Participation in 2016 Inter-laboratory Study on Fish Tissue Reference Materials

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Introduction

Our Laboratory was invited by CIL Cambridge Isotopic Laboratories to take part in a recertification of three certified Fish Tissue Reference Materials. The Materials where Catalog number description: EDF-2524, EDF-2525 and EDF-2526. It consists of a Clean Natural Matrix, A Contained Natural matrix and A Fortified Natural Matrix. We were asked to analyse PCDD/F, DL-PCB, Marker PCB and PBDEE. I had also taken part in the earlier certification study several years ago. This was a good opportunity to ensure that the analysis at our Laboratory equipment is state of the art and that our Accreditated Method even works for this matrix[1].

Materials and methods

The sample ampoules arrived at the 14th of July 2016. Catalog Number and Description:
EDF-2524 Clean Natural Matrix Reference Material (Fish), EDF-2525 Contaminated Natural Matrix Reference Material (Fish), EDF-2526 Fortified Natural Matrix Reference Material (Fish).
The ASE extraction[2] solvent was PAHE (Petroleum ether : Acetone : hexane : diethyl ether, (9:5,5:2,5:1 V/V/V/V)). The ASE 200 extraction program was, 100C, Heat time 5 min, Static time 5 minutes, 2 Static cycles, 1500 PSI, Flush Volume 80-100%. The same extract was used for all measurements. A small part of the extract was taken for the analyses of the PAH compounds before the CAPE Technology clean-up step.

The Internal standards added before the extraction where: EN-1948-4 sampling standard, EN-1948 Extraction Standard for PCDD/F, P48-M-ES, P-48-W-ES for PCB and PBDEE EO-5100-10X.
The cleanup used was CAPE Technology, Silica/Acid Silica + Active Carbon combination[3]. The PBDEE and PCB were analyzed from the combined hexane and hexane: toluene fractions. The PCDD/F were analyzed from the reversed toluene fraction of the Active Carbon column.
Detailed information of the Type of GC System used to quantitate the PCDD/F and WHO[12] PCB, Marker PCB and PBDEE. The instrument is in Jyväskylä.
The Instrument was an Agilent 7010, GC-MS/MS equipped with a Phenomenex ZB 5ms, 60 m, 0,25µm phase, 0,25 mm internal diameter and the Second column was an Agilent DB 5ms, 15 m, 0,25µm phase, 0,25 mm internal diameter. The Injection Volume (µL): 1 and Carrier Gas: Helium. The Flow Rate: 1.1 ml/minute at 180 °C initial oven temperature for PCDD/F, PCB and PBDEE. Ionization Mode and Polarity EI positive.
The Acquisition type used was MMR and the two most intense Precursor Fragments produced for each native compound and labelled Internal standard compound were used. The ratio had to be within ± 20 % to be accepted as a peak representing the compound.
MS Parameters: Transfer line 290 °C, EI-source, 70 eV, source temperature 280 °C, quads Q1 and Q2 150 °C, collision gas 2,25 ml/minute. Injector at 300 °C, Oven program: 180°C, 1 min, 10 °C/min, 100 °C, 16 min, 5 °C/min, 235 °C, 7 min, 5 °C/min, 325 °C, 5 min (PCB) tai 9 min (PCDD/F) and total of 90 minutes for PBDEE.
Range of Precursors and Product Ions
I used the Precursor and Product ions from the pesticide library. The most intense for quantitation and the second best as a qualifier.
Example: 2,3,7,8-TCDD 321.9 to 258.9 and qualifier 2,3,7,8-TCDD 319.9 to 256.9
C^{13}-2,3,7,8-TCDD 333.9 to 269.9 and qualifier C^{13}-2,3,7,8-TCDD 331.9 to 267.9. Expected Ratio 0.91
Software and version used: Mass Hunter B.07.04.2260, 28 Oct. 2015 and Mass Hunter Quantitative Analysis Version 3.07.01 SP1/build 7.1.524.1

Results and discussion

The instrument suitability for these analyses are shown by analyzing a CSL solution containing 40fg/µl tetra PCDD/F and 80fg/µl penta-hexa PCDD/F and 160 fg/µl octa PCDD/F, shown in the Table 1a. The response values used for quantitation are presented for the PCDD/F in the Table 1b and for the PCB in the Table 1c.

Table 1a. Five repeated injections of CSL, stating that the sensitivity requirements are achieved[4].

