

Annex I

SAMPLE Plan 2021 region Valdemingómez, Madrid, Spain

Dioxins (PCDD/F/dl-PCBs), PAH, PFAS emissions

**Biomonitoring
Research Madrid
Spain, 2021**

Eggs
Backyard chicken

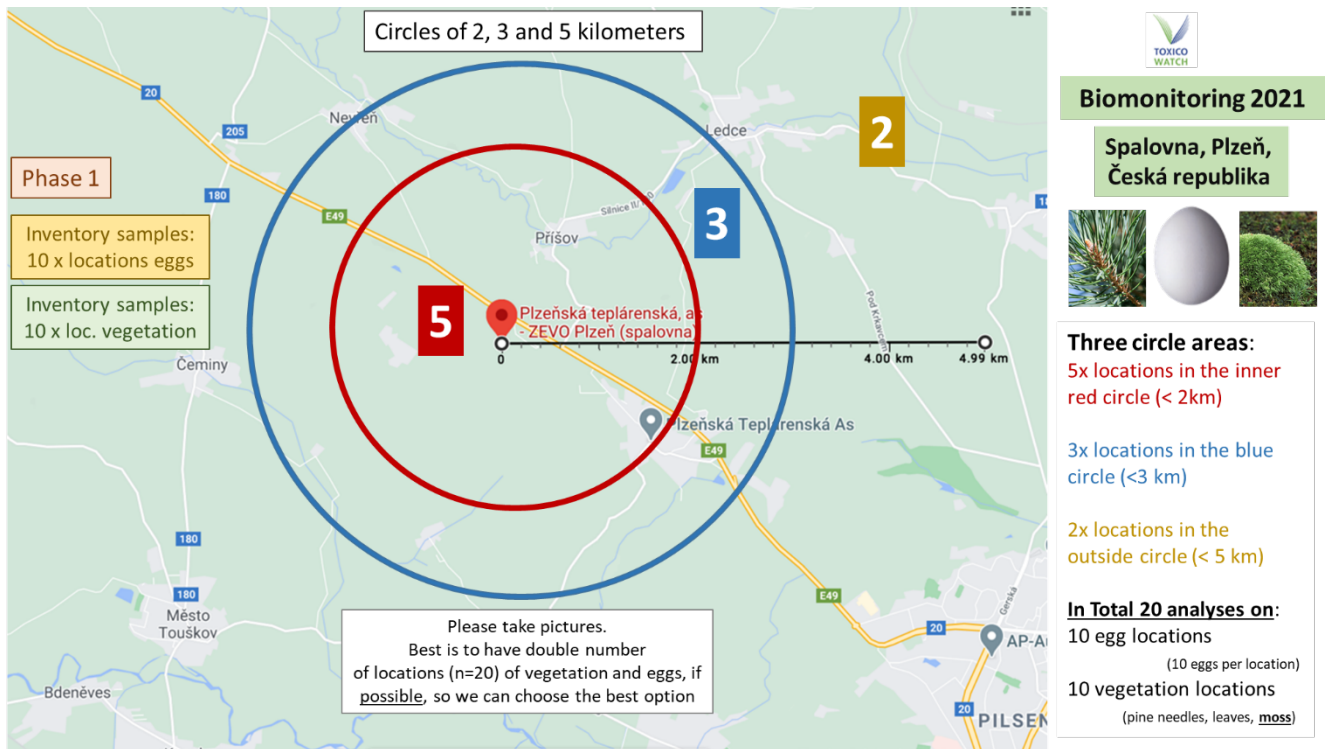
Vegetation
Pine needles
Mosses



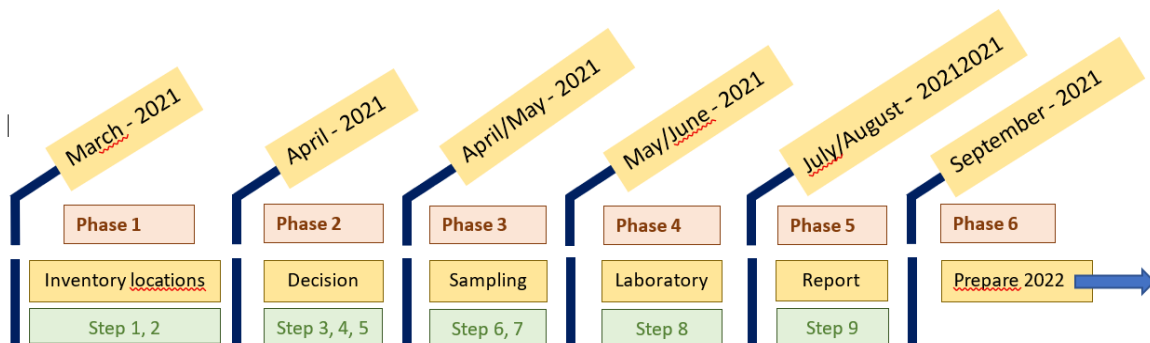
Valdemingómez
waste incinerator



TW Biomonitoring Eggs Backyard Chicken - Madrid / Valdemingómez 2021		
Action	Action	9 Steps EGG sampling
TW	MD	
Phase 1		
Step 1		Inventarisation of suitable Egg locations of backyard chicken < 2, < 3, < 5 km
		5x locations inner circle < 2 km, 3x locations <3 km, 2x locations outside circle < 5 km
		Total: 10 selected vegetation samples
		Please Provide TW Questionnaire Eggs of backyard Chicken to chicken coop owners and send it back to TW
		Locations located IN the winddirection coming undisturbed from the waste incinerator
		Locations at < 2km most wanted for research, at least 5 locations
		Locations <3 km at least 3 locations
		Locations outside circle < 5 km, at least 2 locations for reference research
		TW will check by Google earth and other studies, data
Step 2		Check Chicken Coop owners willingness to Participate, Send back Questionnaire
		Location visit chicken coop owners , please take pictures/photo's of chicken enclosures, fences, building material, like roofs, feed cribs, water containers with <u>ordering of location nr.</u> and <u>coordinates</u> of Egg, Feed, Soil samples
		TW will assist you by mobile phone/video if needed directly on location
		<2 km (5x vegetation locations needed for analyses, if possible 10x locations so we can choose)
		Photo's/pictures needed to be made of:
		1. the whole area chicken coop
		2. enclosure fences
		3. building material chicken enclosure, like roofs, Henhouse, chicken brood boxes
		4. feed cribs, water container suppliers
		5. the chicken coop itself
		SEND BACK the TW Questionnaire for Biomonitoring backyard Chicken Eggs
		< 3 km (3x vegetation locations, if possible 6 x locations so TW can choose)
		3- 5 km (2x vegetation locations, if possible 4 x locations so TW can choose)
Phase 2		
Step 3		Sending all pictures/photo material to ToxicoWatch for study
		with <u>ordering of location+ photo nr.</u> and <u>coordinates</u> of possible egg locations, if needed with TW assistance
		TW will study on all the send photo material of Egg locations together with received questionnaires of Egg locations.
		After studying this photo input, TW will select the best Egg locations options to use for sampling
Step 4		Review with Madrid Team about possibilities of sampling by TW selected
		Egg locations <2,<3, and <5 km (ideal 10 locations), 10 Egg samples/location!
		5x locations inner circle < 2 km, 3x locations <3 km, 2x locations outside circle < 5 km
		Discussion of the selected Egg locations to make definitive selection of sample locations
Step 5		TW Providing Reference lab sample Nr (TW-REF-NR) for Egg samples
		TW provides after studying the photomaterial of the Egg locations <u>TW-REF-NR</u> i.e. TW-MD21-Egg-L01
		The TW-REF-NR is important for the Lab analyses needed to be handled/documentated very strictly
Phase 3		
Step 6		Second visit to selected vegetation locations for COLLECTING SAMPLES
		Collecting the eggs (10 per location) at the selected Egg locations as discussed in Review, step 4.
		The collecting of the Eggs needed AGAIN covered by pictures/video and documented/numbered
		TW assisting by video and mobile during collecting samples
		The photo material needs to be documented with numbers and data, (see Step 2)
		Collected samples needed directly marked with a TW Reference/laboratorium number.
		TW assist if needed by mobile/video connection
		Collected Eggs needed to be stored as prescribed (dry, dark and cool) till shipping to NL
Step 7		Send/shipping all the collected Egg samples to TW as prescribed
		Shipping of the collected Egg samples to the address of Laboratory
		Please take photo of packed Egg sample before shipping to Lab
		TW will provide prescription for sending/shipping
Phase 4		
Step 8		Handling by TW of all the received Egg samples at the Lab
		TW Checking all the received Egg samples on, TW/lab nr/ grams with the photo/video material and other data
		Providing the collected samples to the lab with instructions for analyses
		TW: Proposal discussions with Lab: analyse plan for this collected biomarkers
Phase 5		
Step 9		Lab Analyses results Egg samples, TW Report Madrid Biomonitoring 2021
		TW Studying, working out report MD 21
		TW finalising research Report Madrid Biomonitoring 2021
Phase 6		
		Prepare Biomonitoring Madrid, Spain 2022 on base of the results 2021



The initial biomonitoring sample timeline plan:



		Biomonitoring Vegetation - Madrid / Valdemingómez, 2021
		Evergreen trees & Mosses
Action	Action	9 Steps Vegetation sampling
TW	MD	
Phase 1		
Step 1		Inventarisation of available common vegetation species
		Please check if the vegetation evergreen trees and mosses (preferable <i>Pinus</i> spp) can be found in the area around the incinerator. <i>Pinus</i> is the sole genus in the plant family <i>Pinaceae</i> .
		5x locations inner circle < 2 km, 3x locations <3 km, 2x locations outside circle < 5 km
		Total: 10 selected vegetation samples
		TW will check availability of vegetation by Google maps/earth and other studies, data
Step 2		Team MD Check availability vegetation in the field circle: <2 km , <3km, <5km
		Location visit, making pictures/photo's with ordering of location nr. and coordinates of vegetation samples
		TW will assist you by mobile phone/video if needed directly on location
		Please select vegetation which is easy accessible and approachable, within:
		< 2 km (5x vegetation locations needed for analyses, if possible 10x locations so we can choose)
		< 3 km (3x vegetation locations, if possible 6 x locations so we can choose)
		3- 5 km (2x vegetation locations, if possible 4 x locations so we can choose)
		Photo's/pictures needed to be made of:
		1. the whole tree/shrub/plant in the environment
		2. Close-up from the canopy of the tree
		3. Close-up from the pine needles / Mosses
		4. Close-up from the bark
		5. Extra pictures i.e. leaves, flowers, soil, cones
		Use not only the youngest needles on a twig
		accessible: meaning it is physically possible to enter the needles on the twigs of a tree
		The trees needs to be accessible for collecting the needles mosses + marking the wind direction.
Phase 2		
Step 3		Sending all pictures/photo material to ToxicWatch
		with ordering of location+ photo nr. and coordinates of potential vegetation samples, if needed with TW assistance
		TW will identify the tree species and study on all the send photo material of vegetation.
		After studying this photo input, TW will select the best vegetation sample options to use for biomonitoring 2021
Step 4		Review with MD Team about TW Decision of selected vegetation samples
		Discussion of the selected vegetation locations to make definitive selection of locations for biomonitoring
Step 5		TW Providing TW-Reference-lab (TW-REF-NR) sample numbers
		TW provides after studying the photomaterial of the vegetation TW-REF-NR
		related to A. the location, B. The date of collecting, C. vegetation species and D. winddirection
		The TW-REF-NR is important for the Lab analyses
		and therefore, needed to be handled/documentated very strictly
Phase 3		
Step 6		Second visit to selected vegetation locations for COLLECTING SAMPLES
		Collecting the selected vegetation samples as discussed in Review, step 4.
		the collecting of the samples needed AGAIN covered by pictures/video and documented/numbered
		TW will assist you by video and mobile during collecting samples
		The photo material needs to be documented with numbers and data
		Samples needed marked, so TW can provide a TW-REF-NR/laboratorium number.
		TW will assist you with this as much as possible
		Collected samples needed stored as prescribed (dry, dark and cool) till shipping to NL
Step 7		Sending/shipping all the collected vegetation samples to TW as prescribed
		Shipping of the collected vegetation samples to NL/TW
		as prescribed, TW will provide prescription for sending/shipping
Phase 4		
Step 8		Handling by TW of the received vegetation samples
		Checking all the received vegetation samples on species, TW/lab nr/ grams with the photo/video material and other data
		Providing the collected samples to the lab with instructions for analyses
		TW: Proposal discussions with Lab: analyse plan for this collected biomarkers
Phase 5		
Step 9		Analyse results vegetation samples, TW Report Biomonitoring MD 2021
		TW: Analyse results, studying and work out Report Biomonitoring MD 2021
Phase 6		
		Prepare Biomonitoring 2022 on base of the results 2021

Instructions for shipping samples to ToxicoWatch, The Netherlands

Egg samples:

- 1. Egg boxes need to be:**
 - a. numbered per location**, i.e. egg 1, egg 2, (TW provides TW-REF-NR)
 - the **same for the back-up samples** taken on the locations for **soil**; i.e. soil 1, soil 2, etc. (TW provides TW-REF-NR)
feed; i.e. feed 1, feed 2, etc (TW provides TW-REF-NR)
See list below
 - put **egg boxes in a (HDPE) plastic bag** as used with sampling vegetation
 - put also **number location** on the plastic bag
 - plus **address/coordinates of location on a list**
 - date of sampling of the eggs**
 - each egg box in (HDPE) plastic bag **need to packed with a protected thick layer** of newspapers or if you have available foam or cotton wool to protect breaking of the eggs.
 - TW will measure the weight of the pooled samples
- 2. Place all the wrapped eggboxes careful in a (polystyrene) box together with the backup samples of soil and feed.**
- shipping of the egg samples with a **Track & Trace within 24-48 hours to the Netherlands by a trustful post delivery service**

Vegetation samples:

- 1. Please check if all the bags** of pine needles/leaves and mosses have
 - the **location numbers, date of sampling and coordinates on it or on a list**
For samples : Veg 1, veg 2 , veg 3 etc (TW provides TW-REF-NR)
Mosses 1, Mosses 2, etc(TW provides TW-REF-NR)
 - TW will measure the weight of each sample in NL
 - Store the vegetation samples in (HDPE) bags dark and cool until shipping**
- For shipping put all the (HDPE) plastic vegetation bags in a (polystyrene) box (preferable one big box), strong enough for shipping
 - Close the box well with tided tape
 - Mark the box with text "Handel with care!"
 - TW provided address where to send
 - Preferable a **courier service for quick delivery 24-48 hours**

Please **take pictures of how the sample material is placed in the shipping box(es)** and from the outside of the sealed marked boxes with the address on it and ready for shipping to the Netherlands (NL).

Please **send us the pictures you have taken on the locations during sampling**. TW need the exact coordinates of the samples

Thanks to the Sampling Team for taking care of collecting the biomonitoring samples at the locations!

Annex II & III

Eggs & Vegetation locations, Madrid, Spain - 2021

Dioxins (PCDD/F/dl-PCB), PAH, PFAS emissions

**Biomonitoring
Research Madrid
Spain, 2021**

Eggs
Backyard chicken

Vegetation
Pine needles
Mosses



Valdemingómez
waste incinerator



Biomonitoring
Research Madrid
Spain, 2021

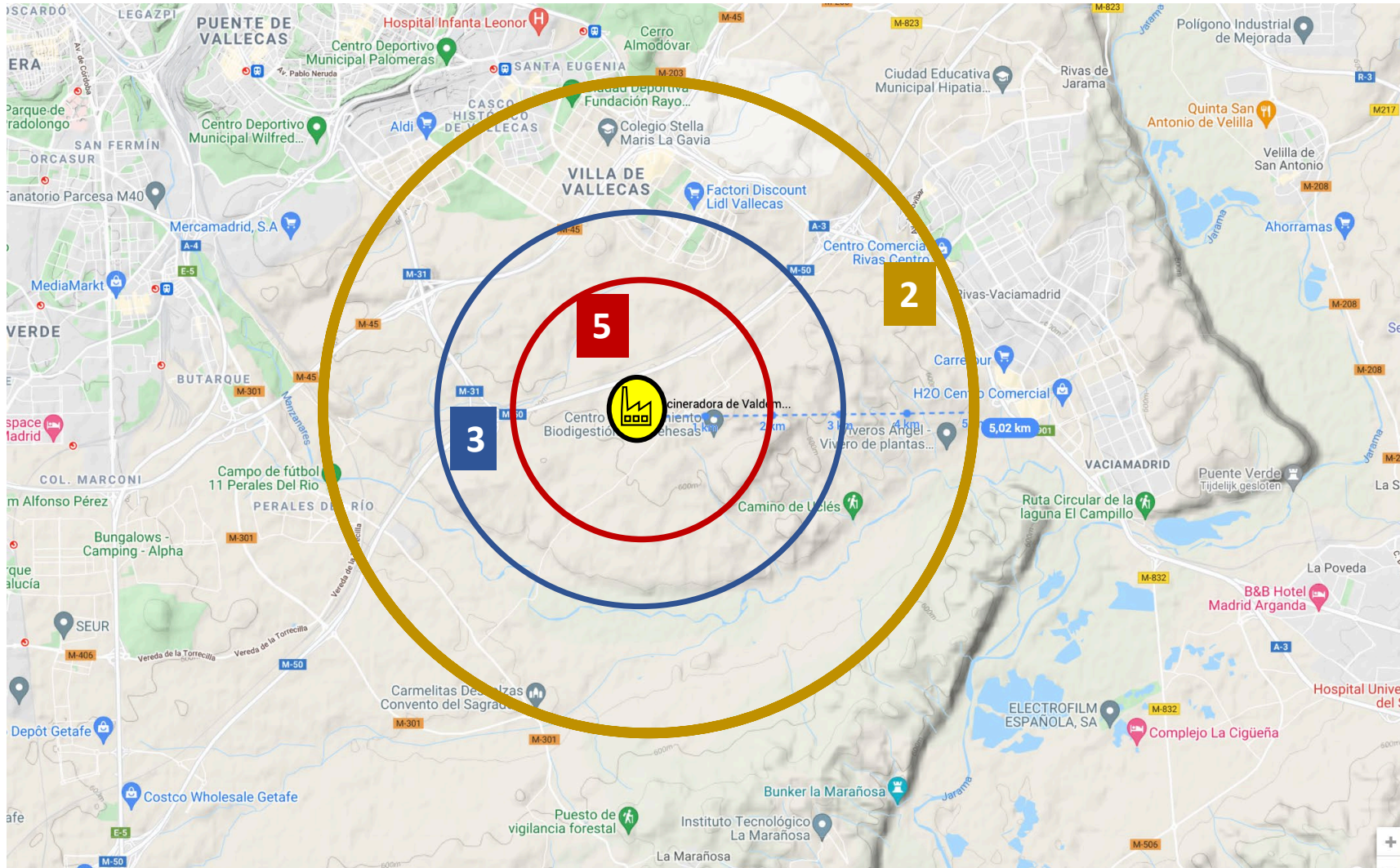
- Eggs
- Backyard chicken
- Vegetation
- Pine needles
- Mosses



Valdemingómez
waste incinerator



Initial sampling plan Madrid - 2021



Biomonitoring 2021

Valdemingómez Madrid, Spain



Three circle areas:
5x locations in the inner red circle (< 2km)

3x locations in the blue circle (< 3 km)

2x locations in the outside circle (< 5 km)

In Total 20 analyses on:
10 egg locations

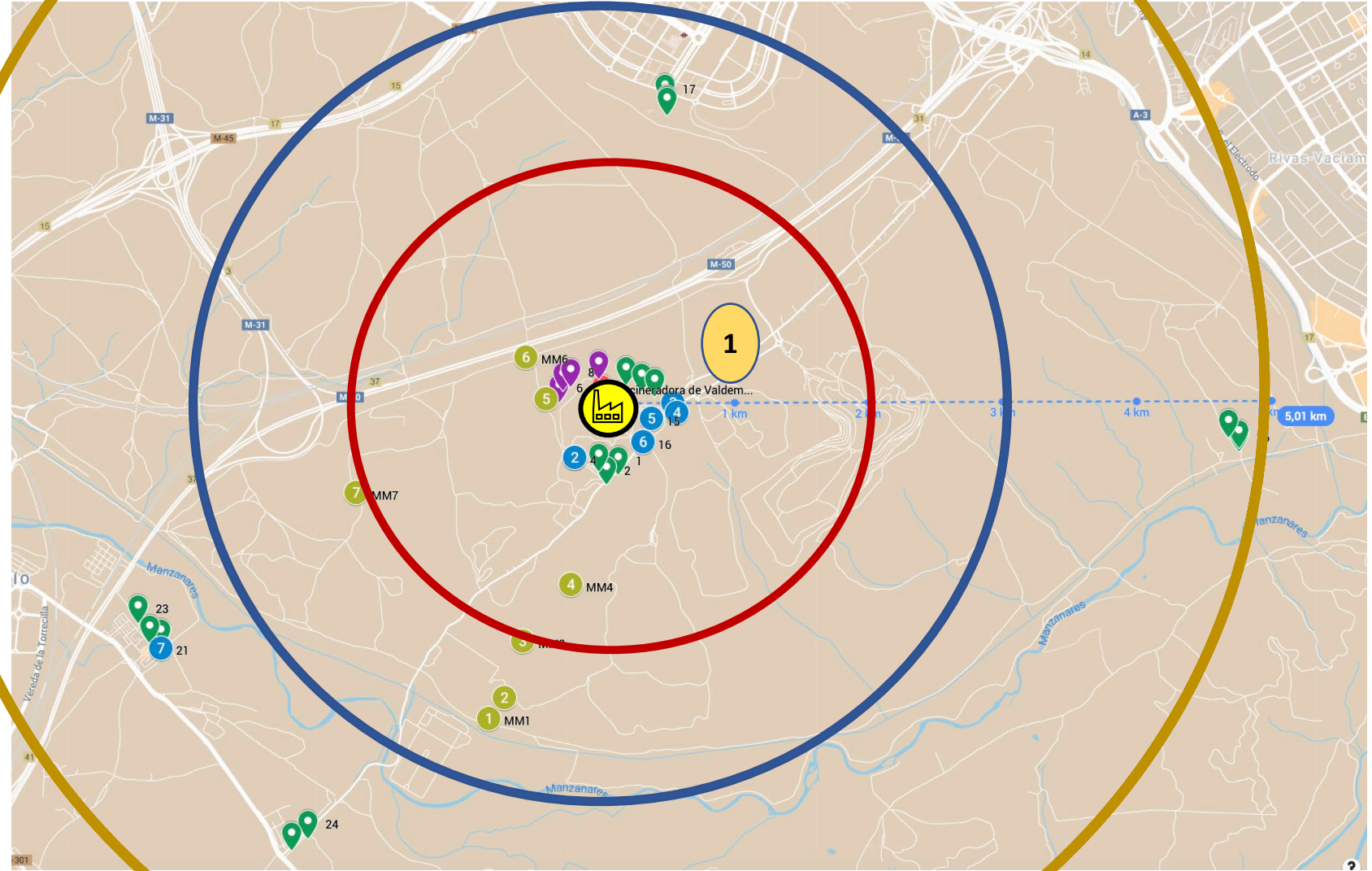
(10 eggs per location)

10 vegetation locations

(pine needles, leaves, moss)

Actual sample locations Madrid - 2021

Pine needles
Leaves
Mosses
Eggs



Mosses

Evergreen tree
Cupressus arizonica

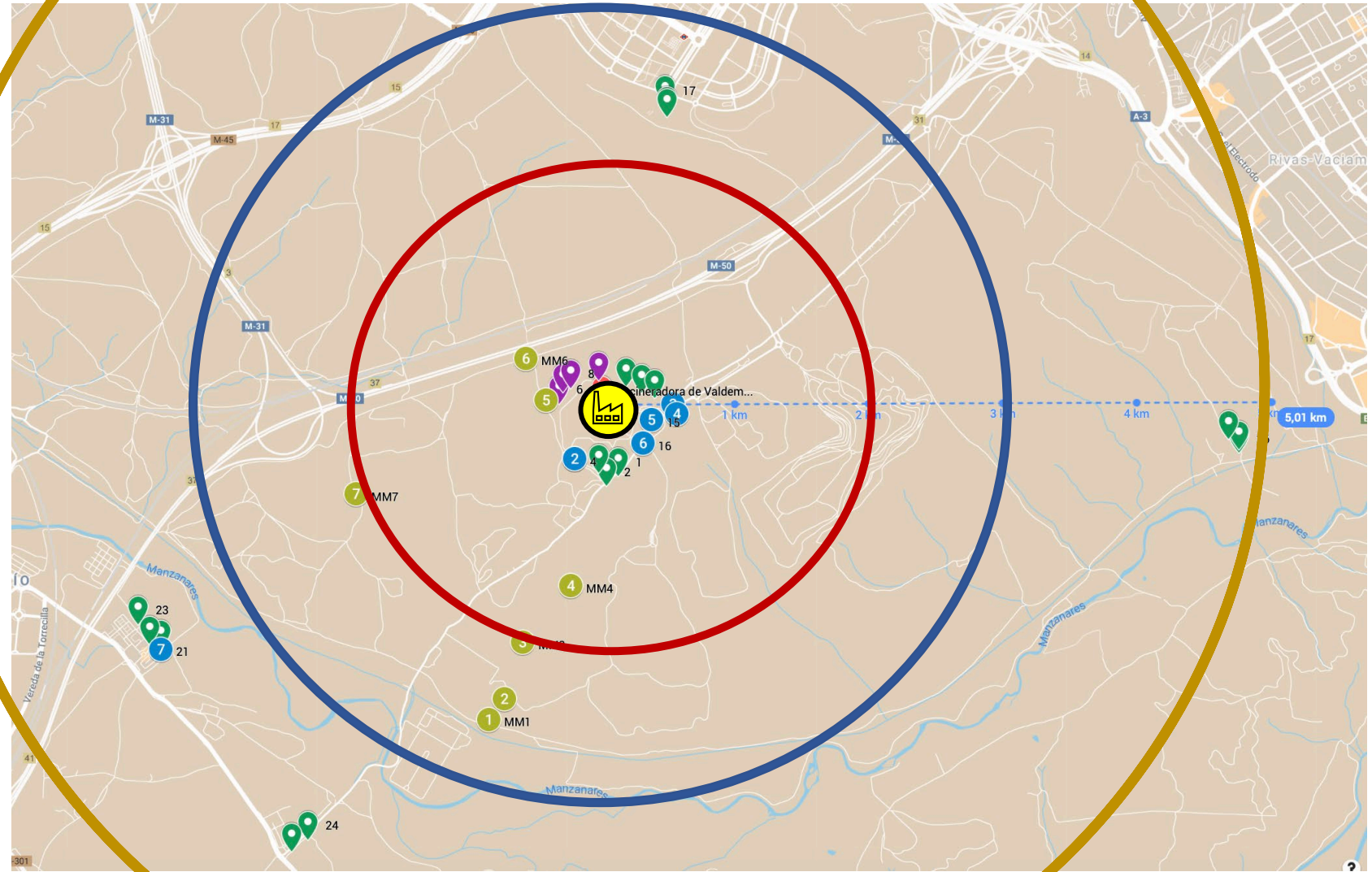
Deciduous tree
Ulmus sp.

