Introduction of Innovative Sampling Media for Biomonitoring of Environmental Loads of Persistent Organic Pollutants (POPs)

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ABSTRACT

Combustion-related industry activity inevitably results in emissions of toxic substances, such as dioxins, PFAS, PAH, also called persistent organic pollutants (POPs) and heavy metals, with a key focus on to what degree this is accompanied by unacceptable pollution in the surrounding environments. Measurements of the environmental load of these and other industrial emissions are today limited in terms of relevant time frame, frequency and target POPs. A comprehensive systematic monitoring is critically needed to support endangered surrounding environments and the health of local populations. Here we present selected studies from the last decade developing a science-backed basis for improving monitoring of industrial emission deposition loads of hazardous POP substances with a special focus on the use of both conventional as well as *innovative* sampling media: soil, water, sediments, *sheep's wool*, *eggshells* of wildlife birds, mosses (*Bryophytes*), pine needles (*Picea abies; Pinus sylvestris*), evergreen tree leaves (*Olea europaea; Quercus ilex*), *liver* (from dead wildlife animals), even *mother's milk*. In this article we highlight some of the challenges involved in using this wide array of sampling media for biomonitoring with respect to representativity, reproducibility (practical monitoring), and relationship to the often highly advanced analytical methods employed. An important result of the work carried out by ToxicoWatch (TW) is that local communities become involved and are actively participating in the practical realisation of relevant research and monitoring projects. With this approach, local communities feel listened to and experience in practice that action is taken to focus their serious concerns through citizen-participation in scientific research and communication between polluting agents, governments, and other relevant organisations. In this way ToxicoWatch acts as a bridge between local communities, industry, and government.

1. Background – ToxicoWatch

European Union (EU) regulations concerning monitoring of industrial emissions of hazardous POP substances currently specify a monitoring frequency *only* amounting to one or two preannounced times yearly (monitoring periods six to eight hours per year (6-16 h/y) on a selected group of chlorinated dioxins (PCDD/F/dl-PCBs) and a limited number of heavy metals *only*, while the important group of other (mixed) halogenated POPs, such as brominated dioxins (PBDD/F), PFAS, mixed halogenated PAH, and chlorinated paraffins are currently *not* included in any mandated monitoring regulation.

Measurements for mandated monitoring of flue gasses are only performed during 'optimal production conditions', in stark contrast to firm evidence that POP emissions are mostly emitted during so-called 'calamities', such as start-ups/shut-downs, technical failures related to insufficiently high temperatures, and wet waste content. Besides such technical failures, toxic outputs without an optimum functioning filter system are a main source of industrial emissions into the surrounding environment according to current Best Available Techniques & Best Environmental Practices (BAT/BEP).

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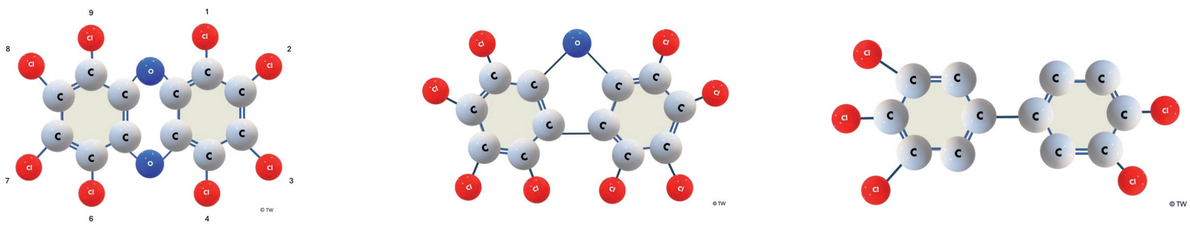
What are dioxins?

Dioxins are formed by halogens: Chlorine (Cl=Chlorine) or other halogens (F=Fluorine, Br=Bromine, I=Iodine), or a mixture of halogens. These halogens are arranged on two benzene rings of carbon atoms (Carbon (C=Carbon), to which 1-8 chlorine or other halogen atoms may be attached. These rings are joined by 2 oxygen (O=Oxygen) atoms, "di-oxy". Furans have one oxygen (O) in the molecular compound and in the case of dl-PCBs, no oxygen (O) atom is present in the molecular compound.

Dioxins are substances, by-products, unintentionally produced during thermal (industrial production) processes, such as waste or co-incineration. In this process, they are mainly formed during incomplete combustion (including low combustion temperatures < 850 C degrees for 2 seconds) incase of waste incineration. By cement kiln incineration with higher temperatures (1100-1600 C degrees), the risk of dioxins emissions occurs during the cool down phase. As well during the heath up in start-up phase waste material of high burning quality (PCB oil, plastics, old car tyers) might be used as well to secure the required high combustion temperatures needed for production of a cement kiln.

Dioxins are Unintentional formed Persistent Organic Pollutants (POPs), and therefore also called UPOPs



OctaChloroDibenzo-p(ara)-Dioxin (OCDD) | OctaChloroDibenzoFuran (OCDF) | Dioxin-like PCB 126

Dioxins PCDD
Polychlorinated dibenzo-p(ara) dioxin

Furans PCDF
PolyChlorinated DibenzoFurans

Dioxin-like PCB (dl-PCB)
PolyChlorinated Biphenyl

Credit: ToxicoWatch; used with permission.

Figure 1: What are dioxins?

In 2019, stricter regulations for waste incinerators were established regarding emissions of POPs in Europe (Neuwahl et al.,2019). However, a glaring loophole to avoid these restrictions is the exemption for permits given before 2019. Thus, 'old' incinerators can still emit high levels of POPs due to these permits. Even new, recently built waste incinerators can get 'off the hook' due to governmental accordance with permits allowed *before* 2019. One of the oldest and largest waste incinerators in Paris, Ivry Paris XIII, which has been operating since 1969, started to be rebuilt in 2024 to modernise the technical equipment and transform the plant into a state-of-the-art Waste-to-Energy (WtE) waste incinerator – but its operation is *still* based on a permit *before* 2019. Although there are various worldwide regulations in play, the major problem is accurate and *transparent* enforcement based on specific knowledge of the targeted POPs.

One of the critical issues for emission control concerns monitoring the deposition of combustion-related industry aerial outputs to the surrounding environment(s). There is a critical need that monitoring of polluting entities be performed by *independent* organisations. This means not being connected to the specific industry branch involved, nor to enforcing government agencies.

In the last two decades, it has become clear that there is insufficient specific knowledge in governmental and enforcement organisations about *which*, and in *what quantity* POP loads are threatening the health of local communities living in the surrounding regions of POP emitting industries. Perhaps not surprisingly what has been revealed – over many decades – are (far) too laxed attitudes towards monitoring conditions (duration, frequency, targeted POPs).

To contribute towards filling this gap, ToxicoWatch (TW), a Public Benefit Organisation, has initiated and continues to develop a science-backed, fully documented biomonitoring approach with which to characterise and measure emissions of dioxins, PFAS, PAH, and heavy metals in the surrounding environment of POP emitting industries. In this article several cases are presented showing how current TW biomonitoring research contribute to informative, transparent documentation and characterisation of POP pollution in the environment. Within this scope, this article has a particular focus on the many kinds of new *innovative sampling media* that has been found useful for this type of biomonitoring research projects.

2. Why is Biomonitoring needed?

Human society is increasingly confronted with pollutants that are persistent, bioaccumulative and extremely toxic, even at very low concentrations. Dioxins, dioxin-like PCBs and PFAS present a significant challenge to human health due to their hormonal effects. Waste incinerators are equipped with comprehensive Air Pollution Control Devices (APCD) designed to eliminate air pollution. However, it should be noted that emissions may occur during emergency situations, when the functionality of these devices may not be fully optimised. These periods are often very short, and even with semi-continuous measurements, it can be challenging to capture them accurately (Arkenbout et al., 2018). Considering this, it may be beneficial to implement structural biomonitoring into the existing monitoring regime, in addition to regular chimney emissions measurements. Biomonitoring, which involves measuring these highly toxic environmental pollutants in various biological media such as eggs of backyard chicken, cow/sheep milk and vegetation, could provide a more comprehensive overview of actual accumulated emissions to the environment. This approach can offer a better understanding of the ongoing POP-emissions, as opposed to a brief measurement in the chimney under ideal conditions.

These Substances of Very High Concern (SVHC) degrade only very slowly, for which reason they are aptly called ‘forever chemicals. Once produced/released/emitted, they remain present in the environment for years, decades, or longer – and *some* toxic substances stay in the human body for life.

3. TWs first biomonitoring initiatives (2013)

In 2013 TW initiated a biomonitoring research program in the surrounding area of the most recent Waste-to-Energy (WtE) incinerator in the Netherlands, REC, operated since 2011. The study focused on the issue of *dioxins occurring in the surrounding environment of this state-of-the-art WtE incinerator*.

3.1 ToxicoWatch's first innovative sampling medium

The first biomonitoring research by ToxicoWatch in 2013 was focused on innovative use of backyard chicken eggs from private chicken coop owners living nearby as a novel sampling medium. There are several challenges for using such a bioaccumulating medium, which is subject to *biomagnification*, *biotransformation*, and *xenobiotic metabolism*.

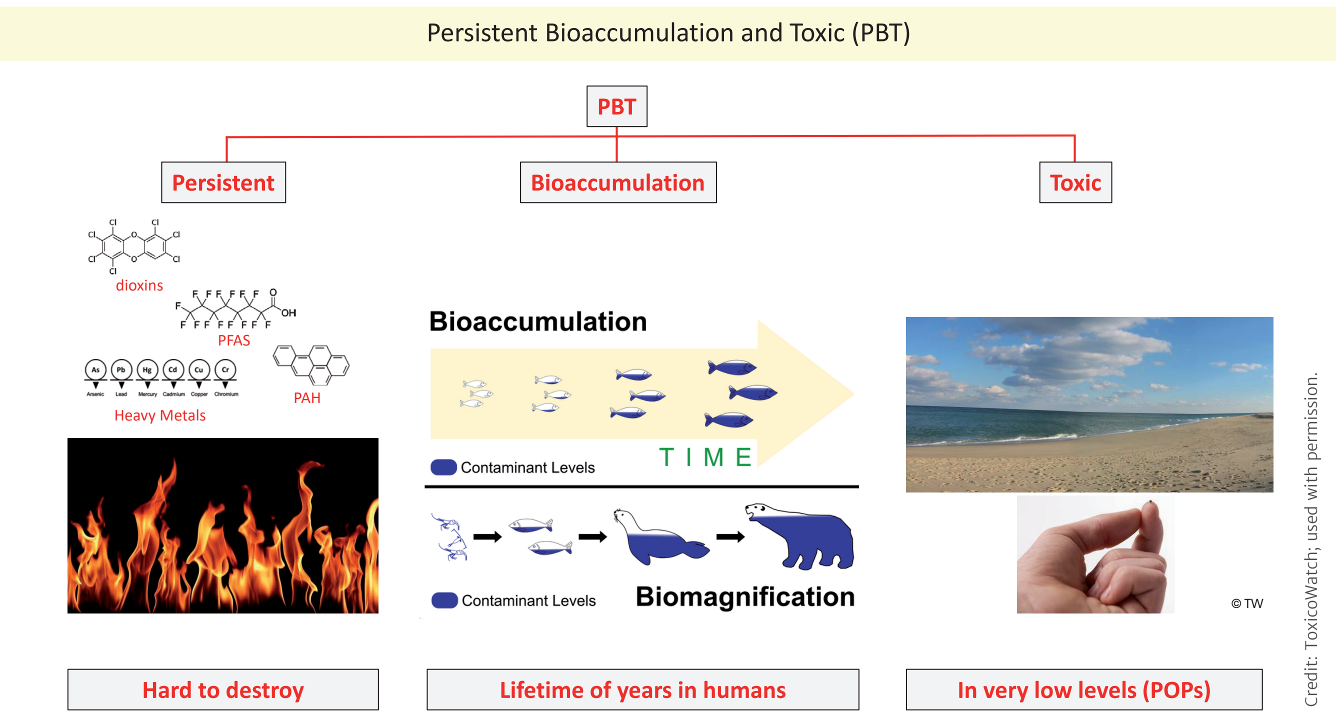
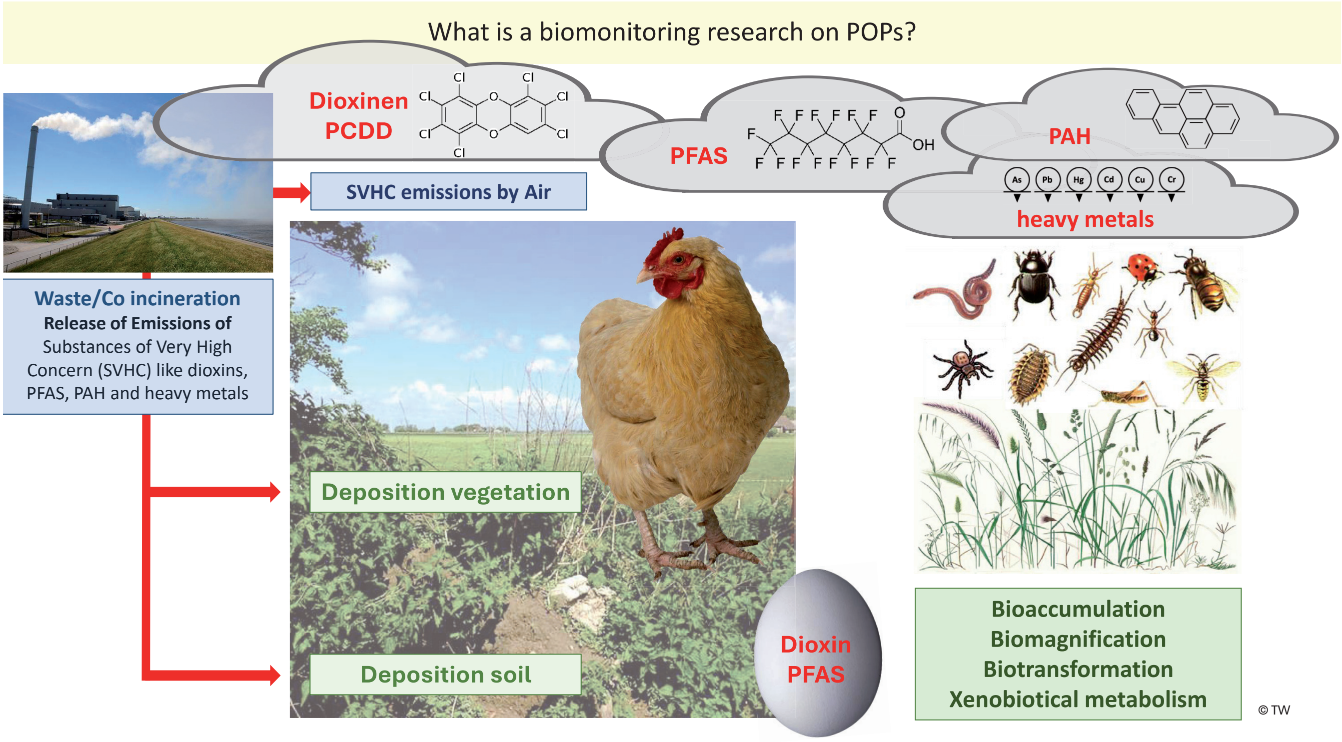


