Magnetic Sector High Resolution GC-MS in Dioxin Analysis "DFS 10th Birthday"

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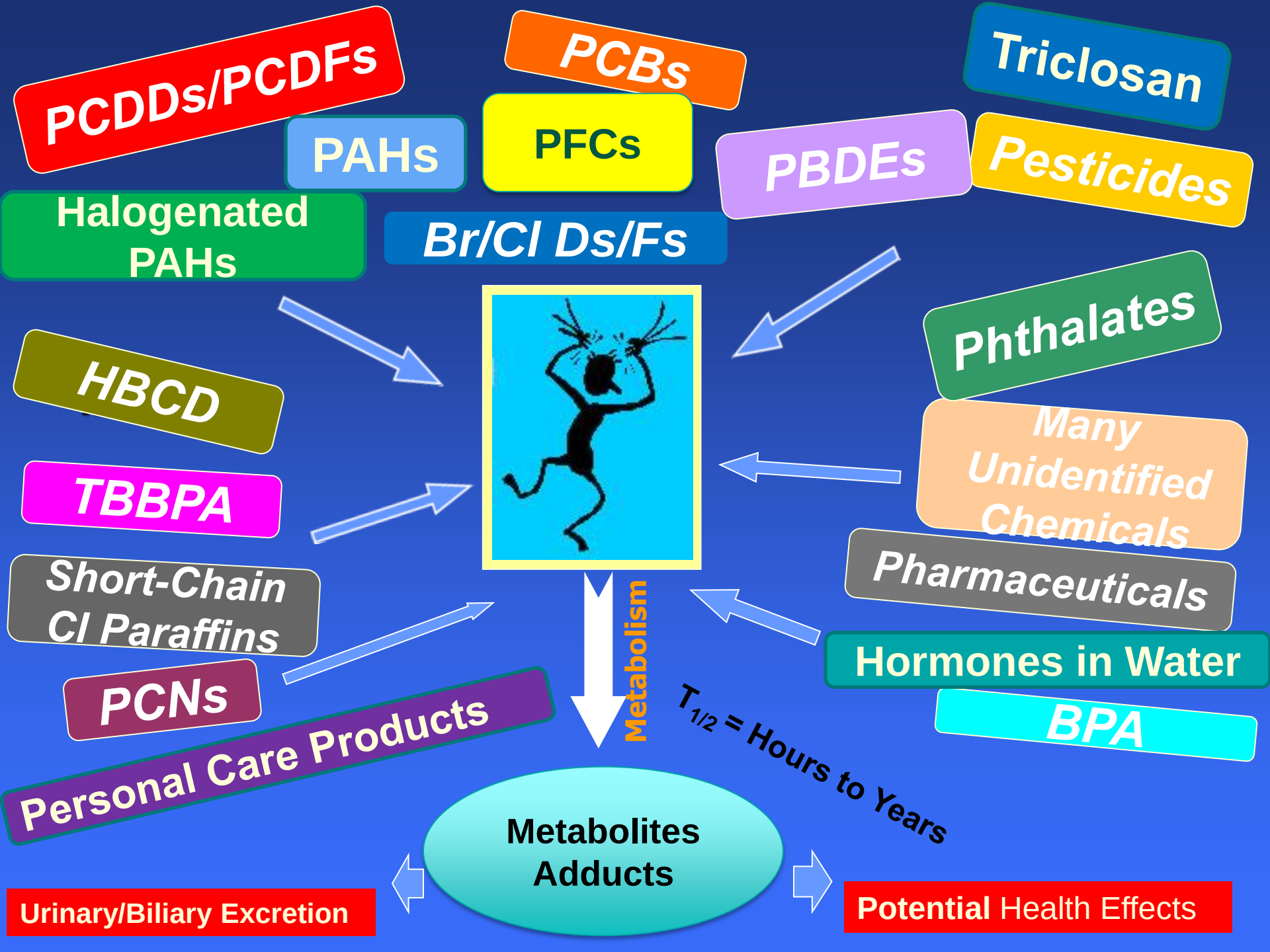
Overview of Presentation

Congratulations on the DFS 10th Birthday

- Personal 44 year “love affair” with HRMS
- Overview of why need to use biomonitoring for human exposure assessment
- Analytical necessity for HRMS to achieve very low detection for human biomonitoring studies
- The new lower detectability introduced by the DFS 10 year ago
- Why we need even better (lower) detection limits
- What does the future look like for the DFS

44 Years of High Resolution Mass Spectrometry

- 1971 Arizona State University (Dr Peter Brown)
  - Varian Atlas SM1B Mattauch-Herzog
    - Field-Ionization Kinetics
- 1975 Stanford University (Dr Carl Djerassi)
  - Varian-Mat 711 HRMS
    - Synthesis and Mass Spec Mechanistic fragmentation studies using isotopic labeling of steroids
- 1979-2008 Centers for Disease Control and Prevention-Retired in 2008
  - Vg ZAB-2F; Vg-70E and 70S; Micromass Ultima;
  - 15 MAT 95XPs; 12 DFSs
- 2009-2015 AXYS Analytical Services-Sidney, BC, Canada


PCDDs/PCDFs
PCBs
Triclosan
PAHs
PFCs
PBDEs
Pesticides
Halogenated PAHs
Br/Cl Ds/Fs
Phthalates
HBCD
Many Unidentified Chemicals
TBBPA
Pharmaceuticals
Short-Chain Cl Paraffins
Hormones in Water
PCNs
BPA
Personal Care Products
Metabolism
T1/2 = Hours to Years
Metabolites Adducts
Urinary/Biliary Excretion
Potential Health Effects

Objectives of Exposure Assessment to Environmental Chemicals

- **Quantification of magnitude, duration, frequency and routes of exposure**
  - Example: Air, water, food, soil, dust, etc.
- **Characterization and enumeration of the exposed population**