Aleppo Pine tree
Pinus halepensis

1 Egg location 1

Vegetation sample locations Madrid - 2021

Pine needles
Leaves
Mosses



Mosses



Evergreen tree
Cupressus arizonica



Deciduous tree
Ulmus sp.



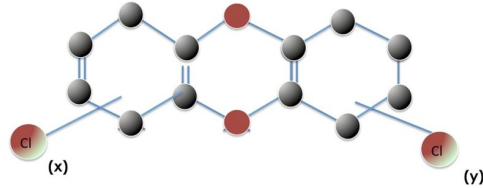
Aleppo Pine tree
Pinus halepensis

GC-MS chlorinated dioxins (PCDD/F/dl-PCB)

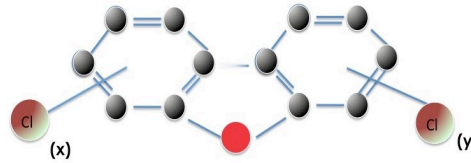
Halogen elements:

Chlorine (Cl)
Bromine (Br)
Fluorine (F)
Iodine (I)

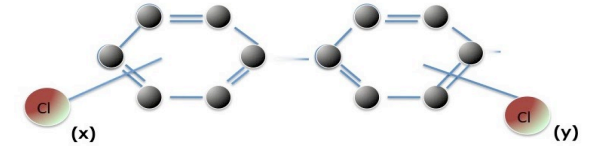
Dioxin
PCDD (75)
n = 7



Furan
PCDF (135)
n = 10



dioxin-like Polychlorobiphenyl
dl-PCB (209)
n = 12



Congeners of chlorinated compounds (chemical GC-MS analysis)

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
HCDF1	1,2,3,4,6,7,8-Heptachlorodibenzofuran	0,01
HCDF2	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

EU regulations for dioxins (PCDD/F/dl-PCB)

Halogens

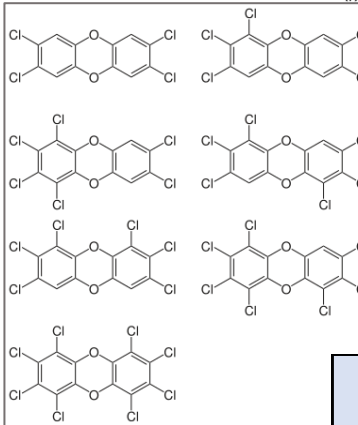
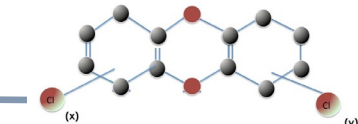
Chlorine (Cl)
Bromine (Br)
Fluorine (F)
Iodine (I)

Chlorine can be substituted by an other halogen

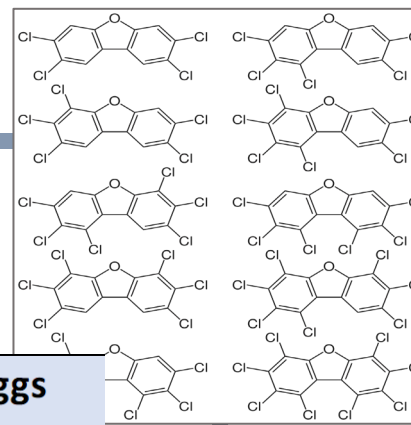
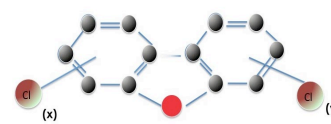
EU regulations only for **PCDD/F** in emissions

EU regulations only on **Chlorinated Persistent Organic Pollutants (POP)**

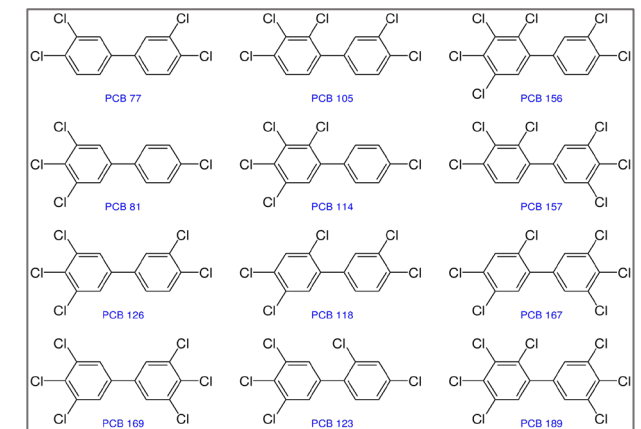
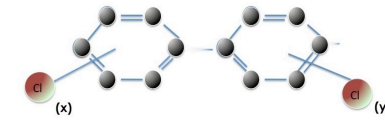
Dioxin
PCDD (75)
n = 7



Furan
PCDF (135)
n = 10



dioxin-like Polychlorinated biphenyl
dl-PCB (209)
n = 12



0.1 ng TEQ/Nm³



EU regulation Eggs

GC-MS pg TEQ/g fat

Action limit PCDD/F **≥ 1.75 pg**

EU regulation Eggs

GC-MS pg TEQ/g fat

Limit PCDD/F **≥ 2.5 pg**

EU regulation Eggs

GC-MS pg TEQ/g fat

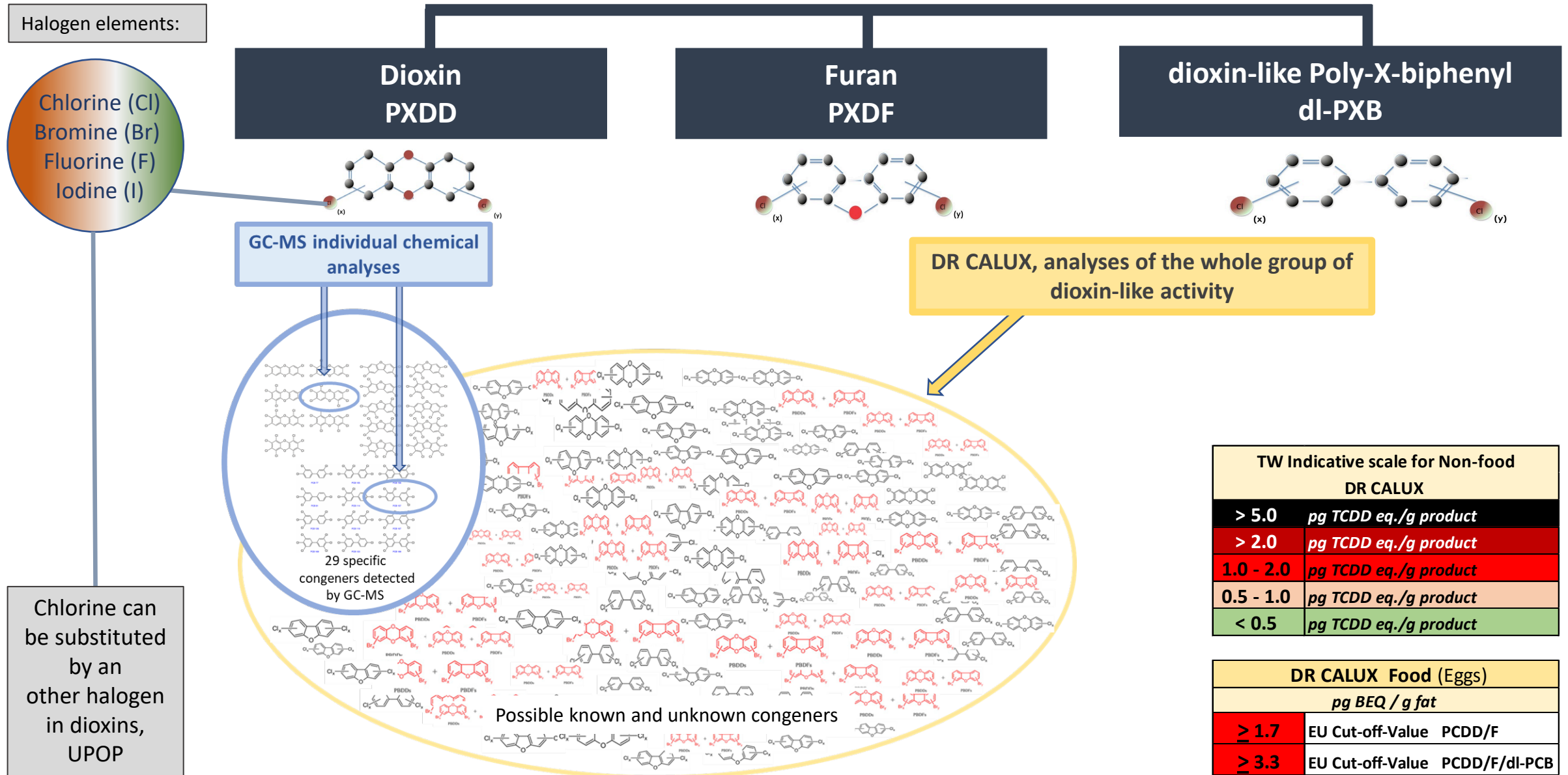
Limit PCDD/F/dl-PCB **≥ 5.0 pg**

EU regulation Eggs

GC-MS pg TEQ/g fat

Action limit dl-PCB **≥ 1.75 pg**

Chemical analysis (GC-MS) vs Bioassay (CALUX)

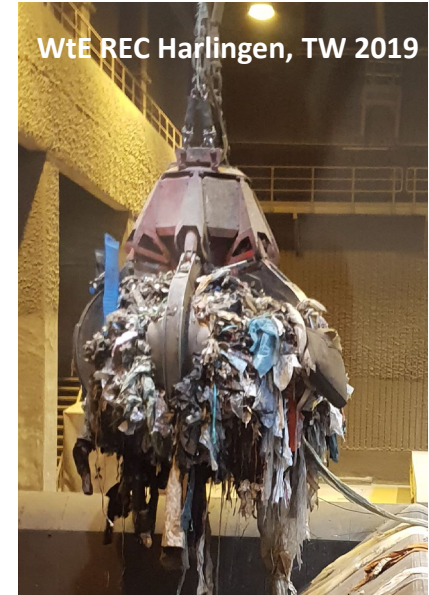
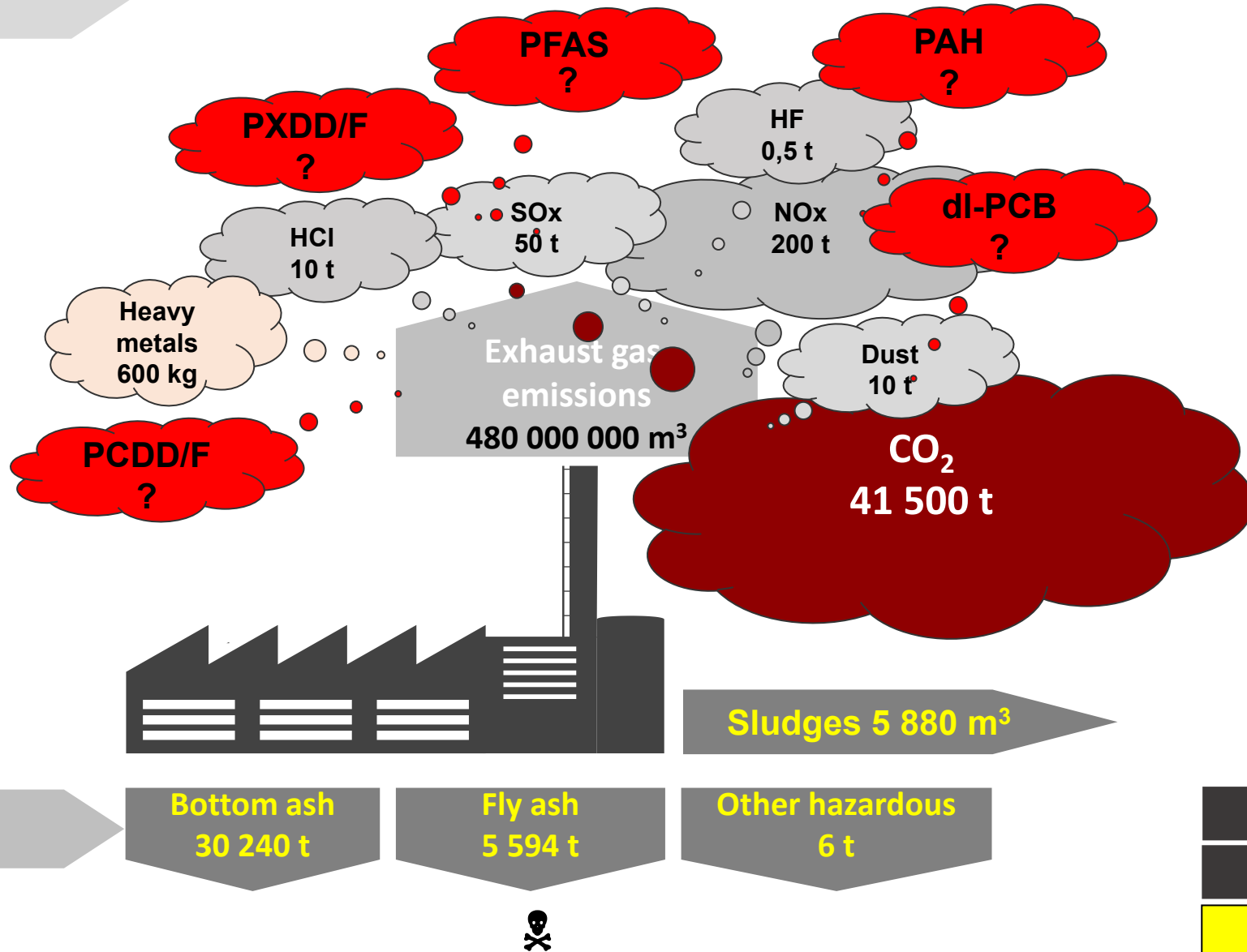


TW Indicative scale for Non-food DR CALUX	
> 5.0	pg TCDD eq./g product
> 2.0	pg TCDD eq./g product
1.0 - 2.0	pg TCDD eq./g product
0.5 - 1.0	pg TCDD eq./g product
< 0.5	pg TCDD eq./g product

DR CALUX Food (Eggs)	
pg BEQ / g fat	
≥ 1.7	EU Cut-off-Value PCDD/F
≥ 3.3	EU Cut-off-Value PCDD/F/dl-PCB

What are the real emissions & residues of a modern waste (WtE) incineration plant ?

Does the emissions of waste incineration comply with EU emission standard ?

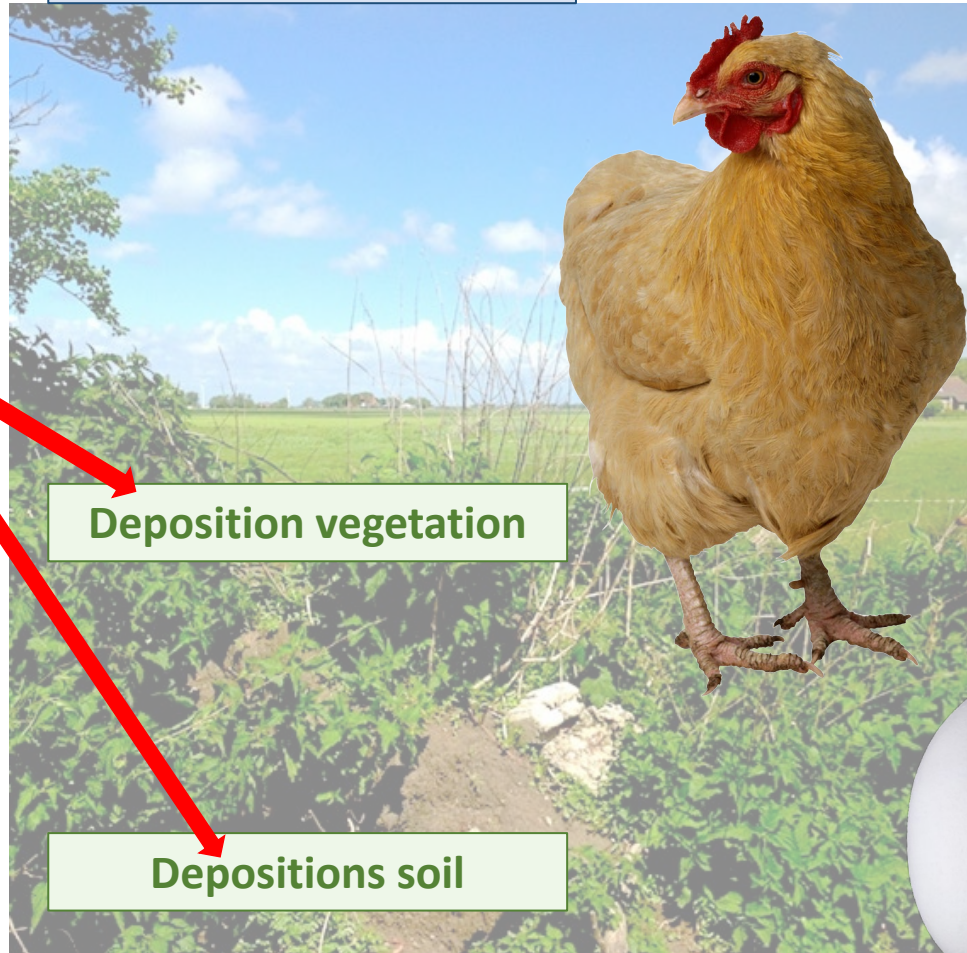


Why use eggs of backyard chicken for biomonitoring?

WtE incineration
Emissions SVHC



Air emissions



Deposition vegetation

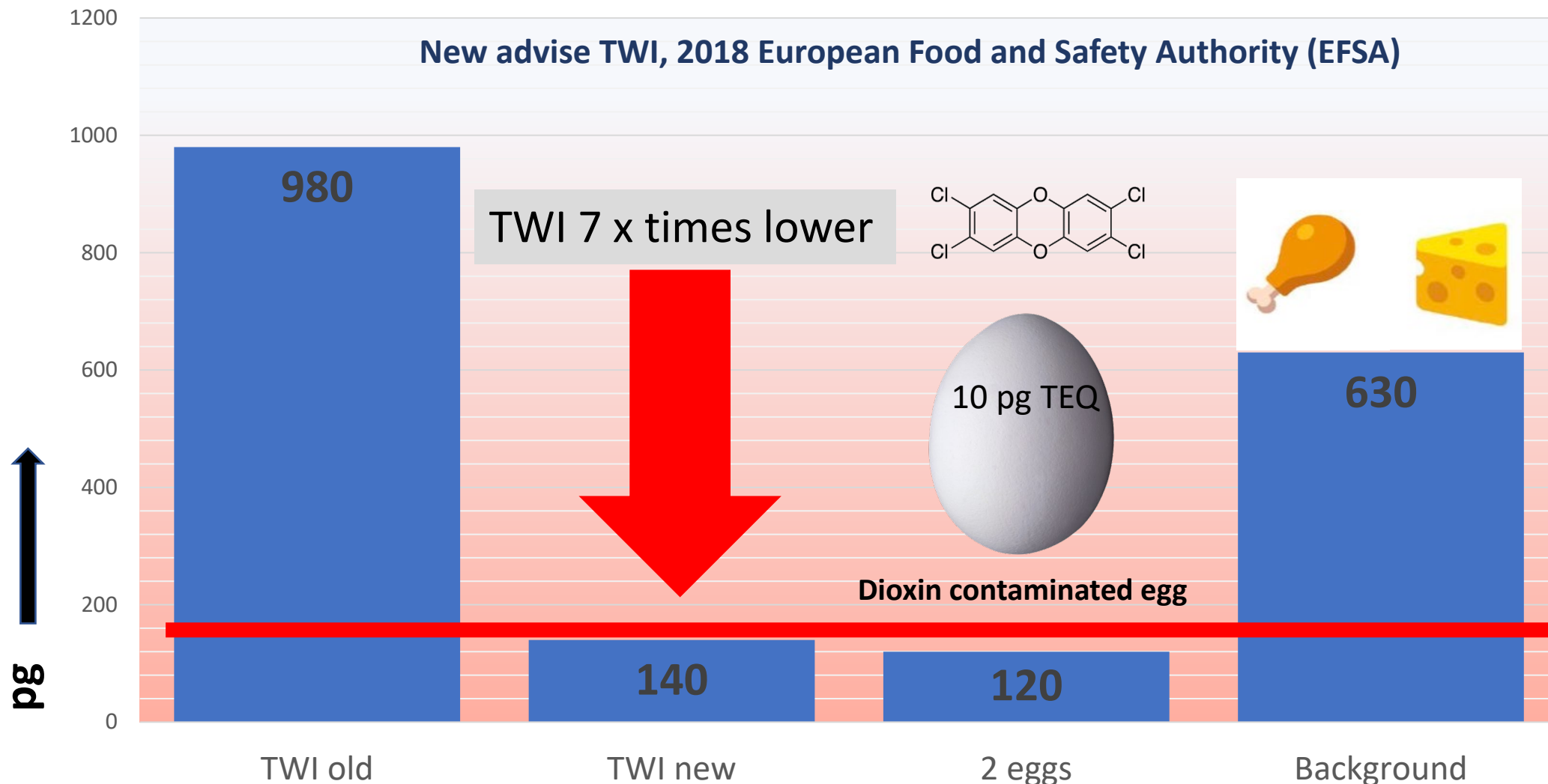
Depositions soil



Bioaccumulation
Biomagnification
Biotransformation
Xenobiotical metabolism

EFSA advise: Tolerable Weekly Intake (TWI) dioxins

■ Person 70 kg



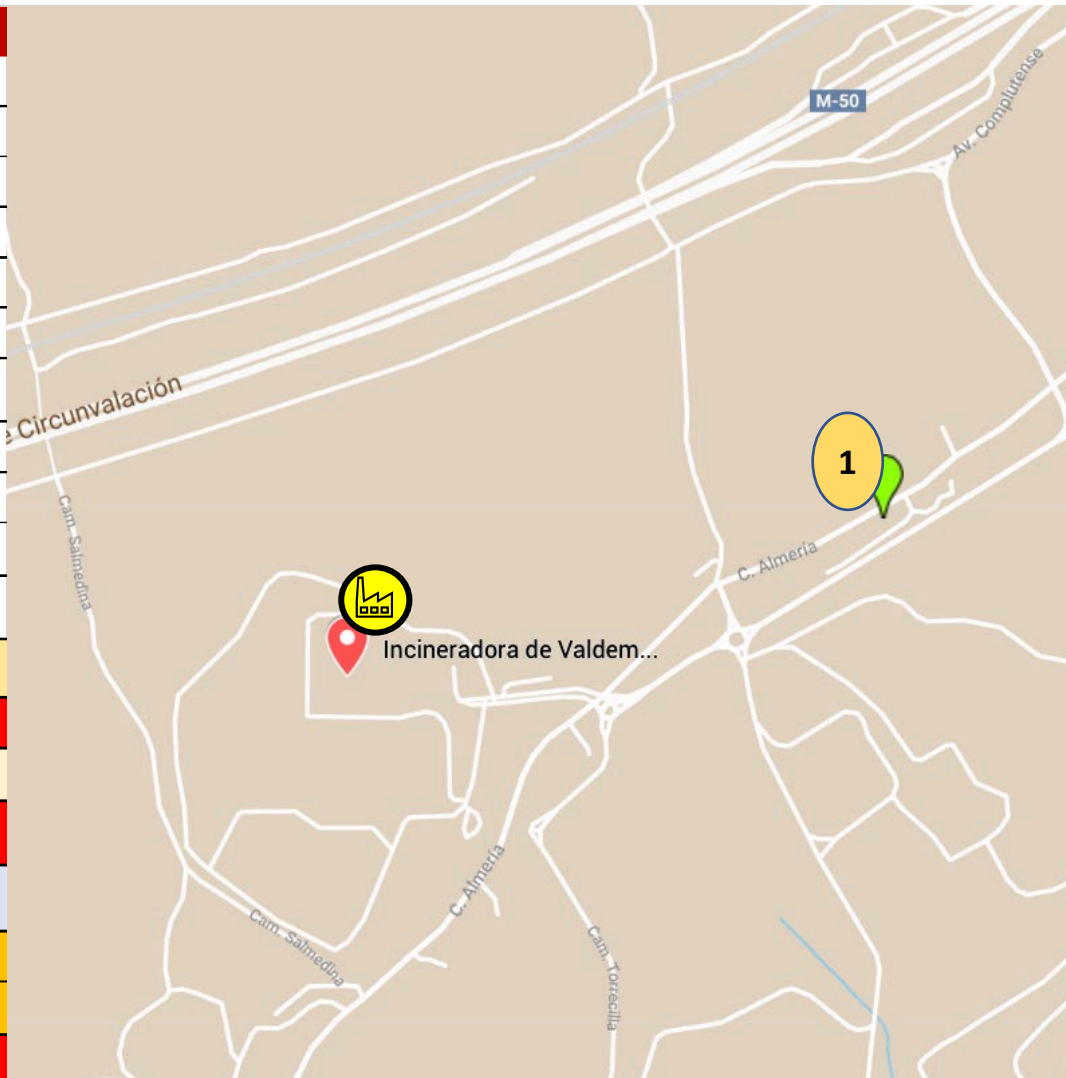
Red line: New TWI EFSA : 2 pg TEQ/kg bodyweight/ PER WEEK (old: 2 pg/day)

Location egg sample Cañada Real, Madrid - 2021



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

TW21-MD-Egg 01	
TW-REF-NR	TW21-MD-Egg 01
Distance	1000
N hens	3
N rooster	
Age	6-12 mnd
Eggs/month	30
Foraging area	
Outdoor fireplace	
All purpose burner	
Pesticides use	
DR CALUX BEQ	
PCDD/F BEQ	6,00
dl-PCB	7,00
PCDD/F/dl-PCB	13,00
GC-MS TEQ	
PCDD/F	4,10
dl-PCB	2,50
PCDD/F/dl-PCB	6,70



