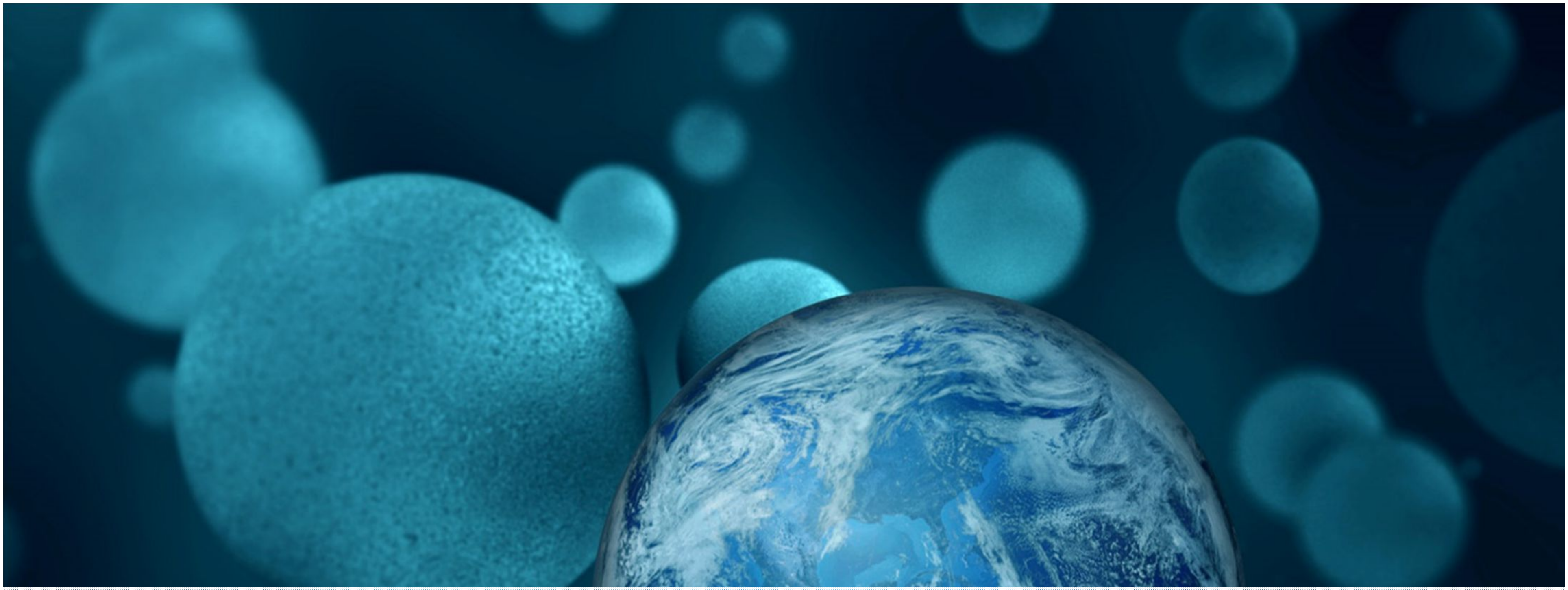


Thermo Fisher SCIENTIFIC

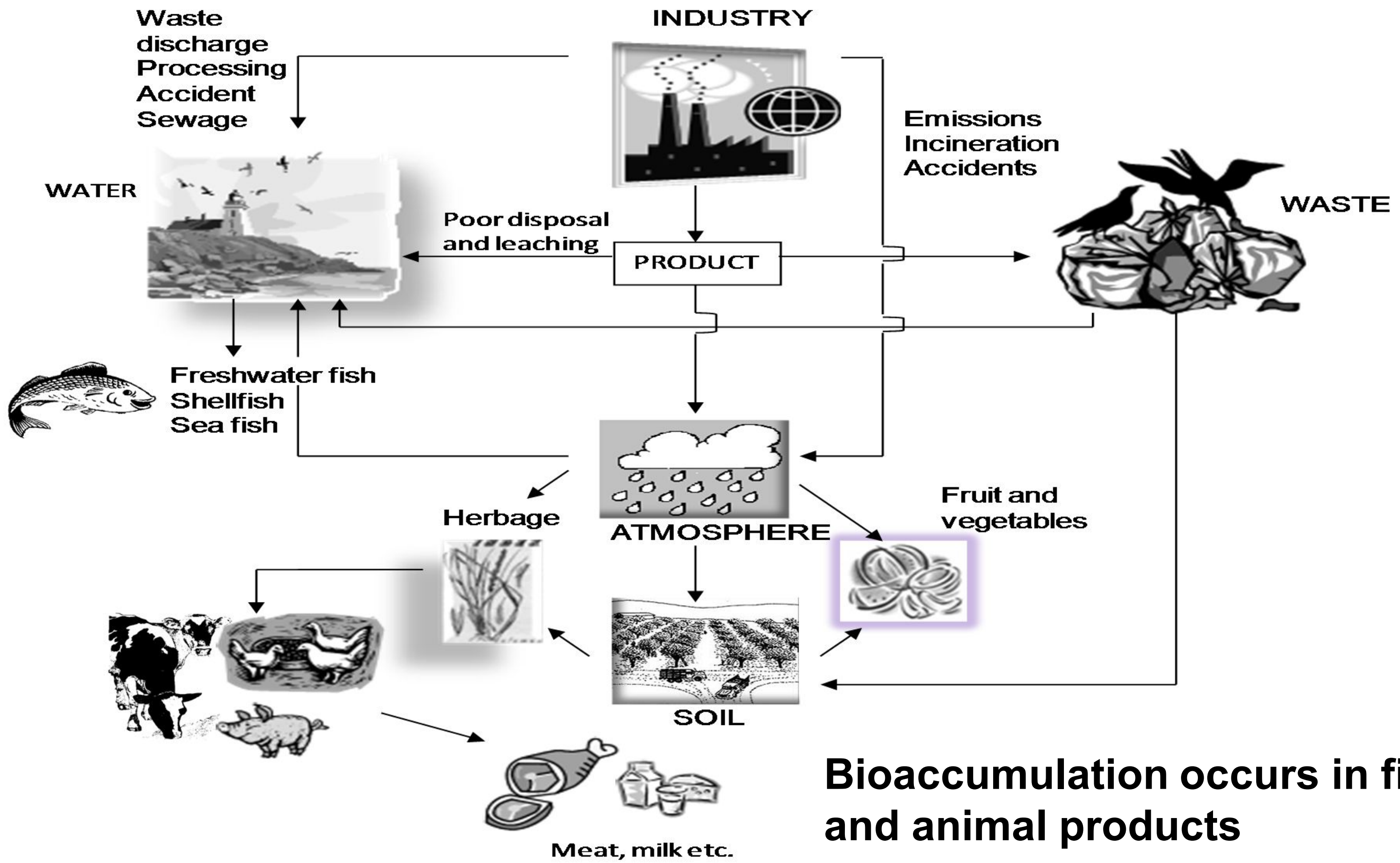
Dioxin and PCBs with Triple Quadruple and HRMS Technology- Setting the New Standard

Michal Godula
Special Solutions Center
Thermo Fisher Scientific

The world leader in serving science

Persistance & Accumulation in the Food Chain

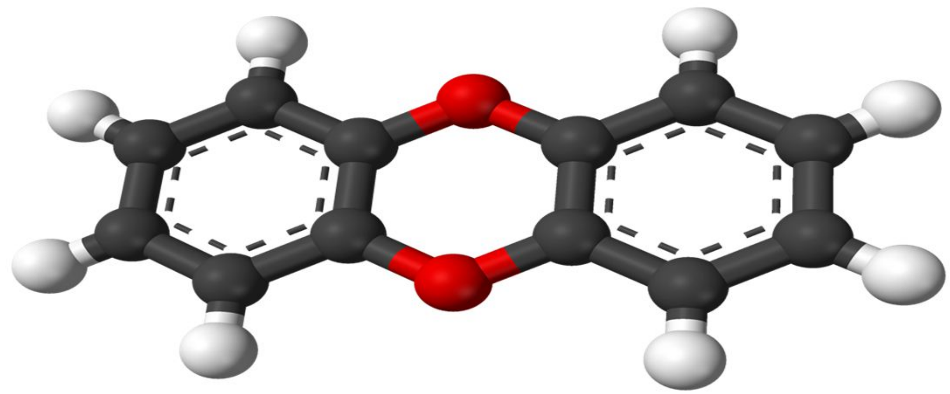
POPs are chemically stable environmental contaminants



Bioaccumulation occurs in fish and animal products

UNEP definition of Persistant Organic Pollutants (POPs)

"…chemical substances that ***PERSIST*** *in the environment,* ***BIOACCUMULATE*** *through the food web, and pose a* ***RISK*** *of causing adverse effects to* ***HUMAN HEALTH*** *and the environment. With the evidence of long-range transport of these substances to regions where they have never been used or produced and the consequent threats they pose to the environment of the whole globe, the international community has now, at several occasions called for urgent global actions to* ***REDUCE AND ELIMINATE RELEASES*** *of these chemicals."*



Dioxins at Trace Levels are **Highly Toxic**

- Tolerable Daily Intake 1 pg/kg/d TEQ

- Amounts typically detected:
  - Food PPT
  - Water PPQ
  - Human Serum PPQ
  - Air fg/m^3

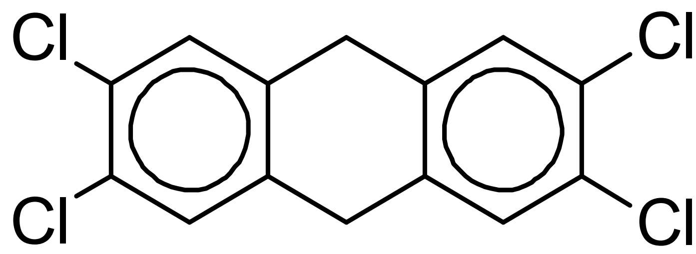
ThermoFisher SCIENTIFIC

Toxicity

- Viktor Yushchenko, President of Ukraine (2005-2010)
- Poisoned in 2004

Basic Concepts of Toxic Equivalency Factor (TEF)

- Dioxin-like toxicity is **cumulative**
  - So we can compare toxicology of various dioxin-like compounds and simplify risk assessment and regulatory control.
- All other compounds with dioxin-like toxicity are compared to 2378 TCDD and given a relative TEF



- Toxic Equivalency Factor (TEF) of 2378-TCDD = 1

Ever Changing TEF Concept

- TEF factors are continuously revised
- TEFs are species dependent
  - Human
  - Fish
  - Avian
  - Mammalian
- Generally we are interested in human exposure

Congener	I-TEF	WHO1998-TEF	WHO2005-TEF
Polychlorinated dibenzo-p-dioxins			
2378-Cl4DD	1	1	**1**
12378-Cl5DD	0.5	1	**1**
123478-Cl6DD	0.1	0.1	**0.1**
123678-Cl6DD	0.1	0.1	**0.1**
123789-Cl6DD	0.1	0.1	**0.1**
1234678-Cl7DD	0.01	0.01	**0.01**
Cl8DD	0.001	0.0001	**0.0003**
Polychlorinated dibenzofurans			
2378-Cl4DF	0.1	0.1	**0.1**
12378-Cl5DF	0.05	0.05	**0.03**
23478-Cl5DF	0.5	0.5	**0.3**
123478-Cl6DF	0.1	0.1	**0.1**
123678-Cl6DF	0.1	0.1	**0.1**
123789-Cl6DF	0.1	0.1	**0.1**
234678-Cl6DF	0.1	0.1	**0.1**
1234678-Cl7DF	0.01	0.01	**0.01**
1234789-Cl7DF	0.01	0.01	**0.01**
Cl8DF	0.001	0.0001	**0.0003**
Non-ortho polychlorinated biphenyls			
PCB-77	-	0.0001	**0.0001**
PCB-81	-	0.0001	**0.0003**
PCB-126	-	0.1	**0.1**
PCB-169	-	0.01	**0.03**
Mono-ortho polychlorinated biphenyls			
PCB-105	-	0.0001	**0.00003**
PCB 114	-	0.0005	**0.00003**
PCB-118	-	0.0001	**0.00003**
PCB-123	-	0.0001	**0.00003**
PCB-156	-	0.0005	**0.00003**
PCB-157	-	0.0005	**0.00003**
PCB-167	-	0.00001	**0.00003**
PCB-189	-	0.0001	**0.00003**

- EU guidelines for food analysis use WHO2005 TEF factors

Persistant Organic Pollutants

- Incineration products
  - Polychlorinated dibenzodioxins –PCDDs (75)
  - Polychlorinated dibenzofurans - PCDFs (135)

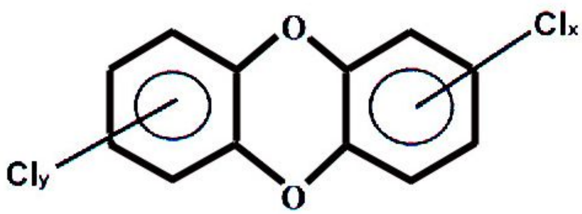
- Environmental contaminants
  - Polychlorinated biphenyls – PCBs (209)
  - Polybrominated diphenyl ethers – PBDEs (209)
  - Chlorinated paraffins
  - Organochlorine pesticides
  - Polyfluorinated ether sulphones

Large numbers of cogeners but of differing toxicity

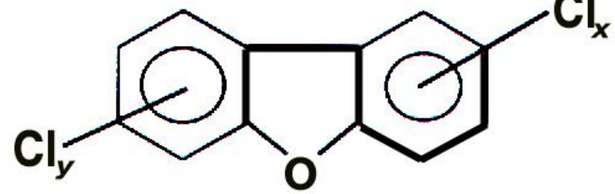
High specificity is required to focus on toxicologically significant POPs

Structural Similarities Requires High Specificity

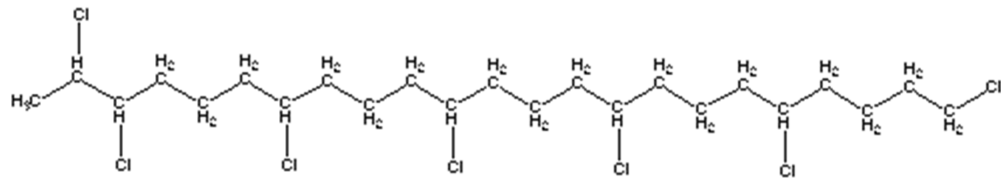
Polychlorodibenzo-p-dioxin



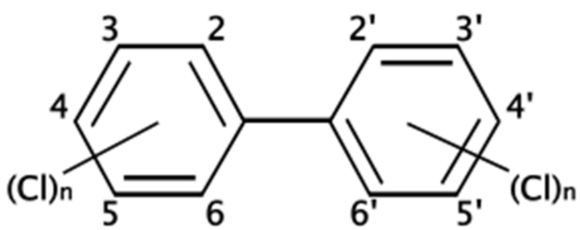
Polychlorodibenzofuran



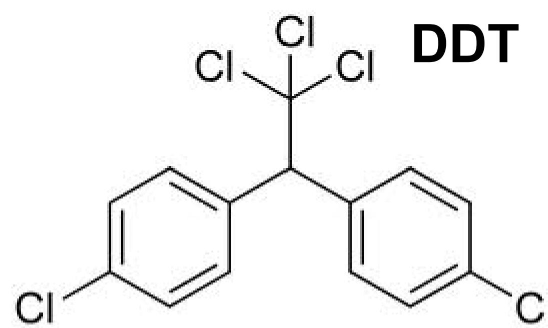
Chlorinated Paraffins



Polychlorinated biphenyls



DDT



EU Regulations for POPs in Food & Feed

- Regulation (EC) No 1881/2006

  For example:

  - Sum of dioxins (WHO-PCDD/PCDF-TEQ)
    - Limits range from 0.75 pg/g fat for vegetable oils to 4.0 pg/g for fish
  - Sum of dioxins & dioxin-like PCBs
    - Limits range from 1.5 pg/g fat for vegetable oils to 8.0 pg/g for fish
  - Separate limits apply to animal feed
- Regulation (EC) No 396/2005

  For example:-

  - DDT (sum of *pp*'-DDT, *op*'-DDT,*pp*'-DDT and *pp*'-TDE (DDD) expressed as DDT
    - Limits range from 0.04 mg/kg for milk 0.05 mg/kg for fruit to 1.0 mg/kg for meat and offal

“Dioxin Incidents” – Economic Impacts

Dioxin and PCB incidents are expensive.

- Egg production enterprise with $5M hens would cost in excess of $30M.
- Broiler enterprise producing 3M broilers per week would exceed $85M.
- Times Beach, Missouri, road dust covered by oil spread. The cleanup cost = $110M million.
- The total costs of the Belgian food crisis are estimated up to $1000M.

