Optimized Sample to Result Workflow of Dioxin and PCBs Analysis in Environmental and Food Using Automated Sample Preparation and GC-MS/MS

Overview

- **Introduction**

- **Sample Prep Workflow**

- **Automated Hyphenated System Configurations for different Matrices and Extractions**

- **Semi Automated Systems**

- **Results using Agilent GC/MS/MS**

FMS, Inc.

- **Founded in 1986 based in USA**

- **Manufactures Total Solution Sample Preparation Systems and Consumables for GC, GC/MS, LC and LC/MS**

- **Manual Dioxin/PCB sample preparation is tedious and comprehensive**

- **FMS provides total solution Dioxin/PCB Automated and Semi-Automated Sample Preparation Systems**
  - **Fast, Consistent, Reliable Results**
  - **Suitable for all kinds of Environmental, Food and Feed samples**
  - **Complies with EPA methods and the criteria of the European legislation**

Laboratory Workflow Breakdown

Sample Prep versus Analytical in Time

Analytical Run

35%

Extraction
Sample Clean up
Concentration

65%

■ Sample Preparation

■ Analytical Run and Analysis
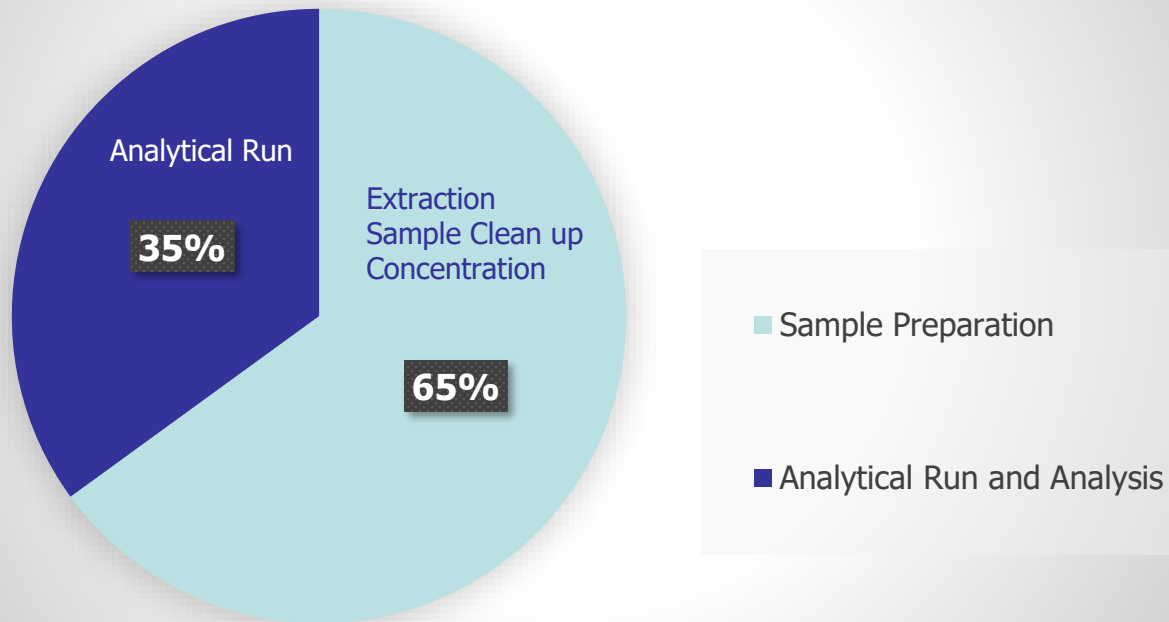
Background

- **POPs (PCDD/Fs, PCBs) continue to attract interest around the world due to strict regulations enforced in many countries**

- **Rapid, quality extraction, sample clean up and analysis is needed for many laboratories processing samples**

- **Processing times and cost are important considerations**

- **In the US, EPA methods SW-846, 1613, 1668 and 8082A are used for PCBs and PCDD/Fs work**

- **Sample extracts mostly in DCM, hexane or toluene**

- **Cleanup for analysis of all 209 PCBs in common in North America - extracts are often in toluene after Soxhlet Extraction**

Challenges of POPs Sample Prep

- **Labor intensive, prone to error**

- **Compliance with regulatory procedures and accreditation (lengthy method validation)**

- **Strict QA/QC requirements**

- **Sample matrix complexity**

- **Native background and interferences (can be orders of magnitude higher than analytes)**

- **Pico/femto-gram analyses require ultra pure extract and excellent instrument sensitivity**

Automated Sample Prep

- **Advantages of Automated Sample Prep**

  - Rapid Turn Around Time: 30 to 45 Minutes for 6 Samples

  - Cleaner Background Interferences: Closed Loop System

  - Quality Results: Certified Pre-packaged Columns

  - Green Technology: Lower solvent and power use

  - QA/QC & Accreditation Requirements: Easier to Manage

  - Computerized Method: Instrumentation based prep

Manual Sample Prep

- **Advantages of Manual Sample Prep**
  - Most labs use a Manual Methods for the following reasons:

    - Viewed as Inexpensive
    - No electronics or mechanical components to fail
    - No down time due to the system failure
    - No service contract
    - No capital equipment cost

FMS
Fluid Management Systems

- Analysis of various matrices for PCDD/Fs and PCBs using extraction, clean up and concentration.

- Soxhlet extraction (typically up to 24-36 h).

- Concentration step

- Preparative multi column chromatography involving various solvents and steps.

