# A Brief 25-year History and Recent Developments in Automated High-Throughput Sample Preparation of Human Specimens for the Measurement of PCDDs, PCDFs, cPCBs, PCNs, PBDDs, PBDFs, PCBs, PBDEs and OC Pesticides by GC/ID-HRMS.

**4th International Seminar on Total Solutions for POPs Analysis**
**Workshop at Dioxin 2011, Brussels, Belgium**

Wayman Turner - Senior Laboratory Scientist – Centers for Disease Control and Prevention Foundation

# Each step is *Important*
## and has its own unique challenges

- Sample collection, storage & handling
- Spiking with $^{13}C$-internal standards
- Pre-cleanup (extraction, SPE etc…)
- Sample Cleanup and Enrichment Procedure
- Sample Evaporation
- Analysis & Quantitation (HRGC/HRMS)
- Data Handling & QA/QC

# Determination of Part-per-Trillion Levels of Polychlorinated Dibenzofurans and Dioxins in Environmental Samples

**Lawrence M. Smith,* David L. Stalling, and James L. Johnson**

*Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, Route 1, Columbia, Missouri 65201*

**The analytical method permits determinations of parts-per-trillion levels and lower of tetrachloro through octachloro congeners of dibenzo-*p*-dioxins and dibenzofurans in various types of biological tissues and sediments. Preliminary tests also indicated the method is applicable to determinations of tetrachloro through hexachloro congeners of ortho-unsubstituted polychlorinated biphenyls. Interferences both from biogenic and from xenobiotic substances are reduced to extremely low levels. The procedure has an extremely low susceptibility to false-positive determinations which could result from the presence of a wide variety of cocontaminants. A modular approach to contaminant enrichment has permitted the integration of seven processes into a two-step procedure, significantly reducing time requirements and the number of sample manipulations, and making the procedure amenable to automation. The reliability and accuracy of the procedure are demonstrated by the results of intralaboratory and interlaboratory studies and by successful analyses of over 200 samples of a wide variety of types.**

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and ortho-unsubstituted polychlorinated biphenyls (non-ortho PCBs) are three structurally and toxicologically related families of anthropogenic chemical compounds that have in recent years been shown to have the potential to cause serious environmental contamination (*1–6*). These substances are trace-level components or byproducts in several large-volume and widely used synthetic chemicals, principally PCBs and chlorinated phenols (*7, 8*), and can also be produced during combustion processes (*3, 9–11*) and by photolysis (*12, 13*). In general, PCDDs, PCDFs, and non-ortho PCBs are classified as highly toxic substances (*14*), although the toxicities are dramatically dependent on the number and positions of the chlorine substituents (*15*). About 10 individual members of a total of 216 PCDDs, PCDFs, and non-ortho PCBs are among the most toxic man-made or natural substances to a variety of animal species (*1–4*). The toxic hazards posed by these chemicals are exacerbated by their propensity to persist in the environment (*16–18*) and to readily bioaccumulate (*19–21*), and although the rate of metabolism and elimination is strongly species dependent (*20*), certain highly toxic isomers have been observed to persist in the human body for more than 10 years (*22*).

The majority of scientific and governmental concerns for the hazards of these compounds have been directed toward analytical methodologies, toxicology, epidemiology, and determination of the disposition in the environment of the single most toxic isomer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) (*1–6, 8*).

More recently, however, investigations into the formation and occurrence of PCDFs suggest that this family of toxic compounds may commonly occur at comparable or greater levels than and could generally pose a greater hazard than PCDDs. PCDFs are often found as cocontaminants in and are readily produced from pyrolysis of PCBs (*7, 23–26*). Most important, the PCDFs produced from pyrolysis of PCBs are predominantly the most toxic isomers, those having a 2,3,7,8-chlorine substitution pattern (*5*). A number of recent fires involving electrical transformers and capacitors have demonstrated the potential for formation of hazardous levels of PCDFs from pyrolysis of PCBs (*26–28, 30*).

In light of these findings and because of the dearth of data pertaining to the occurrence of these compounds in the environment, PCDFs and non-ortho PCBs were included as target compounds in a proposed survey by this laboratory of important U.S. rivers and lakes for PCDDs. The decision to include as many PCDD isomers as possible was based on

# Smith, Stalling & Johnson Schematic

ANALYTICAL CHEMISTRY, VOL. 56, NO. 11, SEPTEMBER 1984 • 1831

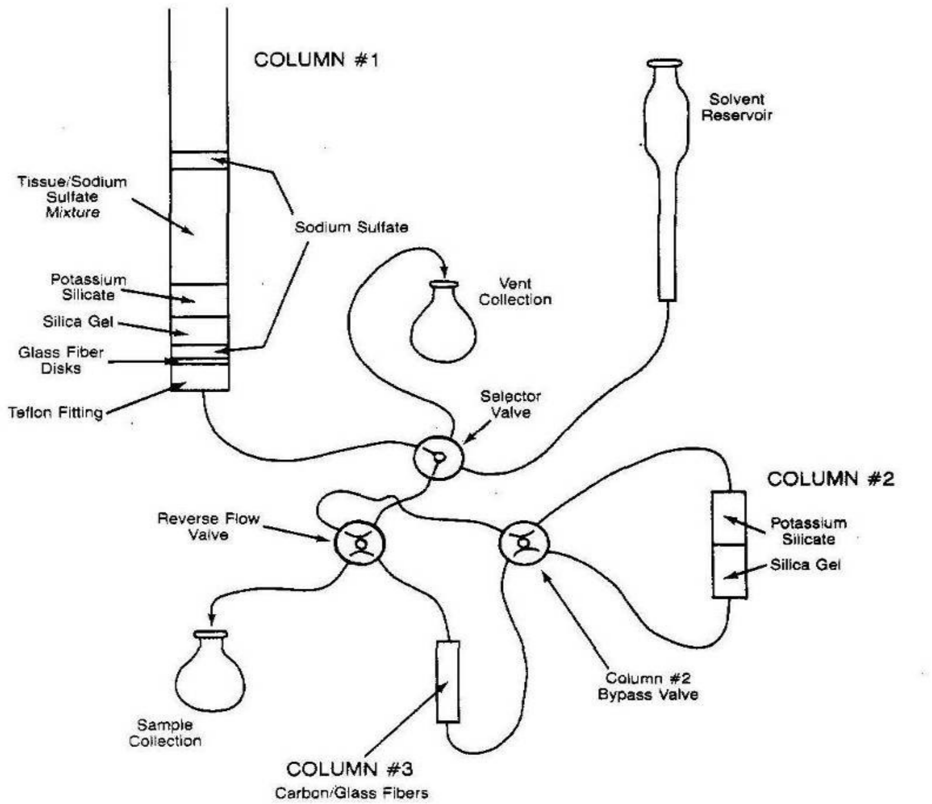


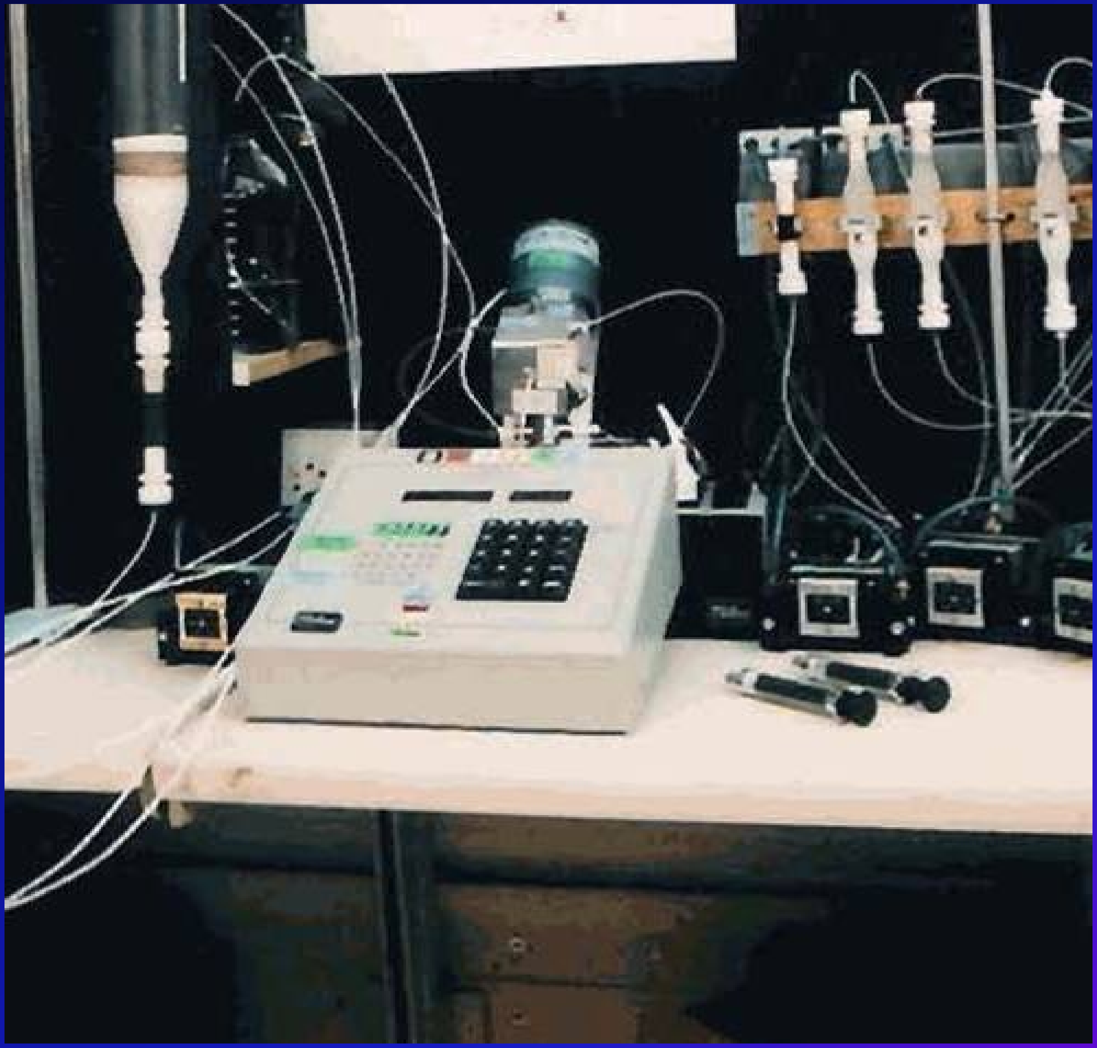

**Figure 2.** Schematic of part I enrichment apparatus.

of the method developed for the determination of PCDDs, PCDFs, and non-ortho PCBs in a variety of environmental matrices.