Repeated “Dioxin” cases from only recent years:

- 1999 Belgian PCB/dioxin in eggs, poultry
- 1999 Clay and zeolithes for feed
- 2000 Choline chloride
- 2004 Potato pulp
- 2005 Hydrochloric acid
- 2007 Indian Guar Gum thickener
- 2008 Irish Pork Meat
- 2008 Italian Mozarella Cheese …
- ... still on today around Naples.
- 2011 German eggs, poultry contaminated from feed

Types of Matrices Requiring POP Analysis

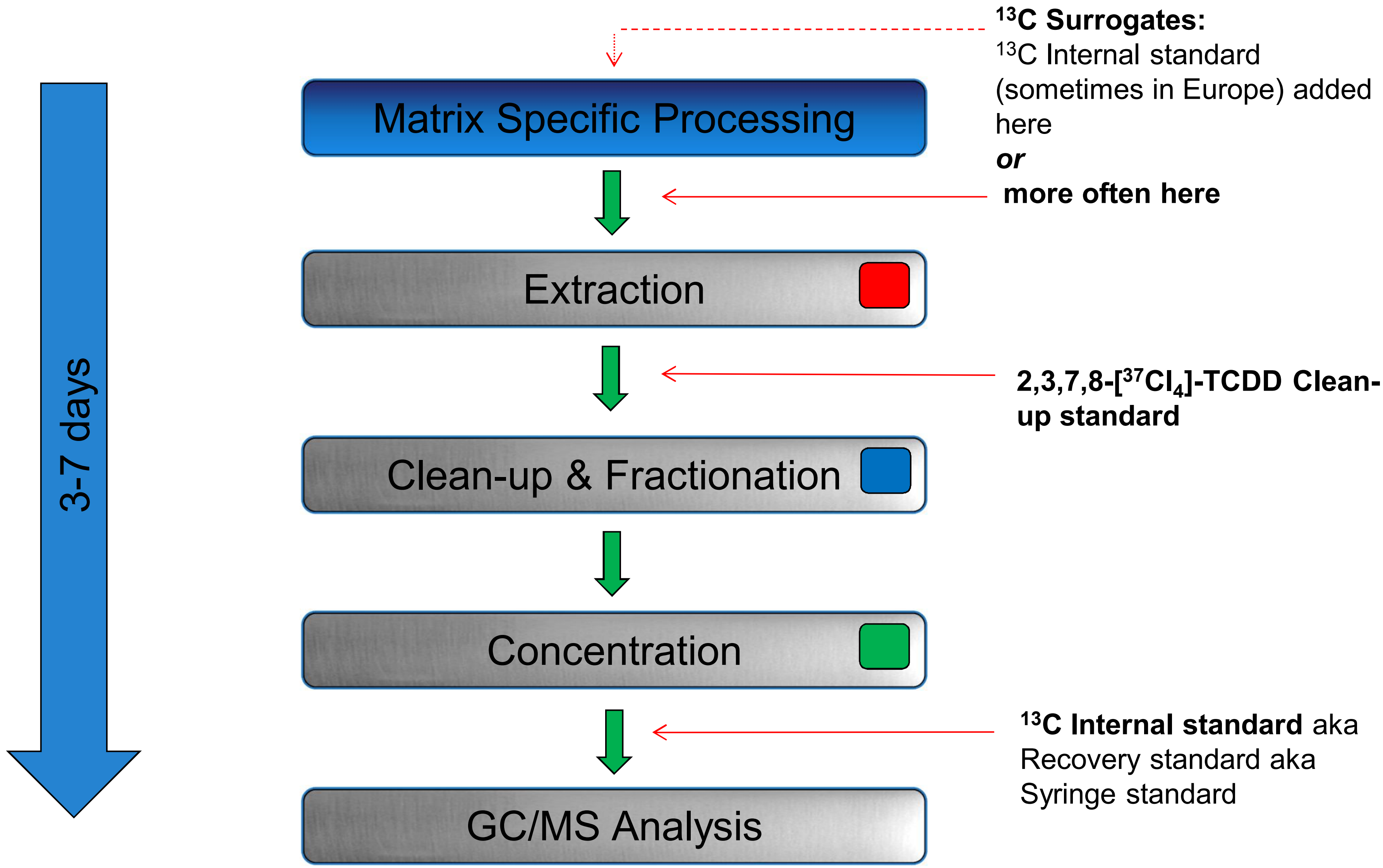
- Agricultural products & foodstuffs
  - Animal & fish products, oils & fats
- Animal feedingstuffs
  - Compound feeds and feed components
- Environmental samples
  - Incinerator fly ash, soil, grass and marine biota
- Biological samples
  - Human and animal blood, human milk & adipose tissue

How To Analyze Dioxins - *Complete POPs Solutions*

Step	Requirement	Solution
Laboratory PPE	Fume hoods, lab coats, gloves, eye protection etc.	Fisher Scientific
Laboratory Reagents	Alumina, Silica, Solvents, Acids	Fisher Scientific
Laboratory Apparatus	Freeze driers, ovens, balances, pipettes, vials, columns	Fisher Scientific
Sample Preparation	Extraction	Thermo Scientific™ Dionex™ ASE350 Accelerated Solvent Extraction
	Extract Fractionation / Purification	CAPE Technologies Sample Preparation Kits*
	Extract Concentration	Thermo Scientific™ Dionex™ Rocket™ Evaporator
Sample Analysis	Chromatography	Thermo Scientific™ Trace 1310 GC, TriPlus RSH & Trace Gold GC/MS columns and consumables
Sample Analysis	GC-HRMS or GC-MS/MS	Thermo Scientific™ DFS™ GC-HRMS or Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS
Data Handling & Reporting	Dioxin Data processing, QA/QC checking &reporting	Thermo Scientific™ TargetQua™n 3 Thermo Scientific™ Xcalibur™
	LIMS	Thermo Scientific™ Nautilus™ LIMS

- Thermo Fisher Scientific supports the entire dioxin analysis workflow

Typical Dioxin Workflow (EPA1613)



Background Info: Confirmation vs Screening

- **Confirmatory Analysis**
  - Unequivocal identification and quantification
  - Results can be used in prosecution

- **Screening Analysis**
  - Indication of levels only
  - Must be followed with confirmatory analysis

ThermoFisher SCIENTIFIC

What's New In EU Regulation For Dioxins?

Past

Commission Regulation (EU) No 252/2012 of 21 March 2012

- Specifies use of GC-HRMS for confirmatory dioxin analysis
- GC-MS/MS was allowed a screening technique.

Present

Commission Regulation (EU) No 589/2014 of 2 June 2014

- Specifies use of **GC-HRMS** **or** **GC-MS/MS** for confirmatory dioxin analysis
- GC-MS/MS is "an appropriate confirmatory method for checking compliance with the maximum", only.
- **GC-HRMS remains the recommended technique for "determination of low background levels in food monitoring, following of time trends, exposure assessment of the population".**

Thermo Fisher SCIENTIFIC

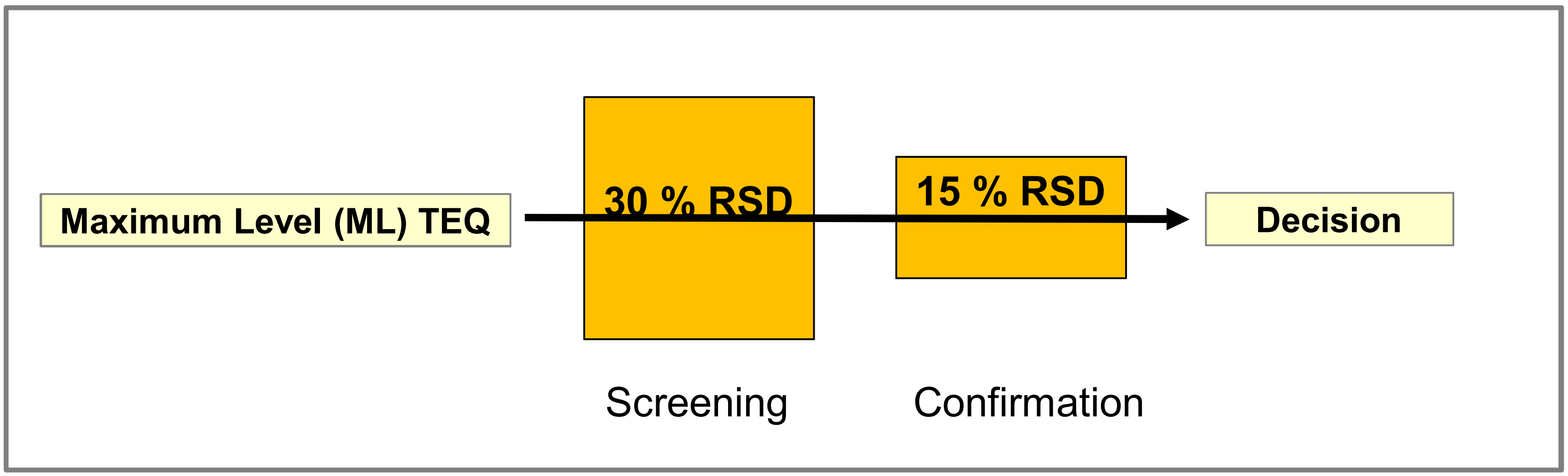
Definitions of Screening and Confirmation

Screening

- High throughput – fast
- Low cost
- Low false negative rate (< 1%)
- False positives acceptable (<5%)
- Relaxed precision (<30 % RSD)
- Sensitivity 25% below of ML

Confirmation

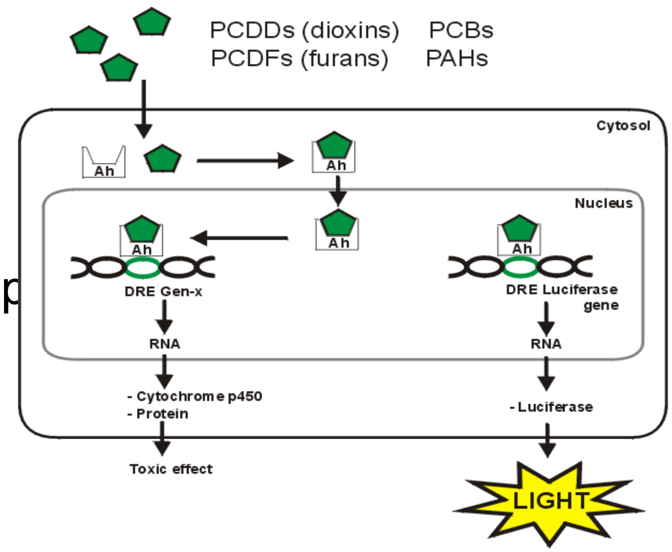
- Unequivocal identification
- High precision (<15 % RSD)
- Higher sensitivity to monitor lower background and action levels.
- High resolution necessary



Source: EU Directives 96/23/EC, 1883/2006

Options for screening POPs in Food & Biological Samples

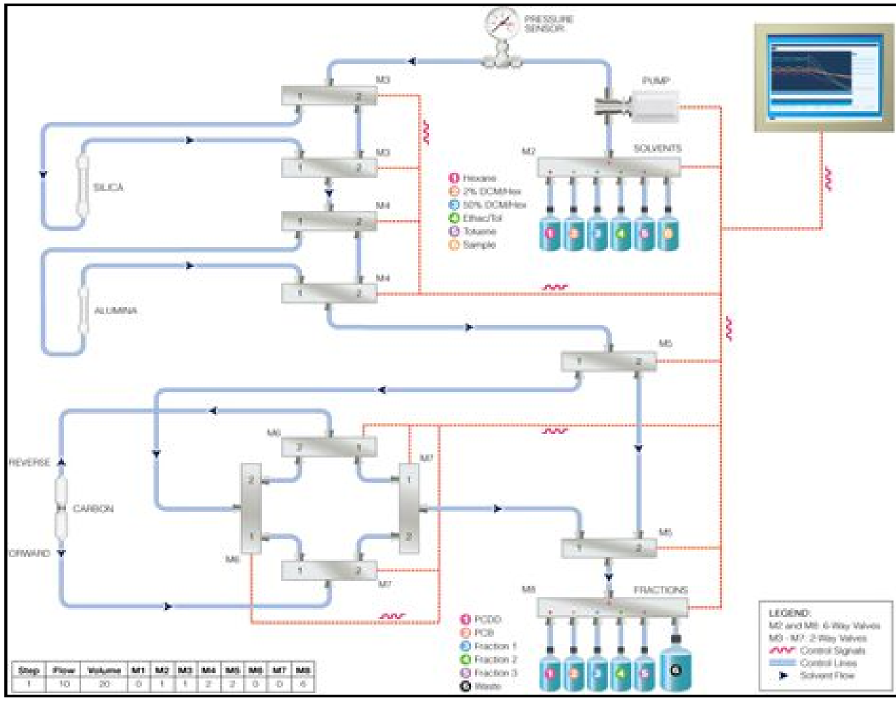
- **DR-CALUX**
  - Receptor-based assay
  - Gives indication of total toxicity of extracts
  - Requires adequate sample concentration & cleanup
  - Lacks flexibility – only PCDDs/PCDFs and PCBs determined



- **GC-MS/MS (also for confirmation)**
  - Instrumental
  - Can measure individual congeners & TEQs
  - Requires adequate sample concentration & cleanup
  - High flexibility – can be used for all POPs as well as other residues & contaminants

ThermoFisher SCIENTIFIC

Extraction & Cleanup for Dioxins & PCBs – HR-GC-MS



Time to response: 3-5 days

Extraction & Cleanup for Dioxins & PCBs – GC-MS/MS

Sample spiking and freeze drying

↓

Fat extraction with hexane using ASE

↓

Concentration to 50 mL

↓

SPE on Extrelut™ column impregnated with sulphuric acid

↓

Alumina clean-up

↓

Elution with DCM

↓

Concentration to 50 µl

↓

GC/MS/MS analysis

Provided by G. Brambilla, ISS Rome, Italy

GC-MS/MS vs. GC-HRMS

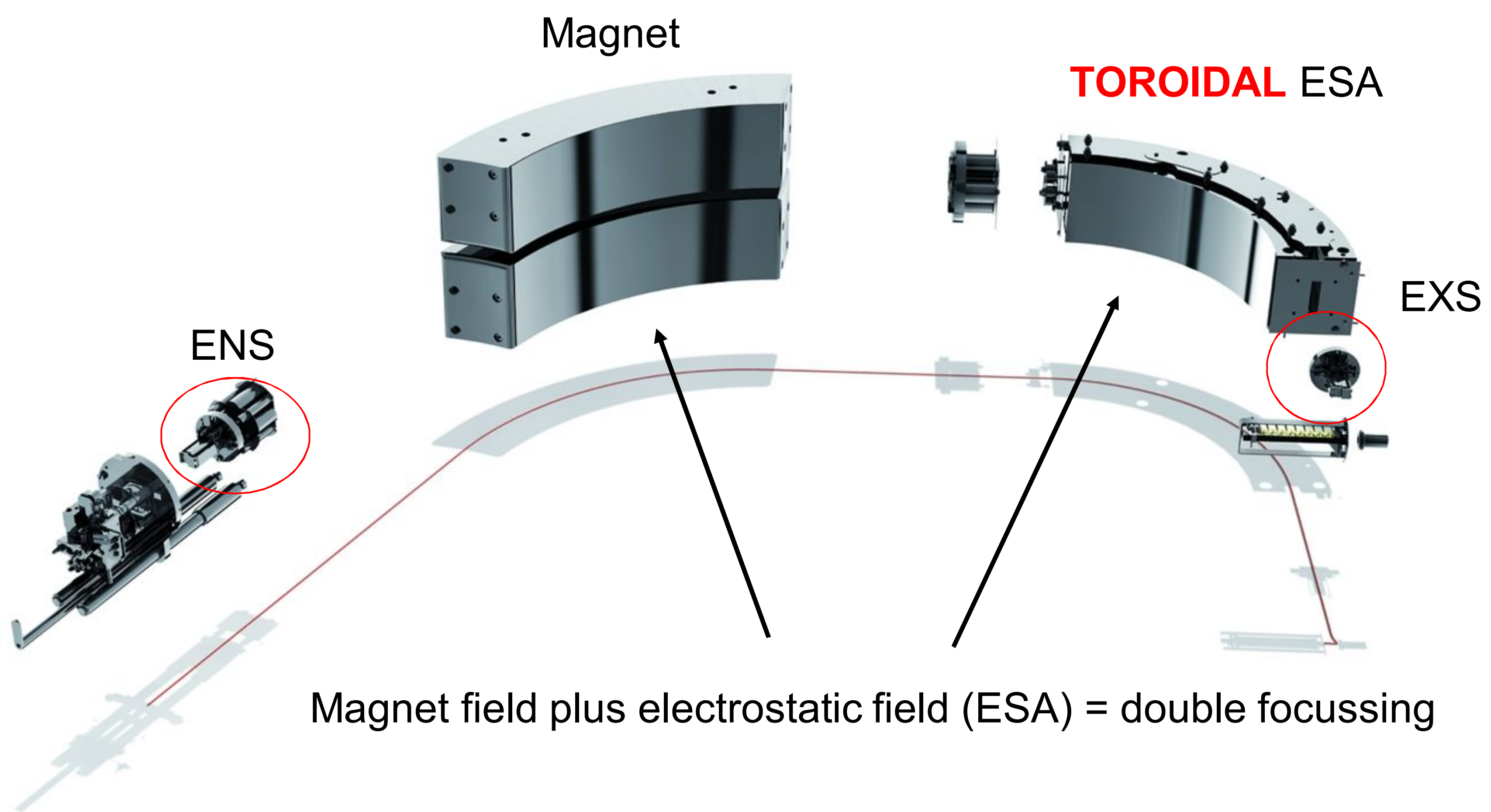
TSQ 8000 Evo	DFS
GC-MS/MS	GC-HRMS
Triple Quadrupole MS	Magnetic Sector HRMS
High performance, easy to use MS/MS for non-experts	High-Resolution Full Scan and SIM
Target Analysis in Complex Matrix	Dioxins, PCBs, Other POPs
Pesticides in Food, Drugs in body fluid	EPA 1613, 8270A, 8290