Human Biomonitoring

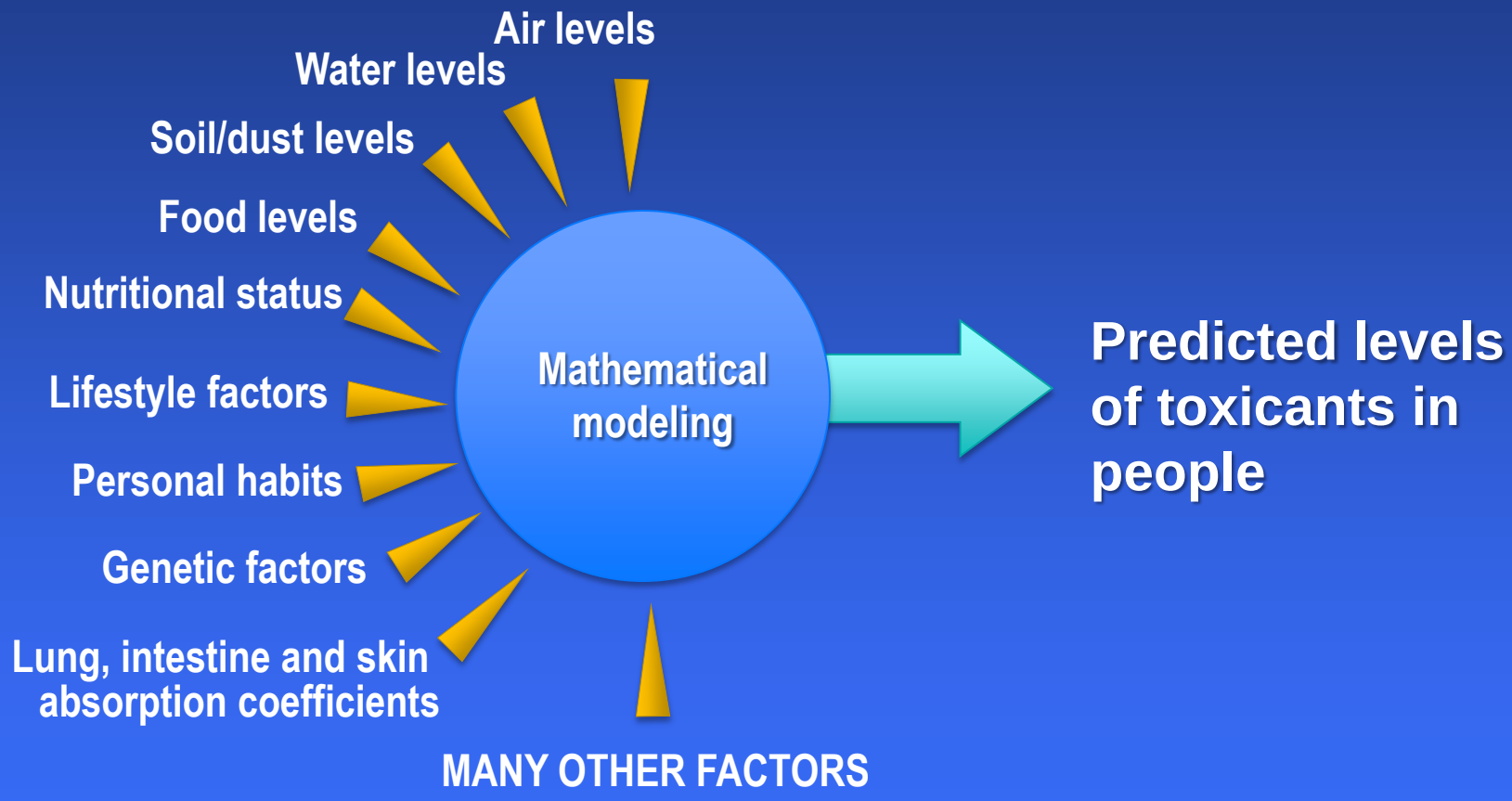
- **Two ways to do human exposure assessment**
  - External dose measurement
    - Modeling to predict internal dose
  - Internal dose measurement
    - Direct measurement of the internal dose

Conventional Exposure Assessment (Indirect)

- Questionnaire data
- Measurement or estimation of concentrations in the various environmental media
- Assumptions of media contact or intake routes—yield a value of applied dose