Figure 2: Key properties (Persistent-Bioaccumulation-Toxic) of Substances of Very High Concern (SVHC)



Credit: ToxicoWatch; used with permission.

Figure 3: The setting of the use of the novel sampling medium backyard chicken eggs for measuring pollution loads of dioxins, PFAS and heavy metals.

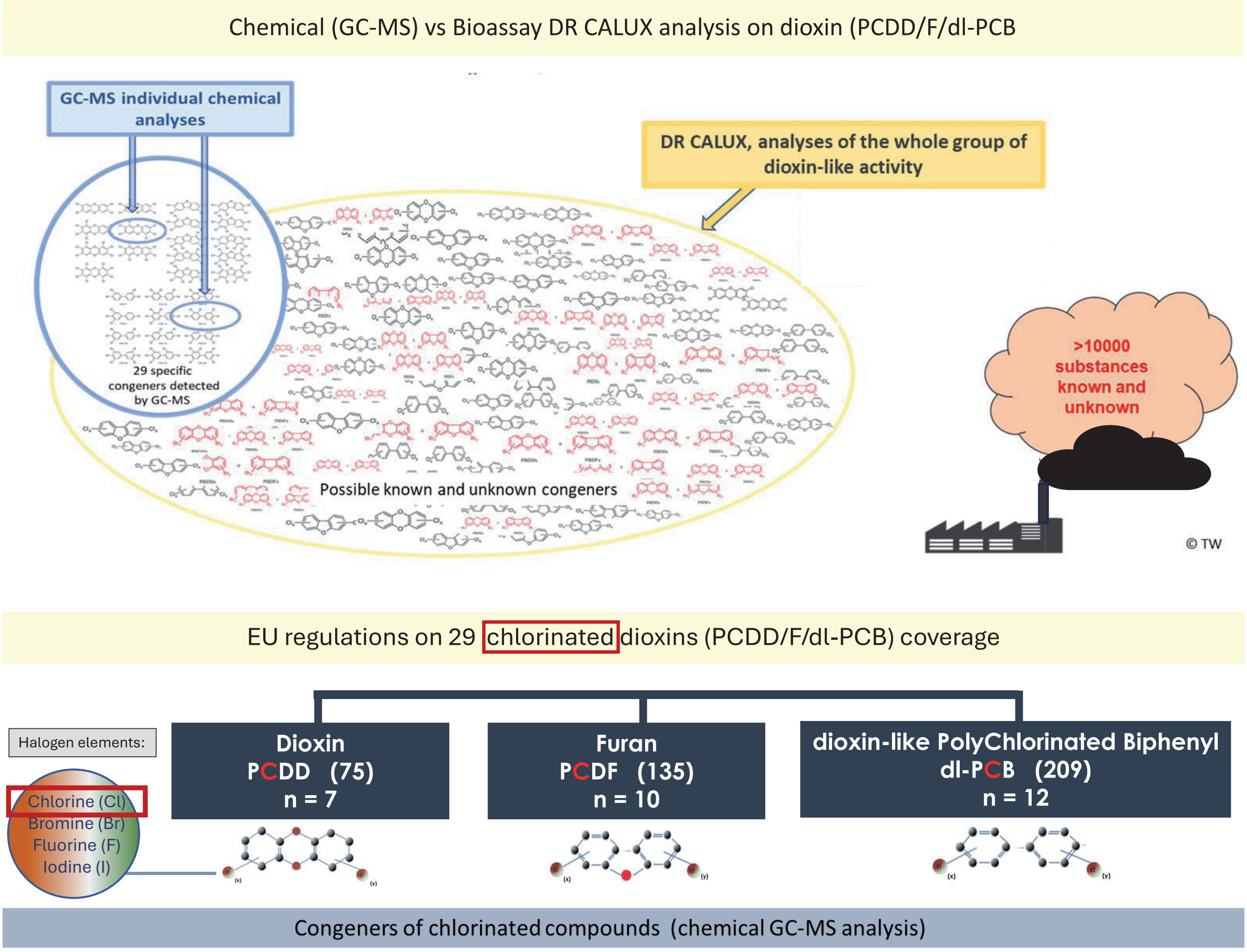
While critical for the scientific understanding of biomonitoring, these analytical aspects are not treated in full here, as ToxicoWatch has discussed these challenges extensively elsewhere in the literature and at relevant scientific conferences (Jovan et al., 2024; Musilova et al., 2024; Qarri et al., 2019; Arkenbout & Esbensen, 2017), see chapter 3.2.

3.2 Bioaccumulation, biomagnification, xenobiotic metabolism

Within the domain of sampling-for-analysis, biomonitoring presents a series of complex challenges regarding the specific analytes used for quantifying deposition load. Whereas most objectives involving sampling organised within the IPGSA community are aimed at analysis and quantification of simple, singular analytes, which are (relatively) easy to quantify e.g., base metals, heavy metals, precious metals, rare earth elements (REE), mineral commodities, food and feed components. Biomonitoring involves quantifying more complex 'analytes', many of which are subject to potential forms of interference in the form of bioaccumulation, biomagnification, and xenobiotic metabolism.

3.3 DR CALUX

The DR CALUX (Dioxin-Responsive Chemical Activated LUciferase gene eXpression) bioassay is a specialized assay used to detect and quantify dioxins, dioxin-like compounds, and other related environmental pollutants. It is based on the use of genetically engineered cell lines that contain a luciferase reporter gene under the control of a dioxin-responsive element. When these cells are exposed to dioxins or dioxin-like compounds, the compounds bind to the aryl hydrocarbon receptor (AhR) within the cells, which then activates the transcription of the luciferase gene. The key features of the DR CALUX bioassay include Sensitivity: The assay is highly sensitive, allowing for the detection of very low concentrations of different types of halogenated dioxins and related compounds. Quantitative Measurement: The amount of light emitted by the luciferase reaction is proportional to the concentration of dioxins present, enabling quantification of these compounds. High Throughput: The assay can be performed in a high-throughput format, making it suitable for screening large numbers of samples quickly and efficiently. Environmental and Food Safety Applications: The DR CALUX bioassay is widely used in environmental monitoring and food safety testing to assess contamination levels of dioxins and similar compounds in various matrices, including soil, water, air, and food products. Generally, the DR CALUX bioassay is an important tool for assessing the presence and potential risk of dioxins and related pollutants in the environment and food supply. CALUX bioassays are novel approaches to fill the data gaps between chemical analyses and the total toxicity of mixtures of substances.



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

Figure 4: Chemical analysis of 29 chlorinated compounds vs bioassay DR CALUX measures a more comprehensive total toxicity of dioxins, not only the EU-regulated 29 chlorinated congeners.

The chemical analysis is based on a limited number of congeners of 29 chlorinated dioxins. CALUX bioassays measure the *biological effect* of a whole *group of toxic substances*. In addition, TW also uses other bioassays, like the PFAS CALUX, which is based on the binding to the thyroid transporter receptor, TTR.

4. First (now famous) results based on backyard chicken eggs

Analytical results of dioxins (PCDD/F/dl-PCBs) in eggs have led to extensive follow-up, and to counter-research by the Dutch Governmental health organisations and industry. For more than two years TW became the sole independent member (representing the concerned population) of the technical working group assembled by delegates of provincial governments and industry/REC management. From activities in a technical work group established by the government and the incinerator plant management, a lot of technical data on emissions of the flue gasses were generated. Remarkably, frequent high dioxin emissions were detected due to technical imperfections in the air pollution control devices (APCD). The outcome of these studies showed that biomonitoring is an essential tool to quantify *realistic loads* of POP deposition in the environment of combustion-related industry.

Counter research on Eggs backyard chicken, RIVM - Dutch government, 2014

Friesland >PCDD/F

	1	2	3	4	5	6	7
	9257	8807	8723	9211	8851	9113	8629
	Noardburgum	Herbaijum	Koudum	Kortehemmen	Tzummarum	Wâlterswâld	Scharne-goutum
2,3,7,8-TCDD	**1,9**	0,97	0,64	0,45	0,64	0,4	0,45
1,2,3,7,8-PeCDD	**2,57**	**1,95**	1,65	1,4	1,23	1,26	0,76
1,2,3,4,7,8-HxCDD	1,78	0,93	1,16	0,89	0,77	0,74	0,44
1,2,3,6,7,8-HxCDD	11,55	3,74	2,66	3,42	2,62	2,67	1,54
1,2,3,7,8,9-HxCDD	**3,21**	1,16	0,69	1,01	0,62	0,94	0,5
1,2,3,4,6,7,8-HpCDD	54,46	77,08	7,54	13,45	9,62	8,2	9,23
OCDD	162,71	100,18	72,22	65,97	165,89	44,5	22,04
2,3,7,8-TCDF	6,5	6,12	3,65	3,6	5	3	3,02
1,2,3,7,8-PeCDF	3,75	2,42	2,27	2,26	2,09	1,89	1,23
2,3,4,7,8-PeCDF	3,64	3,35	2,77	2,87	2,54	2,41	1,57
1,2,3,4,7,8-HxCDF	**3,17**	1,33	1,4	1,68	1,32	1,6	0,77
1,2,3,6,7,8-HxCDF	2,57	1,16	1,03	1,34	0,98	1,38	0,69
2,3,4,6,7,8-HxCDF	2,49	1,22	1,01	1,25	0,98	1,54	0,64
1,2,3,7,8,9-HxCDF	0,24	0,06	0	0,2	0	0,16	0,05
1,2,3,4,6,7,8-HpCDF	10,84	4,29	6,26	4,39	1,85	9,63	1,98
1,2,3,4,7,8,9-HpCDF	0,52	0,16	0,15	0,25	0,15	0,22	0,09
OCDF	3,56	1,13	1,76	1,6	0,68	2,24	0,49
WHO2005-PCDD/F-TEQ (ub)	**9,6**	**6,4**	**4,5**	**4,3**	**4,1**	**3,8**	**2,6**

Eggs as biomarkers of dioxins in the environment:
TW: **Red eggs located near:**
1. **Biomass incinerators** (450 C), producing so called "green gas", heavily promoted as a sustainable energy.
2. **Animal cadaver incineration plant.**

For both: No enforcement and regulation for dioxin/POP emissions.

Figure 5: Map of locations of RIVM, governmental counter-research of backyard chicken eggs in relation to dioxins (PCDD/F (based on Hoogenboom, RLAP, 2016).

Credit: ToxicoWatch; used with permission.