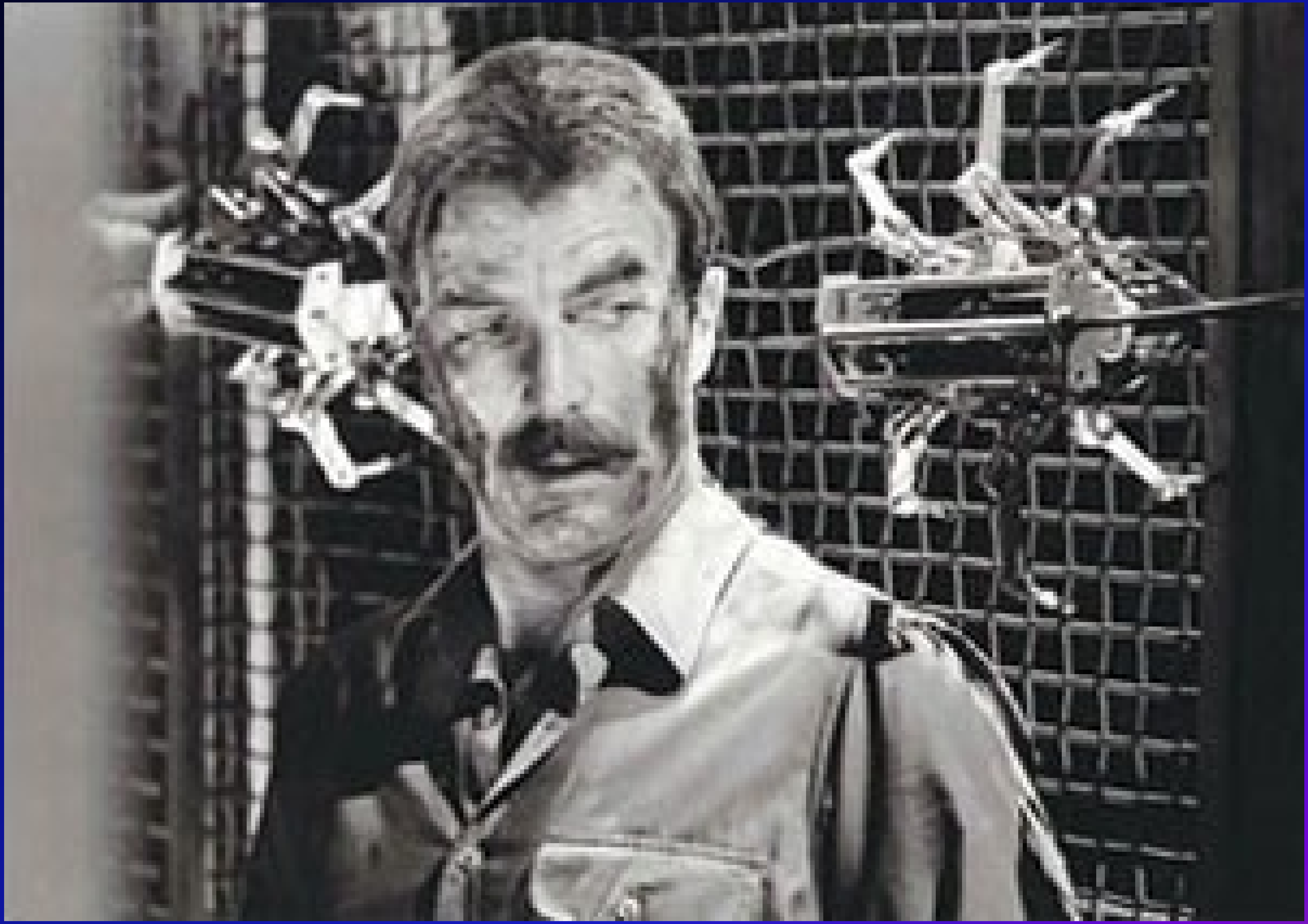
# “Mid-1980’s” First Attempt At “Hand” Automation

# Late-1980’s Automated System

# Eldex Controller



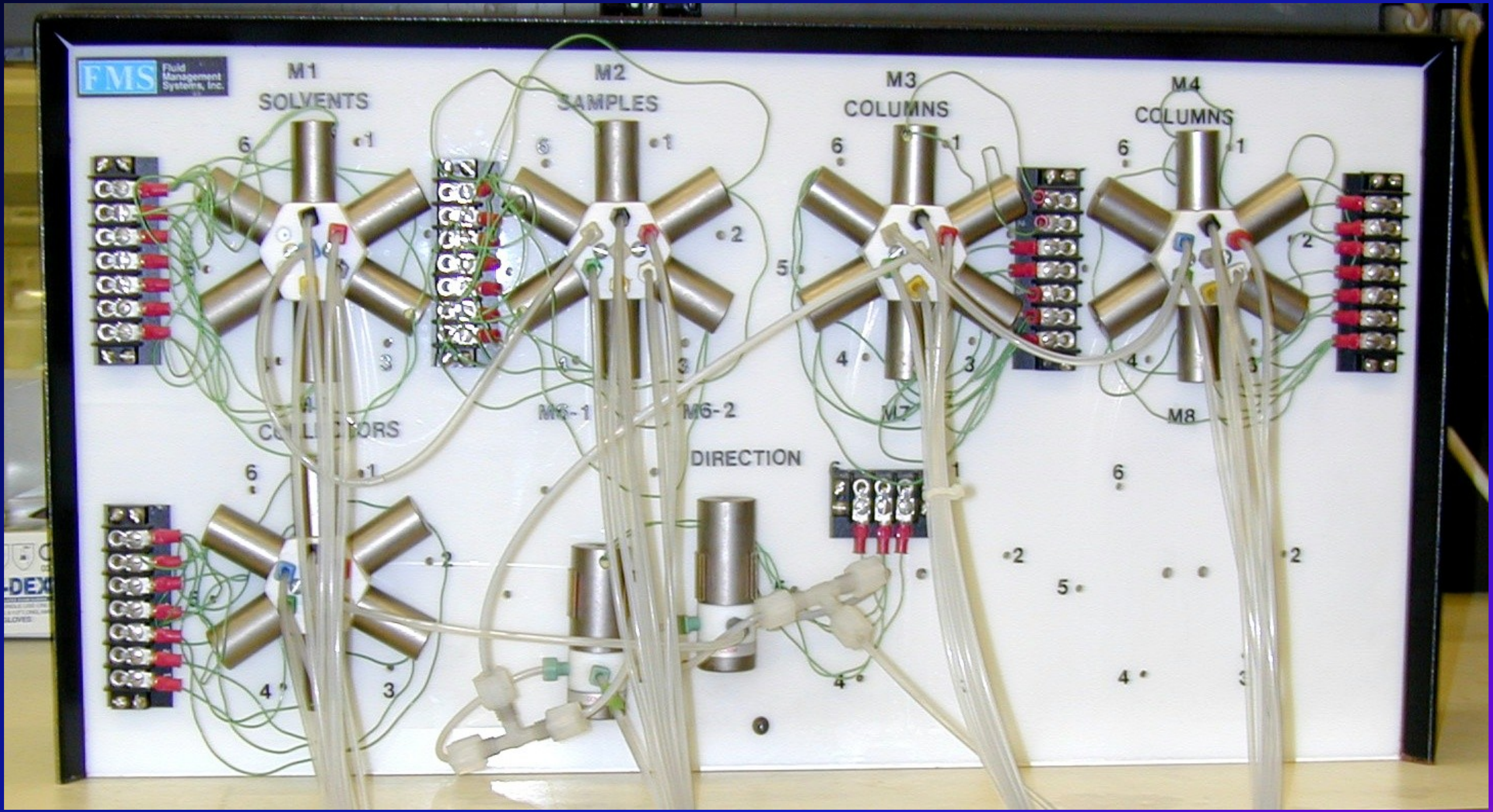
**RUNAWAY** (Tom Selleck) – killer spider robots



