Egg-01 |
| Sample date | 12/06/2021 |
| Distance (m) | 3000 |
| Hens (n) | 7 |
| Age (month) | 12 mnd |
| Eggs/month | 180 |
| DR CALUX (*pg BEQ/g fat*) | |
| PCDD/F BEQ | 4,50 |
| dl-PCB | 1,40 |
| PCDD/F/dl-PCB | 5,90 |
| GC-MS (*pg TEQ/g fat*) | |
| PCDD/F | 2,40 |
| dl-PCB | 1,40 |
| PCDD/F/dl-PCB | 3,80 |

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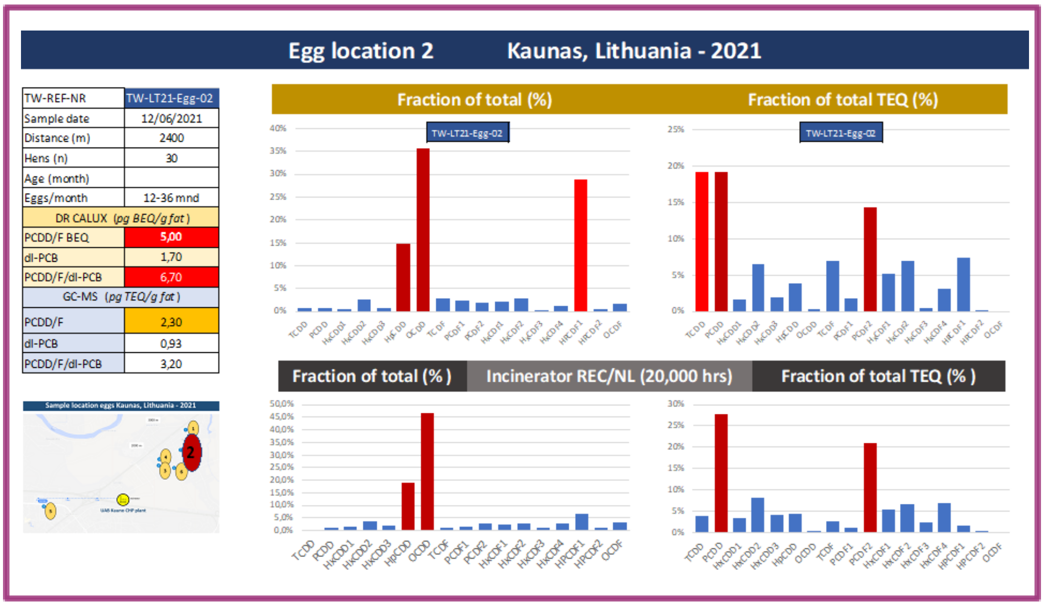
[32] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*

# Egg location 2

The presence of 1,2,3,4,6,7,8-Heptachlorodibenzofuran in combination with the high values of 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD) and Octachlorodibenzo-p-dioxin (OCDD) is a pattern for waste incineration, Figure 24.

**Figure 24: Overview of data, egg location 2 Kaunas – 2021, see Annex II Eggs**



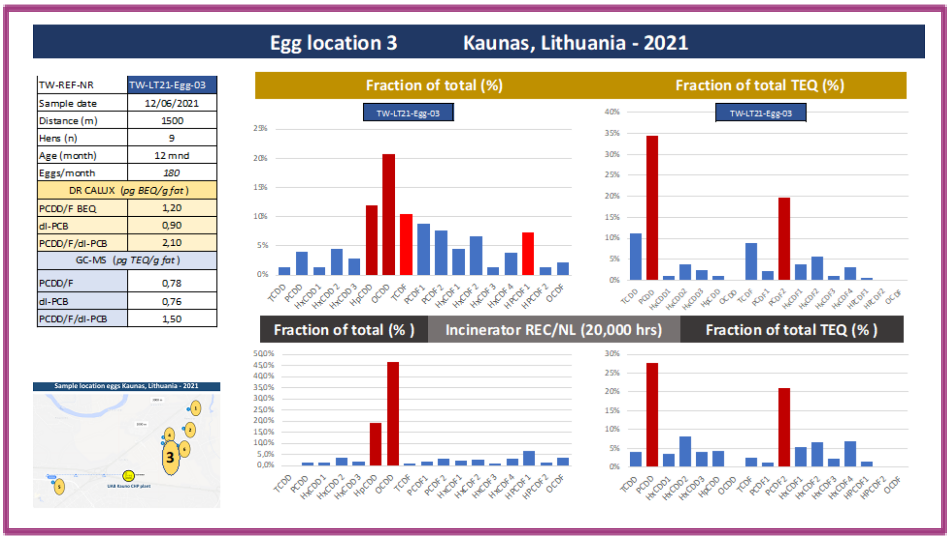
Egg location 2 Kaunas, Lithuania - 2021

| TW-REF-NR | TW-LT21-Egg-02 |
|---|---|
| Sample date | 12/06/2021 |
| Distance (m) | 2400 |
| Hens (n) | 30 |
| Age (month) | |
| Eggs/month | 12-36 mnd |
| DR CALUX (pg BEQ/g fat) | |
| PCDD/F BEQ | 5,00 |
| dl-PCB | 1,70 |
| PCDD/F/dl-PCB | 6,70 |
| GC-MS (pg TEQ/g fat) | |
| PCDD/F | 2,30 |
| dl-PCB | 0,93 |
| PCDD/F/dl-PCB | 3,20 |

# Egg location 3

Location 3 is the nearest egg sample point towards the waste incinerator. The results of the analysis of eggs at this location complies with the regulations of DR CALUX and the GC-MS. The pattern of HpCDD and OCDD can be observed in the concentration patterns here as well (Figure 25). The TEQ profile resembles the TEQ pattern of the waste incinerator in the Netherlands.

**Figure 25: Results of dioxin analysis of egg location 3, Kaunas – 2021**



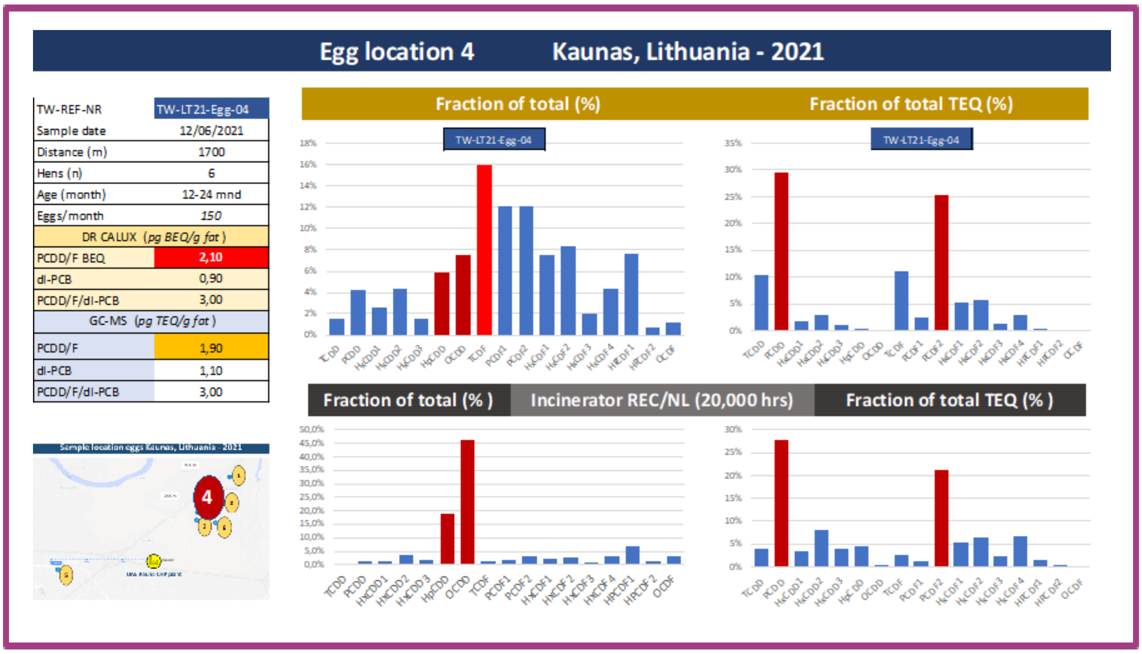
Egg location 3 Kaunas, Lithuania - 2021

| TW-REF-NR | TW-LT21-Egg-03 |
|---|---|
| Sample date | 12/06/2021 |
| Distance (m) | 1500 |
| Hens (n) | 9 |
| Age (month) | 12 mnd |
| Eggs/month | 180 |
| DR CALUX (pg BEQ/g fat) | |
| PCDD/F BEQ | 1,20 |
| dl-PCB | 0,90 |
| PCDD/F/dl-PCB | 2,10 |
| GC-MS (pg TEQ/g fat) | |
| PCDD/F | 0,78 |
| dl-PCB | 0,76 |
| PCDD/F/dl-PCB | 1,50 |

# Egg location 4

Location 4 is characterized by the dominant contribution of 2,3,7,8-Tetrachlorodibenzofuran (TCDF). It is not clear exactly what is causing the increase in this congener. The fraction of furans is high. The ratio PCDF/PCDD is 2.6; for Chen,[33] this is an indication of emissions from the incinerator. With the measured value of 1.9, this location exceeds the EU action limit for dioxins (PCDD/F) and action should be taken to determine the source - Figure 26.

**Figure 26: Results of dioxin analysis of egg location 4, Kaunas – 2021**


Egg location 4 Kaunas, Lithuania - 2021

| | |
|---|---|
| TW-REF-NR | TW-LT21-Egg-04 |
| Sample date | 12/06/2021 |
| Distance (m) | 1700 |
| Hens (n) | 6 |
| Age (month) | 12-24 mnd |
| Eggs/month | *150* |
| DR CALUX (*pg BEQ/g fat*) | |
| PCDD/F BEQ | 2,10 |
| dl-PCB | 0,90 |
| PCDD/F/dl-PCB | 3,00 |
| GC-MS (*pg TEQ/g fat*) | |
| PCDD/F | 1,90 |
| dl-PCB | 1,10 |
| PCDD/F/dl-PCB | 3,00 |

# Egg location 5

Also at egg location five (5) 2,3,7,8-Tetrachlorodibenzofuran (TCDF) is the most prominent congener. Like location one (1,) four (4) and six (6) is also here the fraction of furans high. The ratio PCDF/PCDD is 2.5 and can be an indication of emissions from incineration. With the measured value of 2.2 and 2.1 for dl-PCB, the EU action limits are exceeded and action should be taken to determine the source - Figure 27.

