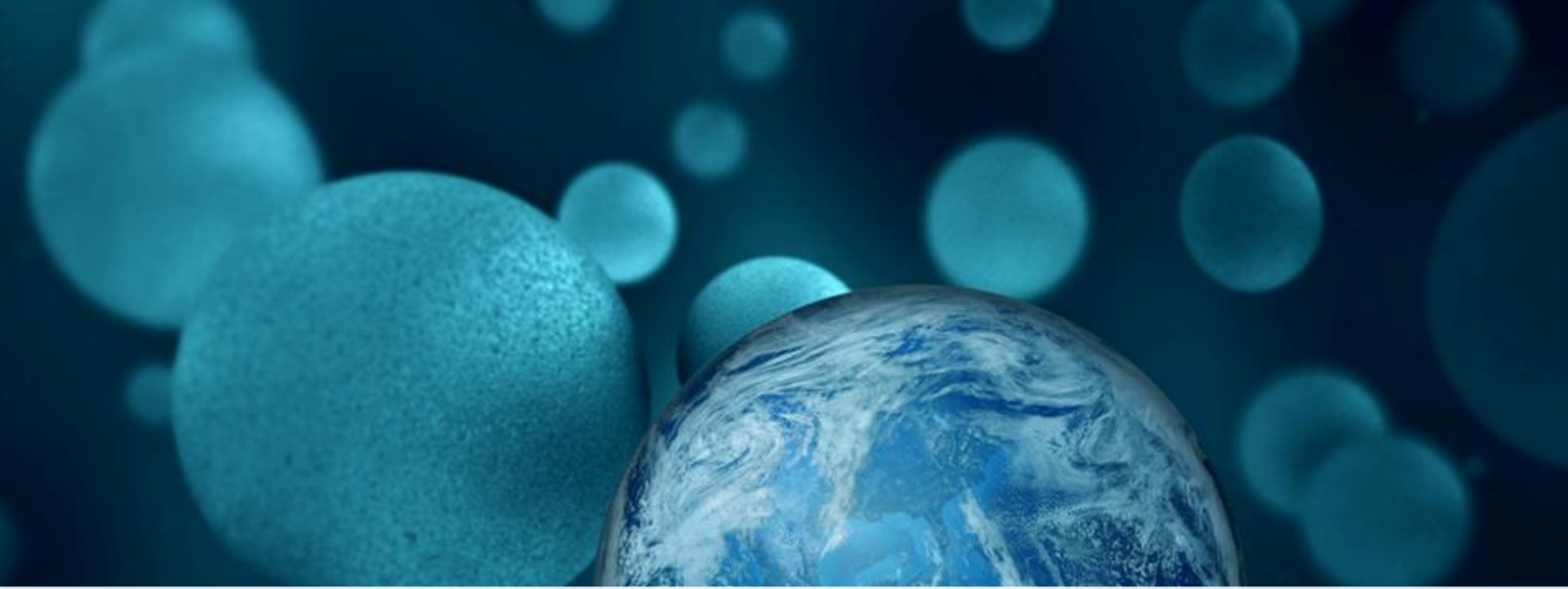

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# Dioxin and PCBs with Triple Quadruple and HRMS Technology- Setting the New Standard

Elsamoul Hamdnalla
Thermo Fisher Scientific

# Agenda

1. **What** are POPs and Dioxins?

2. **Why** do we analyze for Dioxins?

3. **How** do we analyze for Dioxins?
   - Analysis
     - Screening
     - Confirmation
     - New EU legislation



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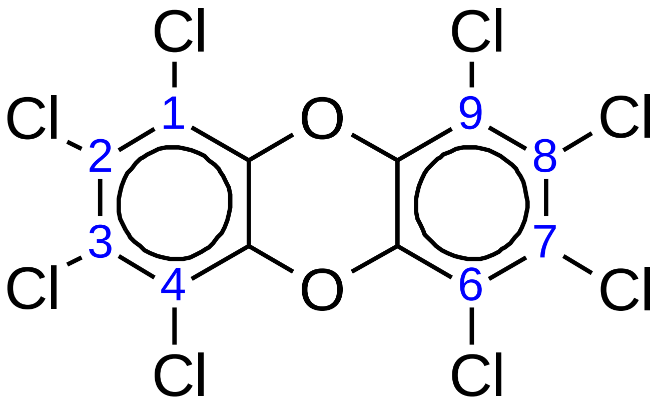
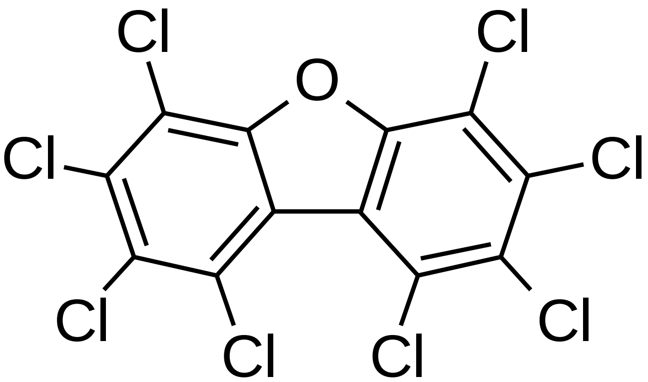
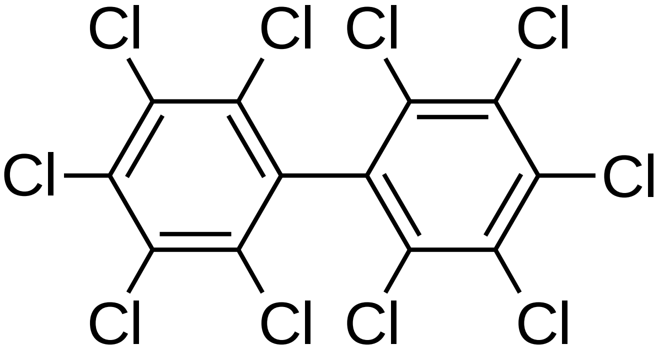
# What is a Dioxin?