# FMS Programmable Pump

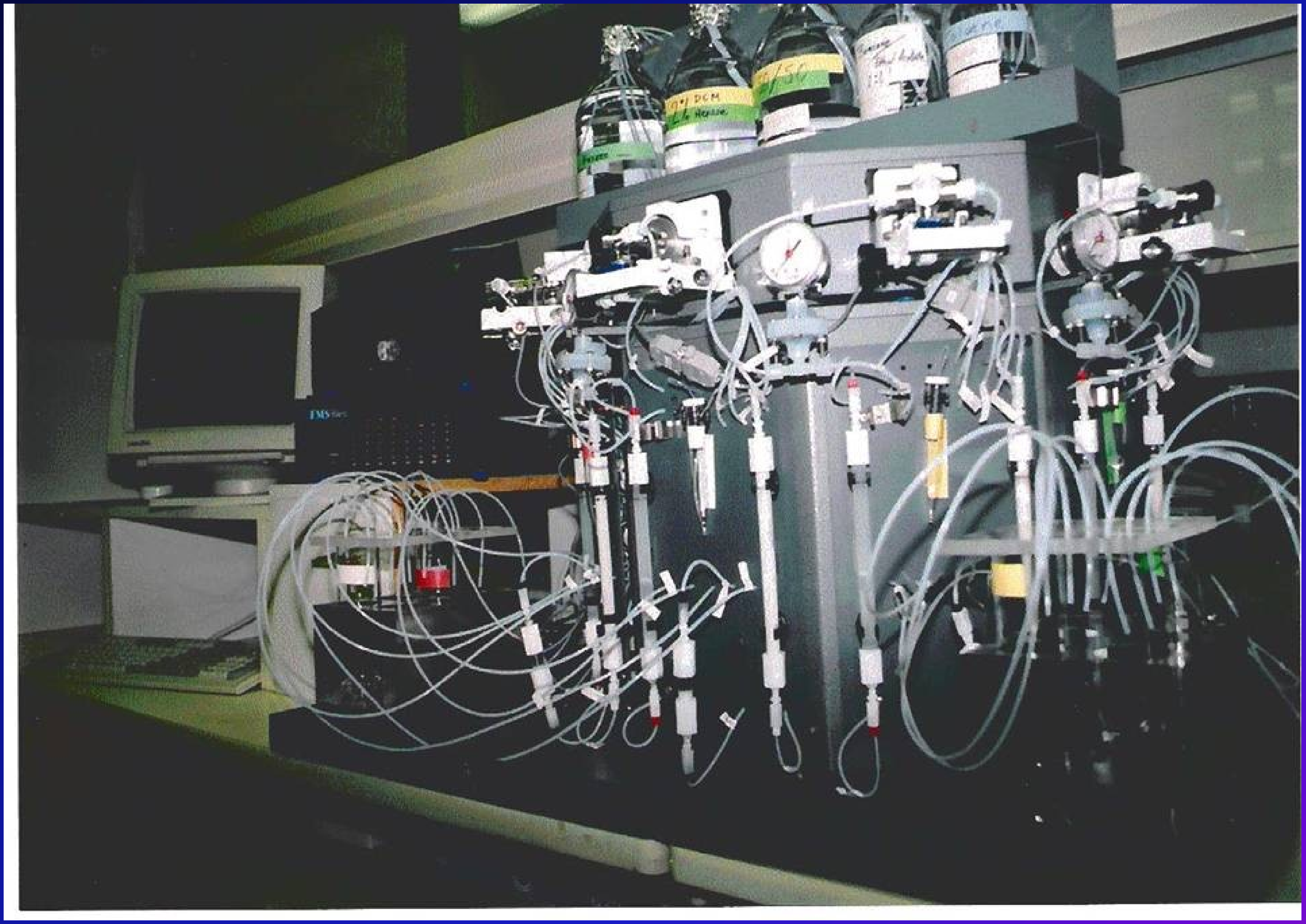
# Early FMS Power-Prep

# Six-way & Two-way Valves



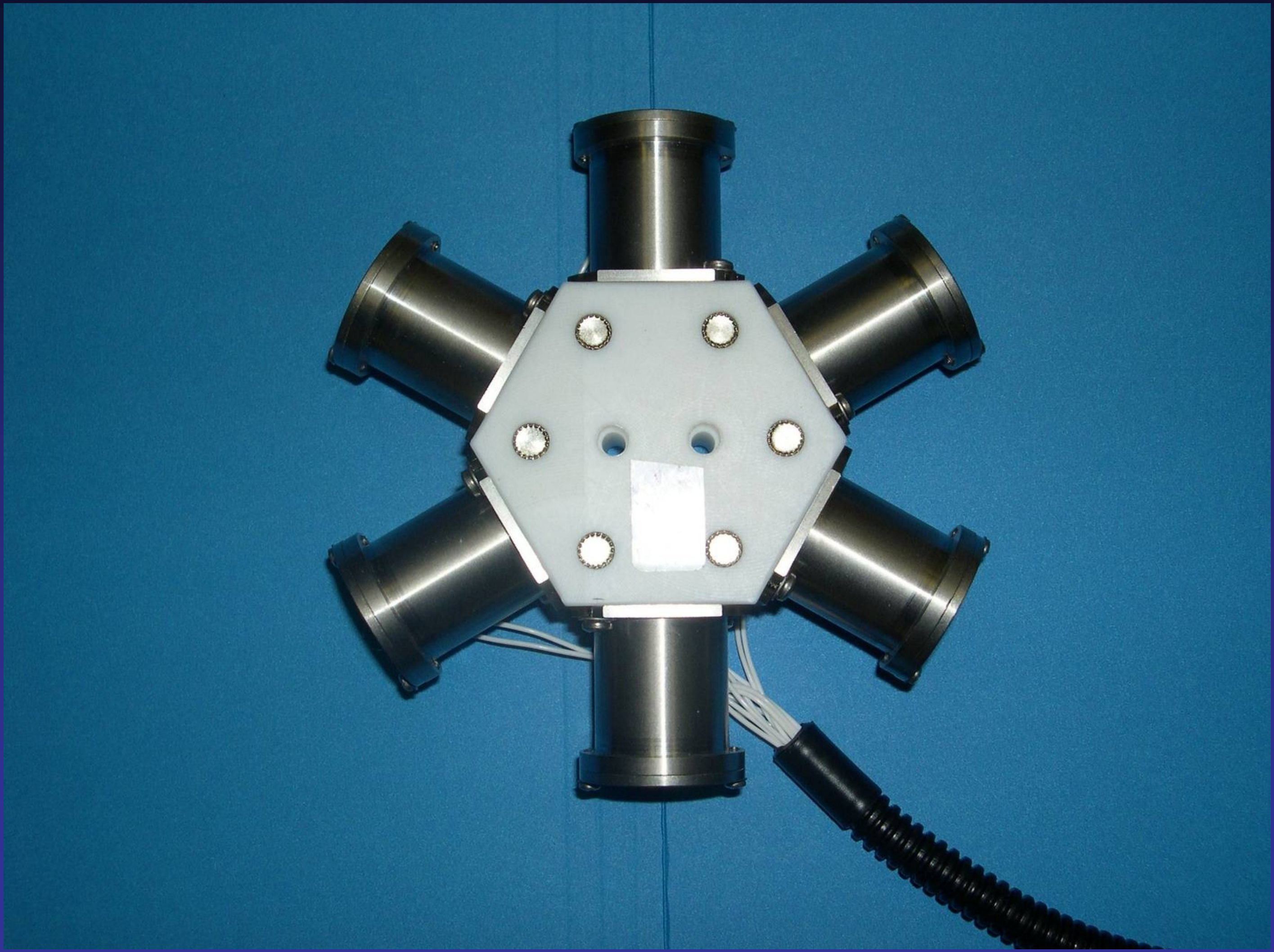

KIMBLE
FOIL

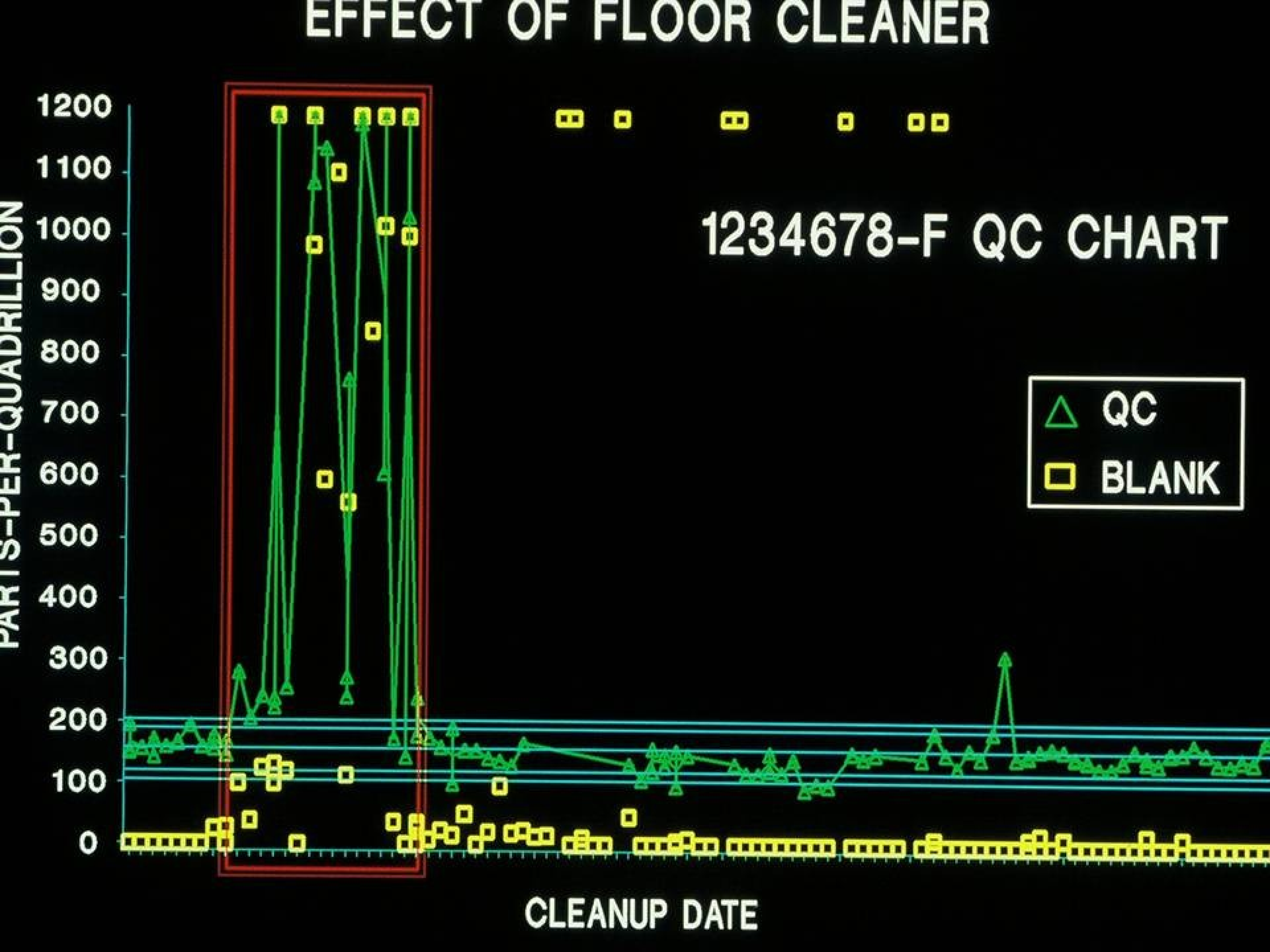
# Early 5-Carbon Column System



# Current FMS Power-Prep

# FMS Six-way Valve




EFFECT OF FLOOR CLEANER
1234678-F QC CHART
PARTS-PER-QUADRILLION
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
QC
BLANK
CLEANUP DATE