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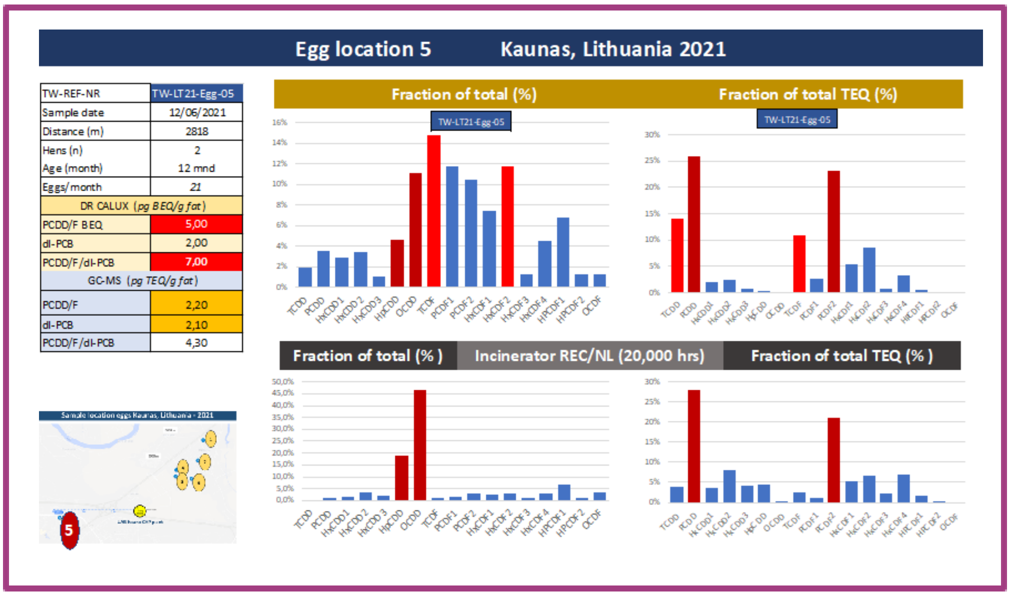
[33] *Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367*

**Figure 27: Results of dioxin analysis of egg location 5, Kaunas – 2021**



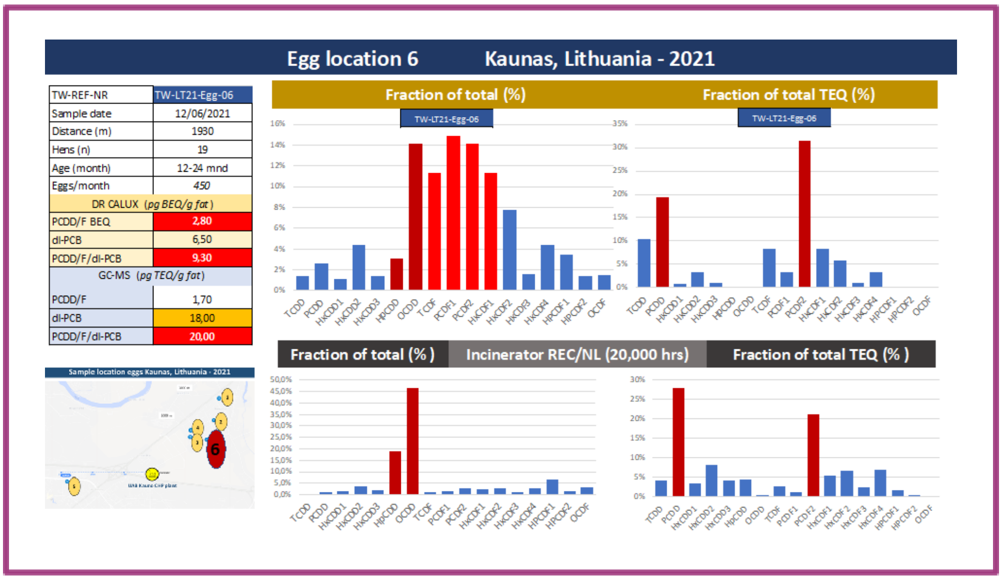
Egg location 5 Kaunas, Lithuania 2021

| | |
|---|---|
| TW-REF-NR | TW-LT21-Egg-05 |
| Sample date | 12/06/2021 |
| Distance (m) | 2818 |
| Hens (n) | 2 |
| Age (month) | 12 mnd |
| Eggs/month | *21* |
| DR CALUX (*pg BEQ/g fat*) | |
| PCDD/F BEQ | 5,00 |
| dl-PCB | 2,00 |
| PCDD/F/dl-PCB | 7,00 |
| GC-MS (*pg TEQ/g fat*) | |
| PCDD/F | 2,20 |
| dl-PCB | 2,10 |
| PCDD/F/dl-PCB | 4,30 |

## Egg location 6

Egg location number 6 does NOT comply with the EU limit of dioxins and dl-PCBs as shown in Figure 28. Consumption of the eggs should be strictly discouraged until the cause of the contamination has been identified. The reason for this high dioxin value is the large presence of 18 pg TEQ/g of dioxin-like PCBs at this location, see next chapter for further explanation. The ratio PCDF/PCDD is 2.6 and can be an indication of emissions from incineration. With the measured value of 18 pg TEQ/g fat for dl-PCB, the EU action limits are exceeded by a factor of 10 and action should be taken to eliminate or reduce the toxic substances at this location. Consumption of eggs should be discouraged.

**Figure 28: Results of dioxin analysis of egg location 6, Kaunas – 2021**



Egg location 6 Kaunas, Lithuania - 2021

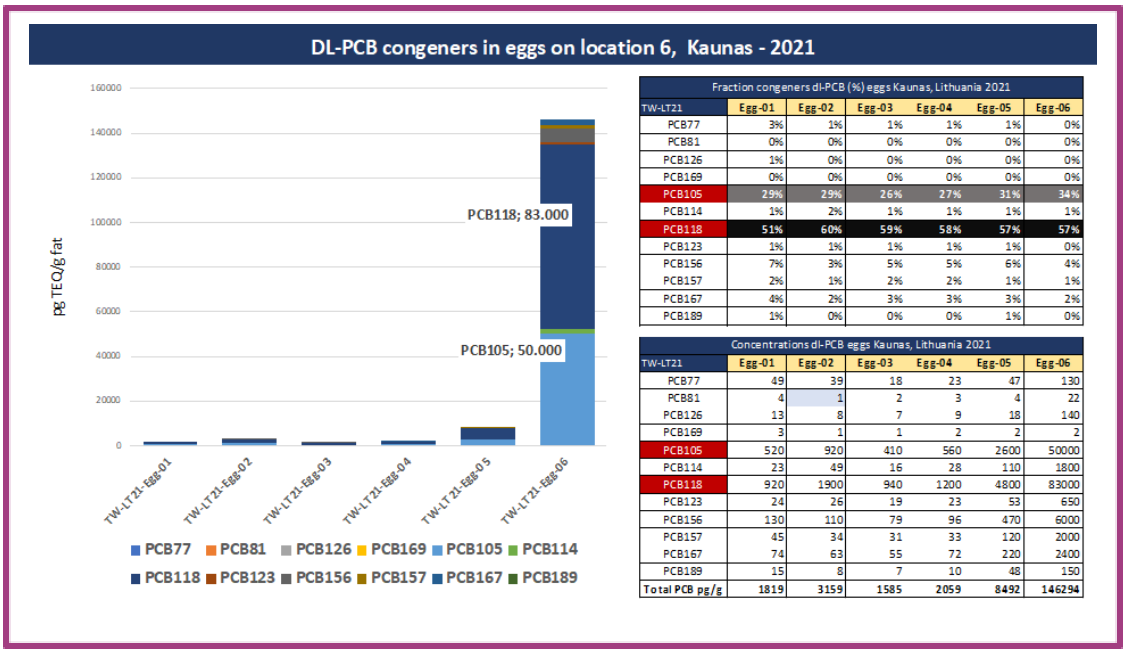
| | |
|---|---|
| TW-REF-NR | TW-LT21-Egg-06 |
| Sample date | 12/06/2021 |
| Distance (m) | 1930 |
| Hens (n) | 19 |
| Age (month) | 12-24 mnd |
| Eggs/month | *450* |
| DR CALUX (*pg BEQ/g fat*) | |
| PCDD/F BEQ | 2,80 |
| dl-PCB | 6,50 |
| PCDD/F/dl-PCB | 9,30 |
| GC-MS (*pg TEQ/g fat*) | |
| PCDD/F | 1,70 |
| dl-PCB | 18,00 |
| PCDD/F/dl-PCB | 20,00 |

# 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 (e.g., release from transformers or capacitors still in use, building materials, stored waste, or contaminated soils) or as unintentional by-products of combustion processes (e.g., waste incineration).

Figure 29 shows the DL-PCB distribution at the egg locations. Location 6 is a strongly elevated level. The graph in this figure shows high levels of PCB 105 and PCB 118 at this location, but resemble a fraction (%) at the other egg locations. A factor of 100 more dioxin-like PCBs are found in the eggs at location 6 compared to egg location three (3), only 430 metres away.

**Figure 29: Dl-PCB distribution on the 6 egg location (pg/g fat)– Kaunas 2021**



Fraction congeners dl-PCB (%) eggs Kaunas, Lithuania 2021

| TW-LT21 | Egg-01 | Egg-02 | Egg-03 | Egg-04 | Egg-05 | Egg-06 |
|---|---|---|---|---|---|---|
| PCB77 | 3% | 1% | 1% | 1% | 1% | 0% |
| PCB81 | 0% | 0% | 0% | 0% | 0% | 0% |
| PCB126 | 1% | 0% | 0% | 0% | 0% | 0% |
| PCB169 | 0% | 0% | 0% | 0% | 0% | 0% |
| PCB105 | 29% | 29% | 26% | 27% | 31% | 34% |
| PCB114 | 1% | 2% | 1% | 1% | 1% | 1% |
| PCB118 | 51% | 60% | 59% | 58% | 57% | 57% |
| PCB123 | 1% | 1% | 1% | 1% | 1% | 0% |
| PCB156 | 7% | 3% | 5% | 5% | 6% | 4% |
| PCB157 | 2% | 1% | 2% | 2% | 1% | 1% |
| PCB167 | 4% | 2% | 3% | 3% | 3% | 2% |
| PCB189 | 1% | 0% | 0% | 0% | 1% | 0% |

Concentrations dl-PCB eggs Kaunas, Lithuania 2021

| TW-LT21 | Egg-01 | Egg-02 | Egg-03 | Egg-04 | Egg-05 | Egg-06 |
|---|---|---|---|---|---|---|
| PCB77 | 49 | 39 | 18 | 23 | 47 | 130 |
| PCB81 | 4 | 1 | 2 | 3 | 4 | 22 |
| PCB126 | 13 | 8 | 7 | 9 | 18 | 140 |
| PCB169 | 3 | 1 | 1 | 2 | 2 | 2 |
| PCB105 | 520 | 920 | 410 | 560 | 2600 | 50000 |
| PCB114 | 23 | 49 | 16 | 28 | 110 | 1800 |
| PCB118 | 920 | 1900 | 940 | 1200 | 4800 | 83000 |
| PCB123 | 24 | 26 | 19 | 23 | 53 | 650 |
| PCB156 | 130 | 110 | 79 | 96 | 470 | 6000 |
| PCB157 | 45 | 34 | 31 | 33 | 120 | 2000 |
| PCB167 | 74 | 63 | 55 | 72 | 220 | 2400 |
| PCB189 | 15 | 8 | 7 | 10 | 48 | 150 |
| **Total PCB pg/g** | **1819** | **3159** | **1585** | **2059** | **8492** | **146294** |

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 continued measurements of the emissions of the incinerator, 10% of the TEQ was found to be related to dioxin-like PCBs, mainly PCB 126.[34] A remark has to be made, that semi-continuous measurements are by far the best in measuring emissions of dioxins during normal operation.[35] 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. A study by Li

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

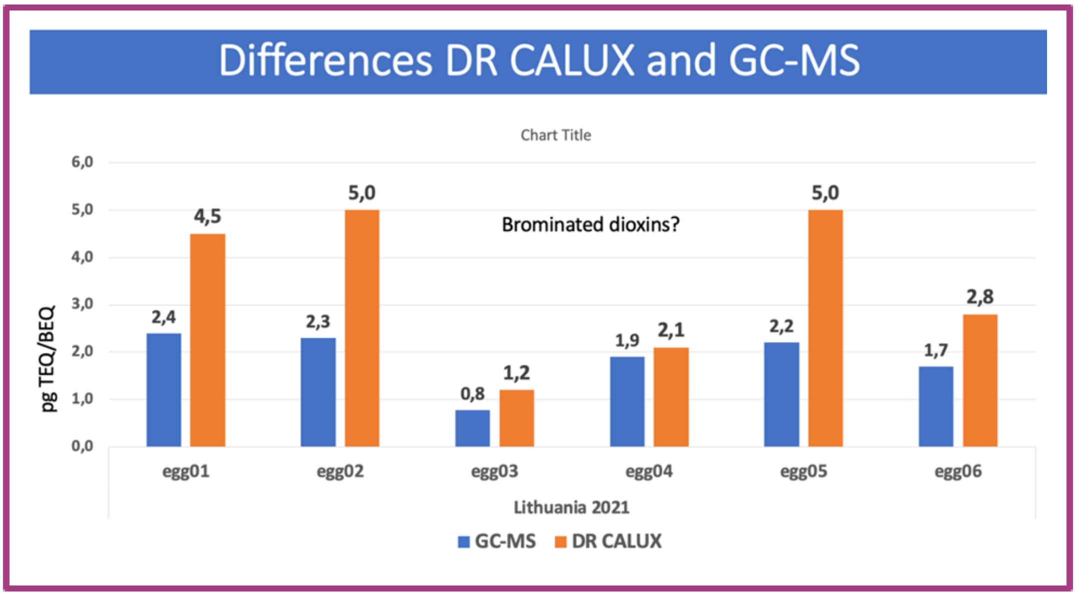
[35] *Arkenbout, A, Olie K, Esbensen, KH, 2018. Emission regimes of POPs of a Dutch incinerator: regulated, measured and hidden issues, Conference paper Dioxin2018*

