

ThermoFisher
S C I E N T I F I C

Routine, regulatory analysis of dioxins and dioxin-like compounds in food and feed samples

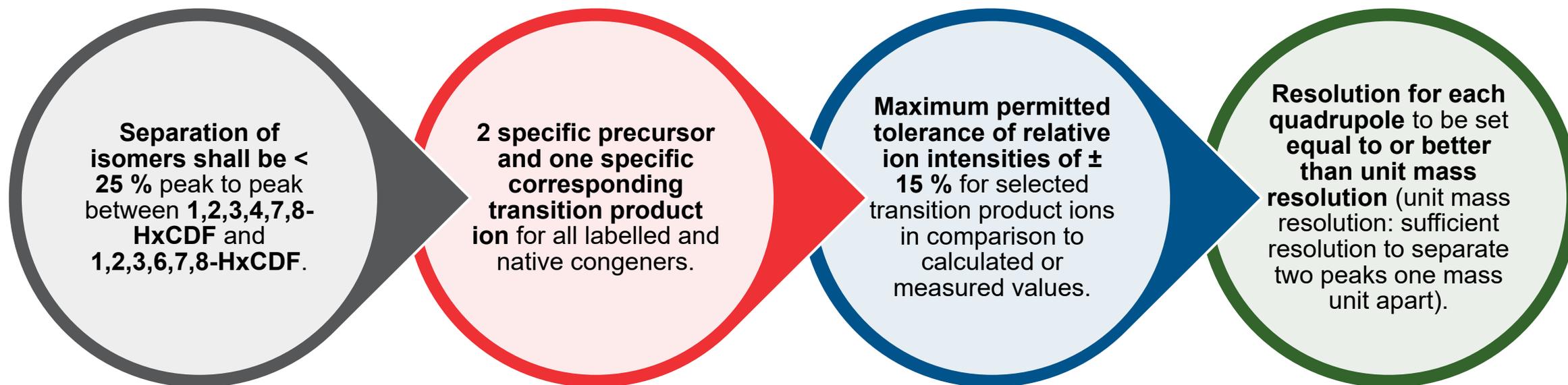
Thermo Fisher Scientific

The world leader in serving science

- Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs) commonly referred to as *dioxins*, and polychlorinated biphenyls (PCBs) are highly toxic substances classed as persistent organic pollutants (POPs).
- Due to their high fat-solubility, dioxins accumulate in the fatty tissues of animals. As a result, more than 90% of human exposure to dioxins is through food, especially meat, dairy, fish etc. Therefore accurate monitoring of food and feed is essential to control dioxin uptake from the food chain.
- In 2014 a change in European Commission regulations, permitted the use gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) to be used as an alternative to gas chromatography-high resolution mass spectrometry (GC-HRMS) for confirmatory analysis and for the control of maximum levels (MLs) and action levels (ALs) in certain food and feed samples

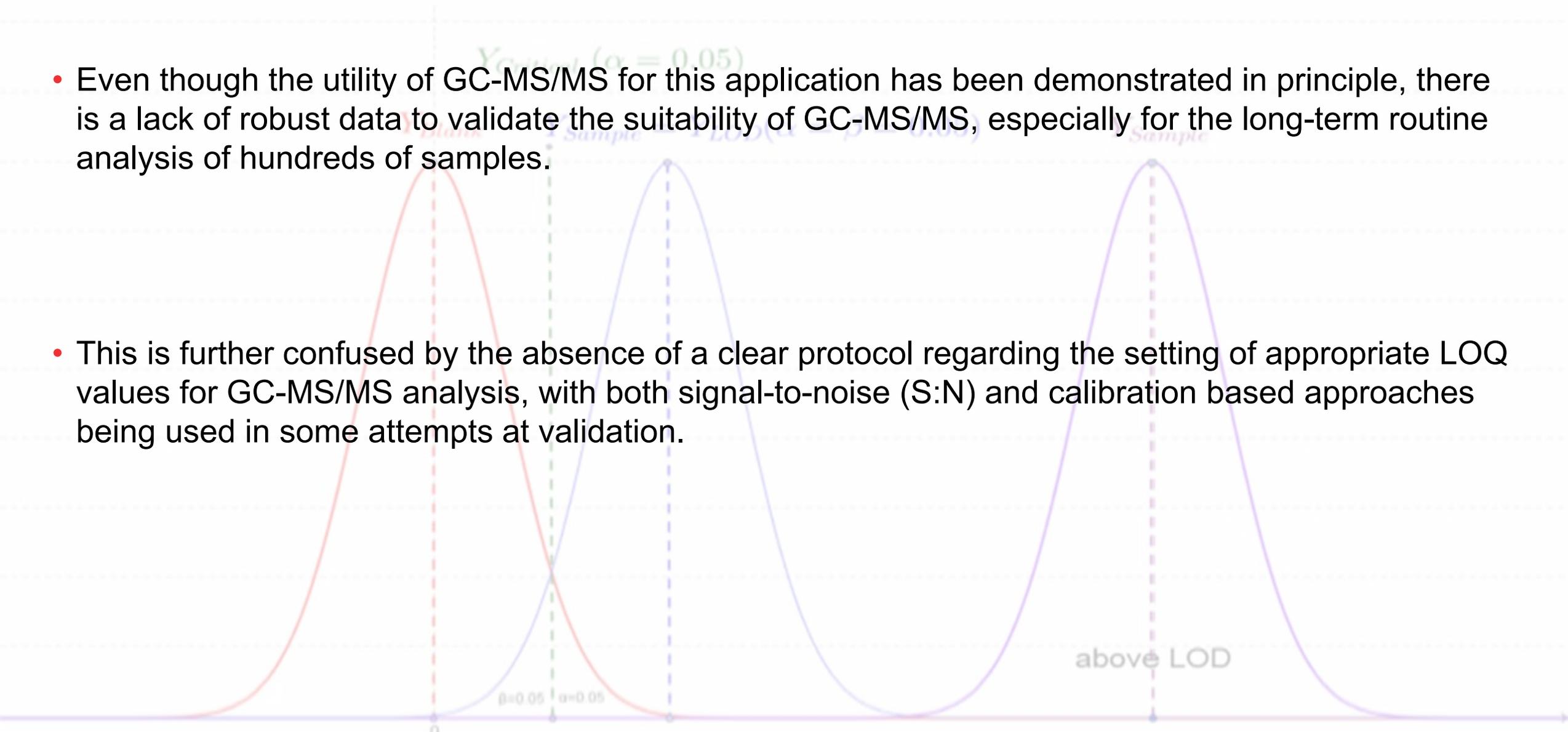
Challenges

- Due to the high toxicity and bioaccumulative nature of dioxins, accurate confirmation and quantification of PCDD/Fs in animal food and feedstuffs is essential to minimise human exposure.
- Current EU legislation requires GC-MS/MS analytical methods to demonstrate compliance at 1/5th of the maximum levels (MLs), the methods must also be able to demonstrate:



Challenges

- Even though the utility of GC-MS/MS for this application has been demonstrated in principle, there is a lack of robust data to validate the suitability of GC-MS/MS, especially for the long-term routine analysis of hundreds of samples.
- This is further confused by the absence of a clear protocol regarding the setting of appropriate LOQ values for GC-MS/MS analysis, with both signal-to-noise (S:N) and calibration based approaches being used in some attempts at validation.



- To demonstrate the utility of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for the routine and regulatory compliant analysis of PCDD/Fs, dioxin-like PCBs and indicator PCBs in food and feed samples.
- To evaluate the performance of two separate TSQ 9000 AEI systems (U.K. and U.S. based) for the analysis of PCDDs, PCDFs, dl-PCBs and non-dioxin-like (indicator) PCBs in solvent standards, food/feedstuff and proficiency test (PT) samples, using a clear, calibration based approach to setting LOQs

Experimental

- For all experiments described below, Thermo Scientific TSQ 9000 triple quadrupole mass spectrometers with AEI sources were used.
- Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system.



GC and Injector conditions – PCDD/Fs and non-ortho PCBs

TRACE 1310 GC PTV

Parameters

Operating Mode:	Large Volume
Injection Volume (µL):	4
Initial Inlet temperature (°C):	75
Carrier Gas, Flow (mL/min):	Helium, 1.2
Splitless Time (min)	1
Split Flow (mL/min)	100
Septum Purge (mL/min)	5 (constant)

PTV Ramp

Settings

	Pressure (Psi)	Rate (°C/s)	Temperature (°C)	Time (min)	Flow (mL/min)
Injection:	-	-	-	0.2	100.0
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0

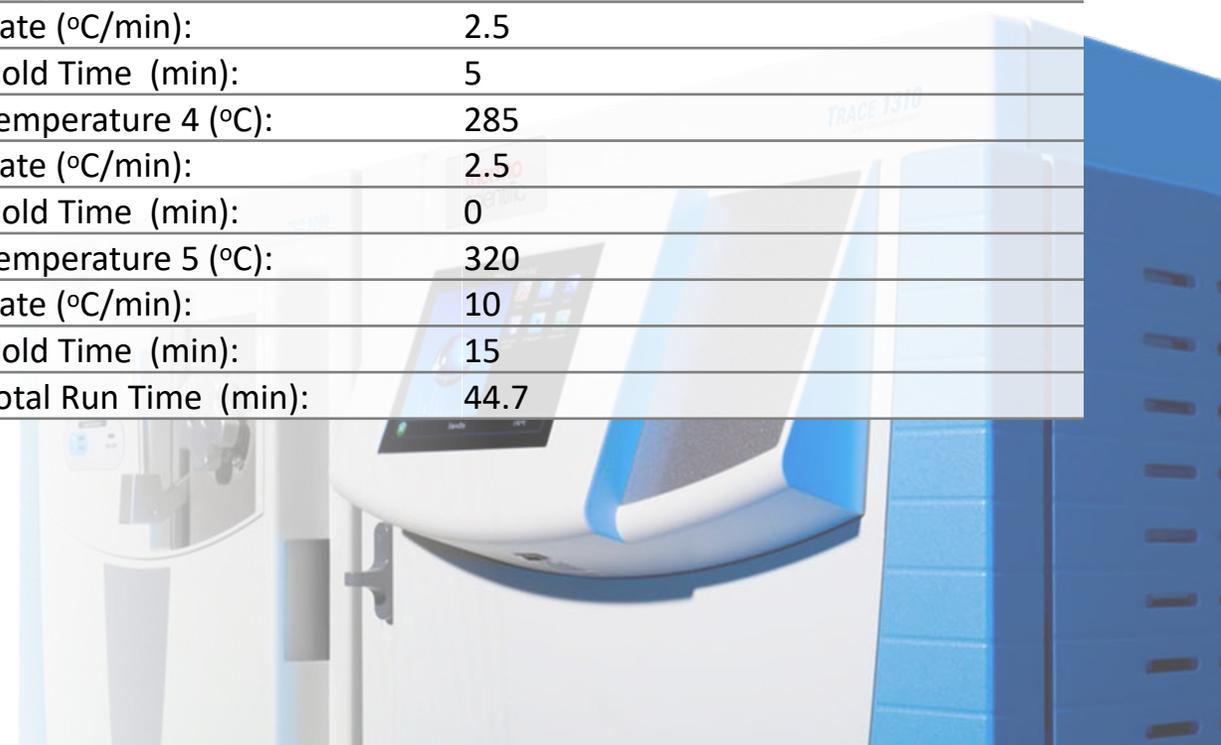
Autosampler settings

Injection Depth (mm)	45
Penetration Speed (mm/s)	100
Injection Speed (µL/s)	1

TRACE 1310 GC

Parameters

Oven Temperature Program:	
Temperature 1 (°C):	120 (initial)
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min):	25
Hold Time (min):	0
Temperature 3 (°C):	260
Rate (°C/min):	2.5
Hold Time (min):	5
Temperature 4 (°C):	285
Rate (°C/min):	2.5
Hold Time (min):	0
Temperature 5 (°C):	320
Rate (°C/min):	10
Hold Time (min):	15
Total Run Time (min):	44.7



GC and Injector conditions - Mono, di-ortho and indicator PCBs

TRACE 1310 GC PTV

Parameters

Operating Mode:	Splitless
Injection Volume (µL):	1
Initial Inlet temperature (°C):	75
Carrier Gas, Flow (mL/min):	Helium, 1.2
Splitless Time (min)	1
Split Flow (mL/min)	100
Septum Purge (mL/min)	5 (constant)

PTV Ramp

Settings

	Pressure (Psi)	Rate (°C/s)	Temperature (°C)	Time (min)	Flow (mL/min)
Injection:	-	-	-	0.2	-
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0