In Europe, although *some* waste incinerators are followed by *limited* biomonitoring, industry unfortunately interferes with this research. Thus, a related study by the Dutch government found elevated dioxins in chicken eggs in *other places* than around the targeted incinerator. First after a legal process lasting two years was TW permitted insight regarding the location of the data obtained by this counter-research, which pointed to potential other dioxin sources, such as *biomass incinerators*. These so-called 'green' biomass waste incinerators act under a much lighter enforcement policy than regular waste incinerators. Biomass plants provide an alternative route for waste deposition. For example, in the Netherlands, a waste incinerator is not allowed to burn impregnated garden wood. The population must pay a fee for discarding impregnated garden fences when brought to municipal collection points, but the treatment of processed/impregnated garden wood is worrisome. According to a municipal spokesperson, treated wood (mostly containing Wolman salts with 2,4-Dinitrophenol, Sodium arsenate, Sodium chromate, and Sodium fluoride) must be delivered to such designated biomass incinerators. But here it is incinerated at *much too low temperatures* in incinerators, which are equipped with much *less efficient* filter systems that in units aimed to destroy the so-called 'Substances of Very High Concern' (SVHC).

5. Pushback

Monitoring with private backyard poultry eggs runs a high risk of adverse interpretation and pushback reactions. In several countries, large-scale media campaigns, run by government agencies, discourage local population from consuming (their own) chicken eggs. Whereas TW employs biomonitoring of backyard chicken eggs as a cost-effective way of measuring air pollution, the government's response places the problem of toxic load on the shoulders of the public. However, the fair intention should be for the relevant government agencies to take measures to identify the *source of pollution* and to *eliminate* or *reduce* unacceptable industrial emissions of hazardous substances. To set the pushback situation it its right perspective we have emphatically stated: "This is not an *egg* problem, but a *pollution* problem."

Below we illustrate the benefits and associated challenges of TW's significantly extended armament of analytic methods, conventional and novel/innovative, targeted and tailored for specific needs.

FACTBOX - Analytical challenges

Sampling biomatrices is not a simple, direct matter. Even sampling of backyard chicken eggs needs to be performed carefully, picking the eggs must be done by the research team first, and/or by TW trained local citizen-participants. It is necessary to perform location inspections before and after sampling, and to conduct a questionnaire interview of the participants involved to avoid interfering factors. TW conducts intensive trainings for this purpose.

Additionally, TW uses mosses from the plant division *Bryophytes*, mostly identified as *Hylocomium splendens* and *Pleurozium schreberi* (local help from professional bryologists is always helpful for specific determination). All mosses, as well lichens, are extremely sensitive to air pollution, for which reason this biomarker is potentially very helpful for TW biomonitoring. In TW's studies, mosses appear to be able to accumulate POPs rapidly; likewise, when toxic waste is discontinued they detox quickly. How the dynamics of xenometabolism is functioning and how the various POPs are taken up is an area of current research.

Figure 6: Complete array of conventional and novel ToxicoWatch biomonitoring sampling media tested in 2013-2025.

6. Innovative bioassay analyses and a broad spectrum of biomatrices for biomonitoring

Following up on the rationale behind the chicken egg approach, TW has further developed many other innovative bioassay analyses for expressing biomarkers to quantify industrial POP emissions. A selection of corresponding sampling media are illustrated in this article: soil, water, sediments, *sheep's wool*, *eggshells* of wildlife birds, mosses (*Bryophytes*), pine needles (*Picea abies; Pinus sylvestris*), evergreen tree leaves (i.e., *Olea europaea*) *liver* (from dead wildlife animals), *mother's milk*. For selected samples, the practical sampling procedures are shown. Figure 6 illustrates the wide range of biomonitoring sampling media that has been tested by Toxicowatch since 2013, see Figure 6 regarding the associated analytical challenges.

Credit: ToxicoWatch; used with permission.

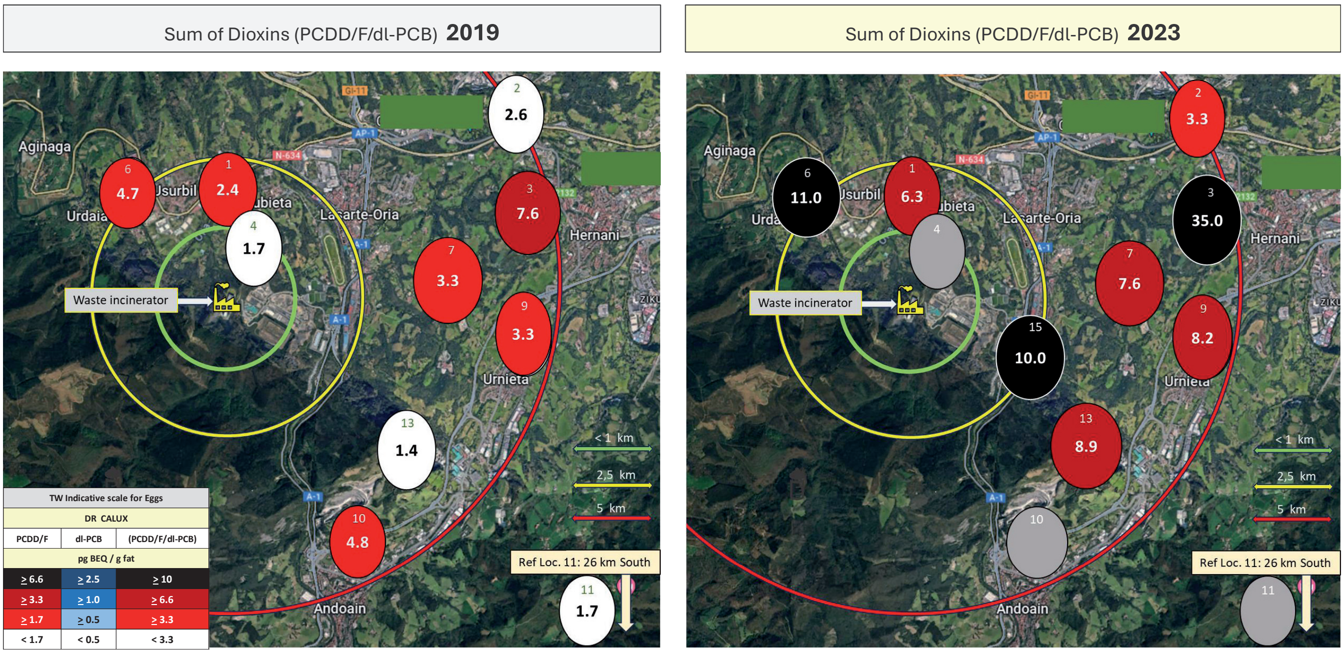


Figure 7: Sum of dioxins (PCDD/F/dl-PCB) results with bioassay DR CALUX in eggs of backyard chicken, Zubieta, Basque country. 'Zero-measurements' in 2019 compared to the results five year later in 2023.

Credit: ToxicoWatch; used with permission.

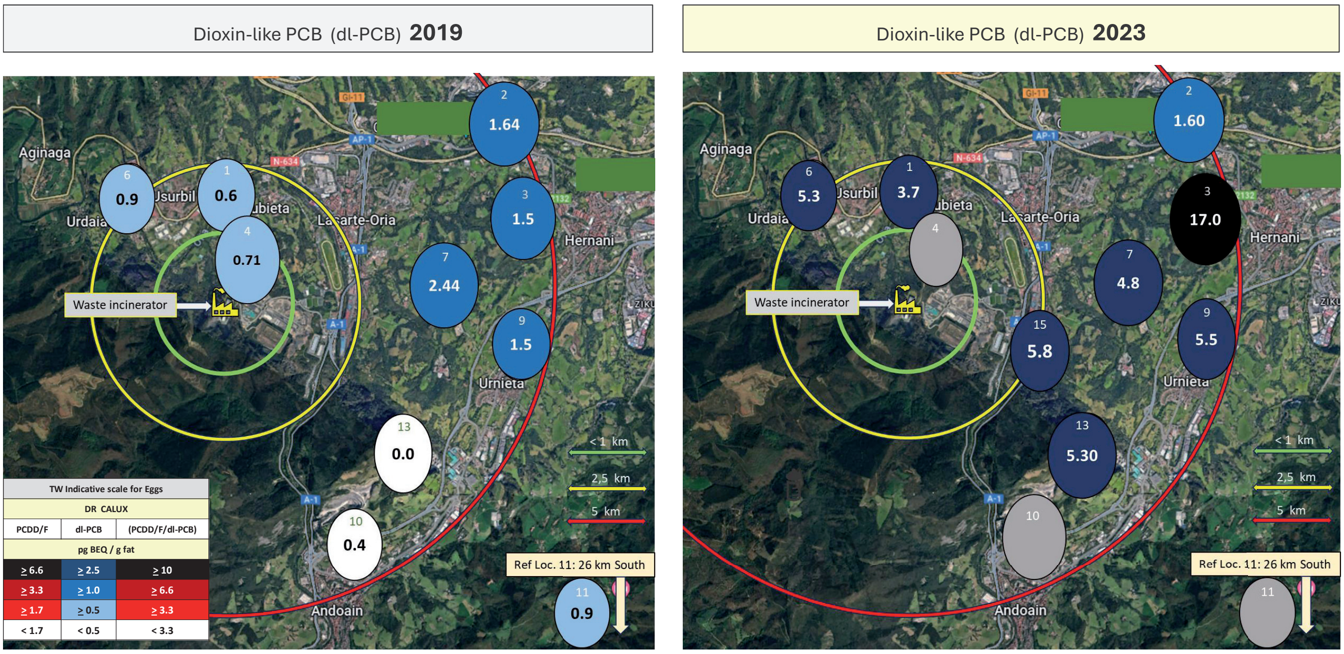
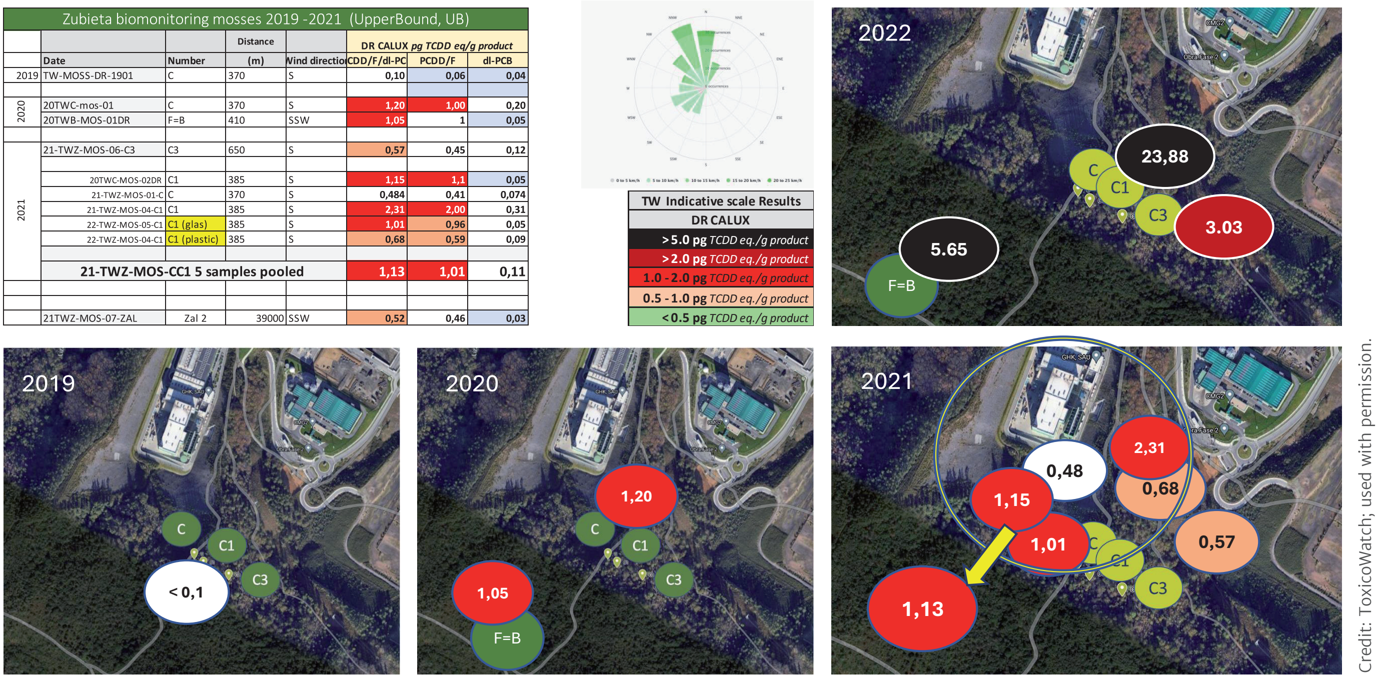


Figure 8: Results dioxin-like (PCBs) with bioassay DR CALUX on eggs of backyard chicken. Comparison of zero-measurements (2019) and five years later in 2023 in the same locations.