(Zhejiang University, Hangzhou, China, 2018) demonstrates high emissions of dioxin-like PCBs during transient phases of start-ups and shutdowns.[36]

## 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 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.[37] The EU regulation covers only the chlorinated dioxins (GC-MS: PCDD/F in TEQ and DR CALUX: PCDD/F in BEQ), see Figures 30, 5 and 6. While more and more scientific publications show the proportion of other halogenated dioxins cannot be neglected and should be integrated into 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 the human body (Jogsten et al 2010).[38]

**Figure 30: Difference between GC-MS and DR CALUX indicates evidence of brominated dioxins**



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[36] *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.*

[37] *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*

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

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 be 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 fewer 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.[39]

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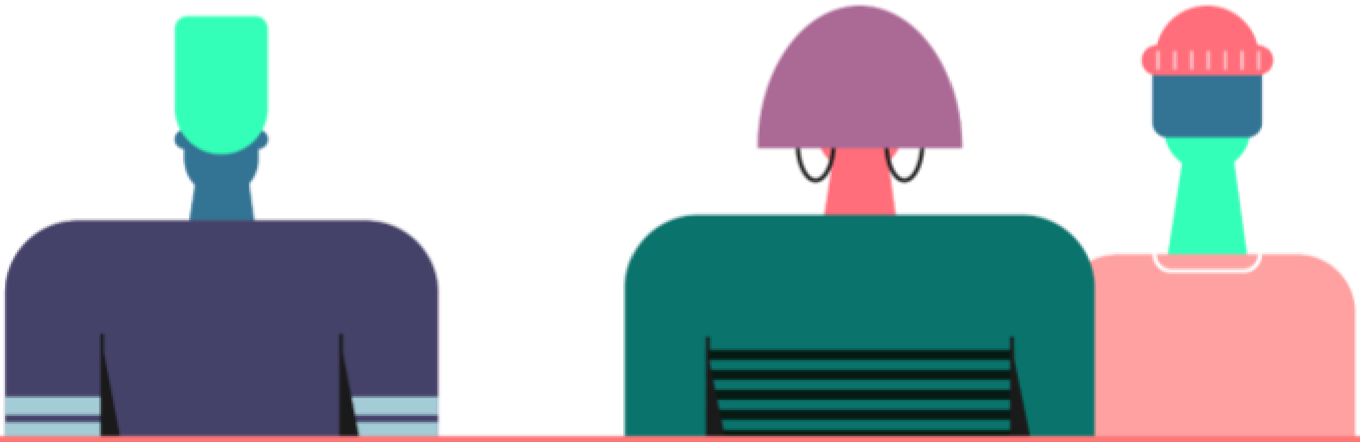
[39] *L. Yang et al. 2021. Environment International 152 (2021) 106450*

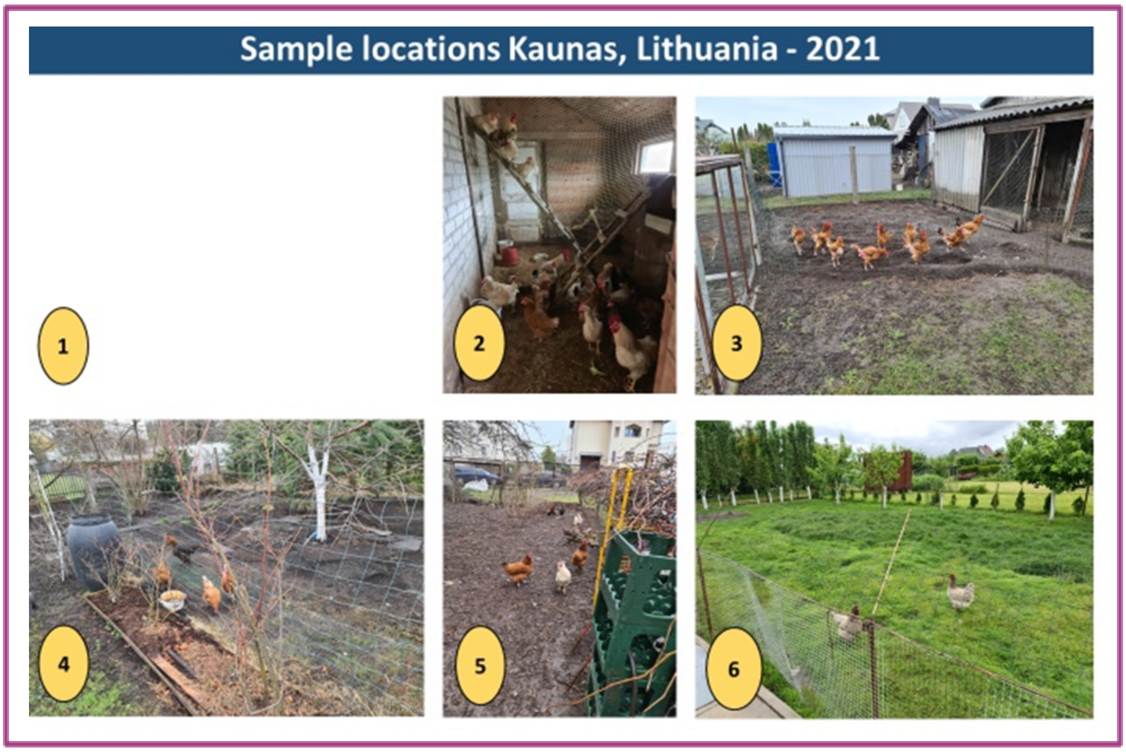


# Summary of analysis results:

## biomonitoring of eggs in Kaunas 2021

Five (5) of the six (6) egg locations (83%) analysed by DR CALUX do not comply with the EU regulations for dioxins in eggs (Figure 31). The results of the chemical analysis with the GC-MS and higher limit values of 2.5 and 5.0 pg TEQ/g fat show 5 out of 6 eggs comply with the limits set by European Union regulations. However, it should be noted that EU regulations are primarily focused on the economic/commercial market. The European Food Safety Authority (EFSA) is more focused on public health. In 2018, this organisation reduced the tolerable weekly intake (TWI) of dioxins (PCDD/F/dl-PCB) by a factor (seven). This reduction has a major impact on food safety standards and action thresholds, and EFSA is also urging a significant lowering of the dioxin limits in EU regulations.

**Figure 31: Six (6) egg locations for biomonitoring research – Kaunas 2021**



The patterns of the dioxins indicate incomplete combustion, which could also be caused by other sources like industry, wood-burning stoves, multi-burners or even illegal backyard burning. There is a lack of technical information on the AEC waste incineration plant in Kaunas, no data on the congener patterns of emissions, and no information on waste inputs. More data is needed to draw any conclusions about a link between the results of this biomonitoring study and the emissions from the waste incinerator in Kaunas. And it is certainly worthwhile to apply continuous dioxin measurements to reassure the population that the incinerator meets all strict environmental requirements.

# Biomonitoring

## of vegetation

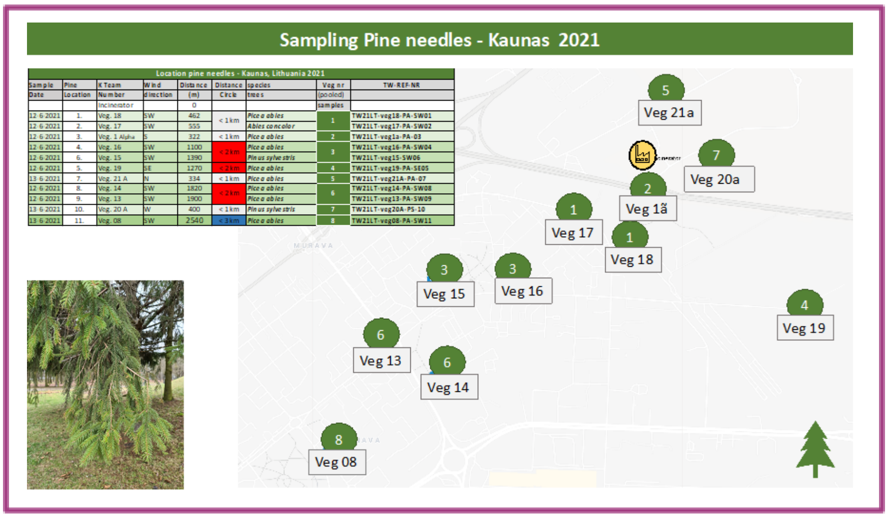
## Pine needles

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 dioxins (PCDD/Fs).[40] Pine needles have an advantage over the use of Polyurethane Foam discs (PUF), which are vulnerable to vandalism.

Pine trees can survive long periods of stressful drought conditions due to the special morphology of pine needles, especially the epicuticular waxes and the distribution of tubular waxes which are species-specific.[41] Meaning the epicuticular wax layer, which helps protect the leaves from the more toxic form of ultraviolet light called UV-B, as well as to prevent water loss of the plant system and risks of pathogen and insect attacks. Dioxins (PCDD/F/dl-PCB) are partitioned in this fatty wax layer because of their lipophilic properties. In fact, lipophilic xenobiotics 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.[42] Persistent organic pollutants are thought to sorb to the cuticular waxes and diffuse into internal leaf (pine needle) compartments.[43] Therefore, plant leaves/ pine needles can be used as a natural sampler for persistent organic pollutants (POPs) in the environment.

Initially, an inventory was made of 22 vegetation sites in the region of Kaunas with different species of pine needles (all visited by the LT sampling team in April). Species *Picea abies* and *Pinus sylvestris* were chosen for biomonitoring of pine needles. Figure 32 shows the sampling with 8 (pooled) samples to obtain an accurate spatial coverage of sampling points in the area around the Kaunas incinerator.

**Figure 32: Vegetation locations for sampling pine needles – Kaunas 2021**



Sampling Pine needles - Kaunas 2021

Location pine needles - Kaunas, Lithuania 2021

| Sample Date | Pine Location | K Team Number | Wind direction | Distance (m) | Distance Circle | species trees | Veg nr (pooled) samples | TW-REF-NR |
|---|---|---|---|---|---|---|---|---|
| | | Incinerator | | 0 | | | | |
| 12-6-2021 | 1. | Veg. 18 | SW | 462 | < 1km | *Picea abies* | 1 | TW21LT-veg18-PA-SW01 |
| 12-6-2021 | 2. | Veg. 17 | SW | 555 | | *Abies concolor* | | TW21LT-veg17-PA-SW02 |
| 12-6-2021 | 3. | Veg. 1 Alpha | S | 322 | < 1km | *Picea abies* | 2 | TW21LT-veg1a-PA-03 |
| 12-6-2021 | 4. | Veg. 16 | SW | 1100 | < 2km | *Picea abies* | 3 | TW21LT-veg16-PA-SW04 |
| 12-6-2021 | 6. | Veg. 15 | SW | 1390 | | *Pinus sylvestris* | | TW21LT-veg15-SW06 |
| 12-6-2021 | 5. | Veg. 19 | SE | 1270 | < 2km | *Picea abies* | 4 | TW21LT-veg19-PA-SE05 |
| 13-6-2021 | 7. | Veg. 21 A | N | 334 | < 1km | *Picea abies* | 5 | TW21LT-veg21A-PA-07 |
| 12-6-2021 | 8. | Veg. 14 | SW | 1820 | < 2km | *Picea abies* | 6 | TW21LT-veg14-PA-SW08 |
| 12-6-2021 | 9. | Veg. 13 | SW | 1900 | | *Picea abies* | | TW21LT-veg13-PA-SW09 |
| 13-6-2021 | 10. | Veg. 20 A | W | 400 | < 1km | *Pinus sylvestris* | 7 | TW21LT-veg20A-PS-10 |
| 13-6-2021 | 11. | Veg. 08 | SW | 2540 | < 3km | *Picea abies* | 8 | TW21LT-veg08-PA-SW11 |