- Can include acid-base-neutral silica, pure acidified silica, alumina, florisil and carbon columns. Use of H_2SO_4 acid mixed with silica; NaOH mixed with silica Neutral Silica

- Fractionation and Concentration

Concentration

**SuperVap®
Concentration and
Evaporation**

**PLE®
Solids and Semi-Solids**

Modular up to 8 Samples Run in Parallel

Direct to Vial

FMS Automated Sample Cleanup

Automated Sample Cleanup

Concentration

PowerPrep® NG
Dioxin & PCB
Sample Cleanup

EconoPrep®
Dioxin & PCB
Sample Cleanup

EP110®
Zero DCM
Dioxin & PCB
Sample Cleanup
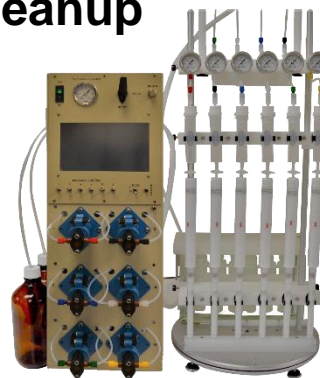
EZPrep +
Dioxin & PCB
Sample Cleanup

SuperVap®
Concentration and
Evaporation

Direct to Vial

Sample Preparation consist of three main instruments:

1- Extraction   2- Sample Clean-Up   3- Concentration

= Total Prep

PLE Extraction   +   **PowerPrep Sample Cleanup**   +   **SuperVAP Concentration**   =   **Total Sample Prep**

Dioxin/PCBs Sample Analysis Workflow
Hours vs Days

Automated Sample Prep Time 1 up to 8 samples = 2hrs

Extraction	+	Concentration	+	Sample Cleanup	+	Concentration	+	GC/MS
30 Min		30 Min		30 Min		30 Min		45Min

Manual Sample Prep Time   = 2 to 4 days

Extraction	Concentration	Sample Cleanup	Concentration	GC/MS
24 hours	60 Min	24 to 48 hours	60 Min	45Min /sample

Automating Sample Prep

- Automated FMS Pressurized Liquid Extraction (PLE) for sample extraction is fast (60 min), efficient (120 ^{o}C, 1500 psi), green (less power), reliable (long track record).

- Users' choice of FMS fully automated or semi-automated clean up system: fast (30-60 min), low solvent usage

- SuperVap Concentrator for Concentration/Evaporation

FMS
Fluid Management Systems

Concentration

**SuperVap®
Concentration and
Evaporation**

PLE®

Solids and Semi-Solids

Direct to Vial

Extraction Procedure

- 1 g sample mixed with Hydromatrix^{TM} to dry, transferred to extraction cells

- Spiked with ^{13}C PCDD/Fs and PCBs standards.

- Void volume filled with Hydromatrix^{TM}.

- Sample Cells filled with 50% mixture Hexane/Methylene Chloride.

- Cells pressurized to 1500 PSI and heated to 120 °C.

- Temperature held for 20 minutes.

- Extraction cells cooled, flushed with solvent (50% cell volume), and nitrogen

- Sent directly to the SuperVap collected in 250 mL tubes and automatically concentrated

Automation

- Advantages of automated sample prep are:

- Reduced time:
  - Automated Pressurized Liquid Extraction (PLE) takes 60 min start-to-finish
  - Manual Soxhlet up to 36 h
  - Modular up to a samples processed in parallel

- Reduced cost: Less labor involved, shorter turnover time per sample, less electricity use for PLE than Soxhlet

- Reduced volume: Less solvent used

- Extract is sent directly to the Concentrator no human interaction

SuperVap Evaporation

- System pre-heated to 45-60 °C.

- Extracts evaporated at stable temperature under 5-6 psi nitrogen.

- Solvent exchange with hexane to eliminate dichloromethane.

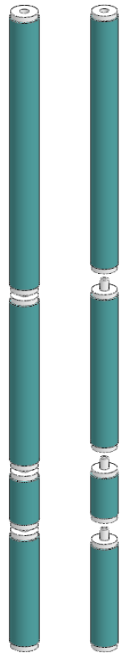- Dichloromethane would interfere with subsequent sample clean up.

- Extracts reduced to a few mls.

Column Kits

Classical Kit
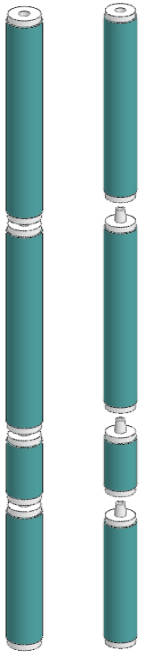
Classical Plus Kit

Mini Kit
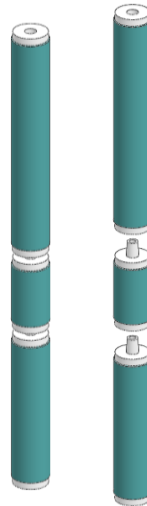
High Capacity Kit
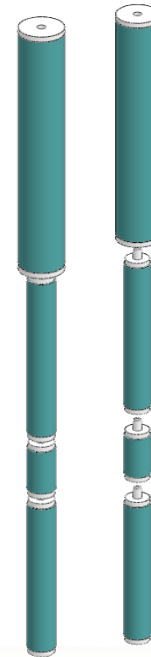
XL Capacity Kit
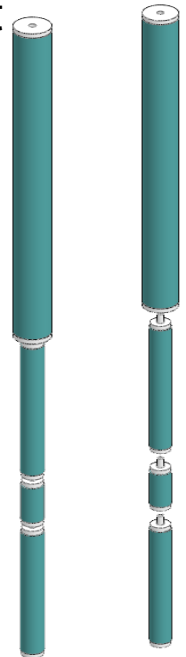
Columns for Sample Cleanup