Zubieta biomonitoring mosses 2019 -2021 (UpperBound, UB)							
			Distance		DR CALUX *pg TCDD eq/g product*		
	Date	Number	(m)	Wind directio	CDD/F/dl-PC	PCDD/F	dl-PCB
2019	TW-MOSS-DR-1901	C	370	S	0,10	0,06	0,04
2020	20TWC-mos-01	C	370	S	1,20	1,00	0,20
	20TWB-MOS-01DR	F=B	410	SSW	1,05	1	0,05
2021	21-TWZ-MOS-06-C3	C3	650	S	0,57	0,45	0,12
	20TWC-MOS-02DR	C1	385	S	1,15	1,1	0,05
	21-TWZ-MOS-01-C	C	370	S	0,484	0,41	0,074
	21-TWZ-MOS-04-C1	C1	385	S	2,31	2,00	0,31
	22-TWZ-MOS-05-C1	C1 (glas)	385	S	1,01	0,96	0,05
	22-TWZ-MOS-04-C1	C1 (plastic)	385	S	0,68	0,59	0,09
	21-TWZ-MOS-CC1 5 samples pooled				1,13	1,01	0,11
	21TWZ-MOS-07-ZAL	Zal 2	39000	SSW	0,52	0,46	0,03

TW Indicative scale Results
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*

Figure 9: Results for dioxins (PCDD/F) in Mosses (Bryophytes), Zubieta, Basque Country 2019-2022.

7. Selected application cases of dioxin results

Since the start of TW biomonitoring activities in 2013, TW has carried out intensive research in seven European countries; most of these studies are multi-year study projects. Selected illustrative studies are presented here below.

Waste incinerator, Zubieta, Basque Country, Spain

In 2019, TW started a so-called 'zero measurement' experiment in the environment of Zubieta, located in the North of Spain, The Basque Country. This is called a 'zero measurement' study because a year later in 2020 a newly built Waste-to-Energy (WtE) waste incinerator would go into production and therefore TW had the unique opportunity to collect environmental POP data *before* industrial emissions would begin. Concerned local communities contacted TW in 2019 to help with biomonitoring by collecting data on dioxins, PAH, PFAS and heavy metals from the following sampling media (biomatrices): i) backyard chicken eggs, ii) vegetation, iii) water, iv) sediment, v) soil and vi) mother's milk. Every year since 2019, samples have been collected at the same locations in this Basque area. This resulted in data that can be used to monitor the temporal evolutions of POPs in the surrounding environment of the waste incinerator.

Figure 7 shows the analytical results for the sum of dioxins (PCDD/F/dl-PCB) in eggs in the vicinity of the newly built waste incinerator. The first year 2019 established the zero-measurement baseline; these relatively low values are shown on the left in Figure 7. A year later, in 2020, the new incinerator was in full production. Elevated dioxins can already be found in the chicken eggs, but after 5 years, in 2023, the amounts of dioxins have increased dramatically, Figure 7 (right).

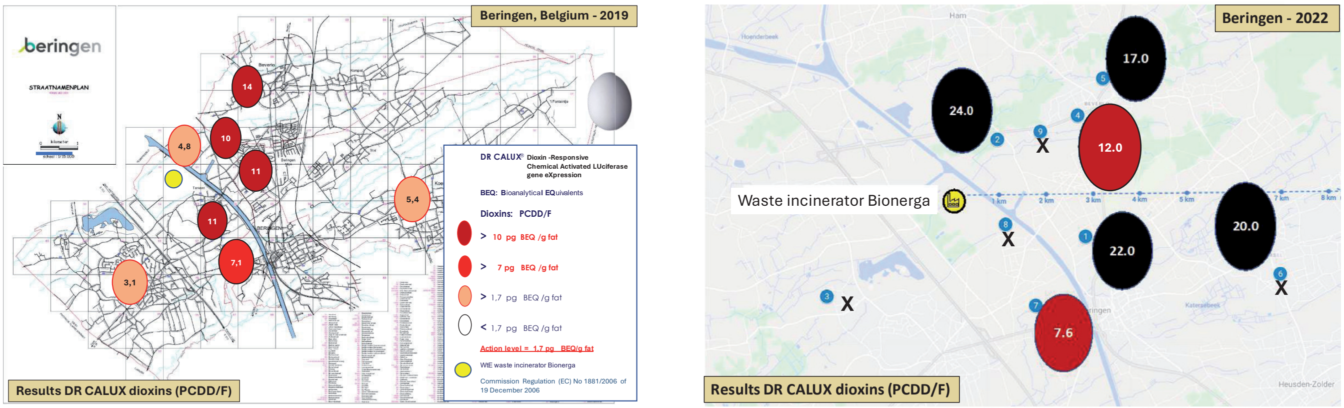
Figure 8 compares the presence of dioxin-like PCB (dl-PCBs) in 2019 and 2023 from the same localities near the waste incinerator in Zubieta, Basque Country. Since the 1970s PCBs have been banned, and it is therefore worrying to measure an increase of these toxic substances after ca. 50 years.

Close to this Zubieta WtE incinerator, mosses (*Bryophytes*) were also collected and analysed for dioxins. Figure 9 shows an example of evolution of dioxin levels in the mosses in the vicinity of the newly built (2020) waste incinerator between 2019-2022: The moss samples (C & B in Figure 9) were collected from the ground 300-500 meters south-west from the incinerator.

7.1 Waste incinerator Bionerga in Beringen, Belgium

In 2019 TW started similar zero-measurements of POP in the surrounding environment of a newly built WtE incinerator Bionerga in Beringen, Belgium, which started production in 2020. The concerned local population, represented by "Leefbaar Tervant", convinced the local government to perform TW biomonitoring for three years (2019-2021/2022) on dioxins (PCDD/F/dl-PCB) in eggs of backyard chicken (Arkenbout & Bouman, 2023). Although the zero measurements already show elevated dioxin values in the backyard chicken eggs in this industrialised area, what is remarkable is the finding of high, significantly above EU-threshold dioxin levels found in the chicken eggs in 2022 (compare with Figure 11).

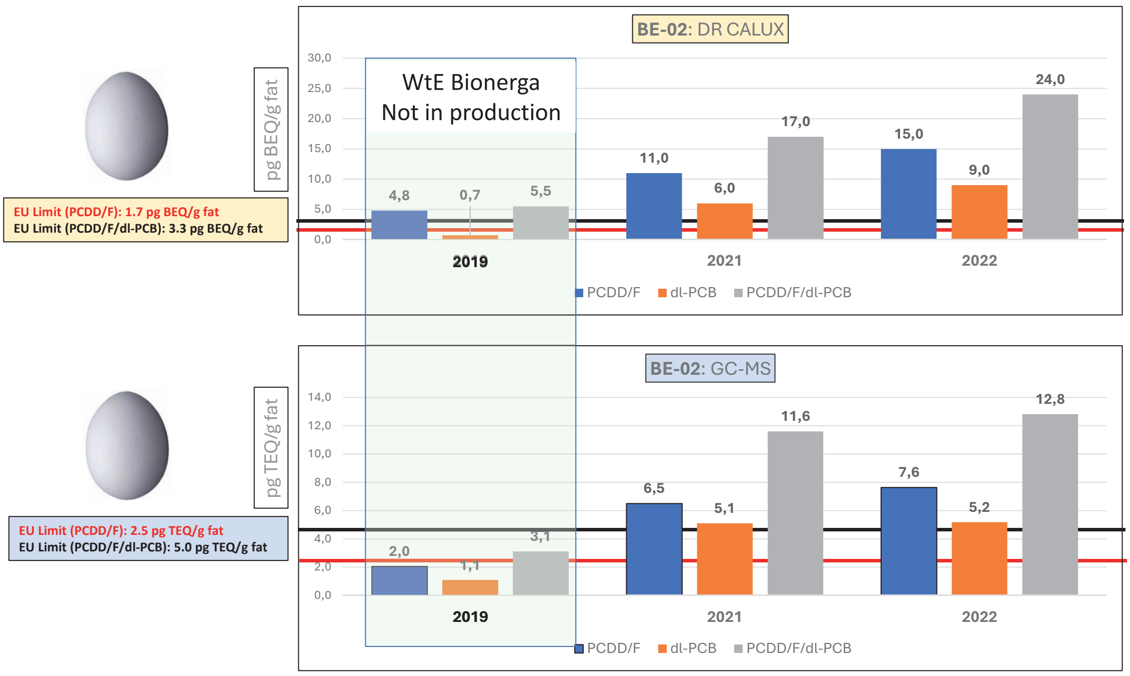
Results Dioxins (PCDD/F) in eggs of backyard chicken, Beringen, Belgium 2019-2022



Credit: ToxicoWatch; used with permission.

Figure 10: Results of dioxins (PCDD/F/dl-PCBs) in eggs of backyard chicken, Beringen, Belgium, which started with zero-measurement 2019 compared to three years later 2022.

EU Limit for Dioxins (PCDD/F) in eggs of backyard chicken, Beringen, Belgium 2019-2022



Credit: ToxicoWatch; used with permission.

Figure 11: EU limit for dioxins in backyard chicken eggs, Beringen, Belgium 2019-2022.

8. Temporal monitoring – analyses of technical data for flue gasses

These newly built waste incineration plants in Zubieta and Beringen have semi-continuous measurement regimen for dioxin and furan (PCCD/F) emissions through long-term flue gas sampling. In principle, this system should be able to provide adequate temporal monitoring, but it appears to be prone to malfunction-

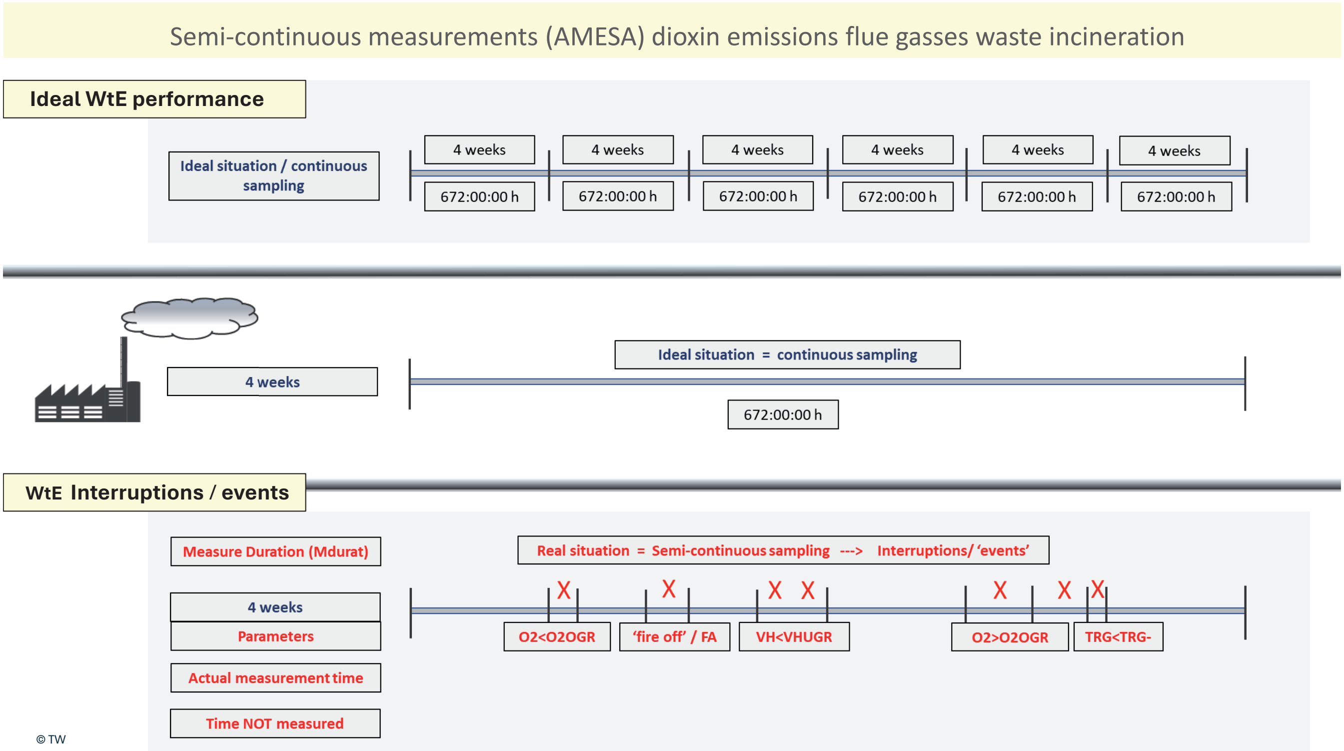


Figure 12: Example of interruptions in the semicontinuous sampling of flue gases of a waste incinerator.

