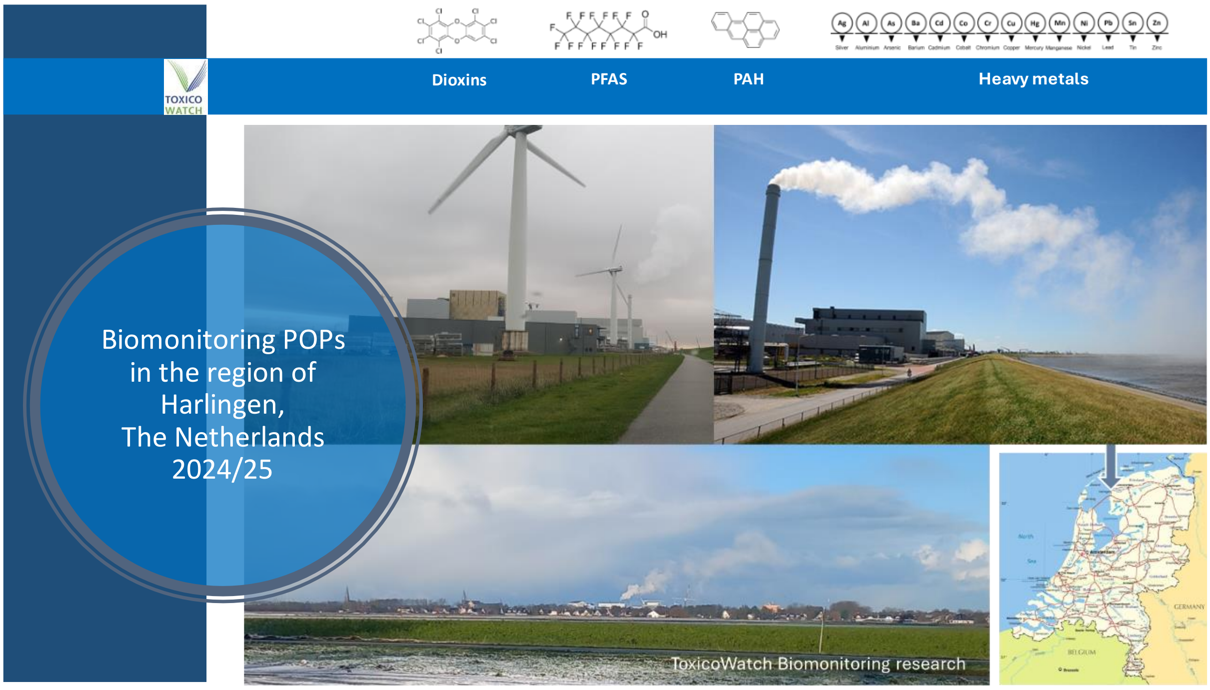
Biomonitoring Persistent Organic Pollutants, POPs in the environment of the WtE waste incinerator REC, Harlingen, the Netherlands 2025

Interim Report 2025



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April, 2025

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

Thanks to the private participants of this research for making this study possible by allowing the TW team collecting samples of their backyard chicken eggs, soil, vegetation, and water streams

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Abbreviations

Abbreviation	Meaning
BAT	Best Available Techniques
BEP	Best Environmental Practice
BEQ	Biological Equivalents
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
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
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
SVHC	Substances of Very High Concern
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)

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1. Introduction

This interim ToxicoWatch (TW) NL Biomonitoring report 2025 presents not only the follow-up of initial biomonitoring results (NL Report 2024) on dioxins, PFAS and heavy metals in eggs and eggshells of backyard chickens, and vegetation – but also an extension of heavy metal analyses on soil, water and mosses (Bryophytes) in the surrounding environment of Waste-to-Energy (WtE) incinerator REC, Harlingen, the Netherlands. Reference samples were collected by TW-team at locations at 15 km northeast of the incinerator, in the dominant wind direction.

Figure 1 depicts a standard scenario, highlighting that the incinerator is equipped with a dry-cleaning system designed to prevent visual plumes on the horizon. Figure 2 presents an infographic summarising the main results of this interim report.

Figure 1: Plume of the WtE incinerator REC, with dry cleaning system, Harlingen, NL

2. Sampling locations

In September 2024, samples were collected from backyard chickens' eggs and eggshells, as well as vegetation: including evergreen tree leaves (*Quercus hispanica*), mosses (*Bryophytes*), grass (*Poaceae*), soil, and water from natural water stream. Sampling locations were situated 400–2000-meters away in the north and east wind directions.

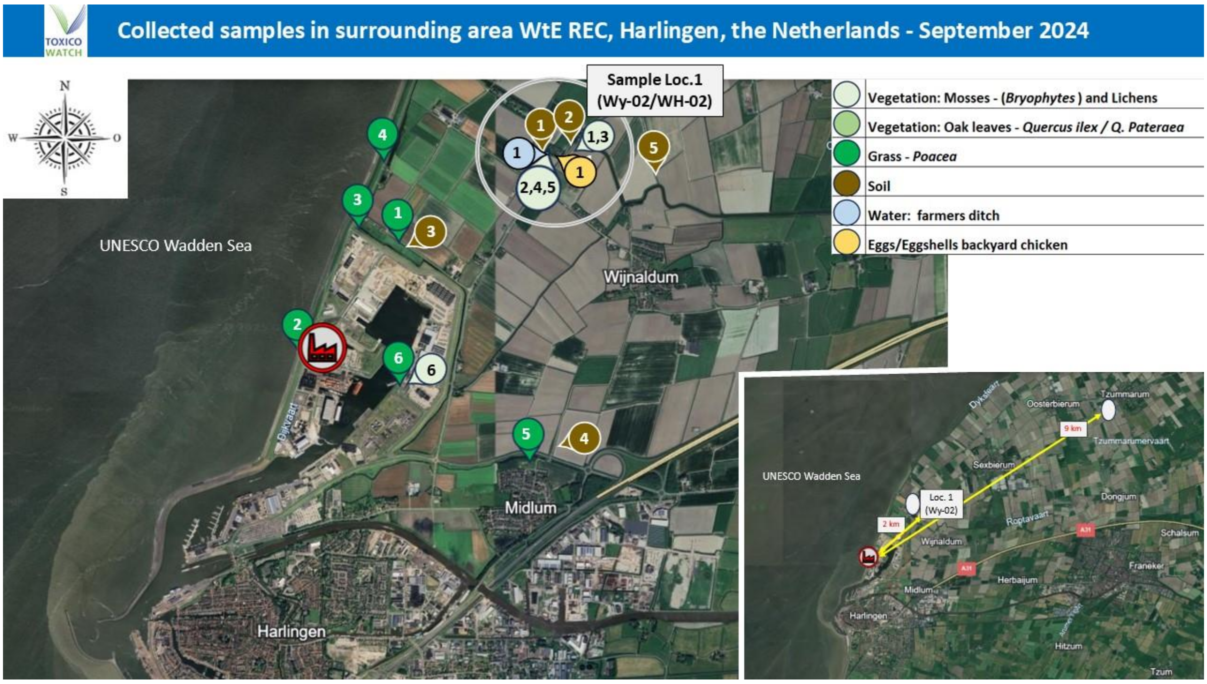


Figure 2: Collected samples Wijnaldum

For 2025, the research area will expand to include six villages within a 3 km radius of the REC waste incinerator: Harlingen, Wijnaldum, Midlum, Sexbierum, Herbaijum and Kimswerd. Additionally, reference samples will be collected from the northern province of *Friesland* in the villages of Tzummarum, (9,5 km north), Zurich (8 km south), Winsum (15 km east) and from the *South Holland province* in Warmond, (150 km southwest).

In this interim report 2025 Harlingen biomonitoring report, backyard chicken eggs and grass samples from Harlingen will be compared with previous data collected at the same locations in 2024 and earlier studies from 2013-2015/2017.

2.1. Backyard chicken Eggs

In September 2024, the TW team collected 12 fresh eggs from a single location. The liquid contents of (yolk and egg white) of 10 eggs were mixed, frozen and stored in HDPE lab containers for further analyses. The team also conducted a questionnaire and a site inspection to identify any potential confounding factors to the sampling location.

Figure 3: Egg location Wijnaldum

2.2. Vegetation

2.2.1. Mosses - Bryophytes

Approximately 100 grams of fresh moss (*Bryophytes)* per sample (N= 5) were collected from the same location as the backyard chicken eggs in September 2024. The samples were immediately stored in HDPE bags and placed in a cool, dark, and dry environment.
The five moss samples were collected from different substrates, like soil in natural habitat, the roof of sheds and the chicken enclosure, at 1800 – 2000 meters from the REC waste incinerator in northeast wind direction.
Mosses from the Bryophytes plant division, mainly *Hylocomium splendens* and *Pleurozium schreberi,* are widely used for biomonitoring persistent organic pollutants (POPs). Like lichens, mosses are highly sensitive to air pollution, making them valuable bioindicators. TW's studies, mosses suggest that rapidly accumulate POPs, and when exposure ceases, they exhibit a fast detoxification process. The mechanisms behind this process are still under investigation. Overall, bryophytes/mosses serve as effective bioindicators, reflecting both the levels and types of pollutants in their habitats.

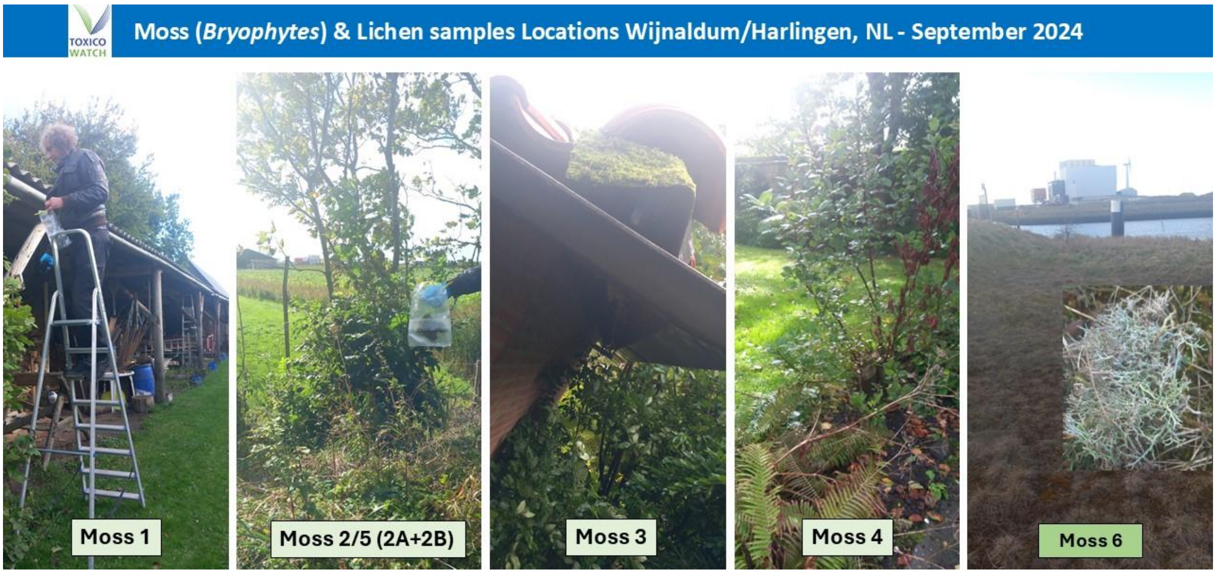


Figure 4: Moss & lichen sample locations

2.2.2. Evergreen leaves of Holm oak andthe deciduous Turkey oak

Two 100-gram samples of oak leaves were collected from: Holm oak (*Quercus ilex*) – Dutch: Steeneik and Sessile oak (*Quercus petraea*) – Dutch: Wintereik.The leaves were gathered directly from the trees in the southeast and northeast wind directions at the same location as the backyard chicken eggs (northeast direction). Evergreen leaves were collected at the height of 1.50 meter from the ground, covering all wind directions around the tree.