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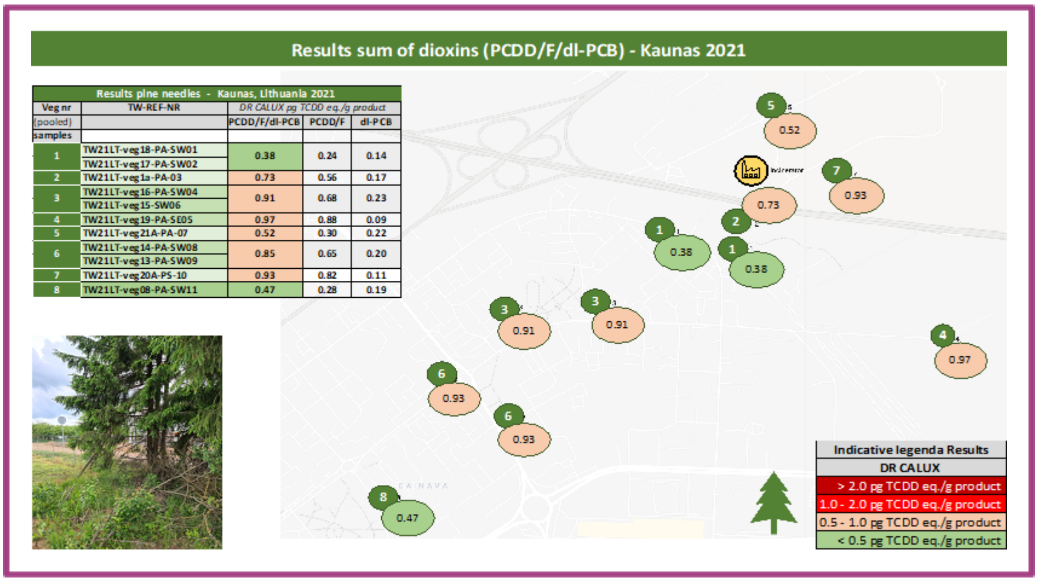
[40] Chen P. et al. (2017). Chemosphere 181 (2017) 360 - 367
[41] Lamppu J., Huttunen S. (2002). Environmental Pollution 122 (2003) 119–126
[42] Moeckel C., 2008. Environ Sci Technol 42:100–105
[43] Barber, JL. (2004). Environ Pollut 128: 99–138

## Results DR CALUX pine needles

The results of the analysis of the pine needles with DR CALUX are shown in Figure 33. It demonstrates a predominantly slight increase in dioxins (PCDD/F) in the vicinity of the waste incinerator, from 0.52 - 0.97 TCDD eq./g product. The exception is the pooled sample at location one (1) (Veg17-PA-SW02 and veg18-PA-SW01) about 500 meters away (SW). The low proportion of dl-PCB in all samples is remarkable. The biggest outlier is location seven (7), at 400 meters East of the incinerator, where 7 times more dioxins (PCDD/F) than dl-PCB are measured. If dioxins (PCDD/F) are measured, it may indicate incomplete combustion which occurs at waste incineration, as TW experience teaches, especially in case of breakdowns, shutdowns and start-ups.

**Figure 33: Sum of dioxins (PCDD/F/dl-PCBs) in Picea Abies – Kaunas 2021**



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

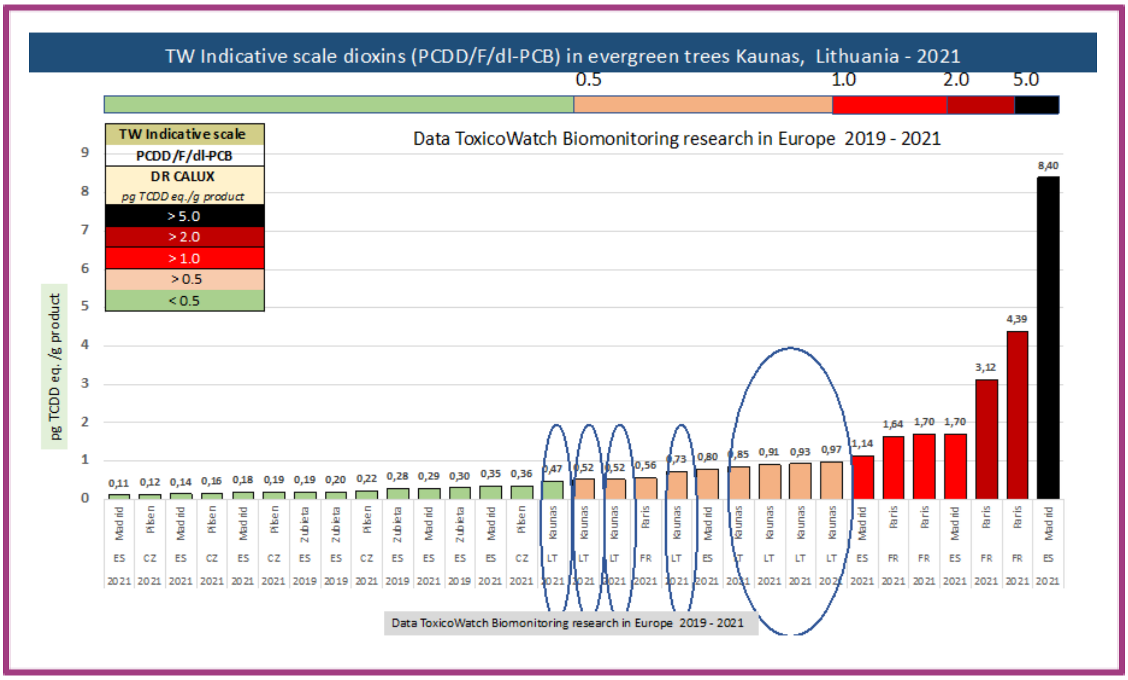
Results pine needles - Kaunas, Lithuania 2021

| Veg nr | TW-REF-NR | *DR CALUX pg TCDD eq./g product* | | |
|---|---|---|---|---|
| (pooled) | | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| samples | | | | |
| 1 | TW21LT-veg18-PA-SW01<br>TW21LT-veg17-PA-SW02 | 0.38 | 0.24 | 0.14 |
| 2 | TW21LT-veg1a-PA-03 | 0.73 | 0.56 | 0.17 |
| 3 | TW21LT-veg16-PA-SW04<br>TW21LT-veg15-SW06 | 0.91 | 0.68 | 0.23 |
| 4 | TW21LT-veg19-PA-SE05 | 0.97 | 0.88 | 0.09 |
| 5 | TW21LT-veg21A-PA-07 | 0.52 | 0.30 | 0.22 |
| 6 | TW21LT-veg14-PA-SW08<br>TW21LT-veg13-PA-SW09 | 0.85 | 0.65 | 0.20 |
| 7 | TW21LT-veg20A-PS-10 | 0.93 | 0.82 | 0.11 |
| 8 | TW21LT-veg08-PA-SW11 | 0.47 | 0.28 | 0.19 |

| Indicative legenda Results |
|---|
| DR CALUX |
| 🟥 > 2.0 pg TCDD eq./g product |
| 🟥 1.0 - 2.0 pg TCDD eq./g product |
| 🟧 0.5 - 1.0 pg TCDD eq./g product |
| 🟩 < 0.5 pg TCDD eq./g product |

The remarkable difference is noted with Pilsen, Czech Rep., where the proportion of dioxins in pine needles is significantly lower than in Kaunas (Figure 34). In the pine needles in Kaunas 0.24-0.88 pg TCDD eq./g product for dioxins (PCDD/F) are measured, and in Pilsen 0.05-0.09 pg TCDD eq./g product.

**Figure 34: Indicative scale – dioxins (PCDD/F/dl-PCB) in pine needles in Europe using DR CALUX**



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

Polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, and toxic (PBT) compounds that have the potential to move far from their original sources. Therefore, routine monitoring of these compounds, in the vicinity of point sources is important. The proportion of dl-PCBs in the pine needles in Kaunas is 0.09 – 0.23 TCDD eq./g pr., Figure 35. In heavily polluted areas such as Madrid and Paris, 0.60 – 1.7 pg TCDD eq./g pr. dl-PCB are measured in pine needles near the incinerator, see Figure 36. In a research by 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.[44] This TW-biomonitoring study on pine needles is performed by the DR CALUX analysis method which gives in general lower results for PCB TEQ. One of the reasons is the use of a relatively high toxicity factor for the TEQ calculation for PCB 126 in the GC-MS analysis. Figure 35 is a comparative scale of other TW-results of DR CALUX measurements in evergreen trees.

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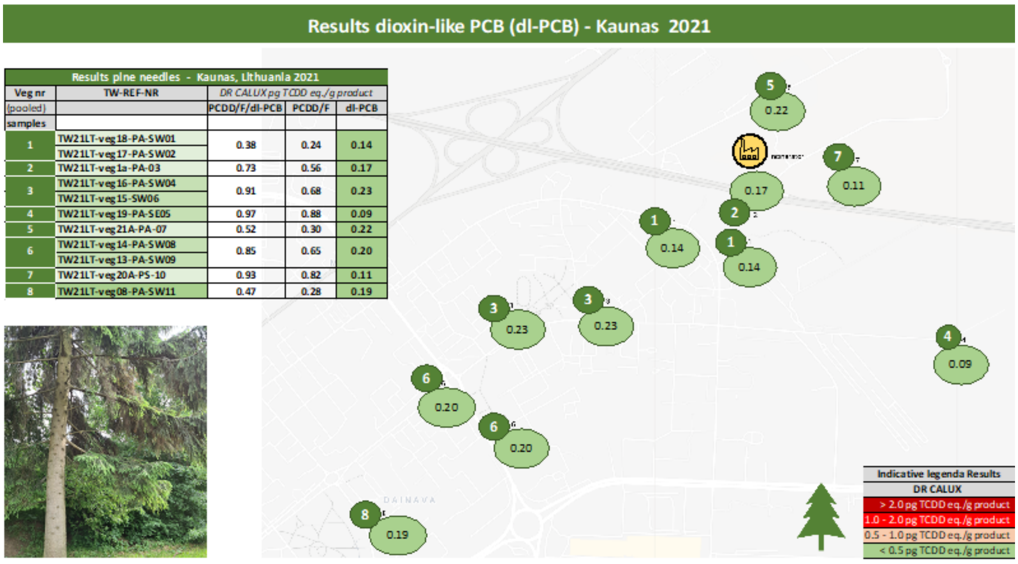
[44] *Holt E. et al. (2016). Spatiotemporal patterns and potential sources of polychlorinated biphenyl (PCB) contamination in Scots pine (Pinus sylvestris) needles from Europe. Environ Sci Pollut Res, DOI 10.1007/s11356-016-7171-6*