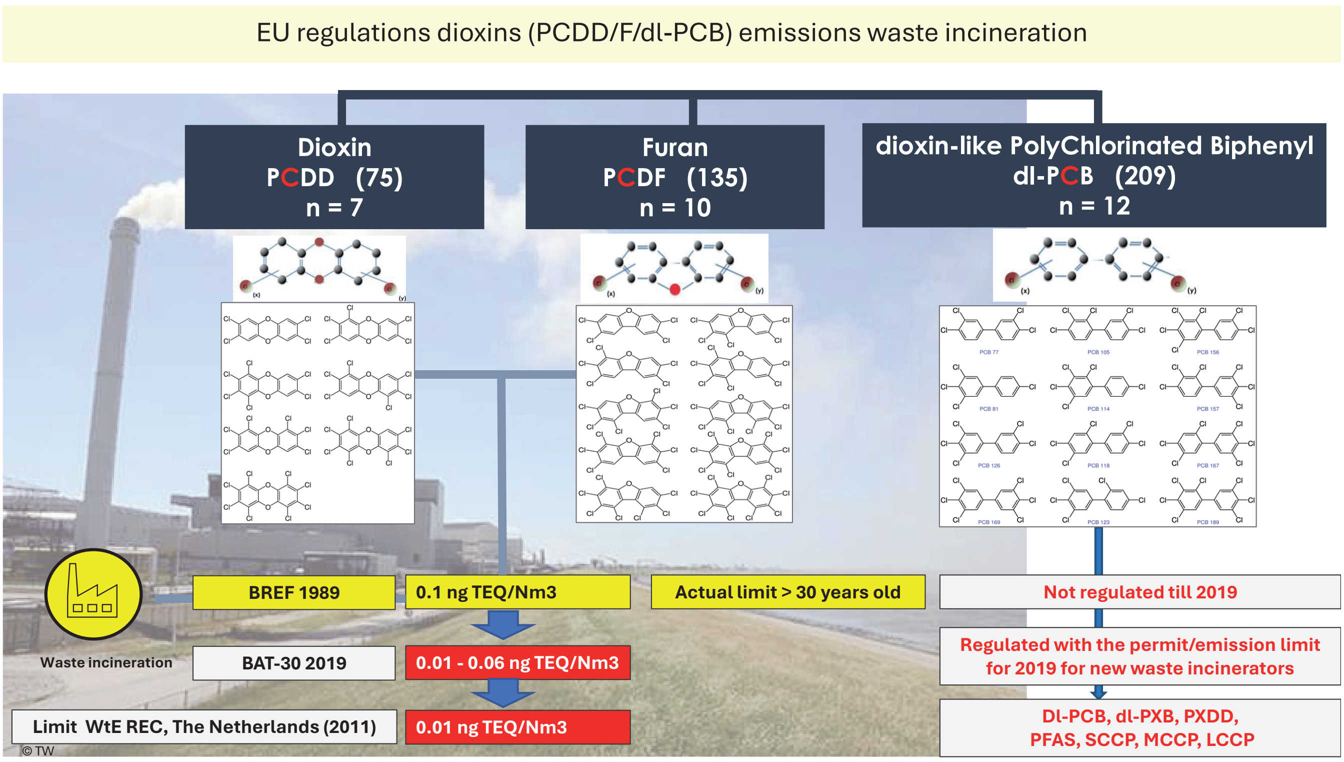


Figure 13: EU regulations: permit thresholds for dioxins emission in flue gasses from waste incinerators.

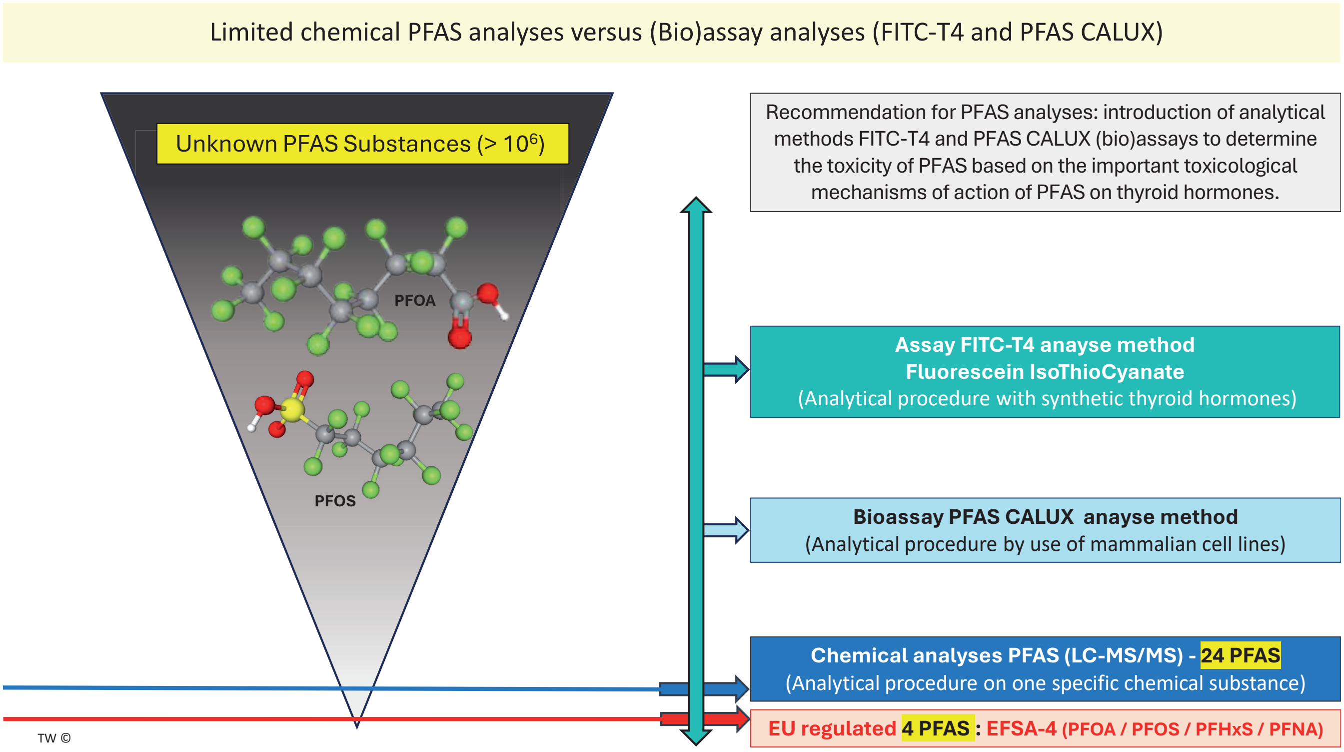
ing exactly at the very moments when measurements are mostly needed. Monitoring is brought to a halt when flue gas flow drops below a given velocity, similarly for temperature, and/or oxygen levels. This unfortunately results in that sampling stops exactly *when there is an enhanced likelihood* for production of elevated levels of toxic substances such as dioxins, PFAS, and PAH – rather the opposite of what is required. Figure 12 shows an example of a similar situation in Paris, France where TW has performed technical research on the semi-continuous minute data of the flue gasses for two years.

9. Temporal monitoring rationale – consequences

Situations deviating from normal operation conditions during waste incineration are referred to as 'Other Than Normal Operating Conditions' (OTNOC). New EU rules specifically address this issue, acknowledging that increased dioxin (PCDD/F/dl-PCB) emissions likely can occur during these events. But the implementation of this new monitoring regime is proving difficult because measuring dioxins during a calamity such as a shutdown or start-up is technologically challenging.
In many countries, there has been hardly any modification of the maximum limit of emissions of dioxins. For example, this limit has since 1989 stood at 0.1 ng TEQ/Nm³. It is only now that some countries are adjusting the limit down to 0.04 - 0.06 ng TEQ/Nmn³ for waste incinerators, according to the Best Available Techniques BAT-30 (Neuwahl, et al., 2019). However, there is still a limited regulatory policy based on analysis only of 17 chlorinated dioxins and not for chlorinated dioxin-like PCBs (regulated with an emissions permit of 2019), nor for brominated or mixed halogenated dioxin - and there is no monitoring of PFAS emissions, Figure 13. From a toxicological point of view this is decidedly *worrying*.

10. PFAS and combustion-related emissions

TW was one of the first entities to find and measure PFAS (2016-2017) in flue gas data from the latest state-of-the-art WtE waste incinerator REC/Harlingen, in the Netherlands. In the following years, TW has conducted analyses for PFAS in *eggs*, *mosses (Bryophytes)*, *pine needles (Picea abies*, *Pinus sylvestris)*, soil, sediment, water, and *wildlife*. The findings of toxic compounds such as dioxins, PFAS and heavy metals, is a clear call-to-action for the responsible authorities to ensure, by (semi-) continuous monitoring of the flue gases, public health safety for the populations living in the surrounding areas.



Credit: ToxicoWatch; used with permission.

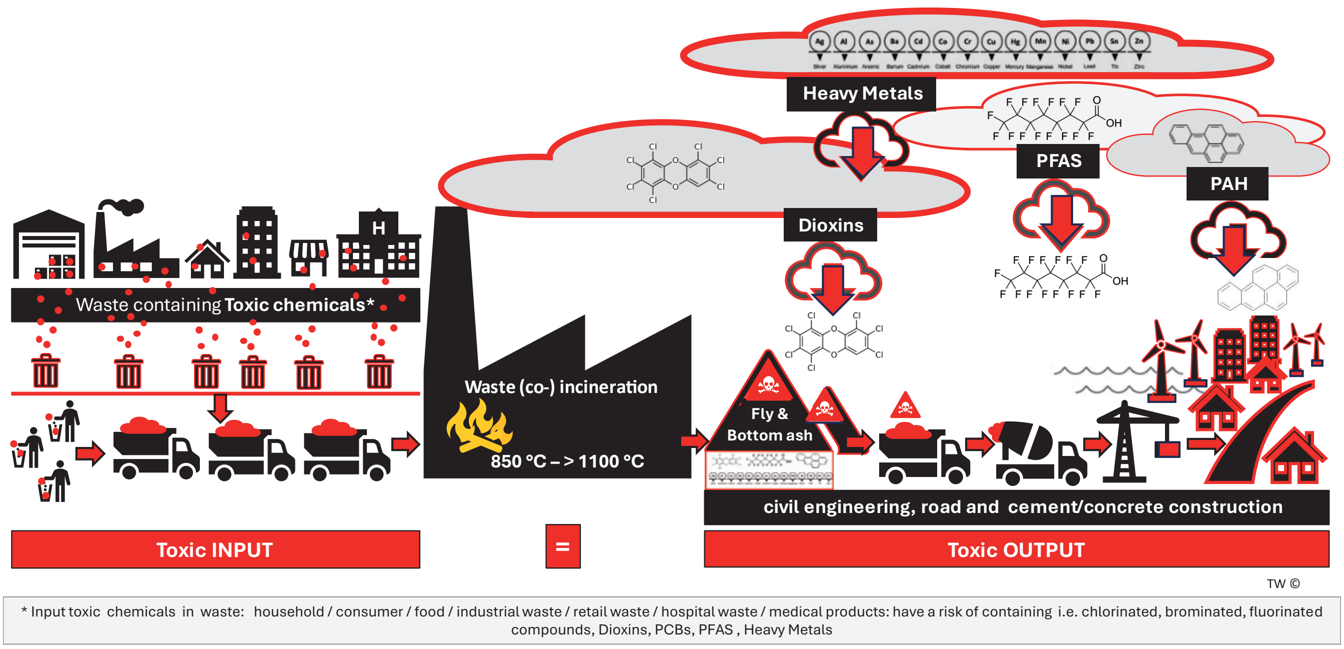
Figure 14: Limited chemical PFAS analyses of fluorinated substances versus (Bio)assay analyses (FITC-T4 and PFAS CALUX).

For broad screening of toxic fluorinated substances TW applies chemical PFAS analyses on 24 fluorinated compounds by use of (bio)assay PFAS CALUX and FTIC-T4, Figure 14. Many PFAS are possible thyroid hormone system disrupting compounds, because they have the capacity a.o. to inhibit the TH thyroxine (T4) from binding to its transport protein transthyretin (de Schepper, et al., 2023).

Approximately 68 million tonnes of municipal solid waste were incinerated in 2017 by all 27 countries in the EU, which generated about 15 million tonnes of municipal solid waste incineration bottom ash (MSWI BA). According to EU's new "Green deal" strategy, these solid wastes are intended to be a valuable resource as secondary raw materials. Thus, after separation of metals, the remaining mineral fraction is mostly reused as unbound aggregates for construction of road base layers and used in civil concrete construction works. However, "the pollution is passed on to future generations," according to the Human Environment and Transport Inspectorate (2019). A full review of bottom ash is given in the report of Arkenbout and Bouman (2025), Figure 15.

