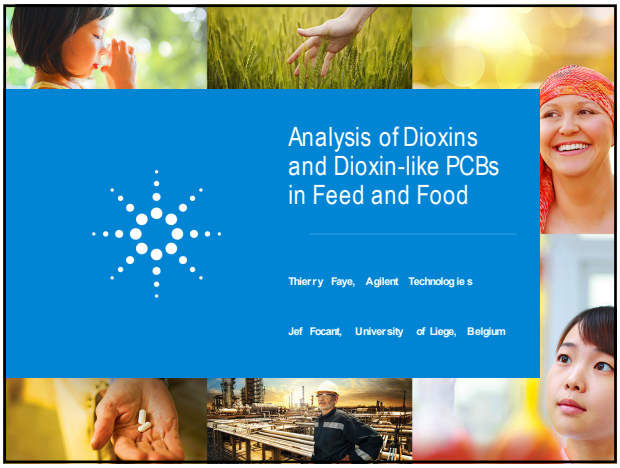
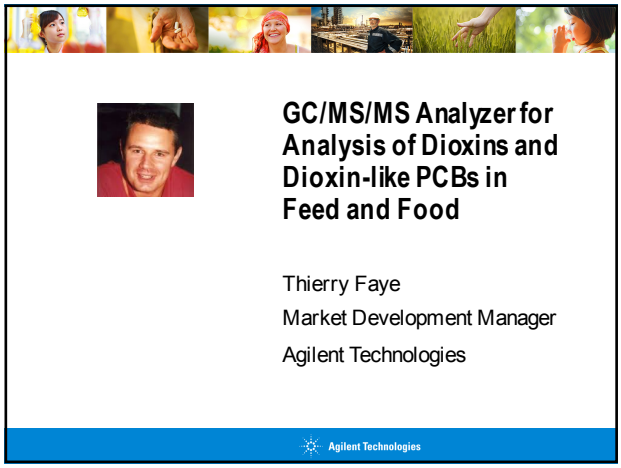
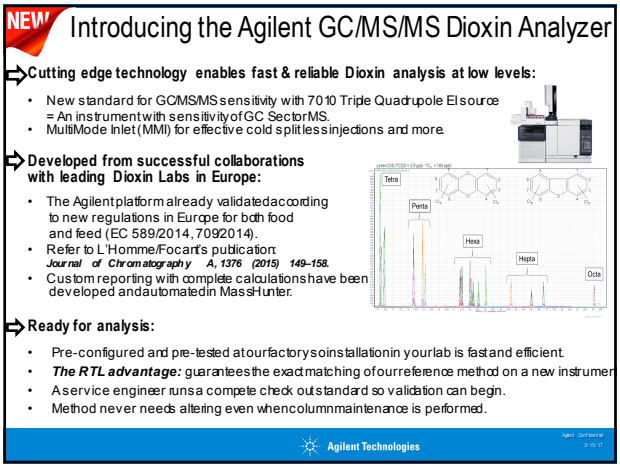
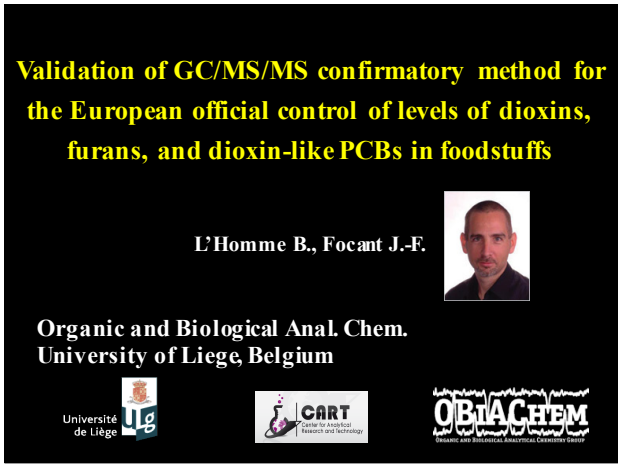
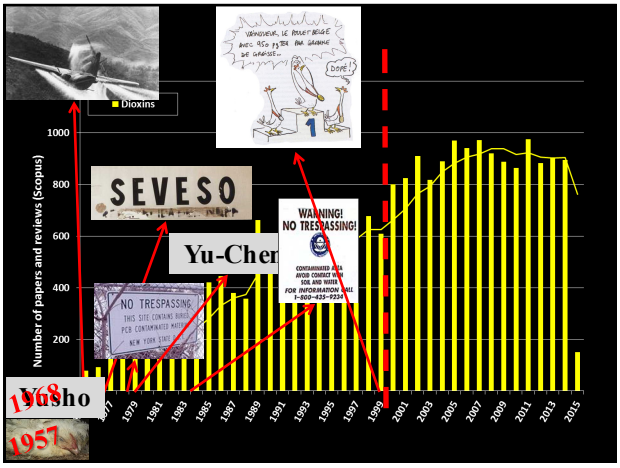
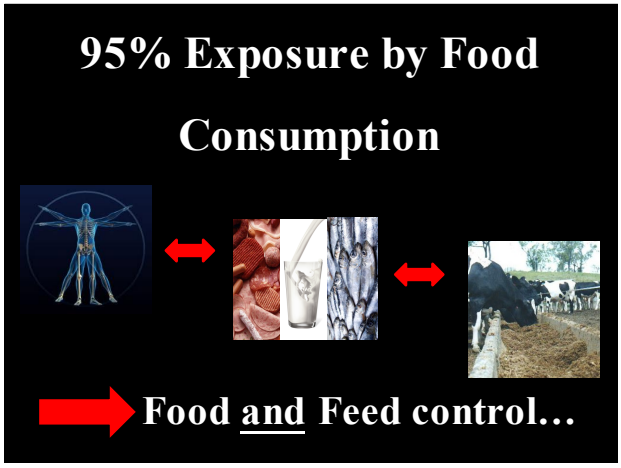