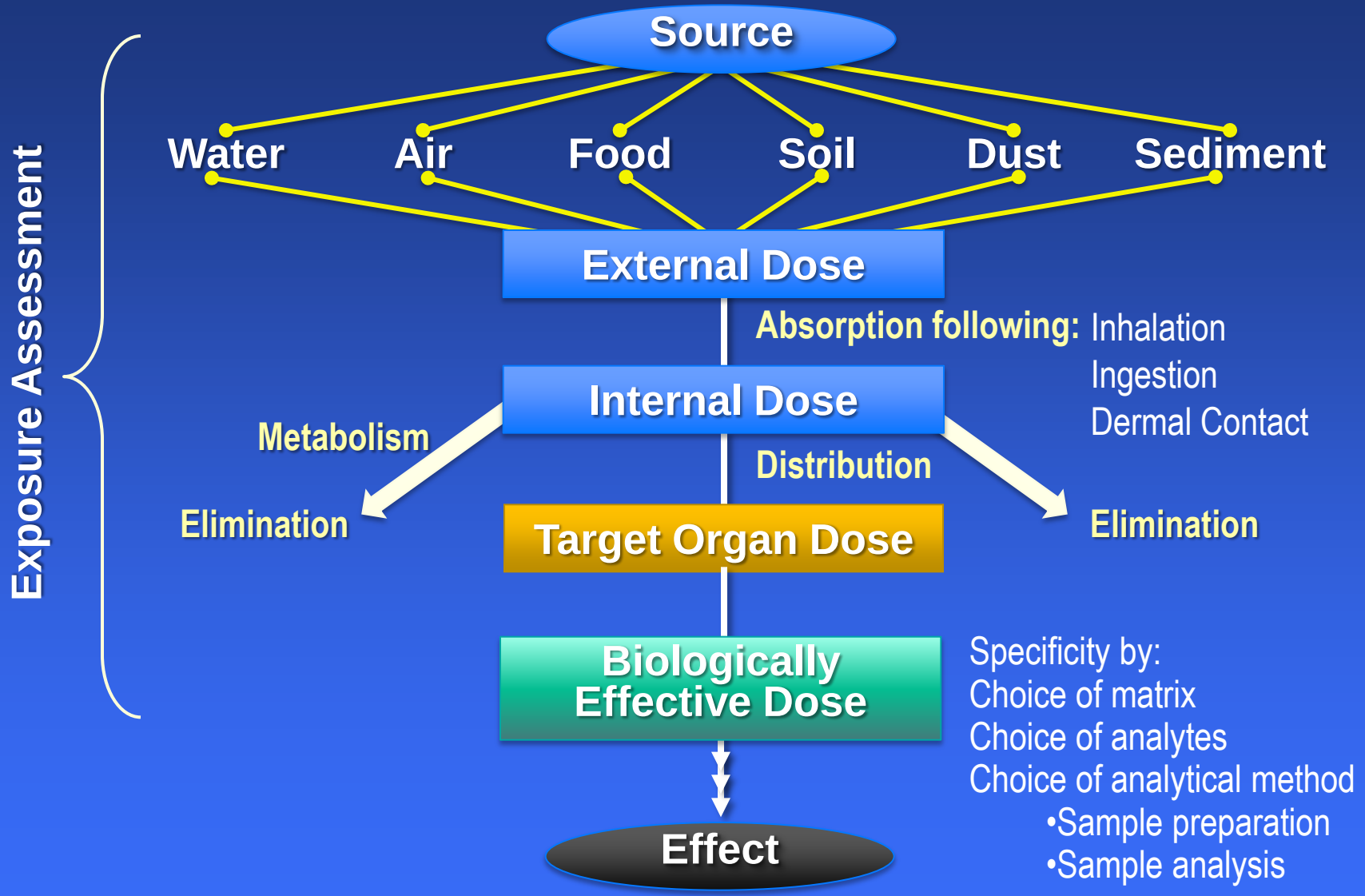

Predicting Levels of Toxicants in People Using Environmental Monitoring Is Very Difficult and Includes Many Assumptions



Biomonitoring Approach to Exposure Assessment

- Provides direct measure of exposure—can integrate exposures from multiple pathways and sources
- Decreases uncertainty inherent in exposure assessment by conventional method
- Provides a more biologically relevant measure of true exposure

Exposure Pathway



INSTEAD OF PREDICTING,

MEASURE LEVELS OF TOXICANTS IN PEOPLE

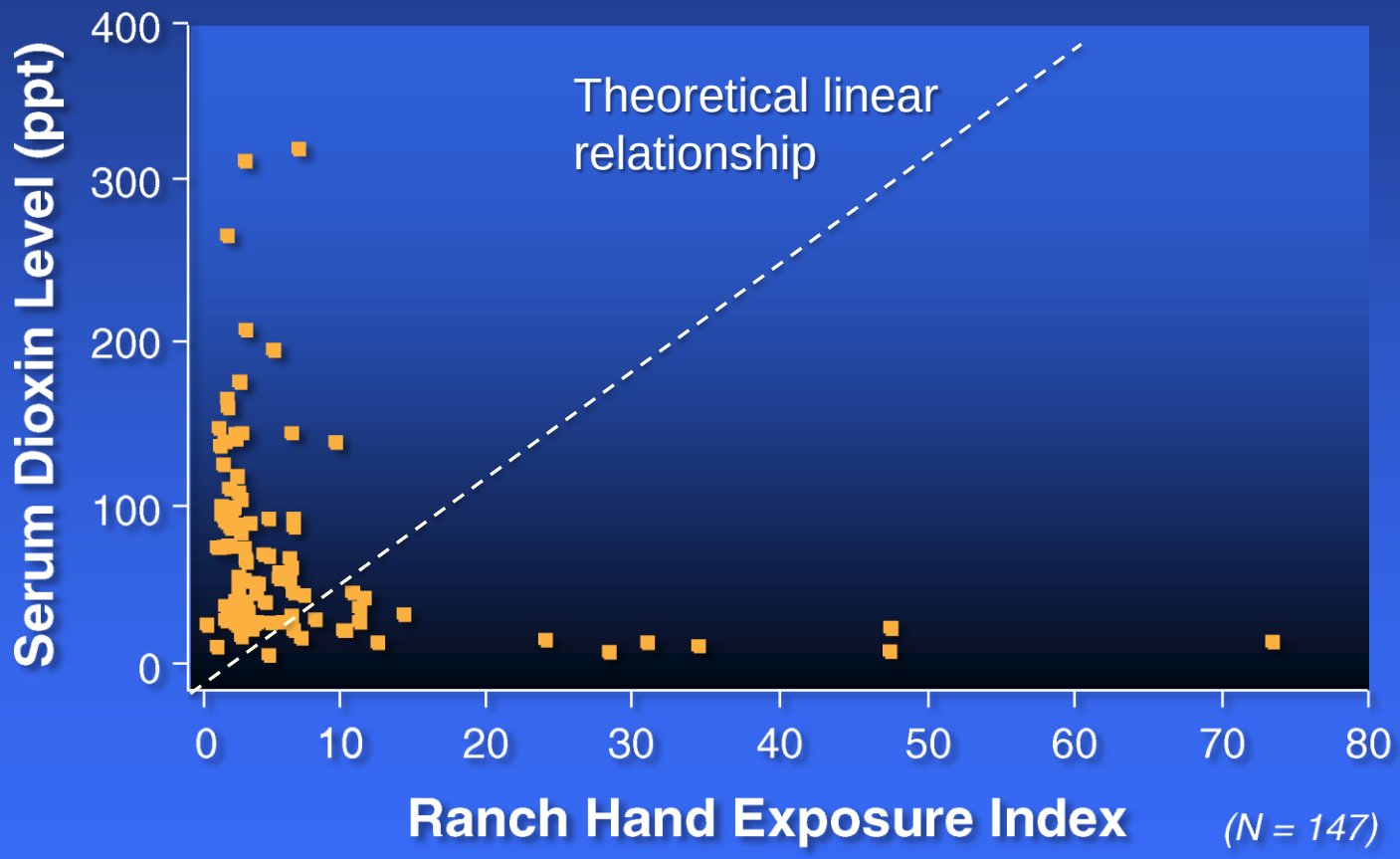
Agent Orange

Herbicide : Defoliant

2,4,5-Trichlorophenoxyacetic Acid *
+
2,4-D in Diesel Oil

* 2,3,7,8-TCDD contaminant

The Agent Orange Vietnam Veteran Ranch Hand Dioxin Exposure Index was Not Correlated with Serum Dioxin Levels