Carbon

Alumina 4g

Alumina 6g

Basic/Neutral

Classical Acid
Mini ABN Acid

Classical Plus Acid

High-Capacity Acid

XL Capacity Acid
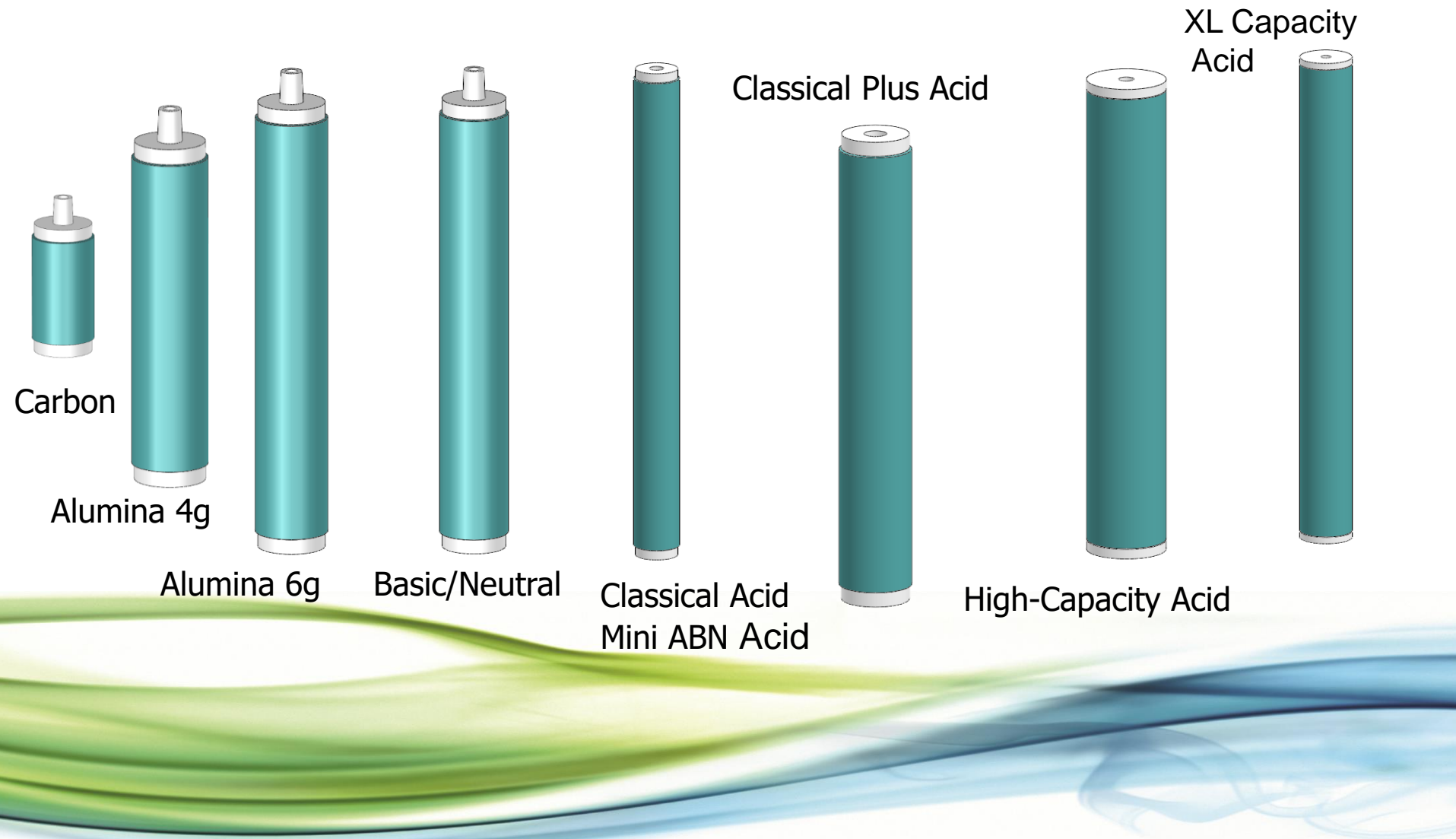
Sample Clean-up Columns

- Dioxin / PCB / PBDE Cleanup and Fractionation
  - Multicolumn Cleanup
    - Silica (Jumbo / Classic/ ABN)
    - Alumina
    - Carbon

- Florisil cleanup

- Custom Clean-up
  - Ability to combine and manufacture any mix of columns

PowerPrep NG
Expandable and Modular

System Flexibility

- With the combination of the PLE, SuperVap and PowerPrep NG you are given the flexibility to automate your extraction, concentration and clean-up for a wide variety of compounds.