Polychlorinated dibenzo-p-dioxin (PCDD)
"Dioxin"
**75 congeners**




Polychlorinated dibenzofuran (PCDF)
"Furan"
**135 congeners**




Polychlorinated Biphenyl (PCB)
**209 congeners**

**29 toxic dioxin and PCB congeners out of total of 419**



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# ‘Dioxins’ – Complex Mixture Of Compounds

- Polychlorinated dibenzodioxins –PCDDs (75)
  - Toxic congeners: 7
- Polychlorinated dibenzofurans - PCDFs (135)
  - Toxic Congeners: 10
- Polychlorinated biphenyls
  - Toxic congeners: 12

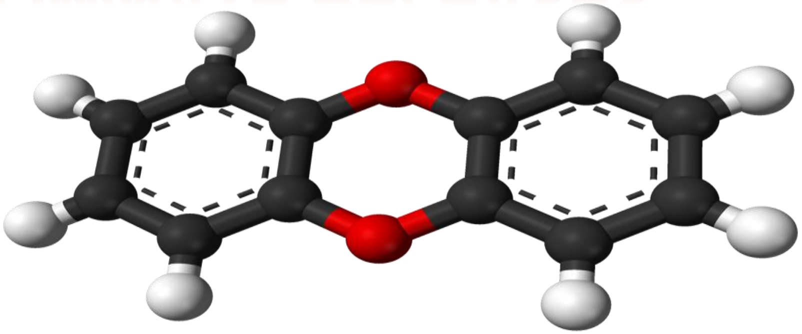
| Compound Group | Mono | Di | Tri | Tetra | Penta | Hexa | Hepta | Octa | Nona | Deca | Total | Toxic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCB | 3 | 12 | 24 | 42 | 46 | 42 | 24 | 12 | 3 | 1 | 209 | **12** |
| PCDD | 2 | 10 | 14 | 22 | 14 | 10 | 2 | 1 | | | 75 | **7** |
| PCDF | 4 | 16 | 28 | 38 | 28 | 16 | 4 | 1 | | | 135 | **10** |
| | | | | | | | | | | | | |
| PBB | 3 | 12 | 24 | 42 | 46 | 42 | 24 | 12 | 3 | 1 | 209 | |
| PBDE | 3 | 12 | 24 | 42 | 46 | 42 | 24 | 12 | 3 | 1 | 209 | |
| PBDD | 2 | 10 | 14 | 22 | 14 | 10 | 2 | 1 | | | 75 | ? |
| PBDF | 4 | 16 | 28 | 38 | 28 | 16 | 4 | 1 | | | 135 | ? |
| PBCDD | 0 | 14 | 84 | 254 | 420 | 452 | 252 | 74 | | | 1550 | ? |
| PBCDF | 0 | 28 | 168 | 496 | 840 | 880 | 504 | 134 | | | 3050 | ? |

# Chloracne


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

# Why Analyze for Dioxins?
## 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 $fg/m^3$

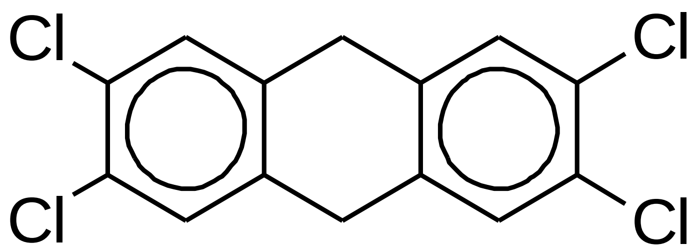
# Toxicity of Dioxins

- Acute toxicity
  - Chloracne
  - Carcinogenic
  - Estrogenic
- President Yushchenko (Ukraine)
  - Suffered from Dioxin intoxication during his election in 2004
  - TCDD 10-12 µg/L in blood
  - 100 **ppb** - 50,000x higher than normal concentrations

- “Dioxin so pure it was made in a laboratory”

# 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

# "Dioxin Incidents" – Economical Impact

Almost all food contamination comes from contaminated animal feed

- 1998 EU Milk from citrus pulp pellet feed
- **1999 Belgium Eggs and poultry – Total cost estimated € 625M**
- 1999 EU Clay and zeolithes for feed
- 2000 EU Choline chloride
- 2002 UK and Eire Feed supplement "Carbosan Copper"
- 2004 EU Potato pulp
- 2005 EU Hydrochloric acid
- 2007 India Guar Gum thickener
- **2008 Ireland Pork – Total cost estimated € 150M**
- 2008 Italy Mozzarella Cheese
- **2010 Germany Eggs & pork – 4700 farms closed.**
  **Current cost estimated > € 300M**
- 2012 Germany - More poultry farms closed (local contamination)
- **2013 ???????**

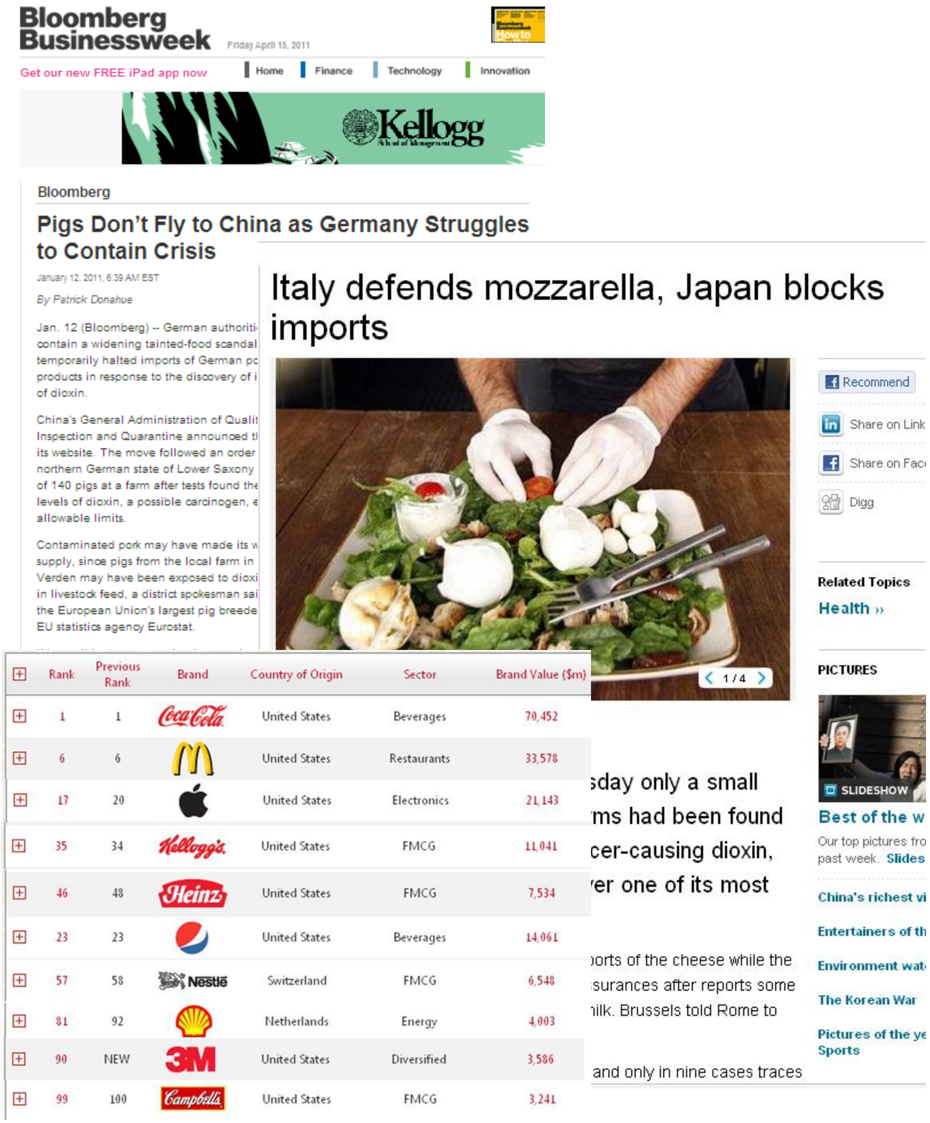

- All exports to/from EU are subject to the same legislation

# Who Tests For Dioxins In Food?

1. Government regulatory laboratories working in food safety and environmental
   - e.g. CVUA Frieburg, CVUA Muenster, FERA, CDC, EPA, FDA