Figure 5: Evergreen leaves sample locations

2.2.3. Grass (*Poaceae)*

Grass (Poaceae) samples were collected in September 2024 from six locations within a 400–1000-meter radius of the waste incinerator REC. Sampling was conducted in the west, north and east wind directions, in areas of open rural fields near the UNESCO Wadden See dyke (west) and agriculture land. All vegetation samples were placed in HDPE lab bags, and stored in a cool, dark, and dry environment until laboratory analysis.

Figure 6: Grass sample locations

Collecting a single species of *Poaceae* is challenging due to:

- The high diversity of grass species.
- The presence of other plants and herbs that thrive in natural grass habitats, creating biodiverse plant communities.

Figure 7: Grass sampling

2.3. Soil

In September 2024, the TW team collected five soil samples:

- Two (2) samples from the same locations as the grass samples.
- One (1) sample from an eastern site.
- Two (2) samples from locations near the backyard egg site.

One sample from the vegetable garden (Egg Location. 1). One from the public verge of agricultural farmland.
All soil samples were taken from the upper 0-5 cm layer, using a stainless-steel scoop, and stored in HDPE bags in a dry, cool and dark environment until laboratory analysis.

Figure 8: Soil sample locations

2.4. Water

One (1) water sample was collected from a natural water stream located west of the backyard chicken egg sampling site. This stream divides the land plots, with neighbouring agricultural land used for commercial flower bulb production and other agricultural activities in recent years.
In September 2024, the TW team collected two (2) 500 ml water samples in HDPE containers directly from the stream. These were stored in a dry, cool and dark until laboratory analyses.

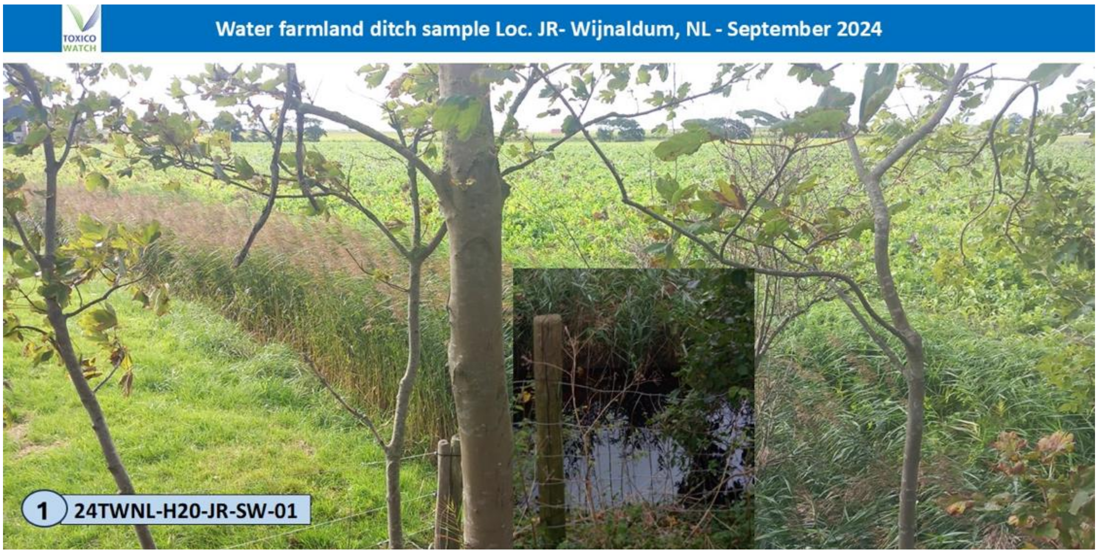


Figure 9: Water ditch sample

3. Analysis Methods

The collected samples were analysed for persistent organic pollutants (POPs) using both chemical analysis and bioassay methods. The targeted substances include:

- Dioxins (PCDD/F/dl-PCB),
- per- and poly-fluoroalkyl substances (PFAS),
- Polycyclic Aromatic Hydrocarbons (PAH),
- Heavy metals)6-14 elements): Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Copper (Co), Chromium (Cr), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Tin (Sn) and Zinc (Zn).

The laboratory analyses for dioxins, PAH and PFAS in this report were conducted by BioDetection Systems in Amsterdam, the Netherlands (NL), an accredited laboratory (RvA L401).
The Chemical analysis of PFAS and heavy metals are performed by the Normec, Groen Agro Control, in Delft, NL.
For PFAS chemical analyses LC-MS/MS for 24 PFAS was employed, while for heavy metals, ICP-MS analyses were used.

3.1. Dioxin Analysis

The bioassay method for dioxins used in this study is DR CALUX® (Dioxin-Responsive Chemical Activated LUciferase gene eXpression), which quantifies chlorinated dioxins, furans (PCDD/F) and dioxin-like PCB, and other substances with dioxin-like activity.

For backyard chicken eggs, in addition to bioassay testing, chemical analyses (GC-MS) were conducted on 29 chlorinated dioxin congeners, in compliance with EU regulations for accredited laboratories. This additional GC-MS analysis was required if DR CALUX results exceeded the EU regulatory limits:

- 1.7 pg TEQ/g fat for PCDD/F
- 3.3. TEQ/g fat PCDD/F/dl-PCB.

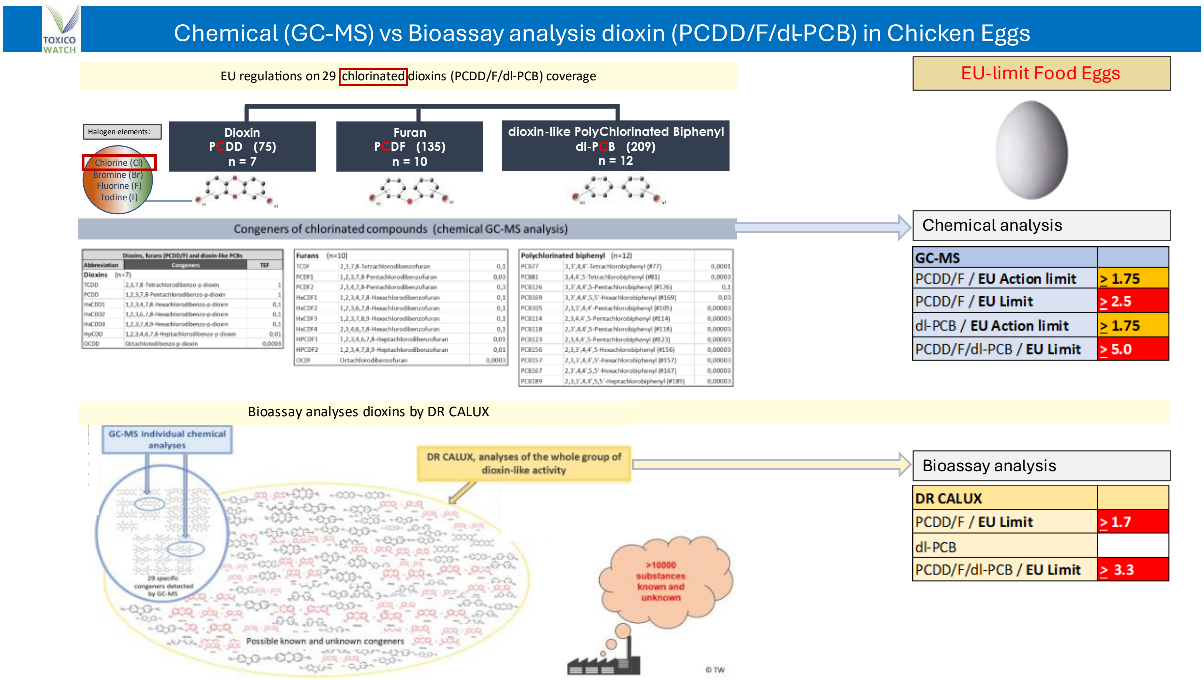


Figure 10: Chemical vs bioassay analyses

3.2. PFAS Analysis

3.2.1. PFAS chemical analysis (LC-MS/MS) - 24 PFAS Substances

Prior PFAS reporter gene analysis, PFAS compounds were extracted using a weak anion exchange (WAX) solid-phase extraction (SPE) cartridge. Approximately 500 ml surface water or 1 litre of WWTP influent/effluent was filtered on glass-fibre filters. WAX-SPE (Oasis WAX, Waters 186002493) columns were conditioned (4 ml MeOH/0.1% NH4OH; 4 mL MeOH; 4 mL super-demi water) after which the indicated volumes of sample were loaded on the columns. After washing the columns (4 mL 25 mM NH4AC pH 4; 8 ml THF/MeOH (75:25)), PFAS were eluted from the WAX- SPE using 4 ml MeOH/0.1% NH4OH. Eluates were evaporated (N2; 45 °C) and reconstituted in 15 µg of DMSO.

The LC-MS/MS analysis in this TW biomonitoring research targeted 24 fluorinated PFAS substances. The table below provides an overview of the PFAS substances and their chain-length classifications.

The European Food & Safety Authority (EFSA) established PFOS and ∑4 PFAS limits in 2018:

- PFOS limit is 1 µg/kg
- Σ4 PFAS (sum of PFOA, PFOS, PFNA, PFHxS): 1.7 µg/kg

∑ 24	(EFSA) ∑ 4	Long Chain C			Middle Chain C						
		C8	C8	C9	C6	Som of					
		PFOS	PFOA	PFNA	PFHxS	∑ 4					
	19	Short Chain C		Middle Chain C							
		C4	C4	C5	C5	C6	C6	C6	C7	C7	
		PFBA	PFBS	PFPeS	PFPeA	PFHxA	4:2FTS	GenX (HFPO-DA)	PFHpA	PFHpS	
		Long Chain C									
		C11	C11	C12	C13	C14	C8	C9	C10	C10	C10
		PFUnDA	PFDoA/PFDODA	PFDoS	PFTrDA	PFTeDA	6:2FTS	PFNS	PFDA	PFDS	8:2FTS

Figure 11: Short, middle and long chain PFAS

When assessing PFAS toxicity, chain length is a critical factor:

- Perfluoroalkyl carboxylic acids (PFCAs): C8 or longer is considered a long-chain PFAS
- Perfluoroalkyl sulfonic acids (PFSAs): C6 or longer is considered a mid-chain PFAS.