- This is an ideal system for:
  - Laboratories that receive varying, different sample types and compounds of interest

  - High volume labs with a consistent daily sample amount load

  - Research laboratories looking to improve efficiency, recoveries and reproducibility

EP-110® Clean Up Zero DCM

Touch Screen Control

Real Time Display of Program Steps

Online evaporator starts during collection

Up to 4 modules, 8 samples parallel

FMS
Fluid Management Systems

- **EP-110 fully automated sample load and elution.**

- **Load Sample Extracts in hexane directly onto the system with no Manual Pretreatment**

- **Easy to perform QC sample simultaneously with a Real sample.**
  - **Run up to 8 samples in parallel**
  - **Expandable up to 4 modules, 2 samples per module**

- **Different column configuration: silica-carbon-alumina.**

- **Uses no DCM, only Hexane and Toluene.**

- **Total Clean Up time 30-45 min.**

- **Low volumes 100-250 mLs.**

- **Direct to SuperVap Concentration**

EconoPrep® for traditional Dioxin and PCB Sample Cleanup

**EconoPrep 4
Capable of running
8 Samples in Parallel**

EconoPrep Features

- **Fully Automated**

- **Low-Cost Dioxin/PCB Sample Cleanup**

- **Runs 2 samples per Module**

- **Expandable up to 4 Modules**

- **Run up to 8 samples in Parallel**

- **Run up to 8 samples in 30 to 45 minutes**

- **Direct to SuperVap Concentration**

EZPrep Plus

**Simple, Quick, Powerful, Low Cost
Automated Dioxins and PCBs
Sample CleanUp**
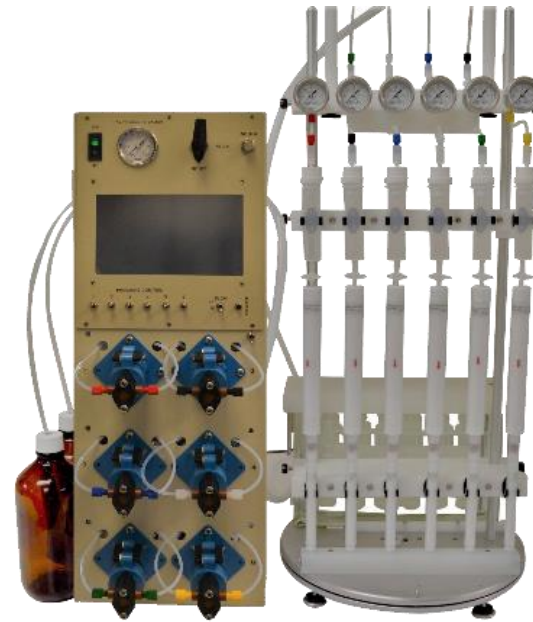
EZPrep Plus

- **Simple to Run -** Simple setup
- **Fast -** 30 to 45 minutes
- **Green Technology -** Zero DCM
- **Closed System -** No exposure to harmful chemicals
- **High Throughput-** 1-6 Samples in Parallel
- **Low Solvent -** As Low as 110 ml Per Sample
- **Reliable -** No Valves to Fail
- **High Fat Capacity -** Up to 10 grams
- **Quality Consumables -** Guaranteed Certified Columns
- **Economical -** Column Kits to Meet Your Sample Matrix
- **Reliable-** Easy to maintain

- **Advantages of Automated Sample Prep**
  - Rapid Turn Around Time: 30 to 60 Minutes for 6 Samples
  - Cleaner Background Interferences: Closed Loop System
  - Quality Results: Certified Pre-packaged Columns
  - Green Technology: Lower solvent and power use
  - QA/QC & Accreditation Requirements: Easier to Manage
  - Computerized Method: Instrumentation based prep
  - Direct to SuperVap Concentration   No Human Interaction

FMS
Fluid Management Systems

Semi-Automated

Concentration

Ezprep 123®
Dioxin & PCB
Sample Cleanup

SuperVap®
Concentration and
Evaporation

Direct to Vial

- **Advantages of Manual Sample Prep**
  - Most labs use a Manual Methods for the following reasons:
    - No electronics or mechanical components to fail
    - No down time due to the system failure
    - No service contract
    - No capital equipment cost

- **Disadvantages of Manual Sample Prep**
  - Open system, background and contamination prone
  - Inconsistent results
  - Labor intensive, require constant monitoring

EZPrep 123 Semi-Automated System Features

- Simple to run, no computerized instrumentation
- Fast: 30 to 60 min
- Closed loop system to give a clean background, low level detection
- Use certified pre-packaged columns
- Green technology, only vacuum pump uses power
- Low solvents, as low as 100 mL for serum
- Economical column kits, choice of low fat and high fat column kits
- Consistent results
- No capital equipment cost
- No electronics or mechanical equipment to fail
- Operator walkaway time

Glass Evaporation tube
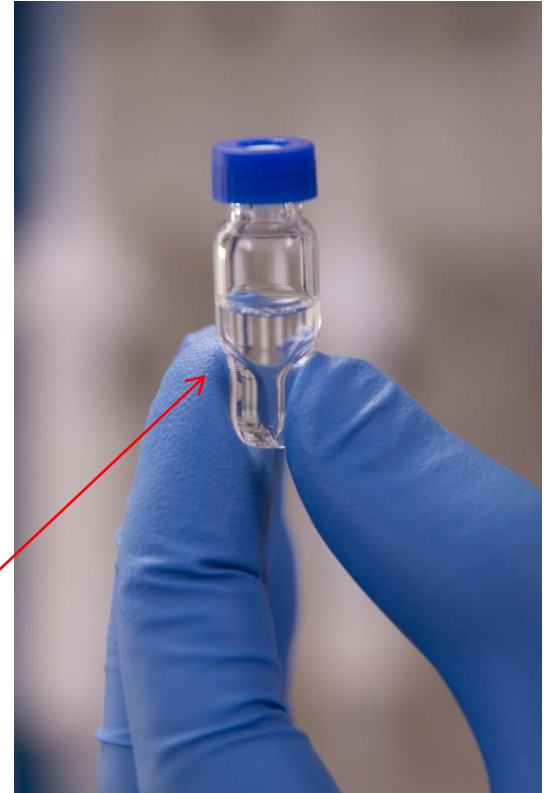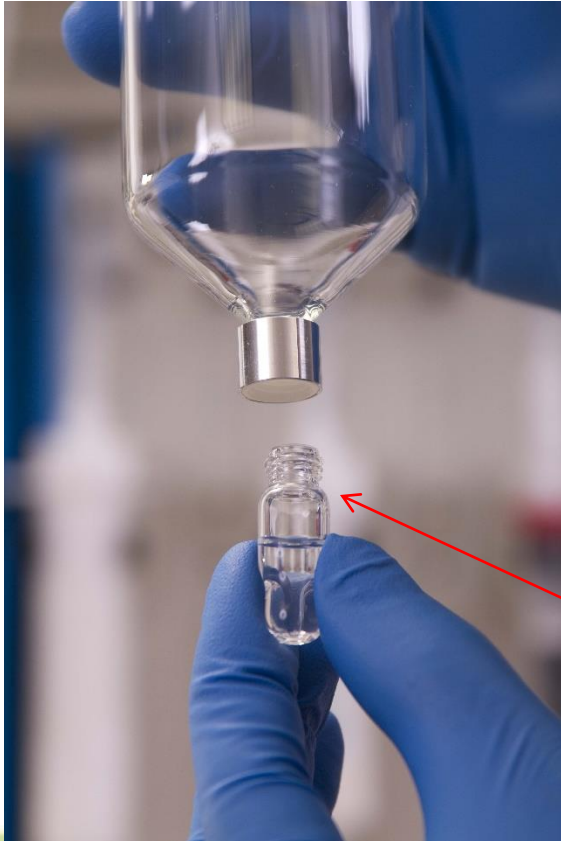
GC vial