Magnetic Sector HRMS: Thermo Scientific DFS

- **Benchtop-like** operation (autotune)
- **Most compact** instrument in its class
- **Toroidal ESA** for highest **sensitivity**
- **Low power** consumption
- **Unique Dual GC** configuration
- **Fit-for-purpose** data evaluation software

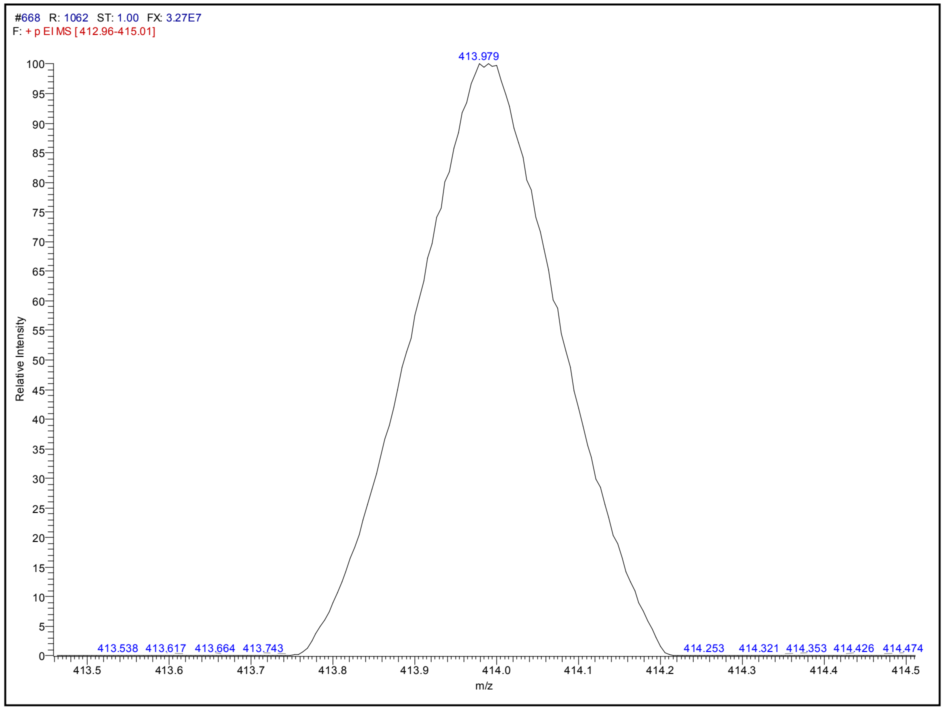
Thermo Fisher SCIENTIFIC

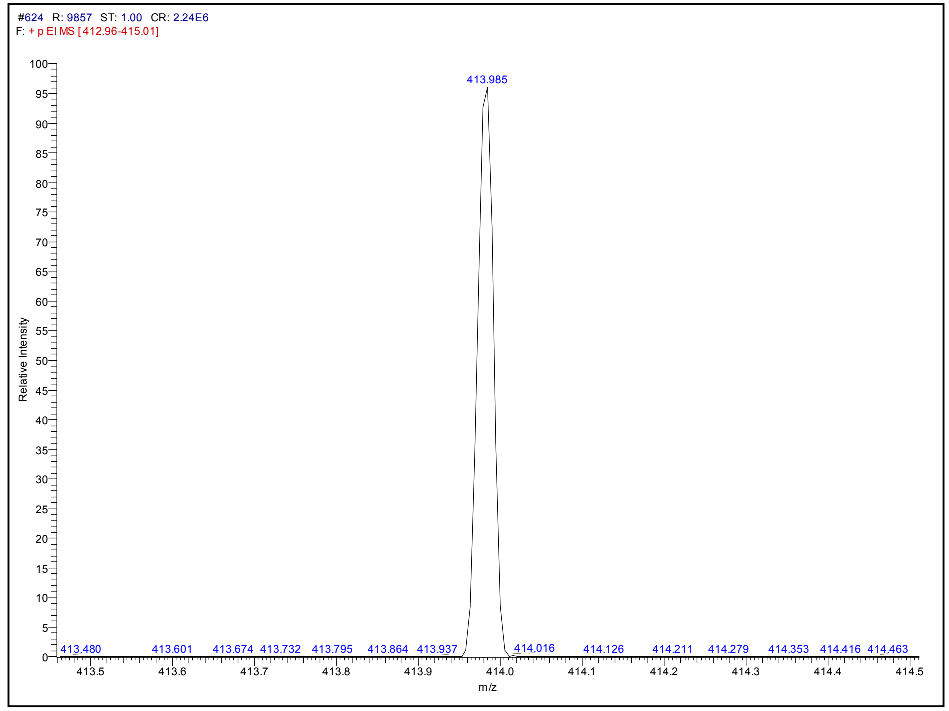
Magnetic Sector HRMS: Ion Optics of the DFS



Magnet field plus electrostatic field (ESA) = double focussing

Magnetic Sector HRMS: Resolution Setting

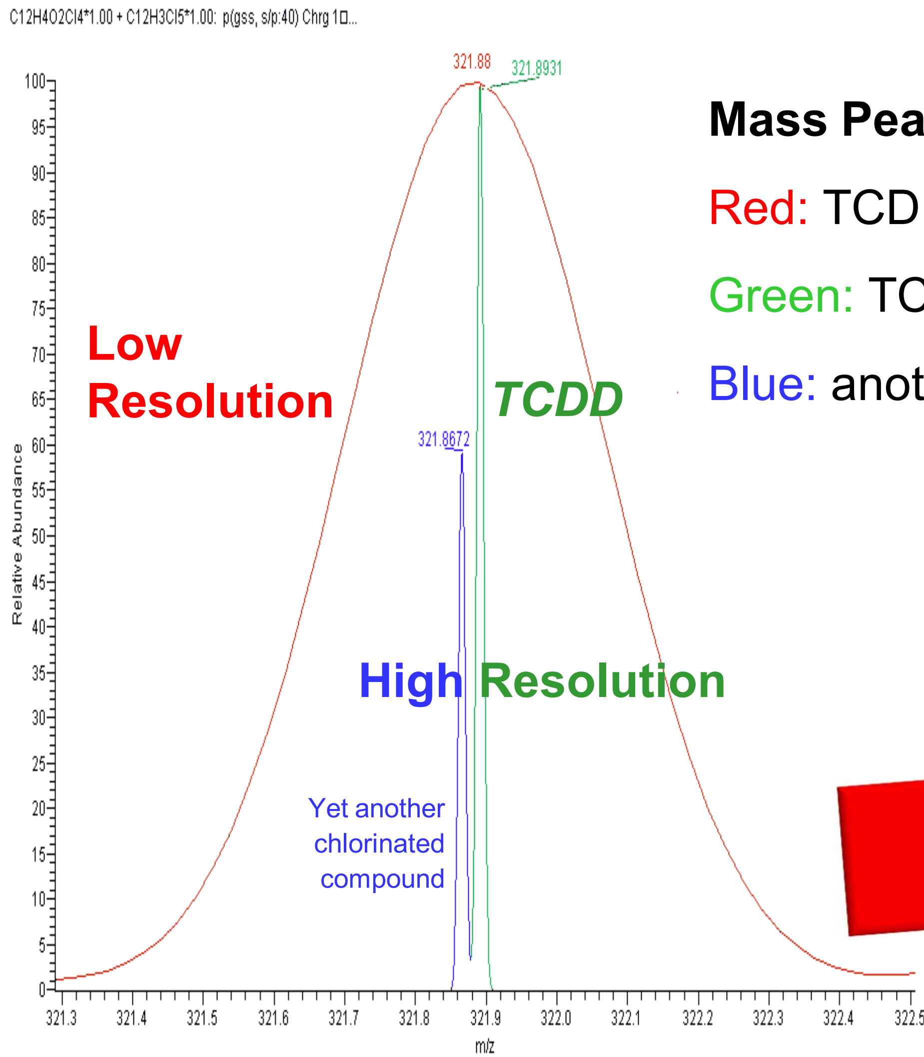




R 1000 **R 10,000**

DFS: Tune peak of FC43 calibrant, mz 414, screenshots at R 1,000 and R 10,000

High Selectivity with 'High Resolution'



Mass Peaks at Different Resolution Settings:

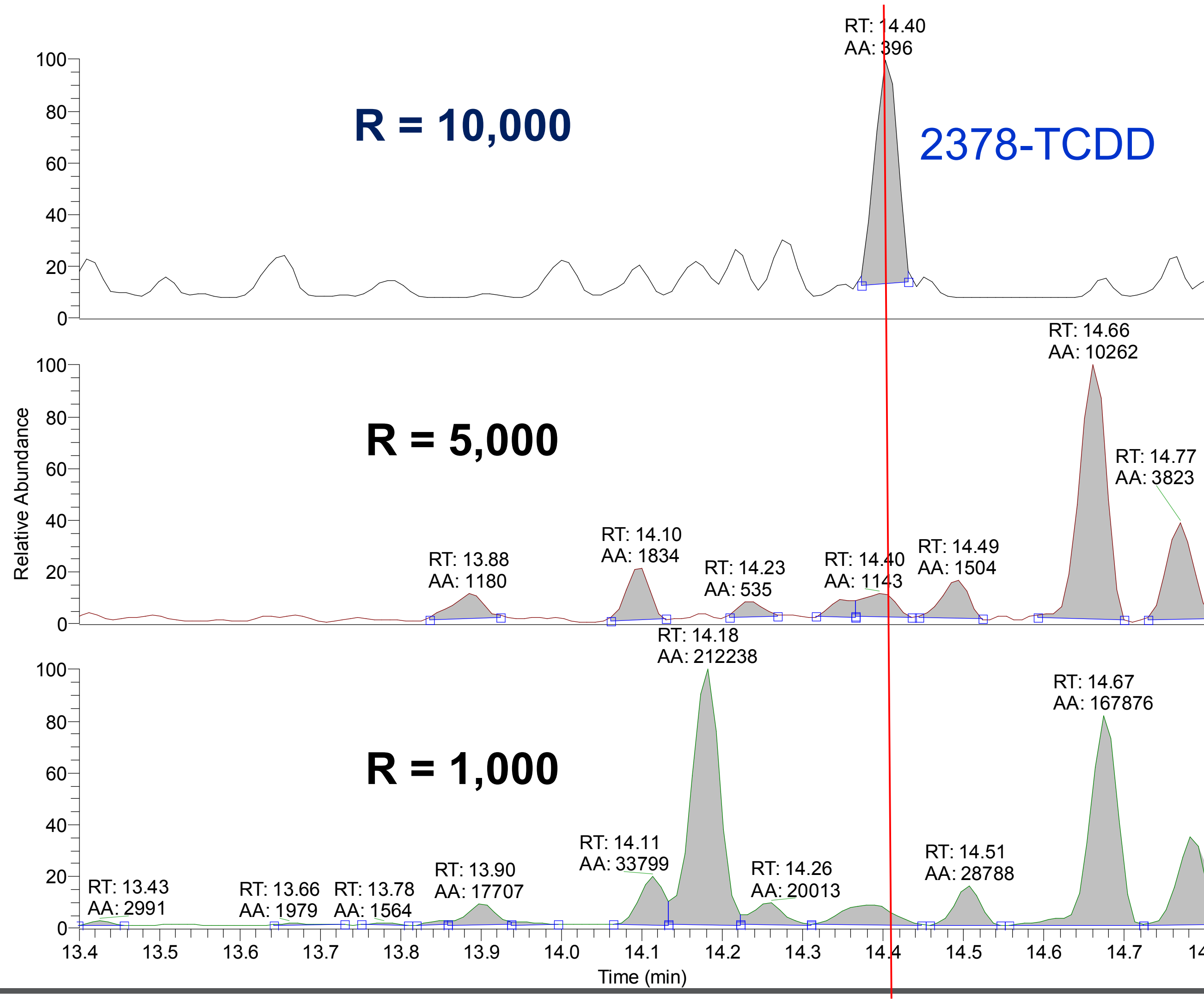
Red: TCDD mass trace at R 1.000

Green: TCDD mass trace at R 10.000

Blue: another chlorinated compound mass trace

Accurate masses are used for target compound analysis

Need for High Resolution GC-MS – Blood Sample



DFS
GC-HRMS

Quantitation Mass
m/z 319.8965

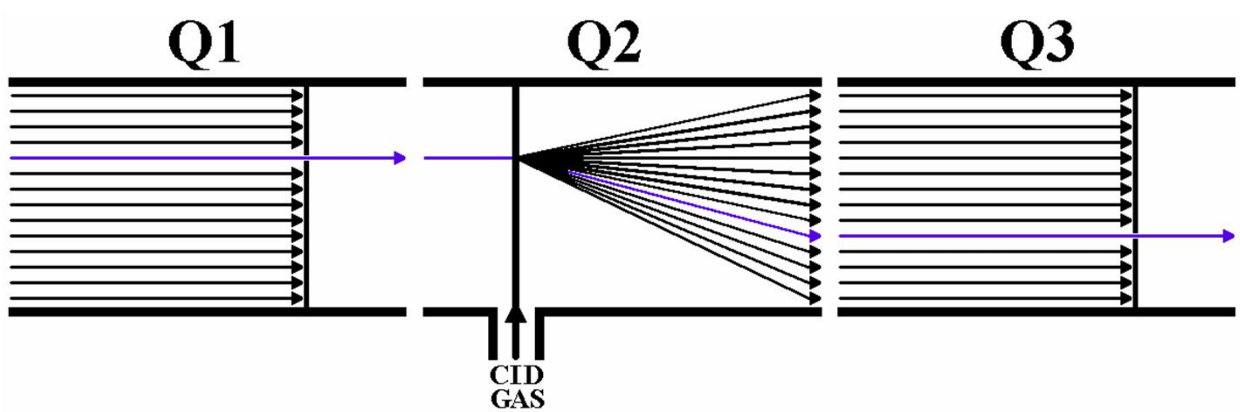
For the native TCDD

Chemical noise from matrix interferences shows up at lower mass resolution

ONLY HRMS at R=10,000 selects the dioxin peak.