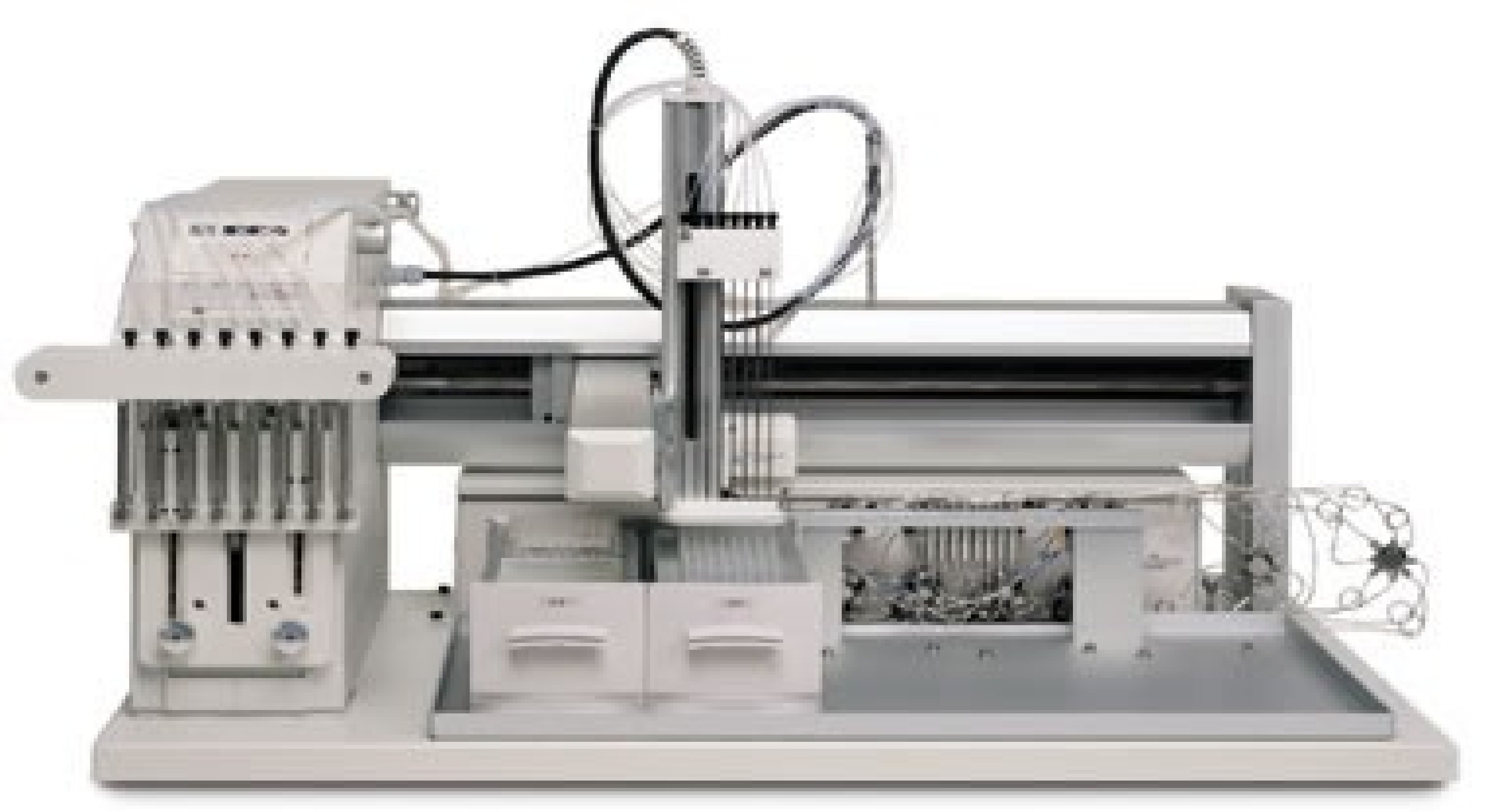

FMS
Fluid Management Systems, Inc.
56-58 Felton St.

# Caliper (Zymark) TurboVap II & LV

## TurboVap II

## TurboVap LV

# Gilson 215 Liquid Handler



Seveso

Agent Orange

Calcasieu Parish, LA

NHANES 1999-2010

Vietnam Experience

NIOSH Workers

VA Chemical Corps

# 1,015,472 Analyses

(PCDDs/PCDFs/cPCBs, PCBs and OCPs)

Alaska Native Villages

World Trade Center

Chapaevsk, Russia

Sierra Vista, AZ

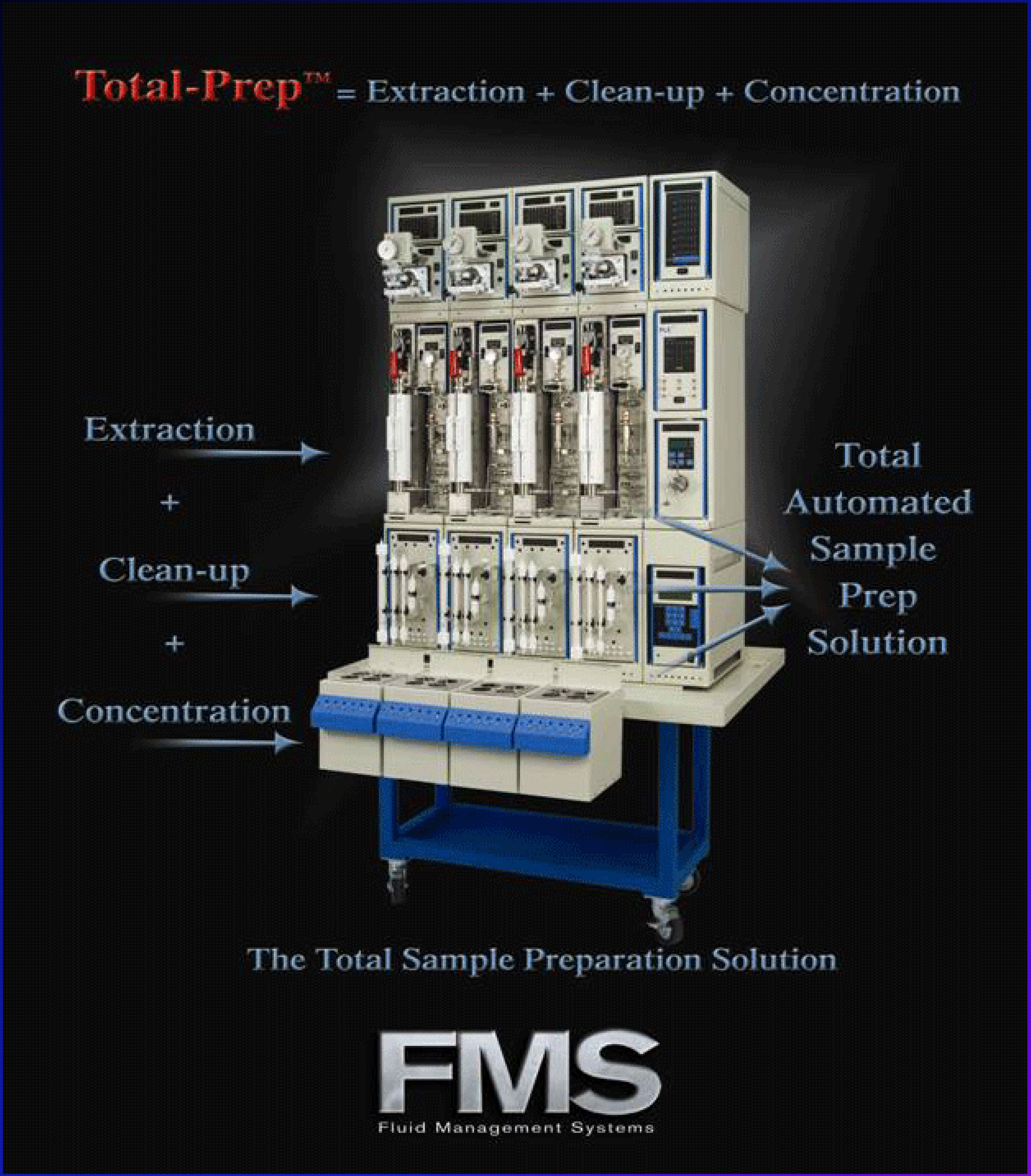
Yucheng

Ranch Hand
- 1982
- 1987
- 1992
- 1997
- 2002

Times Beach, MO

Great Lakes

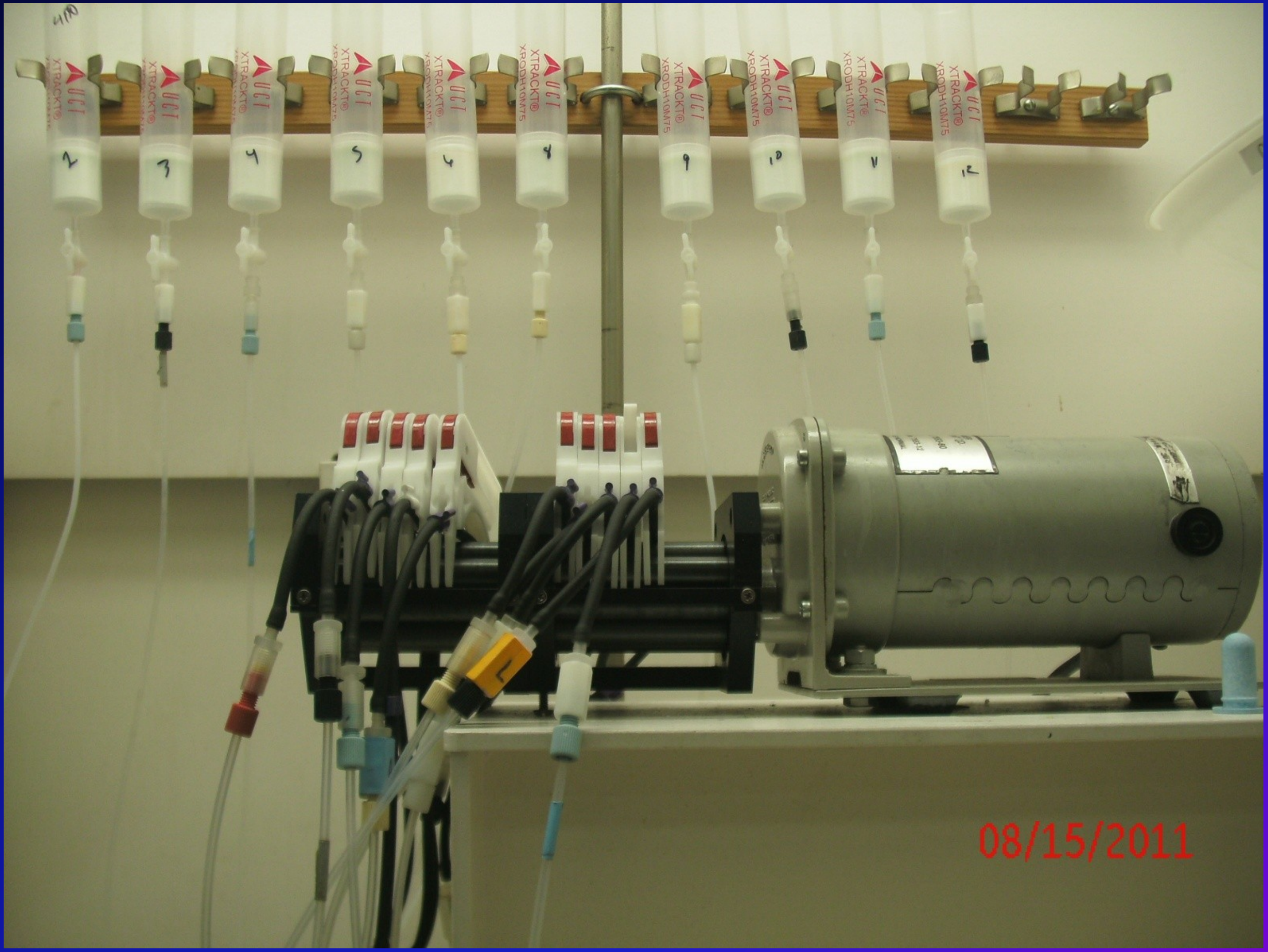
New Zealand Sprayers


Total-Prep™ = Extraction + Clean-up + Concentration
Extraction
+
Clean-up
+
Concentration
Total Automated Sample Prep Solution
The Total Sample Preparation Solution
FMS
Fluid Management Systems