SuperVap Evaporation

- System pre-heated to 45-60 ºC.

- Samples evaporated at stable T under 8 psi nitrogen.

- 1 mL extract vial transferred to GC vial (can have direct-to-vial feature).

- Recovery standards added (nonane/dodecane).

- Extract taken to 10 uL volume with a gentle stream of nitrogen at ambient temperature.

Using the Agilent 7010 GC-MS/MS

Agilent 7010 MS/MS Dioxin Analysis

- **Compliant**
  - Through use of an (ATP) Alternative Test Procedure for the EPA Methods and the new European Union Commission Regulations

- **Excellent Sensitivity**
  - 7010 GC/MS/MS detector meets detection requirements of the EPA methods and European regulations and is 10x more sensitive

- **Cost Effective alternative to High Res Mass Spec Systems**

^{13}C PCDD/F Recoveries across Extraction and Cleanup

	Channel-1	Channel-2	Channel-3	Channel-4	Channel-5	Channel-6	Average	STDEV	RSD (%)
2,3,7,8 TCDF	95	82	83	81	88	92	87	5.7	6.5
2,3,7,8 TCDD	93	88	81	79	87	87	86	4.9	5.8
1,2,3,7,8 PCDF	89	85	77	77	77	87	82	5.5	6.7
2,3,4,7,8 PCDF	97	92	86	81	79	91	88	7.1	8.2
1,2,3,7,8 PCDD	97	91	87	87	91	93	91	3.8	4.2
1,2,3,4,7,8 HxCDF	81	77	73	67	78	75	75	4.7	6.3
1,2,3,6,7,8 HxCDF	74	78	71	63	88	77	75	8.2	10.9
2,3,4,6,7,8 HxCDF	80	77	78	69	90	91	81	8.3	10.3
1,2,3,4,7,8 HxCDD	84	78	73	73	85	97	82	9.1	11.1
1,2,3,6,7,8 HxCDD	80	77	77	68	93	79	79	8.0	10.1
1,2,3,7,8,9 HxCDF	84	76	73	72	78	87	79	6.0	7.7
1,2,3,4,6,7,8 HpCDF	74	71	74	72	74	72	73	1.3	1.8
1,2,3,4,6,7,8 HpCDD	84	80	79	72	79	87	80	5.1	6.3
1,2,3,4,7,8,9 HpCDF	84	74	75	74	87	90	81	7.1	8.8
OCDD	72	77	70	71	87	77	76	6.3	8.4

^{13}C PCB Recoveries across Extraction and Cleanup

	Channel-1	Channel-2	Channel-3	Channel-4	Channel-5	Channel-6	Average	STDEV	RSD (%)
PCB-54	83	76	85	80	73	79	79	4.5	5.7
PCB-104	84	85	80	81	77	84	82	3.1	3.8
PCB-81	79	85	84	75	92	70	81	7.8	9.7
PCB-77	80	82	89	96	96	70	86	10.2	11.9
PCB-123	73	78	72	76	71	73	74	2.6	3.6
PCB-118	73	78	72	76	71	73	74	2.6	3.6
PCB-114	85	83	82	90	75	72	81	6.6	8.2
PCB-105	85	93	73	72	72	75	78	8.7	11.1
PCB-126	103	114	109	110	99	109	107	5.4	5.0
PCB-167	73	76	81	88	90	95	84	8.6	10.2
PCB-156	89	74	72	72	81	82	78	6.8	8.7
PCB-157	80	87	85	86	80	78	83	3.8	4.5
PCB-169	97	97	92	102	108	95	99	5.7	5.8
PCB-189	77	75	96	83	91	101	87	10.5	12.1