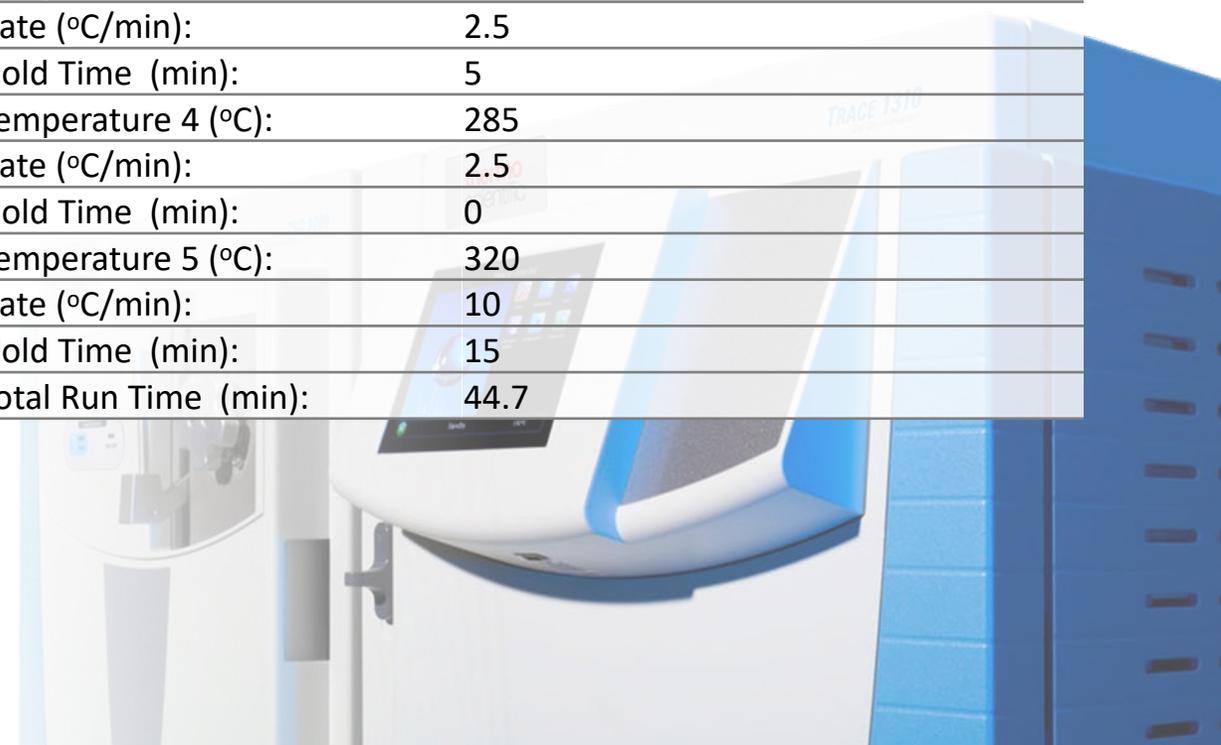
Autosampler settings

Injection Depth (mm)	45
Penetration Speed (mm/s)	100
Injection Speed (µL/s)	1

TRACE 1310 GC

Parameters

Oven Temperature Program:	
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Rate (°C/min):	2.5
Hold Time (min):	0
Temperature 5 (°C):	320
Rate (°C/min):	10
Hold Time (min):	15
Total Run Time (min):	44.7



MS conditions

TSQ 9000 Mass Spectrometer Parameters

Transfer Line (°C):	300
Ionization Type:	EI – with Advanced EI source
Ion Source (°C):	350
Electron Energy (eV):	50
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (x10 – natives HIGH, labelled LOW)
Tuning parameters:	AEI Smart Tune
Collision gas and pressure (psi):	Argon, 70
Resolution :	0.7 (both Q1 and Q3)

Resolution for each quadrupole to be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart).



Standards and samples

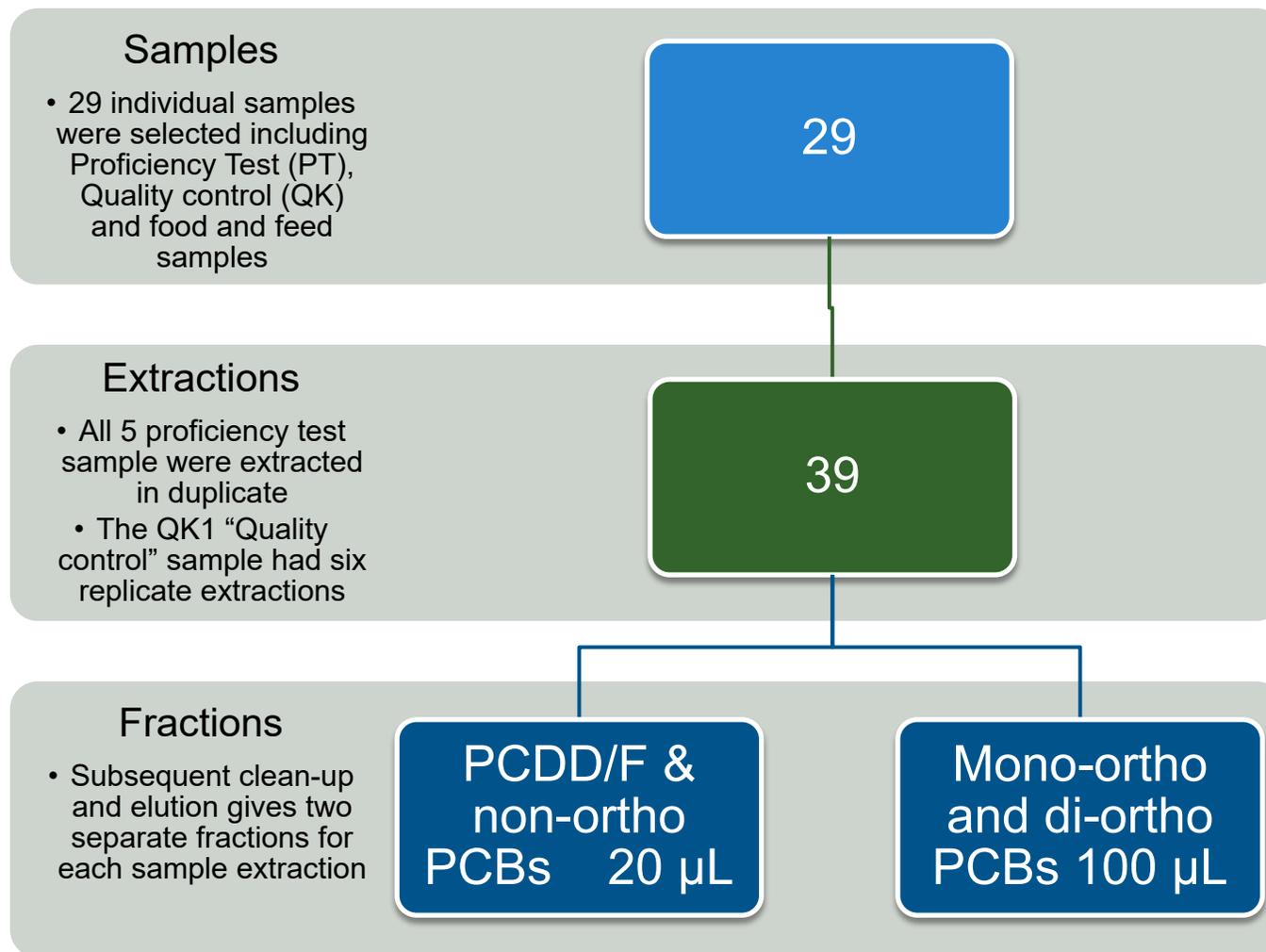
- Food and feedstuff samples (including PT samples) were provided by the EURL for Halogenated POPs in Feed and Food, Freiburg, Germany. A nominal sample intake weight of 2 grams (fat) was used for the samples unless indicated otherwise
- European method EN:1948 standard solutions were utilised for the extraction, calibration and quantitation of PCDD/Fs, dioxin-like PCBs and indicator PCBs.
 - PCDD/Fs
 - EN-1948CVS (calibration and quantitation)
 - EN-1948ES, EN-1948IS (extraction)
 - PCBs
 - WM48-CVS (calibration and quantitation),
 - P48-W-ES, P48-M-ES and P48-RS (extraction)
- All standards were obtained from Wellington Laboratories Inc., Canada.

Sample type	Matrix	Nominal weight taken (g)	Number of replicates	Basis
PT	Pork sausage	2	2	Fat
PT	Whole egg	2	2	Fat
PT	Milk powder	2	2	Fat
PT	Halibut fillet	2	2	Fat
PT	Sugar beet pulp	20	2	Product
QK1	Mixed fat	2	6	Fat
Food	Meat	2	5 (individual)	Fat
Food	Milk	2	4 (individual)	Fat
Food	Fish	2	4 (individual)	Fat
Food	Eggs	2	5 (individual)	Fat
Feed	Fish meal	2	1	Fat
Feed	Grass meal	20	1	Product
Feed	Sepiolite	20	1	Product
Feed	Palm fatty acid distillate (PFAD)	2	1	Product
Feed	Feed fat	2	1	Fat



Samples

- Sample extraction was performed by Twisselmann hot extraction (comparable to Soxhlet) or pressurized liquid extraction.
- Automated clean-up of extracts was performed using a three column (multi-layered acidic silica, alumina, and carbon columns) setup on the DEXTech™ Plus system (LCTech GmbH).



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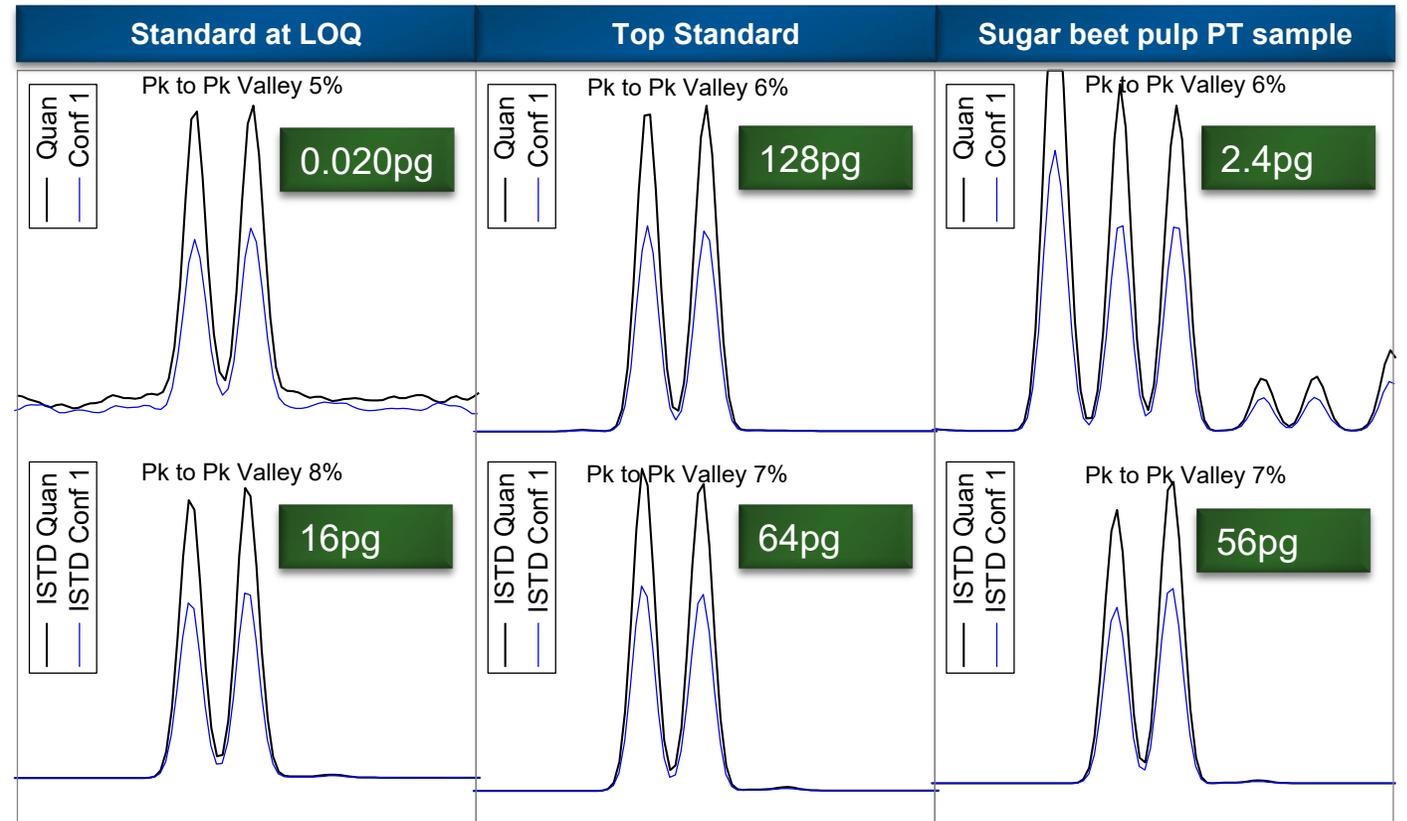


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Chromatography

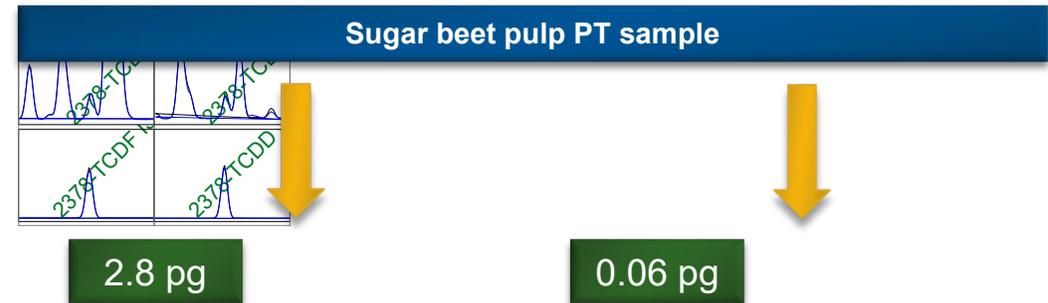
EU regulations specify that the separation of the 1,2,3,4,7,8 and 1,2,3,6,7,8 HxCDF isomers shall be less than 25% peak to peak.