## Figure 35: Dioxin-like PCBs (dl-PCB) in pine needles Picea abies and Pinus sylvestris, Kaunas 2021

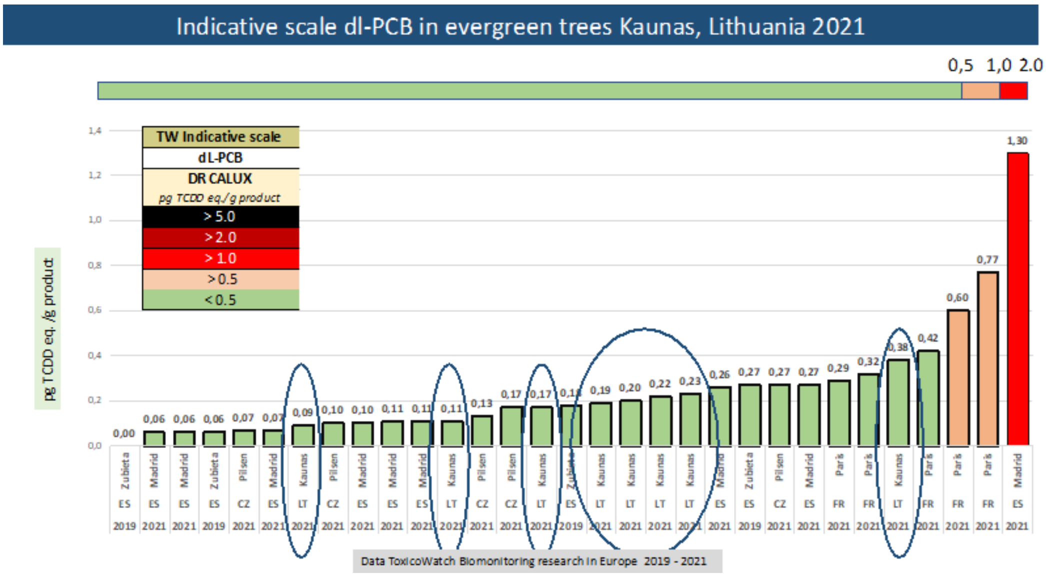


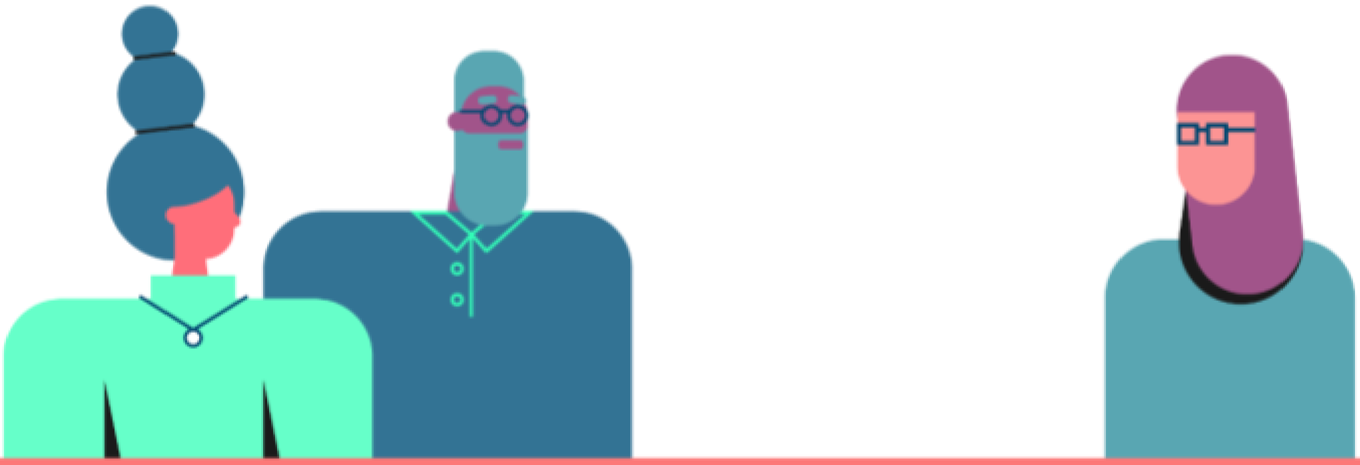
Results dioxin-like PCB (dl-PCB) - Kaunas 2021

| Results pine needles - Kaunas, Lithuania 2021 | | | | |
|---|---|---|---|---|
| Veg nr | TW-REF-NR | DR CALUX pg TCDD eq./g product | | |
| (pooled) | | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| samples | | | | |
| 1 | TW21LT-veg18-PA-SW01<br>TW21LT-veg17-PA-SW02 | 0.38 | 0.24 | 0.14 |
| 2 | TW21LT-veg1a-PA-03 | 0.73 | 0.56 | 0.17 |
| 3 | TW21LT-veg16-PA-SW04<br>TW21LT-veg15-SW06 | 0.91 | 0.68 | 0.23 |
| 4 | TW21LT-veg19-PA-SE05 | 0.97 | 0.88 | 0.09 |
| 5 | TW21LT-veg21A-PA-07 | 0.52 | 0.30 | 0.22 |
| 6 | TW21LT-veg14-PA-SW08<br>TW21LT-veg13-PA-SW09 | 0.85 | 0.65 | 0.20 |
| 7 | TW21LT-veg20A-PS-10 | 0.93 | 0.82 | 0.11 |
| 8 | TW21LT-veg08-PA-SW11 | 0.47 | 0.28 | 0.19 |

| Indicative legenda Results |
|---|
| DR CALUX |
| > 2.0 pg TCDD eq./g product |
| 1.0 - 2.0 pg TCDD eq./g product |
| 0.5 - 1.0 pg TCDD eq./g product |
| < 0.5 pg TCDD eq./g product |

## Figure 36: TW-Indicative scale – dl-PCBs in pine needles in Europe using DR CALUX



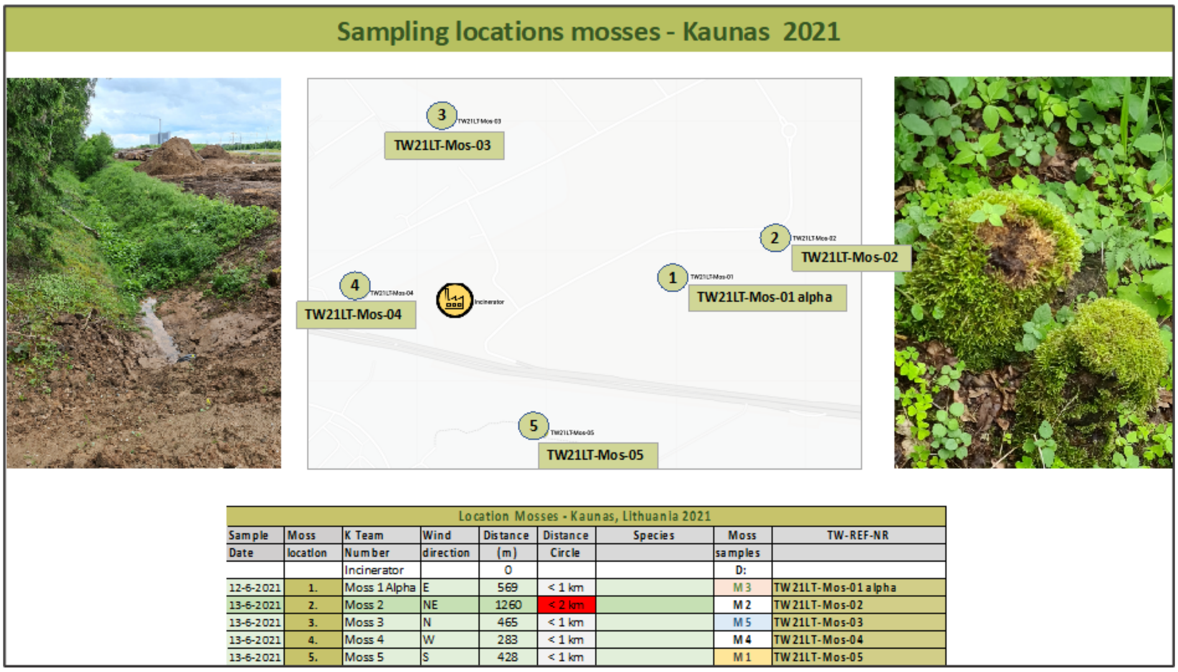


# Mosses

Bryophytes are the non-vascular autotrophic cryptogams with the second-highest conglomeration among land plants after the angiosperms, and nearly 25,000 species were present worldwide. Mosses belong to the kingdom Plantae, and division Bryophyta. Mosses have a crucial ecological role and they represent a largely untapped resource for monitoring and indicating the consequences of pollution in the living environment.

The mosses of the selected five (5) locations are mainly *Hylocomium splendens*, sampled within a 1,000-metre radius of the incinerator (Figure 37). Mosses were sampled in the open field, avoiding the proximity of roads and not under the dense tree canopies to avoid the shedding of leaves.

**Figure 37: Sampling locations of mosses – Kaunas 2021**

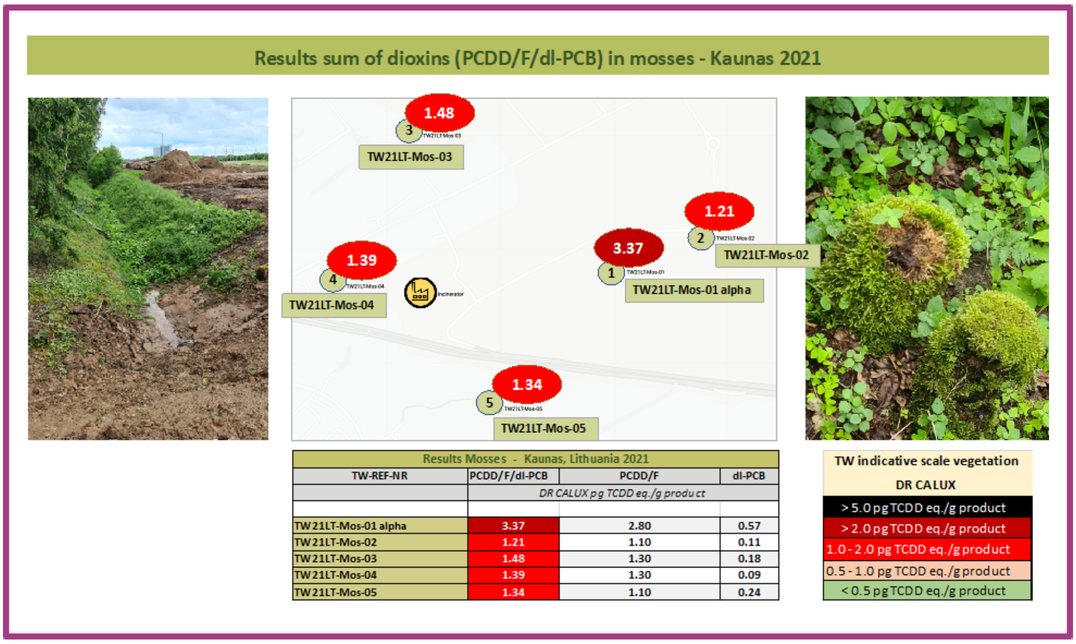


Sampling locations mosses - Kaunas 2021

| Location Mosses - Kaunas, Lithuania 2021 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Date | Moss location | K Team Number | Wind direction | Distance (m) | Distance Circle | Species | Moss samples | TW-REF-NR |
| | | Incinerator | | 0 | | | D: | |
| 12-6-2021 | 1. | Moss 1 Alpha | E | 569 | < 1 km | | M 3 | TW 21LT-Mos-01 alpha |
| 13-6-2021 | 2. | Moss 2 | NE | 1260 | < 2 km | | M 2 | TW 21LT-Mos-02 |
| 13-6-2021 | 3. | Moss 3 | N | 465 | < 1 km | | M 5 | TW 21LT-Mos-03 |
| 13-6-2021 | 4. | Moss 4 | W | 283 | < 1 km | | M 4 | TW 21LT-Mos-04 |
| 13-6-2021 | 5. | Moss 5 | S | 428 | < 1 km | | M 1 | TW 21LT-Mos-05 |

Figure 38 displays the results of the sum of dioxins (PCDD/F/dl-PCBs). At location one (1) to the East of the incinerator, the level of dioxins is 3.37 pg TCDD eq./g, nearly 3 times higher than in the other moss locations. The other four (4) moss locations have similar values (1.21-1.48 pg TCDD eq./g). The colours red and dark red are given according to the TW-indicative legenda, shown at the right. This means that the levels of dioxins detected are high in relation to other research and references. Also, the dl-PCBs at this location are elevated. The fairly low chimney could be a reason for deposition relatively nearby, but additional research is needed. Measurements during OTNOC (as explained previously in this report) could provide some clarity about whether the incinerator contributes to these levels of dioxins and dioxin-like PCBs in the environment.