Analysis of Dioxins
and Dioxin-like PCBs
in Feed and Food
Thierry Faye, Agilent Technologies
Jef Focant, University of Liege, Belgium


GC/MS/MS Analyzer for
Analysis of Dioxins and
Dioxin-like PCBs in
Feed and Food
Thierry Faye
Market Development Manager
Agilent Technologies
Agilent Technologies


NEW
Introducing the Agilent GC/MS/MS Dioxin Analyzer
Cutting edge technology enables fast & reliable Dioxin analysis at low levels:
New standard for GC/MS/MS sensitivity with 7010 Triple Quadrupole EI source
= An instrument with sensitivity of GC Sector MS.
MultiMode Inlet (MMI) for effective cold split less injections and more.
Developed from successful collaborations
with leading Dioxin Labs in Europe:
The Agilent platform already validated according
to new regulations in Europe for both food
and feed (EC 589/2014, 709/2014).
Refer to L'Homme/Focant's publication:
Journal of Chromatography A, 1376 (2015) 149–158.
Custom reporting with complete calculations have been
developed and automated in MassHunter.
Tetra
Penta
Hexa
Hepta
Octa
Ready for analysis:
Pre-configured and pre-tested at our factory so installation in your lab is fast and efficient.
The RTL advantage: guarantees the exact matching of our reference method on a new instrumer
A service engineer runs a compete check out standard so validation can begin.
Method never needs altering even when column maintenance is performed.
Agilent Technologies


Validation of GC/MS/MS confirmatory method for the European official control of levels of dioxins, furans, and dioxin-like PCBs in foodstuffs
L'Homme B., Focant J.-F.
Organic and Biological Anal. Chem.
University of Liege, Belgium
Université de Liège
CART
OBIACHEM


Dioxins
Number of papers and reviews (Scopus)
1000
800
600
400
200
SEVESO
Yu-Cher
Yusho
1968
1957
WARNING! NO TRESPASSING!
NO TRESPASSING
1977
1979
1981
1983
1985
1987
1989
1991
1993
1995
1997
1999
2001
2003
2005
2007
2009
2011
2013
2015


95% Exposure by Food Consumption
Food and Feed control…

Dioxin EU Regulation for food-feed 'started' with the Belgian Dioxin Crisis in 1999…