INTRODUCTION

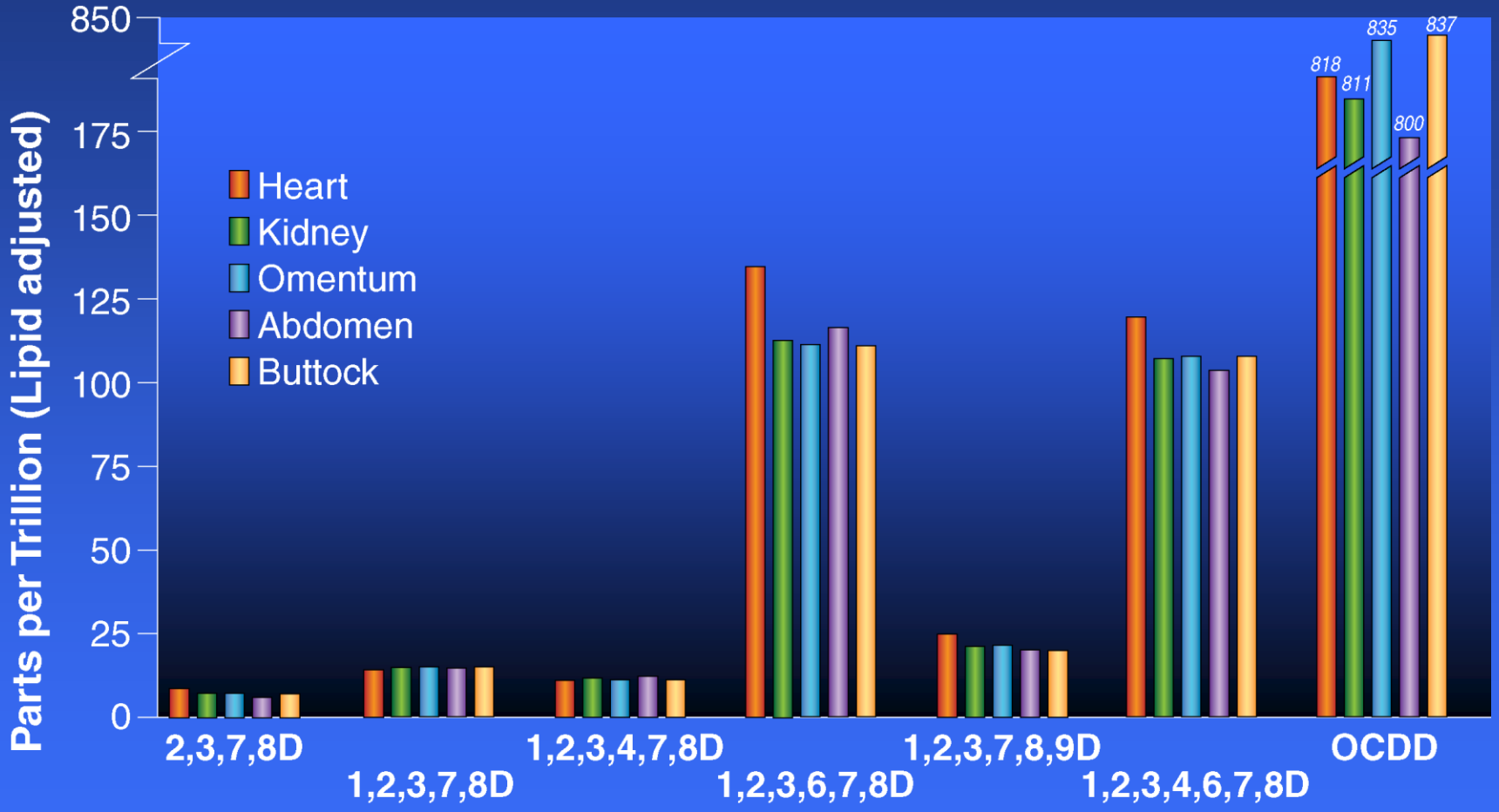
- PCDDs, PCDFs, PCBs and POPs in general are lipophilic compounds
- Bioaccumulate up the food chain to humans
- Can be measured in lipid stores of the human body
- Exposure to humans primarily through food of animal origin (95%)

PCDDs, PCDFs, and PCBs—What We Know

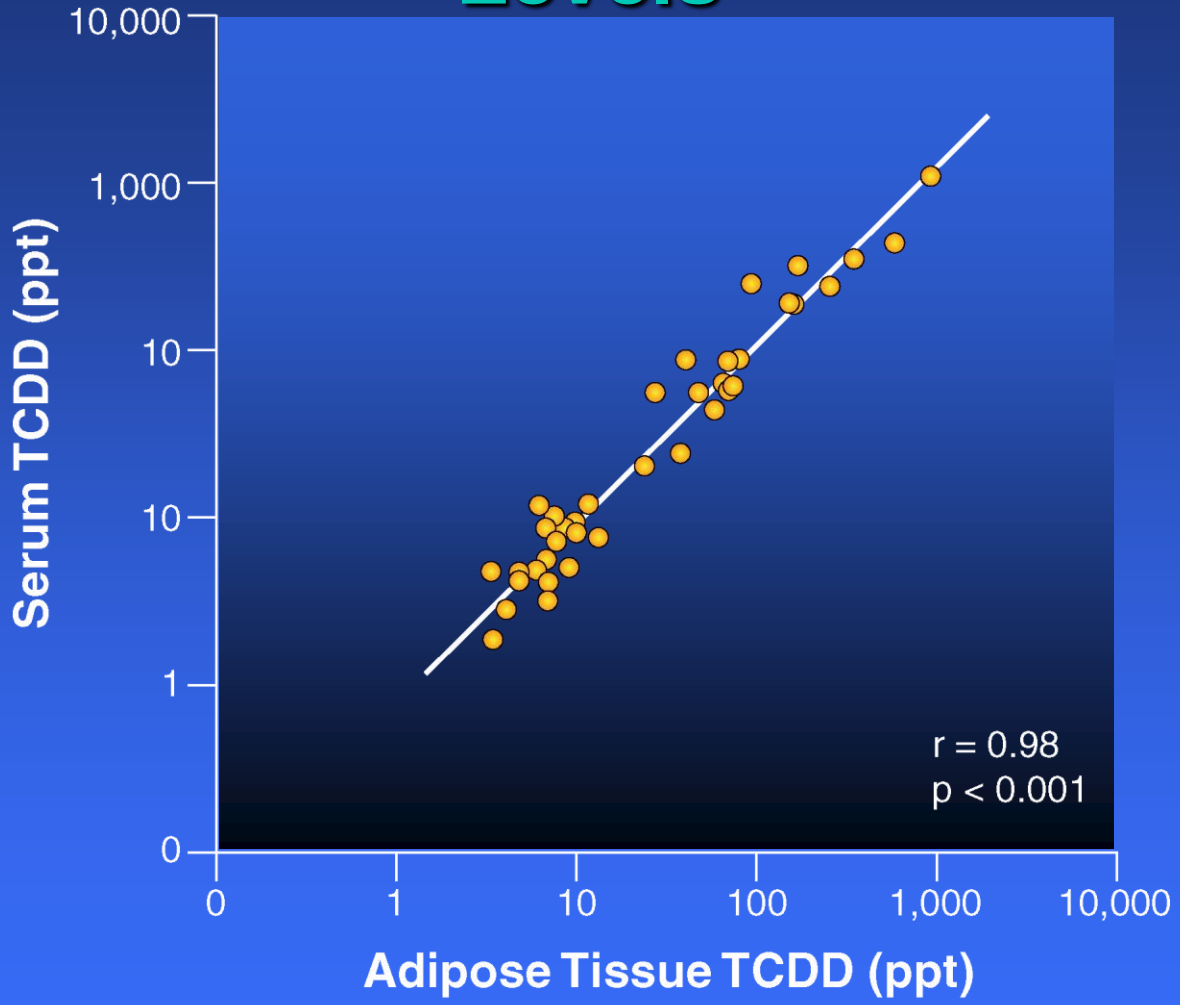
- Ubiquitous environmental contaminants
- Higher in industrialized societies
- Lipophilic compounds
- Distributed equally in the lipid stores of the body
- Increase with age