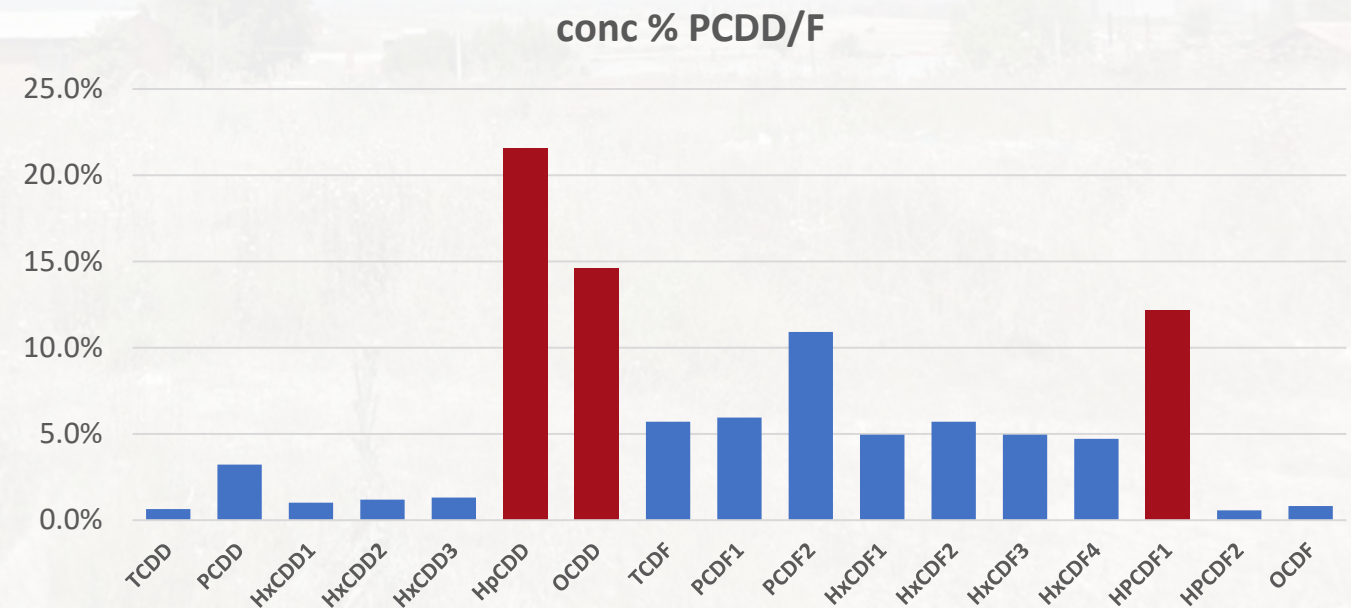
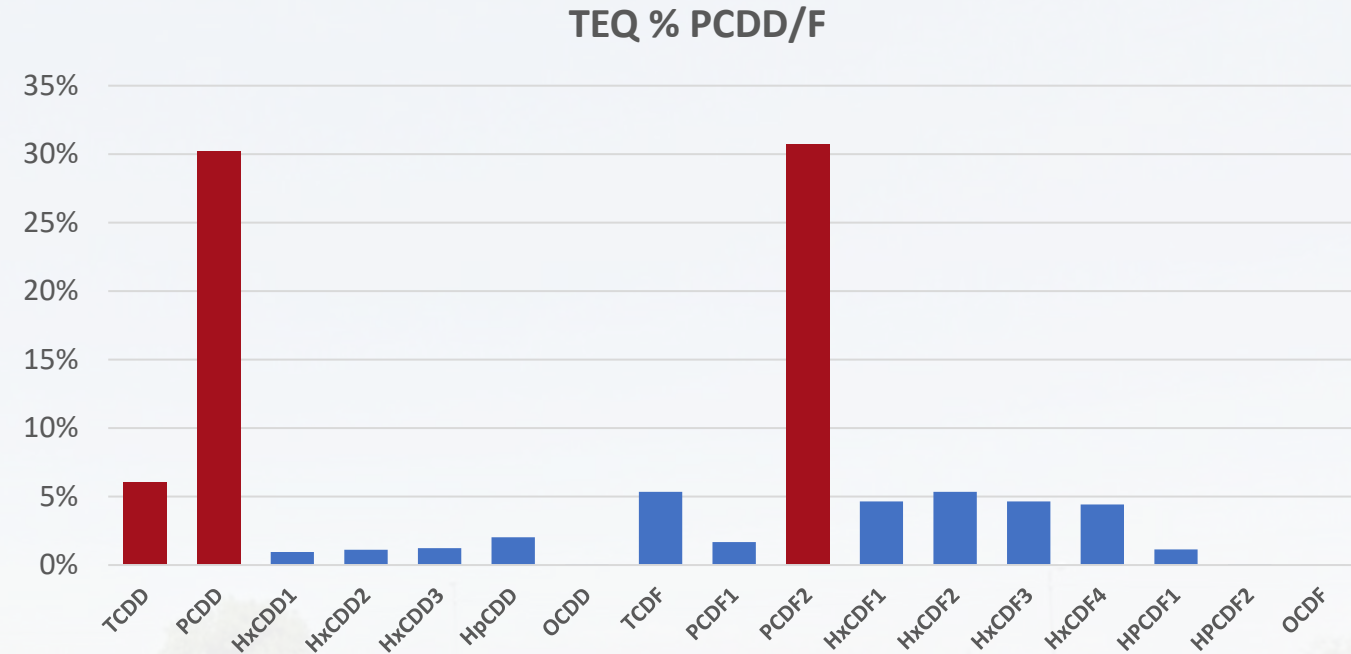
Action-level for PCDD/F and dl-PCB is exceeded

DR CALUX PCDD/F and PCDD/F/dl/PCB are suspect

NOT Complying with EU limit of the sum of PCDD/F/dl-PCBs TEQ.

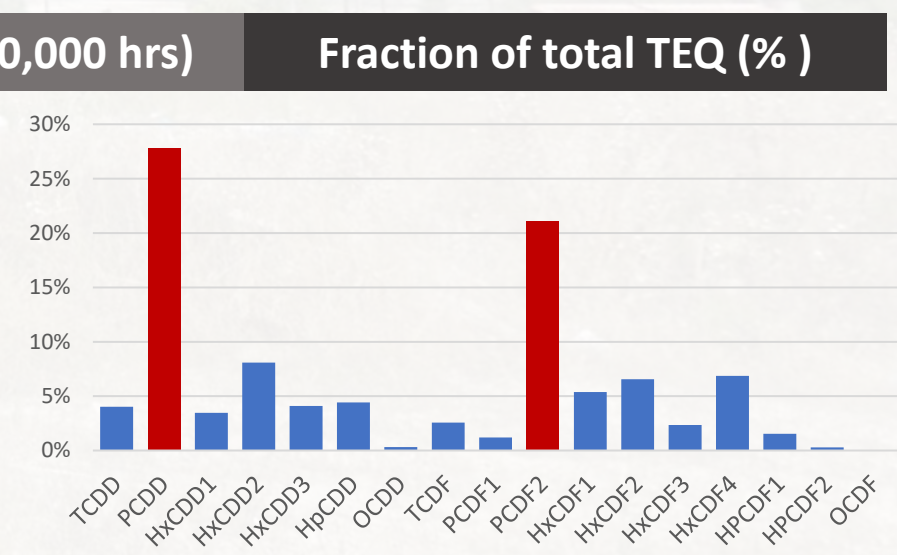
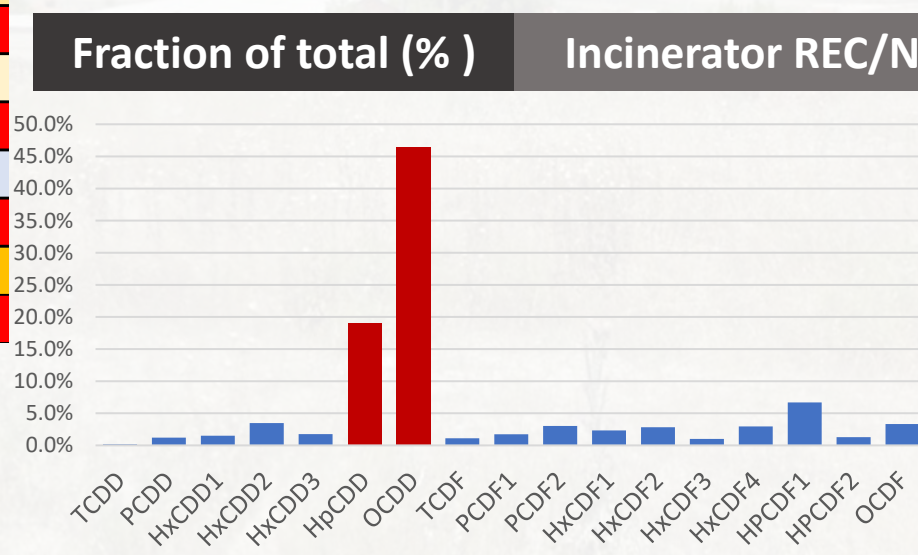
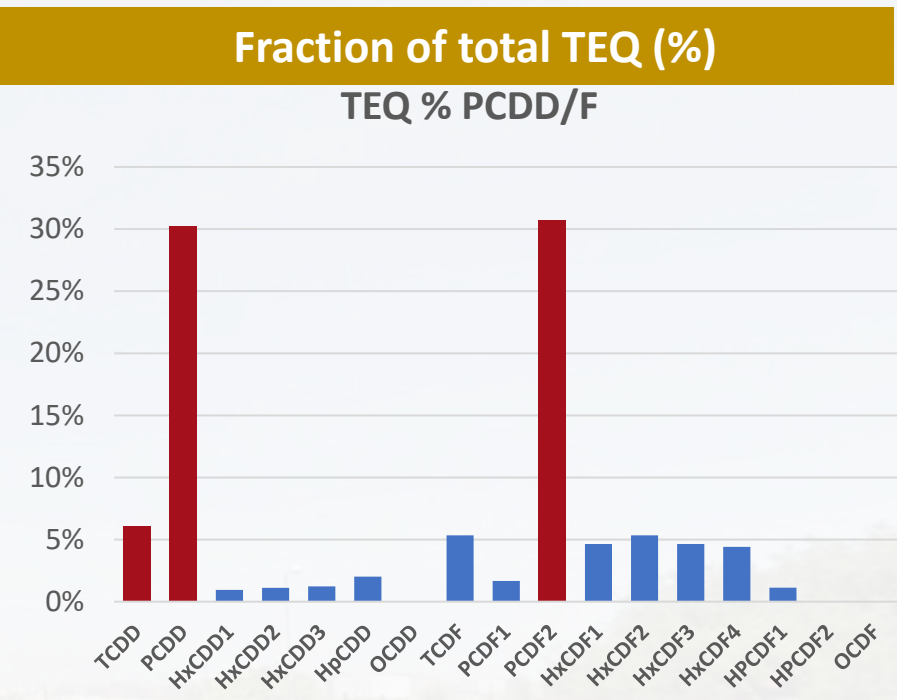
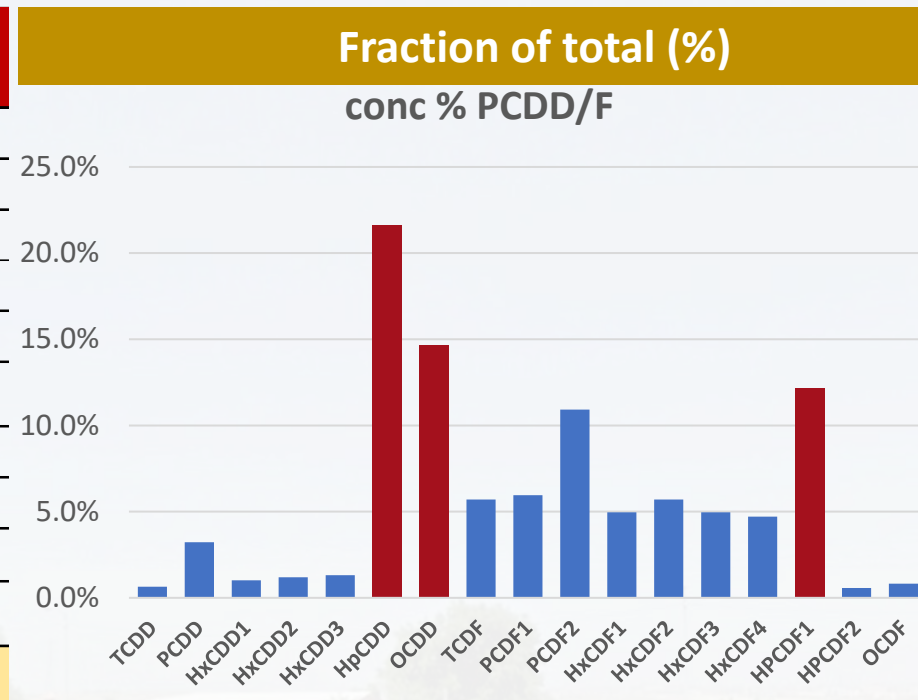
Fingerprints of PCDD/F congeners in eggs, Madrid - 2021

TW21-MD-Egg 01	
TW-REF-NR	TW21-MD-Egg 01
Distance	1000
N hens	3
N rooster	
Age	6-12 mnd
Eggs/month	30
Foraging area	
Outdoor fireplace	
All purpose burner	
Pesticides use	
DR CALUX BEQ	
PCDD/F BEQ	6,00
dl-PCB	7,00
PCDD/F/dl-PCB	13,00
GC-MS TEQ	
PCDD/F	4,10
dl-PCB	2,50
PCDD/F/dl-PCB	6,70



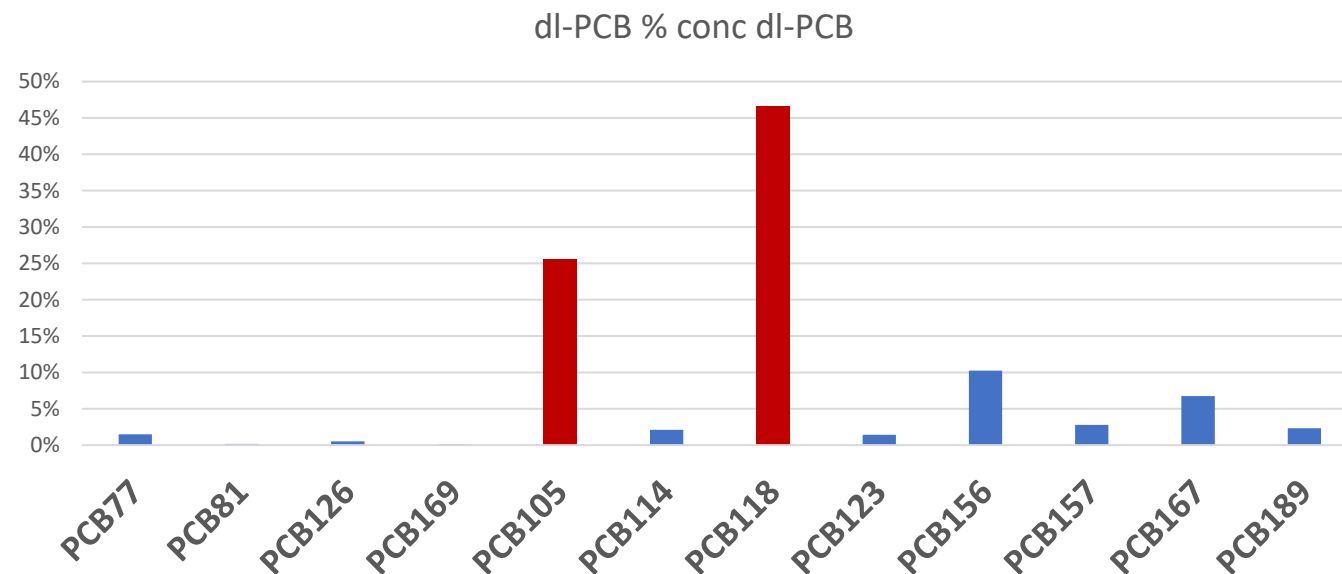
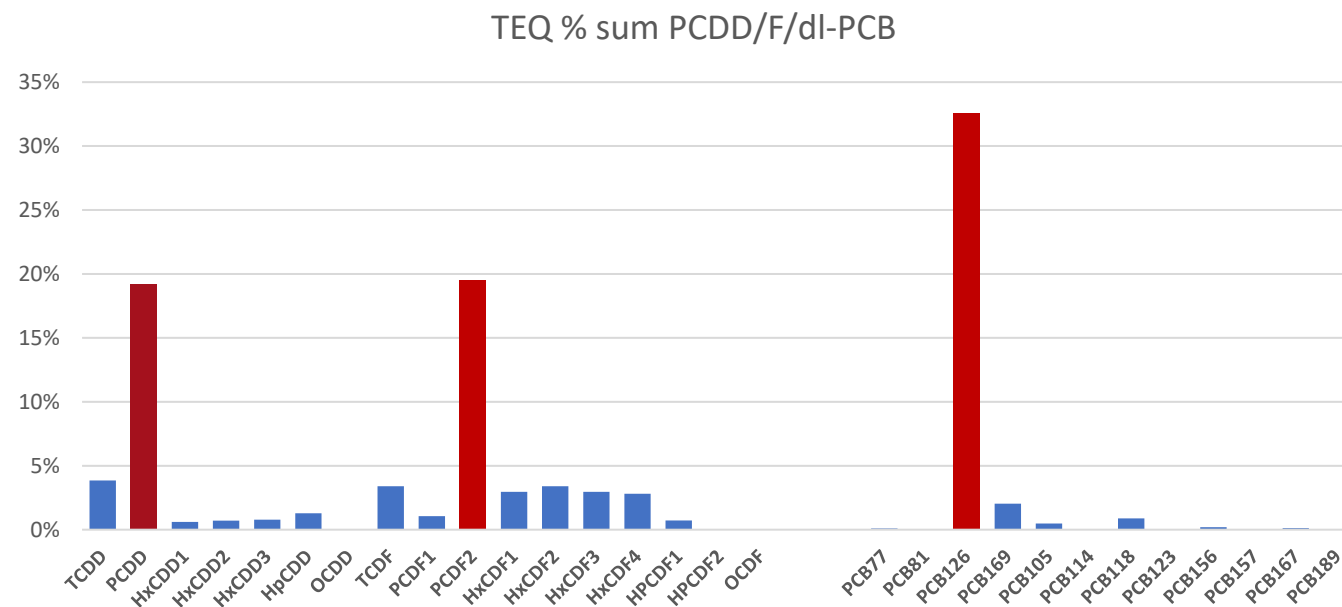
Fingerprints of PCDD/F congeners in eggs, Madrid - 2021

TW21-MD-Egg 01	
TW-REF-NR	TW21-MD-Egg 01
Distance (m)	1000
Hens (n)	3
Rooster (n)	
Age (month)	6-12 mnd
Eggs/month	30
Foraging area	
Outdoor fireplace	
All purpose burner	
Pesticides use	
DR CALUX BEQ	
PCDD/F BEQ	6.00
dI-PCB	7.00
PCDD/F/dI-PCB	13.00
GC-MS TEQ	
PCDD/F	4.10
dI-PCB	2.50
PCDD/F/dI-PCB	6.70



Fingerprints dl-PCB congeners in eggs, Madrid - 2021

Concentrations	Madrid
PCB	TW21-MD-Egg 01
PCB77	64,00
PCB81	7,00
PCB126	22,00
PCB169	4,60
PCB105	1100,00
PCB114	90,00
PCB118	2000,00
PCB123	61,00
PCB156	440,00
PCB157	120,00
PCB167	290,00
PCB189	100,00
Total PCB pg/g fat	4298,60



Biomonitoring Vegetation Madrid - 2021

Pine needles <i>Pinus halepensis</i>				Results Dioxins, PAH, PFAS in Pine needles						
Sample date		Species	(Pooled) Veg Nr	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	1	<i>Pinus halepensis</i>	Pin01	TW-MD21-Veg-01/02	570	8.40	7.10	1.30	220.00	26.00
18-7-2021	2	<i>Pinus halepensis</i>	Pin02	TW-MD21-Veg-10/11/12	280	0.29	0.18	0.11		
18-7-2021	3	<i>Pinus halepensis</i>	Pin03	TW-MD21-Veg-17/18/19	2190	0.18	0.07	0.11		
18-7-2021	4	<i>Pinus halepensis</i>	Pin04	TW-MD21-Veg-20/22/23	3700	0.14	0.08	0.06	8.10	22.00
18-7-2021	5	<i>Pinus halepensis</i>	Pin05	TW-MD21-Veg-24/25	4000	0.35	0.09	0.26		
18-7-2021	6	<i>Pinus halepensis</i>	Pin06	TW-MD21-Veg-26/27/28	4710	0.11	0.05	0.06		

Sampling Pine needles, foliage and leaves, Madrid - 2021

Results Pine needles and leaves, Madrid - 2021

Sample date	species	Weight (gr)	Wind direction	Distance (m)	TW-REF-NR		PCDD/F/dl-PCB	PCDD/F	dl-PCB	PAH	PFAS (FITC-T4)
							DR CALUX	pg TCDD eq./g product	ng BaP eq./g pr.	µg PFOA eq/g pr.	
1	<i>Pinus halepensis</i>	125	S	570	TW-MD21-Veg-01/02	Pin01	8.40	7.10	1.30	220.00	26
2	<i>Pinus halepensis</i>	111	S								
3	<i>Pinus halepensis</i>	102	S								
4	<i>Cupresses arizonica</i>	115	1 S-W	528	TW-MD21-Veg-04/05	Cup02	1.14	0.87	0.27		
5	<i>Cupresses arizonica</i>	148	2 S-W								
6	<i>Ulmus minor</i>	138	W	300	TW-MD21-Veg-06/07/08	Ulmus01	0.12	0.07	0.05		
7	<i>Ulmus minor</i>	144	W								
8	<i>Ulmus minor</i>	179	W								
9	<i>Ulmus minor</i>	149	N	175	TW-MD21-Veg-09	Ulmus02	0.12	0.07	0.05		
10	<i>Pinus halepensis</i>	137	N-E	280	TW-MD21-Veg-10/11/12	Pin02	0.29	0.18	0.11		
11	<i>Pinus halepensis</i>	167	N-E								
12	<i>Pinus halepensis</i>	149	N-E								
13	<i>Cupresses arizonica</i>	169	3 E	400	TW-MD21-Veg-13/14/15	Cup01	1.70	1.60	0.10	380.00	17
14	<i>Cupresses arizonica</i>	80	4 E								
15	<i>Cupresses arizonica</i>		5 E								
16	<i>Cupresses arizonica</i>	89	6 S_E								
17	<i>Pinus halepensis</i>	172	N	2190	TW-MD21-Veg-17/18/19	Pin03	0.18	0.07	0.11		
18	<i>Pinus halepensis</i>	172	N								
19	<i>Pinus halepensis</i>	174	N								
20	<i>Pinus halepensis</i>	211	S-W	3700	TW-MD21-Veg-20/22/23	Pin04	0.14	0.08	0.06	8.10	22
22	<i>Pinus halepensis</i>	122	S-W								
23	<i>Pinus halepensis</i>	155	S-W								
21	<i>Cupressus arizonica</i>	211	7 S-W	3820	TW-MD21-Veg-21	Cup03	0.80	0.73	0.07	31	17
24	<i>Pinus halepensis</i>	193	S-W	4000	TW-MD21-Veg-24/25	Pin05	0.35	0.09	0.26		
25	<i>Pinus halepensis</i>	190	S-W								
26	<i>Pinus halepensis</i>	196	E	4710	TW-MD21-Veg-26/27/28	Pin06	0.11	0.05	0.06		
27	<i>Pinus halepensis</i>	193	E								
28	<i>Pinus halepensis</i>	206	E								

Biomonitoring Vegetation Madrid -2021

Pine needles <i>Pinus halepensis</i>				Results Dioxins, PAH, PFAS in Pine needles						
Sample date		Species	(Pooled) Veg Nr	TW-REF-NR	Distance	PCDD/F/di-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	1	<i>Pinus halepensis</i>	Pin01	TW-MD21-Veg-01/02	570	8.40	7.10	1.30	220.00	26.00
18-7-2021	2	<i>Pinus halepensis</i>	Pin02	TW-MD21-Veg-10/11/12	280	0.29	0.18	0.11		
18-7-2021	3	<i>Pinus halepensis</i>	Pin03	TW-MD21-Veg-17/18/19	2190	0.18	0.07	0.11		
18-7-2021	4	<i>Pinus halepensis</i>	Pin04	TW-MD21-Veg-20/22/23	3700	0.14	0.08	0.06	8.10	22.00
18-7-2021	5	<i>Pinus halepensis</i>	Pin05	TW-MD21-Veg-24/25	4000	0.35	0.09	0.26		
18-7-2021	6	<i>Pinus halepensis</i>	Pin06	TW-MD21-Veg-26/27/28	4710	0.11	0.05	0.06		

leaves <i>Ulmus minor</i>				Results Dioxins, PAH, PFAS in leaves <i>Ulmus minor</i>						
Sample date		Species	(Pooled) Veg Nr	TW-REF-NR	Distance	PCDD/F/di-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	7	<i>Ulmus minor</i>	Ulmus01	TW-MD21-Veg-06/07/08	300	0.12	0.07	0.05		
18-7-2021	8	<i>Ulmus minor</i>	Ulmus02	TW-MD21-Veg-09	175	0.12	0.07	0.05		

Foliage <i>Cupressus arizonica</i>				Results Dioxins, PAH, PFAS in foliage <i>Cupressus Arizonaica</i>						
Sample date		Species	(Pooled) Veg Nr	TW-REF-NR	Distance	PCDD/F/di-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	9	<i>Cupressus arizonica</i>	Cup01	TW-MD21-Veg-13/14/15	400	1.70	1.60	0.10	380.00	
18-7-2021	10	<i>Cupressus arizonica</i>	Cup02	TW-MD21-Veg-04/05	528	1.14	0.87	0.27		
18-7-2021	11	<i>Cupressus arizonica</i>	Cup03	TW-MD21-Veg-21	3820	0.80	0.73	0.07	31.00	

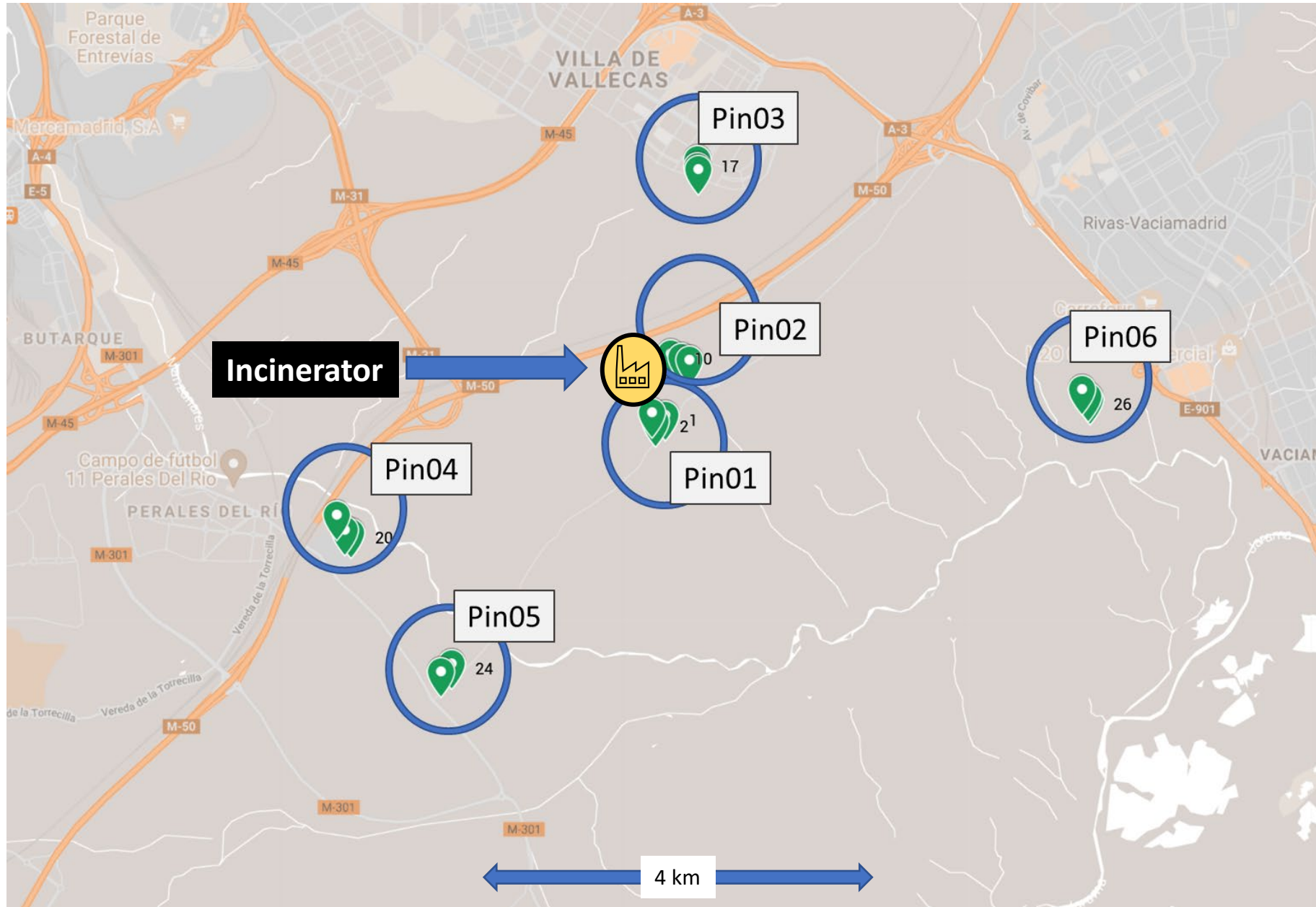
Mosses				Results Dioxins, PAH, PFAS in Mosses						
Sample date		Species	Veg Nr	TW-REF-NR	Distance	PCDD/F/di-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
1-11-2021	1	<i>Mosses</i>	MM1	TW21-MD-M01	2610	2.28	1.30	0.98		
1-11-2021	2	<i>Mosses</i>	MM2	TW21-MD-M02	2350	6.70	3.80	2.90		
1-11-2021	3	<i>Mosses</i>	MM3	TW21-MD-M03	1940	7.80	5.30	2.50		
1-11-2021	4	<i>Mosses</i>	MM4	TW21-MD-M04	1460	10.70	5.30	5.40		
1-11-2021	5	<i>Mosses</i>	MM5	TW21-MD-M05	400	1.55	0.86	0.69		
1-11-2021	6	<i>Mosses</i>	MM6	TW21-MD-M06	572	3.20	1.10	2.10		
1-11-2021	7	<i>Mosses</i>	MM7	TW21-MD-M07	2000	1.42	0.95	0.47		
1-11-2021	8	<i>Mosses</i>	MM8	TW21-MD-M08	30800	0.46	0.36	0.10		