EU Commission Documents

- Council Regulation 2001/102/EC
- Council Regulation (EC) No 2375/2001
- Commission Directive 2002/69/EC
- Commission Directive 2002/70/EC
- Commission Directive 2006/13/EC
- Commission Recommendation
- Commission Regulation …/2006
- Commission … No 1881/2006
- … (EC) No 1883/2006
- … Regulation (EC) No 152/2009
- … mission Regulation (EU) No 1259/2011
- Commission Recommendation 2011/516/EU
- Commission Regulation (EC) No 252/2012
- Commission Regulation (EC) No 277/2012
- Commission Regulation (EC) No 278/2012
- **Commission Regulation (EC) No 589/2014**
- **Commission Regulation (EC) No 709/2014**

http://eur-lex.europa.eu

EU Commission Strategy (food-feed)

- ✓ **Continuous** monitoring
- ✓ **Maximum-Action-(Target)** level strategy
- ✓ **Screening-Confirmatory** approach
- ✓ **RASFF** (high capacities)

➡ Based on state-of-the-art methods

Analytical Methods ?

Evolutive Guidelines

HARMONISED QUALITY CRITERIA FOR CHEMICAL AND BIOASSAYS ANALYSES OF PCDDs/PCDFs IN FEED AND FOOD
PART 1: GENERAL CONSIDERATIONS, GC/MS METHODS

Rainer Malisch [1], Bert Baumann [2], Peter A. Behnisch [3], Richard Canady [4], Daniel Fraisse [5], Peter Fürst [6], Douglas Hayward [7], Ronald Hoogenboom [8], Ronald Hoogerbrugge [2], Djien Liem [2], Olaf Päpke [9], Wim Traag [7], Thomas Wiesmüller [9]

OHC 50 (2001) 53

HARMONISED QUALITY CRITERIA FOR CHEMICAL AND BIOASSAYS ANALYSES OF PCDDs/PCDFs IN FEED AND FOOD
PART 2: GENERAL CONSIDERATIONS, BIOASSAY METHODS

Peter A. Behnisch [1], Randy Allen [2], Jack Anderson [3], Abraham Brouwer [4], David J. Brown [5], T. Colin Campbell [6], Leo Goeyens [7], Robert O. Harrison [8], Ron Hoogenboom [2], Ilse Van Overmeire [7], Wim Traag [7] and Rainer Malisch [1]

OHC 50 (2001) 59

www.dioxin20xx.org

➡ **PBMS**

- GC-IDHRMS *vs* CALUX
- ISO17025
- Validation @ LOQs (@1/5th level of interest)
- ≠Upper-Lowerbound < 20% @1pg TEQ/g fat level
- Z-scores @ PTs
- Recovery rates…

Recent EN 16215

NEN-EN 16215:2012

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16215

April 2012

ICS 65.120

PBMS philosophy

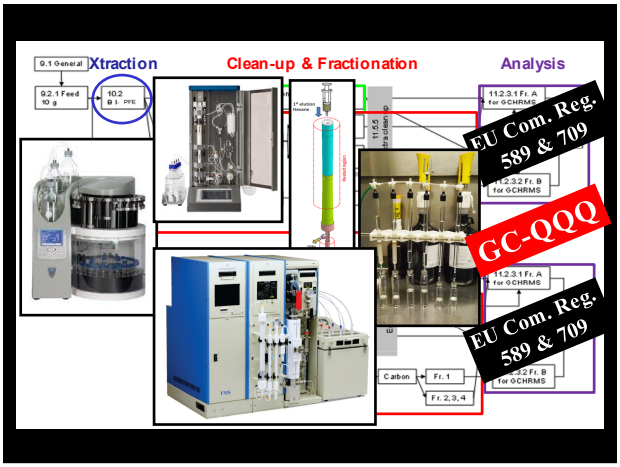
English Version

Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Aliments des animaux - Dosage des dioxines, des PCB de type dioxine et des PCB indicateurs par GC/HRMS

Futtermittel - Bestimmung von Dioxinen und dioxinähnlichen PCBs mittels GC/HRMS und von Indikator-PCBs mittels GC/HRMS

This European Standard was approved by CEN on 9 March 2012.