TCDD Half-life in Different Animal Species and People

Mean Concentration of PCDDs by Adipose Tissue Location (11 individuals)



Serum TCDD Levels are Highly Correlated with Adipose Tissue Dioxin Levels

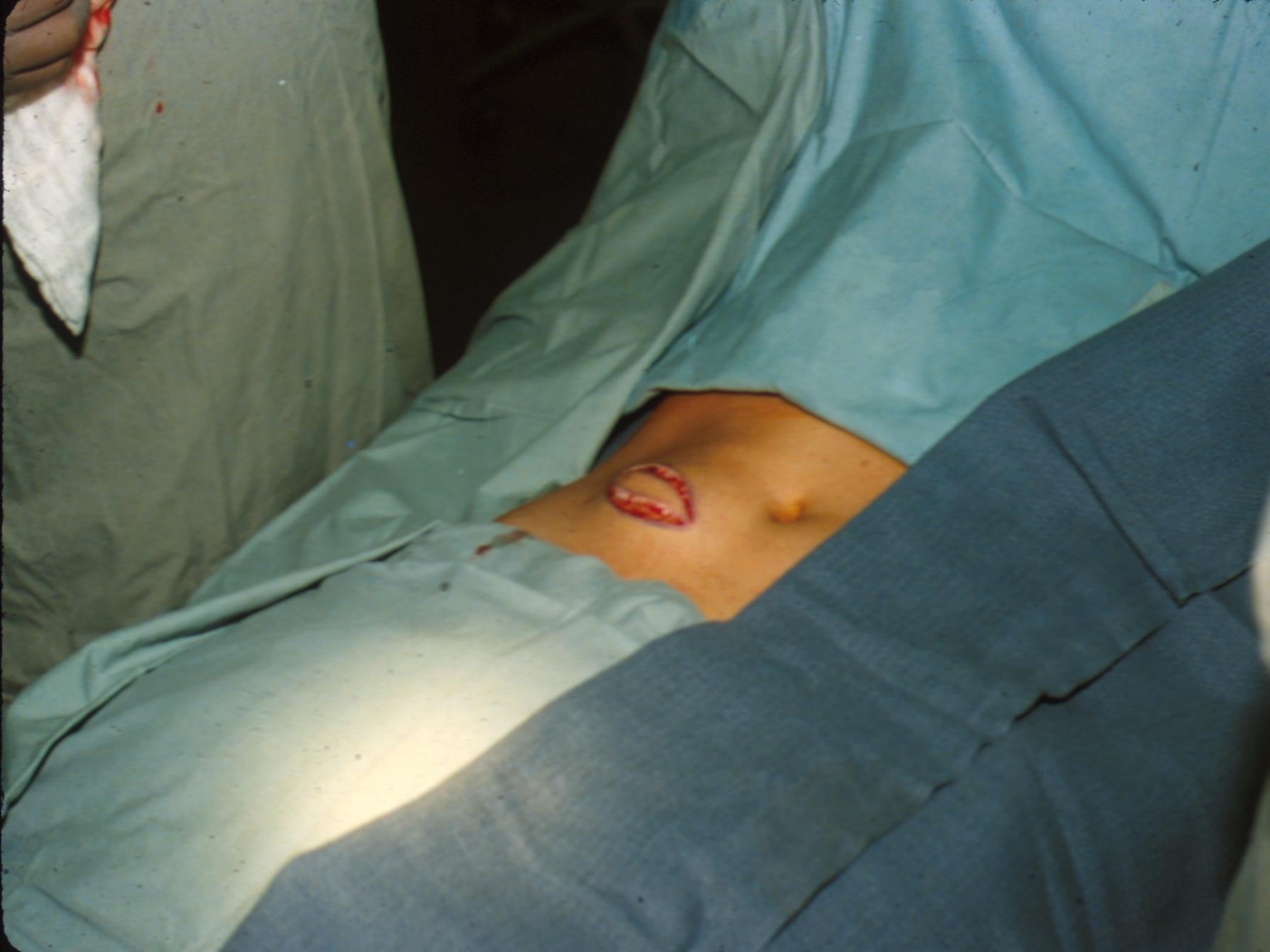