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

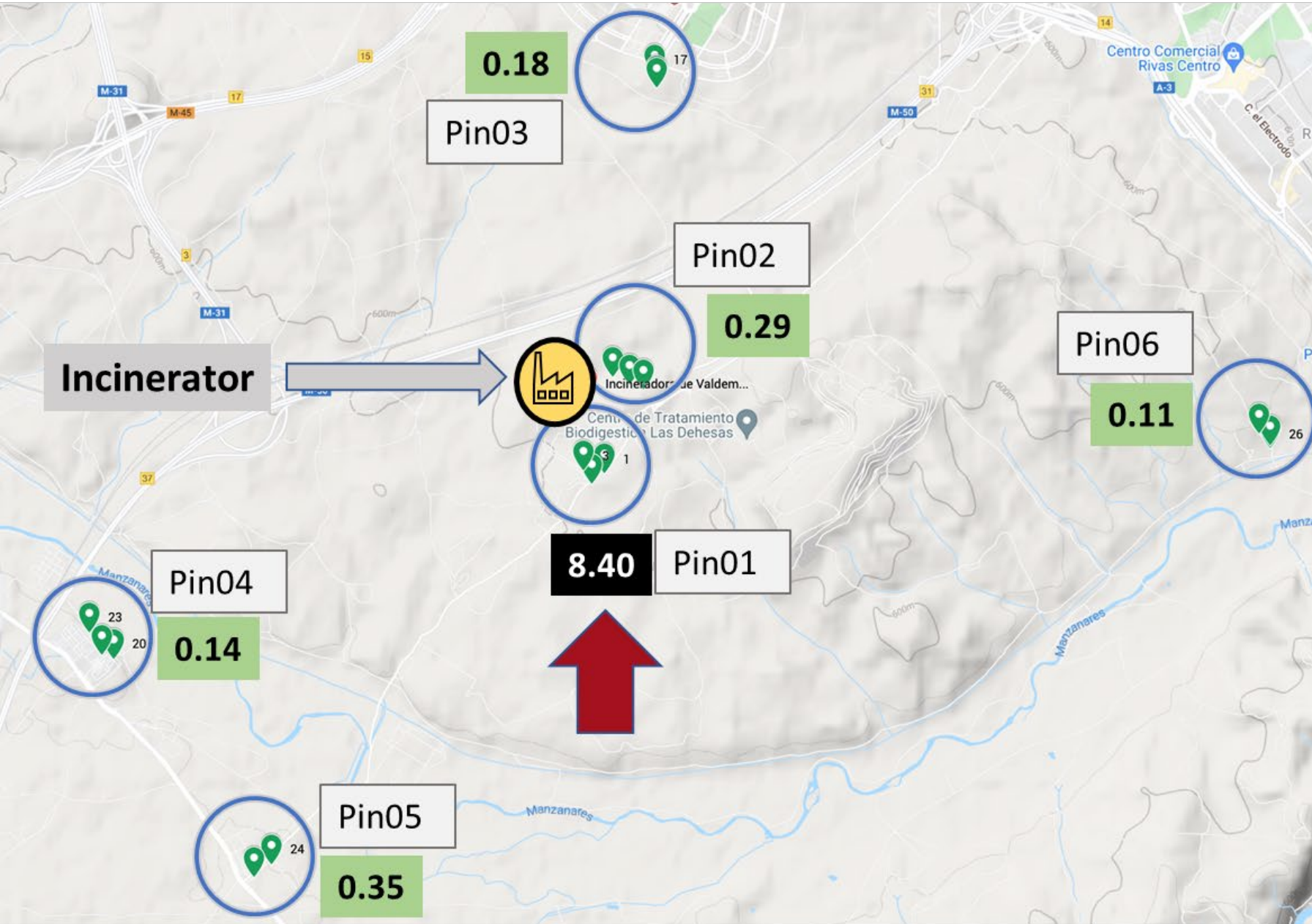
Benzo[a]pyrene equivalent
TW Indicative scale Results
PAH
> 500 ng BaP eq./g product
> 250 ng BaP eq./g product
100-250 ng BaP eq./g product
10-100 ng BaP eq./g product
< 10 ng BaP eq./g product

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

Pine needle, *Pinus Halepensis* locations Madrid - 2021

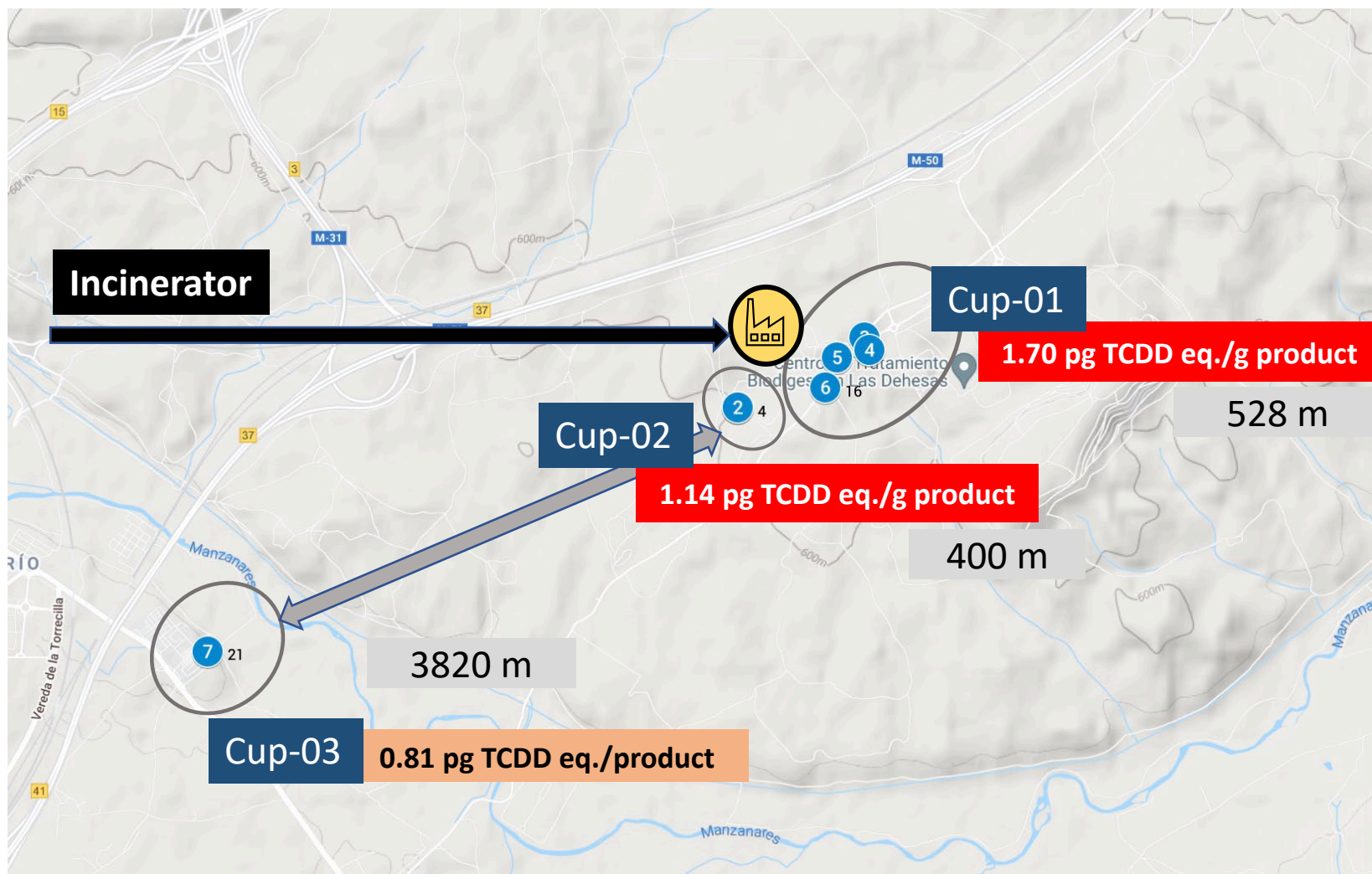


Results sum of dioxins (PCDD/F/dl-PCB) in pine needles - Madrid 2021



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

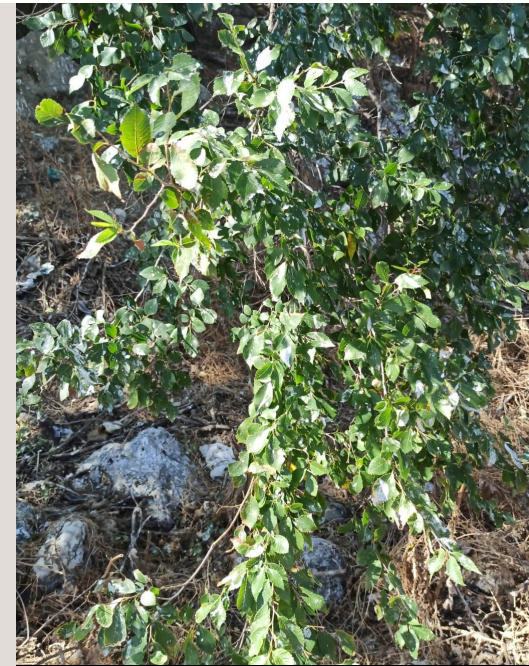
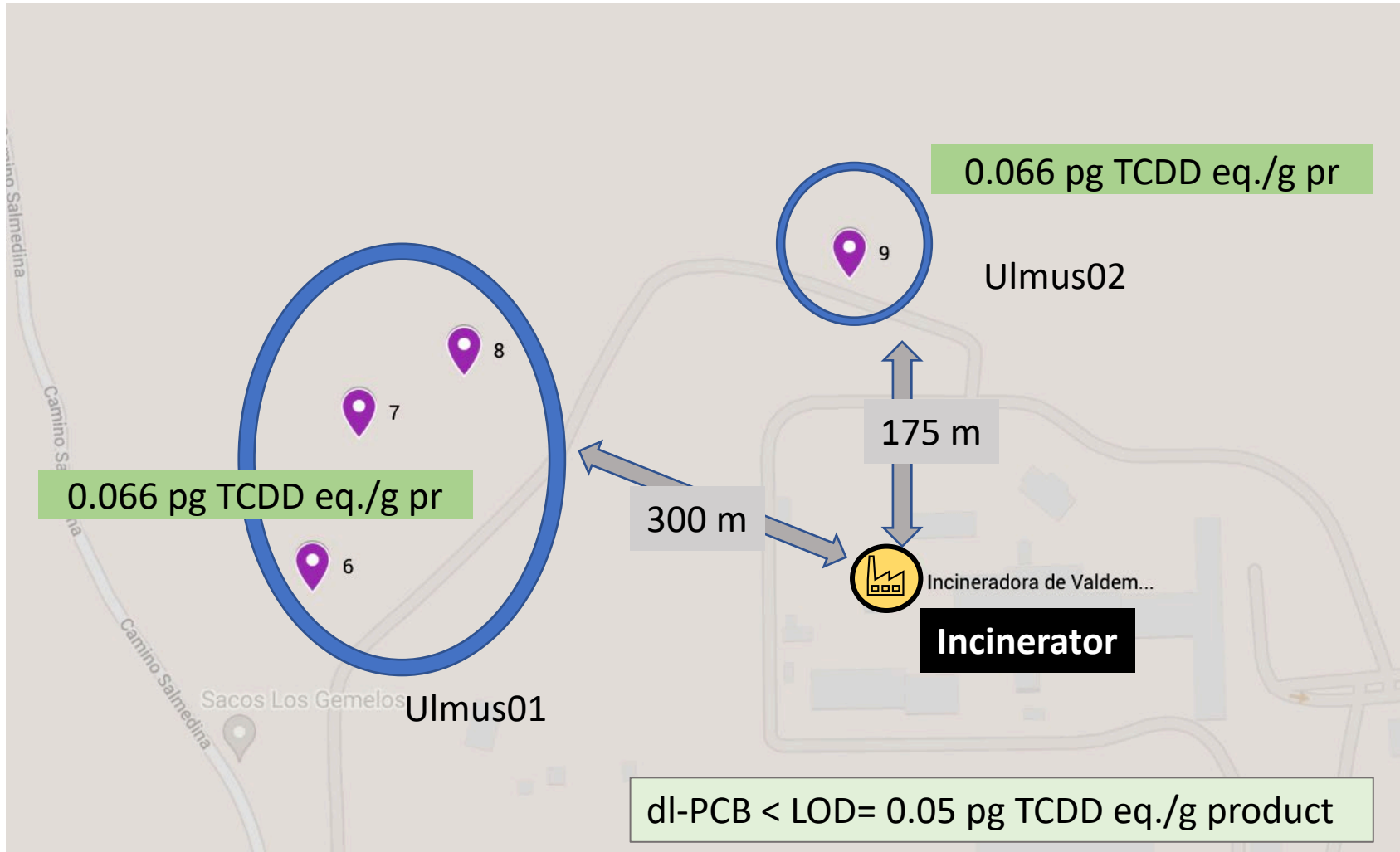
Dioxins in foliage *Cupressus arizonica*, Madrid - 2021



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

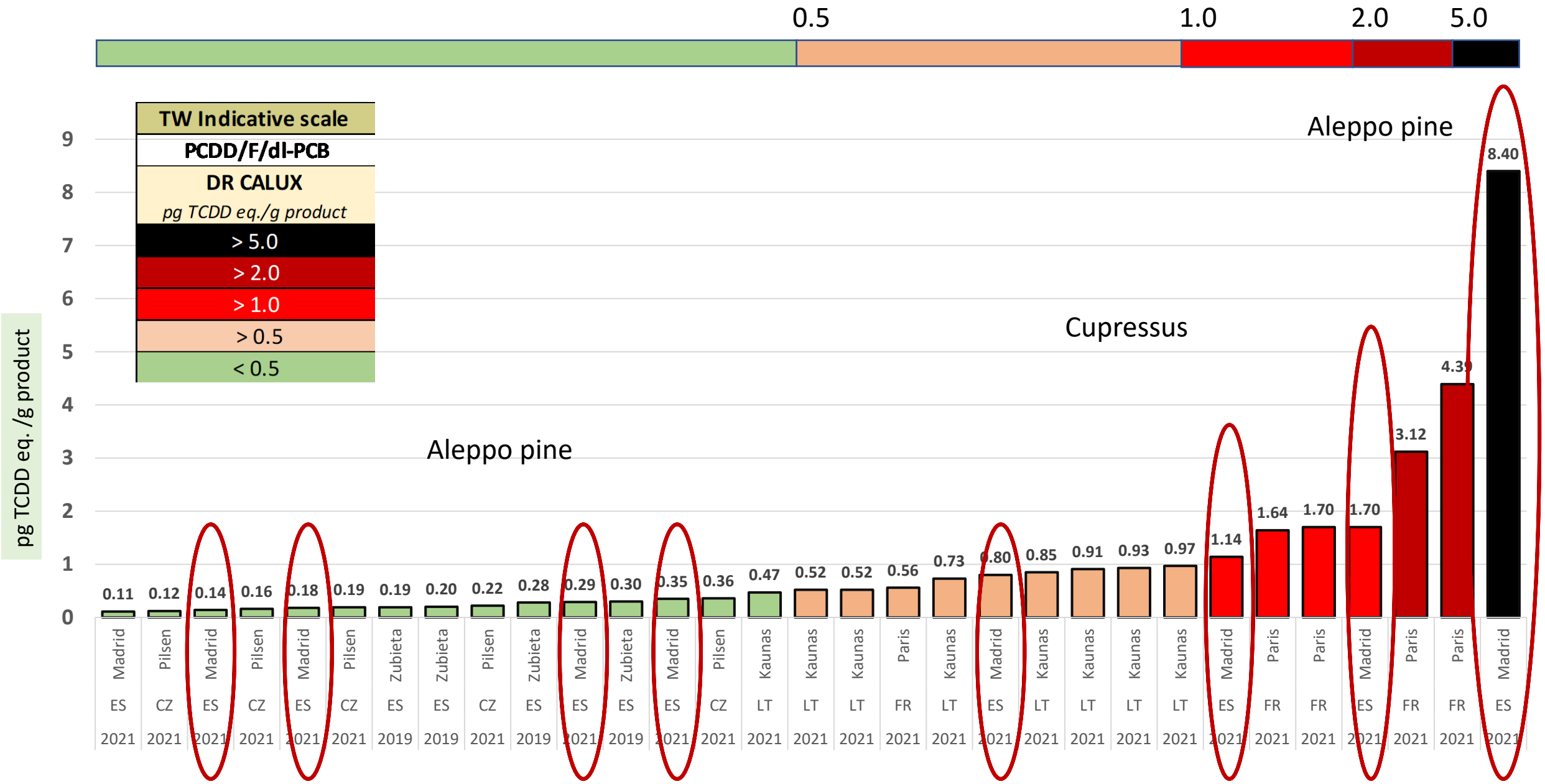
leaves <i>Ulmus minor</i>				Results Dioxins, PAH, PFAS in leaves <i>Ulmus minor</i>						
Sample date		Species	Pooled Veg Nr	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	7	<i>Ulmus minor</i>	Ulmus01	TW-MD21-Veg-06/07/08	300	0.12	0.07	0.05		
18-7-2021	8	<i>Ulmus minor</i>	Ulmus02	TW-MD21-Veg-09	175	0.12	0.07	0.05		
Foliage <i>Cupressus arizonica</i>				Results Dioxins, PAH, PFAS in foliage <i>Cupressus Arizonaica</i>						
Sample date		Species	Pooled Veg Nr	TW-REF-NR	Distance	PCDD/F/dl-PCB	PCDD/F	PCB	PAH	PFAS (FITCH-T4)
					(m)	DR CALUX pg TCDD eq./g product			ng BaP/g pr.	µg PFOA eq/g pr.
18-7-2021	9	<i>Cupressus arizonica</i>	Cup01	TW-MD21-Veg-13/14/15	400	1.70	1.60	0.10	380.00	
18-7-2021	10	<i>Cupressus arizonica</i>	Cup02	TW-MD21-Veg-04/05	528	1.14	0.87	0.27		
18-7-2021	11	<i>Cupressus arizonica</i>	Cup03	TW-MD21-Veg-21	3820	0.80	0.73	0.07	31.00	

Dioxins (PCDD/F) in leaves *Ulmus* (pg TCDD eq./g product)

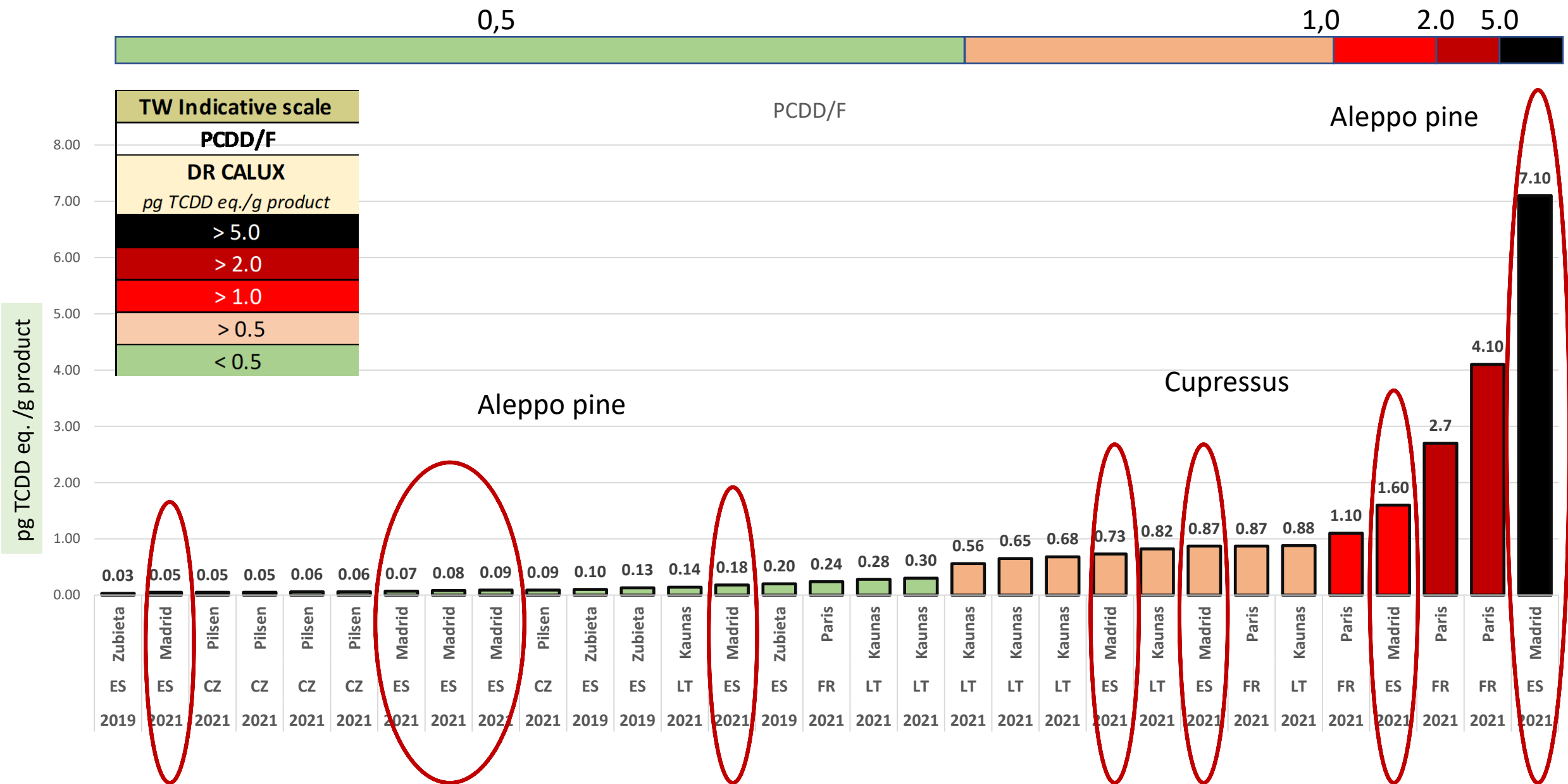


TW Indicative scale Results
DR CALUX
> 5.0 pg TCDD eq./g product
> 2.0 pg TCDD eq./g product
1.0 - 2.0 pg TCDD eq./g product
0.5 - 1.0 pg TCDD eq./g product
< 0.5 pg TCDD eq./g product

TW Indicative scale dioxins (PCDD/F/dl-PCB) in evergreen trees, Madrid - 2021



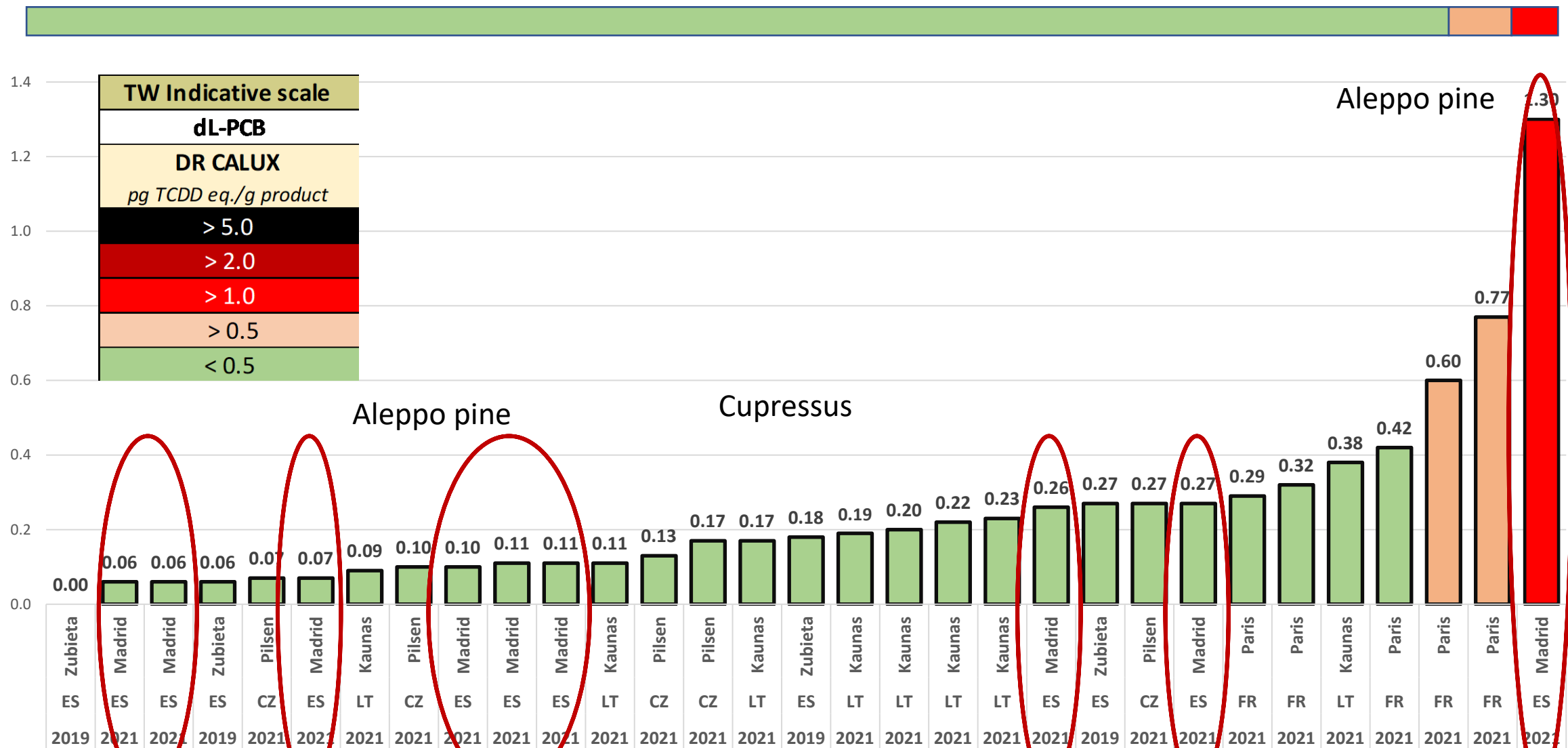
Indicative scale PCDD/F in evergreen trees Madrid - 2021