Triple Quadrupole TSQ8000 Evo for POPs analysis

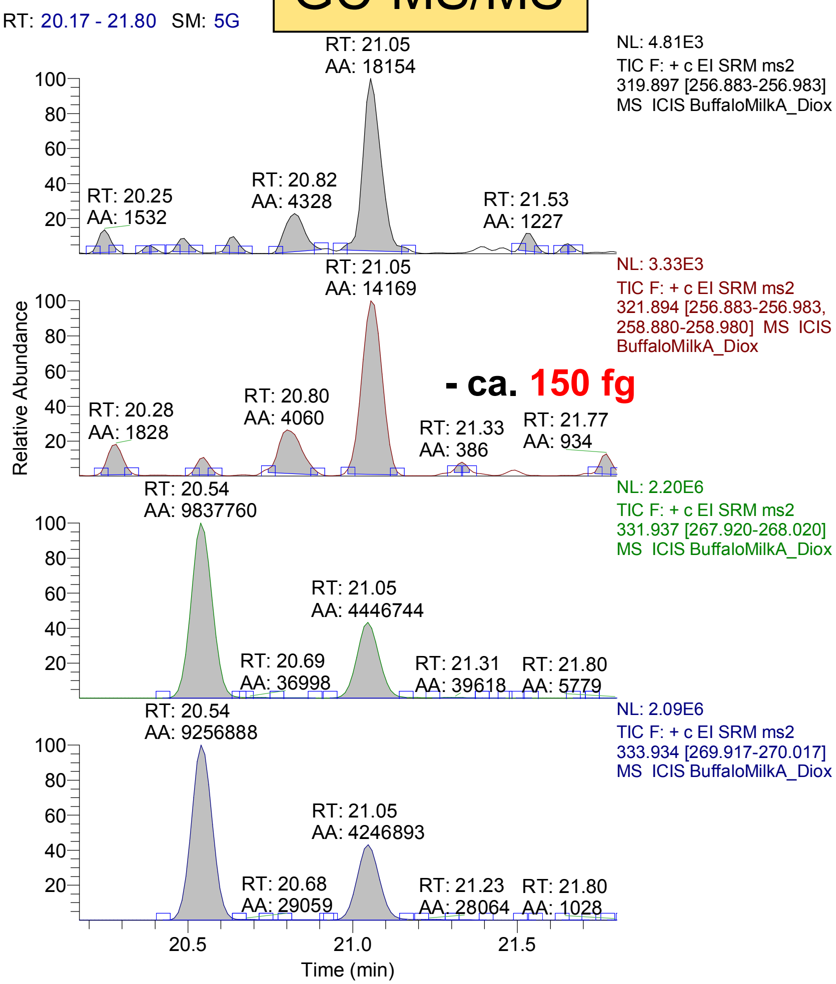
“Monitor a Transition”
from *Precursor in Q1* to *Product Ion in Q3*

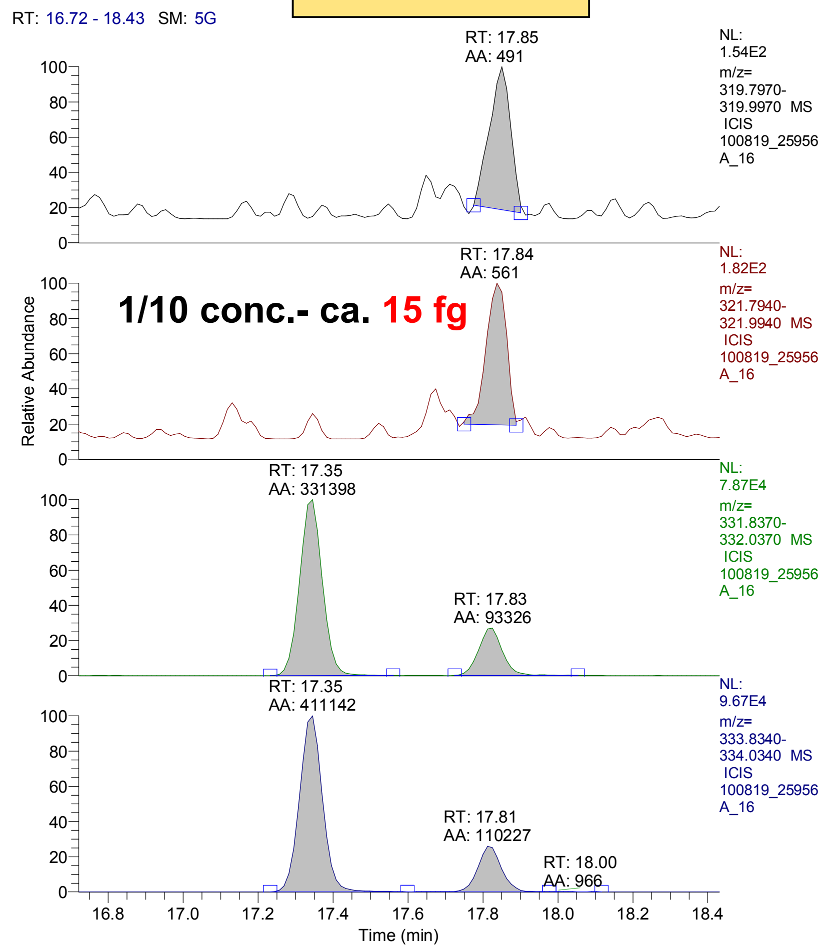


Buffalo Milk Sample, TCDD Mass Traces

GC-MS/MS (5 µl injection PTV solv. split) → GC-HRMS 1/10 concentration

GC-MS/MS → GC-HRMS



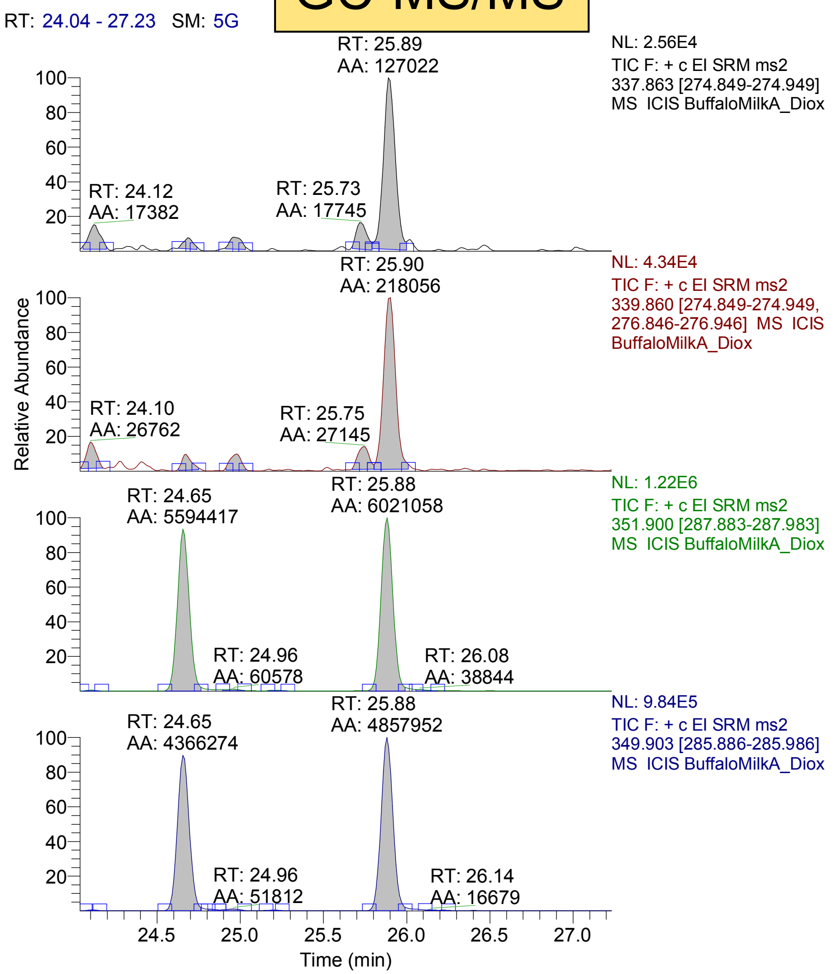


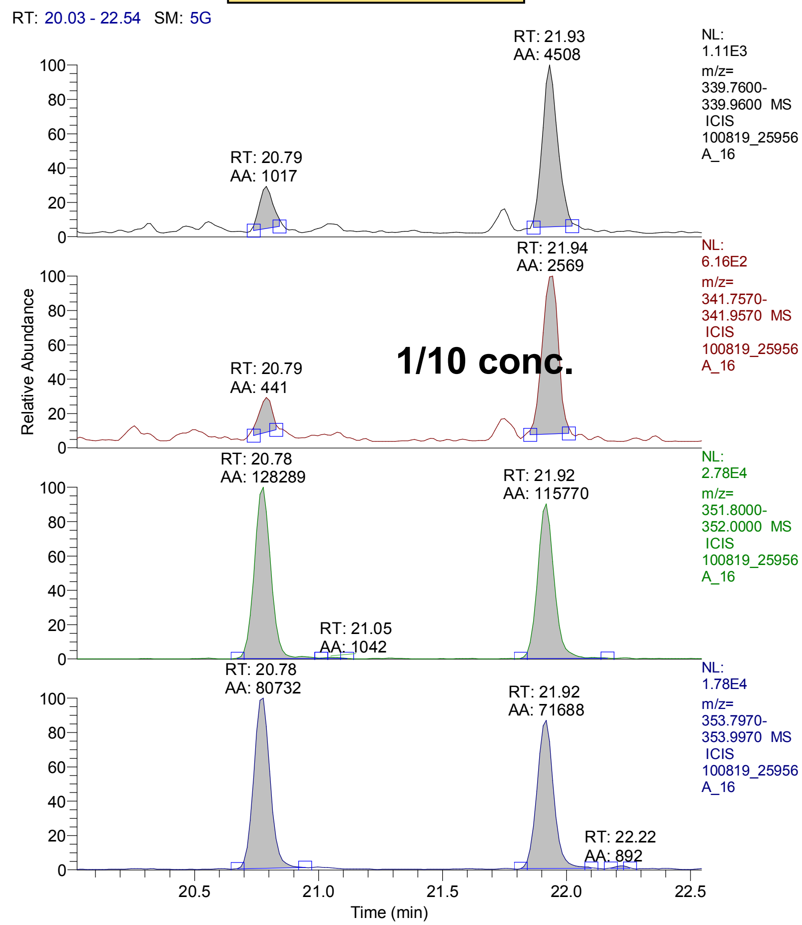
Please note: Peak areas are not comparable between GC-MSMS and GC-HRMS

Buffalo Milk Sample, PCDF

GC-MS/MS (5 µl injection PTV solv. split) → GC-HRMS 1/10 concentration

GC-MS/MS → **GC-HRMS**

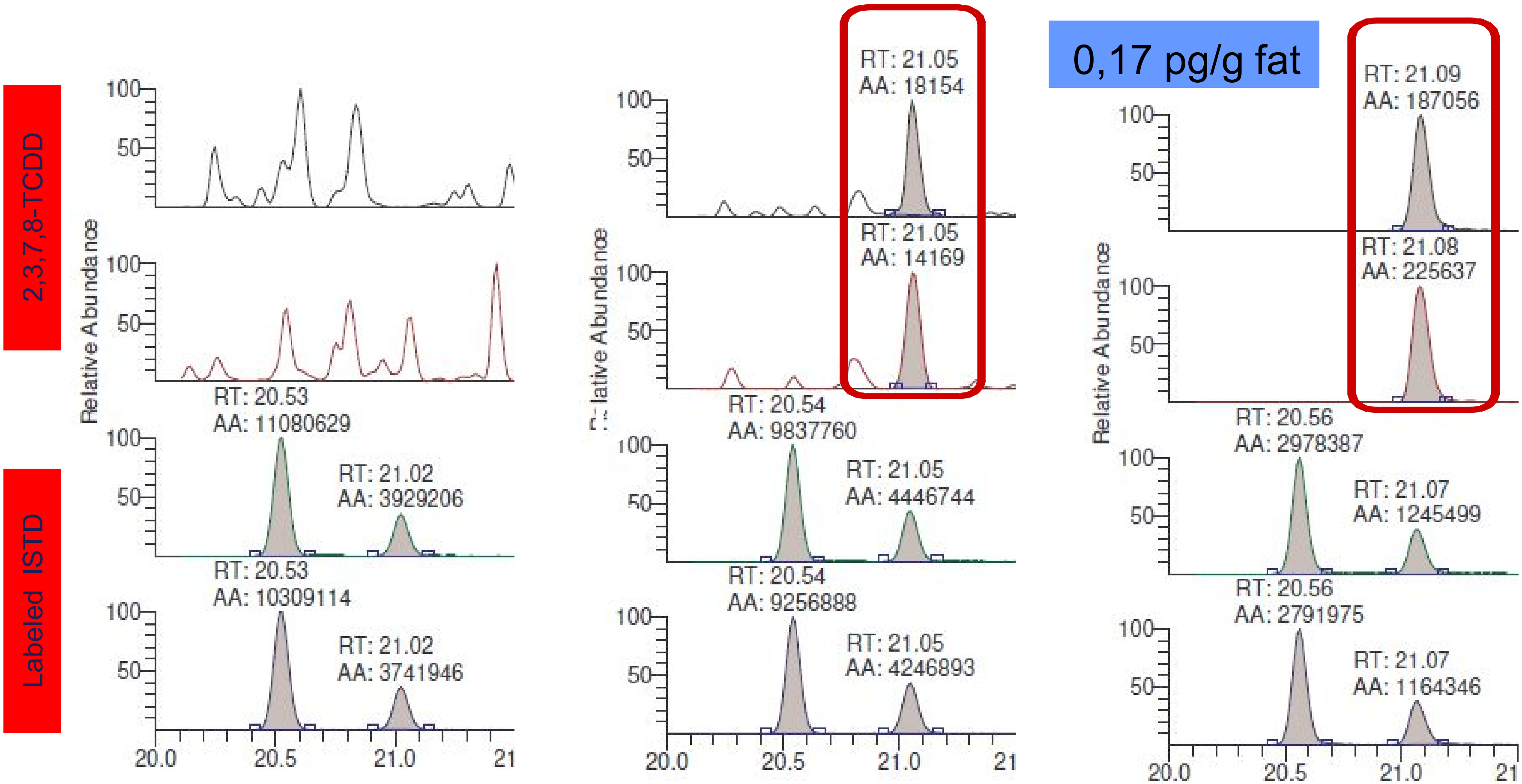




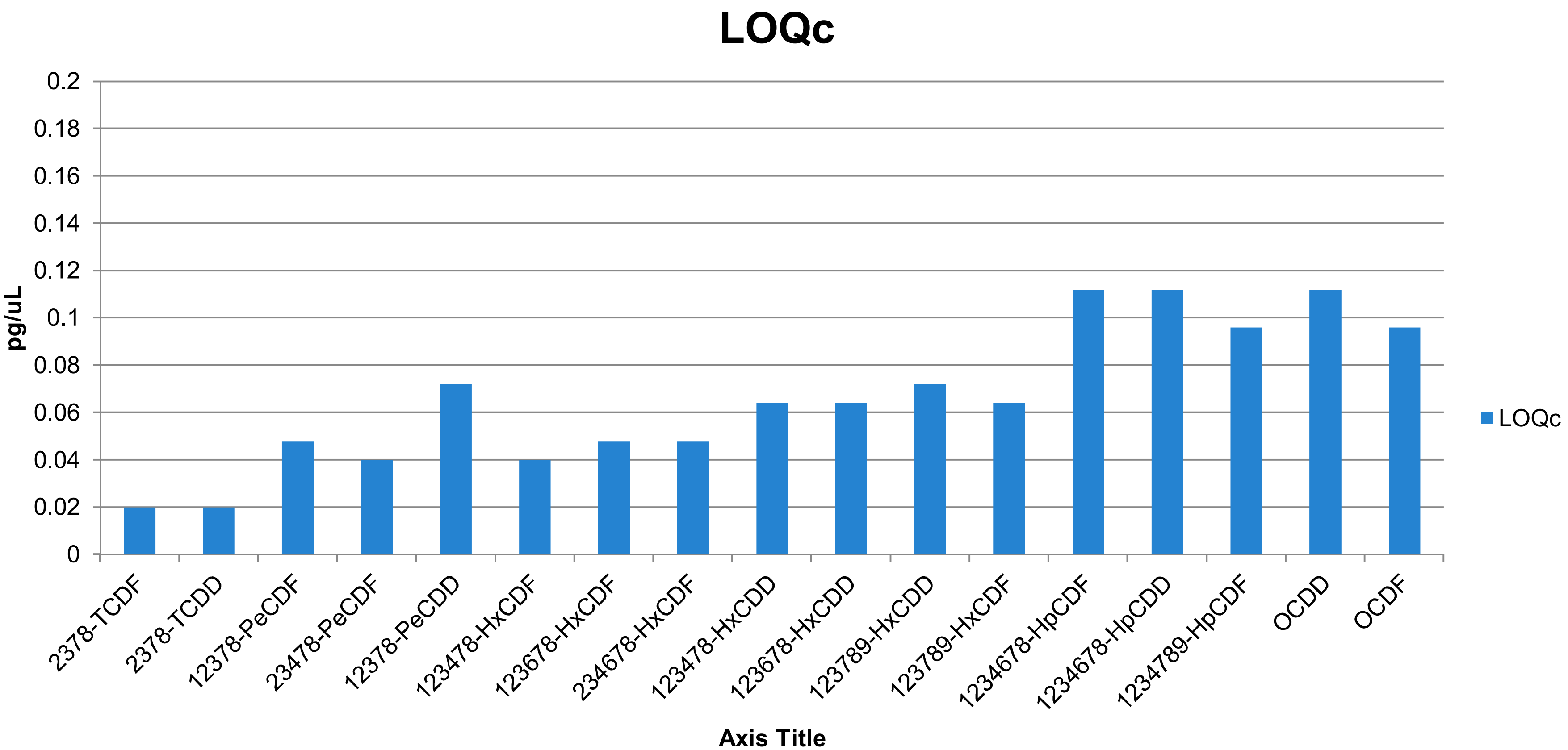
Please note: Peak areas are not comparable between GC-MSMS and GC-HRMS