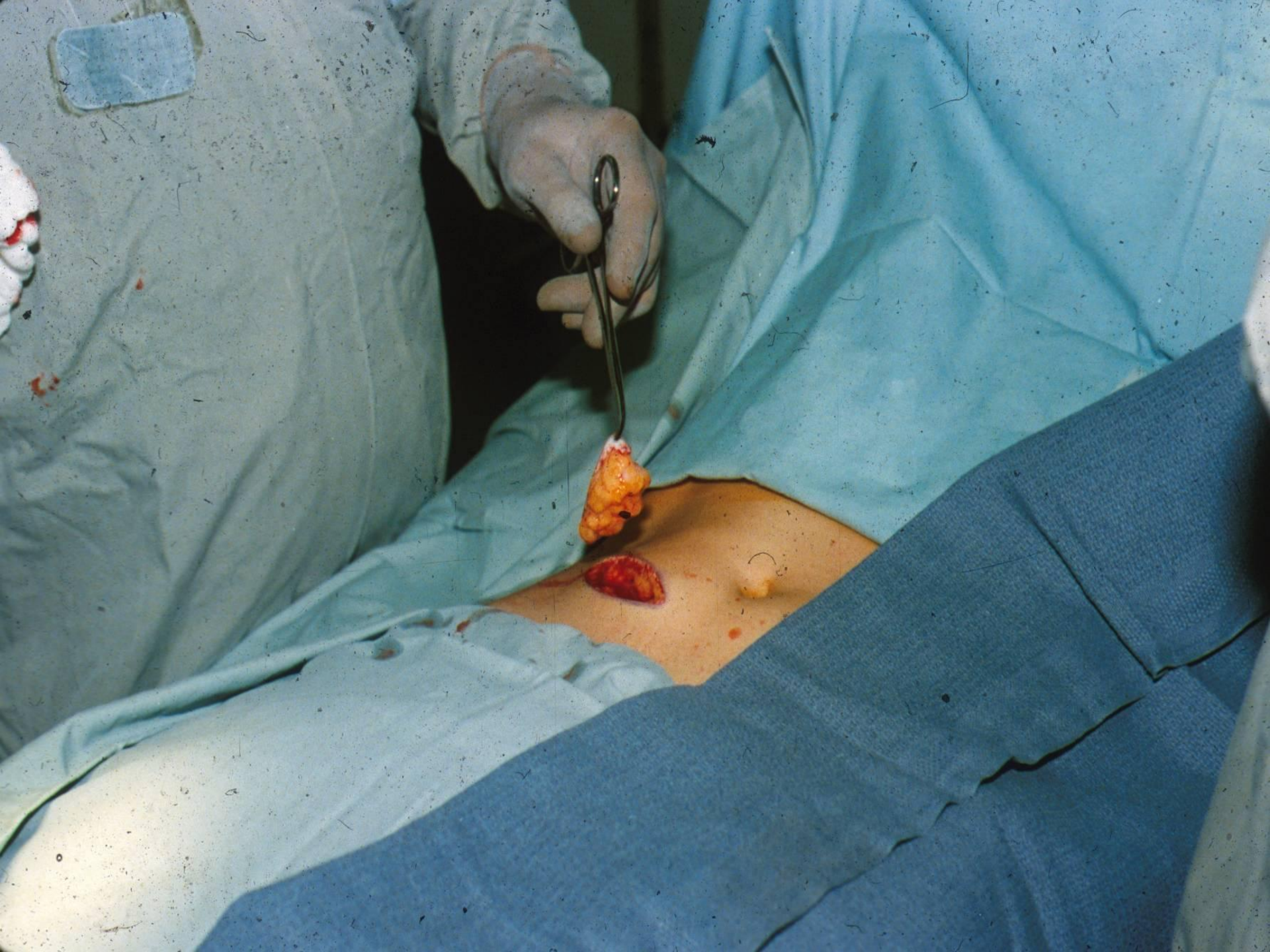
Source: Patterson et al. Arch Environ Contam Toxicol 17:139–143 (1988)

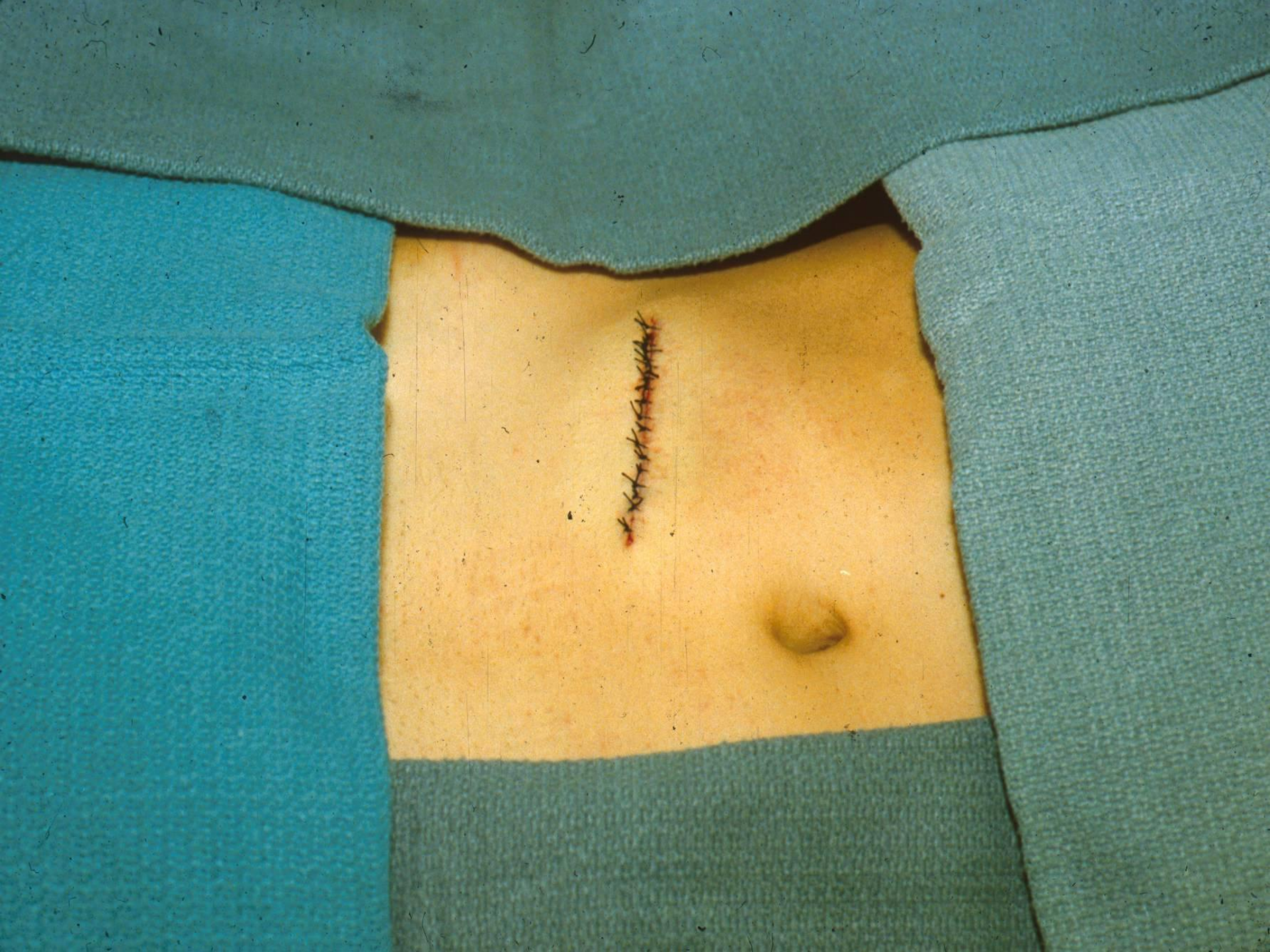
Analytical measurement process for PCDDs, PCDFs, and PCBs in human samples