Sensitivity and stability test for GC-MSMS using five individual injections of CSL: 40fg/µl tetra PCDD/F, 80fg/µl penta-hexa PCDD/F and 160µg/µl octa PCDD/F.										
ZB-5ms, L=60m, ID= 250µm and phase thickness , 0.25µm										
LOT#EN480411CSL		Inj. 1	Inj. 2	Inj. 3	Inj. 4	Inj. 5	Mean value	STDEV	STDEV	Difference from
Exp. Date 04/01/2020	CSL	CSL	CSL	CSL	CSL	CSL	CSL	CSL	CSL	Certified value
Dioxins and furans	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	%	%
2,3,7,8-TCDD	0.04	0.0428	0.0367	0.0443	0.0384	0.0429	0.0410	0.0033	7.99	2.6
1,2,3,7,8-PeCDD	0.08	0.0823	0.0827	0.0793	0.0820	0.0688	0.0790	0.0059	7.43	-1.2
1,2,3,4,7,8-HxCDD	0.08	0.0964	0.0763	0.0714	0.0960	0.0830	0.0846	0.0113	13.41	5.8
1,2,3,6,7,8-HxCDD	0.08	0.0778	0.0765	0.0659	0.0683	0.0866	0.0750	0.0083	11.00	-6.2
1,2,3,7,8,9-HxCDD	0.08	0.0796	0.0867	0.0967	0.0727	0.0968	0.0865	0.0106	12.24	8.1
1,2,3,4,6,7,8-HpCDD	0.16	0.1640	0.1700	0.1850	0.1750	0.1730	0.1734	0.0077	4.44	8.4
1,2,3,4,6,7,8,9-OCDD	0.16	0.1710	0.1720	0.2110	0.1640	0.1890	0.1814	0.0189	10.43	13.4
2,3,7,8-TCDF	0.04	0.0368	0.0303	0.0376	0.0315	0.0348	0.0342	0.0032	9.38	-14.5
1,2,3,7,8-PeCDF	0.08	0.0774	0.0779	0.0774	0.0748	0.0815	0.0778	0.0024	3.08	-2.8
2,3,4,7,8-PeCDF	0.08	0.0807	0.0780	0.0857	0.0794	0.0854	0.0818	0.0035	4.30	2.3
1,2,3,4,7,8-HxCDF	0.08	0.0748	0.0792	0.0698	0.0777	0.0795	0.0762	0.0040	5.29	-4.8
1,2,3,6,7,8-HxCDF	0.08	0.0810	0.0906	0.0803	0.0831	0.0775	0.0825	0.0050	6.00	3.1
2,3,4,6,7,8-HxCDF	0.08	0.0923	0.0866	0.0757	0.0816	0.0710	0.0814	0.0085	10.39	1.8
1,2,3,7,8,9-HxCDF	0.08	0.0756	0.0778	0.0781	0.0689	0.0723	0.0745	0.0039	5.25	-6.8
1,2,3,4,6,7,8-HpCDF	0.16	0.1540	0.1620	0.1700	0.1640	0.1670	0.1634	0.0061	3.71	2.1
1,2,3,4,7,8,9-HpCDF	0.16	0.1560	0.1580	0.1750	0.1660	0.1590	0.1628	0.0078	4.79	1.8
1,2,3,4,6,7,8,9-OCDF	0.16	0.1420	0.1660	0.2080	0.1950	0.1720	0.1766	0.0258	14.59	10.4
Summa PCDD ja PCDF	1.680	1.685	1.707	1.811	1.718	1.740	1.732	0.048	2.8	3.1

Table 1b. Injections of CS1-CS6.

30.9.2016	ZB- 5ms capillary column						mean	standard	standard
	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	value	deviation	deviation
Dioxins and furans	rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	%
2,3,7,8-TCDD	1.04	1.11	0.94	1.10	1.13	1.18	1.08	0.085	7.80
1,2,3,7,8-PeCDD	1.07	1.04	0.98	1.08	1.10	1.15	1.07	0.056	5.28
1,2,3,4,7,8-HxCDD	0.82	0.98	0.86	0.97	0.99	1.04	0.94	0.086	9.10
1,2,3,6,7,8-HxCDD	0.88	0.92	0.85	0.96	0.96	0.98	0.92	0.053	5.79
1,2,3,7,8,9-HxCDD	0.79	0.97	0.78	1.00	0.95	0.92	0.90	0.091	10.06
1,2,3,4,6,7,8-HpCDD	1.11	1.09	1.02	1.11	1.14	1.17	1.11	0.049	4.42
1,2,3,4,6,7,8,9-OCDD	1.29	1.20	1.12	1.20	1.23	1.25	1.22	0.055	4.53
2,3,7,8-TCDF	1.32	1.34	1.15	1.35	1.38	1.42	1.33	0.092	6.91
1,2,3,7,8-PeCDF	1.07	1.13	1.02	1.20	1.20	1.24	1.14	0.085	7.42
2,3,4,7,8-PeCDF	1.15	1.13	1.04	1.16	1.19	1.23	1.15	0.065	5.62
1,2,3,4,7,8-HxCDF	1.07	1.12	1.05	1.13	1.15	1.20	1.12	0.055	4.86
1,2,3,6,7,8-HxCDF	1.09	1.07	0.98	1.08	1.09	1.13	1.08	0.049	4.57
2,3,4,6,7,8-HxCDF	1.06	1.00	0.93	1.03	1.05	1.09	1.03	0.055	5.38
1,2,3,7,8,9-HxCDF	0.83	0.99	0.83	0.91	0.98	0.98	0.92	0.077	8.33
1,2,3,4,6,7,8-HpCDF	1.10	1.05	0.98	1.08	1.10	1.13	1.07	0.051	4.80
1,2,3,4,7,8,9-HpCDF	0.89	0.82	0.64	0.83	0.88	0.95	0.84	0.107	12.86
1,2,3,4,6,7,8,9-OCDF	1.04	1.06	0.97	1.06	1.09	1.11	1.06	0.050	4.75