Although 63.6% of the detected PFAS were long-chain, the three most frequently detected PFAS were short-chain PFAS (Mario di M. et al., 2024).

Long-chain PFAS:

- Strongly associated with cancer, immune suppression, thyroid dysfunction, and developmental abnormalities.
- Persistent in the body due to strong binding to blood proteins, leading to prolonged exposure and amplified health risks.

Short-chain PFAS:

- Cause oxidative stress and membrane damage.
- Generate reactive oxygen species (ROS), leading to lipid peroxidation and cellular membrane damage.

3.2.2. Bioassay PFAS CALUX / PFAS Reporter Gene Bioassay

The PFAS CALUX bioassay was conducted following the conditions previously described by Collet (2020) and Behnisch (2021). Serial dilutions of sample extracts in DMSO were prepared and incubated in Tris-

buffer (pH 8.0) overnight at 40 °C, in the presence of a fixed concentration of transthyretin (TTR) and thyroxine (T4).
To prevent non-specific proteins interference, a serum-free exposure medium was added to the collected eluates containing TTR-bound T4 before being introduced to seeded and pre-incubated TRβ reporter gene cells. After 24 hours, the luciferase activity was measured using a luminometer and reported as total PFOA equivalent per Liter (PFOA eq./l) of processed water (Schepper et al ,2024).

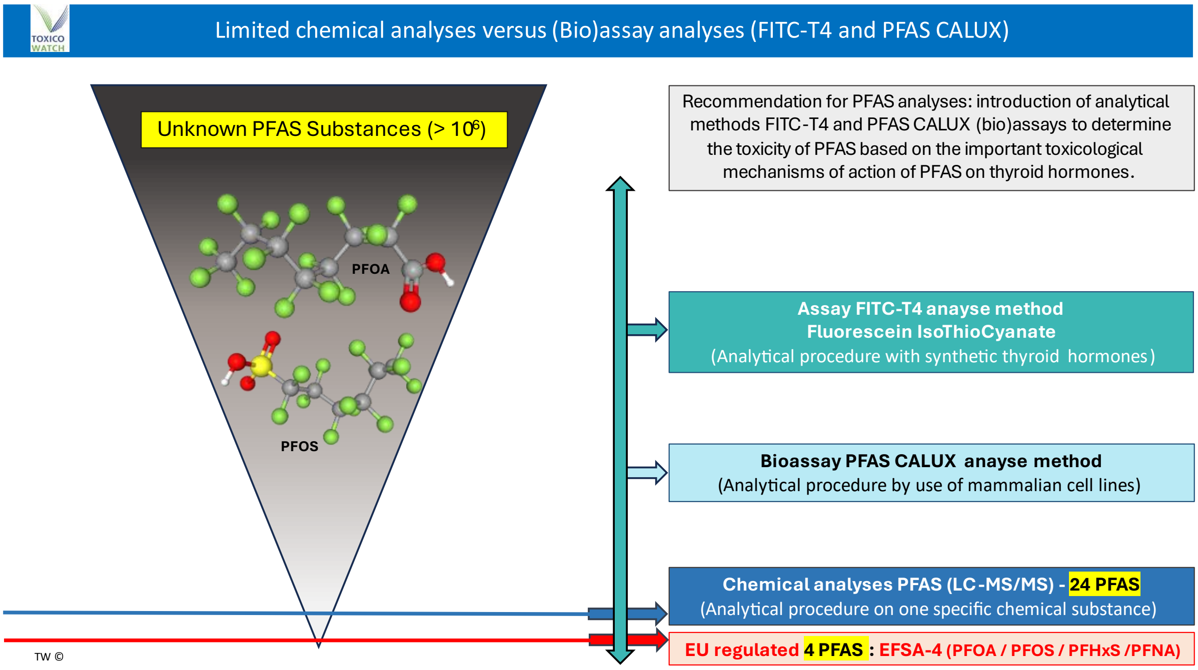
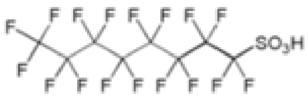
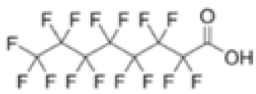


Figure 12: Limited PFAS analyses, chemical, bioassay

Per- and polyfluoroalkyl substances (PFAS) are highly persistent organic pollutants, also known as a ‘forever-chemicals’ due to their extreme resistance to degradation. PFAS production began in the 1940s, and these substances have been widely used in industrial applications and consumer products, including: non- stick coatings, food packaging materials and clothing. Since most PFAS do not break down naturally, they accumulate in the environment over time. Their increasing presence in water, biota, and even human serum has raised global concerns in recent years.



PFOS



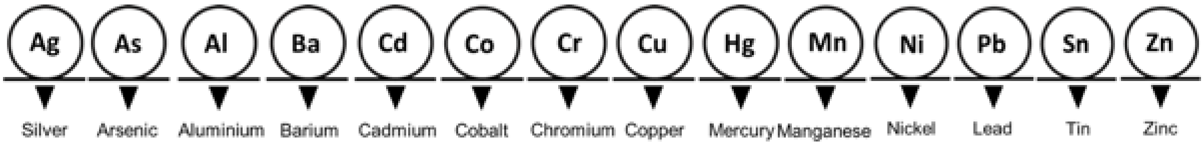
PFOA

As of 2025, addressing PFAS contamination remains a major worldwide challenge, with decades of scientific studies documenting their accumulation. Due to their long-term effects, European Food Safety Authority (EFSA) has established a tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week (EFSA, 2018). In 2022, the Dutch government set a drinking water guideline value for PFAS at 4.4 ng/L, expressed as PFOA equivalents. This value is expected to be legal incorporated into the Drinking Water Decree in the near future.

The effect-based PFAS bioassay (PFAS CLUX/FITC-T4) has demonstrated a strong correlation with the chemical PFAS analysis (LC-MS/MS) converted data. Therefore, in vitro toxicity analysis using the PFAS reporter gene bioassay is considered a reliable strategy for assessing total PFAS content in water samples and evaluating contamination in the environment.

3.3. Heavy Metals

In this biomonitoring research, TW analysed backyard chicken eggshells, soil and mosses (*Bryophytes*) for 5-14 heavy metals (see figure below). The method used by accredited laboratory Normec Groen Agro Control was ICP-MS heavy metals analysis (A068 +A095, Normec method), in accordance with the NEN-EN 13805 and measurement standards conforming to NEN-EN-ISO 17294-2).



4. Analysis Results

Since 2013, when TW conducted its first biomonitoring study on eggs of backyard chickens in the NL, a participation among private chicken coop owners has declined. This trend has continued into 2024, particularly regarding research on dioxins (PCDD/F/dl-PCB) and PFAS.

The main reason for this decline is the detection of dioxins and PFAS in backyard chicken eggs, which led to media attention and negative consumer advisories from the government, discouraging both egg consumption and chicken keeping. As a result, fewer backyard chicken coop owners are willing to participate in biomonitoring studies.

However, it is crucial to emphasize that this is not just a “chicken egg problem”, but an environmental issue. Backyard chicken eggs are highly nutritious and contribute to local biodiversity, but they should not be contaminated with POPs.

Using backyard chicken eggs for biomonitoring is a highly sensitive tool for detecting dioxins and other POPs in the environment. If contamination is found, the next step is to investigate the source of the POP pollution? Could that nearby industrial emissions? TW’s biomonitoring studies serves as an early warning system. If POPs are detected, responsible authorities should act by conducting further transparent investigations and taking appropriate measures.

Due to the reluctance of private chicken coop owners in Harlingen, TW has expanded its monitoring approach. Besides eggs of backyard chickens, TW now analyses vegetation, soil, and water to monitor POPs contamination in an areas. Additional analyses also include chicken feed, materials from chicken enclosures, pesticide use, woodstove emissions.

4.1. Dioxins

4.1.1. Dioxins in Backyard Chicken Eggs

In September 2024, a pooled sample of 10 backyard chicken eggs was analysed using bioassay DR CALUX and chemical analysis (GC-MS). The samples were collected at Wijnaldum (Wy-02), located 2,000-meter northeast of the WtE REC incinerator. This location was previously sampled in 2013 during TW’s first biomonitoring study, and again in 2014, when the Dutch national health service (RIVM) conducted a counter research in response to TW’s findings.

The analysis results of the egg sample collected in September 2024 are **5.60 pg TEQ/g fat** using the bioassay and 2.60 pg TEQ/g fat using the chemical analyses for the sum of PCDD/F/dl-PCB.

The lower values observed in September 2024 biomonitoring maybe be due to the introduction of a completely new, young flock of backyard chickens in 2024. The private chicken coop owner replaced all its chicken after the high PFAS levels (38.4 pg/g) and dioxin levels (9.8 pg TEQ/g) detected in 2023. The 2024 eggs analysis comes from young chickens that had been at the sampling location for less than 12 months, which could explain the reduced contamination levels.

Sample date	bio matrice	Sample year	Country	Graph Code	Research	TW-REF-NR	Loc. Description	Distance (km) WtE REC	wind direction	Analyse method	Lab	DR CALUX pg TEQ/g fat			GC-MS pg TEQ/g fat (mb)		
												PCDD/F	dl-PCB	PCDD/F/dl-PCB	PCDD/F	dl-PCB	PCDD/F/dl-PCB
2013	Eggs	2013	NL	Wy-02	TW	Wijnaldum (H2)	Wijnaldum (H2)	2	N-E	DR CALUX / GC-MS	BDS	0,50	1,20	1,70	0,50	1,20	1,70
2014	Eggs	2014	NL	Wy-02	RIVM	Wijnaldum (H2)	Wijnaldum-H2 (RIVM)	2	N-E	DR CALUX / GC-MS				8,80	2,91	2,52	5,43
2023-09-01	Eggs	2023	NL	Wy-02	TW	23TWH-Eggs-WH-01-5	Wijnaldum (H2)	2	N-E	DR CALUX / GC-MS	BDS	5,20	4,60	9,80	2,40	2,00	4,40
2024-09-28	Eggs	2024	NL	Wy-02	TW	24TWNL-EGG-W-JR-E01	Wijnaldum (H2)	2	N-E	DR CALUX / GC-MS	BDS	2,50	2,70	5,20	1,00	1,60	2,60
						Reference 2024											
2024-02-07	Eggs	2024	NL	War-Ep	TW	24TWN-WMEP-01	Warmond	125	S-W	DR CALUX / GC-MS	BDS	11,00	6,00	17,00	4,50	1,30	5,80
2024-02-07	Eggs	2024	NL	War-OT	TW	24TWN-WMOT-02	Warmond	125	S-W	DR CALUX / GC-MS	BDS	14,00	7,00	21,00	5,60	2,70	8,20
2024-02-21	Eggs	2024	NL	Tzum	TW	24TWN-TZ-egg-01	Tzummarum	10	N-E	DR CALUX / GC-MS	BDS	1,90	1,50	3,40	0,58	0,29	0,87