Separation of isomers shall be < 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF





- The proprietary phase of the TG-Dioxin capillary GC column provides excellent separation of PCDD/F and PCB congeners, particularly the tetra and penta-substituted PCDD/Fs



Sample details – Sugar beet pulp proficiency test 20 g sample intake

- Establishing LOQs in the analysis of PCDD/Fs and PCBs in food and feed is critical.
- Differences in approaches can lead to significant variations in quoted upperbound WHO-PCDD/F-TEQ results, especially where toxic congeners are present at levels close to the LOQ.
- In these experiments, European Union Reference Laboratories guidance was followed and a calibration based approach was used.

$$Sample\ LOQ\ (pg/g) = \sum_{n=PCDD/F}^{17} Min\ Conc_n\ (pg/\mu L) * \left(\frac{Sample\ volume(\mu L)}{Sample\ weight(g) * Recovery\ l\ (\%)} \right)$$

- To demonstrate the sensitivity required to routinely achieve the LOQs applied, a low level standard, at the LOQ, was included in the calibration curve and also injected at regular intervals throughout the batch.

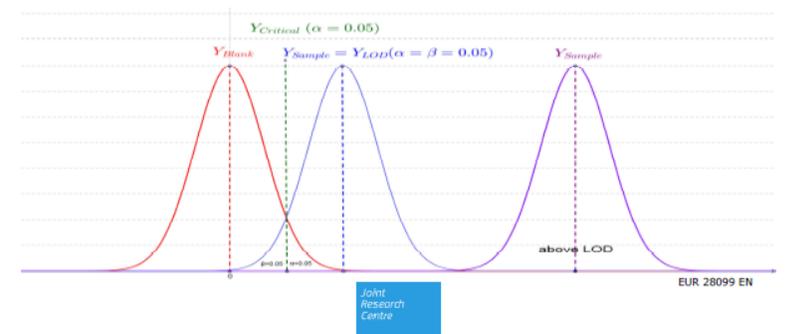


JRC TECHNICAL REPORTS

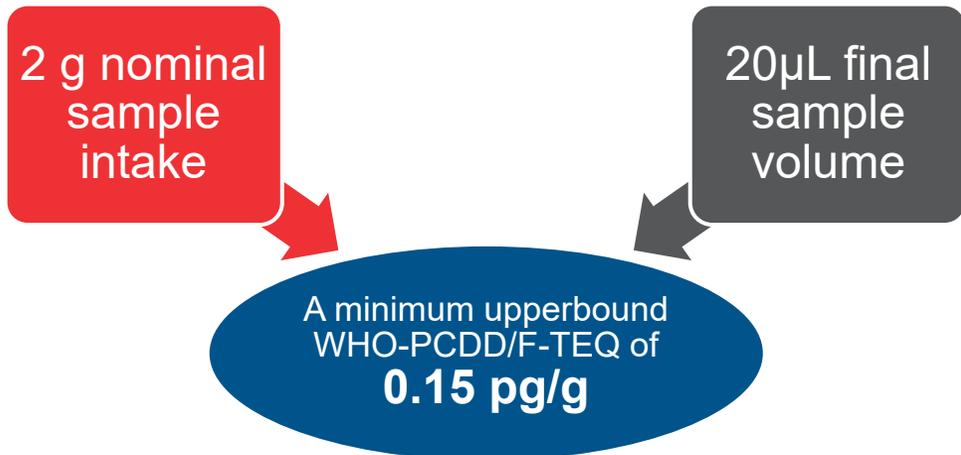
Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food

Thomas Wenzl, Johannes Haedrich, Alexander Schaechtele, Piotr Robouch, Joerg Stroka

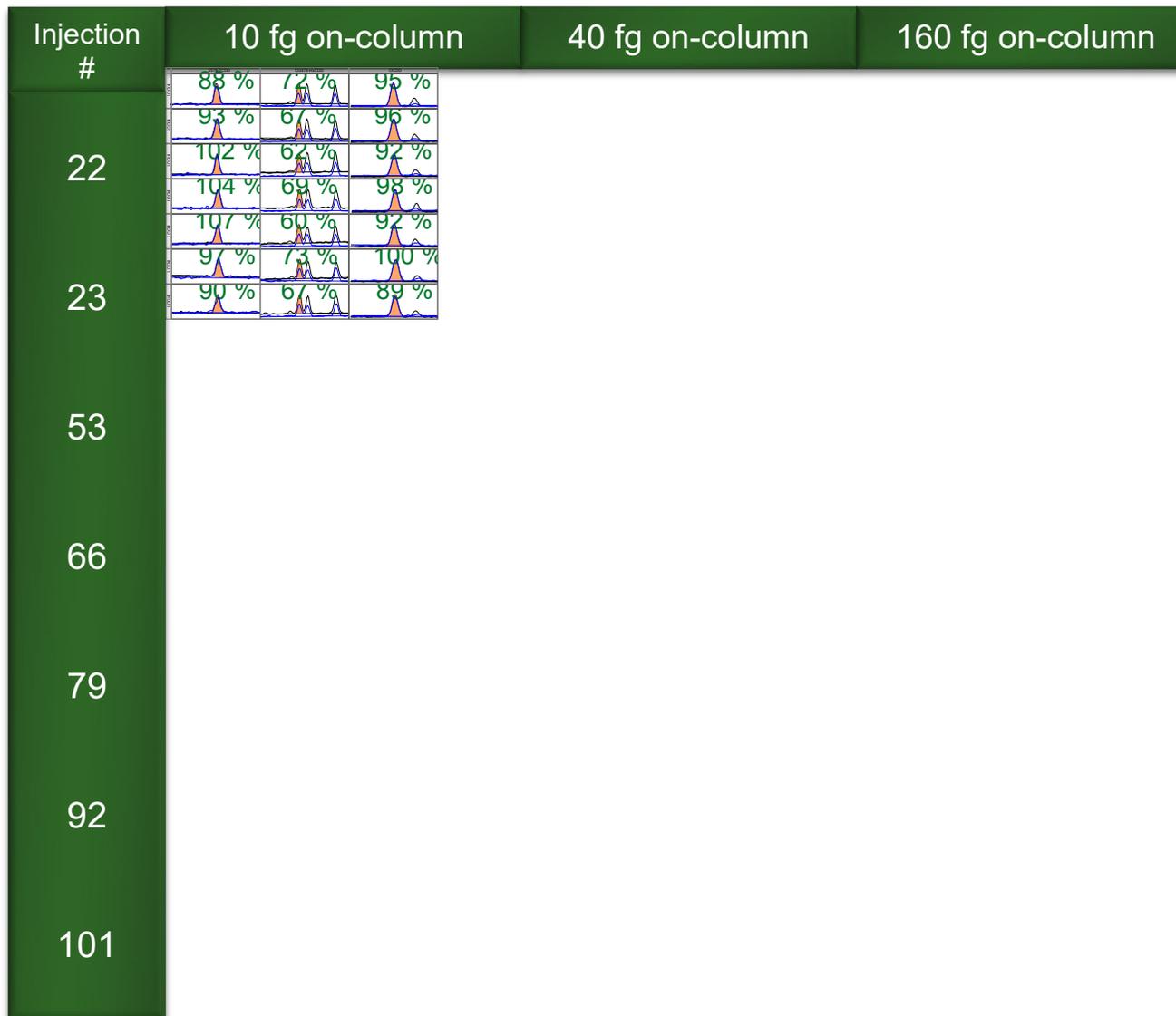
2016



- Injection of the lowest level calibration point and regular assessment of this ensures:



Criteria	Acceptance	Result
Retention time window (for all monitored ions)	'within window' ± 0.1 min	Pass
Relative ion intensities	± 15 %	Pass
Deviation from the average relative response factor	≤ 30 %	Pass



LOQ data...

- As displayed, the deviation from the Response Factor and Ion Ratio are within tolerance for all LOQ injections...

Peak Name	Ret. Time min	Rel. Amnt. Dev. %							IR deviation %						
		LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	
2378-TCDF	20.313	-1.8	11.6	-6.4	11.1	3.3	25.4	-10.8	5.5	-8.5	9.4	-8.4	-7.8	-10.1	3.7
2378-TCDD	20.871	14.0	-5.3	3.6	-7.7	-7.2	12.9	15.3	-6.8	-1.3	7.4	10.2	13.3	2.4	-4.6
12378-PeCDF	24.347	2.3	1.1	1.1	1.1	1.1	1.1	1.1	0.7	0.7	0.7	0.7	0.7	0.7	13.1
23478-PeCDF	25.720	6.1	10.8	8.9	14.1	3.3	7.0	2.6	0.2	-1.9	-5.0	-12.9	0.9	-1.9	-2.8
12378-PeCDD	25.970	4.2	-5.5	5.4	9.9	-6.6	-4.9	4.1	-6.4	7.9	8.9	9.5	13.4	10.1	4.4
123478-HxCDF	29.069	-1.5	5.4	0.3	4.8	8.6	10.4	1.5	-2.9	-5.1	-2.1	-0.8	-0.4	-4.9	7.2
123678-HxCDF	29.180	4.6	-1.3	10.9	13.8	4.7	2.7	9.4	-0.7	6.9	1.3	-7.5	0.4	7.1	-14.8
234678-HxCDF	29.859	-0.7	8.2	0.6	1.1	-1.1	1.3	-0.8	2.4	-5.6	10.6	9.9	3.4	10.0	14.1
123478-HxCDD	29.951	-5.3	3.2	-2.9	5.3	1.8	-1.6	3.1	12.3	5.1	2.4	8.7	-5.7	14.3	5.3
123678-HxCDD	30.039	-1.8	3.7	8.0	11.9	-11.3	-5.8	-5.5	2.9	1.7	-5.8	-4.4	-3.2	3.6	-5.7
123789-HxCDD	30.362	-6.6	10.0	-1.7	17.3	-2.0	3.7	23.3	13.0	9.6	6.9	0.6	6.9	10.7	-10.2
123789-HxCDF	30.725	2.8	7.0	16.7	9.9	10.7	25.8	10.4	-5.9	6.6	-3.5	13.6	-6.8	-7.4	6.1
1234678-HpCDF	32.364	4.1	2.0	4.3	1.0	1.2	3.3	6.5	-0.8	-0.3	-0.1	2.5	-3.0	3.6	-4.5
1234678-HpCDD	33.793	7.0	5.1	1.1	1.1	1.1	1.1	18.1	-3.7	0.7	0.7	0.7	0.7	0.7	-9.3
1234789-HpCDF	34.531	-1.9	-0.3	6.8	3.0	2.6	8.2	9.6	6.8	-0.6	-1.8	1.4	0.3	-0.8	-5.2
OCDD	38.385	0.5	2.5	1.9	3.2	4.7	2.5	5.7	-1.5	0.4	-3.9	1.6	-4.6	4.1	-7.0
OCDF	38.635	0.9	1.6	0.0	2.3	2.7	1.0	4.2	-0.3	0.0	1.5	-2.3	2.1	-3.4	-6.8