# Rotary Evaporator

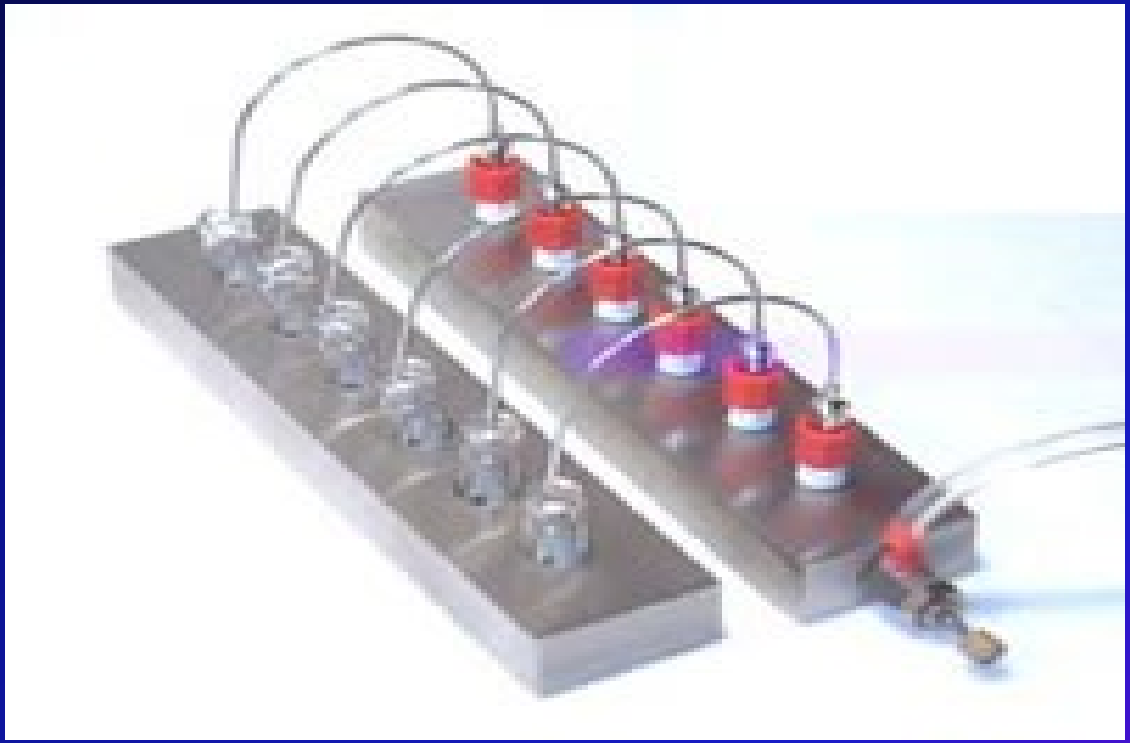
# Separtory Funnel

# Automated C18-SPE



# FMS PowerVap

# Needle Evaporator



# Gilson Microman Pipet

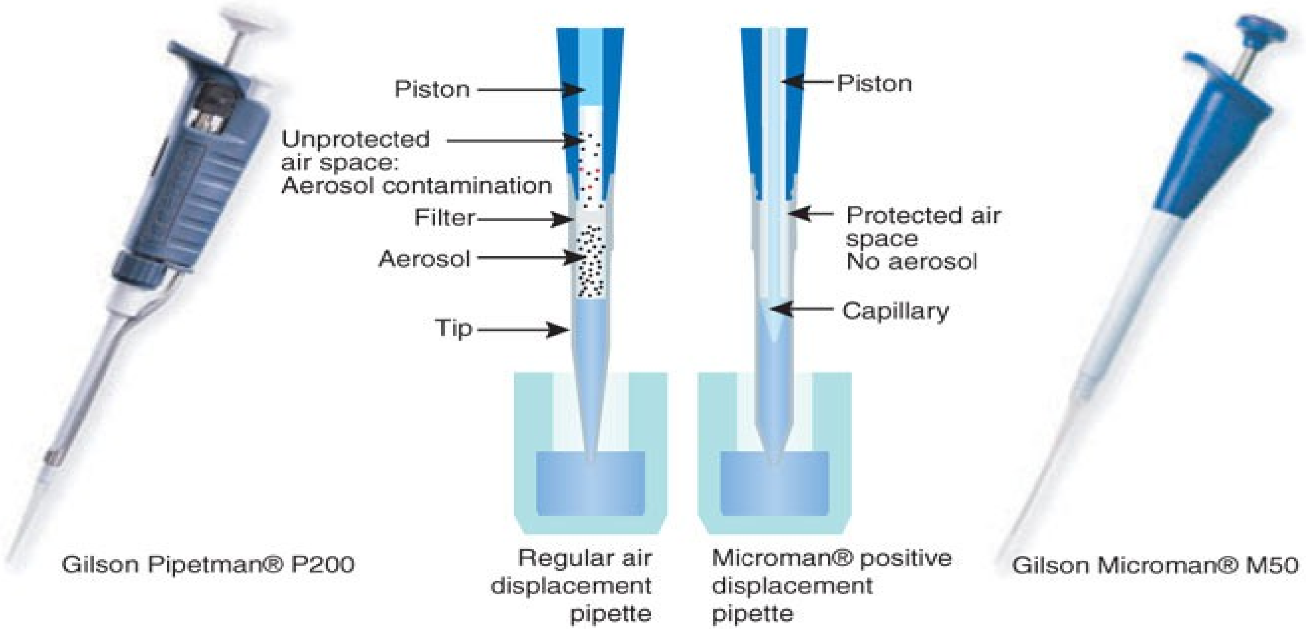
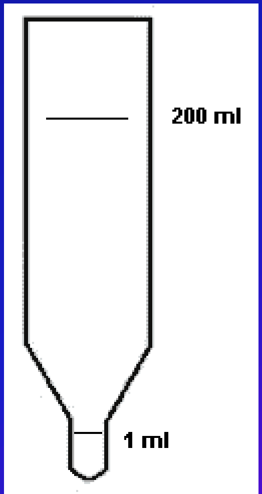



**Figure 1** | Two pipetting concepts: air displacement using standard filter tips, and positive displacement as applied in the Microman pipette.

# Biotage [Zymark] Turbovap II

# Biotage [Zymark] Turbovap LVII

# Total Lipid References

- Akins J.R., Waldrep K., and Bernert J.T. Jr. The Estimation of Total Serum Lipids by a Completely Enzymatic 'Summation' Method. *Clin. Chim. Acta.* 184: 219-226 (1989).

- Phillips D.D., Pirkle J.L., Burse V.W., Bernert J.T., Henderson L.O. and Needham L.L. Chlorinated Hydrocarbon Levels in Human Serum: Effects of Fasting and Feeding. *Arch. Environ. Contam. Toxicol*. 18: 495-500 (1989).

- Bernert J.T., Turner W.E., Patterson D.G. Jr. and Needham L.L. Calculation of Serum "Total Lipid" Concentrations for the Adjustment of Persistent Organohalogen Toxicant Measurements in Human Samples. *Chemosphere* 68: 824-831 (2007)

Short Formula = (2.27*TCHOL + TRIG + 62.3

Long Formula = 1.667*((TCHOL-FCHOL) + FCHOL +TRIG + PLIPID

# NOTHING IS IMPOSSIBLE

*For those who don't have to do the work!*

# Analyte List

## Polychlorinated Dibenzo-p-dioxins and Dibenzofurans

1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)
1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)
2,3,7,8-Tetrachlorodibenzofuran (TCDF)

## Polybrominated Dibenzo-p-dioxins and Dibenzofurans

2,3,7,8-Tetrabromorodienzo-*p*-dioxin (TBDD)
1,2,3,7,8-Pentabromodibenzo-*p*-dioxin (PeBDD)
1,2,3,4,7,8-Hexabromorodibenzo-*p*-dioxin (HxBDD)
1,2,3,6,7,8-Hexabromodibenzo-*p*-dioxin (HxBDD)
1,2,3,7,8,9-Hexabromodibenzo-*p*-dioxin (HxBDD)
1,2,3,4,6,7,8-Heptabromoodibenzo-*p*-dioxin (HpBDD)
1,2,3,4,6,7,8,9-Octabromodibenzo-*p*-dioxin (OBDD)
2,3,7,8,-Tetrabromodibenzofuran (TBDF)
1,2,3,7,8-Pentabromodibenzofuran (PeBDF)
2,3,4,7,8-Pentabromodibenzofuran (PeBDF)
1,2,3,4,7,8-Hexabromodibenzofuran (HxBDF)
1,2,3,6,7,8-Hexabromodibenzofuran (HxBDF)
1,2,3,7,8,9-Hexabromodibenzofuran (HxBDF)
2,3,4,6,7,8,-Hexabromodibenzofuran (HxBDF)
1,2,3,4,6,7,8-Heptabromodibenzofuran (HpBDF)
1,2,3,4,7,8,9-Heptabromodibenzofuran (HpBDF)
1,2,3,4,6,7,8,9-Octabromodibenzofuran (OBDF)

## Dioxin-like Polychlorinated Biphenyls - cPCBs

3,3',4,4'-Tetrachlorobiphenyl (PCB 77)
3,4,4',5-Tetrachlorobiphenyl (PCB 81)
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)

## Polychlorinated Naphthalenes

1,2,3,4-Tetrachlorinated naphthalene (PCN 27)
1,2,3,5,7- and 1,2,4,6,7-Pentachlorinated naphthalene (PNC 52 & 60)
1,2,3,4,5,7- and 1,2,3,5,6,8-Hexachlorinated naphthalene (PNC 64 & 68)
1,2,3,4,6,7- and 1,2,3,5,6,7-Hexachlorinated naphthalene (PNC 66 & 67)
1,2,3,5,7,8-Hexachlorinated naphthalene (PCN 69)
1,2,3,4,5,6,7-Heptachlorinated naphthalene (PCN 73)

# We Have Come a Long Way

## Automation has significantly changed our throughput

- In 1987 one person (original LLE & semi-automated cleanup method) could process 10 samples for TCDD only in 5 days.
- Today one person (using SPE & PowerPrep) can process 60 samples in 5 days for PCDDs/PCDFs/cPCBs + PCNs and PBDDs/PBDFs.
- In addition today one person (using SPE & Rapid Trace) can process 150 samples in 5 days for PCBs (including mPCBs for TEQ), OC pesticides and PBDEs.