TW Indicative scale for Eggs		
DR CALUX		
PCDD/F	dl-PCB	PCDD/F/dl-PCB
pg BEQ or TEQ / g fat		
≥ 6,6	≥ 2,5	≥ 10,0
≥ 3,3	≥ 1,0	≥ 6,6
≥ 1,7	≥ 0,5	≥ 3,3
< 1,7	< 0,5	< 3,3

TW Indicative scale for Eggs		
GC-MS		
PCDD/F	dl-PCB	PCDD/F/dl-PCB
pg TEQ /g fat		
≥ 7,5		≥ 15,0
≥ 5,0		≥ 10,0
≥ 2,5	≥ 1,75	≥ 5,0
< 2,5	< 1,75	< 5,0

Figure 13: Results of dioxin analyses on eggs of backyard chicken

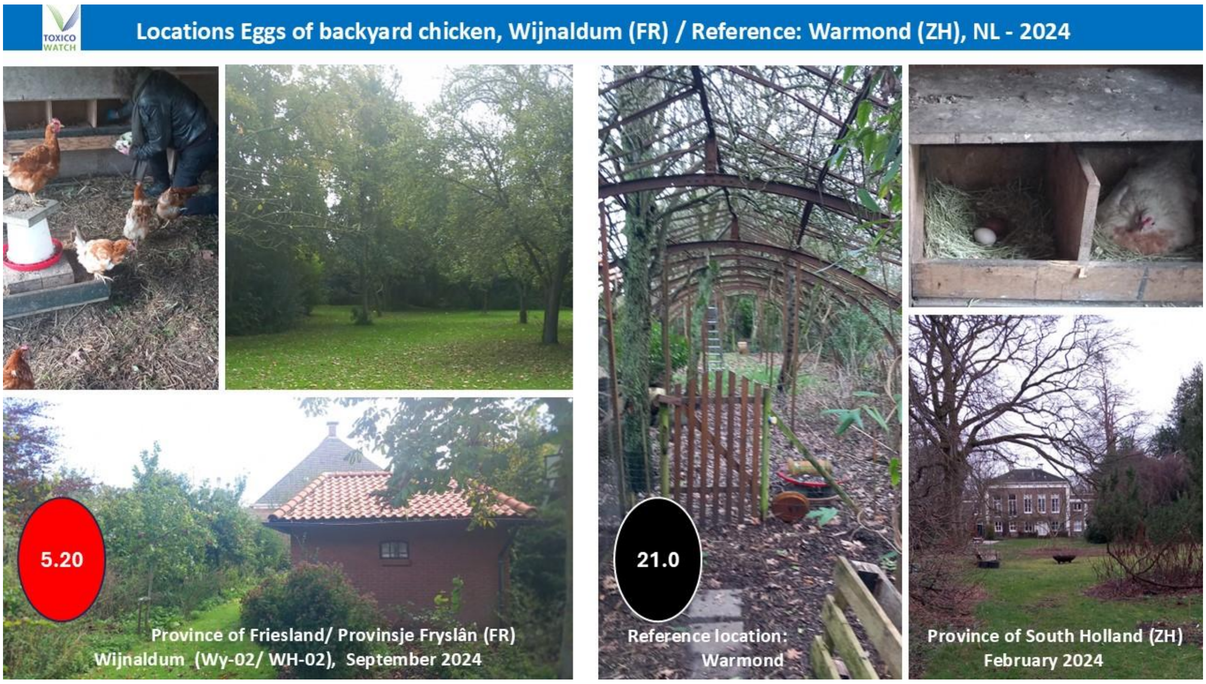


Figure 14: Eggs of backyard chicken

The graphs below show dioxins analyses results in eggs from 2013 to 2024. The most recent analysis in 2024 at location Wijnaldum recorded a value of 5.2 pg TEQ/g fat, which exceeds the EU limit of 3.3 pg TEQ/g fat for the DR CALUX analysis. The mandated chemical GC-MS analysis yielded a value of 2.6 pg TEQ/g fat, which is below the EU limit of 5 pg TEQ/g fat.

In 2013, the first analysis recorded 1.70 pg TEQ/g fat. In 2014 and 2023, elevated levels of dioxins were detected, but no measurements were recorded in the intervening years. The 2024 levels remain three times higher than those recorded in 2013, despite involving a completely new chicken coop.

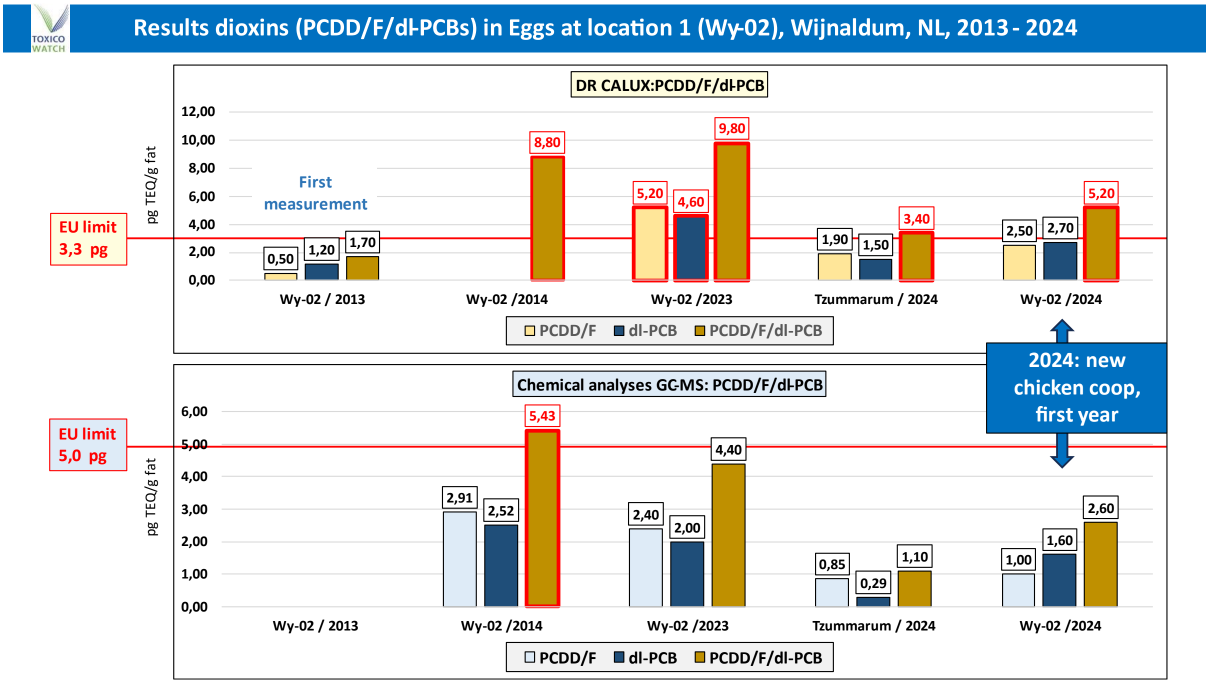


Figure 15: Results dioxins in eggs of location Wijnaldum

In response to these findings, the chicken coop owner took proactive measures by replacing all chickens. The biomonitoring sampling conducted on September 28, 2024, confirmed that the chickens were five months old at the time of sampling.

4.1.1.1. Reference Location: Tzummarum, Province of Fryslân, NL

In Tzummarum, located 9 km from the WtE incinerator REC, backyard chicken eggs were analysed for dioxins in 2023 as a reference for environmental conditions around the incinerator. Notably, the GC-MS aligned with previously measured reference values, whereas the DR CALUX detected 3.40 pg TEQ/g fat. The DR CALUX bioassay measures a broader spectrum of dioxin-like compounds, including, brominated, and mixed halogenated dioxins, beyond the 29 chlorinated congeners assessed by chemical analysis (GC-MS).

Egg location Tzummarum, 9 km distance WtE waste incinerator REC, NL - 2024

Figure 16: Egg location Tzummarum

4.1.2. Dioxins in Grass in the Surrounding Area WtE REC (2024)

The average dioxins (PCDD/F) levels in grass samples collected in 2024 from the north-eastern and eastern regions near the WtE REC ranged between 0.08 and 0.11 pg TEQ/g dw, as analysed using the DR CALUX bioassay. Conducting independent research, such as TW's biomonitoring studies, presents significant challenges. Collecting grass and soil samples from industrial sites without oversight from government-authorised personnel - who typically mediate industry-related inquiries – can be particularly difficult.
For comparison, the RIVM Grass Biomonitoring Research Programme (2014/2015) utilised a chemical analysis (GC-MS) to assess 29 chlorinated dioxin congeners in grass samples.

The figure below shows the RIVM results of dioxin (PCDD/F) analyses in grass from 2014 to 2016. The results have been color-coded using TW's indicative system s to facilitate the interpretation of dioxin values. It clearly shows how factually the data found in 2016 should have been interpreted. There was a high level of dioxin in the environment, which was downplayed in the report by using an incorrect reference

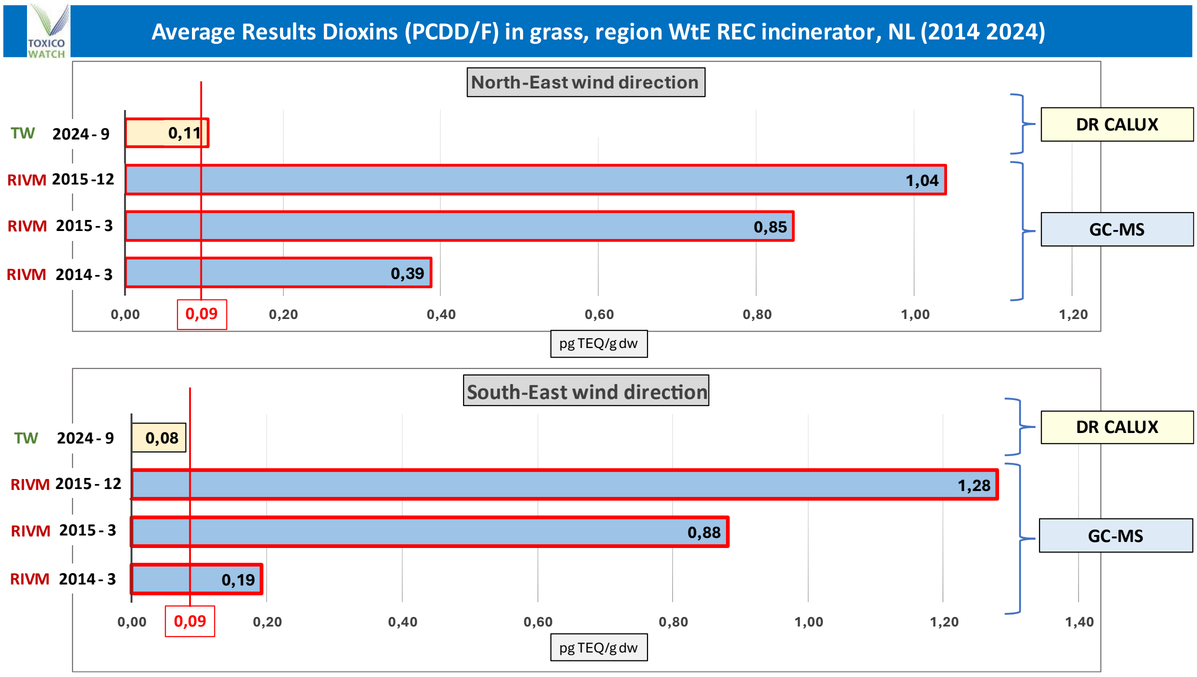


Figure 17: Results TW/RIVM

RIVM: Results Dioxins (PCDD/F) with GC-MS on Grass in the environment of REC Waste incinerator (2014-2016)