≤ 30 %

≤ 15 %

Peak Name	Ret. Time min	Average Peak Amount fg	Stdev Peak Amount fg	RSD Peak Amount %	LOQ amount fg	MDL fg	LOQ 10*StdDev fg	LOD 3*StdDev fg	S/N	LOQ 10/S:N fg	LOD 3/S:N fg
First Injection	First Injection										
2378-TCDF	20.313	10.5	1.2	10.1 %	10.0	3.2	12.4	3.7	37.0	2.7	0.8
2378-TCDD	20.871	10.4	1.0	10.1 %	10.0	3.2	10.5	3.1	25.7	3.9	1.2
12378-PeCDF	24.347	20.7	0.7	3.3 %	20.0	2.1	6.9	2.1	78.2	2.6	0.8
23478-PeCDF	25.720	21.5	0.8	3.8 %	20.0	2.4	8.2	2.5	42.6	4.7	1.4
12378-PeCDD	25.970	20.2	1.3	6.4 %	20.0	4.1	13.0	3.9	22.1	9.0	2.7
123478-HxCDF	29.069	20.8	0.9	4.2 %	20.0	2.6	8.7	2.6	38.9	5.1	1.5
123678-HxCDF	29.180	21.3	1.0	4.9 %	20.0	3.1	10.4	3.1	72.7	2.8	0.8
234678-HxCDF	29.859	20.5	1.0	5.0 %	20.0	3.2	10.3	3.1	48.9	4.1	1.2
123478-HxCDD	29.951	40.7	1.6	3.9 %	40.0	4.9	16.0	4.8	35.2	11.4	3.4
123678-HxCDD	30.039	39.9	3.3	8.3 %	40.0	10.4	33.2	10.0	33.4	12.0	3.6
123789-HxCDD	30.362	42.5	4.4	10.4 %	40.0	13.1	44.2	13.2	26.0	15.4	4.6
123789-HxCDF	30.725	22.4	1.5	6.6 %	20.0	4.2	14.8	4.4	32.0	6.2	1.9
1234678-HpCDF	32.364	41.1	1.1	2.7 %	40.0	3.4	11.2	3.4	72.1	5.6	1.7
1234678-HpCDD	33.793	43.8	2.2	5.1 %	40.0	6.4	22.3	6.7	93.0	4.3	1.3
1234789-HpCDF	34.531	41.6	1.7	4.2 %	40.0	5.2	17.3	5.2	58.7	6.8	2.0
OCDD	38.385	164.8	2.8	1.7 %	160.0	8.4	27.7	8.3	109.4	14.6	4.4
OCDF	38.635	162.9	2.2	1.3 %	160.0	6.8	21.9	6.6	124.8	12.8	3.8

- What about concentrations below the LOQ standard?

Peak Name	Ret.Time min	Rel.Amnt.Dev. %								IR deviation %							
		LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	LOQ/4	
2378-TCDF	20.313	-1.8	11.6	-6.4	11.1	3.3	25.4	-10.8	5.5	-8.5	9.4	-8.4	-7.8	-10.1	3.7		
2378-TCDD	20.871	14.0	-5.3	3.6	-7.7	-7.2	12.9	15.3	-6.8	-1.3	7.4	10.2	13.3	2.4	4.6		
12378-PeCDF	24.347	2.3	1.1	1.4	1.1	1.1	19.2	1.1	0.7	5.1	5.5	3.0	5.9	5.9	3.1		
23478-PeCDF	25.720	2.1	0.9	1.1	1.1	1.1	2.6	2.6	0.2	2.9	-1.1	12.5	0.9	4.9	2.8		
12378-PeCDD	25.970	4.2	-5.5	5.4	9.9	-6.6	-4.9	4.1	-6.4	7.9	8.9	9.5	13.4	10.1	4.4		
123478-HxCDF	29.069	0.9	0.9	0.9	4.9	4.9	10.9	10.9	0.9	4.1	4.1	4.1	4.1	4.1	7.2		
123678-HxCDF	29.180	4.6	-1.3	10.9	13.8	4.7	2.7	9.4	-0.7	6.9	1.3	-7.5	0.4	7.1	4.8		
234678-HxCDF	29.859	-0.7	8.2	0.6	11.1	-2.7	1.3	-0.8	2.4	-5.6	12.6	1.9	3.4	10.9	4.1		
123478-HxCDD	29.951	3.4	3.2	9.1	9.1	9.1	9.1	9.1	1.1	1.1	1.1	1.1	1.1	1.1	5.3		
123678-HxCDD	30.039	-1.8	3.7	8.0	11.9	-11.3	-5.8	-5.5	2.9	1.7	-5.8	-4.4	-3.2	3.6	5.7		
123789-HxCDD	30.362	-6.6	11.7	1.7	11.7	2.0	2.7	2.7	8.5	8.5	6.9	0.6	6.9	10.7	10.2		
123789-HxCDF	30.725	2.8	7.5	16.7	9.5	10.7	25.5	10.4	3.5	6.5	3.5	13.6	-6.8	-7.4	6.1		
123478-HpCDF	32.364	4.1	-2.0	4.3	1.0	1.2	3.3	6.5	-0.8	-0.3	-0.1	2.5	-3.0	3.6	4.5		
1234678-HpCDD	33.793	7.0	3.6	12.5	14.3	7.3	3.5	18.1	-3.7	-1.3	-6.3	-1.8	0.3	2.1	9.3		
1234789-HpCDF	34.531	-1.9	-0.3	6.8	3.0	2.6	8.2	9.6	6.8	-0.6	-1.8	1.4	0.3	-0.8	5.2		
OCDD	38.385	2.8	2.8	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	7.0		
OCDF	38.635	0.9	1.6	0.0	2.3	2.7	1.0	4.2	-0.3	0.0	1.5	-2.3	2.1	-3.4	-6.8		

Although the calculated MDL and S:N LOQ and S:N LOD are interesting, the ion ratios would not be in tolerance at these low levels, and therefore are not practically usable in the routine for confirmation.

Peak Name	Ret.Time min	Average Peak Amount fg	Stdev Peak Amount fg	RSD Peak Amount %	LOQ amount fg	MDL fg	LOQ 10*StdDev fg	LOD 3*StdDev fg	S/N	LOQ 10/S:N fg	LOD 3/S:N fg
2378-TCDF	20.313	10.5	1.2	10.1 %	10.0	3.2	12.4	3.7	37.0	2.7	0.8
2378-TCDD	20.871	10.4	1.0	10.1 %	10.0	3.2	10.5	3.1	25.7	3.9	1.2
12378-PeCDF	24.347	20.7	0.7	3.3 %	20.0	2.1	6.9	2.1	78.2	2.6	0.8
23478-PeCDF	25.720	21.5	0.8	3.8 %	20.0	2.4	8.2	2.5	42.6	4.7	1.4
12378-PeCDD	25.970	20.2	1.3	6.4 %	20.0	4.1	13.0	3.9	22.1	9.0	2.7
123478-HxCDF	29.069	20.8	0.9	4.2 %	20.0	2.6	8.7	2.6	38.9	5.1	1.5
123678-HxCDF	29.180	21.3	1.0	4.9 %	20.0	3.1	10.4	3.1	72.7	2.8	0.8
234678-HxCDF	29.859	20.5	1.0	5.0 %	20.0	3.2	10.3	3.1	48.9	4.1	1.2
123478-HxCDD	29.951	40.7	1.6	3.9 %	40.0	4.9	16.0	4.8	35.2	11.4	3.4
123678-HxCDD	30.039	39.9	3.3	8.3 %	40.0	10.4	33.2	10.0	33.4	12.0	3.6
123789-HxCDD	30.362	42.5	4.4	10.4 %	40.0	13.1	44.2	13.2	26.0	15.4	4.6
123789-HxCDF	30.725	22.4	1.5	6.6 %	20.0	4.2	14.8	4.4	32.0	6.2	1.9
1234678-HpCDF	32.364	41.1	1.1	2.7 %	40.0	3.4	11.2	3.4	72.1	5.6	1.7
1234678-HpCDD	33.793	43.8	2.2	5.1 %	40.0	6.4	22.3	6.7	93.0	4.3	1.3
1234789-HpCDF	34.531	41.6	1.7	4.2 %	40.0	5.2	17.3	5.2	58.7	6.8	2.0
OCDD	38.385	164.8	2.8	1.7 %	160.0	8.4	27.7	8.3	109.4	14.6	4.4
OCDF	38.635	162.9	2.2	1.3 %	160.0	6.8	21.9	6.6	124.8	12.8	3.8

Calibration range

Peak Name	Ret.Time (min)	Number of Points	RF RSD (%)	Coeff.of Determination (R2)	Average RF (Slope)	Range (pg)
2378-TCDF	20.30	16	3.87	0.9995	0.96	0.01 – 64
2378-TCDD	20.86	16	4.72	0.9996	1.04	0.01 – 64
12378-PeCDF	24.34	16	1.66	0.9999	0.93	0.02 – 128
23478-PeCDF	25.71	16	5.36	0.9977	1.03	0.02 – 128
12378-PeCDD	25.96	16	3.60	0.9999	1.05	0.02 – 128
123478-HxCDF	29.06	16	2.98	0.9996	1.02	0.02 – 128
123678-HxCDF	29.17	16	1.95	0.9998	1.00	0.02 – 128
234678-HxCDF	29.86	16	2.83	0.9993	1.02	0.02 – 128
123478-HxCDD	29.94	16	2.49	0.999	1.12	0.04 – 128
123678-HxCDD	30.04	16	2.01	0.9991	1.12	0.04 – 128
123789-HxCDD	30.35	16	3.82	0.9987	1.09	0.04 – 128
123789-HxCDF	30.71	16	3.52	0.9997	0.95	0.02 – 128
1234678-HpCDF	32.35	16	1.78	0.9999	1.03	0.04 – 256
1234678-HpCDD	33.78	16	5.99	0.9968	1.09	0.04 – 256
1234789-HpCDF	34.52	16	1.88	0.9998	1.04	0.04 – 256
OCDD	38.39	16	1.64	1.0000	1.12	0.16 – 256
OCDF	38.64	16	1.34	0.9997	0.94	0.16 – 256
		Max	5.99	1.0000		
		Min	1.34	0.9968		

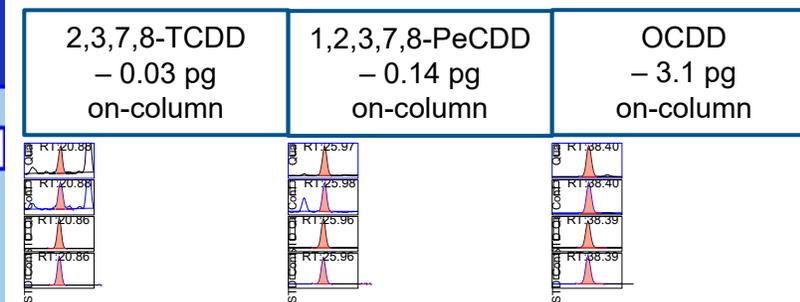
Peak Name	Ret.Time (min)	Number of Points	RF RSD (%)	Coeff.of Determination (R2)	Average RF (Slope)	Range (pg)
PCB 28	11.91	14	2.23	0.9989	1.01	0.1 – 1000
PCB 52	12.49	14	1.81	0.9993	1.06	0.1 – 1000
PCB 101	14.89	14	1.00	0.9999	1.02	0.1 – 1000
PCB 81	16.38	14	1.49	0.9997	1.06	0.04 – 160
PCB 77	16.86	14	1.08	0.9997	1.00	0.04 – 160
PCB 123	17.40	14	2.66	0.9998	0.92	0.02 – 200
PCB 123	17.40	14	2.66	0.9998	0.92	0.02 – 200
PCB 118	17.64	14	1.46	0.9999	0.96	0.1 – 1000
PCB 118	17.64	14	1.46	0.9999	0.96	0.1 – 1000
PCB 114	18.18	14	3.02	0.9989	1.04	0.02 – 200
PCB 114	18.18	14	3.02	0.9989	1.04	0.02 – 200
PCB 153	18.37	14	3.31	0.9996	1.12	0.1 – 1000
PCB 105	18.96	14	5.95	0.9947	0.96	0.02 – 200
PCB 105	18.96	14	5.95	0.9947	0.96	0.02 – 200
PCB 138	19.80	14	1.95	0.9986	1.08	0.1 – 1000
PCB 126	20.90	14	5.69	0.9985	0.95	0.04 – 160
PCB 167	21.52	14	1.74	0.9998	1.15	0.02 – 200
PCB 167	21.52	14	1.74	0.9998	1.15	0.02 – 200
PCB 156	22.90	14	1.98	0.9998	1.14	0.02 – 200
PCB 156	22.91	14	1.97	0.9998	1.14	0.02 – 200
PCB 157	23.12	14	2.41	0.9999	1.11	0.02 – 200
PCB 157	23.12	14	2.41	0.9999	1.11	0.02 – 200
PCB 180	23.43	14	2.33	0.9997	1.03	0.1 – 1000
PCB 169	25.48	14	4.00	0.9999	1.08	0.04 – 160
PCB 189	27.28	14	1.96	0.9989	0.99	0.02 – 200
PCB 189	27.28	14	1.96	0.9989	0.99	0.02 – 200
		Max	5.95	0.9999		
		Min	1.00	0.9947		