Confirmatory Tool

✓ **HRMS sector or MS/MS instruments**

ANALYTICAL CRITERIA FOR USE OF MS/MS FOR DETERMINATION OF DIOXINS AND DIOXIN-LIKE PCBS IN FEED AND FOOD

Kotz A[1], Malisch R[1*], Focant J[2], Eppe G[2], Cederberg TL[3], Rantakokko P[4], Fürst [illegible] L[6], Lovász C[7], Scortichini G[8], Diletti G[8], di Domenico A[9], Ingelido [illegible] [illegible] A[11]

EURL Working group recommendations

[1]European Union Reference Laboratory (EU-RL) [illegible] and Food, Freiburg, Germany; [2]CART, University of Liege, Belgium; [illegible] Veterinary University of Denmark, Soborg, Denmark; [4]National Institute [illegible], Kuopio, Finland; [5]Chemisches und Veterinäruntersuchungsamt [illegible]-Lippe (CVUA-MEL), Münster, Germany; [6]NCSR Demokritos, Athens, Greece; [7]Central Agricultural Office, Budapest, Hungary; [8]Istituto Zooprofilattico Sperimentale del Molise "G. Caporale, Teramo, Italy; [9]Istituto Superiore di Sanità (ISS), Roma, Italy; [10]RIKILT, Wageningen, The Netherlands; [11]The Food and Environment Research Agency (FERA), York, United Kingdom

Organohalogen compound**74** (2012), 156-159

EU Regulation 252/2012 amended by 589/2014 (2nd of June 2014)
EU Regulation 152/2009 amended by 709/2014 (20th of June 2014)

New EU Regulation

L 164/18 EN Official Journal of the European Union 3.6.2014

COMMISSION REGULATION (EU) No 589/2014

of 2 June 2014

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 and repealing Regulation (EU) No 252/2012

(Text with EEA relevance)

27.6.2014 EN Official Journal of the European Union L 188/1

COMMISSION REGULATION (EU) No 709/2014

of 20 June 2014

amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls

(Text with EEA relevance)

(9) In addition to the gas chromatography/high resolution mass spectrometry (GC-HRMS), technical progress and developments have shown that also gas chromatography/tandem mass spectrometry (GC-MS/MS) can be used as a confirmatory method for checking compliance with the maximum level (ML). Regulation (EC) No 252/2012 should therefore be replaced by a new Regulation providing for the use of gas chromatography/tandem mass spectrometry (GC-MS/MS) as an appropriate confirmatory method for checking compliance with the maximum level.

Criteria	PCDD/Fs and DL-PCBs *GC-MS/MS (589/2014)*	NDL-PCBs *GC-MS/MS (589/2014)*
Detectable quantity	-PCDD/F upper femtogram (10^{-15} g) -NO-PCB low picogram (10^{-12} g) -MO-PCB nanogram (10^{-9} g)	NDL-PCB nanogram (10^{-9} g)
Selectivity	-Chromatographic separation of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF <25% valley peak to peak	Relative RT ±0.25% IS vs analyte
MRM transitions	-Monitoring 2 specific precursors with each specific product ion transition for all labeled and unlabeled analytes -Relative ion intensities max ±15% -Resolution MS quadrupoles = unit	-Monitoring at least 1 precursor ion and 2 product ions -Tolerance ratio ±20% if rel. intens. >50% -Tolerance ratio ±25% if rel. intens. 20-50% -Resolution MS quadrupoles = unit
Blank	-Used for LOQ calculation	-Used for LOQ calculation -Blank value <30% of maximul level ML
LOQ	-LOQ calculated from lowest cali. point -lowest concentration point on cali. must give acceptable and consistent deviation to the average RRF -Average RRF calculated for all points -Deviation to average RRF <30%	-ditto
LOQ	-LOQ calculated from average blank level -LOQ < 1/5 of maximum level ML -Difference ub and lb levels <20% ML	-ditto -Diff. ub and lb for sum ind-PCB @ ML <20%
Accuracy Reproducibility	-Demonstrate performances at 0.5 ML, ML, 2 ML -Trueness (accuracy) ±20% -Within-lab reproducibility (RSD) <15%	-Demonstrate performances at 0.5 ML, ML, 2 ML -Trueness for sum ind-PCB @ ML ±30% -Within-lab reproducibility (RSD) <20%
Control	-QC chart for blanks -QC charts control sample	-QC chart for blanks -QC charts control sample
Recovery	-Individual internal std in range 60-120% -Out of range OK if contribu. to TEQ<10%	-Individual internal std in range 50-120% -Out of range OK if contribu. to sum ind PCB<10%
Measurement uncertainty	-Expanded measurement uncertainty -Coverage factor = 2 (CL=95%) -If separate determination of congeners, make sum of separate uncertainty for sum of PCDD/F and DL-PCBs	-Expanded measurement uncertainty -Coverage factor = 2 (CL=95%)