TSQ 8000 Evo- TCDD in Buffalo Milk Samples

Blank(GC-MS/MS) | Buffalo Milk (GC-MS/MS) | Buffalo Milk (GC-HRMS)



Limit of Quantitation (LOQc) for GC-MS/MS

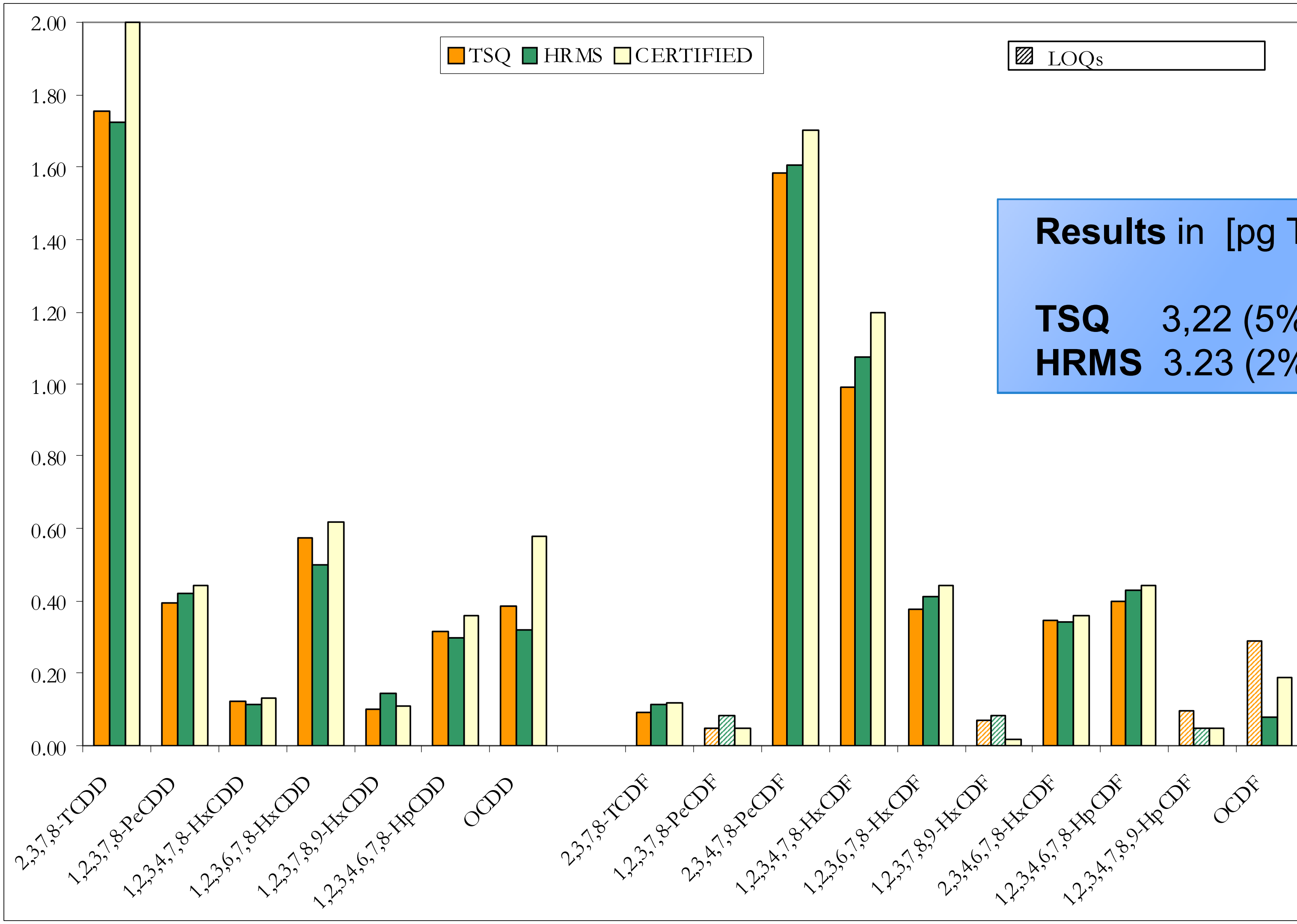


Practically determined LOQc for PCCD/Fs

Serially diluted CS3 standards from CS3/100-CS3/1000

Ion ratio stability within ±15% tolerance

TSQ 8000 Evo GC-MS/MS vs. GC-HRMS Analysis of CRM

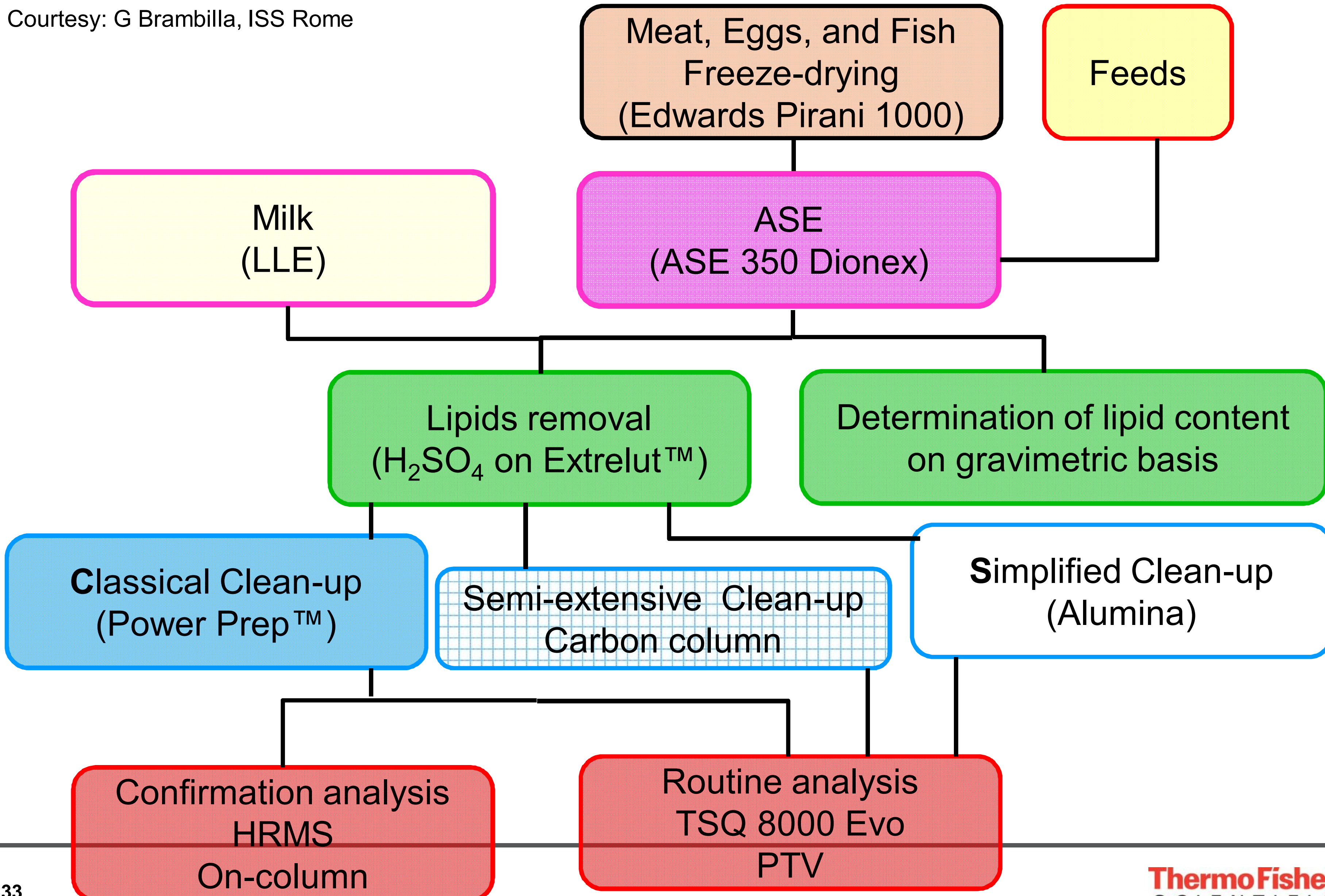


Results in [pg TEQ/g fat]

TSQ 3,22 (5% RSD)
HRMS 3.23 (2% RSD)

Sample preparation proposals

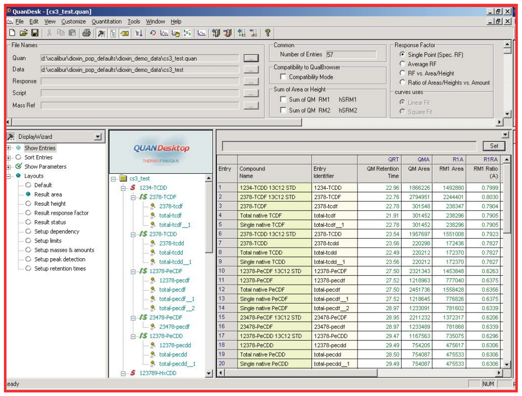
Courtesy: G Brambilla, ISS Rome



TargetQuan - Quantitation Software Suite

Features for dioxin applications:

- Toxicity equivalents (TEQ) - According to NATO and WHO definition, including
  - ***Lower bound calculation***: requires using zero for the contribution of each non-quantified congener
  - ***Medium bound calculation***: requires using half of the limit of quantification
  - ***Upper boundary calculation***: requires using the limit of quantification
- User definable summation
  - Calculated amounts or TEQs
  - Reporting the sum TEQ values
- Isotopic ratio confirmation
  - One quantitation mass and up to two masses based on abundance
- EPA 1613 Rev.B compliant
  - Allows quantification based on average response of selected compound
  - Retention time correction



Thermo Fisher SCIENTIFIC

Applications and Methods

Application		Norm	Thermo Scientific TSQ 8000 Evo GC-MS/MS	Thermo Scientific DFS GC-HRMS
Food	EU Regulation	EU Regulatory Feed Control (at ML)	Approved	Approved
Food	EU Methods	EN 16215	Not in method	Approved
Food	EURL Recommendation	Background food studies (<1/5th EU ML)	Not recommended	Recommended
Clinical	EURL Recommendation	Human studies at trace levels	Not recommended	Recommended
Environmental	US Method	US EPA 8280A	Approved	Not in method
Environmental	EU Method	EN 1948	Not in method	Approved
Environmental	US Method	US EPA 1613 B for strict EPA compliance	Not in method	Approved
Environmental	US Method	US EPA Method 23	Not in method	Approved
Environmental	US Method	US EPA Method 8290	Not in method	Approved
Environmental	Japan Method	JIS K0311	Not in method	Approved
Environmental	Japan Method	JIS K0312	Not in method	Approved

- Any laboratory following EU approach will now be able to use GC-MS/MS for ML compliance control

- Laboratories strictly following US/Japan methods and regulation will continue to use GC-HRMS

Conclusions - Why MS/MS and HRMS for POPs?

- **GC-MS/MS** – Versatile and Easy to use
  - Versatile instrumentation – no need to be dedicated
  - High sensitivity and selectivity for matrix samples
  - Provides TEQ results for PCDD/Fs and DL-PCBs
  - Fast - with high throughput capability, automated runs
- **GC-HRMS** – Unequivocal confirmation
  - Compliant with international regulations
  - Highest sensitivity and precsion for ultra-low level analyses
  - Highly productive routine method – high throughput operation
- **GC-MS/MS + GC-HRMS** – Work seamlessly together
  - Use integrated sample prep workflow for screening and confirmation
  - High precision data using labelled internal standards
  - Common TargetQuan software platform

Supplemental

www.thermofisher.com/pops

ThermoFisher SCIENTIFIC

Search All | Recently Viewed Items | Contact Us | Sign In | Quick Order

Applications & Techniques | Shop All Products | Services & Support | About Us | Cloud

Home › Industrial & Applied Science › Environmental › Water Analysis › Wastewater Analysis › Instruments for Persistent Organic Pollutant (POPs) Analysis

Instruments for Persistent Organic Pollutant (POPs) Analysis

‹ Wastewater Analysis

Instruments for Persistent Organic Pollutant (POPs) Analysis

Automated Discrete Analyzers

Colorimeters

Thermo Fisher Scientific has a strong commitment to supporting laboratories charged with the task of researching and monitoring POPs. Our commitment ensures high productivity, added value solutions for some of the most challenging POPs determinations.

Resources

- Power Plant Water Analysis Products
- Drinking Water Quality Analysis Process Products
- ROSS pH Electrodes Brochure
- SampleManager LIMS™ product brochure

POPs Center of Excellence

Support

Unity Lab Services—Access to our expert service team, information on support plans, parts and consumables resources, and other instrument services.

thermo scientific | applied biosystems | invitrogen | fisher scientific | unity lab services

Sequence/Samples

- Two sequences were analysed on the system

1. PCDD/Fs consisting of:-
   - CSL, CS1→CS6 EN:1948 standards in duplicate
   - Samples (fish and egg) bracketed by blank and CSL injections
   - Total sequence length 103 injection over ~4 days

2. dl-PCBs consisting of: -
   - CS1→CS6 WP-CVS standards in duplicate
   - Samples (fish and egg) bracketed by CS4/100 diluted standards (up to 42 injections)
   - Serial dilutions of WP-CS4 standard (/100, /200, /400) to establish LOQ
   - Total sequence length 72 injections

GC and MS Conditions

TRACE 1310 GC Parameters	
Injection Volume (µL):	**3**
Liner:	LinerGOLD™ double taper (P/N: 453A1345-UI)
Inlet (°C):	280
Carrier Gas, (mL/min):	He, 1.2
Inlet Mode,	Splitless (split flow 120mL/min after 2 min)
Oven Temperature Program:	
Temperature 1 (ºC):	170
Hold Time (min):	0
Temperature 2 (ºC):	250
Rate (ºC/min):	15.4
Hold Time (min):	0
Temperature 3 (ºC):	285
Rate (ºC/min):	2.5
Hold Time (min):	0
Temperature 3 (ºC):	320
Rate (ºC/min):	10
Hold Time (min):	15
Total Run Time (min):	39.7

TSQ 8000 Evo Mass Spectrometer Parameters	
Transfer Line (°C):	300
Ionization Type:	EI
Ion Source(°C):	350
Electron Energy (eV):	40
Acquisition Mode:	Timed SRM with Dwell Time Prioritization
Acquired Masses (Da):	Optimised
Collision Energy (V):	Optimised

Calibration Data PCDD/Fs

EN:1948 CSL, CS1-CS6 PCDD/Fs

Congener (native)	R^2	Mean RF	Std.Dev	RSD(%)
2378-TCDF	0.9999	0.999	0.029	2.9%
2378-TCDD	0.9998	1.131	0.048	4.3%
12378-PeCDF	0.9996	1.046	0.020	1.9%
23478-PeCDF	0.9999	1.086	0.026	2.4%
12378-PeCDD	0.9996	1.121	0.043	3.9%
123478-HxCDF	0.9998	1.153	0.025	2.1%
123678-HxCDF	1.0000	1.152	0.034	3.0%
234678-HxCDF	0.9997	1.117	0.025	2.2%
123478-HxCDD	0.9996	1.175	0.057	4.9%
123678-HxCDD	0.9995	1.087	0.029	2.7%
123789-HxCDD	0.9986	1.062	0.039	3.7%
123789-HxCDF	0.9990	1.058	0.035	3.3%
1234678-HpCDF	0.9999	1.078	0.022	2.1%
1234678-HpCDD	0.9998	1.070	0.034	3.2%
1234789-HpCDF	0.9999	1.112	0.023	2.1%
OCDD	0.9999	1.082	0.035	3.2%
OCDF	0.9998	1.518	0.038	2.5%