- Calibration range of over 5 orders for PCBs
 - All RF RSD <6 % for native congeners
- Duplicate injection per level (8 levels for PCDD/Fs, 7 levels for PCBs)

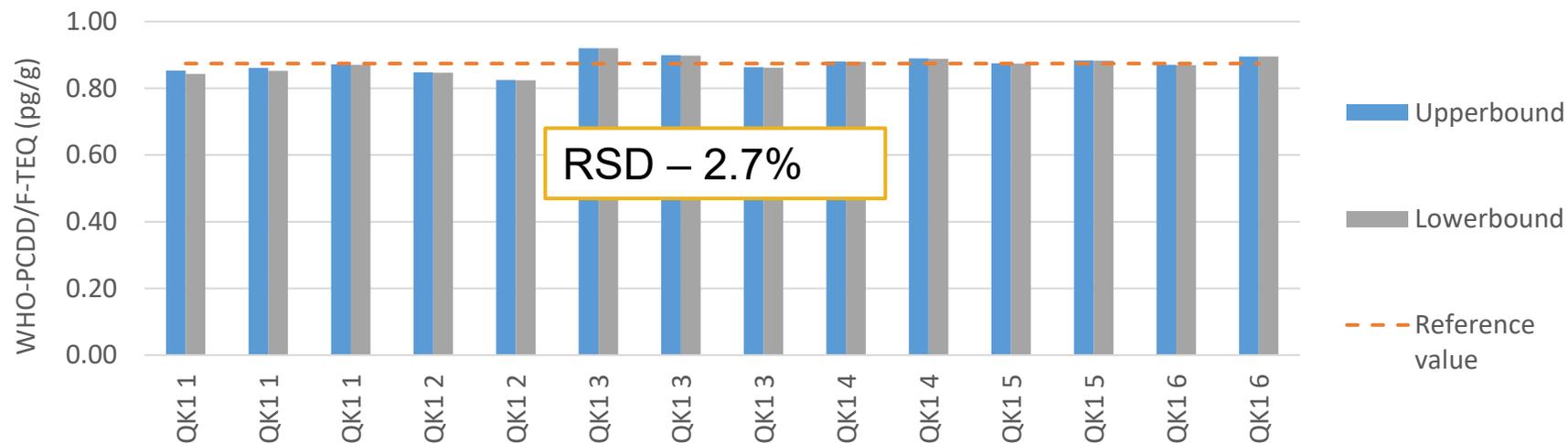
Results – quality control

- Six replicate extractions of a mixed fat quality control sample
 - **QK1** – reference value: 0.87 pg sum WHO-PCDD/F-TEQ
 - split between the two sites and analysed at regular intervals throughout the analytical sequences

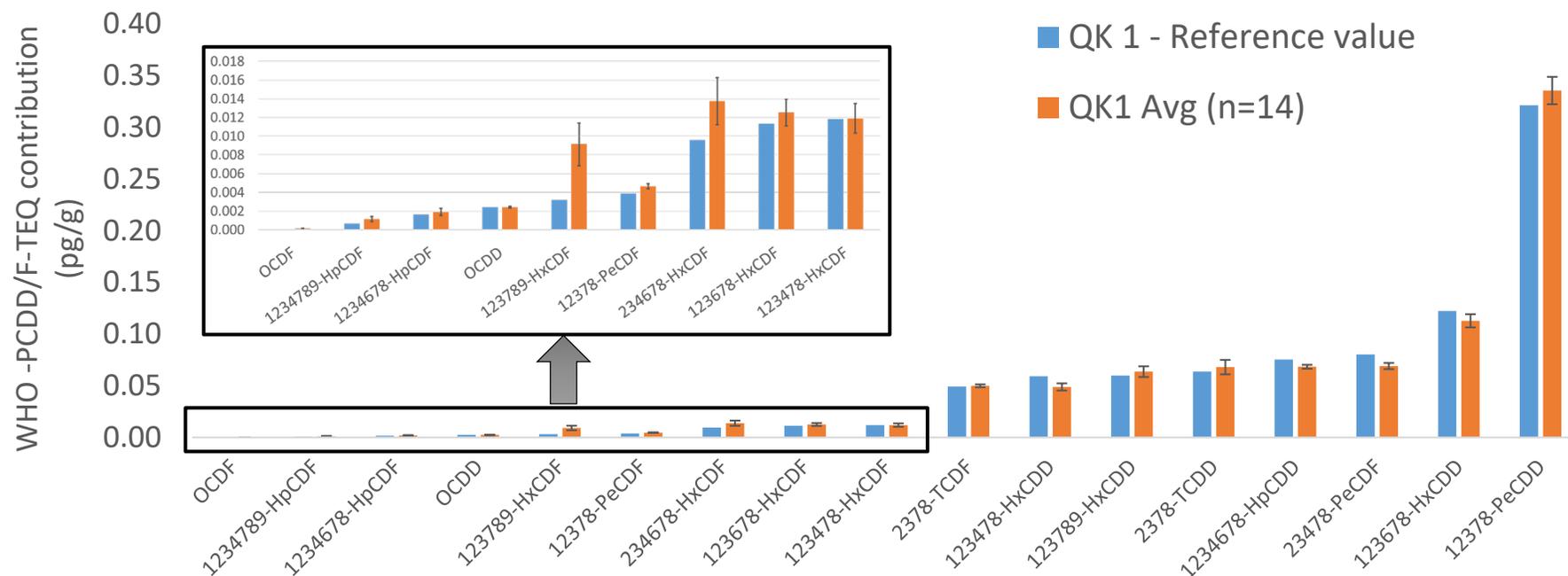
Peak Name	RT min	ISTD recovery %	Target IR %	Actual IR %	IR dev %	Result <LOQ ?	UppBnd	MidBnd	LowBnd	SUM UB pg/g	ML (TEQ pg/g) 1.5
2378-TCDF	20.31	79.2	98.4	96.4	-2.1		0.0493	0.0493	0.0493		
2378-TCDD	20.88	89.2	94.7	105.3	11.1		0.0683	0.0683	0.0683		
12378-PeCDF	24.35	94.2	78.3	81.9	4.6		0.0041	0.0041	0.0041		
23478-PeCDF	25.72	94.8	77.9	78.6	0.8		0.0686	0.0686	0.0686		
12378-PeCDD	25.97	99.7	78.0	75.5	-3.3		0.3410	0.3410	0.3410		
123478-HxCDF	29.07	81.2	63.0	55.9	-11.3		0.0113	0.0113	0.0113		
123678-HxCDF	29.18	87.3	63.0	59.9	-4.8		0.0113	0.0113	0.0113		
234678-HxCDF	29.86	76.8	62.6	68.9	10.0		0.0112	0.0112	0.0112		
123478-HxCDD	29.94	88.4	63.9	65.6	2.6		0.0477	0.0477	0.0477		
123678-HxCDD	30.04	96.0	64.9	65.6	1.0		0.1020	0.1020	0.1020		
123789-HxCDD	30.36	96.0	64.2	65.9	2.6		0.0557	0.0557	0.0557		
123789-HxCDF	30.71	71.2	63.6	74.8	17.7	<LOQ	0.0071	0.0035	0.0000		
1234678-HpCDF	32.36	60.7	80.4	85.3	6.1	<LOQ	0.0017	0.0008	0.0000		
1234678-HpCDD	33.79	94.2	80.7	80.1	-0.7		0.0688	0.0688	0.0688		
1234789-HpCDF	34.52	81.8	79.9	78.9	-1.2	<LOQ	0.0012	0.0006	0.0000		
OCDD	38.40	91.3	96.0	95.0	-1.1		0.0023	0.0023	0.0023		
OCDF	38.67	83.0	94.1	102.5	9.0	<LOQ	0.0001	0.0001	0.0000	0.8518	Below ML



Quality control - precision



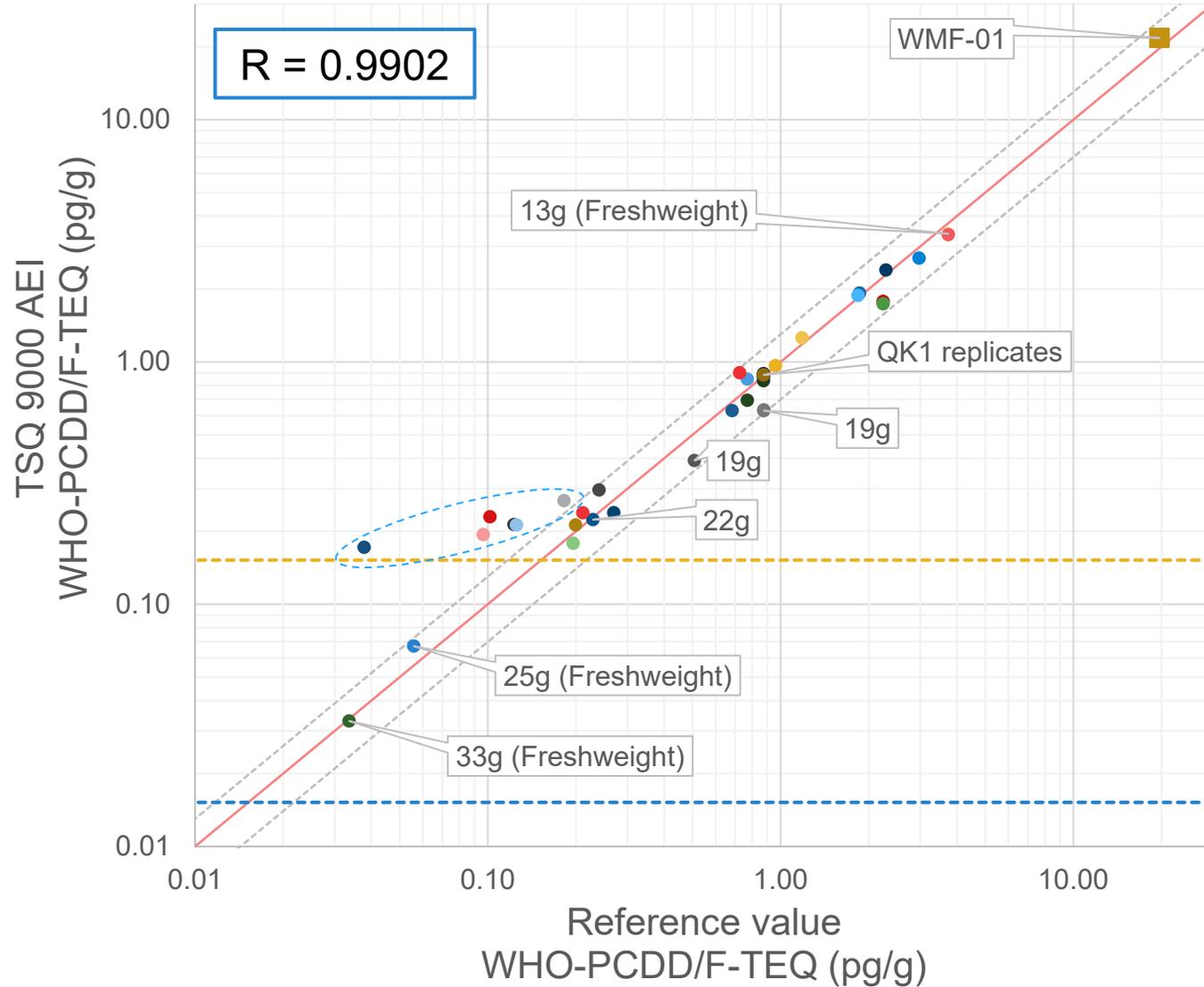
- Replicate analysis of the QK 1 sample over the two sequences showed excellent repeatability and sensitivity.