EN-1948CVS for EN 1948-4. Calibration and verification standards. Producer Wellington. LOT# EN480411CS1-CS6 and EN480411CSL. Exp. date 04/01/2020

Table 1c. The linearity range for the WHO-12 PCB

Measured 18.10.2016								Mean		
ZB-5ms		CS 3	CS 4	CS 5	CS 2	CS 1	CS 6	Values	std dev	std dev
WHO12-PCB		rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	rrfi	%
PCB 81		1.18	1.15	1.25	1.11	1.20	1.15	**1.17**	0.05	4.53
PCB 77		1.09	1.07	1.19	1.08	1.11	1.09	**1.10**	0.05	4.19
PCB 123	penta	0.61	0.62	0.76	0.64	0.73	0.61	**0.66**	0.07	10.19
PCB 118		0.89	0.86	1.05	0.84	1.08	0.88	**0.93**	0.12	12.77
PCB 114		0.92	0.93	1.09	0.97	1.25	0.93	**1.02**	0.14	13.90
PCB 105		0.74	0.74	0.87	0.81	0.80	0.73	**0.78**	0.03	4.29
PCB 126		1.09	1.06	1.23	1.05	1.24	1.08	**1.12**	0.09	8.41
PCB 167	hexa	0.68	0.68	0.60	0.66	0.78	0.68	**0.68**	0.09	13.48
PCB 156		0.97	0.99	1.11	1.05	1.32	0.98	**1.07**	0.14	12.88
PCB 157		0.92	0.99	1.07	0.96	1.22	0.96	**1.02**	0.12	11.43
PCB 169		1.30	1.29	1.14	1.25	1.02	1.29	**1.21**	0.12	9.81
PCB 189	hepta	0.86	0.82	0.98	0.81	1.09	0.82	**0.90**	0.12	13.58

The WHO12-PCB linearity test was performed using EN-1948-4:2010, WM48-CS1-CS6. Calibration and verification standards. Producer Wellington. LOT# WM48112CS1-CS6 and EN480411CSL. Exp. date 08/01/2020

The results are shown in the Table 1c.

Table 2. 2016-CIL Results for PCDD/F for the Fish Tissues.

Dioxins and furans	EDF-2624 ng/kg	EDF-2625 ng/kg	EDF-2626 ng/kg
2,3,7,8-TCDD	0.092	27.1	24.0
1,2,3,7,8-PeCDD	0.18	49.2	4.9
1,2,3,4,7,8-HxCDD	< 0.05	59.7	0.4
1,2,3,6,7,8-HxCDD	0.25	64.6	3.1
1,2,3,7,8,9-HxCDD	< 0.05	79.0	1.3
1,2,3,4,6,7,8-HpCDD	0.30	80.5	0.6
1,2,3,4,6,7,8,9-OCDD	0.65	175.9	1.6
2,3,7,8-TCDF	2.45	20.5	26.4
1,2,3,7,8-PeCDF	0.11	43.0	5.5
2,3,4,7,8-PeCDF	0.18	38.6	15.4
1,2,3,4,7,8-HxCDF	0.20	87.2	8.9
1,2,3,6,7,8-HxCDF	0.17	66.4	2.0
2,3,4,6,7,8-HxCDF	0.069	69.3	1.3
1,2,3,7,8,9-HxCDF	0.22	62.7	0.4
1,2,3,4,6,7,8-HpCDF	0.11	85.3	1.3
1,2,3,4,7,8,9-HpCDF	< 0.1	86.8	0.3
1,2,3,4,6,7,8,9-OCDF	0.37	188.0	1.5
Sum of PCDD and PCDF	5.3	1284	99

Table 3. The Results for Marker PCB from the Fish Tissues.