Analysis of Human Samples for Dioxin-Like Chemicals

- Selective sample preparation
- High resolution gas chromatography/high resolution mass spectrometry (~ $500,000)
- Isotope dilution quantification
- Results reported based on lipid content of the sample







Major Disadvantage in Adipose Tissue Studies

- Surgical procedure required to obtain adipose tissue samples
- **LOW PARTICIPATION RATE**

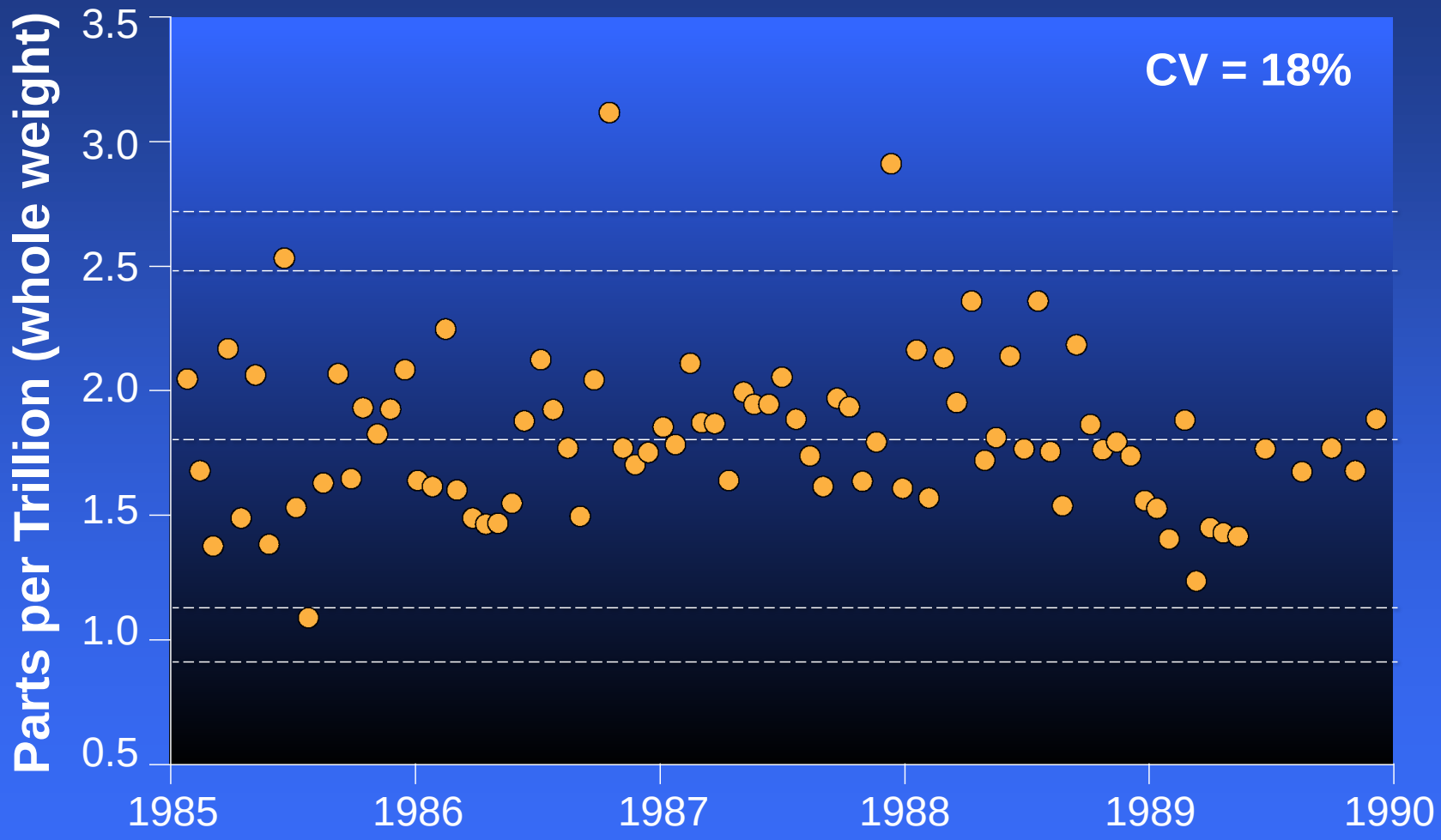
Major Problem in Developing a Serum Method

	Percent Lipid	TCDD Conc.
Adipose Tissue	~ 95	ppt
Serum	~ 0.6	ppq

Major Challenge in Measuring PCDDs, PCDFs, PCBs in Human Samples

- **Need for extensive quality assurance program**
  - To be sure are measuring the correct congener
  - To be sure that the amount measured is correct

Quality Control Chart for 2,3,7,8-Dioxin from 1985–1990



Source: Modified from CDC.