Indicative scale dl-PCB in evergreen trees Madrid, 2021

0,5 1,0 2.0

pg TCDD eq. /g product

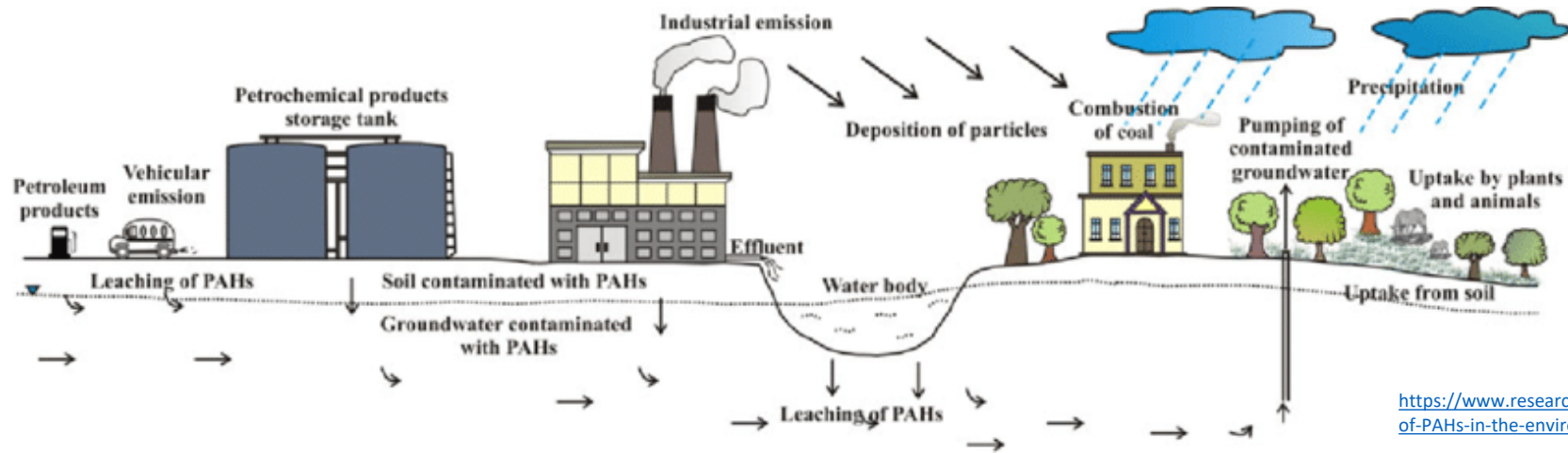


Dioxins in *Aleppo pine needles* near incinerator, Madrid - 2021



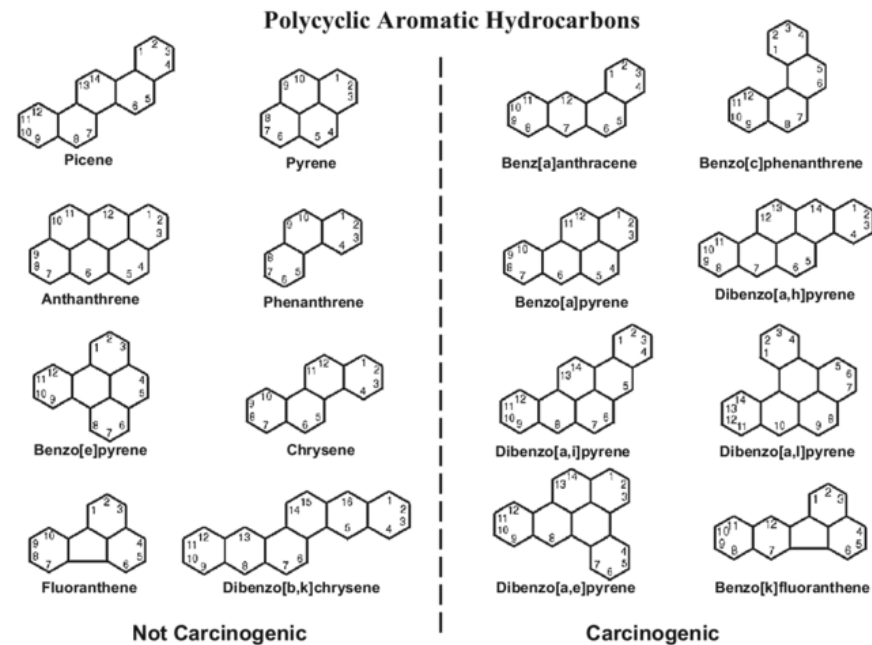
PCDD/F/dl-PCB: 8.40 TCDD eq./g product

Polycyclic Aromatic Hydrocarbons (PAH)

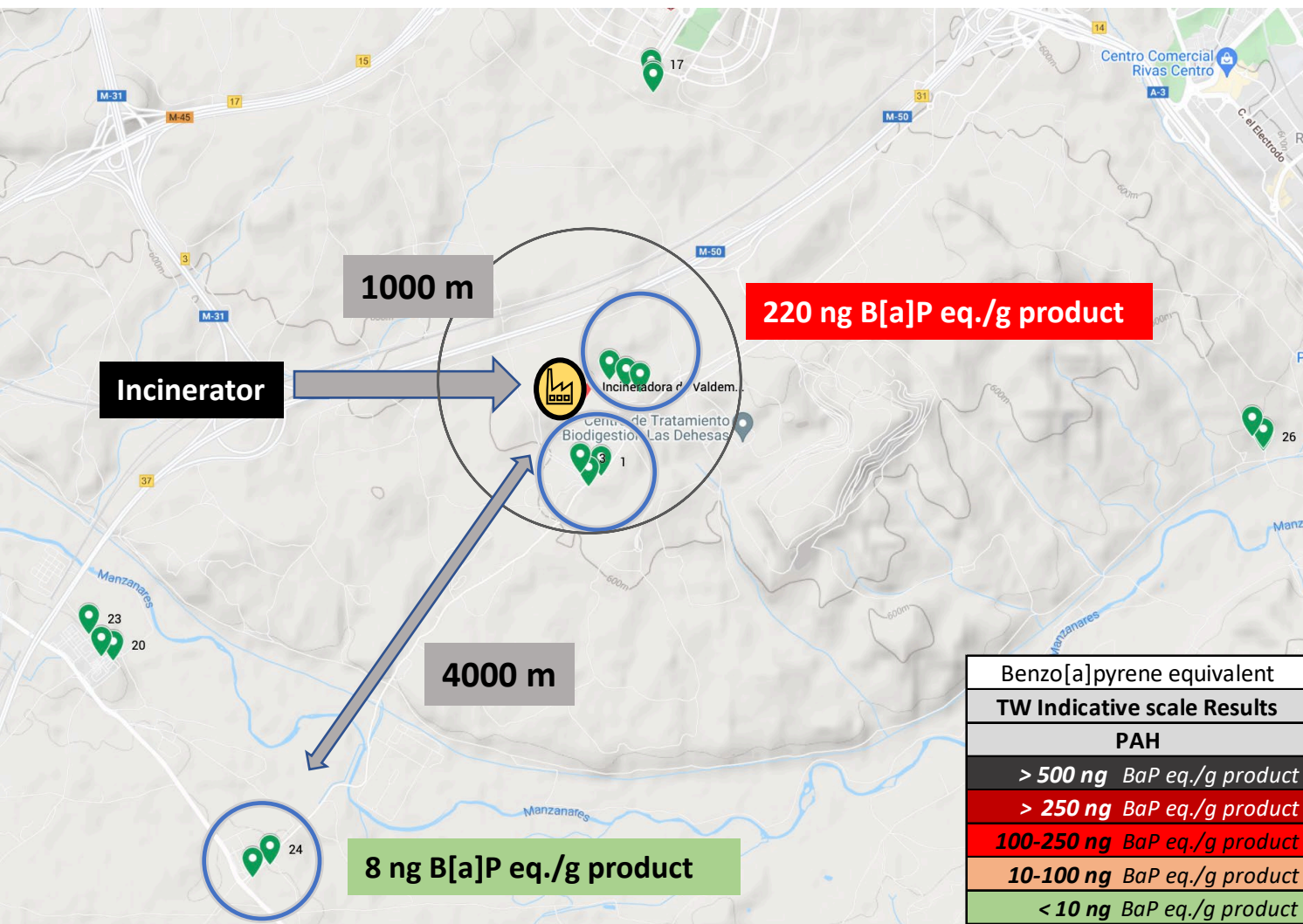


https://www.researchgate.net/figure/Sources-of-PAHs-in-the-environment_fig1_258987468

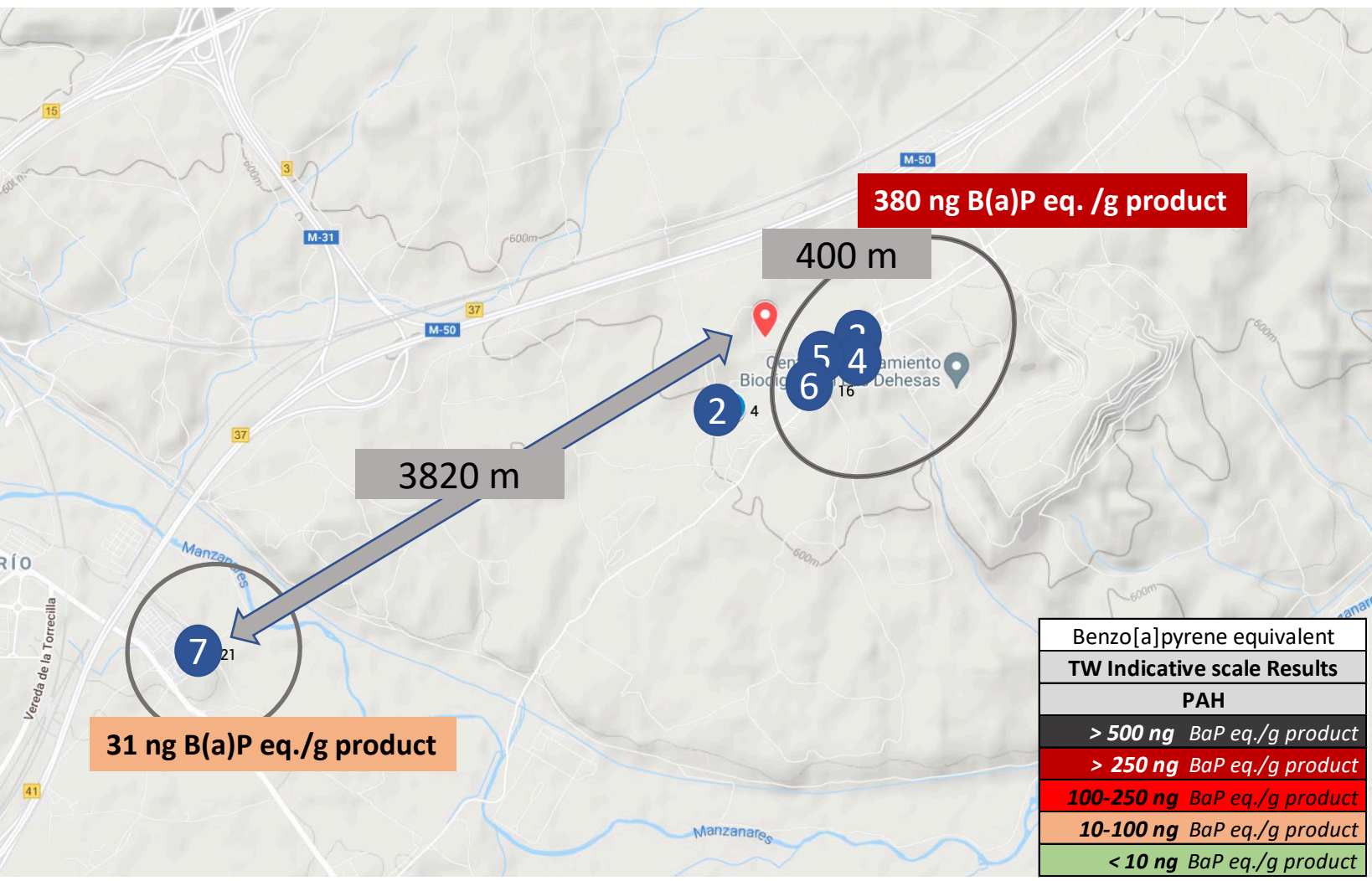
Anthropogenic sources and pathways of PAHs into the environment



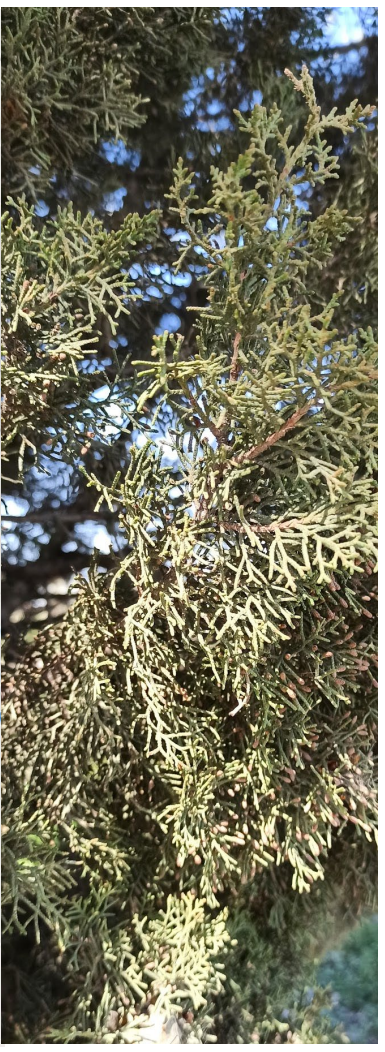
PAH in pine needles Aleppo pine - *Pinus Halepensis*, Madrid - 2021



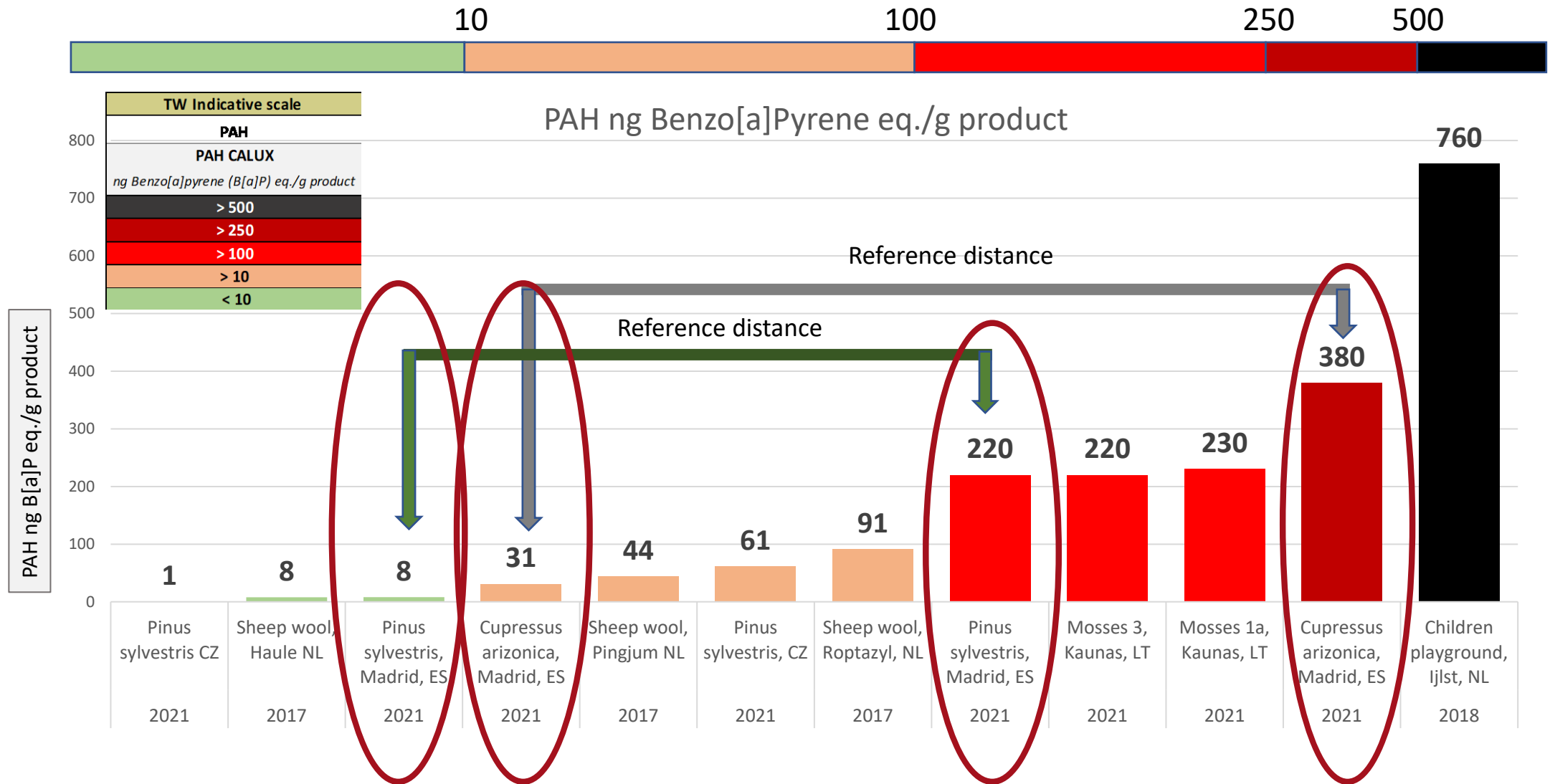
PAH in *Cupressus arizonica*, Madrid - 2021



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



TW Indicative PAH CALUX scale in biomatrices (TW research 2017-2021)



Data ToxicoWatch Biomonitoring research in Europe 2017-2021

PAH distribution Madrid Health (Salud)

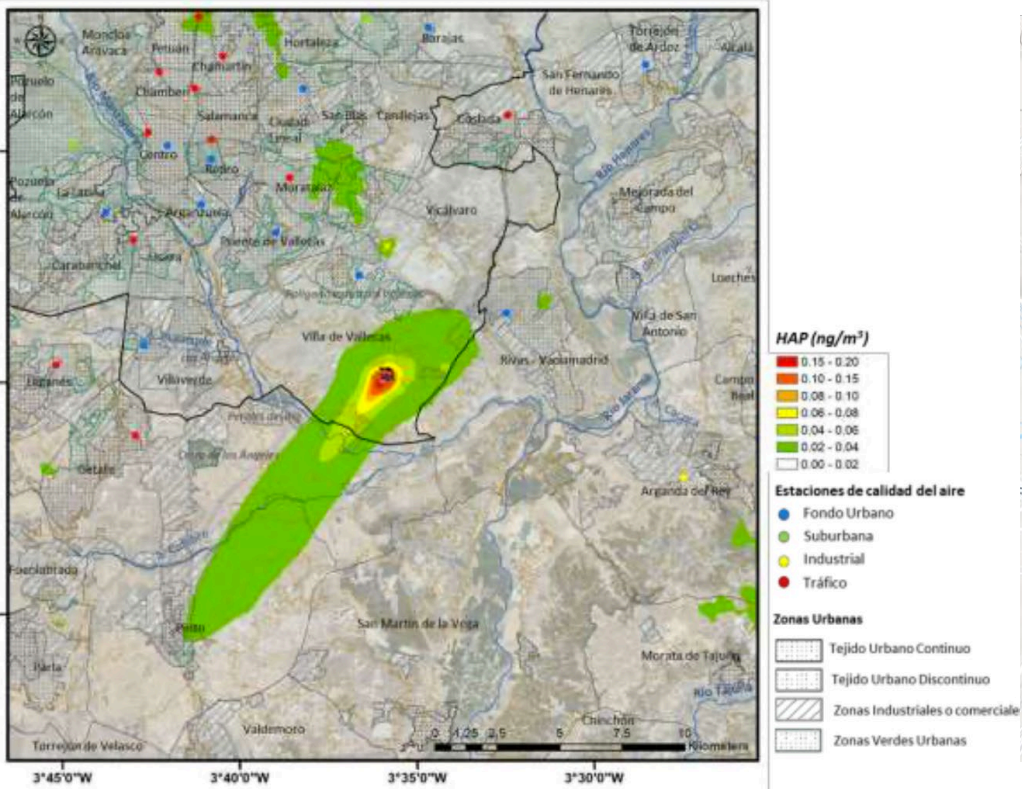
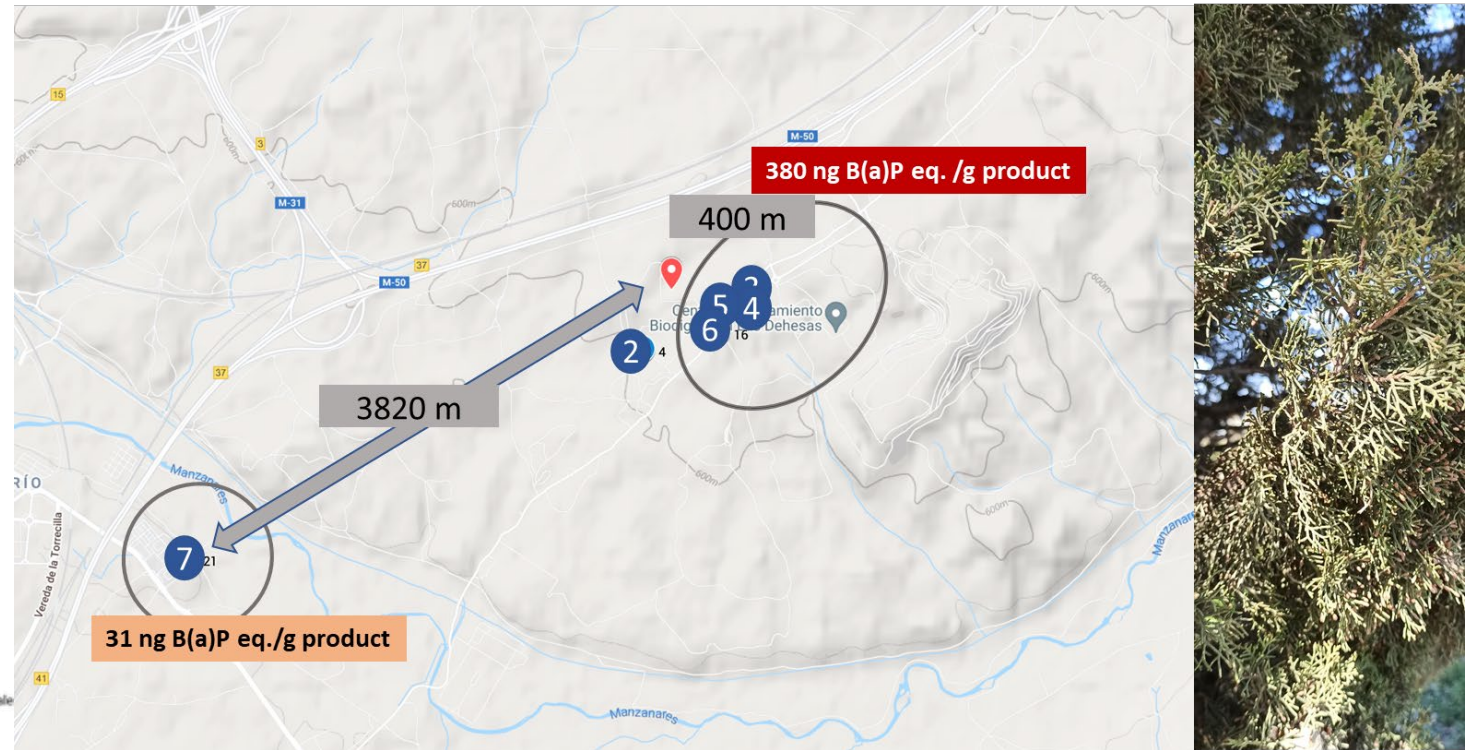
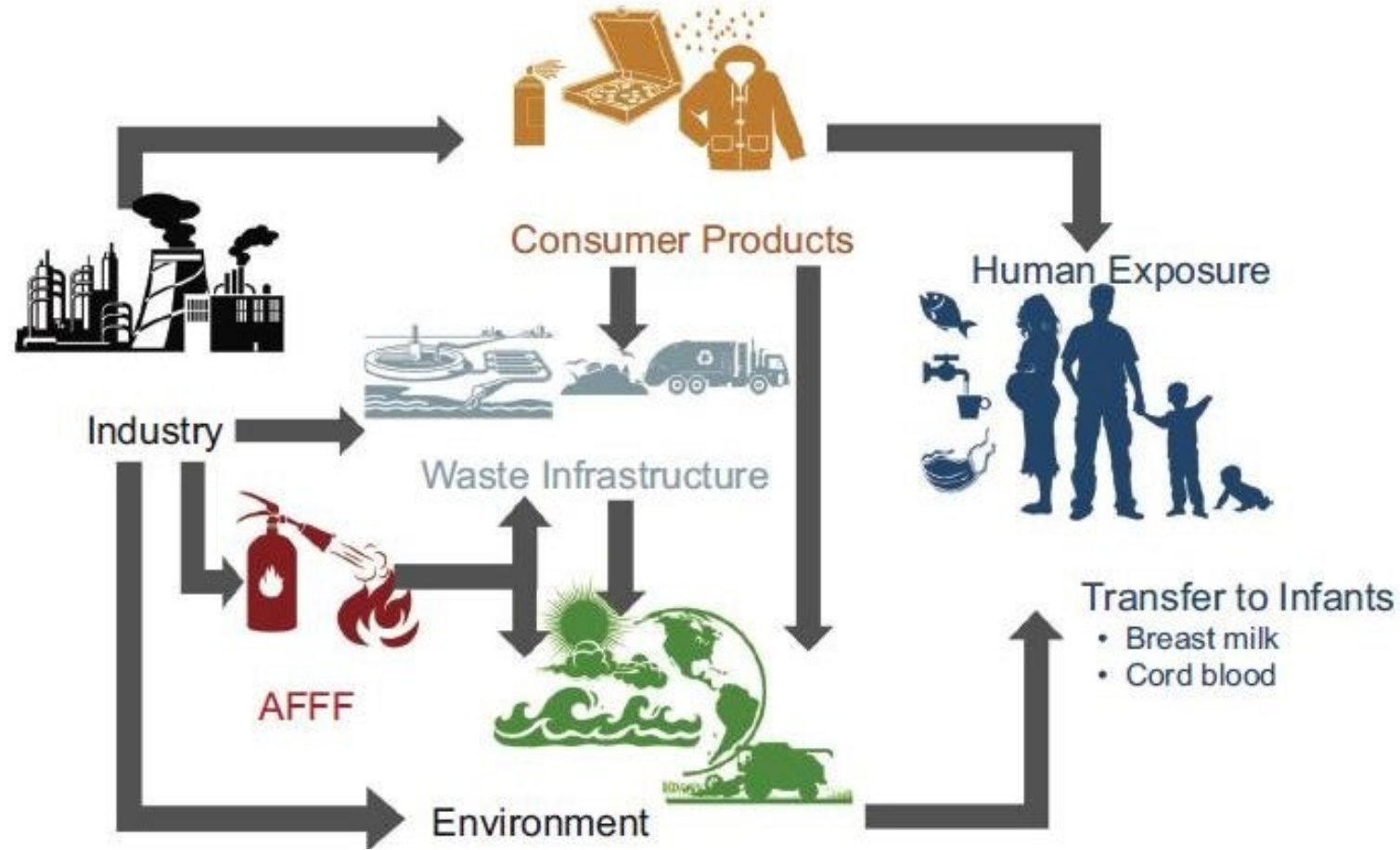