### Figure 38: Sum of dioxins (PCDD/F/dl-PCBs) in mosses – Kaunas 2021

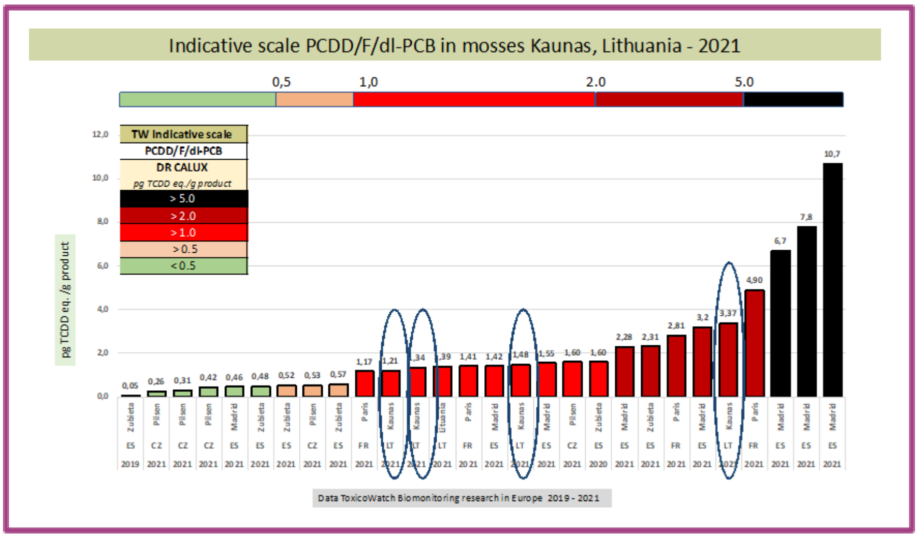

Results sum of dioxins (PCDD/F/dl-PCB) in mosses - Kaunas 2021

| Results Mosses - Kaunas, Lithuania 2021 | | | |
|---|---|---|---|
| TW-REF-NR | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| | DR CALUX pg TCDD eq./g product | | |
| | | | |
| TW21LT-Mos-01 alpha | 3.37 | 2.80 | 0.57 |
| TW21LT-Mos-02 | 1.21 | 1.10 | 0.11 |
| TW21LT-Mos-03 | 1.48 | 1.30 | 0.18 |
| TW21LT-Mos-04 | 1.39 | 1.30 | 0.09 |
| TW21LT-Mos-05 | 1.34 | 1.10 | 0.24 |

| TW indicative scale vegetation<br>DR CALUX |
|---|
| > 5.0 pg TCDD eq./g product |
| > 2.0 pg TCDD eq./g product |
| 1.0 - 2.0 pg TCDD eq./g product |
| 0.5 - 1.0 pg TCDD eq./g product |
| < 0.5 pg TCDD eq./g product |

In the literature (2018),[45] PCDD/F TEQ concentrations are found between 0.024 pg TEQ to 0.81 pg TEQ. Caraballeira et al. (2006)[46] 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 et al. (2016)[47] 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 analysed substances were very low, often close to or below the limits of quantification (LOQ) for the dioxin analyses. In Figure 39, results of the sum of dioxins (PCDD/F/dl-PCB) in mosses compared to other TW biomonitoring studies are presented.

### Figure 39 TW indicative scale overview of dioxins (PCDD/F/dl-PCB) in mosses of Europe (TW research)


Indicative scale PCDD/F/dl-PCB in mosses Kaunas, Lithuania - 2021

Data ToxicoWatch Biomonitoring research in Europe 2019 - 2021

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[45] Dreyer et al. Environ Sci Eur (2018) 30:43 https://doi.org/10.1186/s12302-018-0172-y

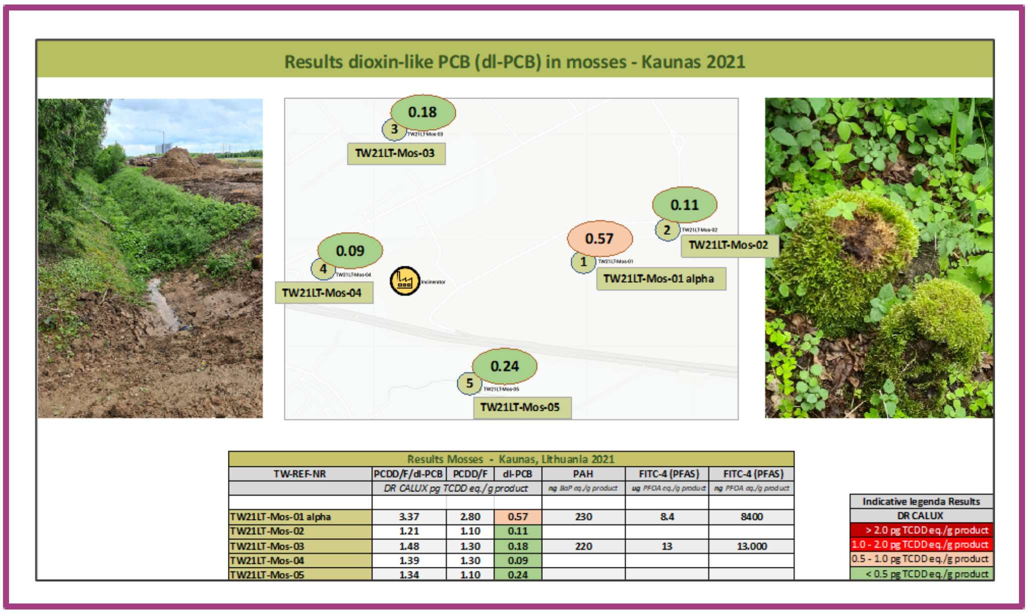
[46] Carballeira A. et al. (2006) Moss: a powerful tool for dioxin monitoring. Atmos Environ 40(30):5776–5786

[47] Danielsson et al. (2016). Persistant organic pollutants in Swedish mosses, IVL-report C 188

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

In parallel with the PCDD/F, the dl-PCB at moss location 1 is also found to be elevated (Figure 39). The value is 0.57 pg TCDD eq./g. Four of the five moss locations have a dl-PCB in the range of 0.09 - 0.24 pg TCDD eq./g.. These values found are low compared to results of other TW biomonitoring surveys, see Figure 40.

**Figure 40: Dioxin-like PCBs (dl-PCBs) in mosses – Kaunas 2021**



Results dioxin-like PCB (dl-PCB) in mosses - Kaunas 2021

Results Mosses - Kaunas, Lithuania 2021

| TW-REF-NR | PCDD/F/dl-PCB | PCDD/F | dl-PCB | PAH | FITC-4 (PFAS) | FITC-4 (PFAS) |
|---|---|---|---|---|---|---|
| | DR CALUX pg TCDD eq./g product | | | ng BaP eq./g product | ug PFOA eq./g product | ng PFOA eq./g product |
| | | | | | | |
| TW21LT-Mos-01 alpha | 3.37 | 2.80 | 0.57 | 230 | 8.4 | 8400 |
| TW21LT-Mos-02 | 1.21 | 1.10 | 0.11 | | | |
| TW21LT-Mos-03 | 1.48 | 1.30 | 0.18 | 220 | 13 | 13.000 |
| TW21LT-Mos-04 | 1.39 | 1.30 | 0.09 | | | |
| TW21LT-Mos-05 | 1.34 | 1.10 | 0.24 | | | |

| Indicative legenda Results |
|---|
| DR CALUX |
| > 2.0 pg TCDD eq./g product |
| 1.0 - 2.0 pg TCDD eq./g product |
| 0.5 - 1.0 pg TCDD eq./g product |
| < 0.5 pg TCDD eq./g product |

The Swedish researchers[48] found a 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 equivalencies (TEQs) of the 2,3,7,8-PCDD/Fs were calculated based on World Health Organization (WHO) toxicity equivalency factors (W-TEFs) (Van den Berg et al., 2006).[49] In this model, the dl-PCB congener PCB 126 has a toxicity equivalency factor of 0,1. This relatively high value must likely be adjusted downwards. The DR CALUX bioassay measures the toxicity of substances on cell culture and measures in general lower dl-PCB values. The dioxins found in the mosses in Kaunas using the DR CALUX bioassay are relatively high when compared with other results in Europe shown in a TW indicative scale of similar biomonitoring projects (see Figure 41).

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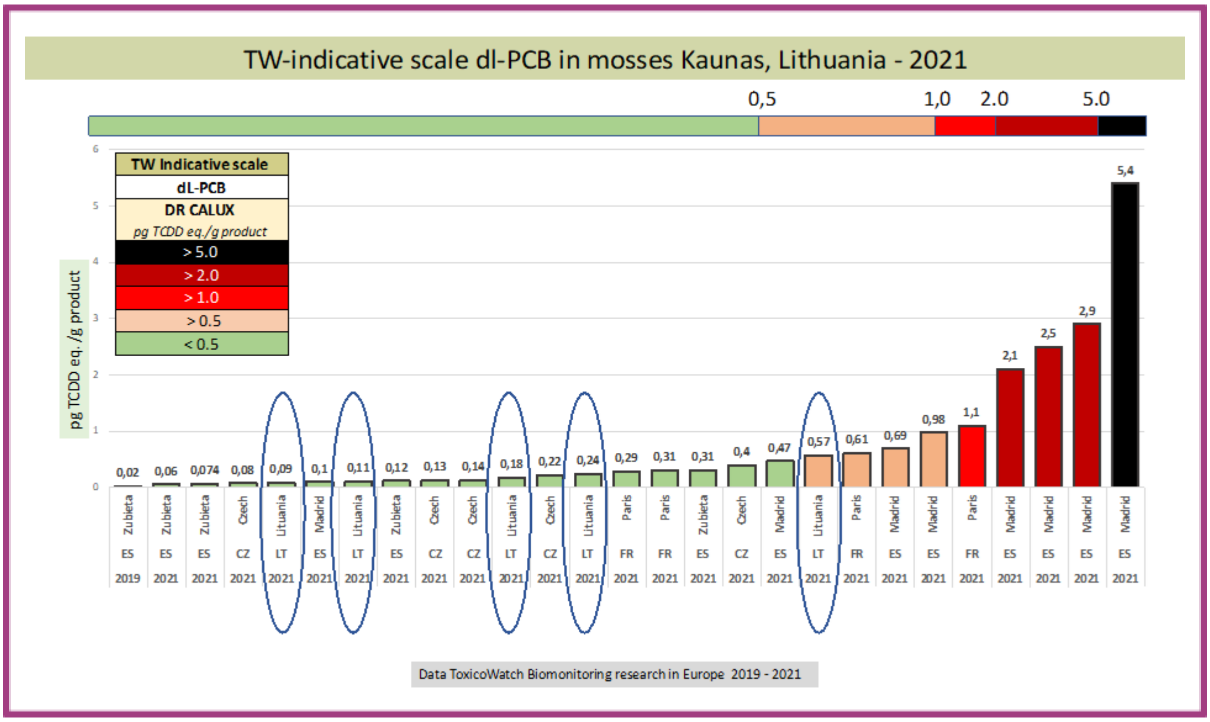
[48] *Danielsson et al. (2016). Persistent organic pollutants in Swedish mosses, IVL-report C 188*

[49] *Van den Berg, M. et al. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.*