RIVM Grass Research GC-MS (pg TEQ/g), 2014 - 2016							
Year	Specification	Sample	TW-REF-NR	Analyse method	GC-MS (MB/dry weight, dw)		
					PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB
2014	Kanaal punt	GRAS	G-13	GC-MS	0,15	0,09	0,23
2014	sibadaweg	GRAS	G-14	GC-MS	0,15	0,08	0,23
2014	hermeskade	GRAS	G-12	GC-MS	0,18	0,13	0,31
2014	kop dijk	GRAS	G-15	GC-MS	0,18	0,15	0,33
2014	Dijk-2	GRAS	G-2	GC-MS	0,19	0,12	0,31
2014	Midgras	GRAS	G-11	GC-MS	0,22	0,19	0,41
2014	Jacob	GRAS	G-8	GC-MS	0,22	0,14	0,36
2014	Dijk-4	GRAS	G-4	GC-MS	0,26	0,16	0,41
2014	Dijk-3	GRAS	G-3	GC-MS	0,27	0,14	0,42
2015	Roptazyl	GRAS	G-16	GC-MS	0,32	0,19	0,51
2014	Dijk-5	GRAS	G-5	GC-MS	0,36	0,21	0,57
2015	Roptazyl	GRAS	G-17	GC-MS	0,42	0,21	0,63
2015	Dijk-1	GRAS	G-1	GC-MS	0,43	0,24	0,67
2014	Dijk-1	GRASS	G-1	GC-MS	0,58	0,33	0,90
2015	Dijk-5	GRAS	G-5	GC-MS	0,60	0,24	0,84
2015	Jacob	GRAS	G-8	GC-MS	0,64	0,31	0,95
2015	Dijk-4	GRAS	G-4	GC-MS	0,83	0,30	1,13
2015	Dijk-2	GRAS	G-2	GC-MS	0,89	0,43	1,32
2015	Dijk-3	GRAS	G-3	GC-MS	0,93	0,42	1,34
2016	dead bird	GRAS	G-6	GC-MS	0,97	0,39	1,36
2016	Dijk-1	GRAS	G-1	GC-MS	0,97	0,21	1,19
2014	dead bird	GRAS	G-6	GC-MS	1,20	0,70	1,90
2016	Jacob	GRAS	G-8	GC-MS	1,22	0,29	1,51
2016	Dijk-4	GRAS	G-4	GC-MS	1,23	0,22	1,45
2016	Dijk-3	GRAS	G-3	GC-MS	1,26	0,29	1,55
2016	Dijk-2	GRAS	G-2	GC-MS	1,27	0,25	1,51
2015	Zijlstra	GRAS	G-7	GC-MS	1,32	0,60	1,92
2016	Zijlstra	GRAS	G-7	GC-MS	1,40	0,25	1,65
2015	dead bird	GRAS	G-6	GC-MS	1,49	0,89	2,38
2015	middle	GRAS	G-10	GC-MS	1,57	0,29	1,86
2016	Dijk-5	GRAS	G-5	GC-MS	1,68	0,23	1,90
2015	ZW pijp	GRAS	G-9	GC-MS	2,23	1,17	3,40

TW Indicative scale		
GC-MS (MB/dry weight, dw)		
PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB
> 1,40	> 1,40	> 2,80
> 0,70	> 0,70	> 1,40
> 0,35	> 0,35	> 0,70
> 0,175	> 0,175	> 0,35
> 0,089	> 0,089	> 0,175
< 0,089	< 0,089	< 0,175

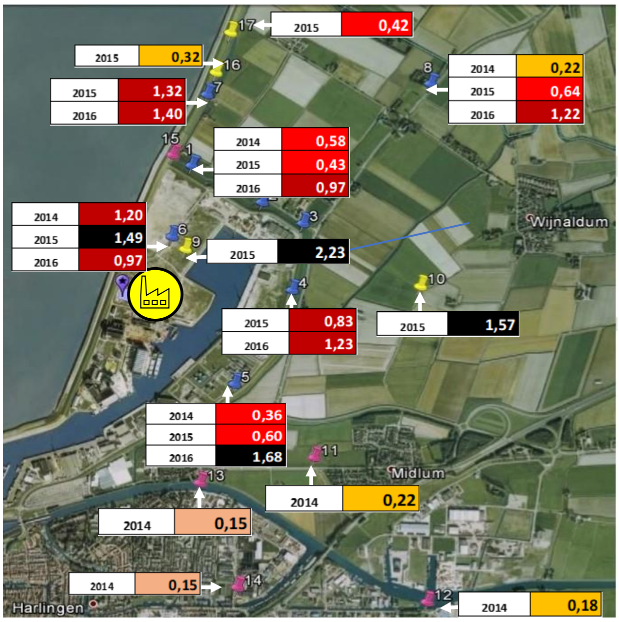


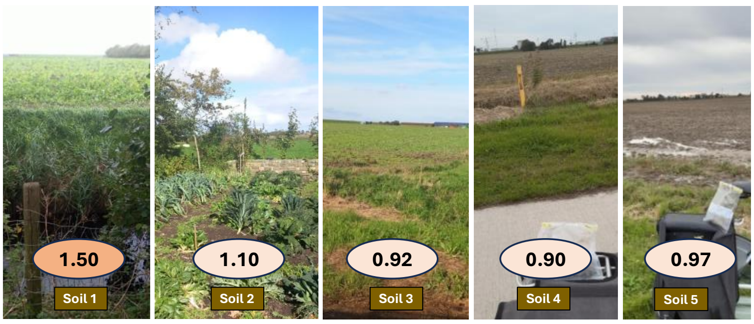
Figure 18: Results dioxins in grass 2014 - 2016

4.1.3. Dioxins in Soil in the Surrounding Area of WtE REC (2013-2024)

Soil samples were collected from five locations in small rural green strips near farmland and directly from farmland. Additionally, one soil sample was taken from a private vegetable garden by TW team in September 2024. All soil samples were analysed for dioxins using the bioassay DR CALUX. Farmland soil in Wijnaldum measured 0.22 pg TEQ PCDD/F/g dw in 2013 and **0.87 – 1.50 pg TEQ eq./g** in 2024**.** A vegetable garden soil in 2024 in Wijnaldum measured **1.10 pg TEQ eq./g dw**.

Results of dioxins (PCDD/F) in SOIL, in environment near WtE incinerator REC, 2024

Biomatrice	Sampling date	Specification	TW-REF-NR	Wind direction	DR CALUX (ub)		
					PCDD/F	dl-PCB	PCDD/F/dl-PCB
Soil - 5	29-9-2024	Pietersbierum	24TWNL-W-SOIL-NRD-05	N-E	0.87	< LOD 0,1	0.97
Soil - 4	29-9-2024	Midlum farmland	24TWNL-ML-SOIL-KAT-04	E	0.9	< LOD 0,1	1.00
Soil - 3	28-9-2024	below dike REC	24TWNL-H-SOIL-KD-03	N	0.92	< LOD 0,1	1.02
Soil - 2	28-9-2024	Garden Wynaldum	24TWNL-W-JR-SOIL-VEG-02	N-E	1.1	< LOD 0,1	1.2
Soil - 1	28-9-2024	Farming Wynaldum	24TWNL-W-JR-SOIL-LB01	N-E	1.5	< LOD 0,1	1.6



TW Indicative scale dioxins in SOIL		
DR CALUX (dry weigh, dw)		
GC-MS		
PCDD/F	dl-PCB	PCDD/F/dl-PCB
pg TEQ eq./g upperbound (ub)		
> 10.0	> 10.0	> 20.0
> 5.0	> 5.0	> 10.0
> 2.5	> 2.5	> 5.0
> 1.25	> 1.25	> 2.5
> 0.75	> 0.75	> 1.5
< 0.75	< 0.75	< 1.5

Figure 19: Results of dioxins in soil

Dioxin levels in the grass samples collected in September 2024 are low and significantly lower than those recorded during the 2015-2016 period. However, dioxin levels in soil have shown an upward trend. The vegetable garden has experienced an approximate 4.5-fold increase, while farmlands exhibited a 7-fold rise in dioxin levels.

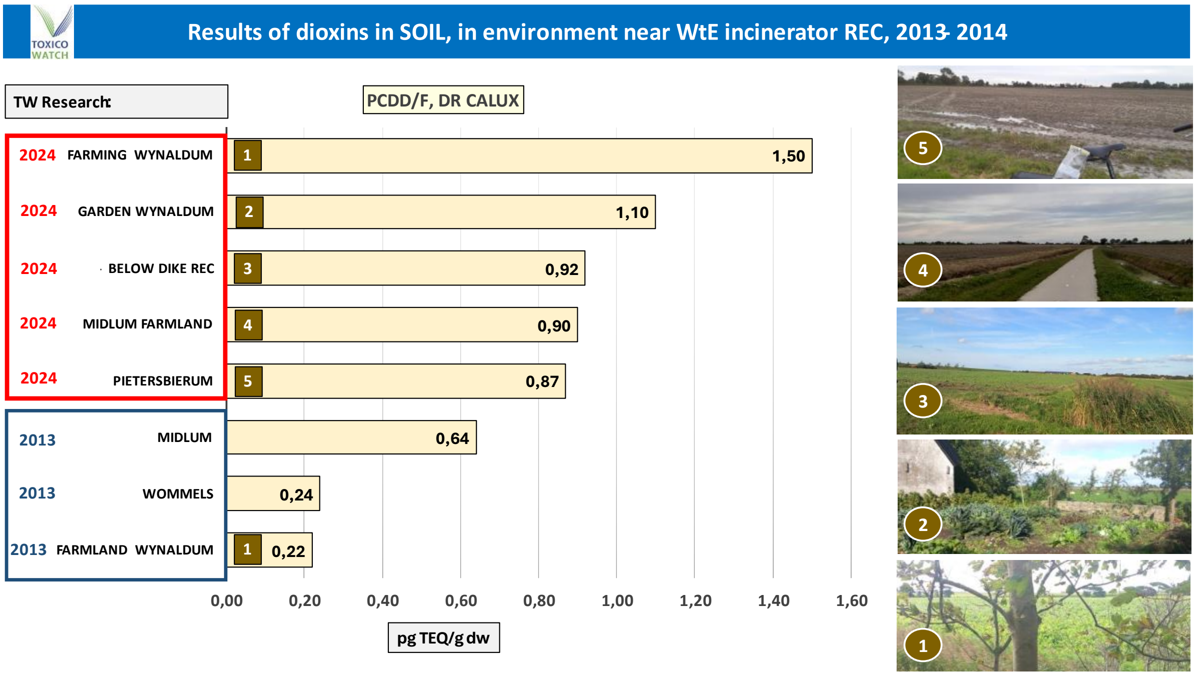


Figure 20: Results of dioxins in soil

Dioxins exhibit high stability and immobility in soil environments. Both biodegradation and chemical degradation processes are either not well established or occur extremely slowly. While photochemical degradation is theoretically possible, it is hypothesised to take place only within the top few millimetres of soil.