2. Contract Testing Organisations - food safety and environmental
   - e.g. Eurofins, SGS, ALS

3. Food conglomerates working for food safety and brand protection
   - e.g. Kraft, Cargil, Coca-Cola, Thames Water



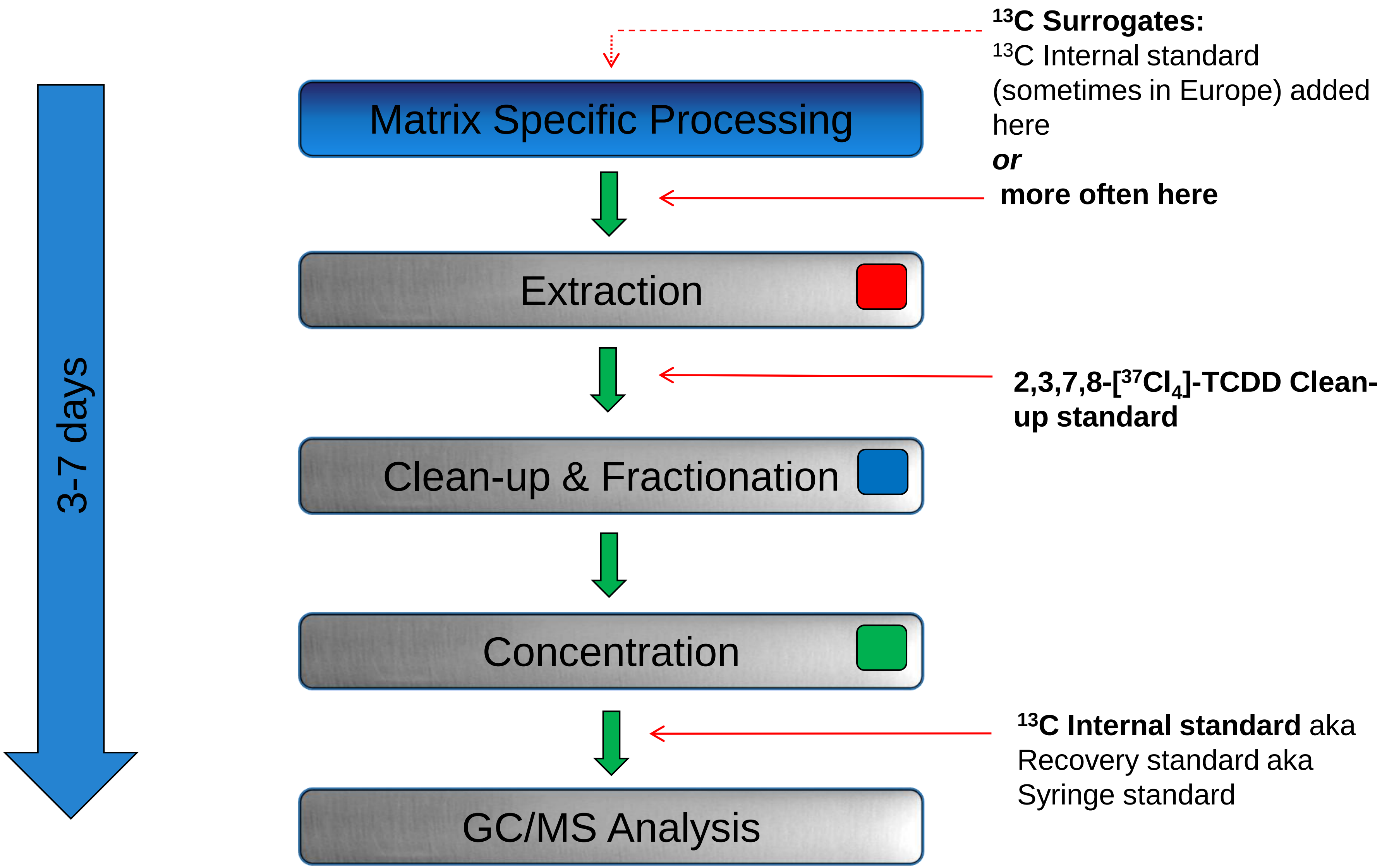

| Rank | Previous Rank | Brand | Country of Origin | Sector | Brand Value ($m) |
|---|---|---|---|---|---|
| 1 | 1 | Coca-Cola | United States | Beverages | 70,452 |
| 6 | 6 | | United States | Restaurants | 33,578 |
| 17 | 20 | | United States | Electronics | 21,143 |
| 35 | 34 | Kellogg's | United States | FMCG | 11,041 |
| 46 | 48 | Heinz | United States | FMCG | 7,534 |
| 23 | 23 | | United States | Beverages | 14,061 |
| 57 | 58 | Nestlé | Switzerland | FMCG | 6,548 |
| 81 | 92 | | Netherlands | Energy | 4,003 |
| 90 | NEW | 3M | United States | Diversified | 3,586 |
| 99 | 100 | Campbell's | United States | FMCG | 3,241 |

# 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)



# Instrumental Analysis: Instrument Characteristics Required For Pops Analysis

1. Chromatography
   - Must be able to separate POP compounds & congeners
2. Sensitivity
   - Compounds typically ppb to ppt concentration in environmental samples
3. Selectivity
   - Must be halogen selective or have resolve analyte from matrix
4. Low price
   - Environmental market is very cost sensitive
5. Matrix tolerance
   - Must be capable of prolonged analysis with
6. Conformity to standard methods for POPs
   - EPA, EN, MCERTS, JIS etc...

- HRGC
- GC-ECD, GC-MS, GC-MS/MS and GC-HRMS
- GC-MS/MS and GC-HRMS
- GC-ECD, GC-MS and GC-MS/MS
- GC-MS/MS and GC-HRMS
- **GC-HRMS and** GC-MS/MS* (*EU)