- Deviation between the upper and lowerbound was no more than 1.2% (for confirmation of exceeding the ML, less than 20% deviation is required)

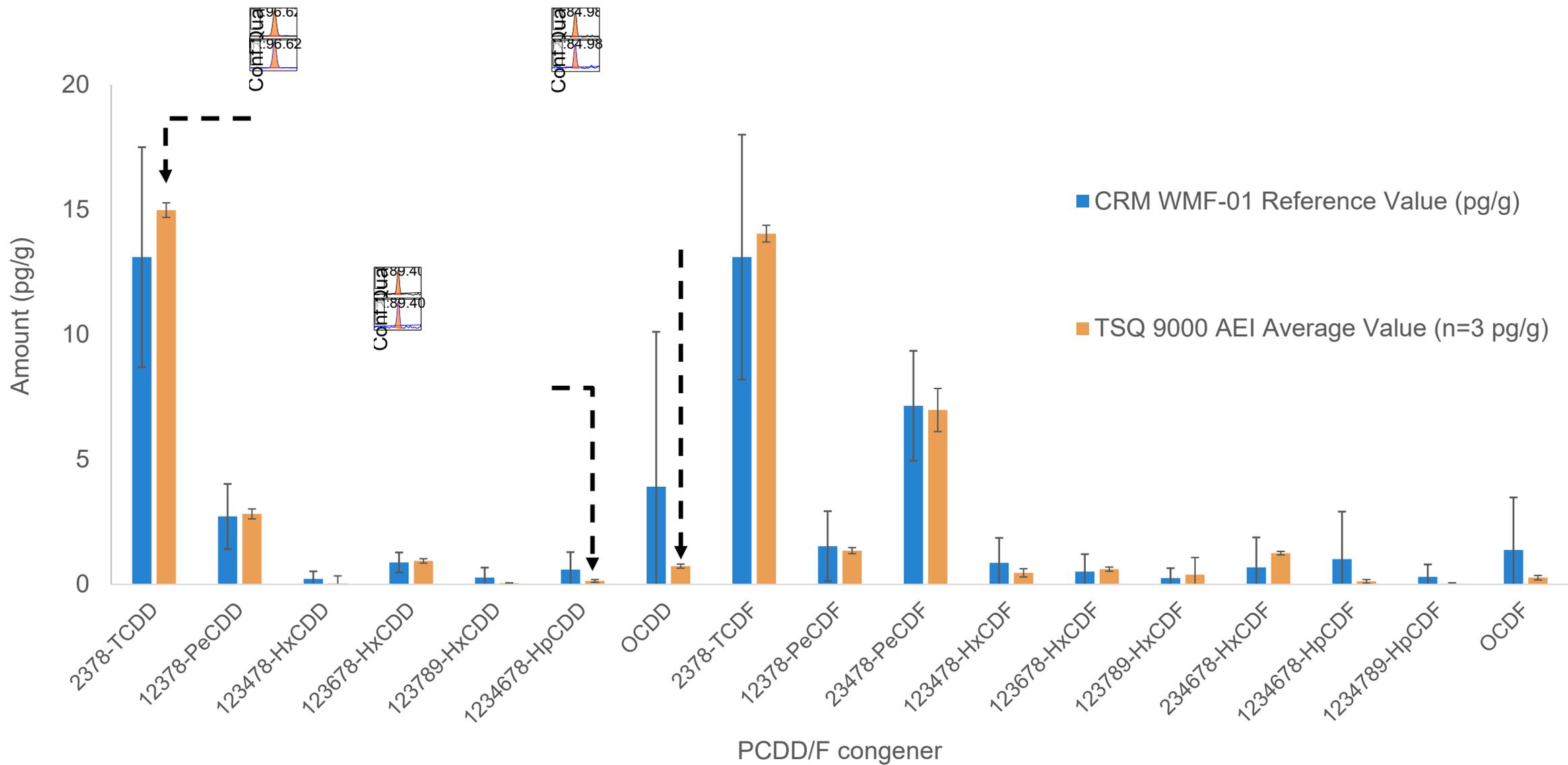
Sample analysis – PCDD/Fs

PCDD/Fs GC-QqQ vs Reference value



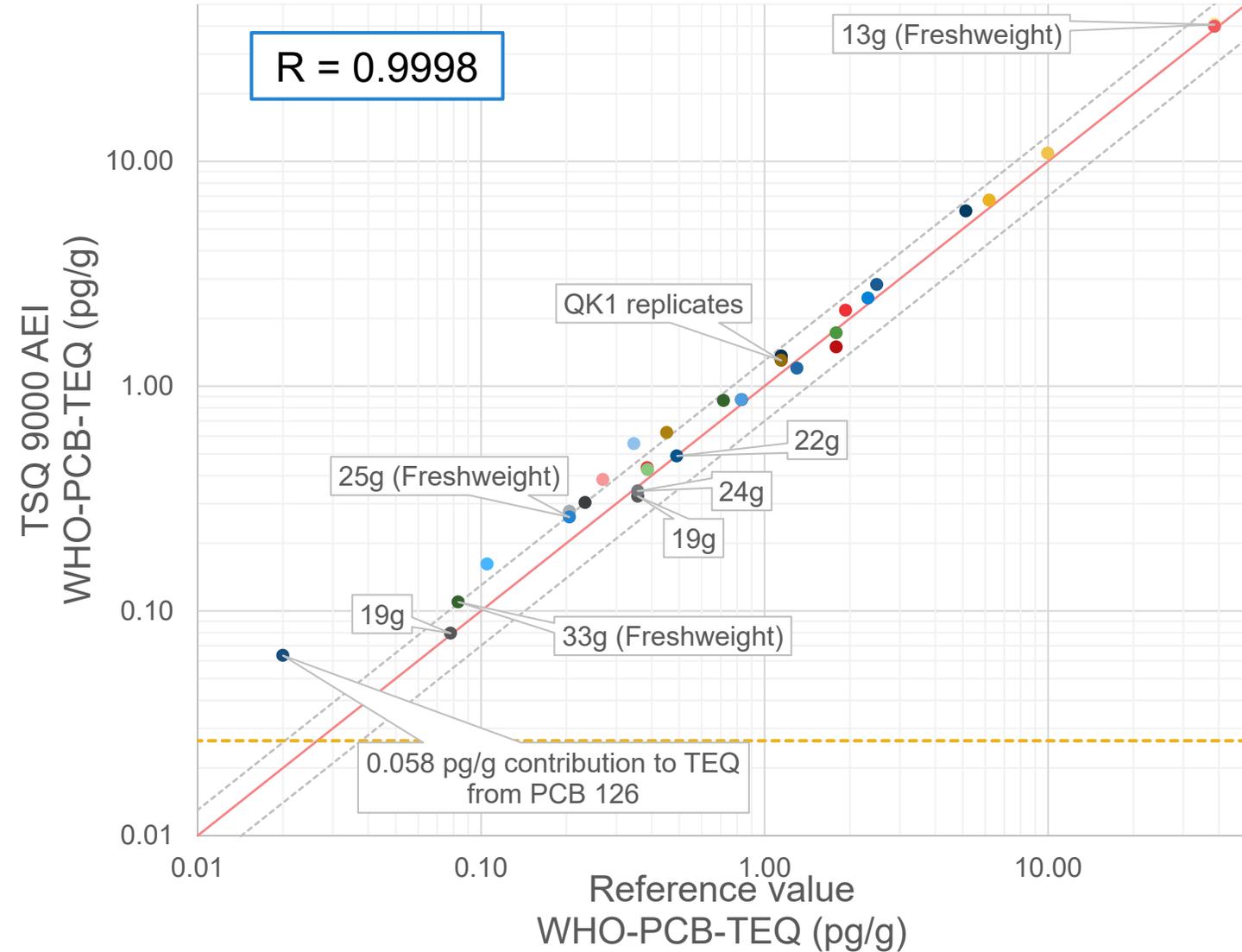
- QK1 - 1 Mixed fat
 - QK1 - 2 Mixed fat
 - QK1 - 3 Mixed fat
 - QK1 - 4 Mixed fat
 - QK1 - 5 Mixed fat
 - QK1 - 6 Mixed fat
 - QK3 Eggs
 - QK5 Fish meal
 - QK6 Fish oil
 - QK7 Fish oil
 - QK8 Meat
 - QK9 Grass meal
 - 1201-PLA 1 Pork sausage
 - 1201-PLA 2 Pork sausage
 - 1202-HEA 1 Whole egg
 - 1302-MIA 1 Milk powder
 - 1302-MIA 2 Milk powder
 - 1501-AFB 1 Sugar beet pulp
 - 1501-AFB 2 Sugar beet pulp
 - 1601-HFA 1 Fish
 - 1601-HFA 2 Fish
 - 1401-SEA Sepiolite
 - 1701-PFA PFAD
 - 1301-FF Feed fat
 - 1302-MIB Milk fat
 - WMF-01
 - 9230 Meat
 - 9253 Meat
 - 9449 Meat
 - 9255 Meat
 - 9367 Milk
 - 9373 Milk
 - 9478 Milk
 - 9182 Eggs
 - 9370 Eggs
 - 9371 Eggs
 - 9487 Fish
 - 9488 Fish
- 2g AEI LOQ (UB)
- 20g AEI LOQ (UB)

WMF-01 replicate analysis



Sample analysis – dl-PCBs

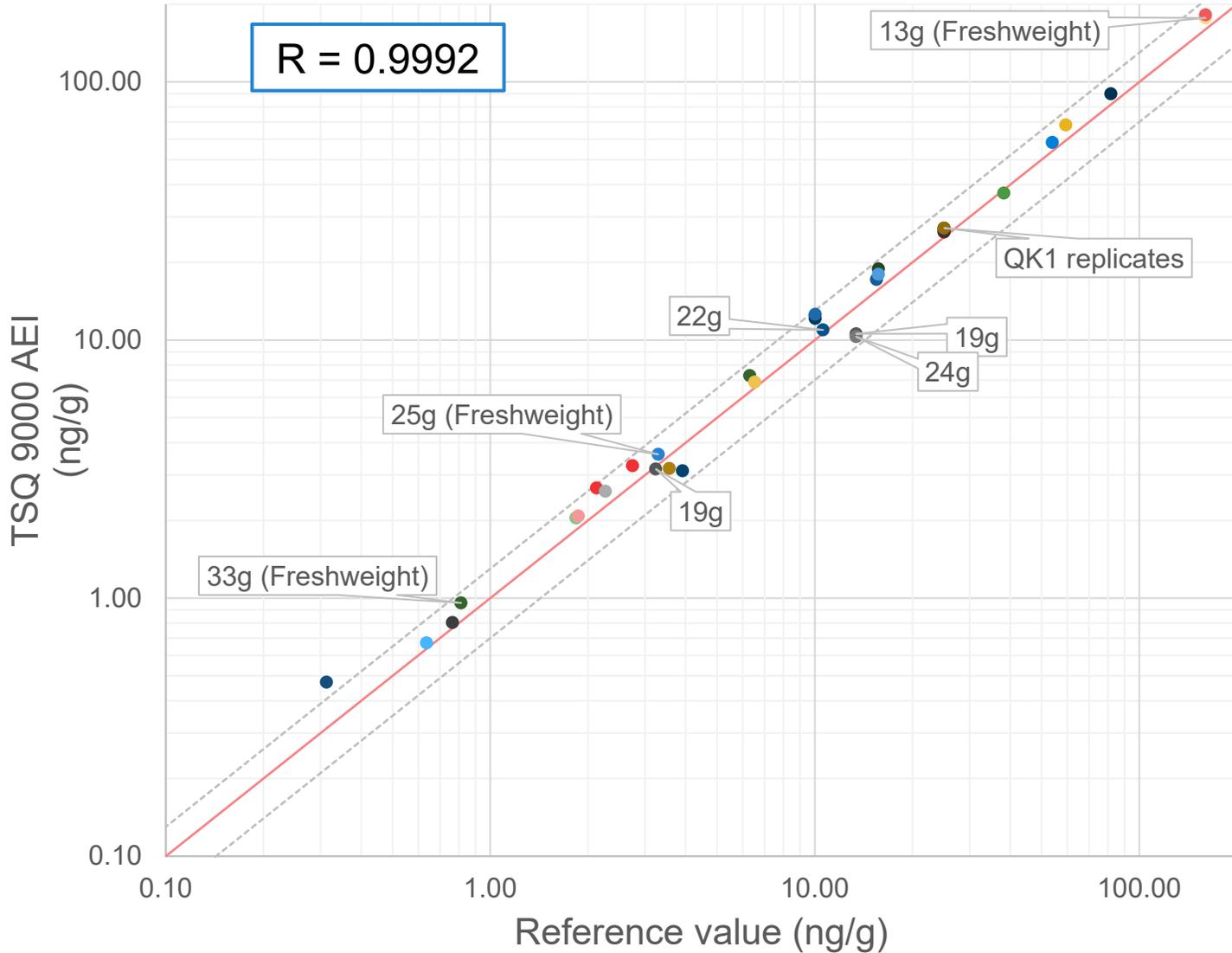
dl-PCBs - TSQ 9000 AEI vs Reference value