From Regulation

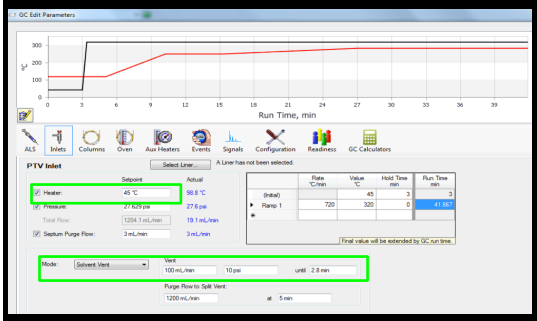
Full validation

Validation for vegetable oil (feed)

Agilent 7000C series Triple Quad GC/MS

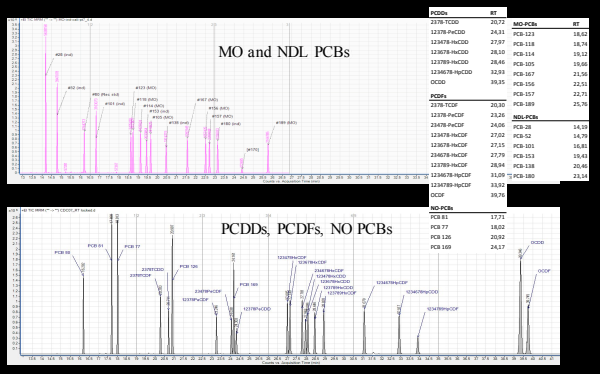
- **MU** — Top-down approach
- **Contro** — Control chart
- **Blanks subtraction** — Average blank / Blanks control chart
- **Reproducibility** — Recovery experiments, QCs
- **Accuracy** — Recovery experiments / Proficiency test
- **LOD/LOQ** — Statistical determination / Matrix related
- **Selectivity, linearity** — MRM, Quant/qual transitions / GC separation, Cali. curve

PTV Injections



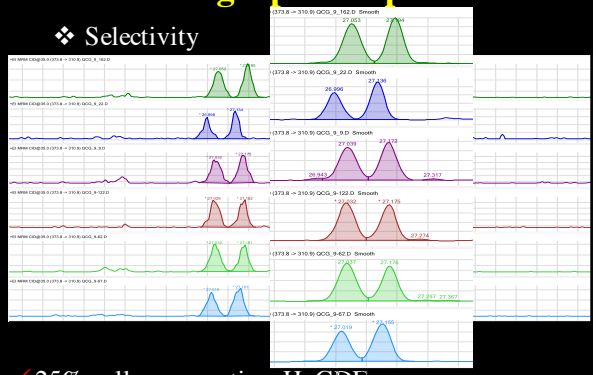
Dioxin fraction runtime = 42min
MO-PCBs fraction runtime = 28min

Chromatographic Profile



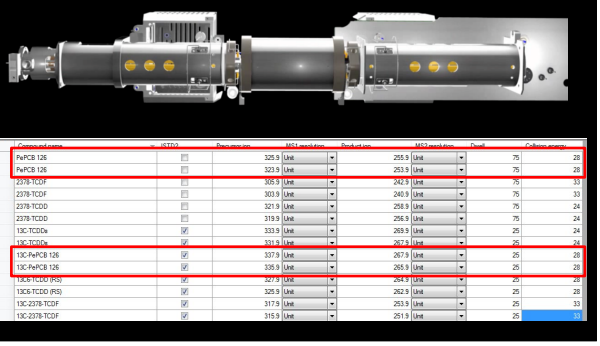
Chromatographic Separation

❖ Selectivity



✓25% valley separation HxCDF

Tandem *in-space* MS



MRM transition Ratio

❖ Quant/Qual transition ratio

EU Reg 709/2014 says:

Tolerance

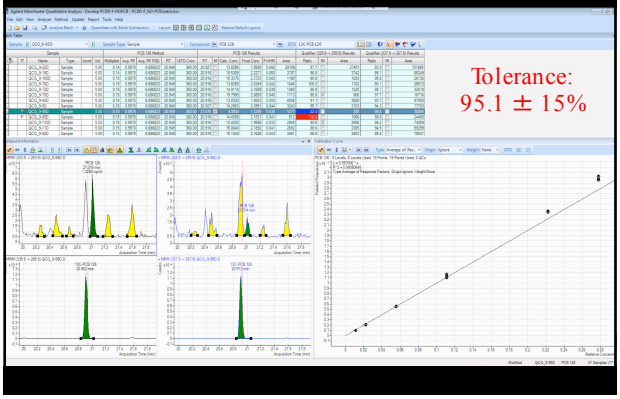
✓ PCDD/Fs, DL-PCBs: 2 specific precursors with each specific product ions

- 2378 TCDD: 319.9 > 256.9, 321.9 > 258.9 ➡ Average ratio = 0.964 ± 15% R(quad)=unit

✓ NDL-PCBs: at least 1 precursor and 2 product ions

- PCB 189: 393.8 > 323.8, 395.8 > 325.8 ➡ Average ratio = 0.627 ± 20% R(quad)=unit

In MassHunter



Proper Estimation of LOQs

❖ LOD/LOQ

ANALYTICAL CRITERIA FOR USE OF MS/MS FOR DETERMINATION OF DIOXINS AND DIOXIN-LIKE PCBS IN FEED AND FOOD

Kotz A[1], Malisch R[1*], Focant J[2], Eppe G[2], Cederberg TL[3], Rantakokko P[4], Fürst P[5], Bernsmann T[6], Leondiadis L[6], Lovász C[7], Scortichini G[8], Diletti G[8], di Domenico A[9], Ingelido AM[9], Traag W[10], Smith F[11], Fernandes A[11]

Approach 1: The LOQ can be calculated from the signal-to-noise ratio as already defined in the current regulations.

Approach 2: As an alternative approach, if the signal-to-noise ratio does not provide reliable results due to a very low, or no discernable noise level, the limit of quantification is based on the calibration curve. The limit of quantification is then defined as the lowest concentration point on a calibration curve that gives an acceptable (≤ 30 %) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor calculated for all points on the calibration curve in each series of samples.

For conversion of the limit of quantification from the calibration curve to the sample, the recovery of the internal standards of the respective congener and the sample intake has to be taken into account.

Organohalogen compound**74** (2012), 156-159

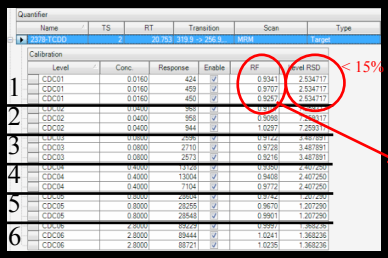
✓ iLOQ = 10*stdev (8 replicate injections – cali point)

✓ LOQ = blank mean + 6*stdev (12 injections – blank)

Validation for vegetable oil (feed)

- iLOD/iLOQ

'Acceptable and consistent deviation to the average RRF'



< 15%

Av RRF_{1-6} = 0.9622

R^2(linear fit) = 0.996(

> 0.990(

Av RRF_1 = 0.9435

Deviation = -1.9%

< 30%

Validation for vegetable oil (feed)

- iLOD/iLOQ

Calibration curve (lowest level)

→ 8 replicates

→ 10*stdev

- LOQ matrix

Procedure blanks

→ 10 independent injections

→ Average + 6*stdev.