Native PCDD/Fs for Initial Demonstration of Capability

natives in pg	spike	IDC-1	IDC-2	IDC-3	IDC-4	Average	RSD (%)	Acceptable window
2,3,7,8 TCDF	400.0	91.8%	97.2%	96.4%	93.4%	94.7%	2.7%	70%-130%
2,3,7,8 TCDD	400.0	93.0%	97.4%	95.4%	94.7%	95.1%	1.9%	70%-130%
1,2,3,7,8 PCDF	2000.0	94.9%	99.9%	95.2%	96.7%	96.7%	2.3%	70%-130%
2,3,4,7,8 PCDF	2000.0	91.9%	96.9%	94.7%	94.4%	94.5%	2.1%	70%-130%
1,2,3,7,8 PCDD	2000.0	96.3%	100.0%	101.5%	98.2%	99.0%	2.3%	70%-130%
1,2,3,4,7,8 HxCDF	2000.0	99.2%	101.9%	99.8%	103.7%	101.2%	2.0%	70%-130%
1,2,3,6,7,8 HxCDF	2000.0	98.8%	101.7%	102.3%	97.0%	100.0%	2.5%	70%-130%
2,3,4,6,7,8 HxCDF	2000.0	99.6%	101.7%	100.7%	102.0%	101.0%	1.1%	70%-130%
1,2,3,4,7,8 HxCDD	2000.0	106.2%	100.4%	99.8%	98.0%	101.1%	3.5%	70%-130%
1,2,3,6,7,8 HxCDD	2000.0	94.5%	107.7%	107.0%	106.1%	103.8%	6.0%	70%-130%
1,2,3,7,8,9 HxCDD	2000.0	105.9%	116.6%	111.6%	107.7%	110.4%	4.3%	70%-130%
1,2,3,7,8,9 HxCDF	2000.0	97.2%	101.8%	99.5%	100.4%	99.7%	2.0%	70%-130%
1,2,3,4,6,7,8 HpCDF	2000.0	94.2%	99.3%	95.6%	93.5%	95.7%	2.7%	70%-130%
1,2,3,4,6,7,8 HpCDD	2000.0	96.8%	99.0%	98.9%	97.9%	98.2%	1.1%	70%-130%
1,2,3,4,7,8,9 HpCDF	2000.0	94.0%	97.3%	97.1%	95.1%	95.9%	1.6%	70%-130%
OCDD	4000.0	97.3%	101.4%	103.3%	101.1%	100.8%	2.5%	70%-130%
OCDF	4000.0	100.7%	104.4%	103.9%	102.0%	102.7%	1.7%	70%-130%

Native spike 400-4000 pg - native amounts reported as percent recovery of spike

Native PCBs for Initial Demonstration of Capability

natives in pg	spike	IDC-1	IDC-2	IDC-3	IDC-4	Average	RSD (%)	Acceptable window
PCB-54	4000.0	90.1%	93.3%	96.5%	94.9%	93.7%	2.9%	70%-130%
PCB-104	4000.0	92.1%	94.5%	96.8%	97.6%	95.3%	2.6%	70%-130%
PCB-81	4000.0	92.3%	93.3%	94.0%	94.1%	93.4%	0.9%	70%-130%
PCB-77	4000.0	97.8%	95.9%	96.3%	98.3%	97.1%	1.2%	70%-130%
PCB-123	4000.0	90.6%	93.4%	95.0%	94.9%	93.5%	2.2%	70%-130%
PCB-118	4000.0	96.3%	107.0%	102.4%	98.8%	101.1%	4.6%	70%-130%
PCB-114	4000.0	88.7%	91.5%	93.1%	94.2%	91.9%	2.6%	70%-130%
PCB-105	4000.0	94.9%	101.0%	99.7%	99.4%	98.8%	2.7%	70%-130%
PCB-126	4000.0	99.7%	101.4%	101.7%	101.4%	101.0%	0.9%	70%-130%
PCB-167	4000.0	90.6%	94.3%	95.7%	96.1%	94.2%	2.7%	70%-130%
PCB-156	4000.0	89.1%	88.2%	78.6%	89.4%	86.3%	6.0%	70%-130%
PCB-157	4000.0	91.5%	90.6%	94.7%	91.9%	92.2%	1.9%	70%-130%
PCB-169	4000.0	91.4%	93.9%	94.1%	94.9%	93.6%	1.6%	70%-130%
PCB-189	4000.0	88.7%	91.0%	92.7%	93.7%	91.5%	2.4%	70%-130%