- QK1 - 1 Mixed fat
- QK1 - 2 Mixed fat
- QK1 - 3 Mixed fat
- QK1 - 4 Mixed fat
- QK1 - 5 Mixed fat
- QK1 - 6 Mixed fat
- QK3 Eggs
- QK5 Fish meal
- QK7 Fish oil
- QK6 Fish oil
- QK8 Meat
- QK9 Grass meal
- 1201-PLA 1 Pork sausage
- 1201-PLA 2 Pork sausage
- 1202-HEA 1 Whole egg
- 1302-MIA 1 Milk powder
- 1302-MIA 2 Milk powder
- 1501-AFB 1 Sugar beet pulp
- 1501-AFB 2 Sugar beet pulp
- 1601-HFA 1 Fish
- 1601-HFA 2 Fish
- 1401-SEA Sepiolite
- 1701-PFA PFAD
- 1301-FF Feed fat
- 1302-MIB Milk fat
- 9230 Meat
- 9449 Meat
- 9255 Meat
- 9367 Milk
- 9373 Milk
- 9182 Eggs
- 9370 Eggs
- 9371 Eggs
- 9487 Fish
- 9488 Fish
- 2g AEI LOQ (UB)

Sample analysis – Indicator PCBs

Indicator PCBs GC-QqQ vs Reference values

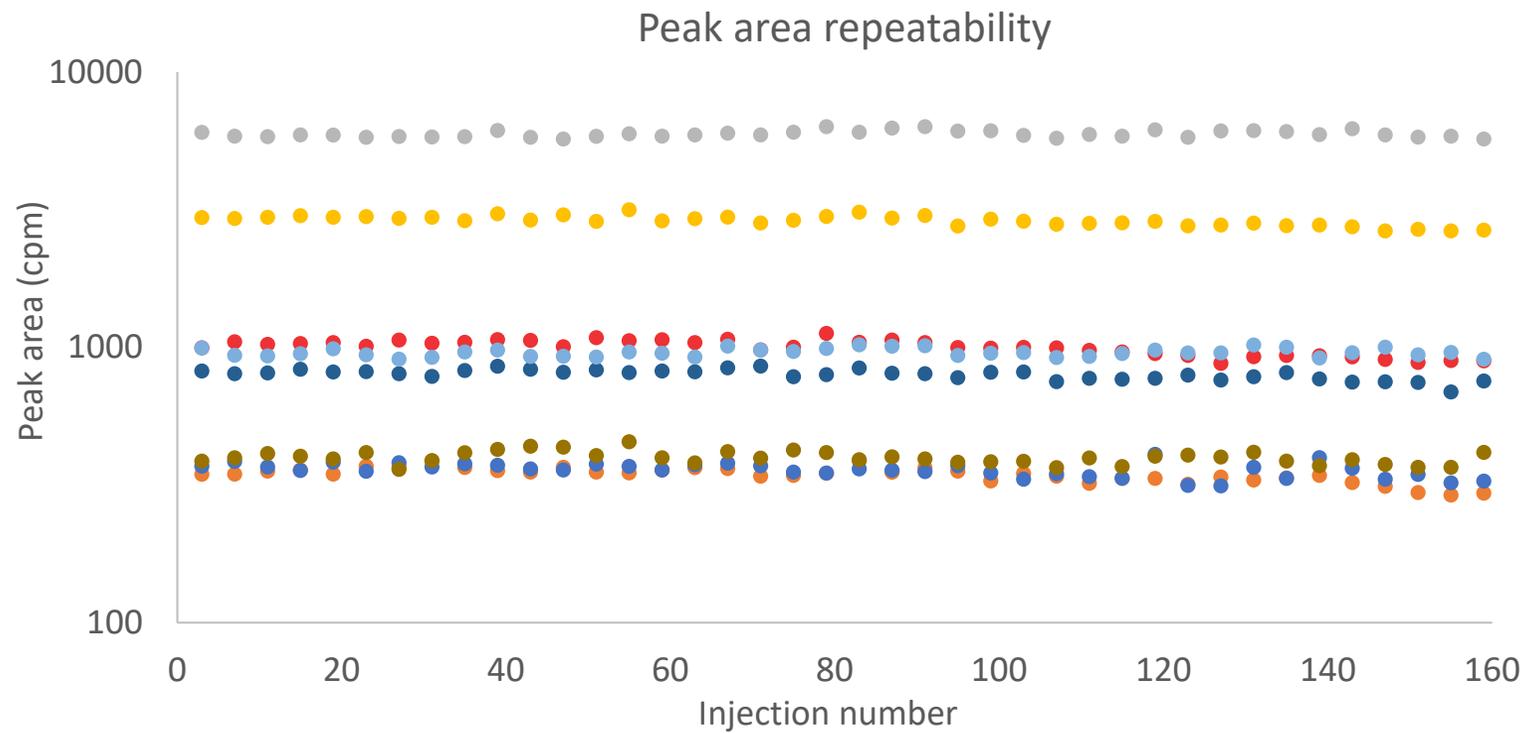


- QK1 - 1 Mixed fat
- QK1 - 2 Mixed fat
- QK1 - 4 Mixed fat
- QK1 - 5 Mixed fat
- QK1 - 6 Mixed fat
- QK3 Eggs
- QK5 Fish meal
- QK6 Fish oil
- QK7 Fish oil
- QK8 Meat
- QK9 Grass meal
- 1201-PLA 1 Pork sausage
- 1201-PLA 2 Pork sausage
- 1202-HEA 1 Whole egg
- 1202-HEA 2 Whole egg
- 1302-MIA 2 Milk powder
- 1501-AFB 1 Sugar beet pulp
- 1501-AFB 2 Sugar beet pulp
- 1601-HFA 1 Fish
- 1601-HFA 2 Fish
- 1401-SEA Sepiolite
- 1701-PFA PFAD
- 1301-FF Feed fat
- 1302-MIB Milk fat
- 9230 Meat
- 9255 Meat
- 9449 Meat
- 9367 Milk
- 9373 Milk
- 9182 Eggs
- 9370 Eggs
- 9371 Eggs
- 9487 Fish
- 9488 Fish

Robustness in sample

- The remaining extract volume for all the UK based sample assessment was combined to give a pooled, mixed matrix sample.
- This pooled matrix sample was then analysed alongside nonane blank and LOQ standard injections. The injection sequence was setup as follows:

- LOQ
 - Blank
 - Pooled matrix
 - Blank (four hour hold)
- Repeat x 40...

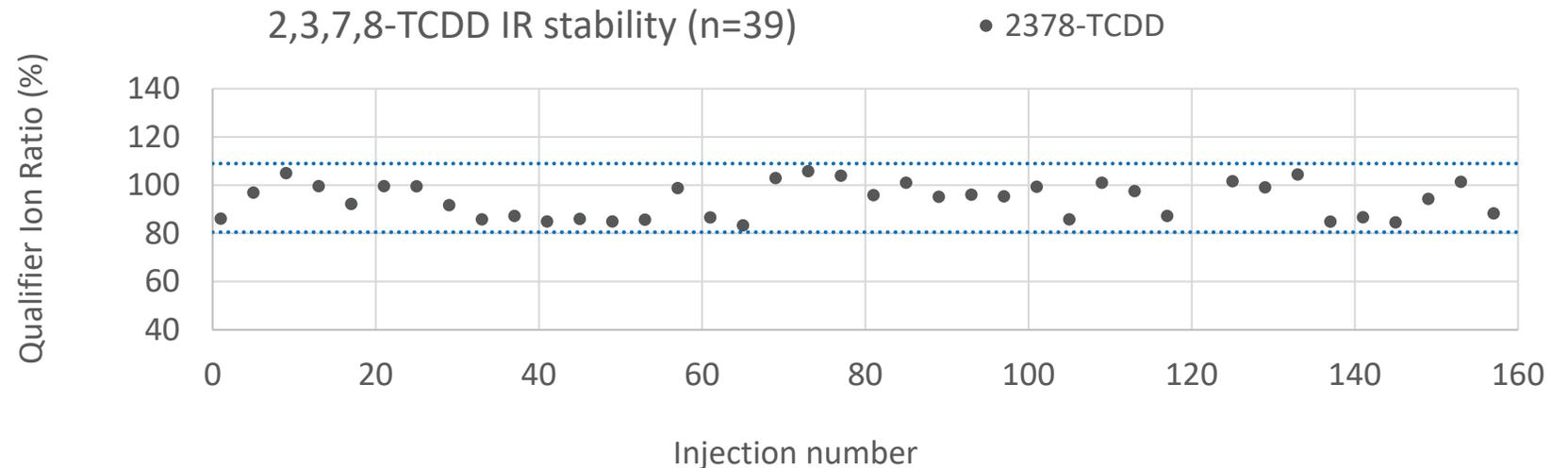
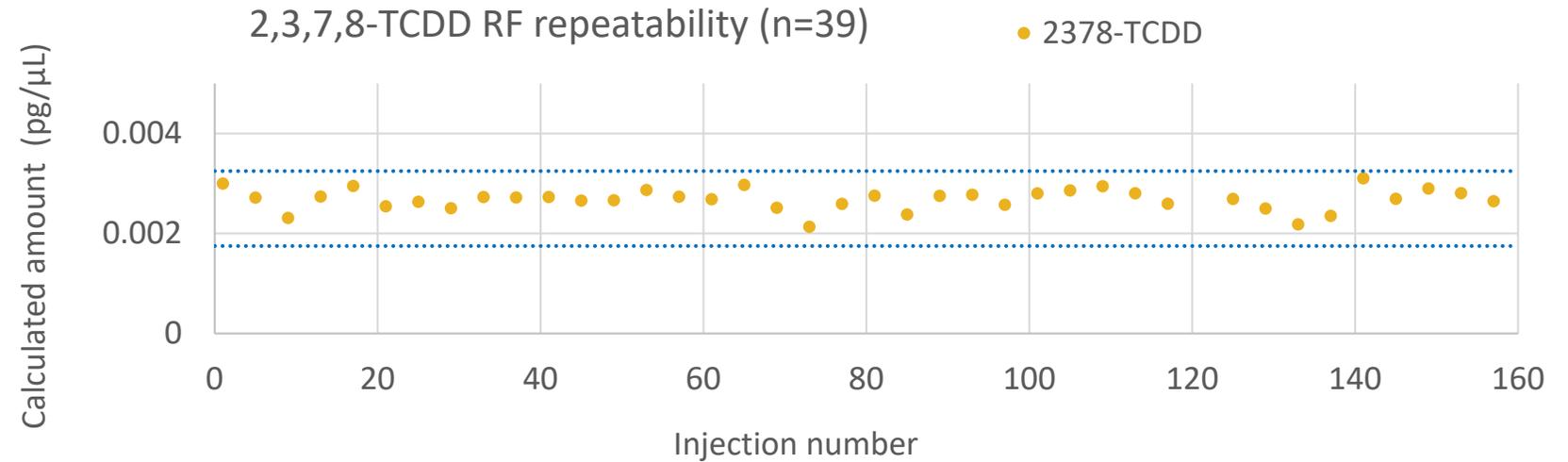
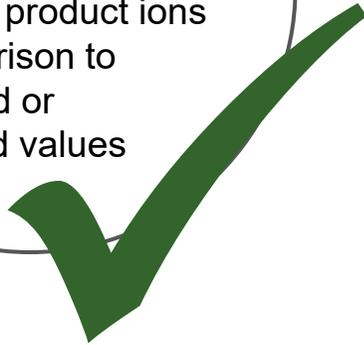


- 2378-TCDD - 6.4% RSD 0.17 pg
- 23478-PeCDF - 4.2% RSD 0.92 pg
- 234678-HxCDF - 4.4% RSD 0.31 pg
- 1234678-HpCDD - 3.5% RSD 0.71 pg
- 12378-PeCDF - 6.3% RSD 0.36 pg
- 12378-PeCDD - 6.0% RSD 0.21 pg
- 123789-HxCDD - 5.3% RSD 0.2 pg
- OCDD - 2.8% RSD 6.12 pg

Robustness at the LOQ

- The TSQ 9000 AEI system delivered consistence performance for the measurement of the LOQ standard, with all congeners falling within the ion ratio tolerance and response factor deviation threshold.