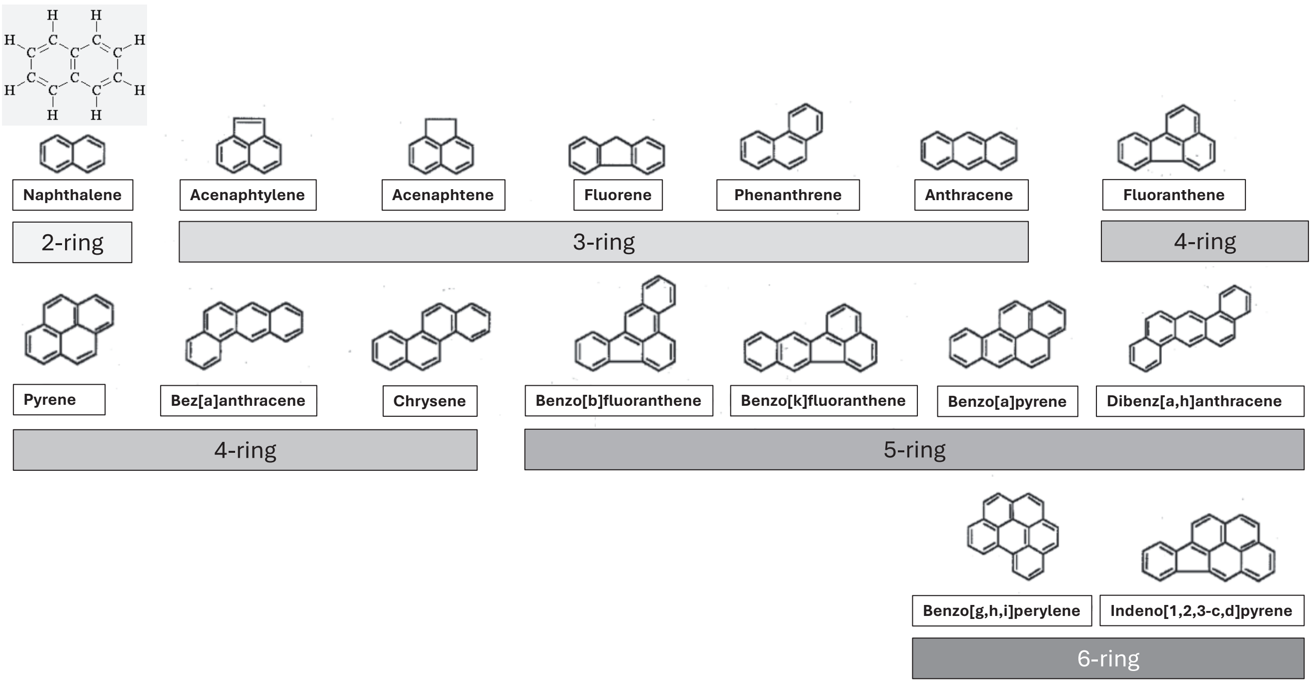
TW biomonitoring research employs chemical analyses of 16 different PAH substances, and if needed (for example triggered by high analytical results) these are extended with PAH bioassay analyses (PAH CALUX), giving a broader scope of the effect of PAH substances.

It is important to note that this group is quite extensive and that a significant proportion of the substances cannot be analysed via conventional chemical methods. Two and three-ring PAH are emitted by petrogenic (petroleum and by products) emissions, like car exhaust gases. Four-ring PAH is pyrogenic source related, like natural forest fires and (natural) burning of biomass. PAH that contains a five or a six-ring are Pyrogenic related, meaning from industrial combustion high temperatures (850-1600 C degrees). An overview of the 16 PAH congeners of chemical analysis is presented in Fig. 16.



Credit: ToxicoWatch; used with permission.

Figure 15: Pathways of PFAS, Substances of Very High Concern (SVHC) from waste content into toxic emissions and residues.



Credit: ToxicoWatch; used with permission.

Figure 16: Congeners (16) of Polycyclic Aromatic Hydrocarbons (PAH) in chemical analyses.

11. Biomonitoring the surrounding environment of the cement kiln, Turňa nad Bodvou, Slovakia

In 2024, a TW biomonitoring program was started concerning possible emissions from a cement kiln in the region of Turňa nad Bodvou in Slovakia, which is fuelled a.o. by co-incineration of PCB oil, old used car tyres and limestone from mining operations. An extensive program was initiated based on samples of backyard chicken eggs/eggshells, wildlife meat from deer, Carp fish (*Cyprinus carpio*), wildlife bird eggshells from Heron (*Ardea*), mosses (*Bryophytes*), pine needles (*Picea abies*), water from natural water stream and wells, sediment from natural water streams and wells; soil was also collected and analysed in accredited labs in the Netherlands.

Substances like dioxins, PFAS, PAHs, and heavy metals are extremely toxic even at very low concentrations and are currently only minimally monitored in the environment of the cement kiln. The population in the vicinity of these facilities lacks transparent emission information from this potential source of pollution. Raw analytical information, preferentially with high resolution, will allow the powerful variographic characterisation well known from the TOS regime (Esbensen 2025). TW started research on the cement kiln in 2023, while the government began environmental biomonitering activities for the first time in 2024.

TW began with analysing fourteen heavy metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn and Zn) in soil and found a children's playground in Dvorníky-Včeláre seriously contaminated with lead (Pb) 110 mg/kg and Arsenic (As) 48 mg/kg; the playground is situated at the short distance of 600 meters from the cement kiln. If/when a child ingests soil from this playground (adsorbed on skin, hand or food, or on food), there is a very likely risk it will exceed the tolerable daily intake of lead and arsenic. The risks of both metals are known to cause adverse neurodevelopmental effects, e.g., reduction of cognition and reasoning abilities (Sprong et al., 2023; Swartjes et al., 2017). Further research is *urgently* needed, as these high levels of heavy metals send a strong warning.

12. Heavy metals in mosses (*Bryophytes*)

As part of the same research and monitoring project, TW has also carried out analysis for the same set of 14 heavy metals (HM) in eggshells, pine needles (*Picea abies*) and mosses (*Bryophytes*).

FACTBOX - Sampling mosses (*Bryophytes*) for biomonitoring

Many organisms in the plant kingdom are commonly called *mosses*, even if belonging to different groups of organisms, like *lichens* (symbionts of algae and fungi) or 'Club moss', 'Spanish moss' (which belongs to the group of vascular plants), and even 'Irish moss', but the latter species belongs to the group of algae. In TW biomonitoring research, only mosses from the phylum *Bryophyta*, specifically from the Class *Bryidae*, which contains most moss species in the world (> 9500), are used and analysed to monitor the aerial load of persistent organic pollutants (POPs).

Credit: ToxicoWatch; used with permission.

Unlike vascular plants, mosses lack specialized vascular tissues such as xylem, conducting cells (uptake water and dissolved minerals from the root system to the rest of the plant, and proving physical support) and phloem, the sieve tube cells (tissue for transport glucoses/energy, located behind the bark tissue), besides true roots, stems, and leaves. This means *bryophytes* rely on direct absorption primarily through their leaflike and stemlike structures, or directly through the cells of their gametophyte body for water and nutrients, and thus, also for POPs.

Their surfaces are in direct contact with the environment, allowing them to absorb substances from the ambient air and water. This characteristic makes mosses effective bioindicators for monitoring environmental pollution, as they constantly accumulate pollutants from their surrounding habitat.

Figure 17: Sampling mosses (*Bryophytes*) for biomonitoring, photos showing moss sampling in natural refence areas in Zubieta, Basque Country, Spain and in Paris, Ivry-sur-Seine in France 2024.

The uptake of persistent organic pollutants (POPs) such as dioxins, polycyclic aromatic hydrocarbons (PAHs), per- and polyfluoroalkyl substances (PFAS) and heavy metals can differ between *bryophyte* species. Several factors contribute to these differences:

1. **Surface area and morphology**: Different *Bryophyte/Bryidae* species have varying surface areas and structural features, which influence the extent of pollutant absorption. Species with a larger surface area can absorb more pollutants.

2. **Habitat Preference**: *Bryophytes* that grow in different environments may be exposed to varying levels of pollutants. For example, species that thrive in urban or industrial areas will encounter higher ambient concentrations of certain pollutants compared to those in more pristine natural environments.

3. **Physiological and biochemical Differences**: Different species may have varying capacities for binding, sequestering, or metabolizing pollutants. These physiological and biochemical differences can affect how pollutants are absorbed, translocated, or stored within the plant.

4. **Cuticle development**: The cuticle is a fatty/wax 'skin' layer of plant leaves. The waxy cuticle and stomata (pores on the leaf surface for gas exchange) are of importance for transport of these xenobiotics and makes these organisms good biomarkers (Matos et al., 2022). While *bryophytes* generally have a poorly developed cuticle, the degree of cuticle development can vary among species, potentially influencing pollutant uptake. The lipid content of the cuticle, which may divers between species, is important for xenobiotic transport.

5. **Water retention capacity**: Species with higher water retention capacities might hold pollutants longer, affecting their uptake and accumulation.

Overall, differences in uptake among *bryophytes*/mosses species must be considered when used as bio-indicators for monitoring environmental POP pollution, as they may also reflect the levels and types of other aerial compounds present in their habitats. Their use for biomonitoring is dependent upon *competent identification* and the use of *composite sampling* involves balanced material increments across year-classes: only on this basis can analytical levels between polluted and reference areas be reliably compared. These challenging aspects of analyte quantification are the object of focused R&D for ToxicoWatch, to be reported in due time.

When the moss samples collected in the surrounding area of the cement kiln are compared with *reference samples* collected at locations in the Slovak Karst National Parc (SKNP) 20 km away, strong local elevations are revealed. At some locations, a factor of 10 more heavy metal loads is observed. If the content of heavy metals in mosses is compared with EU regulations, or to recommended national limits, there is a warning signal for Al, As, Co, Cu, Ni, Pb and Sn. The living environment in this area is under strong pressure and may pose health risks for the local population if/when consuming homegrown vegetables and fruit. These results are grounds for an urgent call for a more intensive and comprehensive monitoring of heavy metals in the environment of the cement kiln industry and other (co-) waste combustion-related industries.

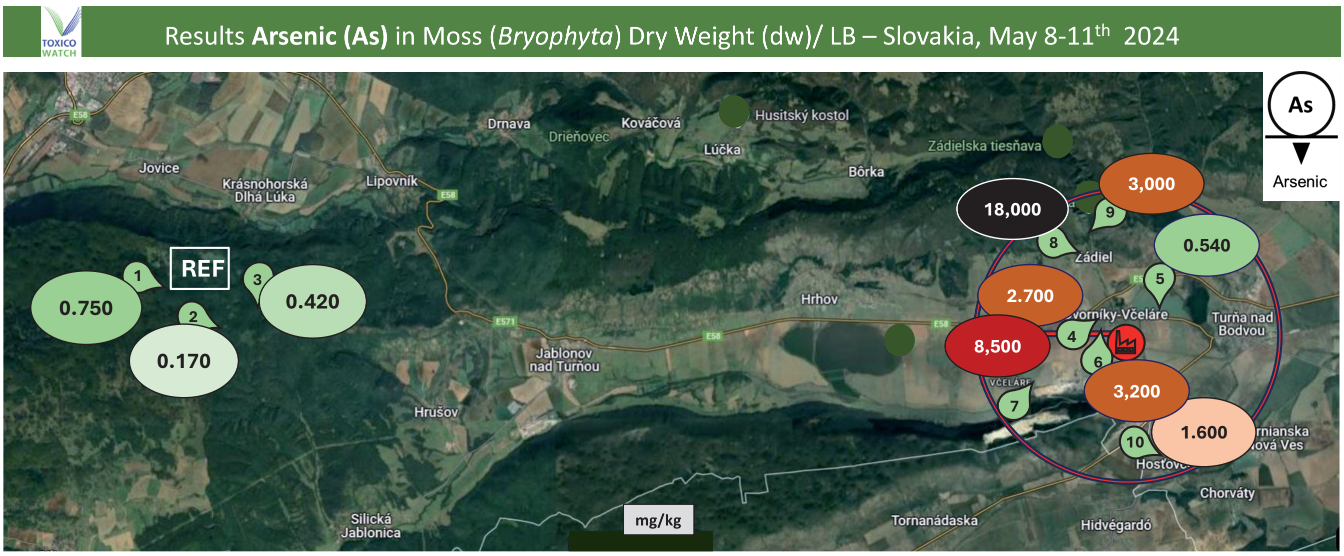
The key issue is whether the observed increased heavy metal concentrations are residues of (historical) agricultural pesticide use or the result of emissions from the cement kiln? To make a judgement about the source of this pollution, uncorrected (semi-) continuous minute-by-minute data from the cement kiln control room is required, including data/measurements during calamities. Monthly monitoring of several hours will be required, but most industry still operates on a measurement regime of only a few hours every six months, announced in advance at that. Given the concentrations of POPs that are detected in the environment, these measurements should be extended with periodic independent biomonitoring to protect the environment and people's health.

Presented below are further examples of TW biomonitoring of heavy metal loads in mosses collected in the vicinity of the cement kiln in the region of Turňa-nad-Bodvou, Slovakia compared with samples in the nature reserve of the Slovak Karst National Park (SKNP) collected using the same field procedures. Concentrations as much as 40 times higher than in the reference samples are observed.

There is a special caution regarding the level of arsenic (As), which in some places is as much as 360 times higher than the safe limit value for arsenic in vegetables to be allowed for consumption, Fig. 18.