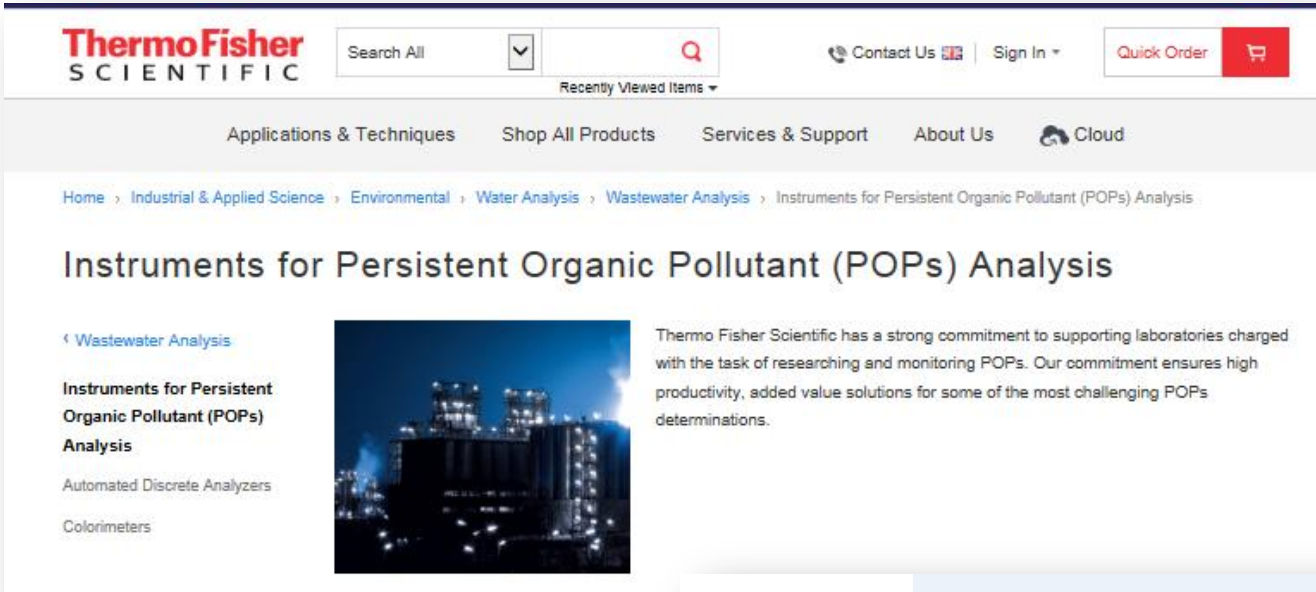
  (for defendable confirmation)

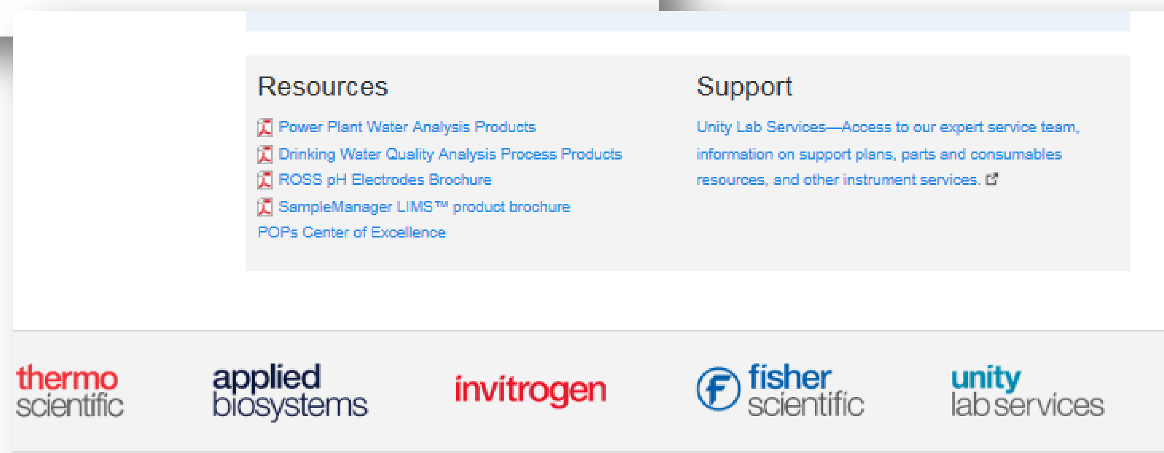
# GC-MS/MS & GC-HRMS

## Analytical Selectivity

| 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 |

# www.thermofisher.com/pops



# 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 (°C): | 170 |
| Hold Time (min): | 0 |
| Temperature 2 (°C): | 250 |
| Rate (°C/min): | 15.4 |
| Hold Time (min): | 0 |
| Temperature 3 (°C): | 285 |
| Rate (°C/min): | 2.5 |
| Hold Time (min): | 0 |
| Temperature 3 (°C): | 320 |
| Rate (°C/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 |

# 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% |

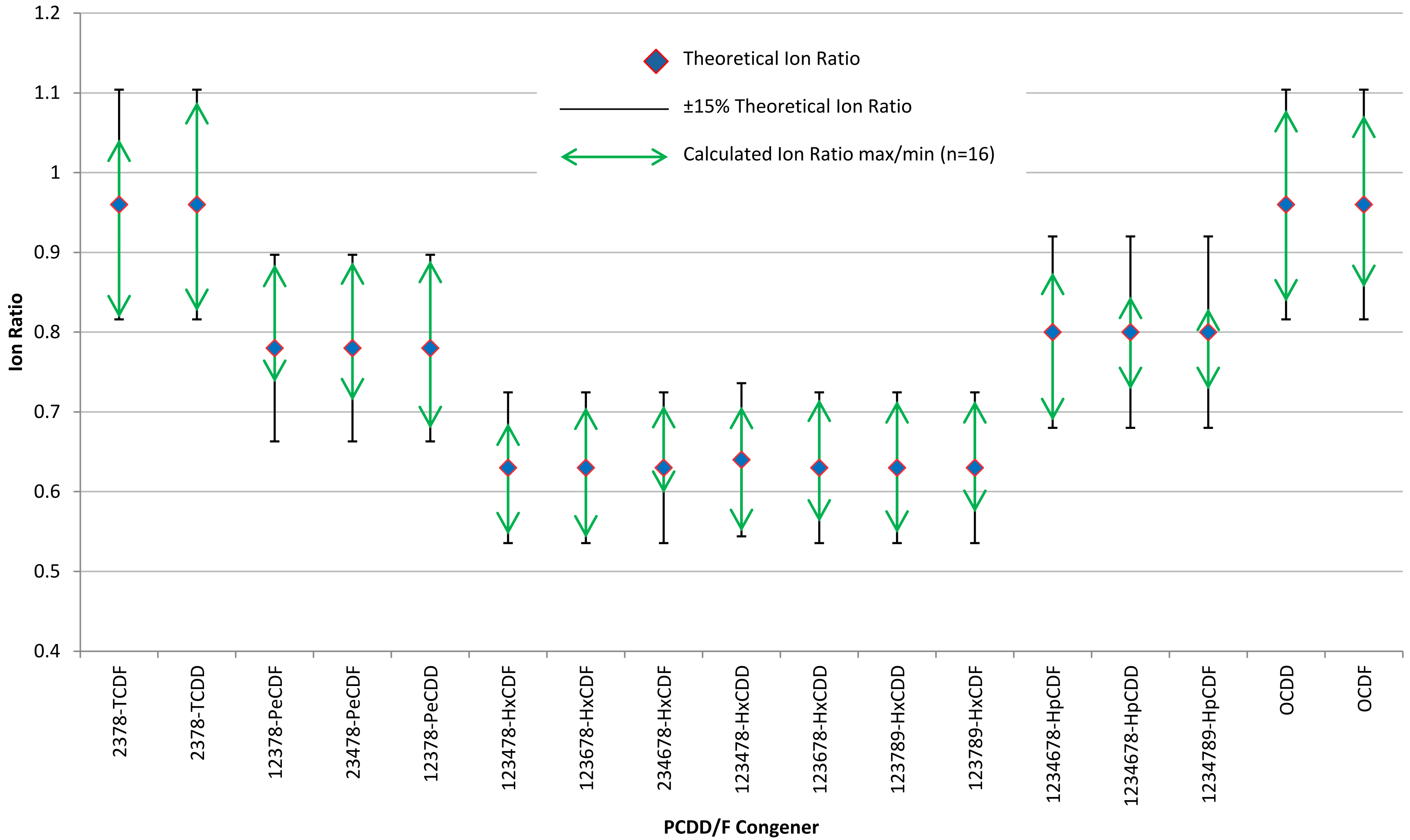
| NATIVE PCDDs & PCDFs | 1948CSL (pg/µl) | 1948CS1 (pg/µl) | 1948CS2 (pg/µl) | 1948CS3 (pg/µl) | 1948CS4 (pg/µl) | 1948CS5 (pg/µl) | 1948CS6 (pg/µl) |
|---|---|---|---|---|---|---|---|
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 0.04 | 0.2 | 0.8 | 4 | 16 | 80 | 320 |
| 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 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 |