NATIVE PCDDs & PCDFs	1948CSL (pg/µl)	1948CS1 (pg/µl)	1948CS2 (pg/µl)	1948CS3 (pg/µl)	1948CS4 (pg/µl)	1948CS5 (pg/µl)	1948CS6 (pg/µl)
2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.04	0.2	0.8	4	16	80	320
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	0.08	0.4	1.6	8	32	160	640
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.08	0.4	1.6	8	32	160	640
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.08	0.4	1.6	8	32	160	640
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.08	0.4	1.6	8	32	160	640
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.16	0.8	3.2	16	64	320	1280
Octachlorodibenzo-p-dioxin	0.16	0.8	3.2	16	64	320	1280
2,3,7,8-Tetrachlorodibenzofuran	0.04	0.2	0.8	4	16	80	320
1,2,3,7,8-Pentachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
2,3,4,7,8-Pentachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
1,2,3,4,7,8-Hexachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
1,2,3,6,7,8-Hexachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
1,2,3,7,8,9-Hexachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
2,3,4,6,7,8-Hexachlorodibenzofuran	0.08	0.4	1.6	8	32	160	640
1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.16	0.8	3.2	16	64	320	1280
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.16	0.8	3.2	16	64	320	1280
Octachlorodibenzofuran	0.16	0.8	3.2	16	64	320	1280
SAMPLING STANDARDS							
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
1,2,3,4,7,8,9-Heptachloro[$^{13}C_{12}$]dibenzofuran	32	32	32	32	32	32	32
EXTRACTION STANDARDS							
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzo-p-dioxin	32	32	32	32	32	32	32
Octachloro[$^{13}C_{12}$]dibenzo-p-dioxin	32	32	32	32	32	32	32
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
2,3,4,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
2,3,4,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16	16	16	16	16	16	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzofuran	32	32	32	32	32	32	32
Octachloro[$^{13}C_{12}$]dibenzofuran	32	32	32	32	32	32	32
SYRINGE STANDARDS							
1,2,3,4-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16	16	16	16	16	16	16

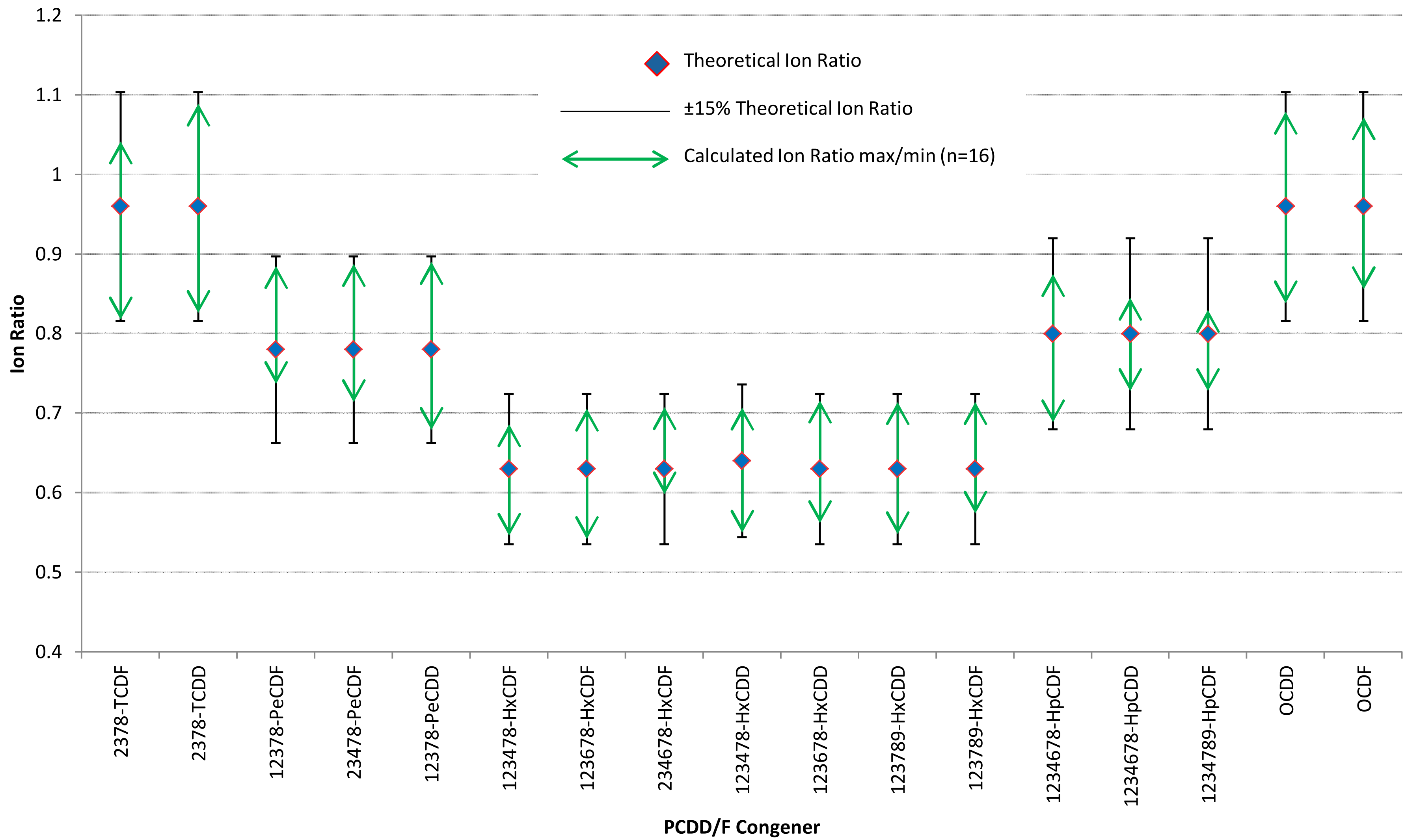
Calibration Data dl-PCBs

WP-CVS (WP-CS1 to WP-CS6) dl-PCBs

Congener (native)	R^2	Mean RF	Std.Dev	RSD(%)
PCB -81- tetrachlorobiphenyl	0.9998	0.998	0.025	2.5%
PCB -77-tetrachlorobiphenyl	0.9999	1.032	0.027	2.6%
PCB -123- pentachlorobiphenyl	1.0000	0.937	0.011	1.2%
PCB -118 pentachlorobiphenyl	1.0000	0.993	0.016	1.6%
PCB -114 pentachlorobiphenyl	1.0000	1.037	0.027	2.6%
PCB -105 pentachlorobiphenyl	1.0000	0.956	0.020	2.1%
PCB - 126-pentachlorobiphenyl	0.9997	0.999	0.049	4.9%
PCB -167- hexachlorobiphenyl	0.9999	1.052	0.020	1.9%
PCB -156- hexachlorobiphenyl	0.9999	1.064	0.027	2.6%
PCB -157- hexachlorobiphenyl	0.9998	1.020	0.022	2.2%
PCB -169- hexachlorobiphenyl	0.9998	1.014	0.025	2.4%
PCB -189- heptachlorobiphenyl	1.0000	1.088	0.019	1.7%

NATIVE PCBs	*IUPAC*	WP-CS1 (ng/ml)	WP-CS2 (ng/ml)	WP-CS3 (ng/ml)	WP-CS4 (ng/ml)	WP-CS5 (ng/ml)	WP-CS6 (ng/ml)	WP-CS7 (ng/ml)
3,3',4,4'-Tetrachlorobiphenyl	*77*	0.1	0.5	2.0	10	40	200	800
3,4,4',5-Tetrachlorobiphenyl	*81*	0.1	0.5	2.0	10	40	200	800
2,3,3',4,4'-Pentachlorobiphenyl	*105*	0.1	0.5	2.0	10	40	200	800
2,3,4,4',5-Pentachlorobiphenyl	*114*	0.1	0.5	2.0	10	40	200	800
2,3',4,4',5-Pentachlorobiphenyl	*118*	0.1	0.5	2.0	10	40	200	800
2',3,4,4',5-Pentachlorobiphenyl	*123*	0.1	0.5	2.0	10	40	200	800
3,3',4,4',5-Pentachlorobiphenyl	*126*	0.1	0.5	2.0	10	40	200	800
2,3,3',4,4',5-Hexachlorobiphenyl	*156*	0.1	0.5	2.0	10	40	200	800
2,3,3',4,4',5'-Hexachlorobiphenyl	*157*	0.1	0.5	2.0	10	40	200	800
2,3',4,4',5,5'-Hexachlorobiphenyl	*167*	0.1	0.5	2.0	10	40	200	800
3,3',4,4',5,5'-Hexachlorobiphenyl	*169*	0.1	0.5	2.0	10	40	200	800
2,3,3',4,4',5,5'-Heptachlorobiphenyl	*189*	0.1	0.5	2.0	10	40	200	800
MASS-LABELLED PCBs								
3,3',4,4'-Tetrachloro[$^{13}C_{12}$]biphenyl	*77L*	50	50	50	50	50	50	50
3,4,4',5-Tetrachloro[$^{13}C_{12}$]biphenyl	*81L*	50	50	50	50	50	50	50
2,3,3',4,4'-Pentachloro[$^{13}C_{12}$]biphenyl	*105L*	50	50	50	50	50	50	50
2,3,4,4',5-Pentachloro[$^{13}C_{12}$]biphenyl	*114L*	50	50	50	50	50	50	50
2,3',4,4',5-Pentachloro[$^{13}C_{12}$]biphenyl	*118L*	50	50	50	50	50	50	50
2',3,4,4',5-Pentachloro[$^{13}C_{12}$]biphenyl	*123L*	50	50	50	50	50	50	50
3,3',4,4',5-Pentachloro[$^{13}C_{12}$]biphenyl	*126L*	50	50	50	50	50	50	50
2,3,3',4,4',5-Hexachloro[$^{13}C_{12}$]biphenyl	*156L*	50	50	50	50	50	50	50
2,3,3',4,4',5'-Hexachloro[$^{13}C_{12}$]biphenyl	*157L*	50	50	50	50	50	50	50
2,3',4,4',5,5'-Hexachloro[$^{13}C_{12}$]biphenyl	*167L*	50	50	50	50	50	50	50
3,3',4,4',5,5'-Hexachloro[$^{13}C_{12}$]biphenyl	*169L*	50	50	50	50	50	50	50
2,3,3',4,4',5,5'-Heptachloro[$^{13}C_{12}$]biphenyl	*189L*	50	50	50	50	50	50	50
INTERNAL STANDARDS: MASS-LABELLED PCBs								
2,3',4',5-Tetrachloro[$^{13}C_{12}$]biphenyl	*70L*	50	50	50	50	50	50	50
2,3,3',5,5'-Pentachloro[$^{13}C_{12}$]biphenyl	*111L*	50	50	50	50	50	50	50
2,2',3,4,4',5'-Hexachloro[$^{13}C_{12}$]biphenyl	*138L*	50	50	50	50	50	50	50
2,2',3,3',4,4',5-Heptachloro[$^{13}C_{12}$]biphenyl	*170L*	50	50	50	50	50	50	50

Ion Ratio Stability



Ion ratio stability for all 17 PCDD/F congeners over 103 consecutive sample/standard injections [n=16 EN:1948 CSL (40fg/µL 2,3,7,8-TCDD)]

ThermoFisher SCIENTIFIC

Ion Ratio Stability

Congener	Ion ratio		Deviation	StDev	RSD(%)
	Theoretical	Calculated Mean			
2378-TCDF	0.96	0.93	-3%	0.063	6.7%
2378-TCDD	0.96	0.95	-2%	0.081	8.5%
12378-PeCDF	0.78	0.82	5%	0.047	5.8%
23478-PeCDF	0.78	0.81	3%	0.054	6.6%
12378-PeCDD	0.78	0.80	2%	0.067	8.4%
123478-HxCDF	0.63	0.62	-2%	0.039	6.3%
123678-HxCDF	0.63	0.63	0%	0.052	8.2%
234678-HxCDF	0.63	0.65	4%	0.039	6.0%
123478-HxCDD	0.64	0.63	-1%	0.054	8.6%
123678-HxCDD	0.63	0.64	1%	0.045	7.0%
123789-HxCDD	0.63	0.63	0%	0.047	7.5%
123789-HxCDF	0.63	0.65	3%	0.041	6.3%
1234678-HpCDF	0.80	0.78	-3%	0.056	7.3%
1234678-HpCDD	0.80	0.79	-1%	0.033	4.2%
1234789-HpCDF	0.80	0.77	-3%	0.034	4.3%
OCDD	0.96	0.94	-2%	0.079	8.4%
OCDF	0.96	0.97	1%	0.068	7.0%

Ion ratio stability over 103 consecutive sample/standard injections (n=16), CSL ran after every 4 matrix sample injections.

Calculated Amount

Congener	Concentration (pg/µL)		Deviation	StDev	RSD(%)
	Nominal	Calculated Mean			
2378-TCDF	0.040	0.043	9%	0.003	7.1%
2378-TCDD	0.040	0.039	-2%	0.003	7.2%
12378-PeCDF	0.080	0.080	0%	0.003	3.8%
23478-PeCDF	0.080	0.079	-1%	0.005	5.9%
12378-PeCDD	0.080	0.077	-4%	0.006	7.8%
123478-HxCDF	0.080	0.084	5%	0.004	5.1%
123678-HxCDF	0.080	0.083	4%	0.004	4.7%
234678-HxCDF	0.080	0.079	-1%	0.005	5.8%
123478-HxCDD	0.080	0.078	-2%	0.007	8.4%
123678-HxCDD	0.080	0.083	4%	0.004	5.0%
123789-HxCDD	0.080	0.082	2%	0.007	8.0%
123789-HxCDF	0.080	0.081	2%	0.008	9.2%
1234678-HpCDF	0.160	0.171	7%	0.009	5.1%
1234678-HpCDD	0.160	0.178	11%	0.007	4.1%
1234789-HpCDF	0.160	0.164	3%	0.010	6.4%
OCDD	0.160	0.176	10%	0.014	7.8%
OCDF	0.160	0.176	10%	0.010	5.5%

Calculated concentration over 103 consecutive sample/standard injections (n=16), CSL ran after every 4 matrix sample injections.

Limit of Confirmation (LOQc)