**Figure 41: TW Indicative scale – dl-PCBs in mosses – Kaunas 2021**

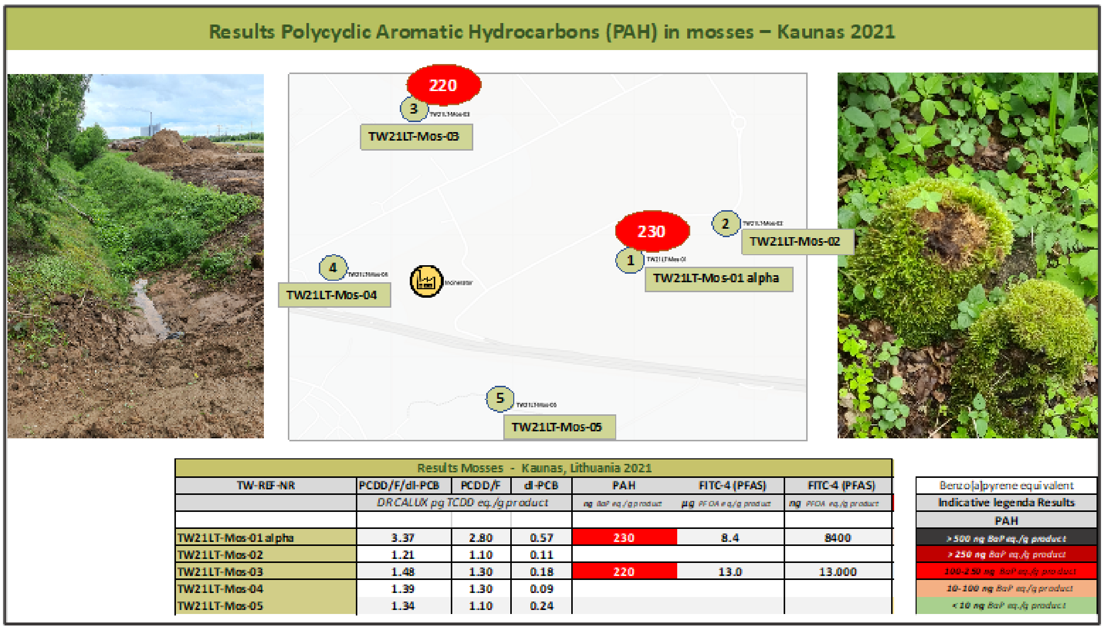


## PAH in mosses

The group of polycyclic aromatic hydrocarbons, PAHs, is a useful biomarker tool to detect certain toxic chemical emissions of thermo-confounders. The high concentrations of toxic congeners of PAHs chemicals in living organisms in both plants and animals (including humans) found by many researchers worldwide have caused great concern over recent years. Compounds from the PAH group may have carcinogenic, mutagenic, teratogenic, and immunosuppressive influences on living organisms. According to the EU directives, concentrations of PAHs in the air should be constantly monitored (EU Directive 2004/107/EC and Regulation 219/2009). The European Union established a health-based standard for PAHs in air equal to 1 ng/m3 B[a]P (Benzo(a)pyrene). In this study, the bioassay of PAH CALUX is used for measuring PAH in environmental moss samples. With this method, the total toxic potency is measured, while the chemical analysis is limited to 4 – 16 PAH congeners. The PAH analysis result is expressed in Benzo[a]Pyrene equivalency (B[a]P eq.).

Figure 42 displays measured values of 230 and 220 ng BAP eq./g at respective locations 1 and 3. In Figure 43, a relative comparison is made with other TW-researches and these results around the incinerator can be classified as high and worrying.

## Figure 42: Polycyclic Aromatic Hydrocarbons (PAHs) in mosses – Kaunas 2021

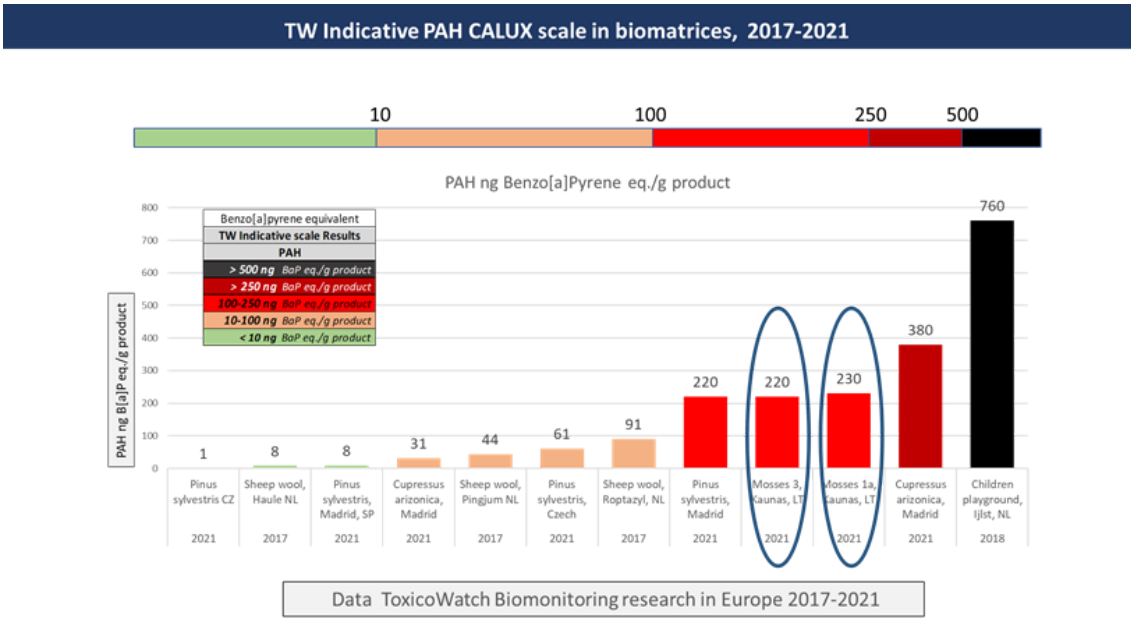


Results Polycyclic Aromatic Hydrocarbons (PAH) in mosses – Kaunas 2021

| Results Mosses - Kaunas, Lithuania 2021 | | | | | | |
|---|---|---|---|---|---|---|
| TW-REF-NR | PCDD/F/dl-PCB | PCDD/F | dl-PCB | PAH | FITC-4 (PFAS) | FITC-4 (PFAS) |
| | DR CALUX pg TCDD eq./g product | | | ng BaP eq./g product | µg PFOA eq./g product | ng PFOA eq./g product |
| | | | | | | |
| TW21LT-Mos-01 alpha | 3.37 | 2.80 | 0.57 | 230 | 8.4 | 8400 |
| TW21LT-Mos-02 | 1.21 | 1.10 | 0.11 | | | |
| TW21LT-Mos-03 | 1.48 | 1.30 | 0.18 | 220 | 13.0 | 13.000 |
| TW21LT-Mos-04 | 1.39 | 1.30 | 0.09 | | | |
| TW21LT-Mos-05 | 1.34 | 1.10 | 0.24 | | | |

| Benzo[a]pyrene equivalent |
|---|
| Indicative legenda Results |
| PAH |
| > 500 ng BaP eq./g product |
| > 250 ng BaP eq./g product |
| 100-250 ng BaP eq./g product |
| 10-100 ng BaP eq./g product |
| < 10 ng BaP eq./g product |

## Figure 43: TW indicative scale PAH CALUX in vegetation biomatrices (TW research)



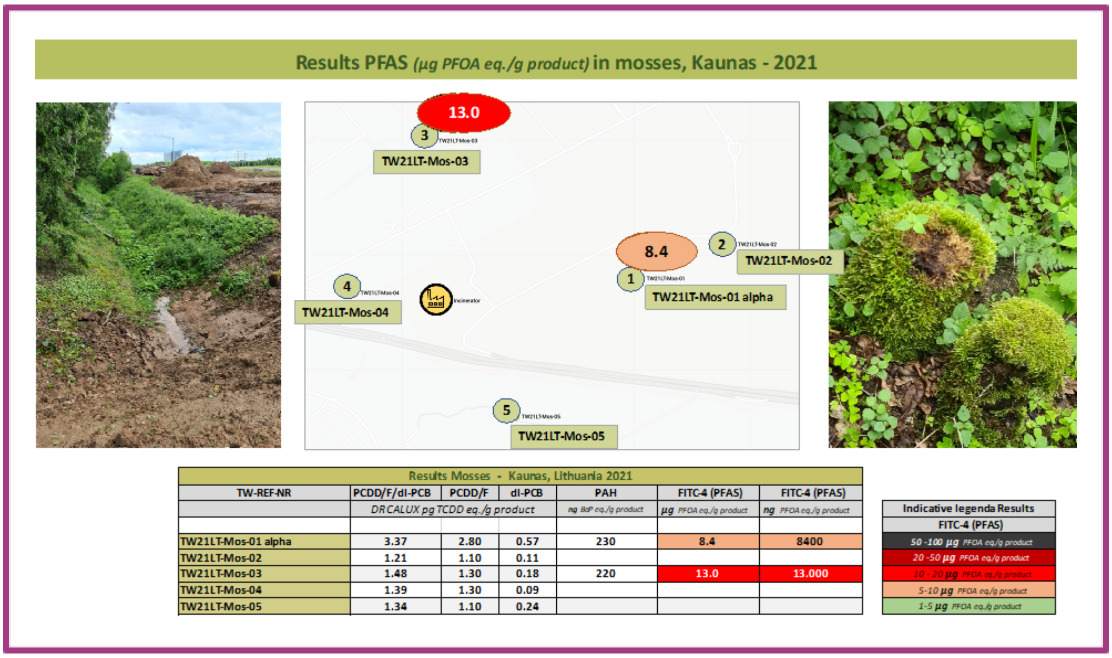
TW Indicative PAH CALUX scale in biomatrices, 2017-2021

Data ToxicoWatch Biomonitoring research in Europe 2017-2021

## PFAS in mosses

To have an impression of PFAS presence in the environment of Kaunas the FITC-T4 analysis method was used on two samples of mosses. The results for moss locations one (1) and three (3) are respectively 13.0 $\mu$g and 8.4 $\mu$g PFOA equivalent per gram product (Figure 44). Expressed in nanograms, these are 13000 and 8400ng PFOA eq./g products. In a Swedish study,[50] 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. What are the consequences of PFAS on the environment, for vegetation, uptake by domestic animals, dairy and meat products, as well as 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.[51] So, the question arises - what is the contribution of waste incineration to the PFAS contamination in the environment of Kaunas?

**Figure 44: PFAS in mosses – Kaunas 2021**



Results PFAS (µg PFOA eq./g product) in mosses, Kaunas - 2021

Results Mosses - Kaunas, Lithuania 2021

| TW-REF-NR | PCDD/F/dl-PCB | PCDD/F | dl-PCB | PAH | FITC-4 (PFAS) | FITC-4 (PFAS) |
|---|---|---|---|---|---|---|
| | DR CALUX pg TCDD eq./g product | | | ng BaP eq./g product | µg PFOA eq./g product | ng PFOA eq./g product |
| | | | | | | |
| TW21LT-Mos-01 alpha | 3.37 | 2.80 | 0.57 | 230 | 8.4 | 8400 |
| TW21LT-Mos-02 | 1.21 | 1.10 | 0.11 | | | |
| TW21LT-Mos-03 | 1.48 | 1.30 | 0.18 | 220 | 13.0 | 13.000 |
| TW21LT-Mos-04 | 1.39 | 1.30 | 0.09 | | | |
| TW21LT-Mos-05 | 1.34 | 1.10 | 0.24 | | | |

| Indicative legenda Results |
|---|
| FITC-4 (PFAS) |
| 50 -100 µg PFOA eq./g product |
| 20 -50 µg PFOA eq./g product |
| 10 - 20 µg PFOA eq./g product |
| 5-10 µg PFOA eq./g product |
| 1-5 µg PFOA eq./g product |

The EFSA advice for a Tolerable Weekly Intake of PFOA is set at 6 nanogram/kg body weight a week. Figure 44 makes a comparison with different biomatrices. In eggs of backyard chicken near the incinerator of Pilsen PFAS is found more than 1000 x the TWI for PFOA. In this Figure the PFAS levels found in the two (2) moss locations are higher, raising concerns about the findings on vegetables in food crops in the area, whether for the market