# 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% |

| | *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) |
|---|---|---|---|---|---|---|---|---|
| **NATIVE PCBs** | | | | | | | | |
| 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)]**

# 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.**



ThermoFisher SCIENTIFIC

# 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)

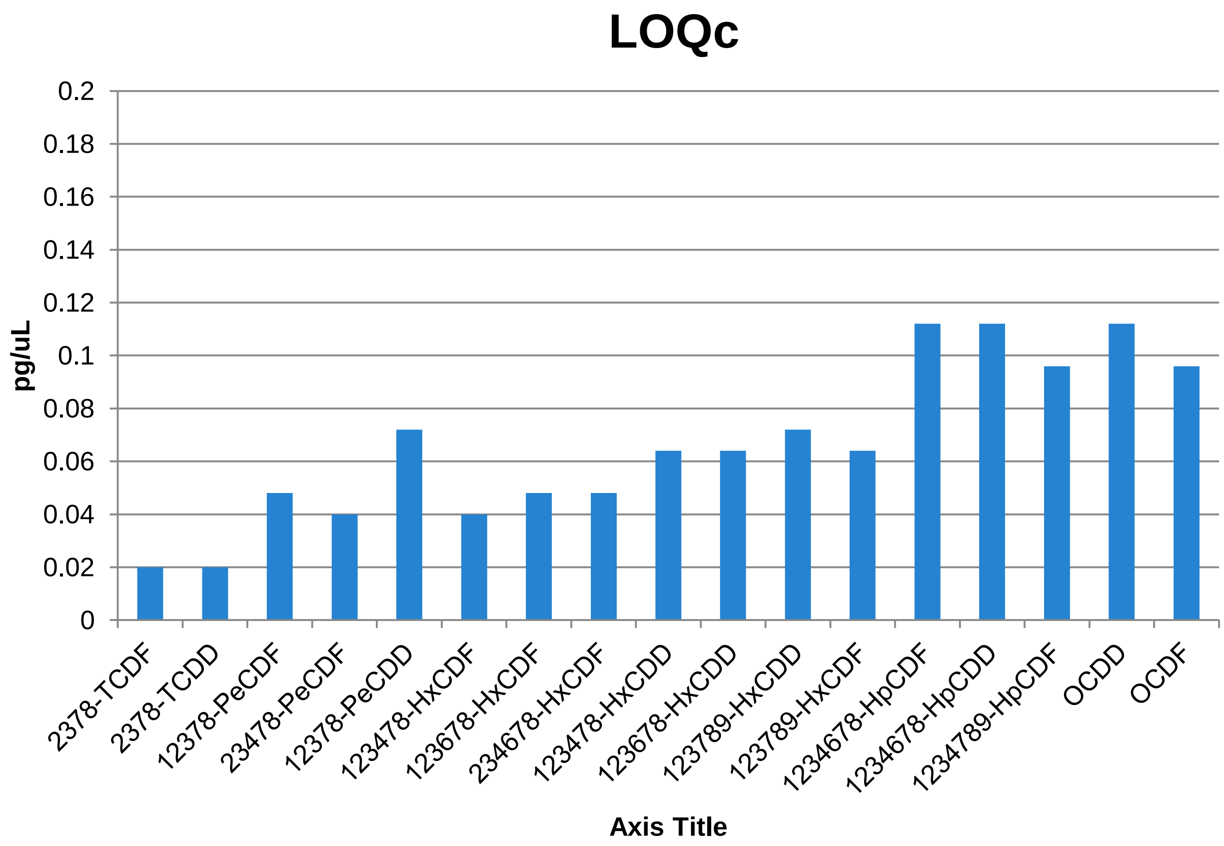


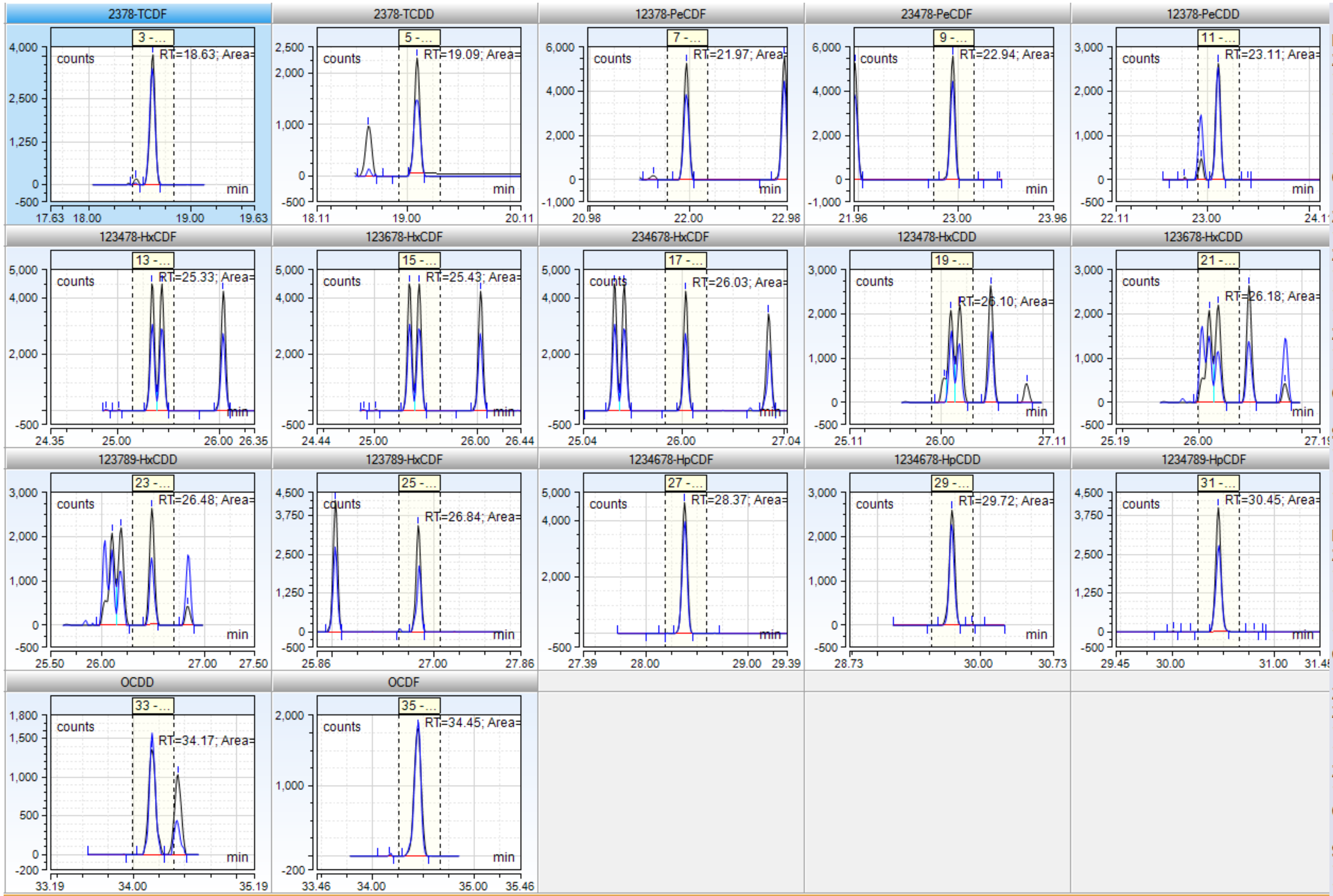

Figure 2. Practically determined LOQc for PCCD/Fs

**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 |

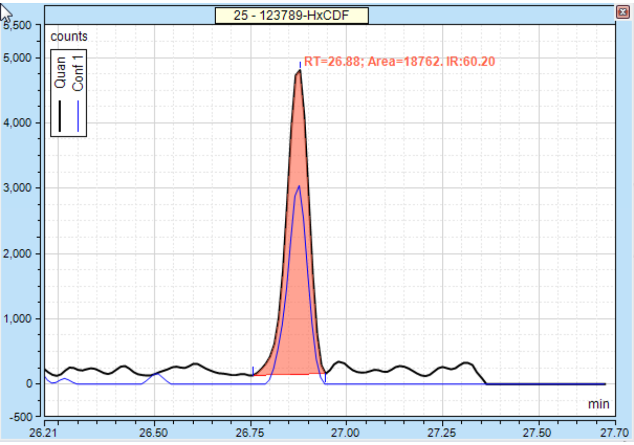