Validation for vegetable oil (feed)

- (i)LOQ

Compound	Lowest cali point				Blanks		
	Avg Conc. pg/µL	Std. Dev.	iLOD pg/µL	iLOQ pg/µL	in blank?	LOQ MS/MS pg/g	LOQ HRMS pg/g
PCB 77	0,2917	0,0037	0,011	0,037	yes	49,66	64,59
PCB 81	0,2935	0,003	0,009	0,030	yes	3,67	9,53
2378-TCDD	0,0152	0,0018	0,005	0,018	no	0,02	0,06
2378-TCDF	0,0162	0,001	0,003	0,010	yes	0,10	0,12
PCB 126	0,2906	0,0077	0,023	0,077	yes	1,37	1,21
23478-PeDF	0,0154	0,0021	0,006	0,021	yes	0,08	0,08
12378-PeDD	0,0169	0,0029	0,009	0,029	no	0,01	0,06
PCB 169	0,3062	0,0071	0,021	0,071	no	0,02	0,27
12378-PeDF	0,0142	0,0022	0,007	0,022	yes	0,56	0,09
123478-HxDF	0,0164	0,0016	0,005	0,016	yes	0,07	0,10
123678-HxDF	0,0169	0,0009	0,003	0,009	yes	0,06	0,06
234678-HxDF	0,0166	0,0007	0,002	0,007	yes	0,08	0,09
123789-HxDF	0,0156	0,002	0,006	0,020	yes	0,18	0,10
123789-HxDD	0,0774	0,0061	0,018	0,062	yes	0,06	0,06
123478-HxDD	0,017	0,0022	0,007	0,022	yes	0,02	0,06
123678-HxDD	0,0426	0,0032	0,010	0,032	yes	0,14	0,08
OCDD	3,5201	0,0465	0,140	0,465	yes	1,99	2,02
OCDF	0,0158	0,0027	0,008	0,027	yes	0,60	0,77

Accuracy & Reproducibility

Spiked materials at 0.5 ML, ML, 2 ML (6 series, 3 days)

PCDD/Fs		Average ng WHO-TEQ/kg	Stdev	RSD %	Target ng WHO-TEQ/kg	Bias %
Spike level	ML/2	0,409	0,029	7,1	0,40	2,36
	ML	0,778	0,045	5,7	0,79	-1,54
	2ML	1,600	0,035	2,2	1,58	1,30

NO-PCBs		Average ng WHO-TEQ/kg	Stdev	RSD %	Target ng WHO-TEQ/kg	Bias %
Spike level	ML/2	0,307	0,028	9,0	0,33	-7,00
	ML	0,595	0,020	3,4	0,65	-8,53
	2ML	1,256	0,021	1,6	1,30	-3,42

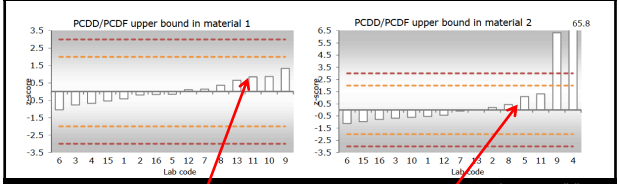
✓ Bias < 20%

✓ Within lab reproducibility < 15%

Accuracy & Reproducibility

Proficiency test (PT)

Material 1	Reported pg/g TEQ	Target Value pg/g TEQ	Accuracy
PCDD/Fs	1.10±0.20	1.01	8,8%
DL-PCBs	0.80±0.19	0.89	-10,3%
Total TEQ	1.90±0.36	1.90	-0,1%
Material 2			
PCDD/Fs	0.55±0.11	0.48	15,6%
DL-PCBs	0.82±0.21	0.85	-3,0%
Total TEQ	1.38±0.26	1.33	3,7%



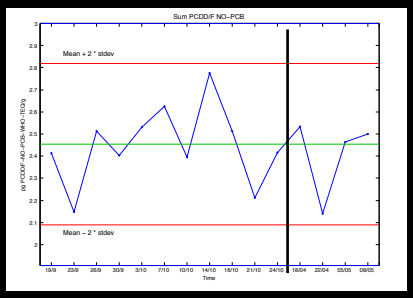
Z score = 0.80

Z score = 0.59

Wim Traag, Rikilt, NL

Within Lab Reproducibility

QC pork fat



MassHunter Report Generator

D:\MassHunter\GCMS\1\data\Develop\PCDD-F-NOP\CBQuantResults\Labrees-PCDD-F-NO.batch.bin