Due to their hydrophobic nature, dioxins do not infiltrate rainwater, leading to permanent accumulation in the top centimetres of soil. Evaporation is the only process that can slightly reduce dioxin levels. Consequently, the half-life of dioxins in soil has been reported to range from several years to multiple decades (De Jong et al., 1990).

Soil and backyard chicken eggs are probably the most sensitive exposure pathways for dl-PCBs and PCDD/Fs from soil to humans. People - especially young children - consuming contaminated eggs can easily exceed health-based safety standards. leading to high exposure levels of POPs, including dioxins. For instance, consuming a single chicken egg per day (assuming an average fat content of 7g) could lead to excessive dioxin intake:

- A 4–5-year-old child (weighing 16 kg) would exceed the Tolerable Daily Intake (TDI) of 2 pg TEQ/kg body weight (bw) bw per day,
- Even if the egg complies with the EU regulatory limit of 5 pg TEQ/g fat for the sum of dioxins (PCDD/F/dl-PCB) (Weber et al., 2018).

4.1.4. Dioxins in Mosses (*Bryopytes*) & Lichens

The analysis results for dioxins (PCDD/F/dl-PCB) in all moss samples collected in 2024 using DR CALUX show elevations on the TW-indication scale, based on the EU action level for food/vegetables[1] (EFSA, 2018).

For comparison, the feed limit values are based on Directive 2002/32/EC, which sets maximum levels of 1.25 pg TEQ/g for PCDD/F/dl-PCB and 0.5 pg TEQ/g for PCDD/F. For DR CALUX analysis, a recalculation 0.66 % was applied.

Dioxin (PCCD/F) Results (DR CALUX) in Moss and Vegetation,NL - September 2024

Sample date	Country	Location specification / REC	Sample	Sample Species	TW-REF-NR	Lab Results BDS (Wet Weight, ww)			Results (mb) Wet Weight (ww)			Moisture (%)	Graph Results (MB) 88% Dry Weight (dw)		
						PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB	PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB		PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB
2024-09-28	NL	Wijnaldum - VEG	Oak leaf - 1	*Quercus ilex* leaf	24TWNL-W-JR_VEG-QI-01	0,085	0,068	0,153	0,085	0,068	0,153	41,7	0,128	0,103	0,231
2024-09-29	NL	Midlum - VEG	Reindeer Moss 6	*Cladonia arbuscula*	24TWNL-ML-KM-SB-01	0,230	0,050	0,280	0,230	0,025	0,255	55,8	0,458	0,100	0,557
2024-09-28	NL	Wijnaldum, garden	Moss - 4	*Brophyta*	24TWNL-W-JR-TU-Mos-04	0,220	0,060	0,280	0,220	0,030	0,250	67,8	0,601	0,164	0,765
2024-09-28	NL	Wijnaldum SW-REC	Moss - 5/2	*Brophyta*	24TWNL-W-JR-RWB-Mos-05	0,360	0,050	0,410	0,360	0,025	0,385	77,6	1,414	0,196	1,611
2024-09-28	NL	Wynaldum, chicken coop	Moss - 3	*Brophyta*	24TWNL-W-JR-KH-Mos-03	0,260	0,050	0,310	0,260	0,025	0,285	85,5	1,578	0,303	1,881
2024-09-28	NL	Wijnaldum gutter North	Moss-1	*Brophyta*	24TWN-W-JRG-Mos-01	1,100	0,250	1,350	1,100	0,250	1,350	65,1	2,774	0,630	3,404

TW Indicative scale Vegetation (FEED)		
DR CALUX dry weight		
PCDD/F	dl-PCB	PCDD/F/dl-PCB
pg TEQ eq./g		
>4.00	>2.67	>6.67
>2.00	> 1.33	>3.33
>1.00	> 0.67	>1.67
>0.50	> 0.33	>0.83

TW Indicative scale Vegetation (FOOD)		
DR CALUX wet weight		
PCDD/F	dl-PCB	PCDD/F/dl-PCB
pg TEQ eq./g		
>1.60	> 0.53	>2.13
>0.80	> 0.27	>1.07
>0.40	> 0.13	>0.53
>0.20	> 0.07	>0.27

Figure 21:Dioxin results in moss and vegetation

The results for the sum of dioxins (PCDD/F/dl-PCB) in 2024 are 0.15 - 1.35 pg TEQ/g ww, and 0.23 – 3.40 pg TEQ/dw 88%. The evergreen Holm oak - *Quercus ilex* showed relatively low dioxin values: 0.23 pg TEQ/g ww and 0.23 pg TEQ/g dw 88%.

For the TW Indicative scale used to assess dioxin levels in mosses (Bryophytes) and lichens, the limit value for dioxins in animal vegetation feed is applied. As shown in the graph below, most moss samples contain dioxin (PCDD/F) concentration exceeding the provisional limit of 0.50 pg TEQ/g dry weight (dw), as determined by the DR CALUX assay. The regulatory limit for polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in feed has remained unchanged since 2002, at 0.75 pg TEQ/ g dw, as per Directive 2002/32/EC. This is despite recommendations from the European Food Safety Authority (EFSA, 2018) to revise and adapt these limits.

[1] 0.3 pg TEQ/g PCDD/F, and 0.1 pg TEQ/g for dl-PCB

4.2. PFAS

4.2.1. PFAS in Backyard Chicken Eggs

A chemical PFAS analysis was performed on a total of 12 eggs collected from Wijnaldum (Egg Location 1), situated 2 km northeast of the WtE REC waste incinerator. The PFAS analysis was performed on a blended sample of 12 egg yolks. Notably, no PFAS were detected in the egg whites, despite the fact that PFAS are water-soluble.

At Egg location 1 (Wijnaldum) in 2023, the PFOS level was 38.4 µg/kg, exceeding the EU limit for PFOS (1 µg/kg) by a factor of 38. This result is particularly noteworthy, as it exceeds even the highest PFOS concentration found in eggs across 64 locations in the Netherlands, which was 24.8 µg/kg (Zafeiriki, 2016).

The EU regulatory limits for PFAS in chicken eggs are:
Sum of 4 PFAS (∑ 4): 1 µg/kg and for sum of 24 PFAS (∑ 24): 1.7 µg/kg.

In September 2024, egg samples from a new, young backyard chicken flock (7 – 12 months old), were collected by the TW team at the same location in Wijnaldum (Egg Location 1). The results showed **lower PFAS level compared to 2023: for the sum of 4 PFAS (∑4) / PFOS is 0,61 µg/kg.** No other PFAS were quantifiable. At the reference backyard chicken egg location in Tzummarum, the PFOS concentration was **0,28 µg PFOS /kg**.

PFAS (LC-MS/MS) in eggs of backyard chicken µg PFOA eq./kg / liter (or ng PFOA eq./g and ng PFOA eq./l) - (Lower bound (Lb))															
				µg PFOA eq. /kg			EFSA-4								
				EFSA-4	Sum	C8	C8	C9	C6	C10	C11	C11	C13	C14	C7	C8	C4
Year	Country	Location	Biomarker	∑ 4 PFAS	∑ 24 PFAS	PFOS	PFOA	PFNA	PFHxS	PFDA	PFUnDA	PFDoA	PFTrDA	PFTeDA	PFHpS	6:2FTS	PFBA
2023	NL	Wijnaldum - 01	Fruit	0,00	0,00												
2023	NL	Wijnaldum - 01	Egg	43,08	48,46	38,40	2,50	1,50	0,68	0,96	0,66	1,10	0,94	0,91	0,58	0,23	
2024	NL	Tzummarum	Egg	0,28	0,28	0,28											
2024	NL	Wijnaldum - 01	Egg	0,61	0,61	0,61											
2022	BE	Antwerp 0-2 km Fluor industry	Egg	43,48	48,06	39,00	0,78	0,30	3,40	0,53	0,70	0,55					2,8
2022	BE	Antwerp 2-4 km Fluor industry	Egg	7,34	10,10	6,50	0,57	0,27		0,66	0,78	0,57					0,75
2022	BE	Antwerp 4 -6 km Fluor industr	Egg	8,84	11,51	4,40	0,57	0,27	3,60	0,52	0,77	0,57					0,81

Figure 22: PFAS congeners in eggs NL and BE.

The table above provides an overview of the chemical PFAS analysis (LC-MS/MS) for ∑24, presenting the results of backyard chicken egg samples collected from Wijnaldum (Egg Location1) in 2013, 2023 and 2024, as well as from the reference location in Tzummarum, in the Netherlands in 2024.

The PFAS concentrations measured in the egg yolk at Wijnaldum (Egg Loc.1) were compared with high values PFAS levels detected near a major international fluorochemical industrial plant in Antwerp, Belgium (R. Lasters et al. 2019). The PFAS concentrations in Wijnaldum (2023) and Antwerp (Belgium) were nearly identical, raising concerns about potential sources of PFAS contamination in Harlingen, where no fluorochemical industry is present. This raises the question of what sources may be responsible for the PFAS deposition observed in this biomonitoring study. Two potential sources include one potential source could be waste incineration, while another possible source could be the use of agricultural pesticides on the neighbouring farmlands.

TW was among the first research entities to measure of PFAS emissions in the flue gases of the WtE incinerator REC using the semi-continuous monitoring system of AMESA. Since 2017, there are no semi-continuous measurements of POPs in the flue gases of the WtE REC.

4.2.2. PFAS in Water and Soil

In 2024, water from a ditch (Water-1) located between the backyard chicken coop (Egg location 1) and the neighbouring farmland – which is commercially used for crop and flower bulb agriculture in spring- measured **0.61 µg PFOA eq./l (= 610 ng PFOA/l)** using chemical analysis (LC-MS/MS). T**his ditch water concentration exceeds the Dutch drinking water limit (4.4 ng PFOA eq./l_ by a factor of 138 (RIVM, 2023).** This Dutch water quality guideline is expected to be formally incorporated into the Dutch Drinking Water Decree as a legal quality requirement in the future (RIVM, 2025).