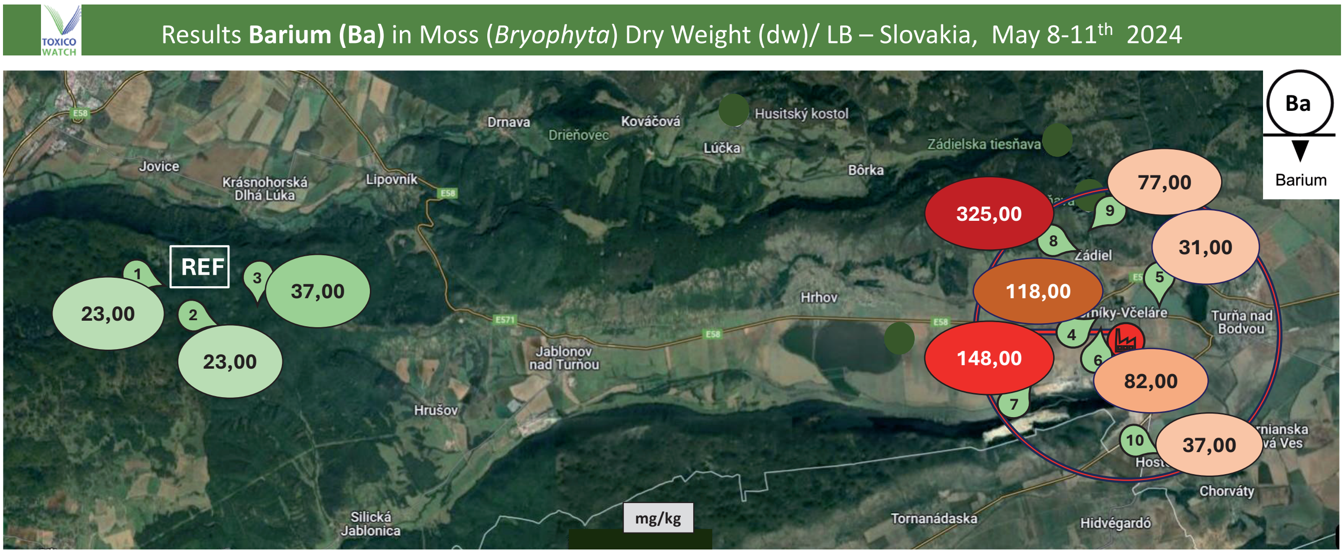
12.1 Barium (Ba)

It is striking that several heavy metals are not measured by default in cement kiln emissions, for instance, Barium (Ba) even though this metal is used in the cement formulation. Cement containing barium (Ba) is used as a binder that resists various types of radiation. Barium (Ba) is extensively used in manufactured materials, including tiles, automobile clutch and brake linings, rubber, brick, paint, glass, and other products. Unusually high concentrations of this metal in soils may indicate anthropogenic activity. Additionally, Barium is commonly found in wastes. For Barium, the Dutch Health Organisation, RIVM, use a tolerable daily intake of 0,6 mg/kg body weight/day, following the approach of the US Agency for Toxic Substances and Disease Registry (ATSDR) in its 2005 report on the toxicological profile for barium, based on animal experiments data (Commission Regulation (EU), 2013).



Credit: ToxicoWatch; used with permission.

Figure 18: Analytical results for Arsenic (As) in mosses (*Bryophytes*) in the environment of the cement kiln, Turňa-nad-Bodvou, Slovakia.



Credit: ToxicoWatch; used with permission.

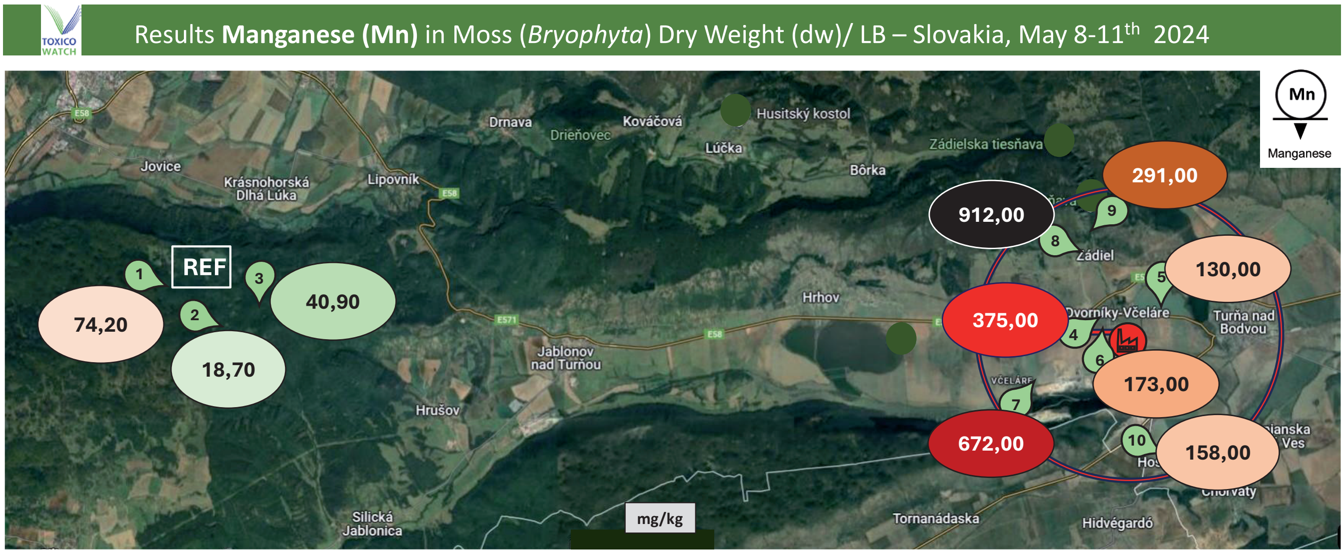
Figure 19: Results Barium (Ba) in mosses (*Bryophytes*) in the environment of the cement kiln, Turňa-nad-Bodvou, Slovakia

The observed Ba levels in mosses in area surrounding the cement kiln in Turňa-nad-Bodvou, Slovakia are presented in Fig.19, locally revealing *very high* levels.

12.2 Manganese (Mn)

Manganese is one of the major trace metals in ordinary Portland cement, which is mainly introduced from alternative fuels and secondary raw feeds during the combustion process of clinker production. Remarkably, no monitoring program is applied for this heavy metal by the cement kiln. Manganese (Mn) emissions and deposition are also related to incinerator processes (Rovira J. et al., 2010).

Long-term exposure to high levels of Manganese can result in serious health effects on the central nervous system such as visual reaction time, hand-eye coordination and hand steadiness. Exposure to higher levels over a long period can result in a syndrome known as *manganism*, which leads to feelings of weakness and lethargy, tremors and psychological disturbances (Kwakye et al., 2015). Figure 20 presents the analytical results for Mn in mosses, with an identical pattern of local *very high* levels as was observed for Ba.



Credit: ToxicoWatch; used with permission.

Figure 20: Results Manganese (Mn) in mosses (*Bryophytes*) in the environment of the cement kiln, Turňa-nad-Bodvou, Slovakia.

12.3 Lead (Pb)

Lead (Pb) is a naturally occurring element and is one of the longest-established poisons, as emphasised by recent excavations in Pompeii and Herculanum: it is today well-known that many Romans lived in a state of constant lead poisoning due to the widespread use of lead in water piping systems. In addition, it was historically in use in orchards. Lead (Pb) is a highly toxic bio accumulative element, which doesn't degrade easily when metabolized. Rădulescu and Lundgren (2019) found measurable cognitive decline (reduced IQ, academic deficits), especially in children. Lead (Pb) exposure can cause *plumbism*, anaemia, nephropathy, gastrointestinal colic, and degenerative central nervous system symptoms. Neurological symptoms include ataxia, encephalopathy, seizure, swelling of the optic nerve, and disorder of consciousness. The EU regulation for the Maximum Level (ML) of lead (Pb) is set at 0.1 mg/kg wet weight (ww) for fruits and roots, and 0.3 mg/kg ww for leafy greens (EU Commission Regulation, 2023).

All moss samples in the TW Slovak research area exceed the EU maximum limit for lead (Pb) in vegetables, in some locations by more than a factor of 1000! The current consensus is that no level of lead (Pb) exposure should be considered as 'safe'.

12.4 Zinc (Zn)

Zinc is a heavy metal, which is not monitored by the cement kiln. But zinc is overwhelmingly present in waste streams, according to TW analytical results of water and sediment samples in 2024. A major source of zinc is car tyres. Because cement kiln plants also burn used car tyres, and despite included in the European Green Deal, Zinc will be emitted as a highly volatile metal. Atmospheric emissions of Zinc from anthropogenic sources are an important source which can enter the human body by dispersion, deposition, assimilation by plants and transfer through the food chain, which can result in adverse human health effects. Li et al., (2024) found that excessive exposure to Zn from the ambient air can cause chronic bronchitis, peritonitis, emphysema, asthma and lung cancer. At some locations in *Turňa-nad-Bodvou* TW found extremely high levels of zinc (Zn) in the biomonitoring samples, exceeding the average concentration of zinc (Zn) in vegetables at certain locations by a factor of 10 – 2000 (Fig. 21).

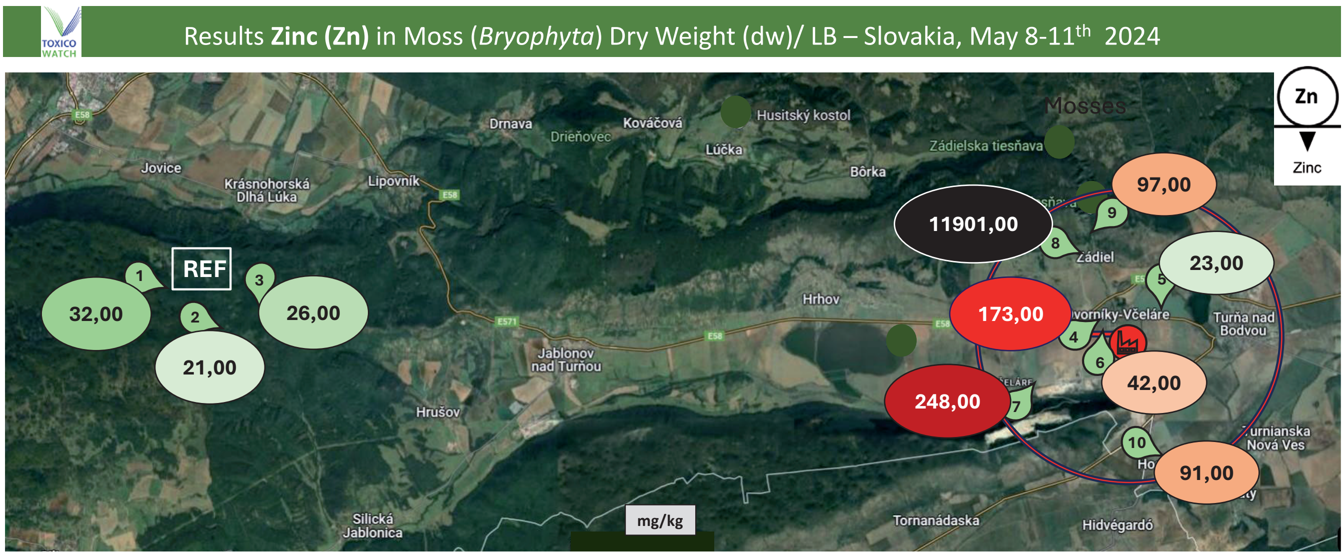


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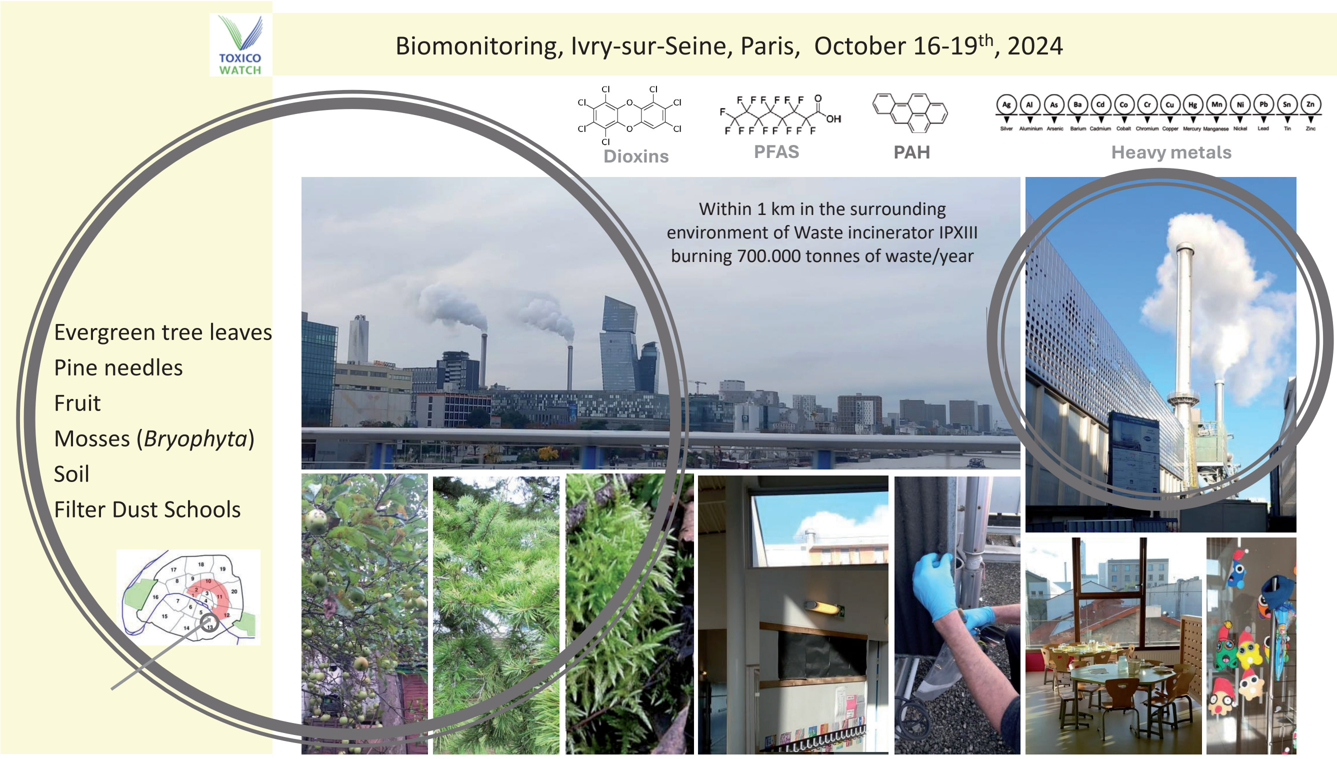
Figure 21: Zn results in mosses (*Bryophytes*) in the environment of the cement kiln, Turňa-nad-Bodvou, Slovakia.