Native spike 4000 pg - native amounts reported as percent recovery of spike

Native PCDD/Fs Method Detection Limit

MDL study	native spike ppt	ppt MDL-1	ppt MDL-2	ppt MDL-3	ppt MDL-4	ppt MDL-5	ppt MDL-6	ppt MDL-7	ppt MB	ppt STDEV	ppt MDL
2,3,7,8 TCDF	0.40	0.35	0.30	0.30	0.28	0.32	0.39	0.32	0.09	0.04	0.12
2,3,7,8 TCDD	0.40	0.39	0.25	0.38	0.30	0.35	0.40	0.31	0.08	0.05	0.17
1,2,3,7,8 PCDF	2.00	1.84	1.36	1.73	1.42	1.57	2.01	1.63	0.41	0.23	0.72
2,3,4,7,8 PCDF	2.00	1.85	1.27	1.71	1.36	1.57	1.94	1.66	0.41	0.24	0.77
1,2,3,7,8 PCDD	2.00	1.82	1.44	1.76	1.29	1.77	1.99	1.57	0.37	0.24	0.76
1,2,3,4,7,8 HxCDF	2.00	2.00	1.40	1.91	1.46	1.76	2.02	1.96	0.36	0.26	0.82
1,2,3,6,7,8 HxCDF	2.00	1.93	1.31	1.67	1.35	1.73	1.96	1.62	0.39	0.25	0.80
2,3,4,6,7,8 HxCDF	2.00	1.89	1.26	1.74	1.38	1.53	2.15	1.78	0.38	0.31	0.96
1,2,3,4,7,8 HxCDD	2.00	1.85	1.24	1.70	1.27	1.86	1.89	1.62	0.37	0.28	0.87
1,2,3,6,7,8 HxCDD	2.00	1.84	1.17	1.89	1.40	1.78	2.10	1.77	0.36	0.31	0.99
1,2,3,7,8,9 HxCDD	2.00	1.86	1.30	1.88	1.48	1.76	2.27	1.88	0.40	0.31	0.98
1,2,3,7,8,9 HxCDF	2.00	1.91	1.35	1.84	1.30	1.77	1.95	1.82	0.37	0.27	0.84
1,2,3,4,6,7,8 HpCDF	2.00	1.84	1.61	1.95	1.41	1.76	2.10	1.71	0.60	0.22	0.71
1,2,3,4,6,7,8 HpCDD	2.00	1.83	1.46	1.85	1.52	2.04	1.89	1.86	0.51	0.21	0.66
1,2,3,4,7,8,9 HpCDF	2.00	1.96	1.28	1.64	1.43	1.77	2.24	1.62	0.57	0.32	1.02
OCDD	4.00	4.01	2.81	3.98	2.92	4.63	4.59	3.88	1.03	0.72	2.27
OCDF	4.00	4.10	2.50	3.58	2.64	3.51	4.26	3.74	0.93	0.68	2.12

Native PCBs
Method Detection Limit

| MDL study		native spike ppt	ppt MDL-1	ppt MDL-2	ppt MDL-3	ppt MDL-4	ppt MDL-5	ppt MDL-6	ppt MDL-7	ppt MB	ppt STDEV	ppt MDL
22'66'-T4CDB	PCB-54	10.00	8.39	8.89	8.75	8.26	8.77	8.00	7.84	n.d.	0.41	1.28
22'466'-PeCB	PCB_104	10.00	7.75	8.54	8.02	7.71	7.76	7.54	7.59	0.55	0.34	1.08
344'5-Te-PCB	PCB_81	10.00	8.28	9.44	8.71	8.91	8.40	8.64	8.78	0.75	0.38	1.19
33'44'-Te_PCB	PCB_77	10.00	8.06	8.04	7.98	8.72	10.66	10.66	10.99	1.35	1.40	4.40
2'344'5-Pe-PCB	PCB_123	10.00	7.59	8.78	9.16	8.33	9.16	7.30	8.33	0.60	0.73	2.29
23'44'5-Pe-PCB	PCB_118	10.00	8.88	9.16	9.14	9.40	9.33	11.24	11.92	5.25	1.20	3.76
2344'5-Pe-PCB	PCB_114	10.00	7.64	8.85	9.02	8.03	8.74	8.81	8.75	n.d.	0.51	1.60
233'44'-Pe-PCB	PCB_105	10.00	8.66	8.26	8.79	7.81	8.16	8.06	9.44	2.50	0.55	1.73
33'44'5-Pe-PCB	PCB_126	10.00	7.83	8.86	8.53	8.29	8.11	8.15	8.14	0.70	0.33	1.05
23'44'55'-Hx-PCB	PCB_167	10.00	8.46	10.10	8.20	8.60	9.54	8.33	8.42	0.95	0.72	2.27
233'44'5-Hx-PCB	PCB_156	10.00	9.56	7.91	7.32	7.17	6.67	8.41	8.61	0.70	0.99	3.11
233'44'5'-Hx-PCB	PCB_157	10.00	10.03	8.18	10.27	8.35	11.58	9.40	9.61	0.60	1.17	3.67
33'44'55'-Hx-PCB	PCB_169	10.00	8.39	8.71	8.41	8.18	7.31	8.20	7.71	0.85	0.47	1.48
233'44'55'-Hp-PCB	PCB_189	10.00	8.33	9.48	9.39	8.36	9.71	8.64	8.71	0.50	0.57	1.79