Figura 23. Concentración anual media de HAP

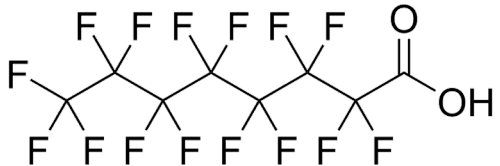
PAH in *Cupressus arizonica*, Madrid - 2021



PFAS (Per- and polyfluoroalkyl substances)

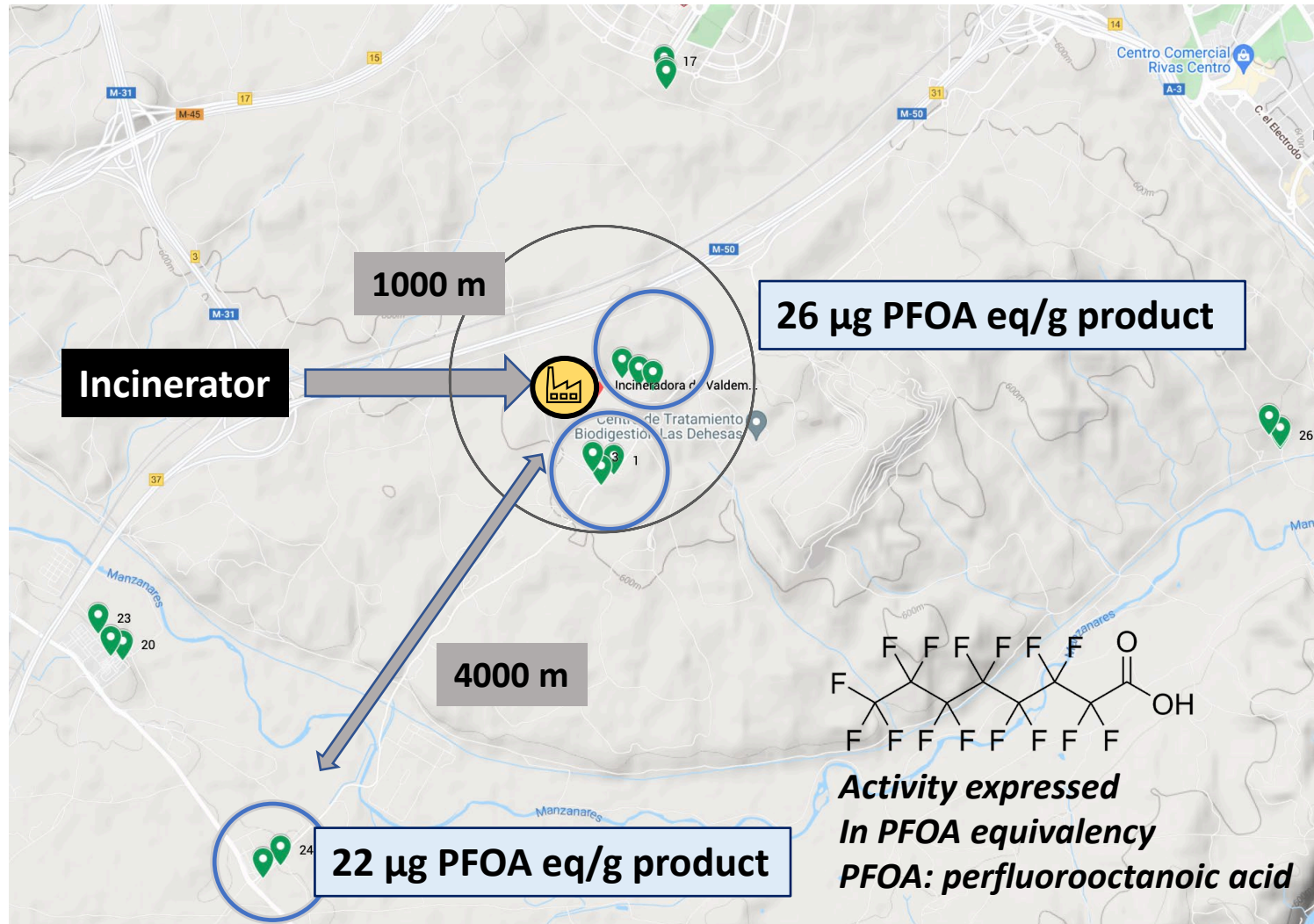


Overview of PFAS exposure pathways to the human population and the environment. Source: (Sunderland et al. 2019).



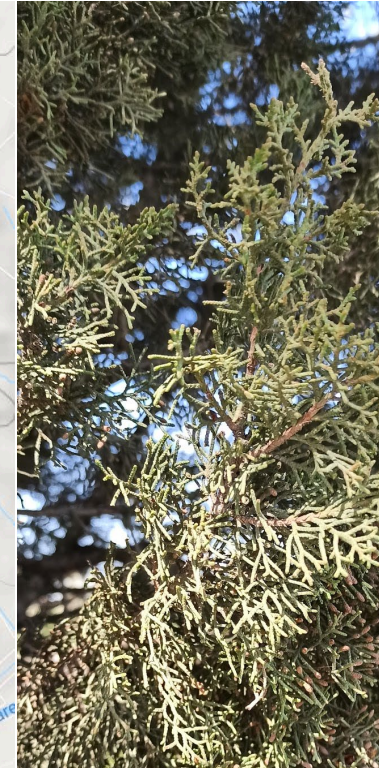
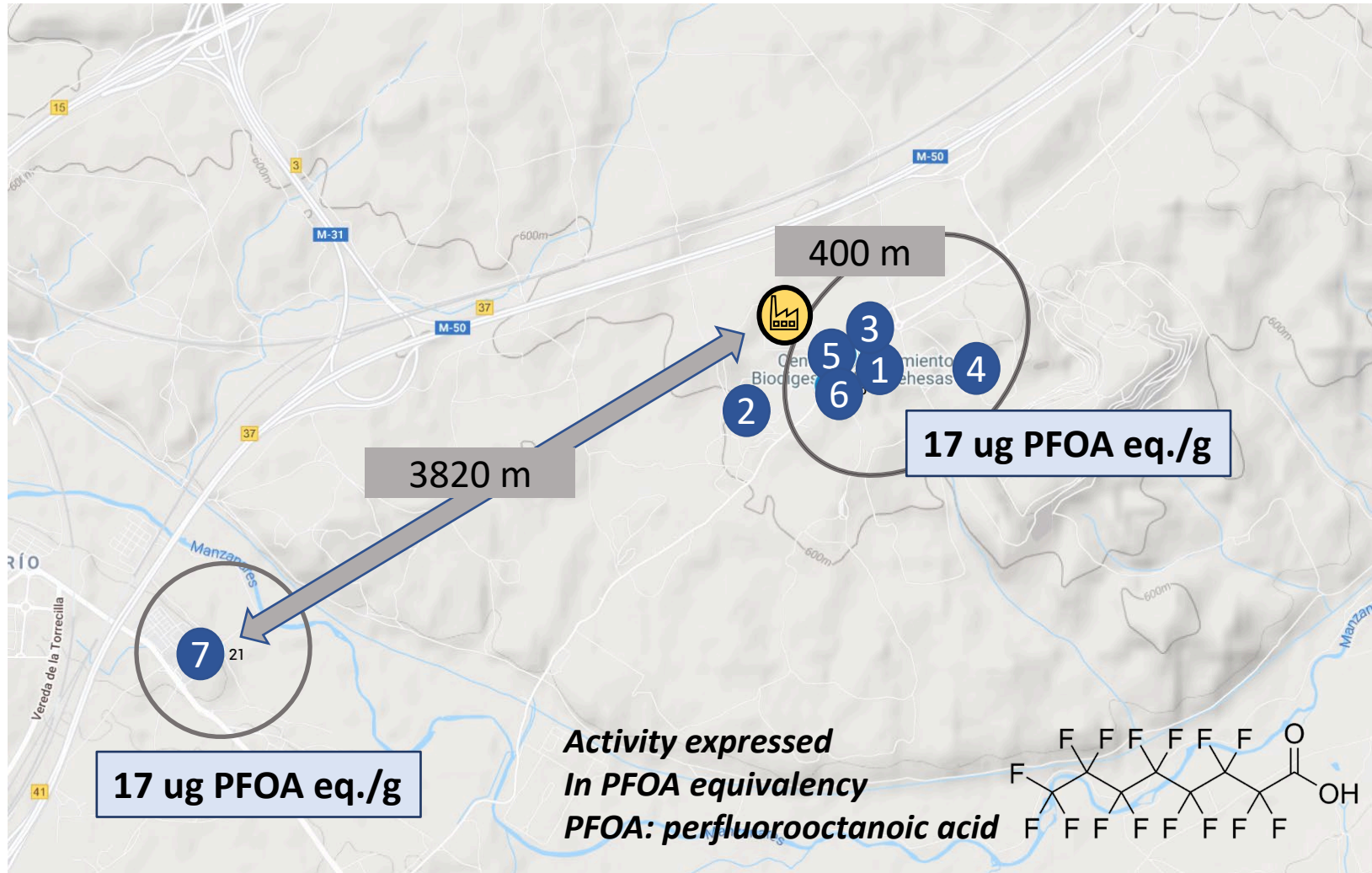
These are man-made substances that do not naturally occur in the environment. Examples of PFAS are GenX, PFOA perfluoro octanoic acid and PFOS perfluorooctane sulfonates. PFASs are used in many products. As a result, and due to emissions and incidents, these substances have ended up in the environment and are now found in, among other things, soil, dredging spoil and surface water. (<https://www.rivm.nl/en/pfas>)

PFAS in pine needles of *Pinus halepensis*, Madrid - 2021



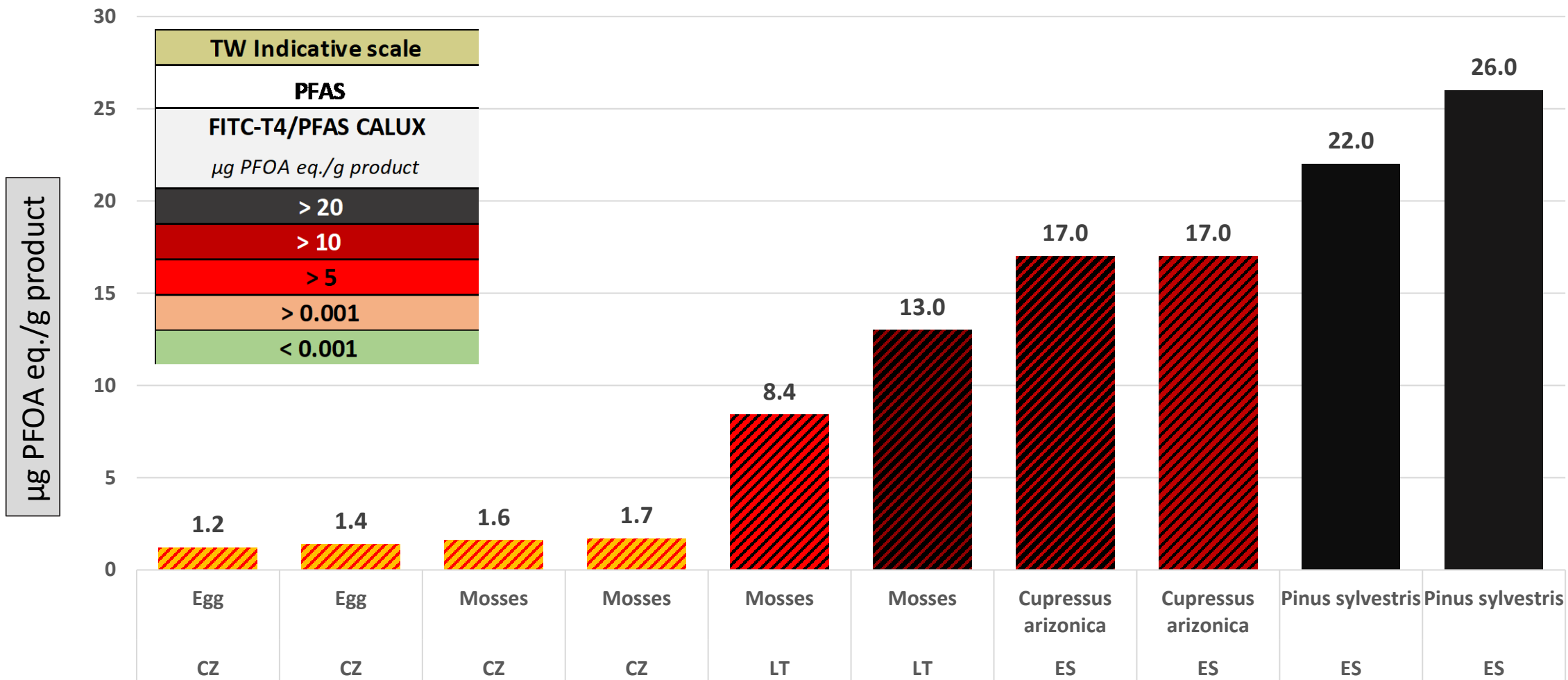
TW Indicative scale Results
FITC-4 (PFAS)
50 -100 µg PFOA eq./g product
20 -50 µg PFOA eq./g product
10 - 20 µg PFOA eq./g product
5-10 µg PFOA eq./g product
1-5 µg PFOA eq./g product

PFAS in *Cupressus arizonica*, Madrid - 2021



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

TW indicative scale PFAS (μg PFOA eq./g product) in divers biomatrices



PFAS a serious threat for human health

If a person take one cup pine needle tea of 10 grams pine needles, taken from the area around the incinerator Valdemingómez, Madrid.

In this biomonitoring research on pine needles in the area around the incinerator, the analyse results with **bioassay FITC-T4** are **22 µg PFOA eq./gr product** at location Pin01, on 570 m distance.

For a person drinking one cup pine needle tea of this location means: an intake of $10 \times 22 = 220 \mu\text{g PFOA} = 220.000 \text{ ng PFOA}$

EFSA 2018: Tolerable Weekly Intake (TWI) = 6 ng PFOA/kg body weight (bw) per week

Meaning: a person of 70 kg has a TWI of $70\text{kg} \times 6 \text{ ng} \times 7 \text{ days (week)} = 2940 \text{ ng PFOA/week}$

A cup of pine needle tea of location Pin01 has $220 \mu\text{g} = 220.000 \text{ ng PFOA}$

This means that the body burden for a person who drinks one cup tea with these pine needles once a week, an exceeding TWI with a factor 75 for PFOA will be the body burden.

If the person drinks this tea every day: the TWI exceeds with a factor > 500 .

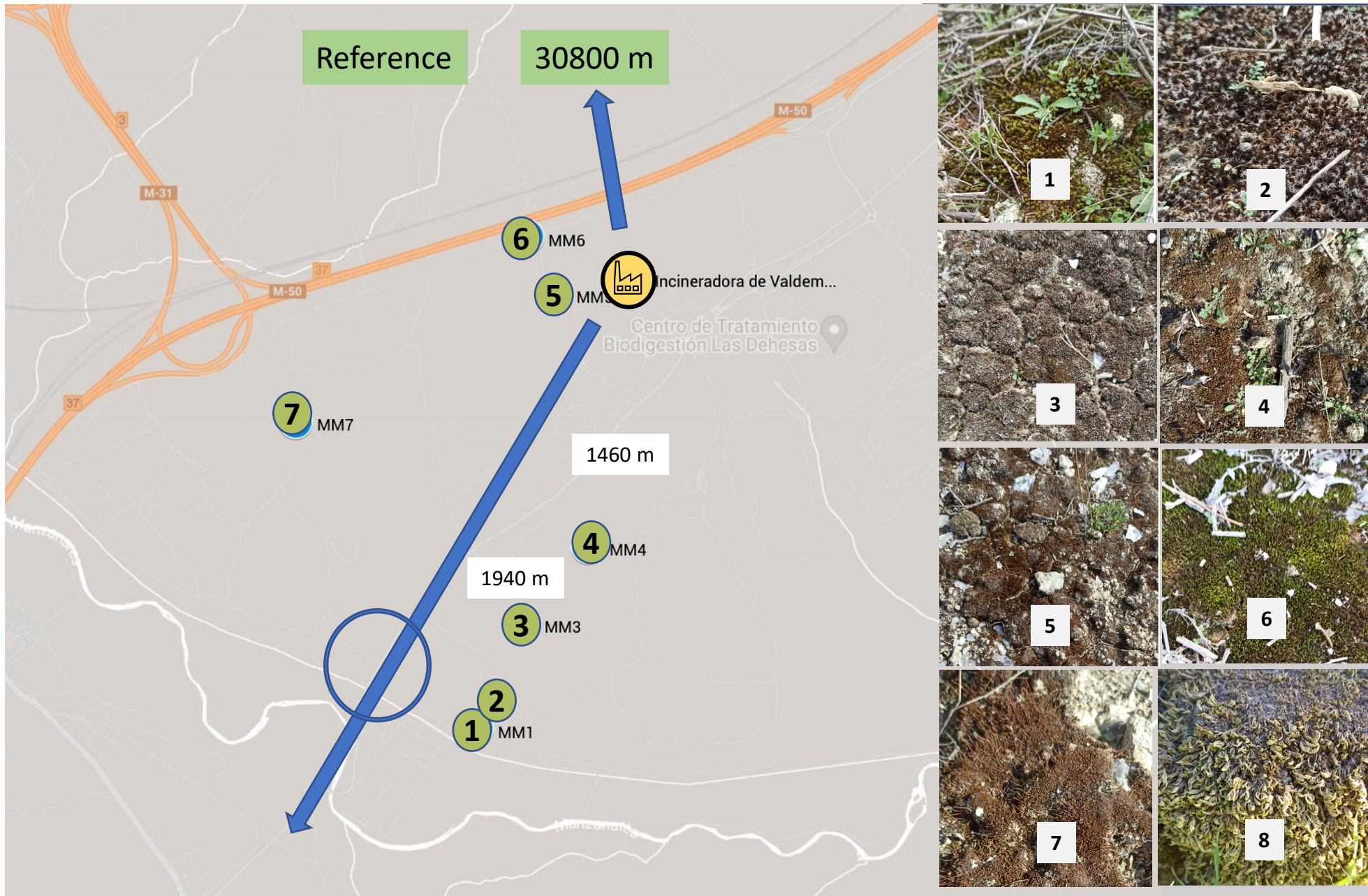
This example shows how the human population, by not knowing about daily health risks of PFAS contaminated food and water intake, have to deal with the serious possibility of an unknown body burden of fluorinated “for-ever-chemicals” of their food and water intake and with that a major threat for human health and the environment.

The question needs to be asked: What about the PFAS content of our food like (home grown) vegetables and our daily water supply in general and especially around a waste incinerator?

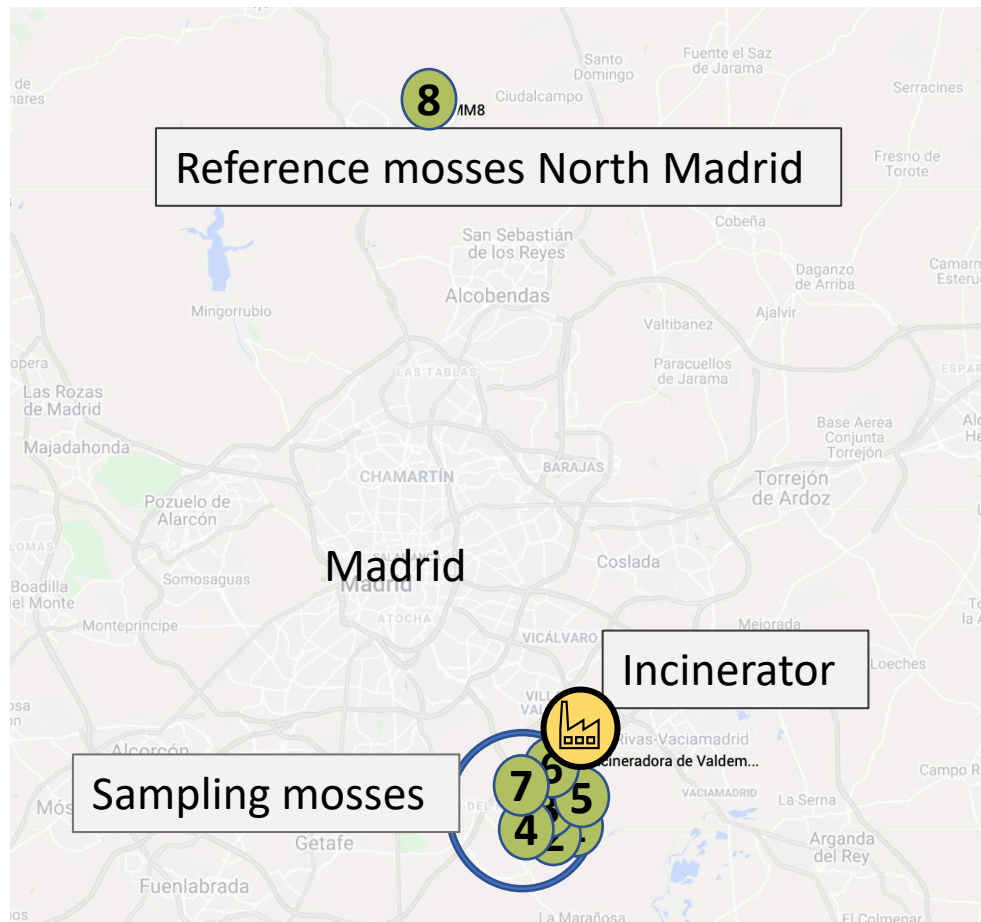
Results Dioxins (PCDD/F/dl-PCB) in Mosses, Madrid - 2021

Mosses , Madrid 2021						Results Mosses madrid 2021				
Sample nr	sample date	Biomarker	Weight (gr)	Wind direction	Distance (m)	TW-REF-NR	Veg nr	PCDD/F/dl-PCB	PCDD/F	dl-PCB
								DR CALUX (pg TCDD eq./g product)		
1	1-11-2021	Mosses		S	2610	TW21-MD-M01	M01	2.28	1.30	0.98
2	1-11-2021	Mosses		S	2350	TW21-MD-M02	M02	6.70	3.80	2.90
3	1-11-2021	Mosses		S	1940	TW21-MD-M03	M03	7.80	5.30	2.50
4	1-11-2021	Mosses		S	1460	TW21-MD-M04	M04	10.70	5.30	5.40
5	1-11-2021	Mosses		W	400	TW21-MD-M05	M05	1.55	0.86	0.69
6	1-11-2021	Mosses		W	572	TW21-MD-M06	M06	3.20	1.10	2.10
7	1-11-2021	Mosses		W	2000	TW21-MD-M07	M07	1.42	0.95	0.47
8	1-11-2021	Mosses		N	30800	TW21-MD-M08	M08	0.46	0.36	0.10

Sample locations mosses, Madrid - 2021



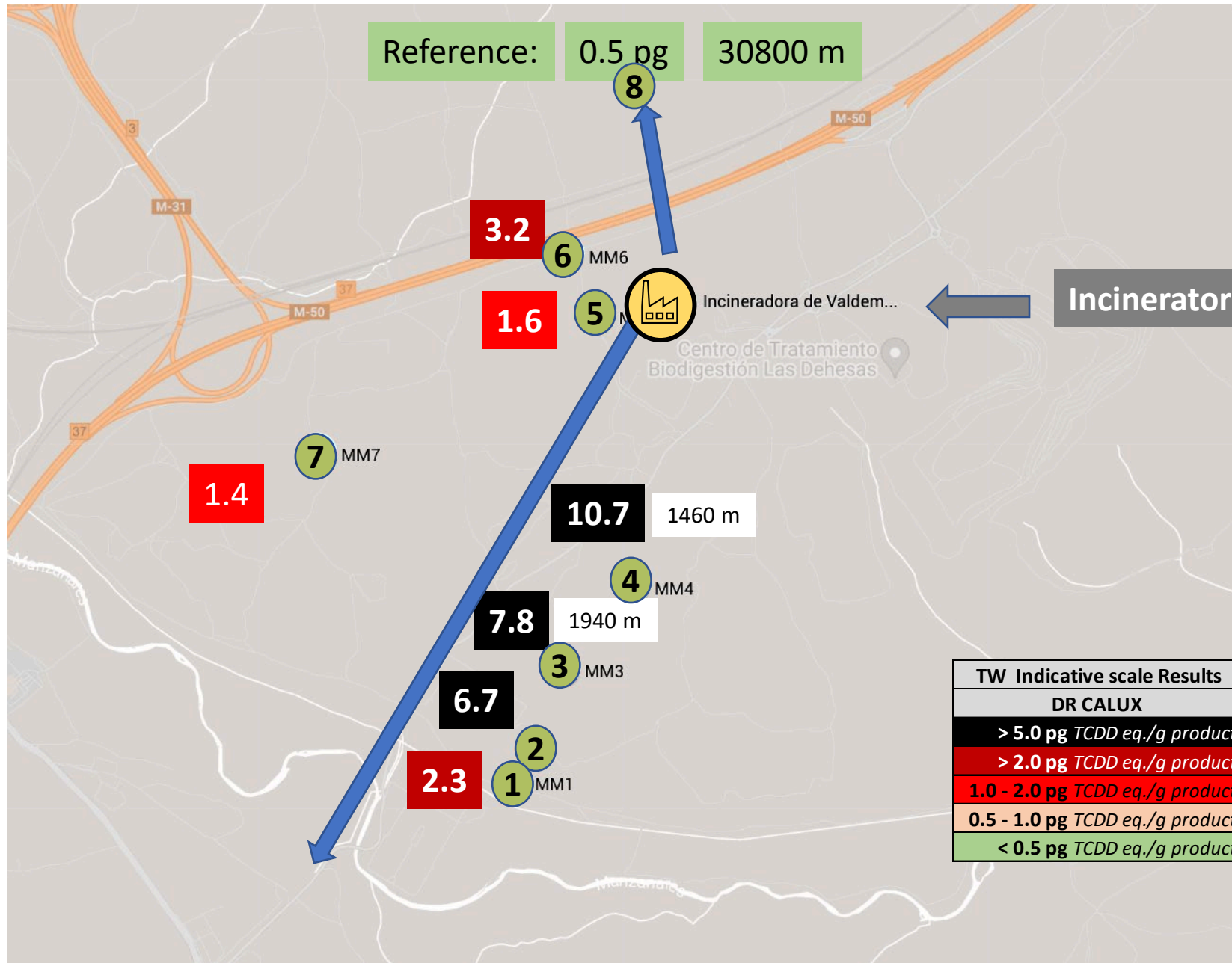
Reference location mosses Castillo de Viñuelas, Madrid - 2021