Credit: ToxicoWatch; used with permission.

Figure 22: TW Biomonitoring research in Paris, Ivry-sur-Seine, 2024-2025.

13. Biomonitoring of POP emissions on primary school filters, Paris

In 2025, TW will conduct biomonitoring research in Paris (dioxins, PFAS, PAH and heavy metals) using dust collected from primary school filters, constructed to filter outside air led into school buildings situated in the surrounding area of the rebuilt waste incinerator Ivry Paris XIII. TW will use vegetation and soil sampling media on the school grounds to compare dust contamination levels from the filters. It is unique that TW has obtained approval from the primary school management to deploy dust filters on the roof of the school building in the vicinity of the waste incinerator. Three years earlier, in 2021, TW started biomonitoring on privately held backyard chickens, mosses and other vegetation in this section of Paris to determine general dioxin contamination levels in the urban region of the urban capital. In addition to analyses of dioxins and PFAS, in 2025, TW's project will also focus on heavy metals in mosses (*Bryophytes*), pine needles (*Picea abies* and *Pinus sylvestris*), and in the soil with the aim of comparing loads as determined from these diverse biomonitoring sampling media with the analytical results from the primary school dust filters. Results will be presented later in 2025 in relevant outreach publications.

14. Perspective

The last 13 years of TW performing biomonitoring research in the surrounding environment of POP emitting industries has clearly demonstrated the need for, and value from, continuing of this kind of studies and monitoring to get a better science-backed understanding of pollution levels in contaminated industrial regions. Use of innovative bioaccumulating sampling media began in 2013 and has provided a challenging R&D focus point ever since. So far, a wide range of sampling media has been tested out, with selected results presented here.

The use of conventional as well as novel sampling media is a highly challenging scientific and technological field in constant development, which is addressed with all available efforts. It is of special interest for the readership of this journal that the representativity of the sampling procedures used/developed must be thoroughly monitored and documented, especially for the innovative sampling media. This is carried out by parallel practical sampling campaigns based on Replication Experiments (RE) and variographic characterisation, well-know within the Theory of Sampling (TOS) (Esbensen, 2024).

However, it must be mentioned that such scientific baseline documentation constitutes quite a load on a Not-for-Profit public organisation running on sponsorships from the public. Lots of voluntary work is still needed and more funding from concerned stakeholders (civil, industrially based and governmental) is very much welcomed.

ZERO WASTE EUROPE BIOMONITORING

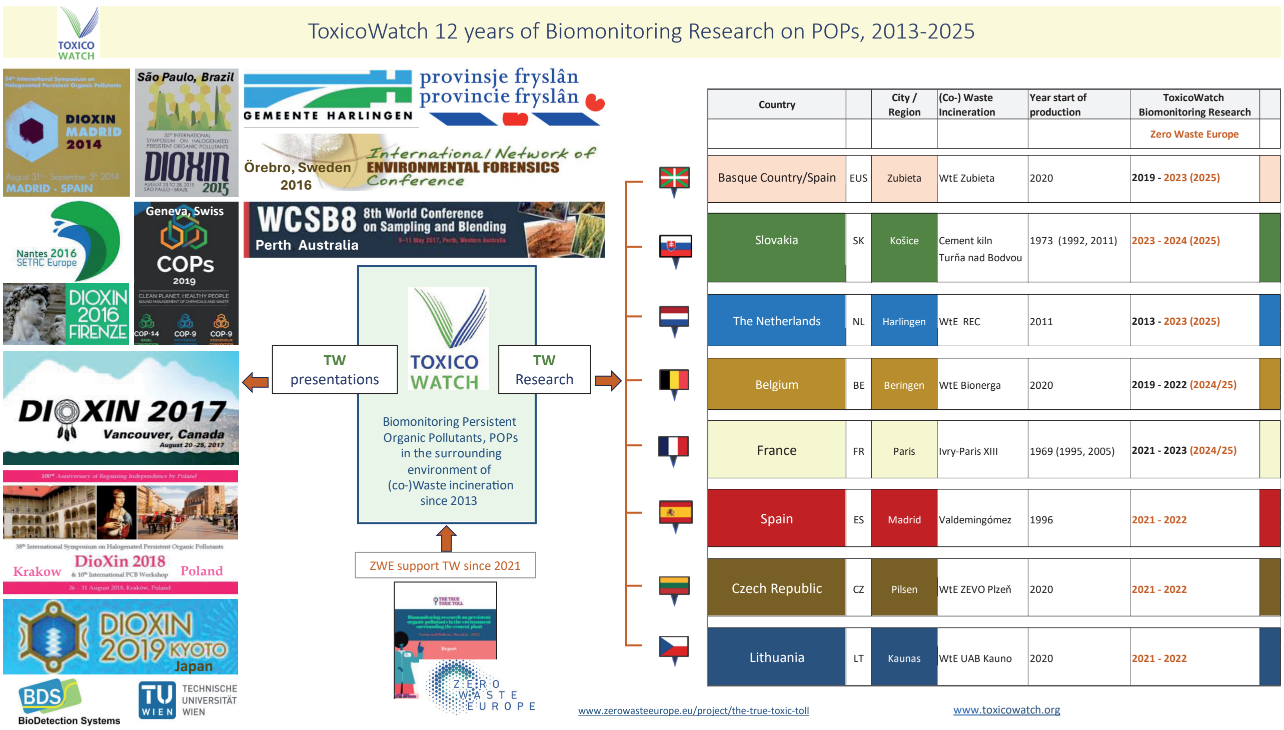
This video shows how biomonitoring is becoming a vital tool in the demand for environmental transparency. Learn how you can join this growing movement for change in your own community.

youtu.be/YFQWpDjC2xs

15. ToxicoWatch and 'Zero Waste Europe' support

TW started research on demand of concerned local people and local politic parties in Harlingen, The Netherlands in 2013 by TW's own initiative an independent biomonitoring study on dioxins in backyard chicken eggs in the surrounding environment of the newly built WtE waste incinerator REC (2011). The high documented level of dioxins in these eggs resulted in national attention with several documentaries on national Dutch TV and counter research from the Ministry of Health (VROM). From 2014 till 2019 TW has presented these results at Dioxin conferences and other relevant toxicological conferences and symposia (SETAC, INEF and BDS) about the NL research findings as well of TW biomonitoring in other European countries on POP in relation to emissions of waste incineration. TW attended, based on its research, the Basel Rotterdam Stockholm conventions (BRS COPs 2017 & 2019). Since 2021 *Zero Waste Europe* in Brussels has supported TW financially regarding multi-year biomonitoring research projects and on POPs in in surrounding areas of (co-) waste incineration in several European countries (see Fig. 23 and video on the left).

Additionally, Toxicowatch is engaged in a continuing educational outreach activity bridging between local populations, industry and governments (Fig. 24).



Credit: ToxicoWatch; used with permission.

Figure 23: TOXICOWATCH biomonitoring research outreach 2013-2025 (www.toxicowatch.org)

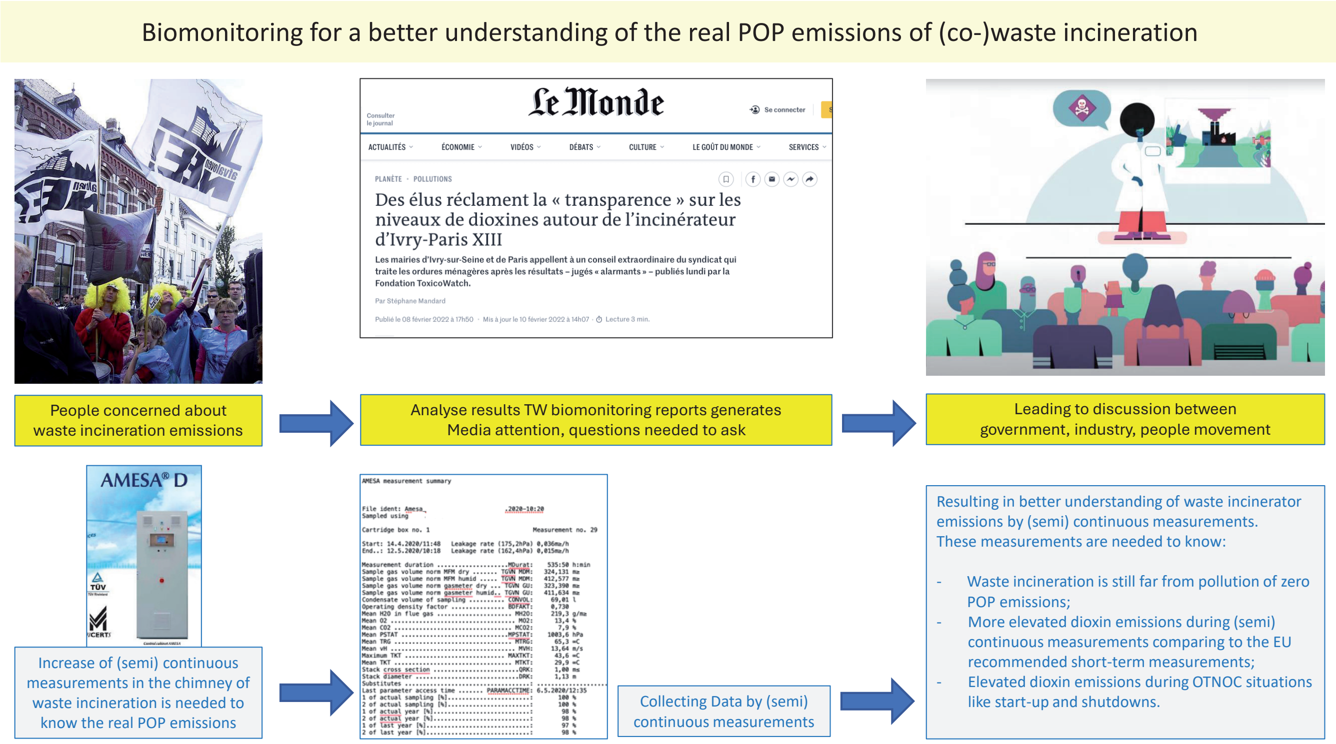


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Figure 24: Biomonitoring for a better understanding of the real POP emissions of (co-) waste incineration.

16. Conclusions

It can be concluded that biomonitoring is essential as a tool for monitoring the *real* deposition of industrial emissions of hazardous POP substances in the surrounding (human) environment. An important result of TW's work and research is that local communities become involved and are actively participating in the practical realisation of highly relevant research projects. With this approach, local communities feel listened to, and experience in practice that action is taken to focus their serious concerns through citizen-participation, scientific research, and communication with governments and other relevant organisations. In this way, TW acts as a bridge between local communities, industry, and government.

Authorities must prioritise elimination of harmful POP industrial emissions, on behalf of a reasonable and fair precautionary principle, to give human and environmental health priority over industry management focused on economic profit-oriented considerations. This master principle should guide every effort to address the significant environmental and public health concerns illustrated. If unmitigated these concerns will even harm businesses and and national economies as well.

It is of critical importance to maximise *dissemination* of the results of relevant biomonitoring studies as well as encourage further citizen-driven counter activities against harmful industrial emissions. Interested parties and stakeholders are referred to TOXICOWATCH' homepage: www.toxicowatch.org

TOXICOWATCH is grateful for the opportunity to present some of its R&D work to the international sampling community.

LEARN MORE

- **zerowasteeurope.eu/project/the-true-toxic-toll**
- **toxicowatch.org**

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