13C PCB various matrices

13C Dioxins various matrices
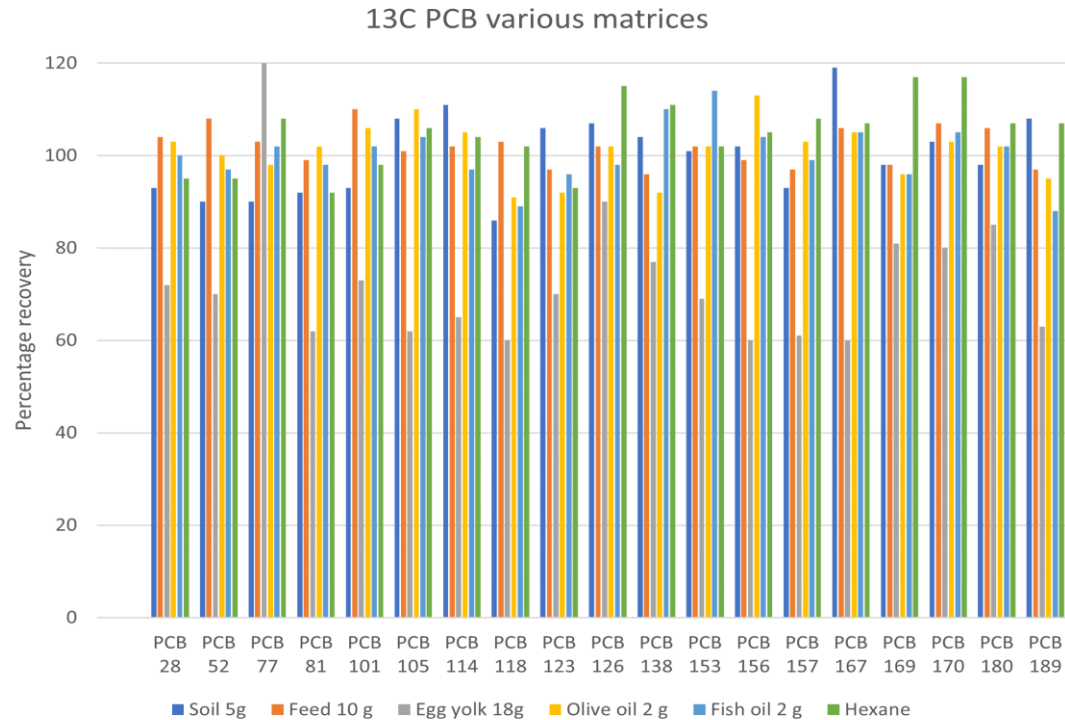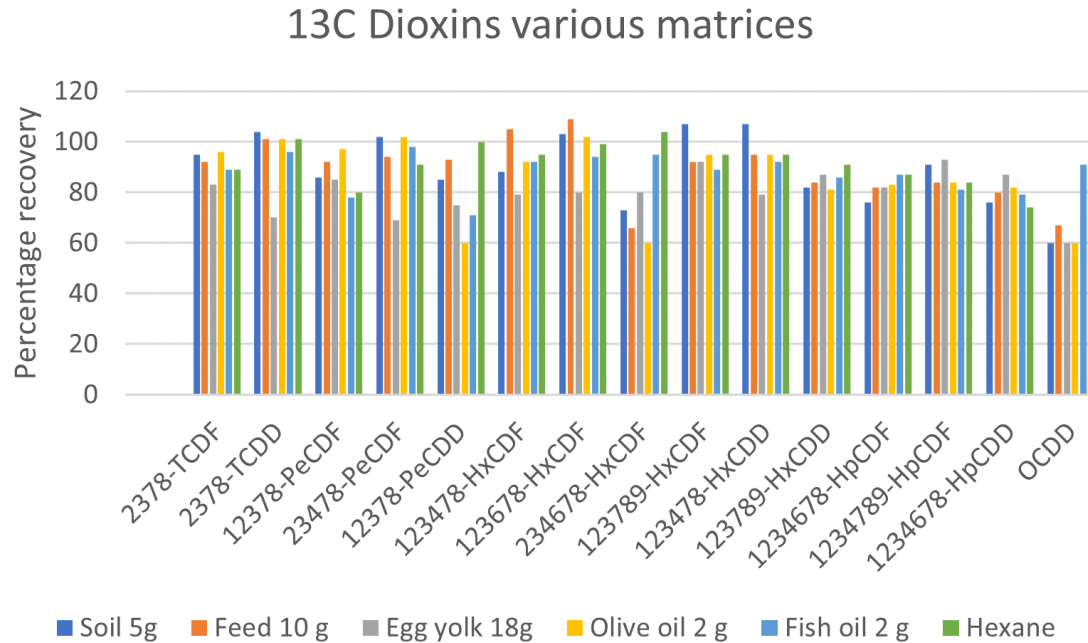
Comparison of Native Data with Automated System

PCBs comparison 2 g fish oil
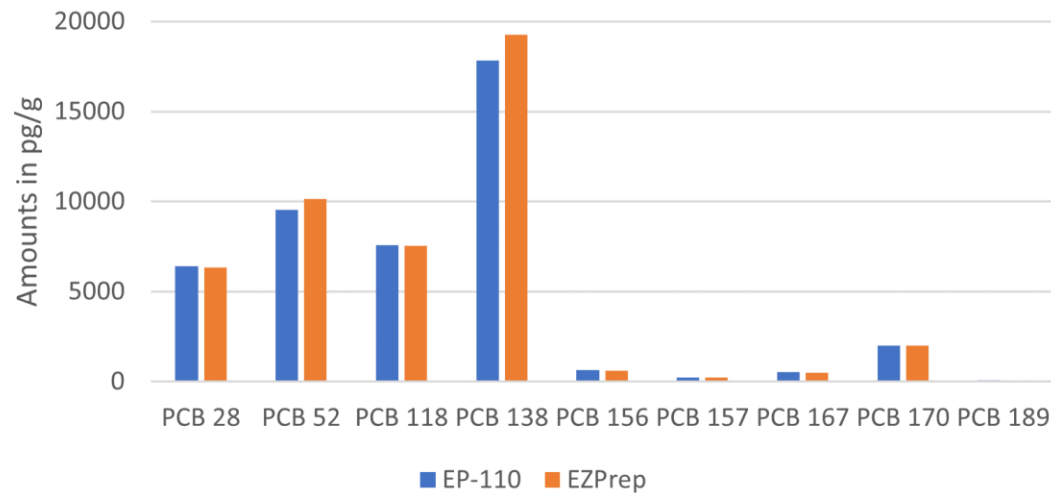
- ## **Dioxin/PCB/PBDE (POPs) workflow**

  - Fully Automated Extraction/Semi-Automated Sample Cleanup/Concentration and Agilent 7010 GC/TQ
    - Easy to Use, Low-Cost Sample Prep Workflow
    - Provide SOPs for the entire process
    - FMS will provide Installation and training

  - Fully Automated Extraction/Sample Cleanup/Concentration and Agilent 7010 GC/TQ
    - Fully Automated, Unattended, Expandable from 2 to 8 samples
    - Provide SOPs for the entire process
    - FMS will provide Installation and training

Questions?

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