# Chromatography – Lowest Calibration Standard (EN:1948 CSL)

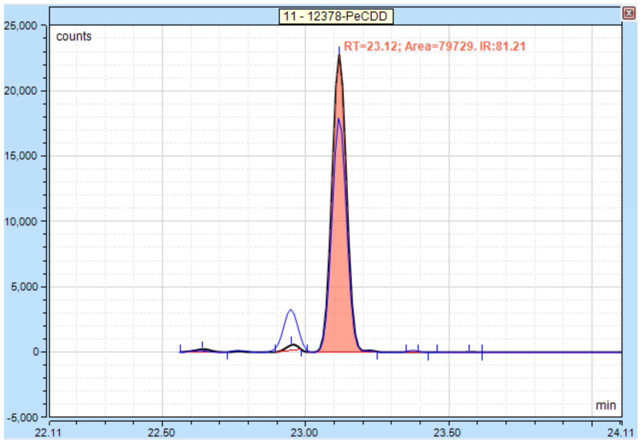


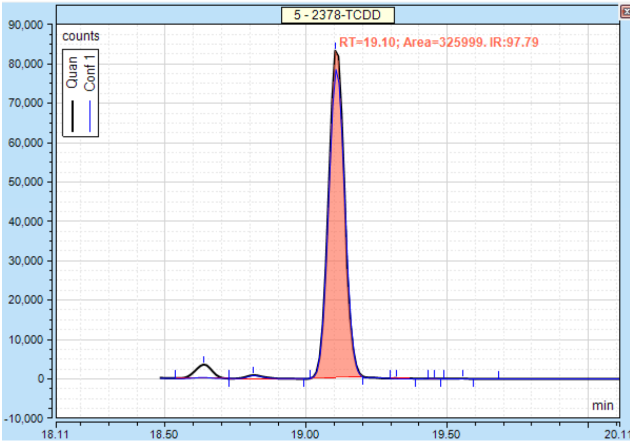

| | 1948CSL (pg/µl) |
|---|---|
| **NATIVE PCDDs & PCDFs** | |
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 0.04 |
| 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 0.08 |
| 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 0.08 |
| 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin | 0.08 |
| 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin | 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