TW's biomonitoring research primarily focuses on the north-east, due to the prevailing southwest wind direction, which is particularly strong along the coastline. **Soil sample 1** (verge near farm/agricultural land and Egg loc 1., at 2 km from REC and the **soil sample 3** near the incinerator REC (1,100 m from REC), PFAS chemical analyses (LC-MS/MS) **measured respectively 100 and 570 ng** PFOA eq./g dw.

For reference, the Dutch PFAS limit for soil, as recommended by RIVM, is 0.8 ng PFOA/g dm. These results indicate a significant PFAS load in the environment surrounding the REC waste incinerator.

In the 2025 follow-up TW-biomonitoring research, a broader range of biomarker samples will be taken, including from eastern and southern regions to provide comparative environmental data. However, as previously discussed in the WUR biomonitoring study, identifying suitable reference locations presents certain challenges in establishing accurate environmental baselines.

PFAS in SOIL & Water, in environment near WtE incinerator REC, 2024

				µg PFOA eq. /kg		
				EFSA-4	Sum	C8
Year	Country	Location	Biomarker	∑ 4 PFAS	∑ 24 PFAS	PFOS
2023	NL	Wijnaldum - 01	Fruit	0,00	0,00	
2023	NL	Wijnaldum - 01	Egg	43,08	48,46	38,40
2024	NL	Tzummarum	Egg	0,28	0,28	0,28
2024	NL	Wijnaldum - 01	Egg	0,61	0,61	0,61
2022	BE	Antwerp 0-2 km Fluor industry	Egg	43,48	48,06	39,00
2022	BE	Antwerp 2-4 km Fluor industry	Egg	7,34	10,10	6,50
2022	BE	Antwerp 4 -6 km Fluor industr	Egg	8,84	11,51	4,40

				PFAS CALUX		
			dry weight (dw)	blanco	µg PFOA eq./g	ng PFOA eq./g
2024	NL	Wijnaldum - 01	Soil - 1		0,10	110
2024	NL	Wijnaldum - 01	Soil - 3		0,57	570
					µg PFOA eq./l	ng PFOA eq./l
2024	NL	Wijnaldum - 01	Water - 1	1,1, (-0,48)	0,62	620

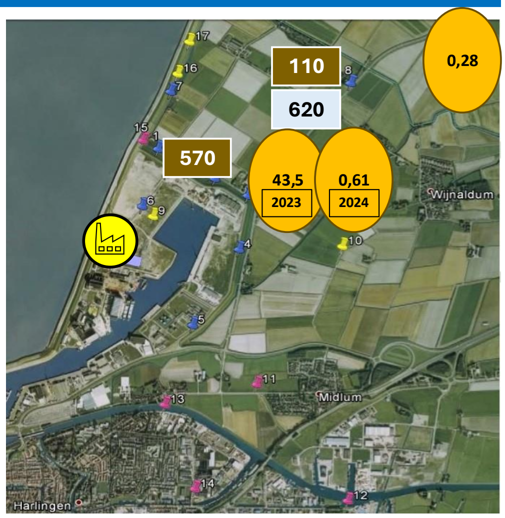


Figure 23: PFAS in soil and water.

Biomonitoring of POPs/PFAS should be integrated into waste incineration monitoring protocols to improve understanding of health risks and POP emissions. Incinerators can serve as potential sources of PFAS emissions due incomplete combustion of PFAS containing waste (see Figure 24 below).

Elevated concentrations of perfluoroalkyl substances (PFAS) have been detected in the eggs of free-range chickens collected 2 km from the REC, aligning with the predominant wind direction. The PFAS concentrations in these eggs is comparable to those found in eggs produced under the plume of a major fluorochemicals industry, such as in Antwerp, Belgium. These findings raise serious concerns regarding PFAS contamination at Egg location 1.

In France, the government is considering measurement campaigns to assess atmospheric emissions of PFAS from waste incineration and co-incineration plants. The goal is to determine whether combustion effectively eliminates PFAS and to improve overall understanding of the thermal degradation of these "eternal" pollutants.

By mid--2025 and late 2027. 49 PFAS compounds will be measured using the US OTM-45 method. This enhanced PFAS monitoring in France, underscores the need for similar initiatives in the Netherlands, particularly within the WUR/LTO biomonitoring program, to investigate PFAS contamination in the environment surrounding the REC waste incinerator.

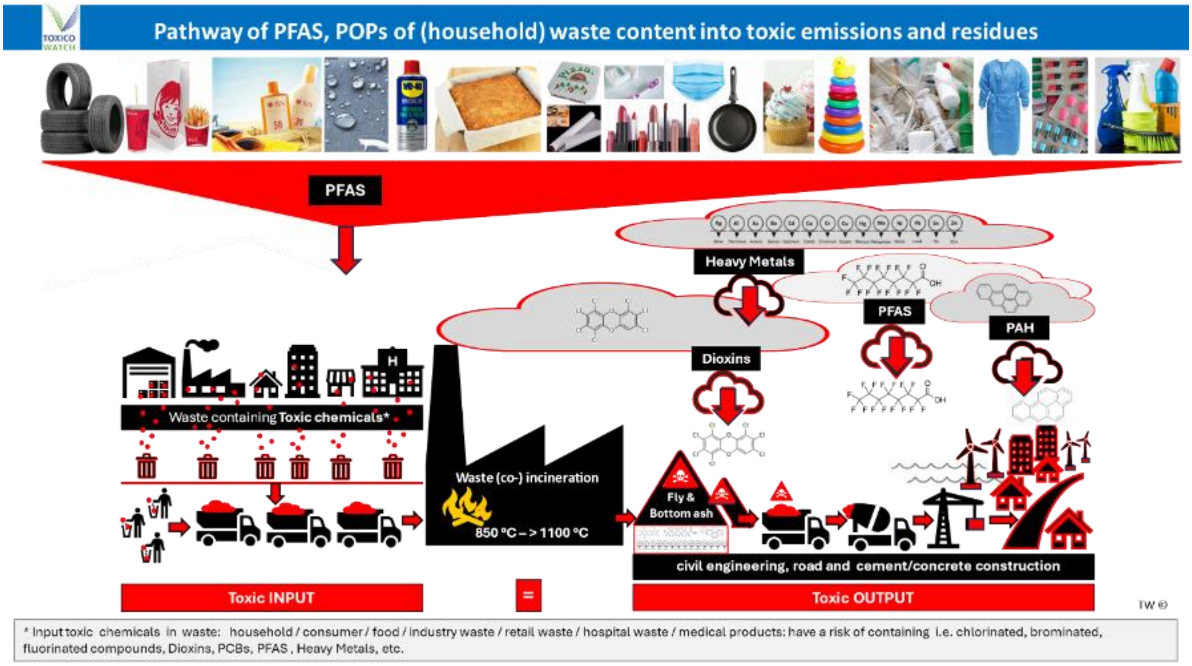


Figure 24: Pathway of PFAS

4.3. Heavy Metals

4.3.1. Heavy Metals in Soil

Dioxins (PCDD/Fs) should integrate into a more holistic assessment of pollution, particularly in relation to human exposure and health impacts. These assessment should also take into account emissions and deposition of heavy metals into the environment.

As part of this study, TW analysed 14 heavy metals in soil samples. The table below shows the analysis results, and a TW heatmap was compiled based on literature data or EU regulatory limits for lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and chromium (Cr).

The concentration of silver (Ag) in soil sample 4, located in the vicinity of Midlum's farmland, and sol sample 2 from the vegetable garden in Wijnaldum (Egg loc. 1), was found to be particularly elevated, with levels of 0.84 mg/kg and 0.21 mg/kg, respectively. These values are 28.0 and 7.0 times higher than the reference values. The lack of data on silver (Ag) emissions from waste incineration is a notable concern. Of particular concern is monovalent silver (Ag+) due to its antimicrobial properties. Silver nanoparticles have been incorporated into a variety of products, including clothing, plant protection products and fertilisers (Eckelman 2007). During the incineration process, these nanoparticles may be released into the environment, raising potential environmental and health concerns.

A study was conducted to analyse the mercury (Hg) concentrations in the soil of the vegetable garden at Wijnaldum (Egg location 1). The results showed that Hg levels were 15.2 times higher than the reference values. Elevated Hg concentrations were also detected in the moss samples (Bryophytes) collected from the same location, reinforcing concerns about Hg contamination in the area.

		Results Heavy Metals [14] in SOIL, mg/kg, (dw) September 2024															
Sample date	Location	TW-REF-NR	Loc. Nr	1 Ag	2 Al	3 As	4 Ba	5 Cd	6 Co	7 Cr	8 Cu	9 Hg	10 Mn	11 Ni	12 Pb	13 Sn	14 Zn
				Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
2024-09-29	Midlum	24TWNL-ML-SOIL-KAT-04	Soil 4	0,84	13877,00	14,00	33,00	0,17	5,10	33,00	22,00	0,039	342,00	18,00	20,00	1,20	44,00
2024-09-28	Midlum	24TWNL-H-SOIL-KD-03	Soil 3	0,14	9606,00	16,00	27,00	0,22	4,30	26,00	29,00	0,046	293,00	15,00	24,00	1,70	39,00
2024-09-28	Wijnaldum	24TWNL-W-JR-SOIL-VEG-02	Soil 2	0,21	8069,00	6,40	43,00	0,45	4,00	21,00	30,00	0,50	364,00	13,00	51,00	1,70	130,00
2024-09-28	Wijnaldum	24TWNL-W-JR-SOIL-LB01	Soil 1	0,13	9877,00	9,00	20,00	0,20	4,10	26,00	24,00	0,05	303,00	14,00	46,00	1,10	42,00

		Exceeding X Factor Heatmap of the Results Heavy Metals [14] in SOIL, September 2024															
Sample date	Exceeding factor	TW-REF-NR	Loc. Nr	1 Ag	2 Al	3 As	4 Ba	5 Cd	6 Co	7 Cr	8 Cu	9 Hg	10 Mn	11 Ni	12 Pb	13 Sn	14 Zn
				Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
	> 100,0	TW-Literature ref.		0,03	4000,00	5,00	22,00	0,31	8,00	13,00	9,00	0,03	500,00	9,80	29,00	2,83	47,00
	50,0 - 100,0																
2024-09-29	10,0 - 50,0	24TWNL-ML-SOIL-KAT-04	Soil 4	28,0	3,5	2,8	1,5	0,5	0,6	2,5	2,4	1,2	0,7	1,8	0,7	0,4	0,9
2024-09-28	5,0 - 10,0	24TWNL-H-SOIL-KD-03	Soil 3	4,7	2,4	3,2	1,2	0,7	0,5	2,0	3,2	1,4	0,6	1,5	0,8	0,6	0,8
2024-09-28	2,0 - 5,0	24TWNL-W-JR-SOIL-VEG-02	Soil 2	7,0	2,0	1,3	2,0	1,5	0,5	1,6	3,3	15,2	0,7	1,3	1,8	0,6	2,8
2024-09-28	1,5 - 2,0	24TWNL-W-JR-SOIL-LB01	Soil 1	4,3	2,5	1,8	0,9	0,6	0,5	2,0	2,7	1,5	0,6	1,4	1,6	0,4	0,9

Figure 25: Heavy metals in soil

4.3.2. Heavy Metals in Mosses (*Bryophytes*)

Moss (*Bryophytes*) sampled from the vicinity of the incinerator contains remarkably high concentrations of aluminium (Al), arsenic (As), copper (Cu), mercury (Hg), nickel (Ni) and lead (Pb). The **reference value of aluminium (Al) in vegetables is set at 27.5 mg/kg dw** (González-Weller, 2010). Aluminium is potentially neurotoxic, with health effects linked to neurological disorders, cognitive decline, and dementia or Alzheimer's disease (González-Weller, 2010). The findings of this study indicate an aluminium accumulation factor of 26 to 560 in moss (Bryophytes), which is a cause for concern and warrants further research.