Maximum permitted tolerance of relative ion intensities of $\pm 15\%$ for selected transition product ions in comparison to calculated or measured values



Chromeleon acquisition, processing and reporting

Run Finished

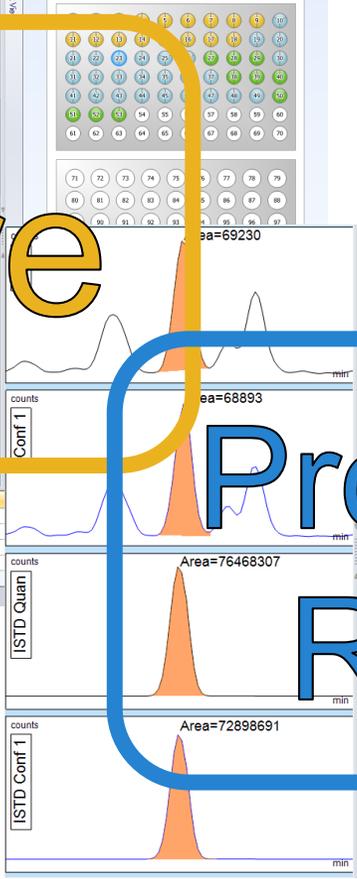
EURL Sample analysis - non-ortho and PCDD-Fs

Save Studio Print Up Insert Row Fill Down Lock Filtering Grouping Custom Columns Find Next

<UKRUN-HRTY.QD> (Not Connected)

#	MS Quantitation	Name	Type	Analyte_Type	Level	ML	CM7 list/Level	Weight	Dilution	LOG...
25	Disabled	LOQ2	Calibration Standard	PCDD/F	PCDD/F LO...		PCDD/F LOQ2	1.0000	1.0000	
26	Disabled	LOQ	Calibration Standard	PCDD/F	PCDD/F LOQ		PCDD/F CS1	1.0000	1.0000	
27	Disabled	LOQ2	Calibration Standard	PCDD/F	PCDD/F LO...		PCDD/F CS1	1.0000	1.0000	
28	Disabled	LOQ2	Calibration Standard	PCDD/F	PCDD/F LO...		PCDD/F CS1	1.0000	1.0000	
29	Disabled	LOQ2	Calibration Standard	PCDD/F	PCDD/F LO...		PCDD/F CS1	1.0000	1.0000	
30	Disabled	CS1	Calibration Standard	PCDD/F	PCDD/F CS1		PCDD/F CS1	1.0000	1.0000	
31	Disabled	CS1	Calibration Standard	PCDD/F	PCDD/F CS1		PCDD/F CS1	1.0000	1.0000	
32	Disabled	CS2	Calibration Standard	PCDD/F	PCDD/F CS2		PCDD/F CS1	1.0000	1.0000	
33	Disabled	CS2	Calibration Standard	PCDD/F	PCDD/F CS2		PCDD/F CS1	1.0000	1.0000	
34	Disabled	CS3	Calibration Standard	PCDD/F	PCDD/F CS3		PCDD/F CS1	1.0000	1.0000	
35	Disabled	CS3	Calibration Standard	PCDD/F	PCDD/F CS3		PCDD/F CS1	1.0000	1.0000	
36	Disabled	CS4	Calibration Standard	PCDD/F	PCDD/F CS4		PCDD/F CS1	1.0000	1.0000	
37	Disabled	CS4	Calibration Standard	PCDD/F	PCDD/F CS4		PCDD/F CS1	1.0000	1.0000	
38	Disabled	Blank	Unknown	PCDD/F				1.0000	1.0000	
39	Disabled	Blank	Unknown	PCB				1.0000	1.0000	
40	Disabled	Blank	Unknown	PCB & PCDD/F				1.0000	1.0000	
41	Disabled	Blank	Unknown	PCB & PCDD/F				1.0000	1.0000	
42	Disabled	1201 PLA 1	Unknown	PCB & PCDD/F	Pork_meat_PC...	Non-ortho PCB	2.0480	20.0000	Min_C	
43	Disabled	1201 PLA 1	Unknown	PCB & PCDD/F	Pork_meat_PC...	Non-ortho PCB	2.0480	20.0000	Min_C	
44	Disabled	9373	Unknown	PCB & PCDD/F	Milk_PCDDF	Non-ortho PCB	2.1700	20.0000	Min_C	
45	Disabled	9373	Unknown	PCB & PCDD/F	Milk_PCDDF	Non-ortho PCB	2.1700	20.0000	Min_C	
46	Disabled	Blank	Unknown	PCB & PCDD/F	Fish_PCDDF	Non-ortho PCB	1.9930	20.0000	Min_C	

Acquire



Process & Review

Peak Name	RT min	ISTD recovery %	Target IR %	Actual IR %	IR dev %	Result <LOQ?	UppBnd	MidBnd	LowBnd	SUM UB pg/g	ML check
2378-TCDF	20.30	76.0	98.4	99.5	1.1	0.0147	0.0147	0.0147			
2378-TCDD	20.86	80.0	94.7	83.1	-12.3	0.0308	0.0308	0.0308			
12378-PeCDF	24.34	82.9	78.3	74.0	-5.5	0.0034	0.0034	0.0034			
23478-PeCDF	25.71	85.1	77.9	78.2	0.4	0.3050	0.3050	0.3050			
123478-HxCDF	29.06	80.5	63.0	65.2	3.6	0.0785	0.0785	0.0785			
123678-HxCDF	29.17	85.6	63.0	66.9	6.3	0.0319	0.0319	0.0319			
234678-HxCDF	29.85	74.8	62.6	64.8	3.5	0.0262	0.0262	0.0262			
123478-HxCDD	29.94	84.2	63.9	64.4	0.8	0.0172	0.0172	0.0172			
123678-HxCDD	30.03	79.1	64.9	65.1	0.2	0.0332	0.0332	0.0332			
123789-HxCDD	30.35	79.1	64.2	72.7	13.2	<LOQ	0.0000	0.0000			
123789-HxCDF	30.72	67.7	63.7	61.5	-5.0	0.0000	0.0000	0.0000			
234678-HxCDF	32.72	70.5	67.4	68.4	-2.6	0.0092	0.0092	0.0092			
1234678-HxCDF	32.78	78.5	67.5	78.5	11.0	0.0000	0.0000	0.0000			
123478-HxCDF	34.78	77.2	74.9	74.9	-1.8	<LOQ	0.0000	0.0000			
123478-HxCDF	34.78	77.2	74.9	74.9	-1.8	<LOQ	0.0000	0.0000			
OCDF	38.64	71.9	94.1	100.2	6.5	<LOQ	0.0002	0.0001	0.0000	0.70	Below ML

1201 PLA 1 Sample type: Pork_meat_PCDDF - ML 1.0000

Injection results

Run Time (min): 44.700

Injection Details

Injection Name: QK1 1

Injection Volume: 25

Injection Type: PCDD/Fs - PTV

Calibration Level: Non-ortho PCDD/Fs - PTV

Instrument Method: Non-ortho PCDD/Fs

Dilution Factor: 20.0000

Sample Weight: 1.9870

Lipid content (%): 100.000

Dry Mass (%): 100.000

Injection Date: 25/09/2018 10:01:00

Peak Name	Retention Time min	Quantitation Ion	Qualification Ion	Quan Area counts*min	Qual Area counts*min	Target Ratio %	Ion Ratio %	ISTD Rec %	Amount pg/g	<LOQ?	WHO TEF	UB WHO TEF pg/g	MB WHO TEF pg/g	LB WHO TEF pg/g
12378-TCDF	20.30	303.9/240.9	305.9/242.9	2823.1	2720.7	79.2	84.29	0.4829	0.0483	0.0483	0.1000	0.0483	0.0483	0.0483
12378-TCDD	20.86	319.9/256.9	321.9/258.9	254.9	268.3	94.75	105.26	89.2	0.0683	0.0683	1.0000	0.0683	0.0683	0.0683
12378-PeCDF	24.34	339.9/276.9	337.9/274.9	724.7	652.3	78.23	85.79	94.2	0.1832	0.0000	0.0000	0.0000	0.0000	0.0000
12378-PeCDD	25.71	339.9/276.9	337.9/274.9	1337.5	1059.9	77.94	75.45	94.8	0.2388	0.0000	0.0000	0.0000	0.0000	0.0000
12378-HxCDF	29.06	355.9/292.9	353.9/290.9	1116.3	842.3	78.00	75.45	99.7	0.3410	1.0000	0.3410	0.3410	0.3410	0.3410
123678-HxCDF	29.17	373.9/310.9	371.9/308.9	532.7	297.5	62.88	55.86	81.2	0.1125	0.1000	0.0113	0.0113	0.0113	0.0113
123678-HxCDD	29.17	373.9/310.9	371.9/308.9	476.6	348.7	62.87	59.96	87.3	0.1130	0.1000	0.0113	0.0113	0.0113	0.0113
234678-HxCDF	32.72	388.9/325.9	386.9/323.9	1489.3	976.7	63.04	62.55	80.4	0.4771	0.0000	0.0477	0.0477	0.0477	0.0477
123478-HxCDF	30.03	389.9/326.9	387.9/324.9	3460.3	2269.9	64.04	65.69	96.9	1.0200	0.1000	0.1020	0.1020	0.1020	0.1020
123789-HxCDF	30.35	389.9/326.9	387.9/324.9	1639.3	1039.3	64.04	65.69	96.9	0.5514	0.1000	0.0557	0.0557	0.0557	0.0557
123789-HxCDD	30.35	373.9/310.9	371.9/308.9	3071.3	235.5	63.04	65.69	71.1	0.0666	<LOQ	0.0000	0.0000	0.0000	0.0000
1234678-HxCDF	32.78	407.9/344.9	405.9/342.9	472.6	403.2	60.31	65.31	89.0	0.1654	<LOQ	0.0000	0.0000	0.0000	0.0000
1234678-HxCDD	33.78	423.9/360.9	421.9/358.9	1782.0	1383.2	60.31	65.31	89.0	0.8788	0.0000	0.0888	0.0888	0.0888	0.0888
1234789-HxCDF	34.51	407.9/344.9	405.9/342.9	116.0	75.7	60.31	65.31	89.0	0.0012	0.0000	0.0012	0.0012	0.0012	0.0012
OCDF	38.66	457.9/394.9	455.9/392.9	1166.5	94.9	94.9	91.3	91.3	0.0000	<LOQ	0.0000	0.0000	0.0000	0.0000
OCDF	38.66	441.8/378.8	443.8/380.8	281.2	288.3	94.1	102.53	83.0	0.1155	<LOQ	0.0000	0.0001	0.0001	0.0001
SUM WHO-PCDD/F-TEQ (pg/g)									0.852		0.847	0.847	0.842	0.842

WHO-PCDD/F TEQ (2005) Upperbound pg/g: 0.852

WHO-PCB TEQ (2005) Middlebound pg/g: 0.847

WHO-PCDD/F TEQ (2005) Lowerbound pg/g: 0.842

Report



Conclusions

- The results of this study demonstrate that the TSQ 9000 GC- MS/MS system, configured with the AEI source and controlled using Chromeleon CDS software can deliver routine-grade performance for the confirmation of PCDD/Fs, dioxin-like PCBs and indicator PCBs in food and feedstuffs.
- Successful validation of method performance criteria (LOQ, precision, accuracy and calibration) was carried out on two separate TSQ 9000 AEI systems, in two geo-locations.
- The sensitivity achieved with the TSQ 9000 AEI system allowed for Upperbound WHO-PCDD/F-TEQ (pg/g) values as low as 0.15 (for a 2 g sample intake weight and 20 μ L final sample volume), meeting the 1/5th maximum level requirements for all but the most challenging matrices.
- The outstanding linear range and accurate quantitative performance generated excellent comparative data to the EURL reference data supplied, with calibration data showing RF %RSD of <6 over more than 4 orders of magnitude for all native congeners.

Conclusions

- Minimizing user intervention has been demonstrated by running over 2 weeks with no maintenance (such as source cleaning, liner replacement, tuning or analytical column trimming) allowing maximum uptime and sample throughput.
- Chromeleon 7.2 CDS software provides an integrated platform, with the ability to automatically setup, easily acquire, process and report compliant data in a fully regulated environment, eliminating the need for using external spreadsheet programs. Chromeleon eWorkflows™, available from [AppsLab](#), also provide error-free execution of each analysis to meet SOP requirements, further simplifying the user experience.