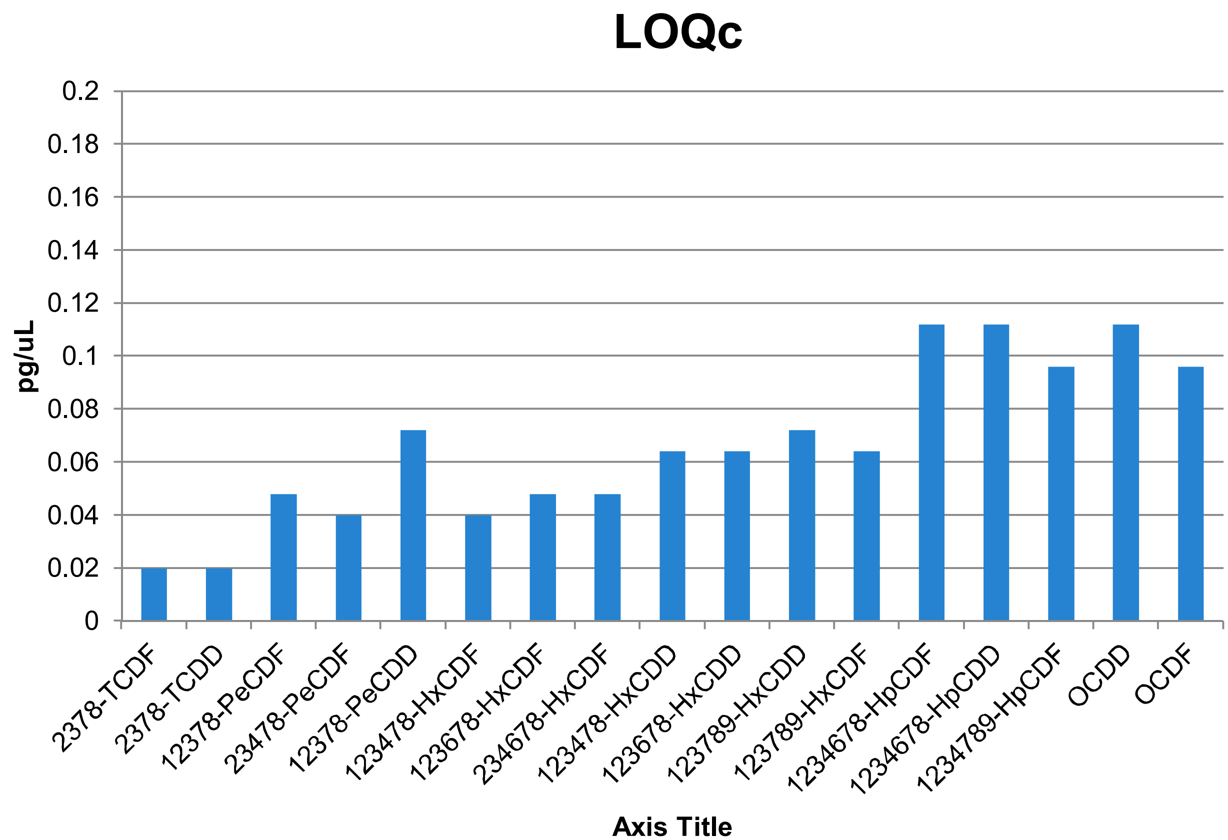


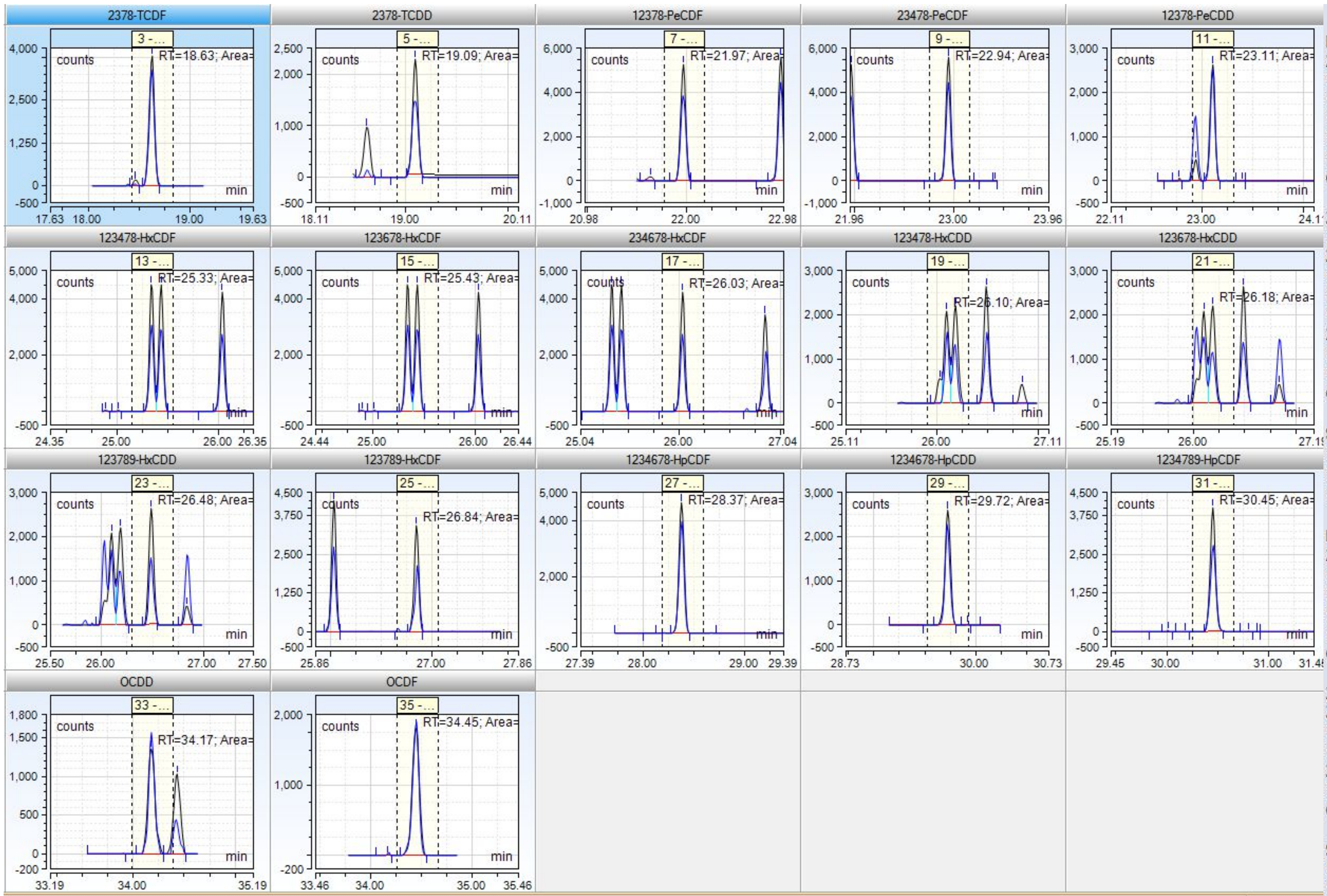
Figure 2. Practically determined LOQc for PCCD/Fs

Serially diluted CS3 standards from CS3/100-CS3/1000

Ion ratio stability within ±15% tolerance

	1948CS3 (pg/µl)
NATIVE PCDDs & PCDFs	
2,3,7,8-Tetrachlorodibenzo-p-dioxin	4
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	8
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	8
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	8
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	8
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	16
Octachlorodibenzo-p-dioxin	16
2,3,7,8-Tetrachlorodibenzofuran	4
1,2,3,7,8-Pentachlorodibenzofuran	8
2,3,4,7,8-Pentachlorodibenzofuran	8
1,2,3,4,7,8-Hexachlorodibenzofuran	8
1,2,3,6,7,8-Hexachlorodibenzofuran	8
1,2,3,7,8,9-Hexachlorodibenzofuran	8
2,3,4,6,7,8-Hexachlorodibenzofuran	8
1,2,3,4,6,7,8-Heptachlorodibenzofuran	16
1,2,3,4,7,8,9-Heptachlorodibenzofuran	16
Octachlorodibenzofuran	16
SAMPLING STANDARDS	
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,7,8,9-Heptachloro[$^{13}C_{12}$]dibenzofuran	32
EXTRACTION STANDARDS	
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzo-p-dio	32
Octachloro[$^{13}C_{12}$]dibenzo-p-dioxin	32
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzofuran	16
2,3,4,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
2,3,4,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzofuran	32
Octachloro[$^{13}C_{12}$]dibenzofuran	32
SYRINGE STANDARDS	
1,2,3,4-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16

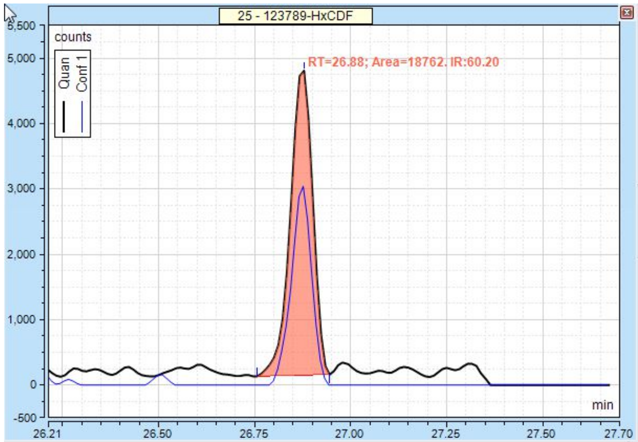
Chromatography – Lowest Calibration Standard (EN:1948 CSL)



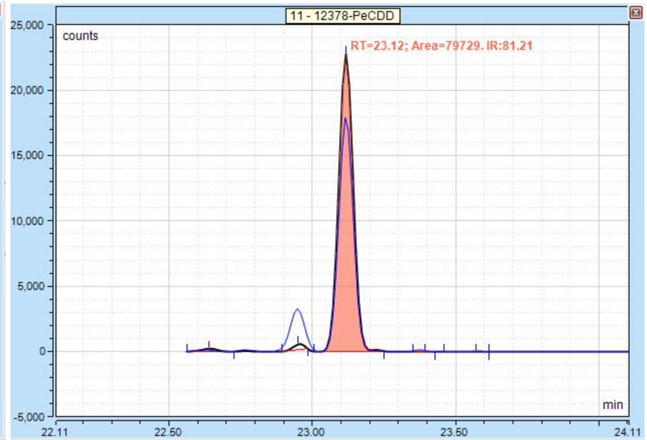
	1948CSL (pg/µl)
NATIVE PCDDs & PCDFs	
2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.04
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	0.08
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.08
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.08
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.08
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.16
Octachlorodibenzo-p-dioxin	0.16
2,3,7,8-Tetrachlorodibenzofuran	0.04
1,2,3,7,8-Pentachlorodibenzofuran	0.08
2,3,4,7,8-Pentachlorodibenzofuran	0.08
1,2,3,4,7,8-Hexachlorodibenzofuran	0.08
1,2,3,6,7,8-Hexachlorodibenzofuran	0.08
1,2,3,7,8,9-Hexachlorodibenzofuran	0.08
2,3,4,6,7,8-Hexachlorodibenzofuran	0.08
1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.16
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.16
Octachlorodibenzofuran	0.16
SAMPLING STANDARDS	
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,7,8,9-Heptachloro[$^{13}C_{12}$]dibenzofuran	32
EXTRACTION STANDARDS	
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzo-p-dioxin	32
Octachloro[$^{13}C_{12}$]dibenzo-p-dioxin	32
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzofuran	16
2,3,4,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
2,3,4,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	16
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzofuran	32
Octachloro[$^{13}C_{12}$]dibenzofuran	32
SYRINGE STANDARDS	
1,2,3,4-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	16

Natives only

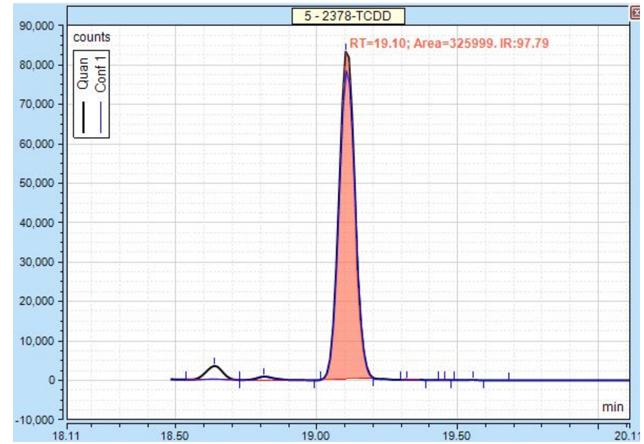
Sample – Selected Chromatography And Calculations



123789-HxCDF – "below" LOQc



12378-PeCDD – 0.162 pg/g WHO-TEQ



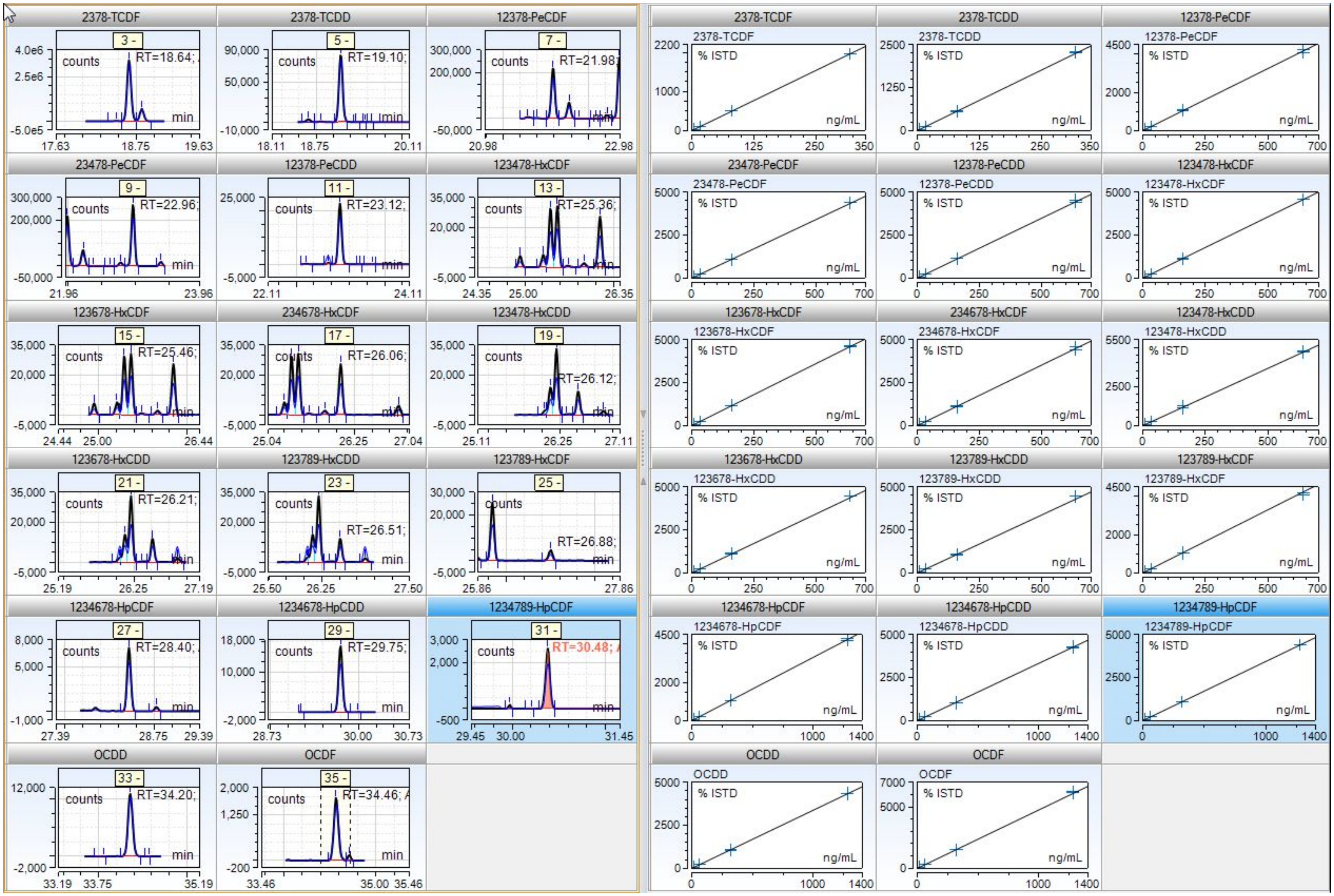
2378-TCDD – 0.193 pg/g WHO-TEQ

Peak Name	Retention Time min	Amount pg/µL	LOQc pg/uL	WHO-TEF (2005)	WHO-TEQ(2005) pg/g	Peak Confirmation
2378-TCDF	18.642	12.8690	0.02	0.1	0.512	Valid
2378-TCDD	19.103	0.4864	0.02	1	0.193	Valid
12378-PeCDF	21.977	0.7828	0.048	0.03	0.009	Valid
23478-PeCDF	22.956	2.1316	0.04	0.3	0.254	Valid
12378-PeCDD	23.117	0.4070	0.072	1	0.162	Valid
123478-HxCDF	25.356	0.1276	0.04	0.1	0.005	Valid
123678-HxCDF	25.456	0.1242	0.048	0.1	0.005	Valid
234678-HxCDF	26.056	0.1160	0.048	0.1	0.005	Valid
123478-HxCDD	26.118	0.0938	0.064	0.1	0.004	Valid
123678-HxCDD	26.207	0.2346	0.064	0.1	0.009	Valid
123789-HxCDD	26.507	0.0825	0.072	0.1	0.003	Valid
123789-HxCDF	26.878	0.0301	0.064	0.1	0.001	nd<LOQc
1234678-HpCDF	28.396	0.1036	0.112	0.01	0.000	nd<LOQc
1234678-HpCDD	29.747	0.4449	0.112	0.01	0.001	Valid
1234789-HpCDF	30.475	0.0453	0.096	0.01	0.000	nd<LOQc
OCDD	34.197	0.4003	0.112	0.0003	0.000	Valid
OCDF	34.457	0.0417	0.096	0.0003	0.000	nd<LOQc
				Sum	**1.163**	**pg/g**