That's all Folks!

# Polychlorinated Dibenzo Dioxins and Furans

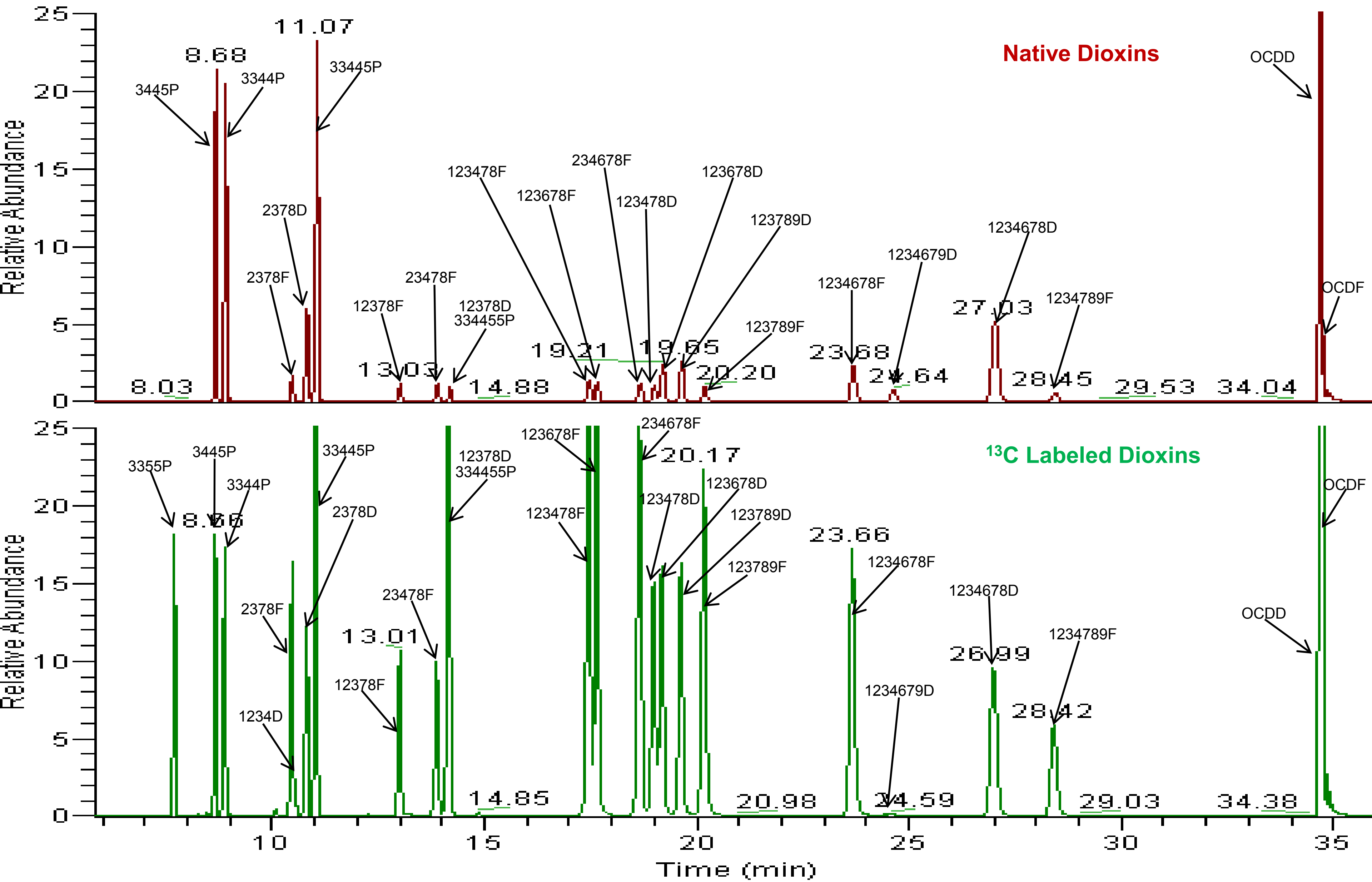



Figure 3

# Polychlorinated Naphthalens

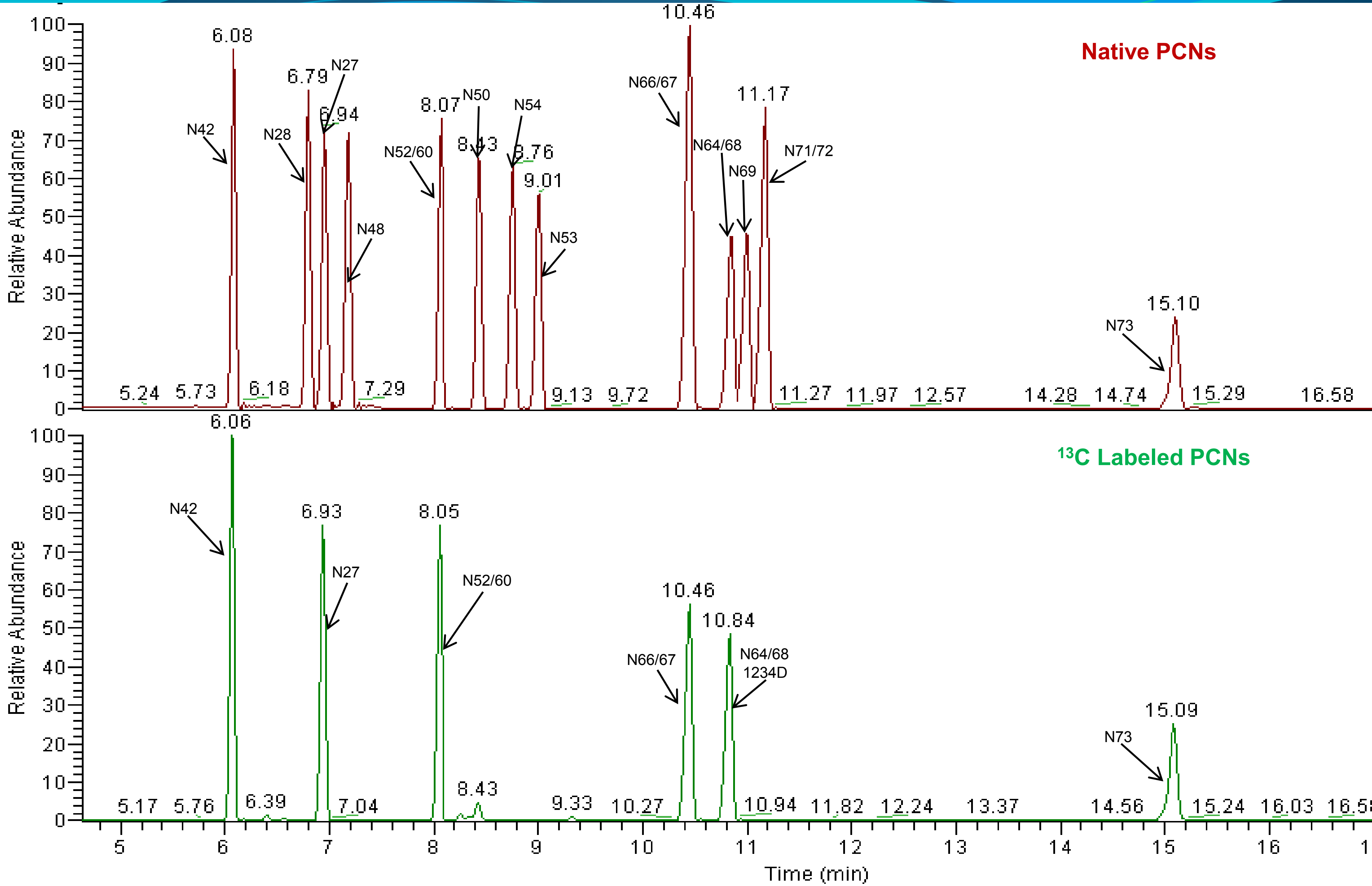



Figure 4

# Pesticides 2010

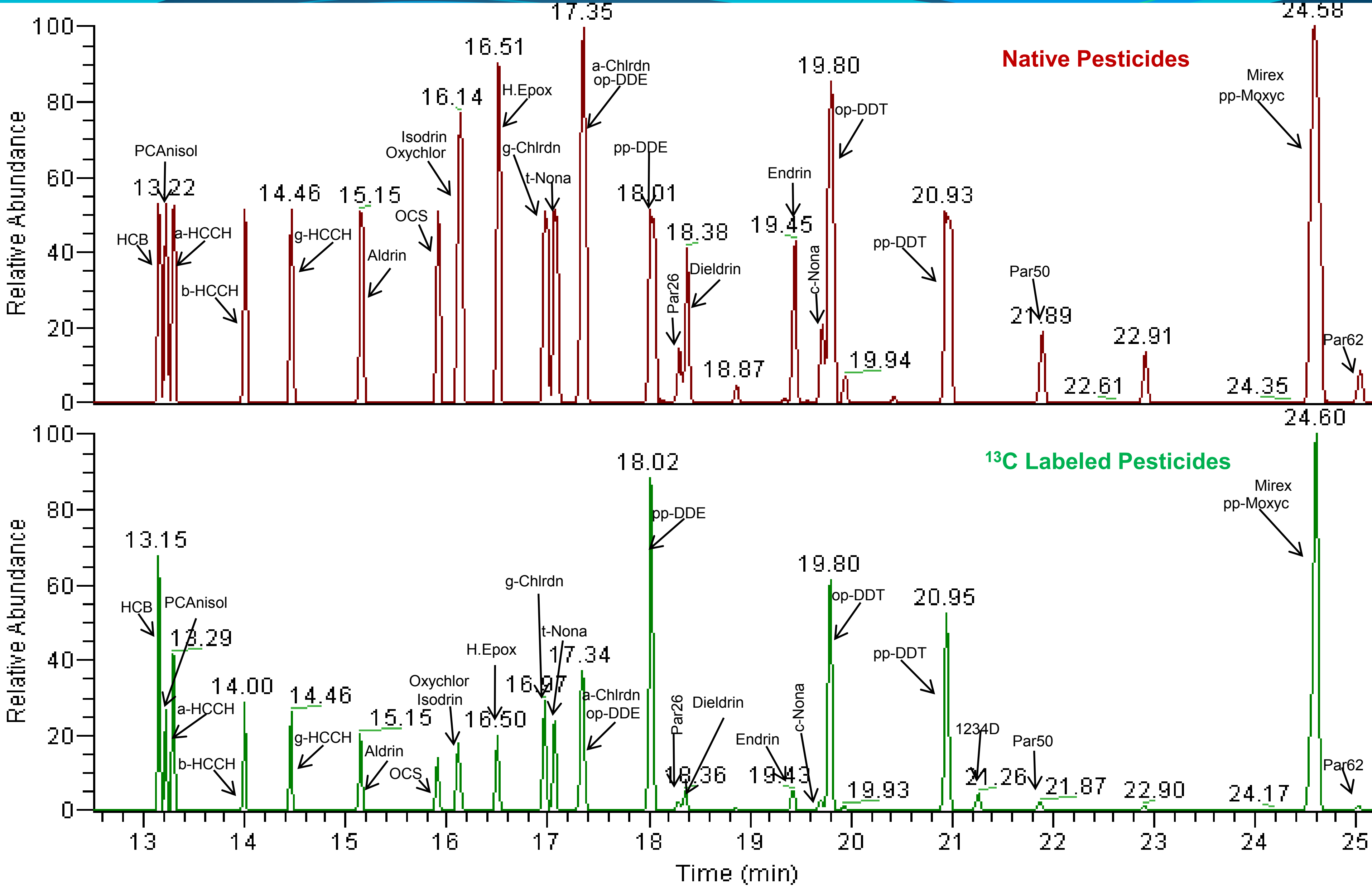
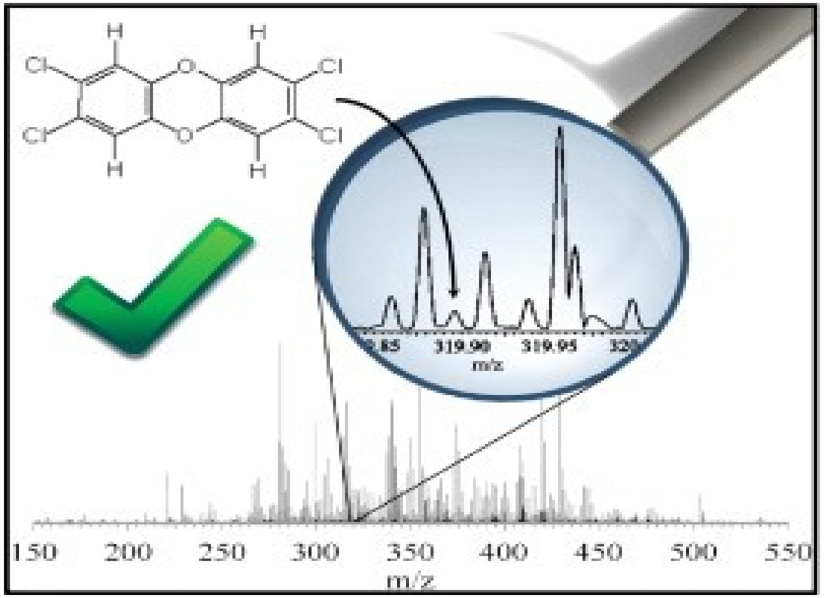


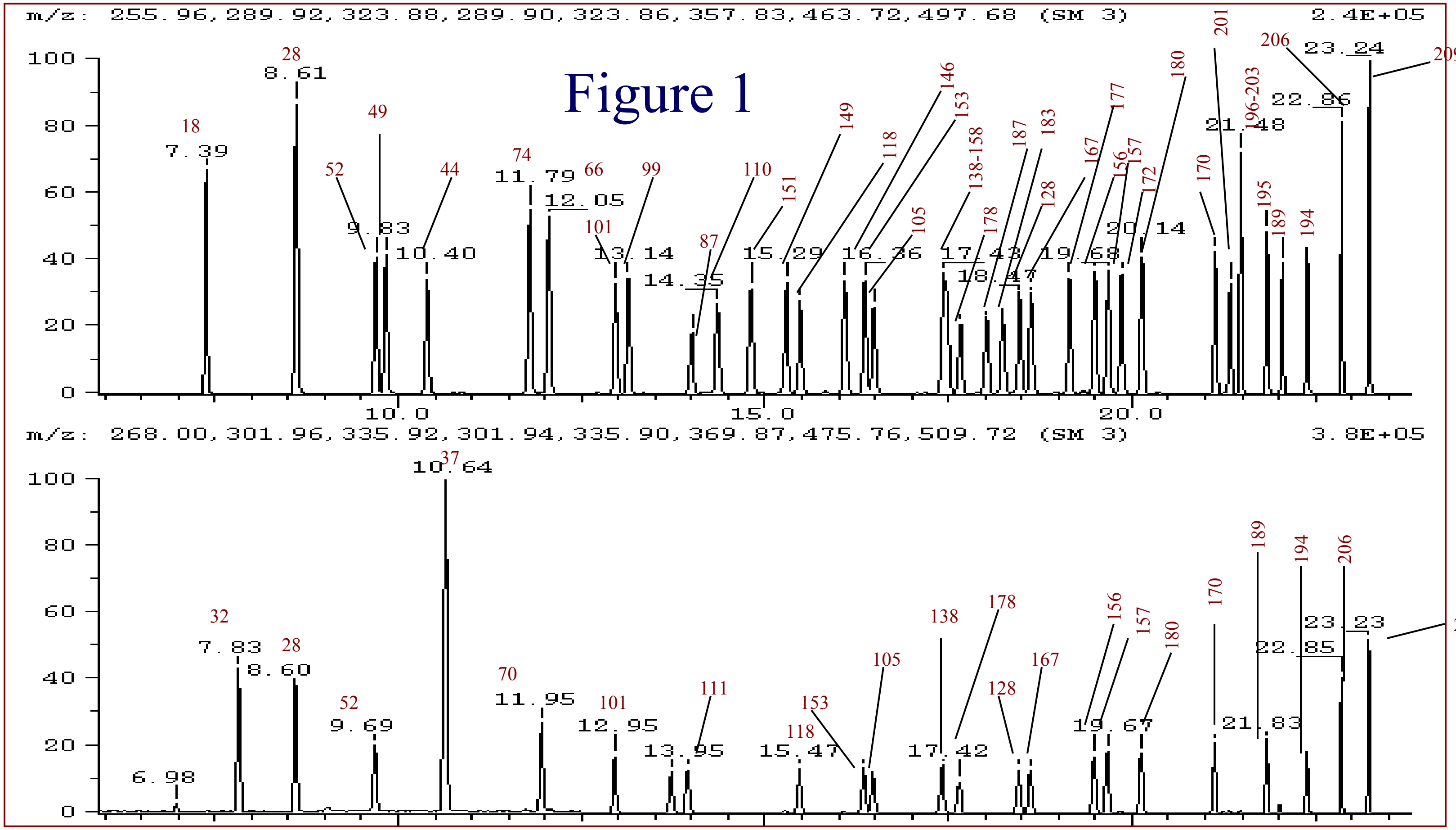

**Figure 2**

# Observation from the 'real world' by an analytical chemist who retired after 57 years from NIST ...

It has been estimated that concentrations of 1 in $10^{18}$ of almost any substance can be expected to be present in almost any sample. This translates to approximately a thousand molecules. In: *Quality Assurance of Chemical Measurements* by John K. Taylor (1987, p48).