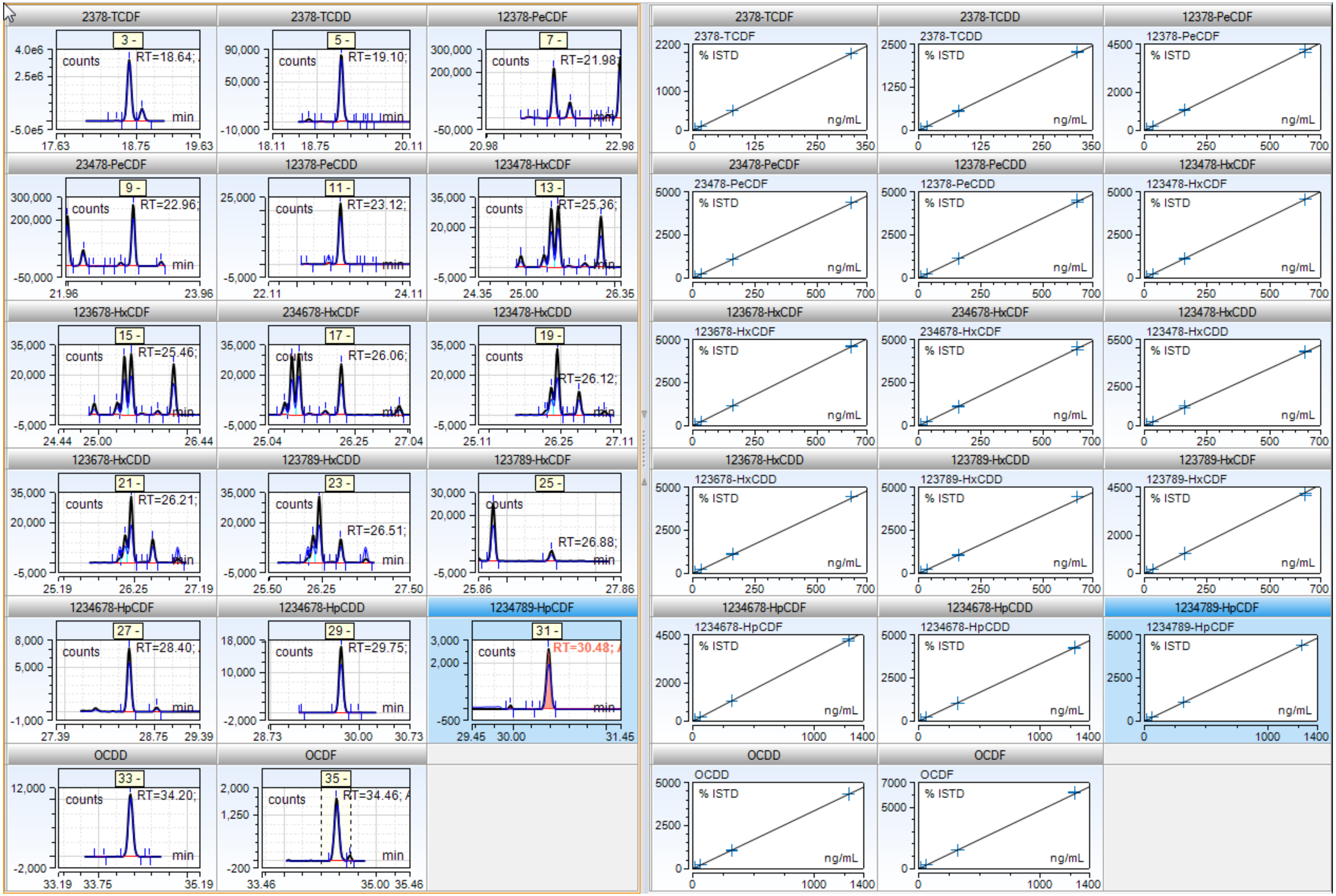
12378-PeCDD – 0.162 pg/g WHO-TEQ

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** |

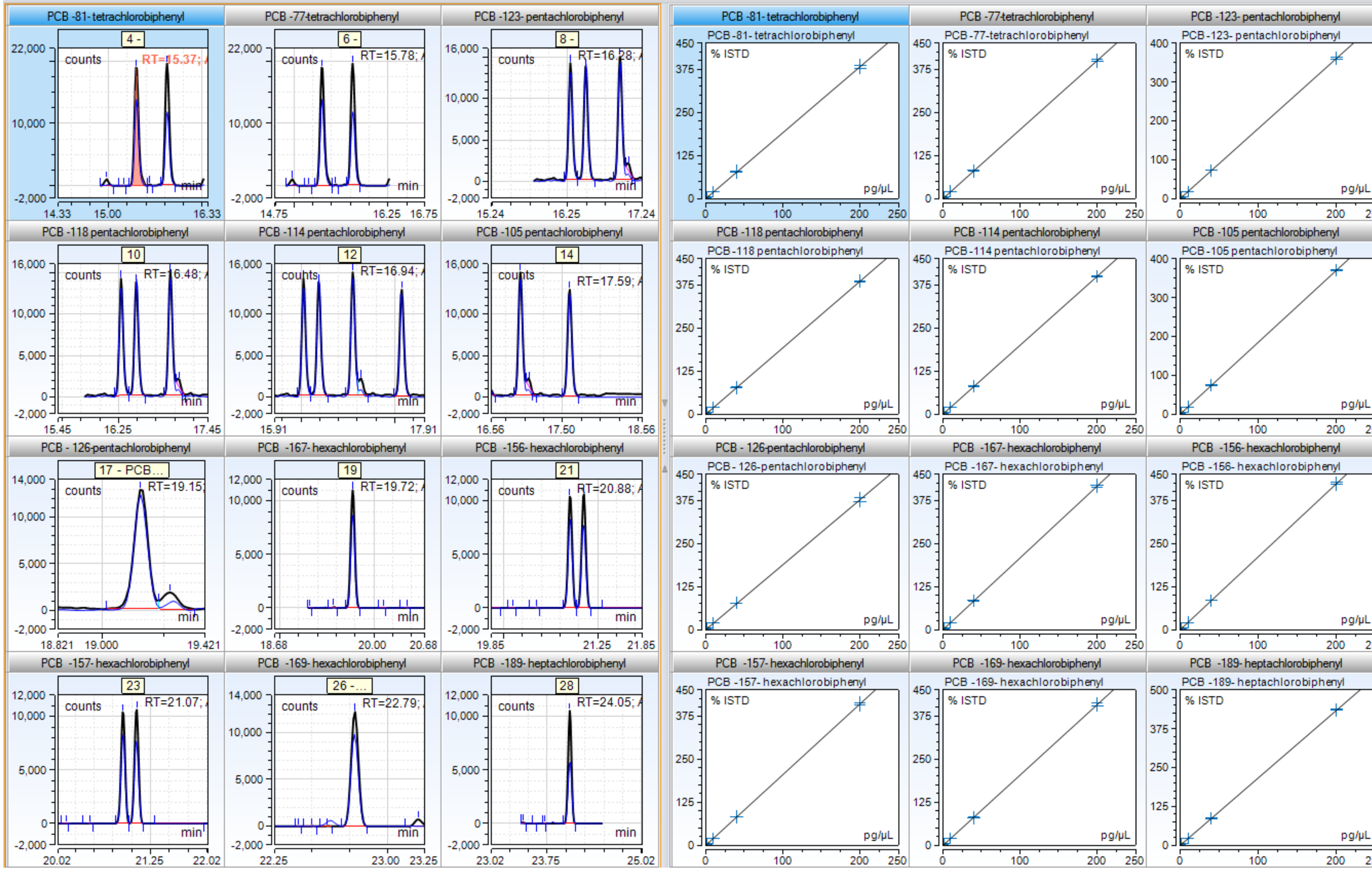
Fish sample WHO-TEQ (2005) – 78.6g Fresh weight – HR-GCMS 1.29pg/g

# 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)



# 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

- **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?