Fish sample WHO-TEQ (2005) – 78.6g Fresh weight – HR-GCMS 1.29pg/g

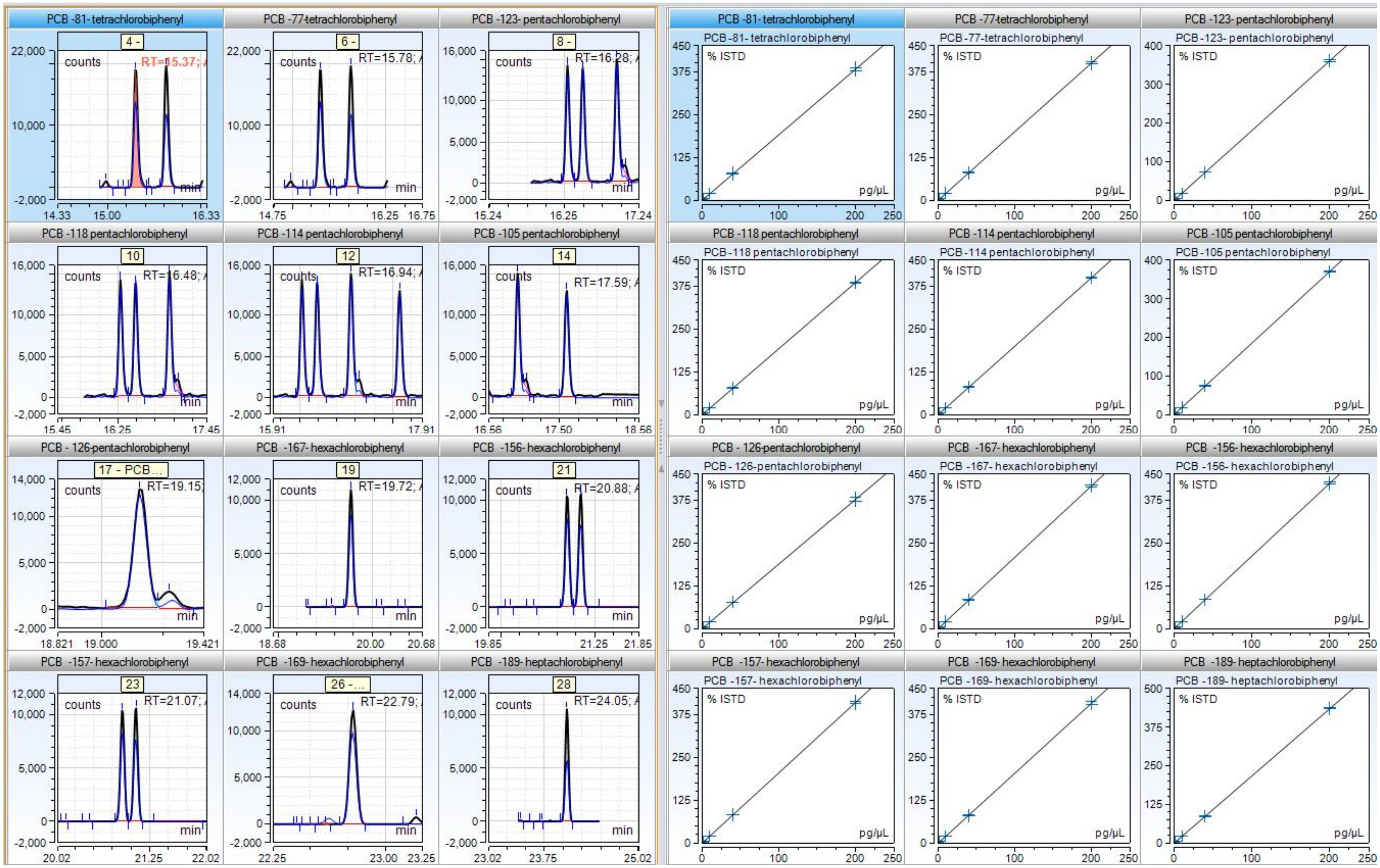
Thermo Fisher SCIENTIFIC

Sample Chromatography And Calibration – PCDD/Fs



Fish sample WHO-TEQ (2005) 1.16pg/g – 78.6g Fresh weight

Standard Chromatography And Calibration – dl-PCBs (WP-CS1)



Thermo Fisher SCIENTIFIC

Dioxin Analysis Legislation - Approaches to Dioxin Analysis

- Europe
  - Performance based approach.
  - Regulated technology and defined performance and quality criteria.
  - Methods are provided as a guide and can be modified.
  - Levels in food are regulated with strict maximum limits.
  - Emission levels are regulated.

- North America & Japan
  - Method based approach
  - Regulatory agencies (such as USEPA) provide complete methods to be followed.
  - Deviations to the method are possible. But must be documented closely.
    - Significant deviations from methods can result in the analysis being challenged or becoming unsellable in market.
  - Levels in food are not regulated.
  - Emission guidelines are given.

- ROW adopt variety of approaches,
- dependent on regulatory and/or business requirement

Background Info: Confirmation vs Screening

- **Confirmatory Analysis**
  - Unequivocal identification and quantification
  - Results can be used in prosecution

- **Screening Analysis**
  - Indication of levels only
  - Must be followed with confirmatory analysis

What Has Been Happening In EU Dioxin Analysis?

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[5], 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]

[1]European Union Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food, Freiburg, Germany; [2]CART, University of Liege, Belgium; [3]National Food Institute, Technical University of Denmark, Søborg, Denmark; [4]National Institute for Health and Welfare (THL), Kuopio, Finland; [5]Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL), Münster, Germany; [6]NCSR Demokritos, Athens, Greece;[7]Central Agricultural Office, Budapest, Hungary; [8]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale, Teramo, Italy; [9]Istituto Superiore di Sanitá (ISS), Roma, Italy ; [10]RIKILT, Wageningen, The Netherlands; [11]The Food and Environment Research Agency (FERA), York, United Kingdom

http://www.dioxin20xx.org/pdfs/2012/1041.pdf

- 2009–2012 GC-MS/MS systems were evaluated from several vendors
- Conclusion:
  - "GC-MS/MS systems with sufficient sensitivity" can be "used as confirmatory methods"
  - "However, for the determination of low background levels the application of GC-HRMS is required" e.g. <20% of Maximum Level (ML)

Does MS/MS Agree with HRMS?

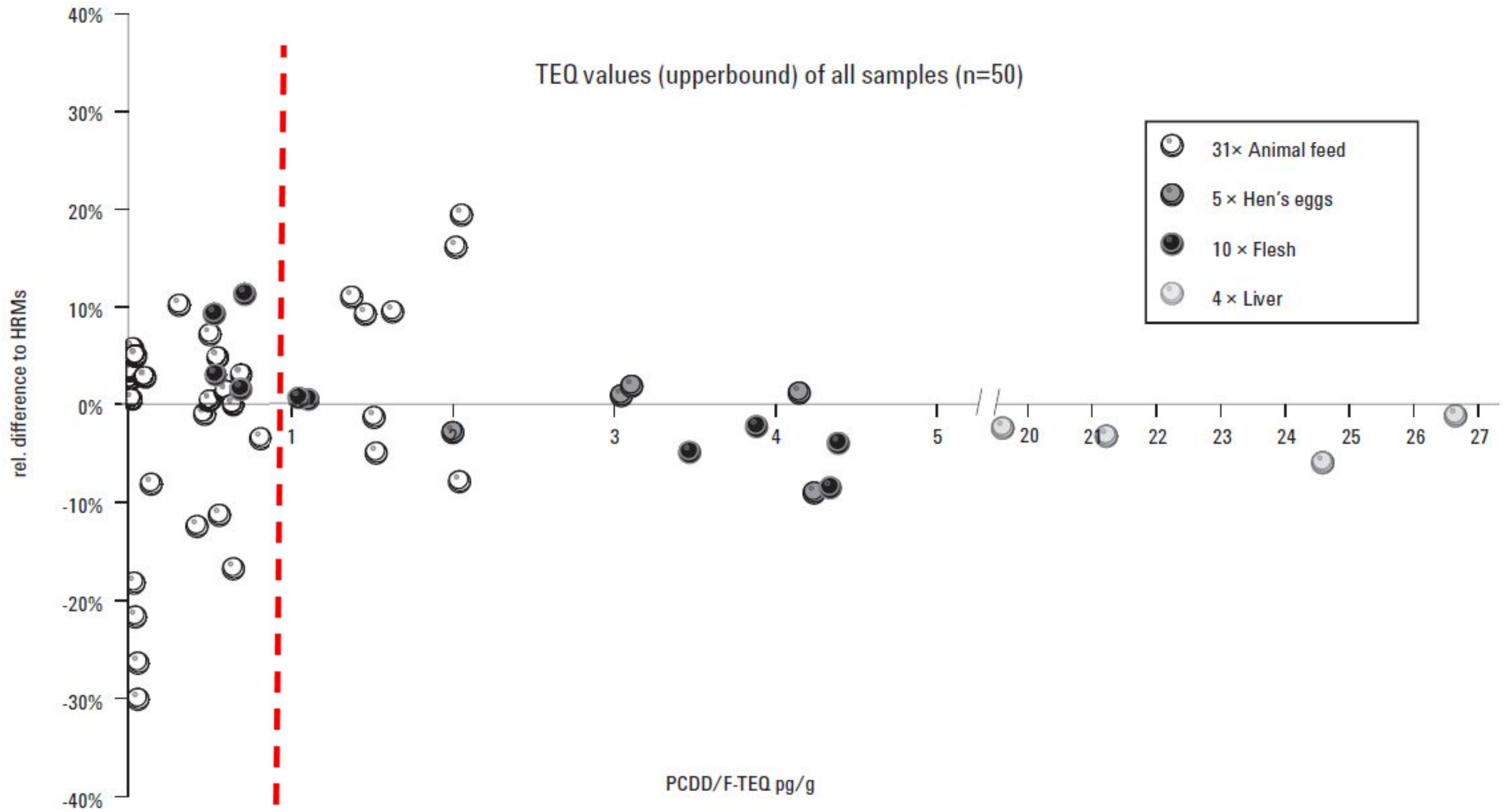
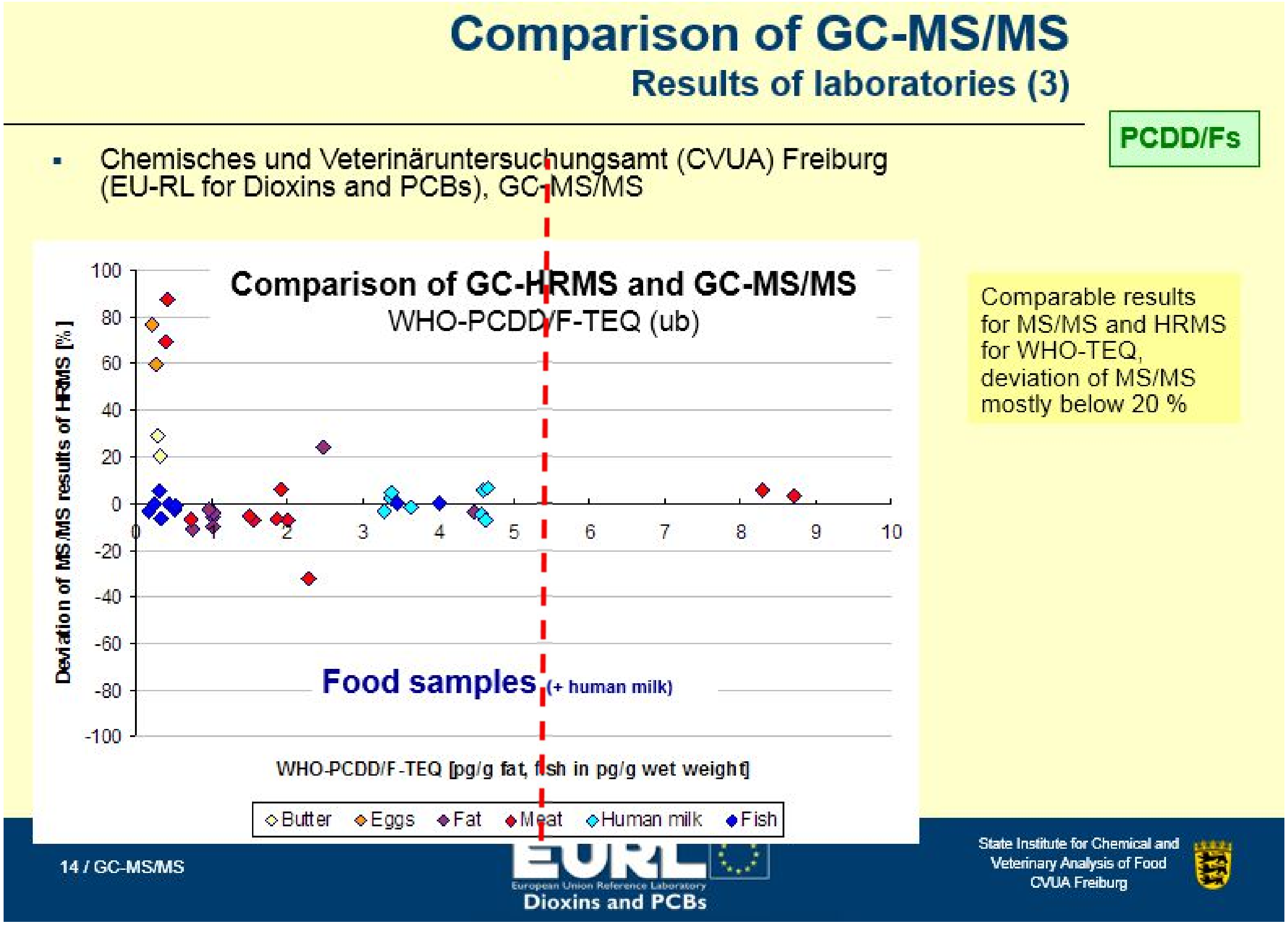


Figure 11. Comparative results (upperbound concentration values) for 50 food and feed samples analyzed by GC/HRMS and GC/MS/MS.

- At EU ML results are comparable.
- Below EU ML results are not comparable.

Does MS/MS Agree with HRMS?



- At EU ML results are comparable.
- Below EU ML results are not comparable.

What's New In EU Regulation For Dioxins?

Past

Commission Regulation (EU) No 252/2012 of 21 March 2012

- Specifies use of GC-HRMS for confirmatory dioxin analysis
- GC-MS/MS was allowed a screening technique.

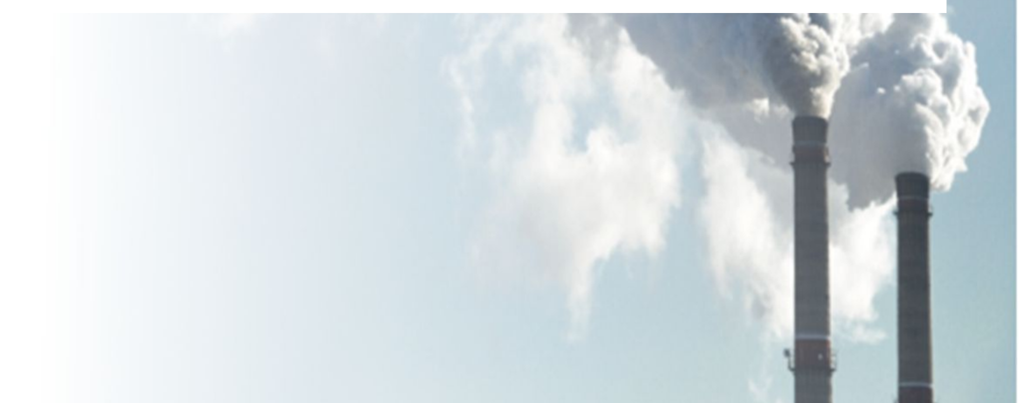
Present

Commission Regulation (EU) No 589/2014 of 2 June 2014

- Specifies use of **GC-HRMS** **or** **GC-MS/MS** for confirmatory dioxin analysis
- GC-MS/MS is "an appropriate confirmatory method for checking compliance with the maximum", only.
- **GC-HRMS remains the recommended technique for "determination of low background levels in food monitoring, following of time trends, exposure assessment of the population".**

Compliance With New EU Dioxin Regulation

European Commission GC-MS/MS Confirmatory Performance Criteria	TSQ 8000 Evo GC-MS/MS Capabilities	Compliance Confirmed
Two specific precursor ions with two specific production ions	All recommended methods developed **as defined in criteria** Fully optimized by AutoSRM	**Yes**
Tolerance of ion ratios within ± 15%	**< 10%** measured at EPA 1613 CSL level (n=14)	**Yes**
Resolution of each quadrupole equal to or better than unit mass resolution	All recommended methods developed **Q1 and Q3 at 0.7 Da**	**Yes**
The % RSD of the five (or more) Relative Response Factors (RRFs) for each unlabeled PCDD/PCDF and labelled internal standards must not exceed 20%	6 point curve EPA 1613 CSL-CS4 **2%**	**Yes**



Applications and Methods

Application		Norm	Thermo Scientific TSQ 8000 Evo GC-MS/MS	Thermo Scientific DFS GC-HRMS
Food	EU Regulation	EU Regulatory Feed Control (at ML)	Approved	Approved
Food	EU Methods	EN 16215	Not in method	Approved
Food	EURL Recommendation	Background food studies (<1/5th EU ML)	Not recommended	Recommended
Clinical	EURL Recommendation	Human studies at trace levels	Not recommended	Recommended
Environmental	US Method	US EPA 8280A	Approved	Not in method
Environmental	EU Method	EN 1948	Not in method	Approved
Environmental	US Method	US EPA 1613 B for strict EPA compliance	Not in method	Approved
Environmental	US Method	US EPA Method 23	Not in method	Approved
Environmental	US Method	US EPA Method 8290	Not in method	Approved
Environmental	Japan Method	JIS K0311	Not in method	Approved
Environmental	Japan Method	JIS K0312	Not in method	Approved

- Any laboratory following EU approach will now be able to use GC-MS/MS for ML compliance control
- Laboratories strictly following US/Japan methods and regulation will continue to use GC-HRMS