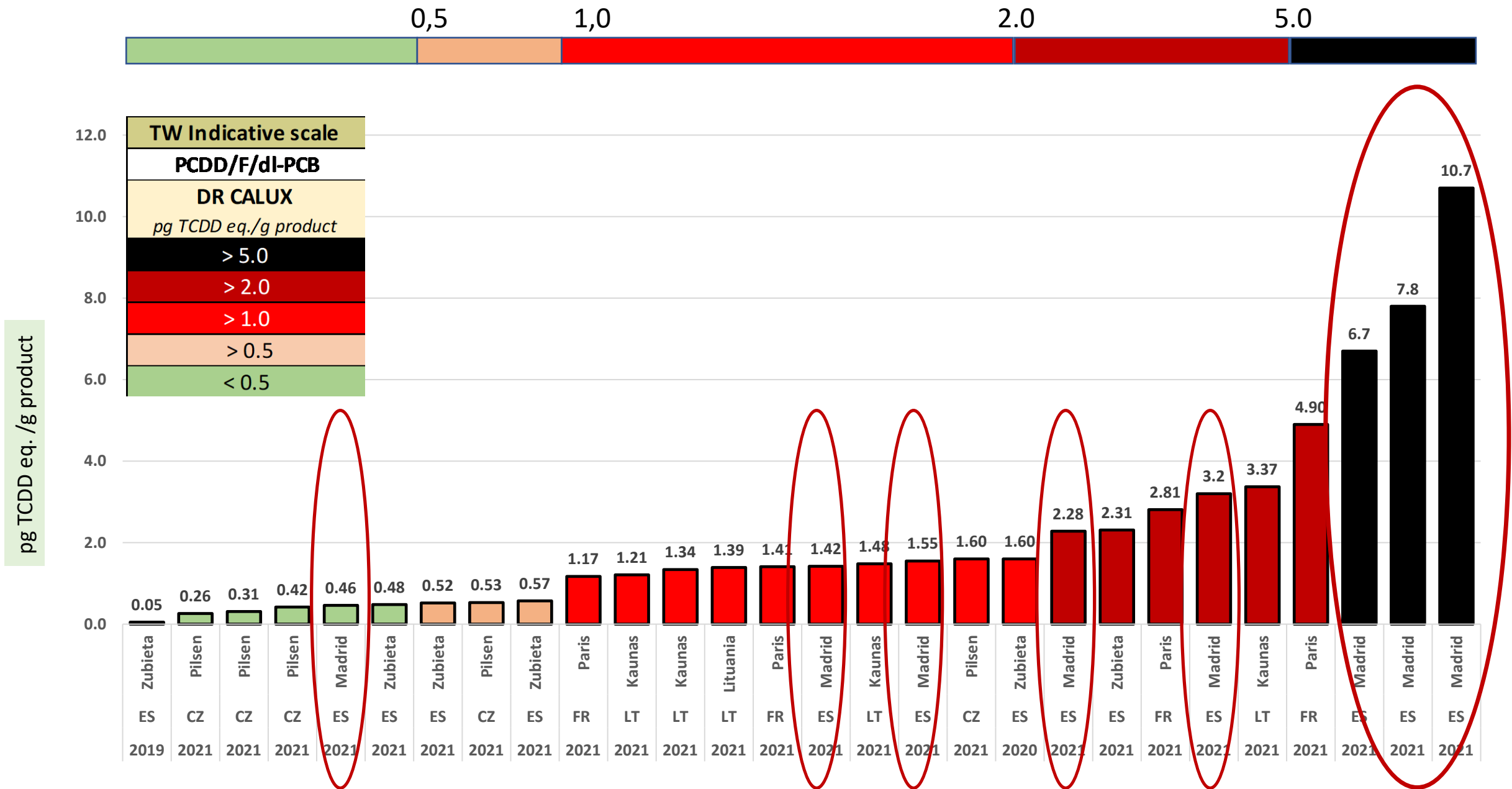
Sample locations *Mosses*, Madrid - 2021



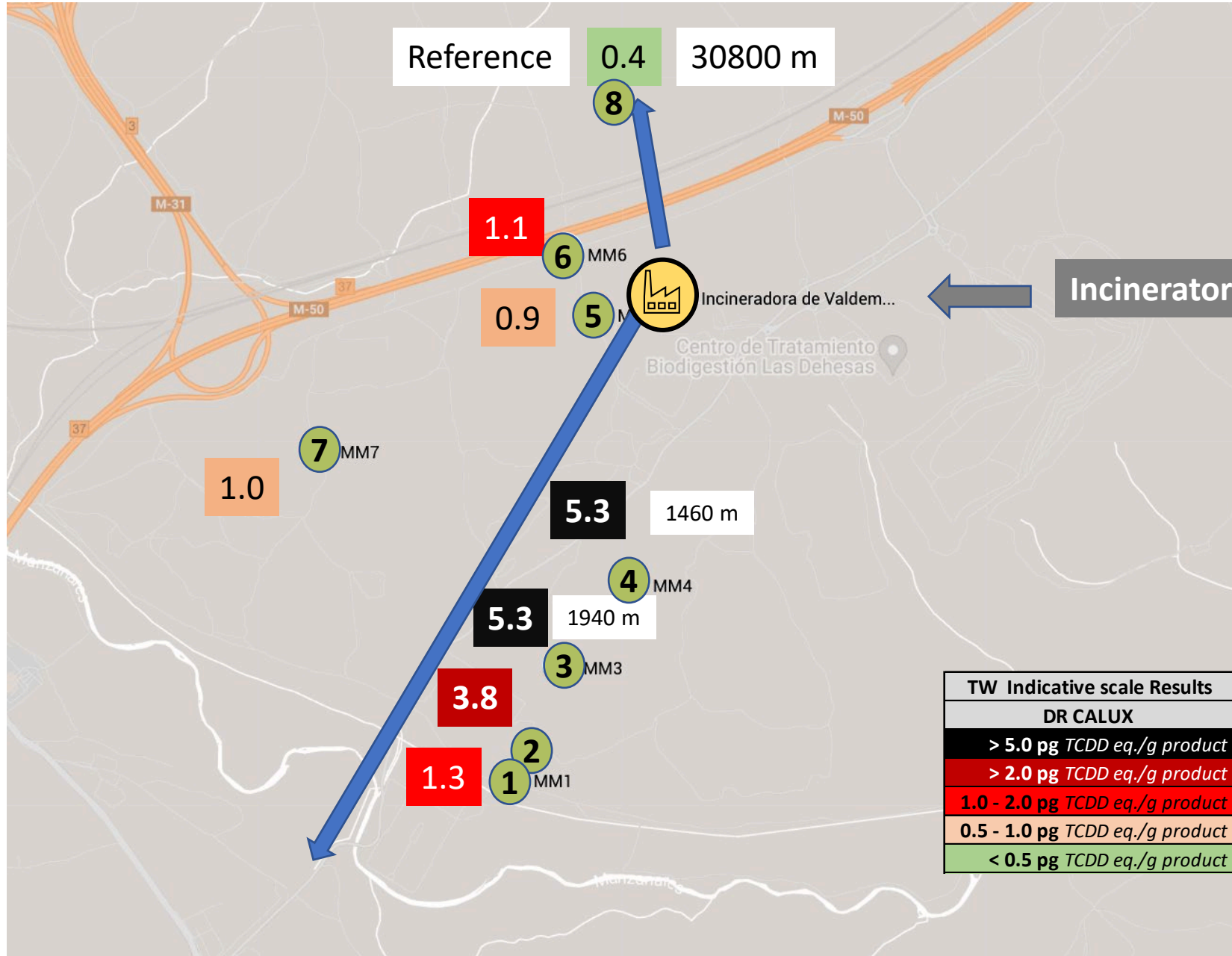
Results sum of Dioxins (PCDD/F/dl-PCB) in *Mosses* Madrid - 2021



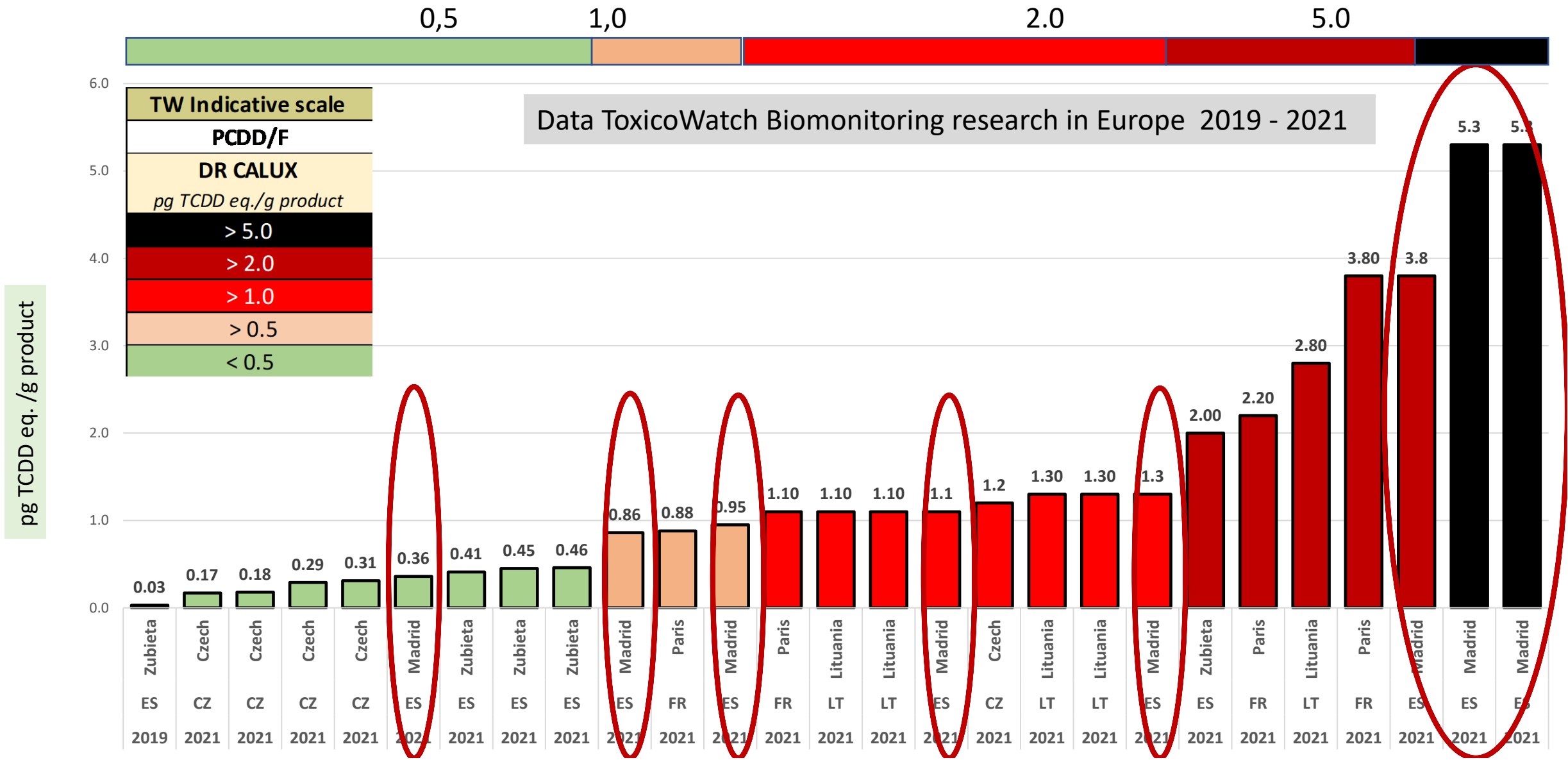
Indicative scale PCDD/F/dl-PCB mosses Madrid, Spain 2021



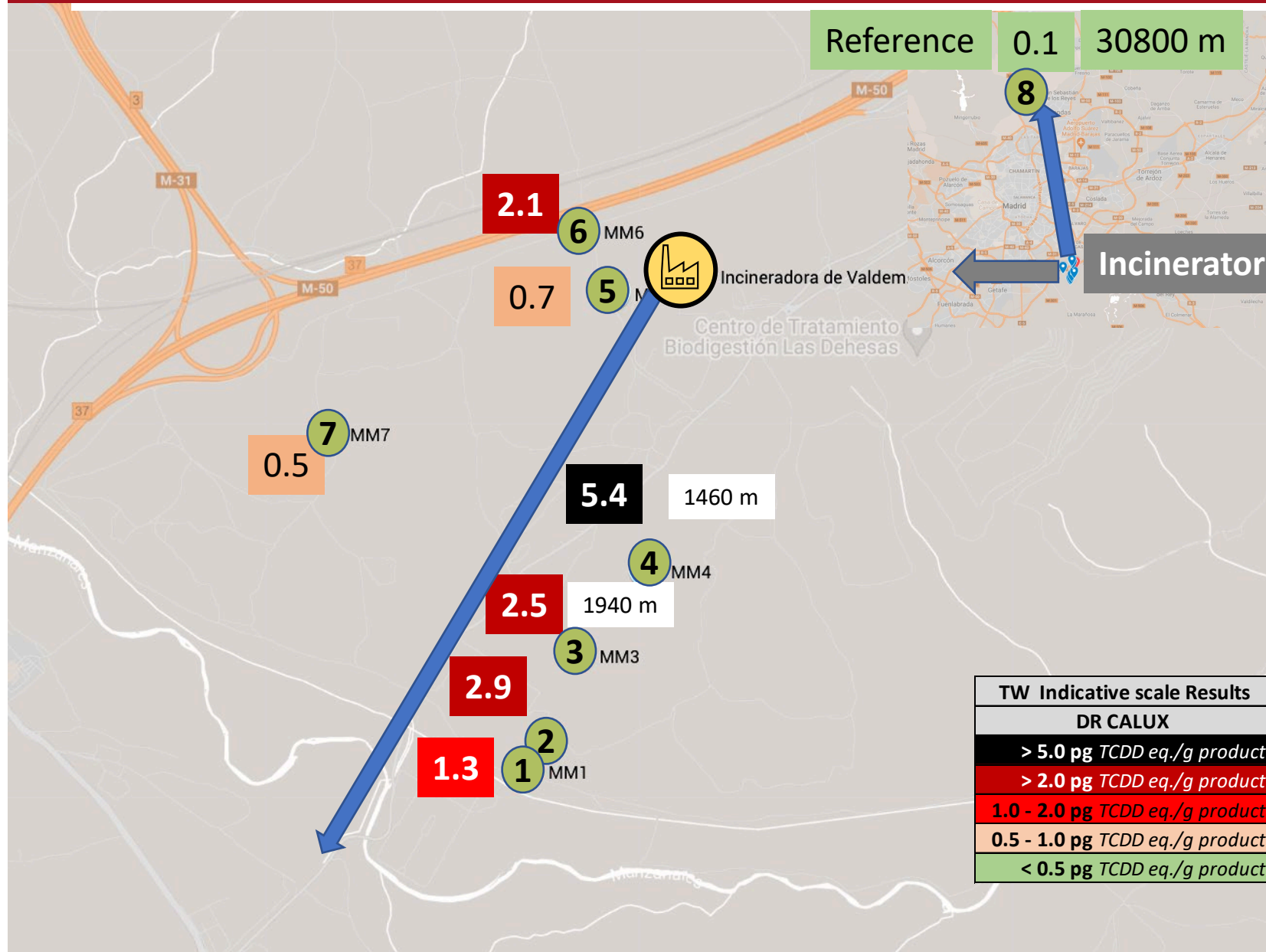
Dioxins (PCDD/F) in *Mosses*, Madrid - 2021



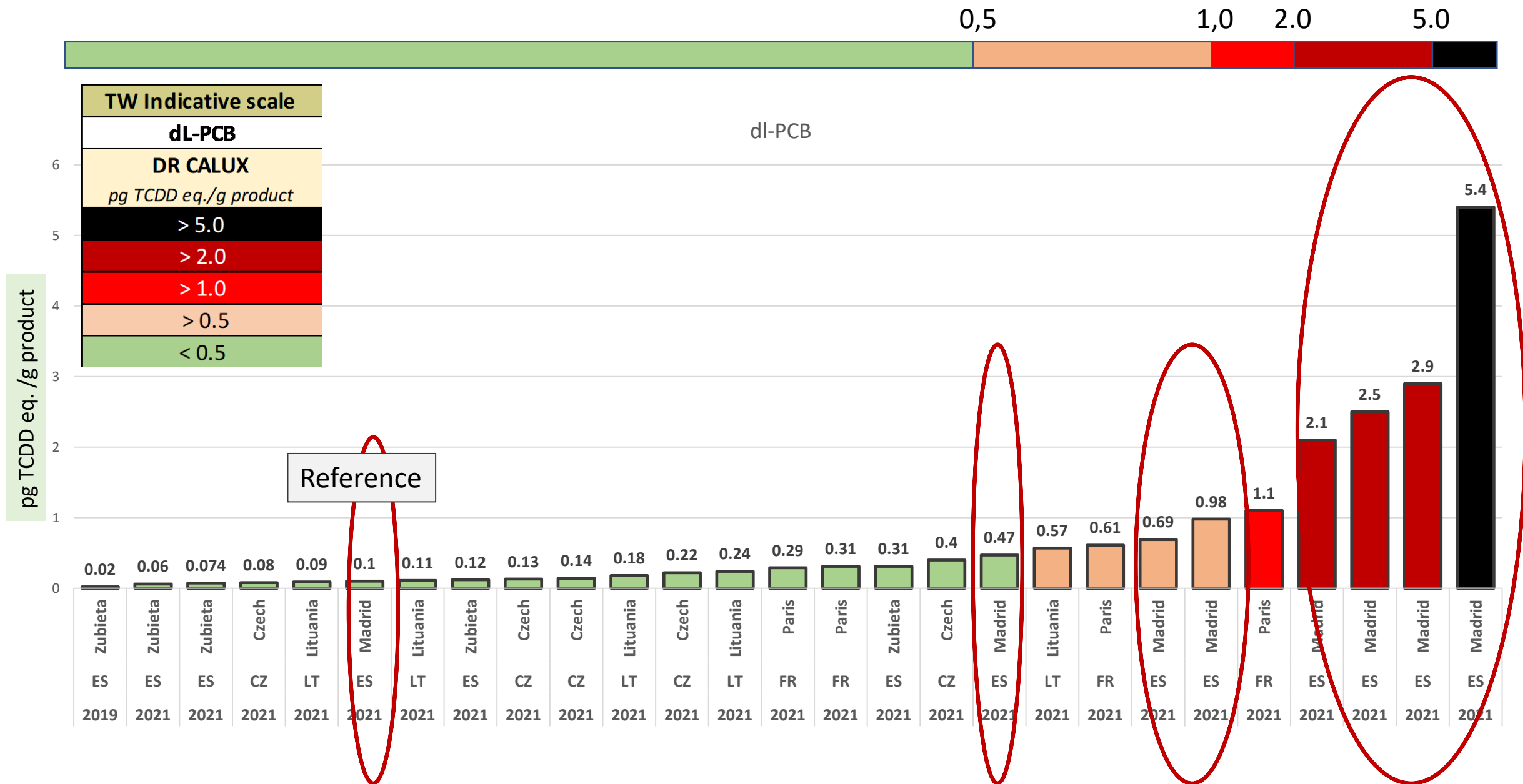
Indicative scale PCDD/F in mosses Madrid, Spain



Biomonitoring of dl-PCB in *mosses*, Madrid - 2021



TW-indicative scale dl-PCB in mosses Madrid - 2021



Sample locations *Mosses*, Madrid - 2021



Sample locations *Mosses*, Madrid - 2021



A person wearing a green polo shirt, a backpack, and a face mask is crouching in a field of tall grass and shrubs, likely collecting samples. The background shows a dense line of green trees and a fence.

Dioxins (PCDD/F/dl-PCB):
1.7 pg TCDD eq./g pr.
in
Pinus halepensis

Dioxins (PCDD/F/dl-PCB):
Reference
0.1 pg TCDD eq./g pr.


PAH:
380 ng BaP eq./g pr.
in
Cupressus arizona


PAH:
reference
0.5 ng BaP eq./g pr.


Dioxins (PCDD/F/dl-PCB):
10.7 pg TCDD eq./g pr.
in
Mosses


Dioxins (PCDD/F/dl-PCB):
Reference
0.5 pg TCDD eq./g pr.


Results biomonitoring - Madrid, Spain 2021


- 

DR CALUX : Factor 75 more dioxins (PCDD/F) in pine needles near the incinerator
- 

PAH CALUX : Factor 10 more PAH evergreen trees near the incinerator
- 

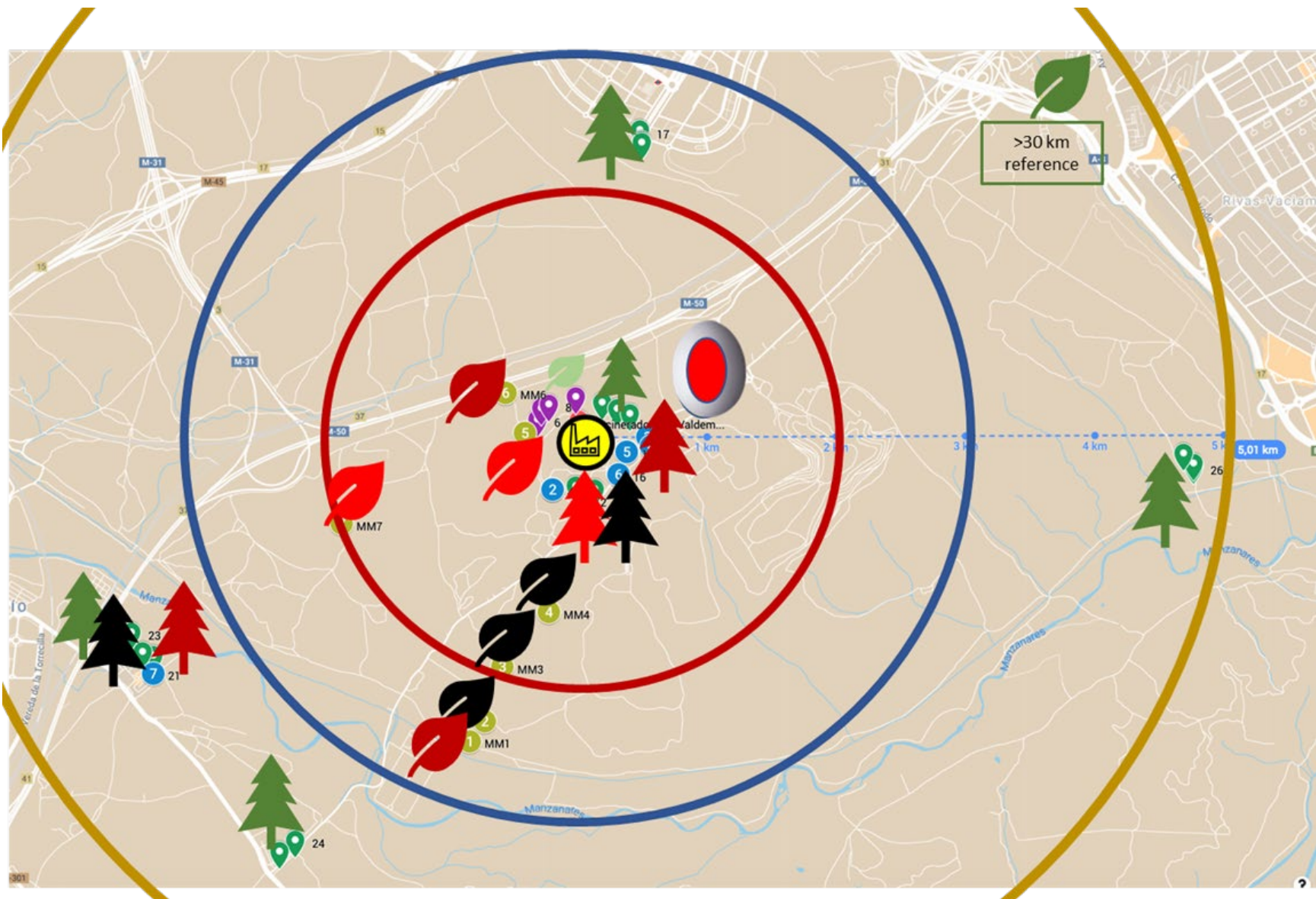
DR CALUX : Factor 20 more dioxins and factor 50 more dl-PCBs in *mosses*
- 

DR CALUX: High levels of dioxins (PCDD/F) in *Mosses* South West
- 

DR CALUX: Eggs do not comply the EU limits dioxins and dioxin like PCBs for safe egg consumption
- 

GC-MS: Congener patterns of the dioxins are identical with those from a waste incinerator

Legenda color, see Figure below



Highest values in TW research data 2017-2021 for dioxins (PCDD/F/ dl-PCBs), PAHs, and PFAS

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

Short-term vs long-term measurements WtE

Short-term

Sampling: 0,1 % of a year



- 12 hours measurement period (2 x 6 hours)
- Only under steady state conditions
- Pre-announced
- Only PCDD/F

EU Regulatory



Long-term

Sampling: 95 % of a year



Should be EU Regulatory:

Continuous measurements in chimney WtE

Including Other Than Normal Conditions (OTNOC)

Analyses of other UPOPs (PFAS, PAH, PXDD/F)

Analysis report

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Authorized by:

Snezana Zeljkovic
 Principle analyst

Date report (dd-mm-yyyy):

30-08-2021

Responsible person BDS:

Emiel Felzel
 Head of Testing Laboratory



Information about report

The results of examination refer exclusively to the checked samples.

Results are given in table 1.

Sample characteristics are given in table 2.

The measurement uncertainty for CALUX method is typically below 30%. For the calculation a coverage factor of 1 is used.