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

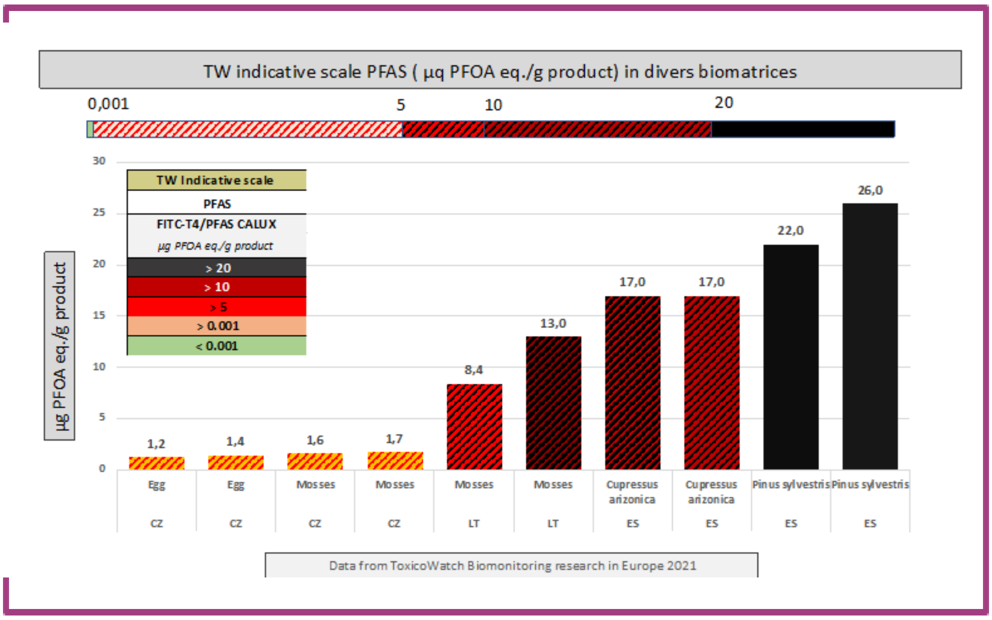
[51] *Arkenbout, A, 2018. Long-term sampling emission of PFOS and PFOA of a Waste-to-Energy incinerator*

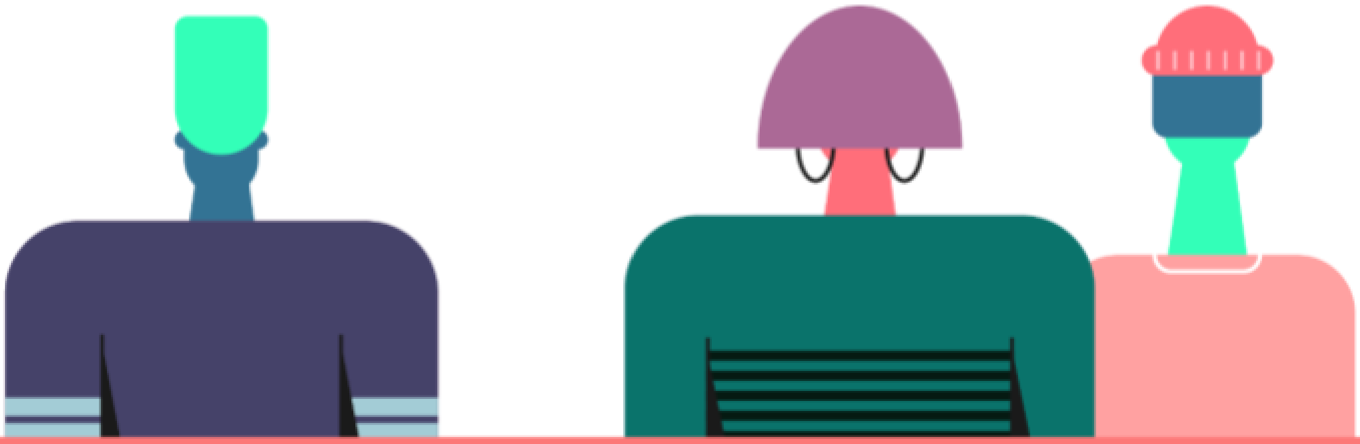
or private use. Additional research on PFAS is needed for a better understanding of how these findings can be interpreted.

The previous section explained how the analytical research of PFAS is lagging. 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 14). The FITC-4 is an analytical method measuring the total toxicity of a mixture of different PFAS substances (page 16). 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 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 45, 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 45: TW-Indicative scale for PFAS in various biomatrices – 2021**



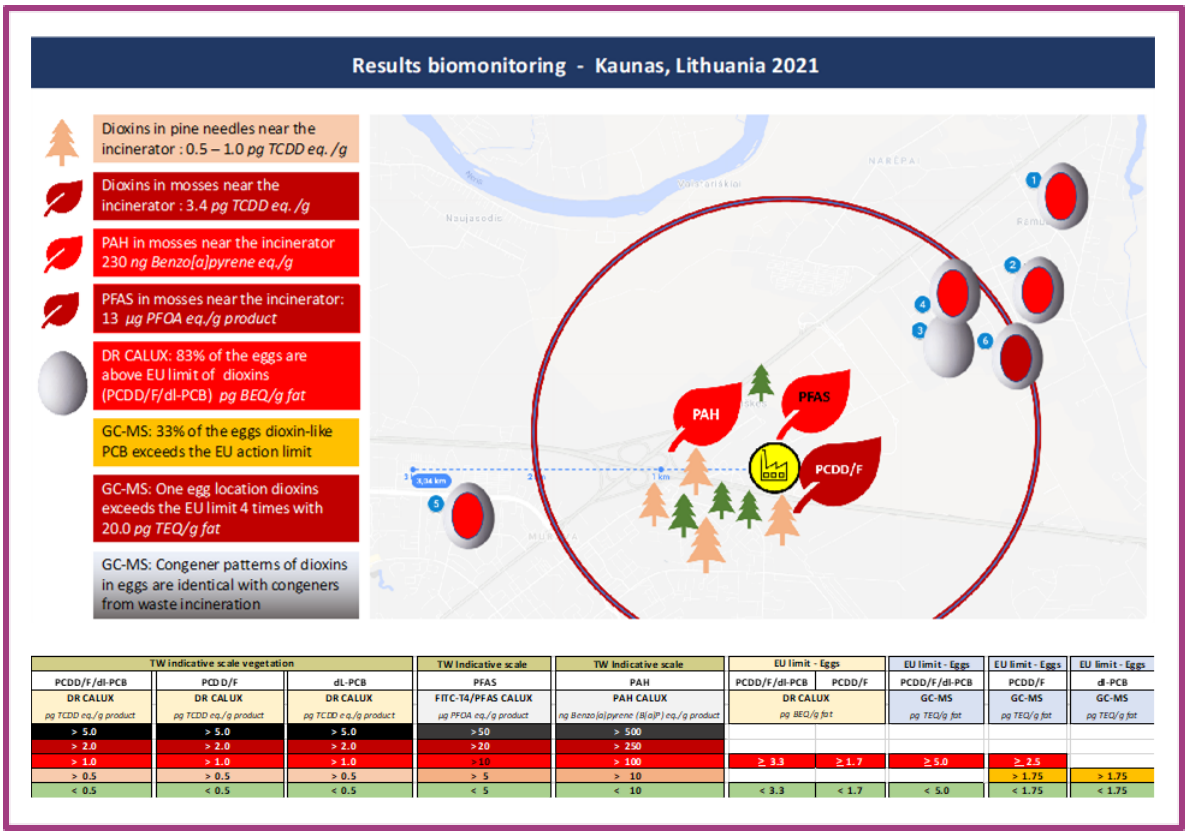


# Conclusion

The newly built Kaunas WtE (waste) incinerator has been in operation since November 2020. The first round of biomonitoring research on biomarkers of backyard chicken eggs, pine needles and mosses in the region around the incinerator has taken place. This year in 2022, the biomonitoring study around the WtE (waste) incinerator will be continued.

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 83% of the eggs (5 out of 6) with levels exceed the EU action limits for food safety as regulated in the EU Directive 2013/711/EU. The chemical analysis GC-MS shows one egg location exceeds the EU food limits with a factor of four (4). The EU action limit exceeds for dioxins (PCDD/F) on four (4) egg locations. Two egg locations exceeded the GC-MS action limit for dioxin-like PCBs (dl-PCB). The EU regulations urge for action on these egg locations to find out the source of persistent organic pollutant contamination, to eliminate or at least do the utmost to reduce dioxins (PCDD/F) to a 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 solely 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) by the EFSA in 2018. This health advice is still not yet implemented by governments in the EU. Since private consumption of backyard chicken eggs is as high as 450 eggs per month, according to the questionnaire from the chicken coop owners, this is a serious health risk.

**Figure 46: Conclusion: Results of biomonitoring – Kaunas, Lithuania 2021**



**Results biomonitoring - Kaunas, Lithuania 2021**

Dioxins in pine needles near the incinerator : 0.5 – 1.0 *pg TCDD eq. /g*

Dioxins in mosses near the incinerator : 3.4 *pg TCDD eq. /g*

PAH in mosses near the incinerator 230 *ng Benzo[a]pyrene eq./g*

PFAS in mosses near the incinerator: 13 *µg PFOA eq./g product*

DR CALUX: 83% of the eggs are above EU limit of dioxins (PCDD/F/dl-PCB) *pg BEQ/g fat*

GC-MS: 33% of the eggs dioxin-like PCB exceeds the EU action limit

GC-MS: One egg location dioxins exceeds the EU limit 4 times with 20.0 *pg TEQ/g fat*

GC-MS: Congener patterns of dioxins in eggs are identical with congeners from waste incineration

| TW indicative scale vegetation | | | TW Indicative scale | TW Indicative scale | EU limit - Eggs | | EU limit - Eggs | EU limit - Eggs | EU limit - Eggs |
|---|---|---|---|---|---|---|---|---|---|
| PCDD/F/dl-PCB | PCDD/F | dL-PCB | PFAS | PAH | PCDD/F/dl-PCB | PCDD/F | PCDD/F/dl-PCB | PCDD/F | dl-PCB |
| DR CALUX | DR CALUX | DR CALUX | FITC-T4/PFAS CALUX | PAH CALUX | DR CALUX | | GC-MS | GC-MS | GC-MS |
| *pg TCDD eq./g product* | *pg TCDD eq./g product* | *pg TCDD eq./g product* | *µg PFOA eq./g product* | *ng Benzo[a]pyrene (B[a]P) eq./g product* | *pg BEQ/g fat* | | *pg TEQ/g fat* | *pg TEQ/g fat* | *pg TEQ/g fat* |
| > 5.0 | > 5.0 | > 5.0 | >50 | > 500 | | | | | |
| > 2.0 | > 2.0 | > 2.0 | >20 | > 250 | | | | | |
| > 1.0 | > 1.0 | > 1.0 | >10 | > 100 | ≥ 3.3 | ≥ 1.7 | ≥ 5.0 | ≥ 2.5 | |
| > 0.5 | > 0.5 | > 0.5 | > 5 | > 10 | | | | > 1.75 | > 1.75 |
| < 0.5 | < 0.5 | < 0.5 | < 5 | < 10 | < 3.3 | < 1.7 | < 5.0 | < 1.75 | < 1.75 |

The results of the analysis of the vegetation, pine needles and mosses show elevation of dioxin levels in the vicinity of the waste incinerator. Moss samples, within a 1,000-meter radius of the WtE incinerator, demonstrate high levels of dioxins (PCCD/F/dl-PCB) of 1.21 - 3.37 pg TCDD equivalent/g.

Together with the results of high PAH levels of 230 ng Benzo[a]Pyrene equivalent/g and 8.4–13 $\mu$g PFOA equivalent/g, the mosses are considered to be seriously contaminated with persistent organic pollutants. Elevated dioxin patterns are found South-East in pine needles, moderately high when these results are compared to other environments near waste incinerators.

The EU regulations urge for action on this egg location to find out the source of persistent organic pollutant contamination, to eliminate or at least do the utmost to reduce dioxins (PCDD/F) to a 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 solely 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) by the EFSA in 2018. This health advice is still not yet implemented by governments in the EU. Since private consumption of backyard chicken eggs can be high, this poses a serious health risk.

Measurements of the flue gasses could verify the fingerprints, congener patterns, found in the eggs and most important quantify the emitted POPs during Other Than Normal Operation Conditions (OTNOC). The region of Kaunas shows an environment under threat by contamination of substances of very high concern in eggs of backyard chicken, pine needles, and mosses. This biomonitoring gives a warning signal for the contribution of emissions of the waste incinerator into the environment with toxic substances such as dioxins (PCDD/F), dioxin-like PCBs, PAHs and PFAS.

The biomonitoring project will be continued in 2022.

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