Marker PCB	EDF-2524 ng/kg	EDF-2525 ng/kg	EDF-2526 ng/kg
PCB 28	196	9299	312
PCB 52	563	26055	372
PCB 101	1049	76249	521
PCB 153	2431	264450	577
PCB 138	1784	199718	468
PCB 180	428	94112	109
PCB 170	183	33793	36

Table 4. The Results for WHO12-PCB from the Fish Tissues.

Koplanar PCB	EDF-2524 ng/kg	EDF-2525 ng/kg	EDF-2526 ng/kg
PCB 81	4.8	118	1.5
PCB 77	12	1770	436
PCB 123	30	2463	10
PCB 118	832	108750	308
PCB 114	21	1533	7.0
PCB 105	258	36310	82
PCB 126	4.8	794	374
PCB 167	39	8910	15
PCB 156	63	10219	16
PCB 157	20	3095	6.2
PCB 169	1.1	1331	631
PCB 189	5.3	1116	1.7

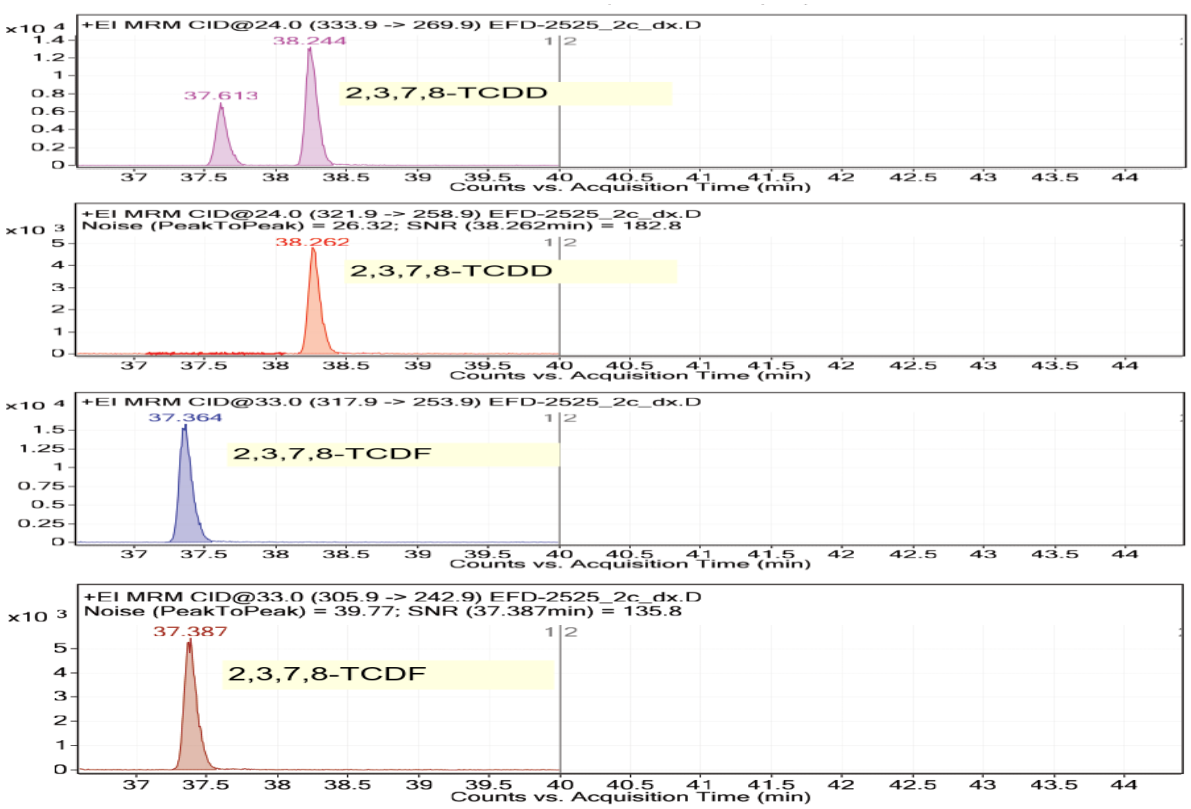

Figure 1. An example of the MRM ions measured in the TCDD/F region of the extract from EDF-2525. The C^{13}-labelled congener is on top and the native congener below for TCDD respective TCDF.

From the Tables 2-4 and Figure 1 above, we can determine, that the concentrations of the materials agree with the earlier certificated values. From the tables 1a-1c we can also determine that sensitivity and linearity Requirements of EU Regulation 709/2014 are met. An example of the chromatography is also shown in the the Figure 1. The organizer of the study also provided a report showing that the materials are recertified using these results, together with the results of the other 11 participating laboratories. All these results are found in the published CIL Report[5].

As a conclusion, we can establish that a GC-MSMS Instrument is well capable of analyzing these compounds at the concentration levels present in these samples.

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References

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