It is noteworthy that aluminium is not included in the monitoring programme of WUR, and it not commonly analysed in standard commercial heavy metals laboratory tests. Additionally, aluminium was detected in eggshells from Location Wijnaldum (Egg Location 1), at a concentration of 4.9 mg/kg. However, interpretating this finding is challenging due to the lack of available literature on aluminium levels in eggshells.

Of a particular concern is the accumulation factor of lead (Pb) in moss (Bryophytes), which ranges from 240 - 1410. Scientific research has demonstrated that even low levels of lead exposure can have adverse effects on young children's health. The accumulation of lead in moss via atmospheric deposition is a serious concern, particularly, given that lead is not subject to monitoring by the waste incineration industry (REC).

Lead (Pb) exposure can impair brain development in young children, potentially resulting in reduced Intelligence Quotient (IQ). In response to new findings, the European Food Safety Authority (EFSA) concluded that the previous health-based limit value for lead (Pb), the Provisional Tolerable Weekly Intake of 25 µg/kg body weight/week, was no longer safe (EFSA, 2010). Consequently, eliminating all exposure to lead (Pb) is now recognised as a public health priority.
Given these risks, it would be prudent for the waste incineration industry and government agencies to include lead (Pb), cadmium (Cd) and mercury (Hg) in their monitoring programmes to better assess. And mitigate potential health impacts.

	Results Heavy Metals [14] in Mosses (*Bryophytes*), mg/kg, (dw) September 2024															
Location	TW-REF-NR	Loc. Nr	1 Ag	2 Al	3 As	4 Ba	5 Cd	6 Co	7 Cr	8 Cu	9 Hg	10 Mn	11 Ni	12 Pb	13 Sn	14 Zn
			Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
Wijnaldum	24TWN-W-JRG-Mos-01	Moss 1	0,120	15414,00	4,000	69,00	0,740	7,300	70,00	57,00	4,000	391,00	76,00	141,000	4,800	335,00
Wijnaldum	24WNL-W-JR-RWB-Mos-05	Moss 2/5	0,019	983,00	0,760	29,00	0,180	0,490	17,00	14,00	0,073	364,00	1,60	24,000	0,560	68,00
Wijnaldum	24TWNL-W-JR-KH-Mos-03	Moss 3	0,120	727,00	1,900	20,00	0,640	2,800	2,00	36,00	0,073	72,40	7,10	52,000	0,460	102,00

	Exceeding X Factor Heatmap of the Results Heavy Metals [14] in Mosses (*Bryophytes*), mg/kg, (dw) September 2024															
Exceeding factor	TW-REF-NR	Loc. Nr	1 Ag	2 Al	3 As	4 Ba	5 Cd	6 Co	7 Cr	8 Cu	9 Hg	10 Mn	11 Ni	12 Pb	13 Sn	14 Zn
			Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
> 100,0	Ref. veg mg/kg (EU)		0,03	27,5	0,05	45,7	0,2	0,05	1,3	1,22	0,03	70	0,33	0,1	0,053	6,1
50,0 - 100,0																
10,0 - 50,0	24TWN-W-JRG-Mos-01	Moss 1	4,0	560,5	80,0	1,5	3,7	146,0	53,8	46,7	133,3	5,6	230,3	1410,0	90,6	54,9
5,0 - 10,0	24WNL-W-JR-RWB-Mos-05	Moss 2/5	0,6	35,7	15,2	0,6	0,9	9,8	13,1	11,5	2,4	5,2	4,8	240,0	10,6	11,1
2,0 - 5,0	24TWNL-W-JR-KH-Mos-03	Moss 3	4,0	26,4	38,0	0,4	3,2	56,0	1,5	29,5	2,4	1,0	21,5	520,0	8,7	16,7
1,5 - 2,0																

Figure 26: Heavy metals in moss

The heatmap is based on a literature review of heavy metal concentrations in vegetables. In this TW study, cadmium (Cd) levels in mosses (Bryophytes) ranged from 180 to 640 µg/kg, which is three times higher than the 208 µg/kg recorded by the WUR biomonitoring programme in the environment of the waste incinerator REC.

Similarly, while the maximum mercury (Hg) concentration recorded by the WUR biomonitoring/REC was 6.4 µg/kg, the TW study recorded a concentration of 4,000 µg/kg of mercury (Hg) in mosses (Bryophytes).

5. Conclusion

The results of the sampling in September 2024 are presented in this interim biomonitoring report on persistent organics pollutants (POPS) in the environment surrounding the WtE waste incinerator REC in Harlingen.

In 2024, **dioxins were found in backyard chicken eggs, soil and mosses.** DR CALUX bioassay analysis for dioxins (PCDD/F/dl-PCBs) in backyard chicken eggs confirmed continued contamination, with a measured concentrations of **5.20 pg TEQ/g fat, exceeding the EU limit of 3.3 pg TEQ/g fat**. This result was obtained from a newly established chicken coop, after replacing older chickens (2/3 years). While no elevated dioxins were found in grass, dioxin levels in soil showed sevenfold (7 x) increase compared to 2013. Additionally, high dioxin levels in mosses (*Bryophytes*) were recorded at 1200 meters from WtE incinerator REC, raising further concerns.

The **PFAS results in eggs, soil and water are especially concerning.** In 2023, backyard chicken eggs contained **48.3** µg/kg of PFAS. After replacing the chickens (< 12 months old), the **2024 analysis showed reduced levels of 0.61 µg/kg PFOS**. However, the specific PFAS congeners detected in 2023 were comparable to those found near the major fluorochemical plant in Antwerp, Belgium. This raises critical question: How can such high levels of PFAS occur in Harlingen, a primarily agricultural environment?

Using innovative PFAS CALUX bioassay analysis, **110 and 570 ng PFOA eq./g dw** were measured in soil in the vicinity of the incinerator. For reference, the Dutch government's recommended limit for soil is 0.8 ng PFOA/g dm (0.8 µg PFOA/kg dw), meaning the detected PFAS levels exceed the limit by a factor of 700.

In 2024, water samples from a ditch contained **610 ng PFOA eq. /l**, **exceeding the Dutch drinking water limit of 4.4 ng PFOA eq./l (RIVM, 2023) by a factor of 138.** These findings underscore the need for investigation of PFAS contamination in the environment surrounding the REC waste incinerator.

Heavy metal analysis showed significantly elevated concentrations in soil and mosses. Key findings include:

- Mercury (Hg) in the vegetable garden soil at Wijnaldum (egg location 1) measured 15 times higher than the reference values.
- The cadmium (Cd) in mosses (*Bryophytes*) range from 180 to 640 µg/kg, which is three times higher than the WUR biomonitoring programme result for the waste incinerator REC.
- Of particular concern is the high accumulation factor of lead (Pb), which ranges from 240 to 1410 across all three samples. Elevated levels were also observed for aluminium (Al), arsenic (As), and nickel (Ni). Additionally, the findings revealed notably high concentrations of cobalt (Co) and mercury (Hg).

After 12 years of biomonitoring, the main conclusion is that environment surrounding the WtE waste incinerator REC remains contaminated with dioxins, PFAS, and heavy metals. Further research is needed to determine the extent to which the waste incineration contribute to this contamination. This can be achieved by resuming semi-continuous POP-measurements in flue gases, providing critical insights into emissions sources and environmental impact.

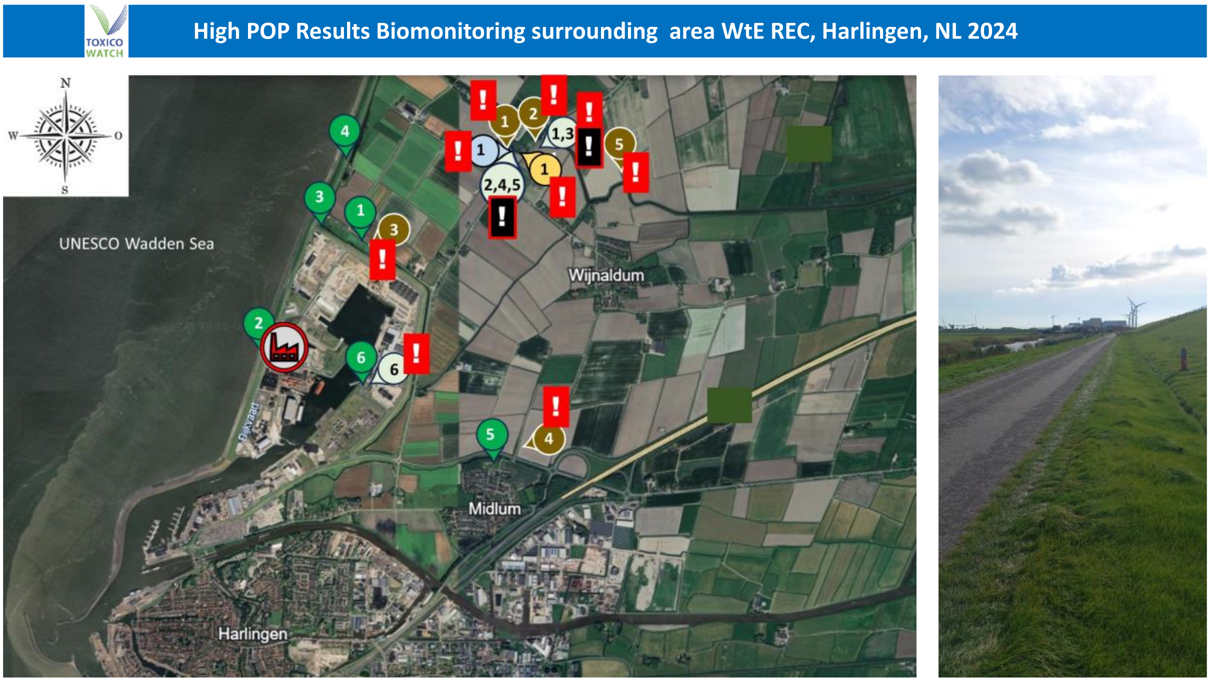


Figure 27: Infographic results biomonitoring Harlingen, The Netherlands, 2024

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Annex 3: Challenging biomonitoring