Accreditation ISO 17025 (RvA L401) is not applicable for activities described in this report

Date of the performance of the test: 30-08-2021

Table 1 sample analysis results

No.	Client code	Method	Parameter	Result	Conclusion	Cut off	Unit
1	pin01	DR CALUX	dl-PCBs (separated TEQ)	1.3	---	n.a.	pg TEQ / gram product
2	pin01	DR CALUX	PCDD/PCDF (separated TEQ)	7.1	---	n.a.	pg TEQ / gram product
3	pin02	DR CALUX	dl-PCBs (separated TEQ)	0.11	---	n.a.	pg TEQ / gram product
4	pin02	DR CALUX	PCDD/PCDF (separated TEQ)	0.18	---	n.a.	pg TEQ / gram product
5	pin03	DR CALUX	dl-PCBs (separated TEQ)	0.11	---	n.a.	pg TEQ / gram product
6	pin03	DR CALUX	PCDD/PCDF (separated TEQ)	0.074	---	n.a.	pg TEQ / gram product
7	pin04	DR CALUX	dl-PCBs (separated TEQ)	0.055	---	n.a.	pg TEQ / gram product
8	pin04	DR CALUX	PCDD/PCDF (separated TEQ)	0.080	---	n.a.	pg TEQ / gram product
9	pin05	DR CALUX	dl-PCBs (separated TEQ)	0.26	---	n.a.	pg TEQ / gram product
10	pin05	DR CALUX	PCDD/PCDF (separated TEQ)	0.092	---	n.a.	pg TEQ / gram product
11	pin06	DR CALUX	dl-PCBs (separated TEQ)	0.057	---	n.a.	pg TEQ / gram product
12	pin06	DR CALUX	PCDD/PCDF (separated TEQ)	LOQ <0.05	---	n.a.	pg TEQ / gram product
13	ulmus01	DR CALUX	dl-PCBs (separated TEQ)	LOQ <0.05	---	n.a.	pg TEQ / gram product
14	ulmus01	DR CALUX	PCDD/PCDF (separated TEQ)	0.066	---	n.a.	pg TEQ / gram product
15	ulmus02	DR CALUX	dl-PCBs (separated TEQ)	LOQ <0.05	---	n.a.	pg TEQ / gram product
16	ulmus02	DR CALUX	PCDD/PCDF (separated TEQ)	0.066	---	n.a.	pg TEQ / gram product
17	cup01	DR CALUX	dl-PCBs (separated TEQ)	0.10	---	n.a.	pg TEQ / gram product
18	cup01	DR CALUX	PCDD/PCDF (separated TEQ)	1.6	---	n.a.	pg TEQ / gram product
19	cup02	DR CALUX	dl-PCBs (separated TEQ)	0.27	---	n.a.	pg TEQ / gram product
20	cup02	DR CALUX	PCDD/PCDF (separated TEQ)	0.89	---	n.a.	pg TEQ / gram product
21	cup03	DR CALUX	dl-PCBs (separated TEQ)	0.073	---	n.a.	pg TEQ / gram product
22	cup03	DR CALUX	PCDD/PCDF (separated TEQ)	0.73	---	n.a.	pg TEQ / gram product

For results below the limit of quantification (LOQ), behind the less than sign the limit of quantification is given

n.a.= no cut off according to EU guideline in BEQ established, maximal levels applicable if available

Table 2 sample characteristics

No.	Client code	BDS code	Matrix	ISO17025 (RvAL401)	Date arrival	Sealed
1	pin01	41122	Not defined	no	12-08-2021	
2	pin01	41122	Not defined	no	12-08-2021	
3	pin02	41123	Not defined	no	12-08-2021	
4	pin02	41123	Not defined	no	12-08-2021	
5	pin03	41124	Not defined	no	12-08-2021	
6	pin03	41124	Not defined	no	12-08-2021	
7	pin04	41125	Not defined	no	12-08-2021	
8	pin04	41125	Not defined	no	12-08-2021	
9	pin05	41126	Not defined	no	12-08-2021	

10	pin05	41126	Not defined	no	12-08-2021
11	pin06	41127	Not defined	no	12-08-2021
12	pin06	41127	Not defined	no	12-08-2021
13	ulmus01	41128	Not defined	no	12-08-2021
14	ulmus01	41128	Not defined	no	12-08-2021
15	ulmus02	41129	Not defined	no	12-08-2021
16	ulmus02	41129	Not defined	no	12-08-2021
17	cup01	41130	Not defined	no	12-08-2021
18	cup01	41130	Not defined	no	12-08-2021
19	cup02	41131	Not defined	no	12-08-2021
20	cup02	41131	Not defined	no	12-08-2021
21	cup03	41132	Not defined	no	12-08-2021
22	cup03	41132	Not defined	no	12-08-2021

For the method DR CALUX and the sum parameter PCDD/PCDF (separated TEQ) the used method is extraction with organic solvents; the extracts are cleaned on an acid silica column and separation is done with a florisil column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure) and benchmarked against 2,3,7,8-TCDD. The DR CALUX analysis is done according to p-bds-051

For the method DR CALUX and the sum parameter dl-PCBs (separated TEQ) the used method is extraction with organic solvents; the extracts are cleaned on an acid silica column futher clean-up is done with a florisil column; The cleaned extracts are dissolved in DMSO; Seperation is done with alumina; ; the DR CALUX Analysis is done according to p-bds-051extraction with organic solvents; the extracts are cleaned on an acid silica column and separation is done with a alumina column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure) and benchmar

Analysis report

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Authorized by:

Snezana Zeljkovic
 Principle analyst

Date report (dd-mm-yyyy):

01-11-2021

Responsible person BDS:

Emiel Felzel
 Head of Testing Laboratory



Information about report

The results of examination refer exclusively to the checked samples.

Results are given in table 1.

Sample characteristics are given in table 2.

The measurement uncertainty for CALUX method is typically below 30%. For the calculation a coverage factor of 1 is used.

Accreditation ISO 17025 (RvA L401) is not applicable for activities described in this report

Date of the performance of the test: 01-11-2021

Table 1 sample analysis results

No.	Client code	Method	Parameter	Result	Conclusion	Cut off	Unit
1	TW21-MD-M01	DR CALUX	dl-PCBs (separated TEQ)	0.98	---	n.a.	pg TEQ / gram product
2	TW21-MD-M01	DR CALUX	PCDD/PCDF (separated TEQ)	1.3	---	n.a.	pg TEQ / gram product
3	TW21-MD-M02	DR CALUX	dl-PCBs (separated TEQ)	2.9	---	n.a.	pg TEQ / gram product
4	TW21-MD-M02	DR CALUX	PCDD/PCDF (separated TEQ)	3.8	---	n.a.	pg TEQ / gram product
5	TW21-MD-M03	DR CALUX	dl-PCBs (separated TEQ)	2.5	---	n.a.	pg TEQ / gram product
6	TW21-MD-M03	DR CALUX	PCDD/PCDF (separated TEQ)	5.3	---	n.a.	pg TEQ / gram product
7	TW21-MD-M04	DR CALUX	dl-PCBs (separated TEQ)	5.4	---	n.a.	pg TEQ / gram product
8	TW21-MD-M04	DR CALUX	PCDD/PCDF (separated TEQ)	5.3	---	n.a.	pg TEQ / gram product
9	TW21-MD-M05	DR CALUX	dl-PCBs (separated TEQ)	0.69	---	n.a.	pg TEQ / gram product
10	TW21-MD-M05	DR CALUX	PCDD/PCDF (separated TEQ)	0.86	---	n.a.	pg TEQ / gram product
11	TW21-MD-M06	DR CALUX	dl-PCBs (separated TEQ)	2.1	---	n.a.	pg TEQ / gram product
12	TW21-MD-M06	DR CALUX	PCDD/PCDF (separated TEQ)	1.1	---	n.a.	pg TEQ / gram product
13	TW21-MD-M07	DR CALUX	dl-PCBs (separated TEQ)	0.47	---	n.a.	pg TEQ / gram product
14	TW21-MD-M07	DR CALUX	PCDD/PCDF (separated TEQ)	0.95	---	n.a.	pg TEQ / gram product
15	TW21-MD-M08	DR CALUX	dl-PCBs (separated TEQ)	0.10	---	n.a.	pg TEQ / gram product
16	TW21-MD-M08	DR CALUX	PCDD/PCDF (separated TEQ)	0.36	---	n.a.	pg TEQ / gram product

n.a.= no cut off according to EU guideline in BEQ established, maximal levels applicable if available

Table 2 sample characteristics

No.	Client code	BDS code	Matrix	ISO17025 (RvAL401)	Date arrival	Sealed
1	TW21-MD-M01	41620	Not defined	no	18-10-2021	
2	TW21-MD-M01	41620	Not defined	no	18-10-2021	
3	TW21-MD-M02	41621	Not defined	no	18-10-2021	
4	TW21-MD-M02	41621	Not defined	no	18-10-2021	
5	TW21-MD-M03	41622	Not defined	no	18-10-2021	
6	TW21-MD-M03	41622	Not defined	no	18-10-2021	
7	TW21-MD-M04	41623	Not defined	no	18-10-2021	
8	TW21-MD-M04	41623	Not defined	no	18-10-2021	
9	TW21-MD-M05	41624	Not defined	no	18-10-2021	
10	TW21-MD-M05	41624	Not defined	no	18-10-2021	
11	TW21-MD-M06	41625	Not defined	no	18-10-2021	
12	TW21-MD-M06	41625	Not defined	no	18-10-2021	
13	TW21-MD-M07	41626	Not defined	no	18-10-2021	
14	TW21-MD-M07	41626	Not defined	no	18-10-2021	
15	TW21-MD-M08	41627	Not defined	no	18-10-2021	
16	TW21-MD-M08	41627	Not defined	no	18-10-2021	

For the method DR CALUX and the sum parameter PCDD/PCDF (separated TEQ) the used method is extraction with organic solvents; the extracts are cleaned on an acid silica column and separation is done with a florisil column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure) and benchmarked against 2,3,7,8-TCDD. The DR CALUX analysis is done according to p-bds-051

For the method DR CALUX and the sum parameter dl-PCBs (separated TEQ) the used method is extraction with organic solvents; the extracts are cleaned on an acid silica column futher clean-up is done with a florisil column; The cleaned extracts are dissolved in DMSO; Seperation is done with alumina; ; the DR CALUX Analysis is done according to p-bds-051extraction with organic solvents; the extracts are cleaned on an acid silica column and separation is done with a alumina column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure) and benchmar



Analysis report

Client:

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 Head of Testing Laboratory

Date report (dd-mm-yyyy):

26-11-2021

Responsible person BDS:

Emiel Felzel
 Head of Testing Laboratory

Information about report

The results of examination refer exclusively to the checked samples.

Results are given in table 1.

Sample characteristics are given in table 2.

The measurement uncertainty for CALUX method is typically below 30%. For the calculation a coverage factor of 1 is used.

If an analysis is accredited by ISO17025 (RvA L401) is indicated by a yes or a no

Date of the performance of the test: 26-11-2021

Table 1 sample analysis results

No.	Client code	Method	Parameter	Result	Conclusion	Cut off	Unit
1	TW21-MD-Egg 01 (Madrid)	DR CALUX	PCDD/PCDF (BEQ; semi)	6.0	suspected	1.7	pg BEQ / gram fat
2	TW21-MD-Egg 01 (Madrid)	DR CALUX	PCDD/PCDF and dl-PCBs (BEQ; semi)	13	suspected	3.3	pg BEQ / gram fat

For the suspected sample(s) to be non-compliant, the concentration has to be determined by a confirmatory method

Table 2 sample characteristics

No.	Client code	BDS code	Matrix	ISO17025 (RvAL401)	Date arrival	Sealed
1	TW21-MD-Egg 01 (Madrid)	41835	Food, egg(product)	yes	22-11-2021	
2	TW21-MD-Egg 01 (Madrid)	41835	Food, egg(product)	yes	22-11-2021	

For the method DR CALUX and the sum parameter PCDD/PCDF (BEQ; semi) the used method is shake extraction with organic solvents (hexane); the extracts are cleaned on an acid silica column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure). The response of the sample is corrected for the background and subsequently corrected for the apparent bioassay recovery with a reference sample at the level of interest. The evaluation was done on the maximum level for PCDD/F, from which a cut off value has been established (2/3 of maximum level) to determine if a sample is compliant or suspected. As a maximum level the level of the matrix as described in the table above is used. After the evaluation an estimation is given of the sample in the form of a BEQ outcome. The DR CALUX analysis is done according to p-bds-051.

For the method DR CALUX and the sum parameter PCDD/PCDF and dl-PCBs (BEQ; semi) the used method is shake extraction with organic solvents (hexane); the extracts are cleaned on an acid silica column. The cleaned extracts are dissolved in DMSO. The DR CALUX activity is determined (24h exposure). The response of the sample is corrected for the background and subsequently corrected for the apparent bioassay recovery with a reference sample at the level of interest. The evaluation was done on the maximum level for PCDD/F and dl-PCBs, from which a cut off value has been established (2/3 of maximum level) to determine if a sample is compliant or suspected. As a maximum level the level of the matrix as described in the table above is used. After the evaluation an estimation is given of the sample in the form of a BEQ outcome. The DR CALUX analysis is done according to p-bds-051.

For the method DR CALUX and the sum parameter dl-PCBs (BEQ; semi) the used method is

All DR CALUX analysis results comply with EU requirements as indicated in Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs. Maximal levels according to COMMISSION REGULATION (EU) 2015/704 of 30 April 2015.



Analysis report

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Date report (dd-mm-yyyy):

06-12-2021

Responsible person BDS:

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Information about report

The results of examination refer exclusively to the checked samples.

All analysis results comply with EU requirements as indicated in Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs. Maximal levels according to COMMISSION REGULATION (EU) 2015/704 of 30 April 2015.

For the analyses on dioxins/furans/dl-PCBs/ndl-PCB the sample is extracted with organic solvents (hexane); the extracts are cleaned on an acid silica column/alumina/florisil/carbon. For recovery calculation all ¹³C labeled congeners are added. The concentrations are determined by GC-MS/MS.

Information about sample

BDS sample number 41879
Client identification TW21-MD-Egg 01 (Madrid)
Sample received on 29-11-2021
Start of test 30-11-2021
End of test 02-12-2021
Matrix Food, egg(product)

Judgement

Non-compliant for maximal level limit (expressed as WHO PCDD/F TEQ) taking into account expanded measurement uncertainty.
Sample TW21-MD-Egg 01 (Madrid) is above the maximal level of 2.5 pg TEQ / gram fat.

Non-compliant for maximal level limit (expressed as WHO PCDD/F + dl-PCBs TEQ) taking into account expanded measurement uncertainty.
Sample TW21-MD-Egg 01 (Madrid) is above the maximal level of 5 pg TEQ / gram fat.

Test results:

WHO sum parameters (accredited under RvA L401)

WHO PCDD/F TEQ excl. LOQ 2005	4.1	pg TEQ / gram fat	U+/-	24%
WHO PCDD/F TEQ incl. LOQ 2005	4.1	pg TEQ / gram fat	U+/-	24%
WHO dl-PCBs TEQ excl. LOQ 2005	2.5	pg TEQ / gram fat	U+/-	24%
WHO dl-PCBs TEQ incl. LOQ 2005	2.5	pg TEQ / gram fat	U+/-	24%
WHO PCDD/F + dl-PCBs TEQ excl. LOQ 2005	6.6	pg TEQ / gram fat	U+/-	23%
WHO PCDD/F + dl-PCBs TEQ incl. LOQ 2005	6.7	pg TEQ / gram fat	U+/-	23%

Dioxins/furans (accredited under RvA L401)

2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.26	pg / gram fat	U+/-	44%
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	1.3	pg / gram fat	U+/-	31%
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.41	pg / gram fat	U+/-	44%
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.48	pg / gram fat	U+/-	46%
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.53	pg / gram fat	U+/-	41%
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	8.7	pg / gram fat	U+/-	34%
Octachlorodibenzo-p-dioxin	5.9	pg / gram fat	U+/-	49%
2,3,7,8-Tetrachlorodibenzofuran	2.3	pg / gram fat	U+/-	27%
1,2,3,7,8-Pentachlorodibenzofuran	2.4	pg / gram fat	U+/-	31%
2,3,4,7,8-Pentachlorodibenzofuran	4.4	pg / gram fat	U+/-	29%
1,2,3,4,7,8-Hexachlorodibenzofuran	2.0	pg / gram fat	U+/-	37%

1,2,3,6,7,8-Hexachlorodibenzofuran	2.3	pg / gram fat	U+/-	25%
1,2,3,7,8,9-Hexachlorodibenzofuran	LOQ (<0.2)	pg / gram fat	U+/-	41%
2,3,4,6,7,8-Hexachlorodibenzofuran	1.9	pg / gram fat	U+/-	32%
1,2,3,4,6,7,8-Heptachlorodibenzofuran	4.9	pg / gram fat	U+/-	25%
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.23	pg / gram fat	U+/-	28%
Octachlorodibenzofuran	0.33	pg / gram fat	U+/-	37%

dl-PCBs (accredited under RvA L401)

3,3',4,4'-Tetrachlorobiphenyl (#77)	64	pg / gram fat	U+/-	39%
3,4,4',5-Tetrachlorobiphenyl (#81)	7.0	pg / gram fat	U+/-	32%
3,3',4,4',5-Pentachlorobiphenyl (#126)	22	pg / gram fat	U+/-	26%
3,3',4,4',5,5'-Hexachlorobiphenyl (#169)	4.6	pg / gram fat	U+/-	53%
2,3,3',4,4'-Pentachlorobiphenyl (#105)	1100	pg / gram fat	U+/-	51%
2,3,4,4',5-Pentachlorobiphenyl (#114)	90	pg / gram fat	U+/-	32%
2,3',4,4',5-Pentachlorobiphenyl (#118)	2000	pg / gram fat	U+/-	44%
2,3',4,4',5'-Pentachlorobiphenyl (#123)	61	pg / gram fat	U+/-	36%
2,3,3',4,4',5-Hexachlorobiphenyl (#156)	440	pg / gram fat	U+/-	36%
2,3,3',4,4',5'-Hexachlorobiphenyl (#157)	120	pg / gram fat	U+/-	37%
2,3',4,4',5,5'-Hexachlorobiphenyl (#167)	290	pg / gram fat	U+/-	35%
2,3,3',4,4',5,5'-Heptachlorobiphenyl (#189)	100	pg / gram fat	U+/-	37%

Recovery Dioxins/furans

2,3,7,8-Tetrachlorodibenzo-p-dioxin	67.2%
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	85.1%
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	160.1%
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	168.1%
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	137.7%
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	124.7%
Octachlorodibenzo-p-dioxin	177.7%
2,3,7,8-Tetrachlorodibenzofuran	77.9%
1,2,3,7,8-Pentachlorodibenzofuran	76.8%
2,3,4,7,8-Pentachlorodibenzofuran	80.8%
1,2,3,4,7,8-Hexachlorodibenzofuran	132.6%
1,2,3,6,7,8-Hexachlorodibenzofuran	126.4%
1,2,3,7,8,9-Hexachlorodibenzofuran	131.7%
2,3,4,6,7,8-Hexachlorodibenzofuran	116%
1,2,3,4,6,7,8-Heptachlorodibenzofuran	128.5%
1,2,3,4,7,8,9-Heptachlorodibenzofuran	122.5%
Octachlorodibenzofuran	143.8%

Recovery dl-PCBs

3,3',4,4'-Tetrachlorobiphenyl (#77)	58.5%
3,4,4',5-Tetrachlorobiphenyl (#81)	73.6%
3,3',4,4',5-Pentachlorobiphenyl (#126)	66.7%
3,3',4,4',5,5'-Hexachlorobiphenyl (#169)	133.3%
2,3,3',4,4'-Pentachlorobiphenyl (#105)	79.5%
2,3,4,4',5-Pentachlorobiphenyl (#114)	60.7%
2,3',4,4',5-Pentachlorobiphenyl (#118)	86.3%
2,3',4,4',5'-Pentachlorobiphenyl (#123)	60.9%
2,3,3',4,4',5-Hexachlorobiphenyl (#156)	98.4%
2,3,3',4,4',5'-Hexachlorobiphenyl (#157)	99.2%
2,3',4,4',5,5'-Hexachlorobiphenyl (#167)	87.4%
2,3,3',4,4',5,5'-Heptachlorobiphenyl (#189)	113.7%

Annex 5 :

From the Report of impact on health of emissions from the Valdemingómez technology park” Madrid 2019 of Madrid Health

ANEXO IV - ESTUDIO DE DISPERSIÓN DE CONTAMINANTES ATMOSFÉRICOS DE LA PLANTA DE VALORIZACIÓN ENERGÉTICA DE RESIDUOS "LAS LOMAS" EN EL PARQUE TECNOLÓGICO DE VALDEMINGÓMEZ

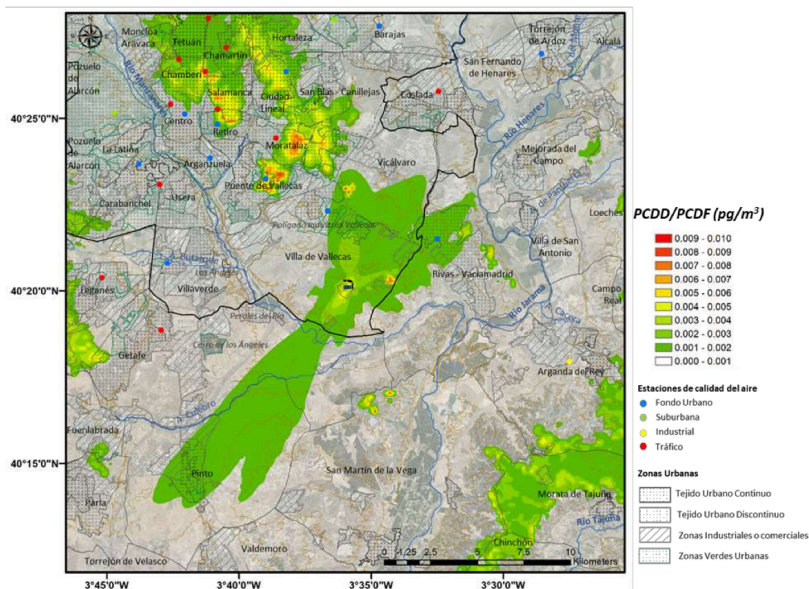


Figura 22. Concentración máxima en 24 horas de PCDD y PCDF

And the model PAH emissions by the incinerator

ANEXO IV - ESTUDIO DE DISPERSIÓN DE CONTAMINANTES ATMOSFÉRICOS DE LA PLANTA DE VALORIZACIÓN ENERGÉTICA DE RESIDUOS "LAS LOMAS" EN EL PARQUE TECNOLÓGICO DE VALDEMINGÓMEZ

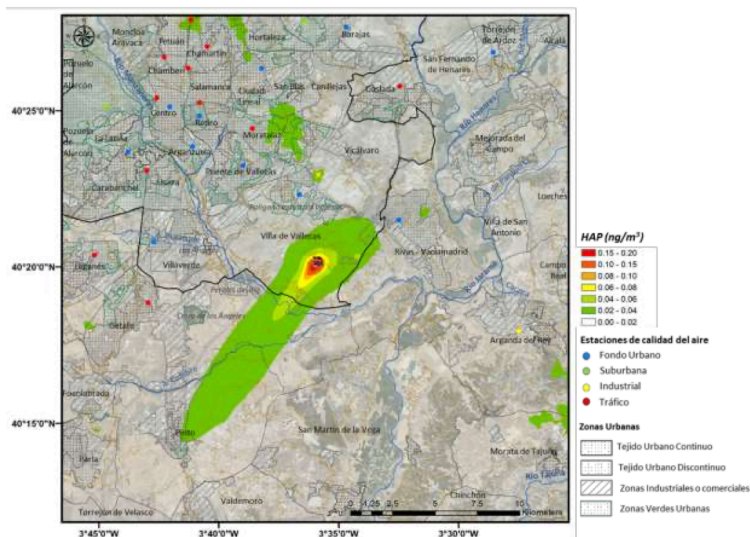


Figura 23. Concentración anual media de HAP

PAH and relative potency

PAH researches are difficult to compare because different methods like EPA 16, where 16 PAH are analysed, or PAH4 is used, where only 4 PAH re measured. The PAH CALUX, measures the total toxicity of PAH, expressed in Benzo(a) pyrene equivalency.

Table 1. Overview of the Relative Potencies^a

PAH	accession number	MW	REP (M/M)	list	IARC classification	TEF
naphthalene	91-20-3	128	<0.0001	EPA	2B	0.001
acenaphthylene	208-96-8	152	<0.0001	EPA		0.001
acenaphthene	83-32-9	154	<0.0001	EPA	3	0.001
fluorene	86-73-7	166	<0.0001	EPA	3	0.001
phenanthrene	85-01-8	178	<0.0001	EPA	3	0.001
anthracene	120-12-7	178	<0.0001	EPA	3	0.01
fluoranthene	206-44-0	202	<0.0001	EPA	3	0.001
pyrene	129-00-0	202	<0.0001	EPA	3	0.001
benzo[<i>c</i>]fluorene	205-12-9	216	<0.0001	EU	3	
benzo[<i>ghi</i>]perylene	191-24-2	276	<0.0001	EPA, EU	3	0.01
cyclopenta[<i>cd</i>]pyrene	27208-37-3	226	0.0003	EU	2A	
dibenzo[<i>a,l</i>]pyrene	191-30-0	302	0.002	EU	2A	
dibenzo[<i>a,h</i>]pyrene	189-64-0	302	0.2	EU	2B	
dibenzo[<i>a,i</i>]pyrene	189-55-9	302	0.2	EU	2B	
dibenzo[<i>a,e</i>]pyrene	192-65-4	302	0.3	EU	2B	
benz[<i>a</i>]anthracene	56-55-3	228	0.3	EPA, EU	2B	0.1
chrysene	218-01-9	228	0.8	EPA, EU	2B	0.01
benzo[<i>a</i>]pyrene	50-32-8	252	1	EPA, EU	1	1
benzo[<i>j</i>]fluoranthene	205-82-3	252	1.3	EU	2B	
dibenz[<i>a,h</i>]anthracene	53-70-3	278	1.3	EPA, EU	2A	5
indeno[1,2,3- <i>cd</i>]pyrene	193-39-5	276	1.3	EPA, EU	2B	0.1
5-methylchrysene	3697-24-3	242	1.4	EU	2B	
benzo[<i>k</i>]fluoranthene	207-08-9	252	3.7	EPA, EU	2B	0.1
benzo[<i>b</i>]fluoranthene	205-99-2	252	5.0	EPA, EU	2B	0.1
2,3,7,8-TCDD	1746-01-6	322	5.0		1	

^aREP; in relation to benzo(a)pyrene of 16 EPA-PAHs in the PAH CALUX, along with an indication whether they are recommended for screening by the European Union (EU) or the U.S. Environmental Protection Agency (EPA), their IARC classification (1=carcinogenic to human; 2A = probably carcinogenic to humans; 2B = possibly carcinogenic to humans; 3 = not classifiable as carcinogenic to humans) and TEF-values (relative to BaP) according to Nisbet and LaGoy.⁶

Relative potency factor PFAS CALUX and FITC-T4

Table 3

Potency factors of tested PFASs compounds in the TTR-TRβ CALUX® bioassay.

Compound	Potency factor IC ₅₀ -based	Potency factor PC ₈₀ -based
	(PFOA = 1)	(PFOA = 1)
PFBA	0.0012	0.0018
PFPeA	0.048	0.080
PFHxA	0.16	0.19
PFHpA	1.3	1.4
PFOA	1.0	1.0
PFNA	0.48	0.32
PFDCa	0.12	0.12
PFBS	0.10	0.052
PFHxS	2.5	1.6
PFHpS	2.0	1.0
PFOS	3.0	2.0
H4PFOS (6:2 FTS)	0.033	0.019
PFOSA	1.2	0.72