Batch Data Path			
Analysis Time	2014-06-12T10:33:58.462+02:00	**Analyst**	admin
Report Time	2014-06-12T15:30:36.076+02:00	**Reporter**	admin
	2014-05-12T16:56		Labrees-PCDD-F-NO.batch.bin
Last Calib Update		**Batch**	
2014-06-12T10:33:58.462+02:00	2014-06-12T15:30:36.076+02:00	2014-05-12T16:56:08.348.58.4+02:00	

Sample Name	14.4441_diox-1	**Type**	Sample	**Vial**	62
Vol. [µl]	5	**Comment**	Diox		

Compound	RT [min]	Conc [ng/ml]	TEQ Conc [ng/ml]	LOQ	Upper Bound [ng/ml]	Medium Bound [ng/ml]	Lower Bound [ng/ml]	WHO-TEF 2005
2378-TCDD	20,94	<LOQ	<LOQ	0,0200	0,02000	0,01000	0,00000	1
12378-PeCDD	24,27	<LOQ	<LOQ	0,0300	0,03000	0,01500	0,00000	1
123478-HxCDD	28,02	0,0376	0,00376	0,0200	0,00376	0,00376	0,00376	0,1
123678-HxCDD	28,15	<LOQ	<LOQ	0,1400	0,01400	0,00700	0,00000	0,1
123789-HxCDD	28,51	<LOQ	<LOQ	0,0600	0,00600	0,00300	0,00000	0,1
1234678-HpCDD	33,08	<LOQ	<LOQ	0,4400	0,00440	0,00220	0,00000	0,01
OCDD	39,46	4,9895	0,00150	1,9900	0,00150	0,00150	0,00150	0,0003
2378-TCDF	19,90	<LOQ	<LOQ	0,1000	0,01000	0,00500	0,00000	0,1
12378-PeCDF	23,26	<LOQ	<LOQ	0,5600	0,01680	0,00840	0,00000	0,03
23478-PeCDF	24,08	<LOQ	<LOQ	0,0800	0,02400	0,01200	0,00000	0,3
123478-HxCDF	27,05	<LOQ	<LOQ	0,0700	0,00700	0,00350	0,00000	0,1
123678-HxCDF	27,19	<LOQ	<LOQ	0,0600	0,00600	0,00300	0,00000	0,1
123789-HxCDF	29,06	<LOQ	<LOQ	0,1800	0,01800	0,00900	0,00000	0,1
234678-HxCDF	27,84	<LOQ	<LOQ	0,0800	0,00800	0,00400	0,00000	0,1
1234678-HpCDF	31,29	<LOQ	<LOQ	0,5500	0,00550	0,00275	0,00000	0,01
1234789-HpCDF	34,01	<LOQ	<LOQ	0,0200	0,00020	0,00010	0,00000	0,01
OCDF	39,89	<LOQ	<LOQ	0,6000	0,00018	0,00009	0,00000	0,0003
PCB 77	18,01	<LOQ	<LOQ	49,6600	0,00497	0,00248	0,00000	0,0001
PCB 81	18,01	6,7691	0,00203	3,6700	0,00203	0,00203	0,00203	0,0003
PCB 126	21,04	<LOQ	<LOQ	1,3700	0,13700	0,06850	0,00000	0,1
PCB 169	24,22	<LOQ	<LOQ	0,0700	0,00210	0,00105	0,00000	0,03
Total TEQ					**0,32144**	**0,16436**	**0,00729**	
Sum TEQ PCDD/F					**0,17534**	**0,09030**	**0,00526**	
Sum TEQ PCBs					**0,14610**	**0,07406**	**0,00203**	

Sum TEQ PCDD/F 0,175 ±0,035
Sum TEQ PCBs 0,146 ±0,035

Take Home Message

- ✓ PTV-GC/MS/MS accepted as a confirmatory tool under EU Regs
- ✓ Full validation on challenging matrix
- ✓ MS/MS, but still dioxin analyses…
- ✓ MS/MS & sectors to be properly perceived