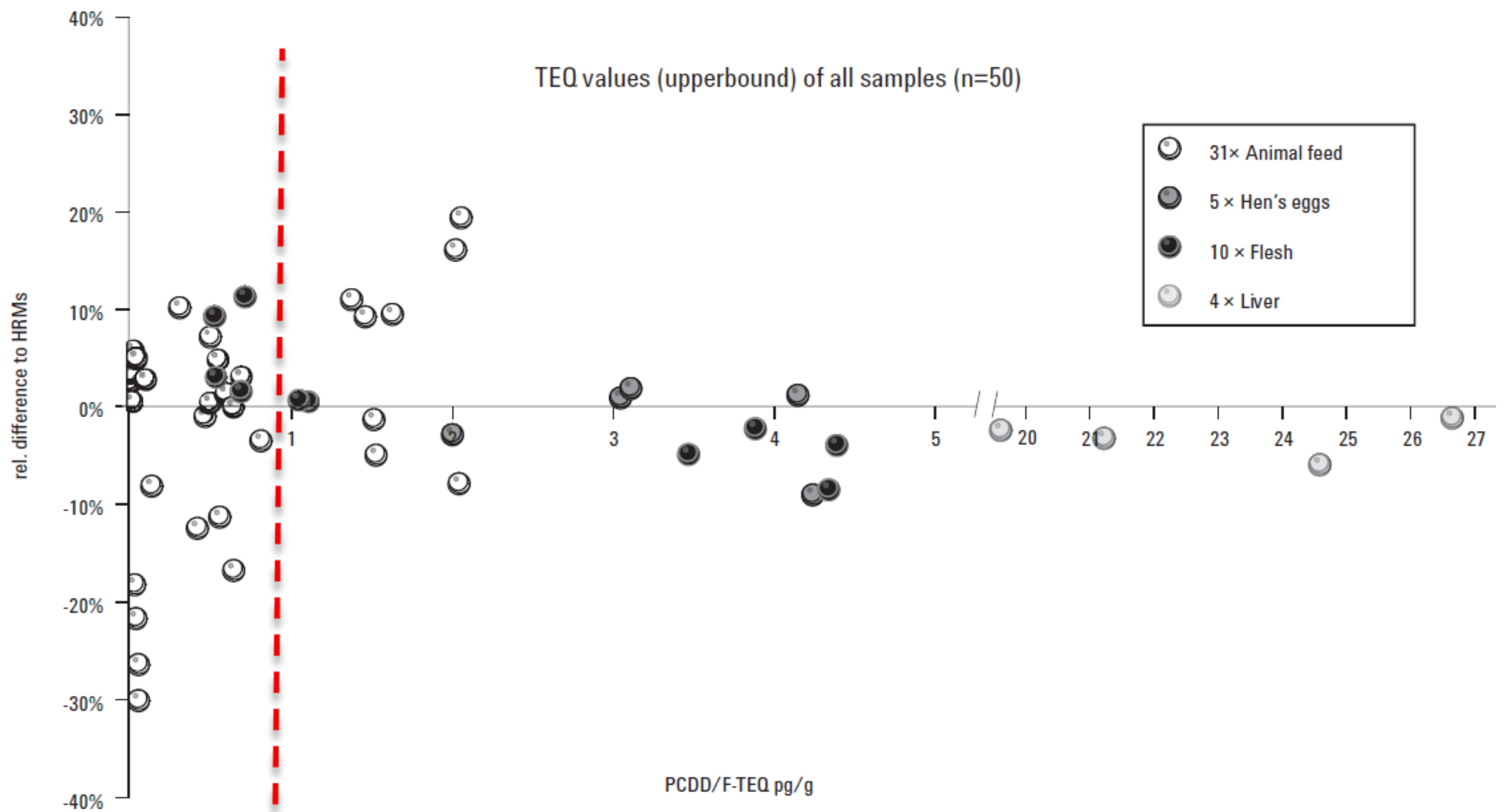
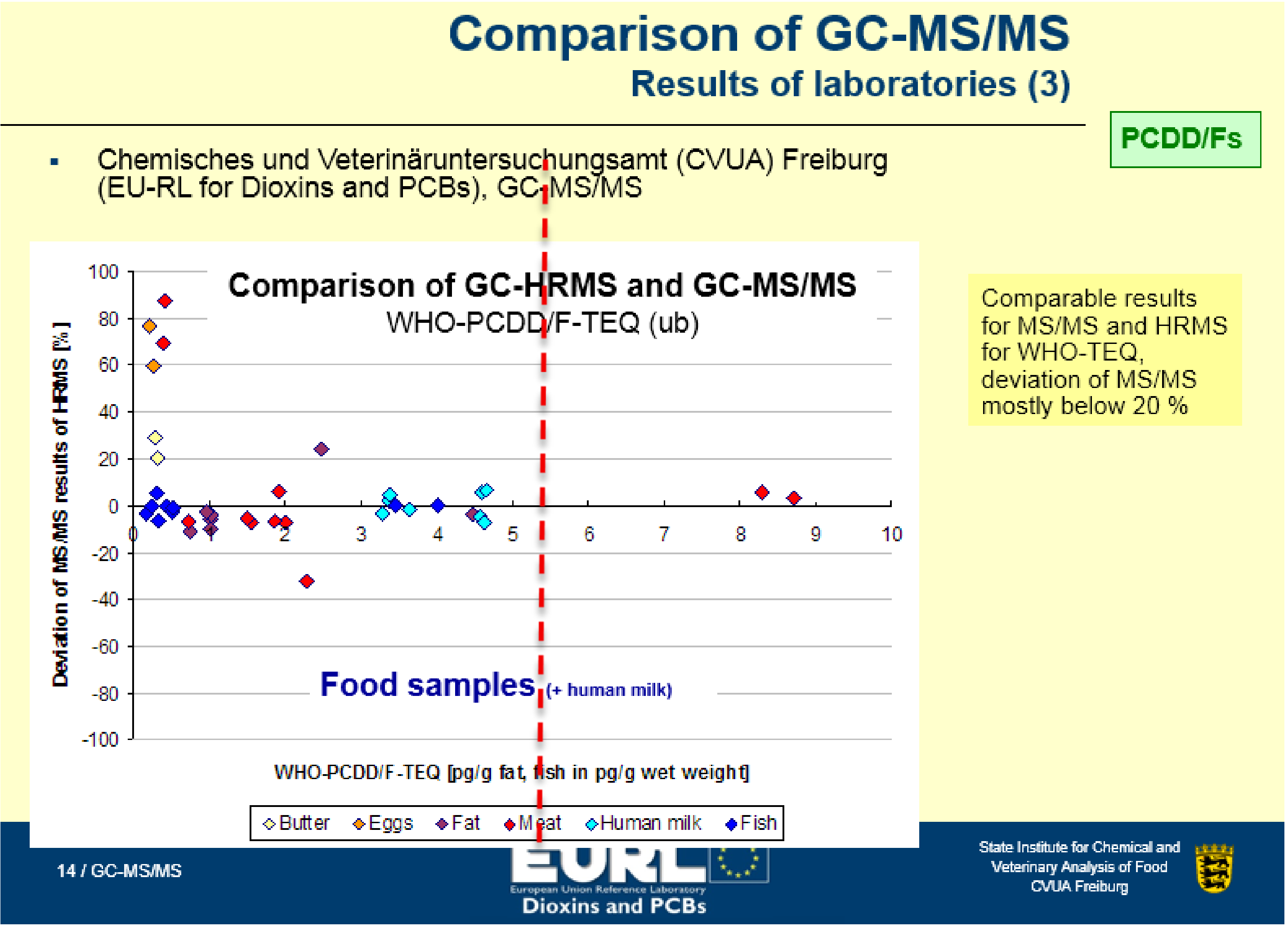



*Figure 11. Comparative results (upperbound concentration values) for 50 food and feed samples analyzed by GC/HRMS and GC/MS/MS.*

- 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