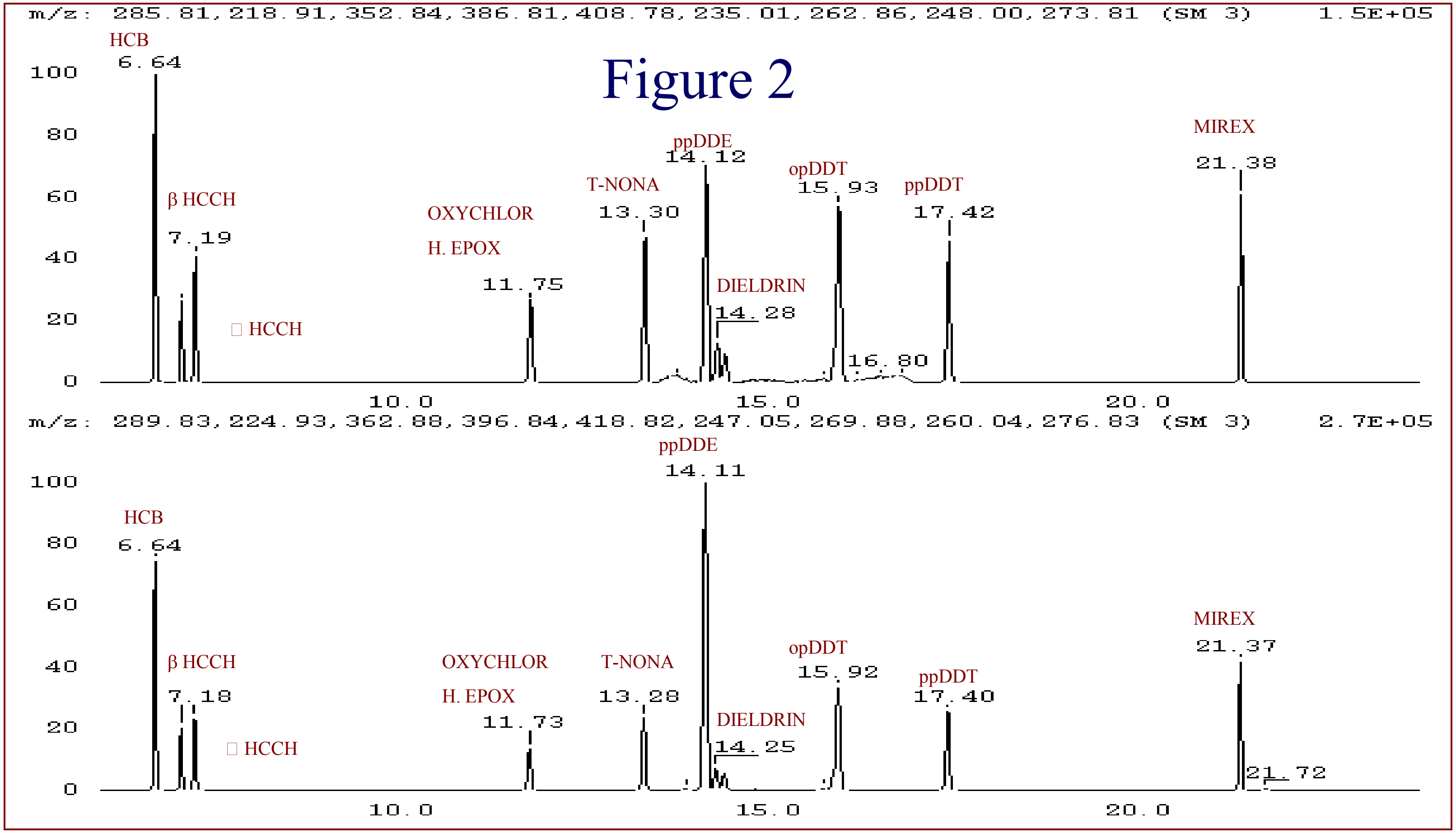


# Figure 1




Top : Native PCBs

Bottom: $^{13}$C-labeled PCBs




# Figure 2

Top : Native Pesticides

Bottom: $^{13}$C-labeled Pesticides

# LOD (pg/g Lipid) for TCDD MAT95 vs. DFS

Assumptions: 70% Recovery & Total Lipid 0.6 %

| SWEIGHT | MAT95 | DFS |
|---|---|---|
| 5 | 1.786 | 0.595 |
| 10 | 0.893 | 0.298 |
| 15 | 0.595 | 0.198 |
| 20 | 0.446 | 0.149 |
| 25 | 0.357 | 0.119 |
| 30 | 0.298 | 0.990 |
| 35 | 0.255 | 0.085 |
| 40 | 0.223 | 0.074 |
| 45 | 0.198 | 0.066 |
| 50 | 0.179 | 0.060 |

# High Resolution Mass Spectrometers

- Micromass 70E (Missouri Adipose)
- Micromass 70SE (Missouri Serum)
- Micromass Autospec
- Micromass Autospec Ultima
- Thermo Electron MAT95 XL (Apr ‘00)
- Thermo Electron  MAT95 XP
- Thermo Fisher DFS (May ‘06)
- 2-D GC (GCxGC)/HRMS (Future?)

# Thermo Fisher DFS

# Extra Slides


Reynolds
FOIL
M1
SOLVENTS
M2
SAMPLES
M5
COLLECTOR
A
50/50
C
D
brand
Methanol
FLAMMABLE
KEEP FIRE AWAY

# DOXPOPs Analyte List

| | |
|---|---|
| **Polychlorinated Dibenzo-p-dioxins and Dibenzofurans** | **Dioxin-like Polychlorinated Biphenyls - mPCBs** |
| 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p* -dioxin (OCDD) | 2,4,4'-Trichlorobiphenyl (PCB 28) |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-*p* -dioxin (HpCDD) | 2,3',4,4'-Tetrachlorobiphenyl (PCB 66) |
| 1,2,3,4,7,8-Hexachlorodibenzo-*p* -dioxin (HxCDD) | 2,4,4',5-Tetrachlorobiphenyl (PCB 74) |
| 1,2,3,6,7,8-Hexachlorodibenzo-*p* -dioxin (HxCDD) | 2,3,3',4,4'-Pentachlorobiphenyl (PCB 105) |
| 1,2,3,7,8,9-Hexachlorodibenzo-*p* -dioxin (HxCDD) | 2,3,3',4,4'-Pentachlorobiphenyl (PCB 114) |
| 1,2,3,7,8-Pentachlorodibenzo-*p* -dioxin (PeCDD) | 2,3',4,4',5-Pentachlorobiphenyl (PCB 118) |
| 2,3,7,8-Tetrachlorodibenzo-*p* -dioxin (TCDD) | 2',3,4,4',5-Pentachlorobiphenyl (PCB 123) |
| | 2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156) |
| **Polychlorinated Dibenzo-p-dioxins and Dibenzofurans** | 2,3,3',4,4',5'-Hexachlorobiphenyl (PCB 157) |
| 1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF) | 2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167) |
| 1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF) | 2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189) |
| 1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF) | |
| 1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF) | **Non-dioxin-like Polychlorinated Biphenyls** |
| 1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF) | 2,2'3,5'-Tetrachloro biphenyl (PCB 44) |
| 1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF) | 2,2',4,5'-Tetrachloro biphenyl (PCB 49) |
| 1,2,3,7,8-Pentachlorodibenzofuran (PeCDF) | 2,2',5,5'-Tetrachlorobiphenyl (PCB 52) |
| 2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF) | 2,2',3,4,5'-Pentachlorobiphenyl (PCB 87) |
| 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) | 2,2',4,4',5-Pentachlorobiphenyl (PCB 99) |
| 2,3,7,8-Tetrachlorodibenzofuran (TCDF) | 2,2',4,5,5'-Pentachlorobiphenyl (PCB 101) |
| | 2,3,3',4',6-Pentachlorobiphenyl (PCB 110) |
| **Polybrominated Dibenzo-p-dioxins and Dibenzofurans** | 2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128) |
| 2,3,7,8-Tetrabromorodienzo-*p* -dioxin (TBDD) | 2,2',3,4,4',5' and 2,3,3',4,4',6-Hexachlorobiphenyl (PCB 138 & 158) |
| 1,2,3,7,8-Pentabromodibenzo-*p* -dioxin (PeBDD) | 2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146) |
| 1,2,3,4,7,8-Hexabromorodibenzo-*p* -dioxin (HxBDD) | 2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149) |
| 1,2,3,6,7,8-Hexabromodibenzo-*p* -dioxin (HxBDD) | 2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151) |
| 1,2,3,7,8,9-Hexabromodibenzo-*p* -dioxin (HxBDD) | 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) |
| 1,2,3,4,6,7,8-Heptabromoodibenzo-*p* -dioxin (HpBDD) | 2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170) |
| 1,2,3,4,6,7,8,9-Octabromodibenzo-*p* -dioxin (OBDD) | 2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172) |
| 2,3,7,8,-Tetrabromodibenzofuran (TBDF) | 2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177) |
| 1,2,3,7,8-Pentabromodibenzofuran (PeBDF) | 2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178) |
| 2,3,4,7,8-Pentabromodibenzofuran (PeBDF) | 2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180) |
| 1,2,3,4,7,8-Hexabromodibenzofuran (HxBDF) | 2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183) |
| 1,2,3,6,7,8-Hexabromodibenzofuran (HxBDF) | 2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187) |
| 1,2,3,7,8,9-Hexabromodibenzofuran (HxBDF) | 2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194) |
| 2,3,4,6,7,8,-Hexabromodibenzofuran (HxBDF) | 2,2',3,3',4,4',5,6-Octachlorobiphenyl (PCB 195) |
| 1,2,3,4,6,7,8-Heptabromodibenzofuran (HpBDF) | 2,2',3,3',4,4',5,6' and 2,2',3,4,4',5,5',6-Octachlorobiphenyl (PCB 196 & 203) |
| 1,2,3,4,7,8,9-Heptabromodibenzofuran (HpBDF) | 2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)<br>**(change in nomenclature; previously referred to as PCB 201)** |
| 1,2,3,4,6,7,8,9-Octabromodibenzofuran (OBDF) | 2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206) |
| | 2,2',3,3',4,4',5,5',6,6'-Decachloro biphenyl (PCB 209) |
| **Dioxin-like Polychlorinated Biphenyls - cPCBs** | |
| 3,3',4,4'-Tetrachlorobiphenyl (PCB 77) | **Organochlorine Pesticides** |
| 3,4,4',5-Tetrachlorobiphenyl (PCB 81) | Hexachlorobenzene |
| 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) | *beta* -Hexachlorocyclohexane |
| 3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169) | *gamma* -Hexachlorocyclohexane |
| | *p,p'*- DDT |
| **Polychlorinated Naphthalenes** | *p,p'* -DDE |
| 1,2,3,4-Tetrachlorinated naphthalene (PCN 27) | Oxychlordane |
| 1,2,3,5,7- and 1,2,4,6,7-Pentachlorinated naphthalene (PNC 52 & 60) | *trans* -Nonachlor |
| 1,2,3,4,5,7- and 1,2,3,5,6,8-Hexachlorinated naphthalene (PNC 64 & 68) | Heptachlor Epoxide |
| 1,2,3,4,6,7- and 1,2,3,5,6,7-Hexachlorinated naphthalene (PNC 66 & 67) | Mirex |
| 1,2,3,5,7,8-Hexachlorinated naphthalene (PCN 69) | Aldrin |
| 1,2,3,4,5,6,7-Heptachlorinated naphthalene (PCN 73) | Dieldrin |
| | Endrin |
| **Toxaphenes** | alpha-Hexachlorocyclohexane (HCCH) |
| Parlar 26<br>2-Endo,3-exo,5-endo,6-exo,8b,8c,10a,10c-octachlorobornane | *cis* -Chlordane (or alpha) |
| Parlar 50<br>2-Endo,3-exo,5-endo,6-exo,8b,8c,9c,10a,10c-nonachlorobornane | *trans* -Chlordane (or gamma) |
| Parlar 62<br>2,2,5,5,8c,9b,9c,10a,10b-nonachlorobornane | *cis* -Nonachlor |
| | *o,p* '-DDE |
| | Octachlorosytrene |
| | Pentachloroanisole |