All persons from industrialized societies have levels of PCDDs, PCDFs, and PCBs in their bodies

Therefore, before what is ‘abnormal’ can be determined, what is ‘normal’ must be defined

National Report on Human Exposure to Environmental Chemicals

- **What it is:**
  - An ongoing (every 2 years) biomonitoring assessment of the exposure of the U.S. population to selected environmental chemicals
- **Matrices monitored: Urine, blood and its components**

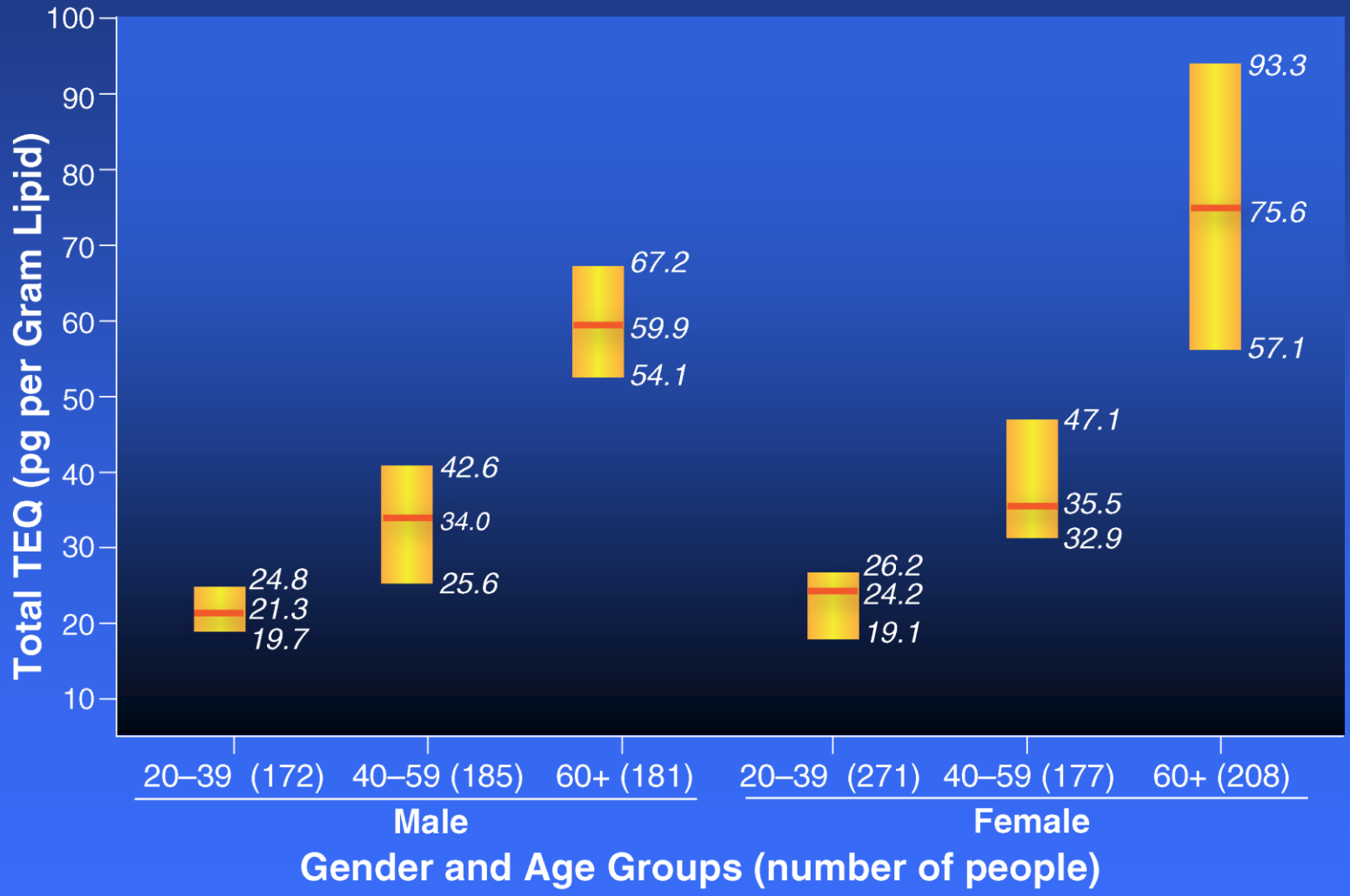


Chemicals in 4th Report ~265 Chemicals

- Metals
- Polychlorinated biphenyls, dioxins, and furans
- Organochlorine pesticides
- Carbamate pesticides
- Organophosphorous pesticides
- Pyrethroid pesticides
- Herbicides
- Polycyclic aromatic hydrocarbons
- Phthalates
- Phytoestrogens
- Pest repellants
- Cotinine
- Perfluorinated chemicals
- Brominated flame retardants
- VOCs
- Perchlorate
- Bisphenol A and alkylated phenols
- Triclosan, parabens, acrylamide
- Sunscreen agent
- Speciated arsenic

www.cdc.gov/exposurereport

NHANES 2001/2002 U.S. Reference Range 90th Percentile for Gender and Age Group Total TEQ (2005 TEFs) (95% Confidence Intervals)



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AUTOMATION IS ABSOLUTELY ESSENTIAL TO CONDUCT *LARGE SCALE* EXPOSURE ASSESSMENTS OR EPIDEMIOLOGICAL STUDIES

MATERIALS AND METHODS

- Integrated, automated sample extraction (SPE), clean-up (Power-Prep), and automated evaporation systems manufactured by Fluid Management Systems (FMS) in Waltham, MA

- Extracts measured by isotope-dilution GC-high resolution mass spectrometry on MAT 95XPs and more recently Thermo Scientific DFS HRMS


H303-4
Hexanes

"DFS 10th BIRTHDAY"

- Prior to 2006 the Centers for Disease Control and Prevention used the MAT 95XP HRMS instruments for all the human analytical measurments
- In 2006, the Centers for Disease Control and Prevention purchased their first Thermo Scientific DFS instrument
- Over the recent years the MAT 95XP instruments were all replaced with 12 Thermo Scientific DFS instruments

Thermo Scientific DFS
Happy 10th Birthday!!

…and our customers confirm it!! – Study by CDC

CDC Talk and Poster at Dioxin 2006 in Oslo

THE USE OF VARIOUS GAS CHROMATOGRAPHY AND MASS SPECTROMETRY TECHNIQUES FOR HUMAN BIOMONITORING STUDIES

Patterson, DG Jr.[1], Welch, SM[1], Focant J-F[2], Turner, WE[1]

[1]Division of Laboratory Science, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Atlanta, Georgia 30341; [2]Mass Spectrometry Laboratory, University of Liege, Allee de la Chimie 3, B-6c Sart-Tilman, B-4000 Liege, Belgium

- DFS up to **5 times** more sensitive than MAT95 on standards
- DFS up to **3 times** more sensitive than MAT95 on real sample

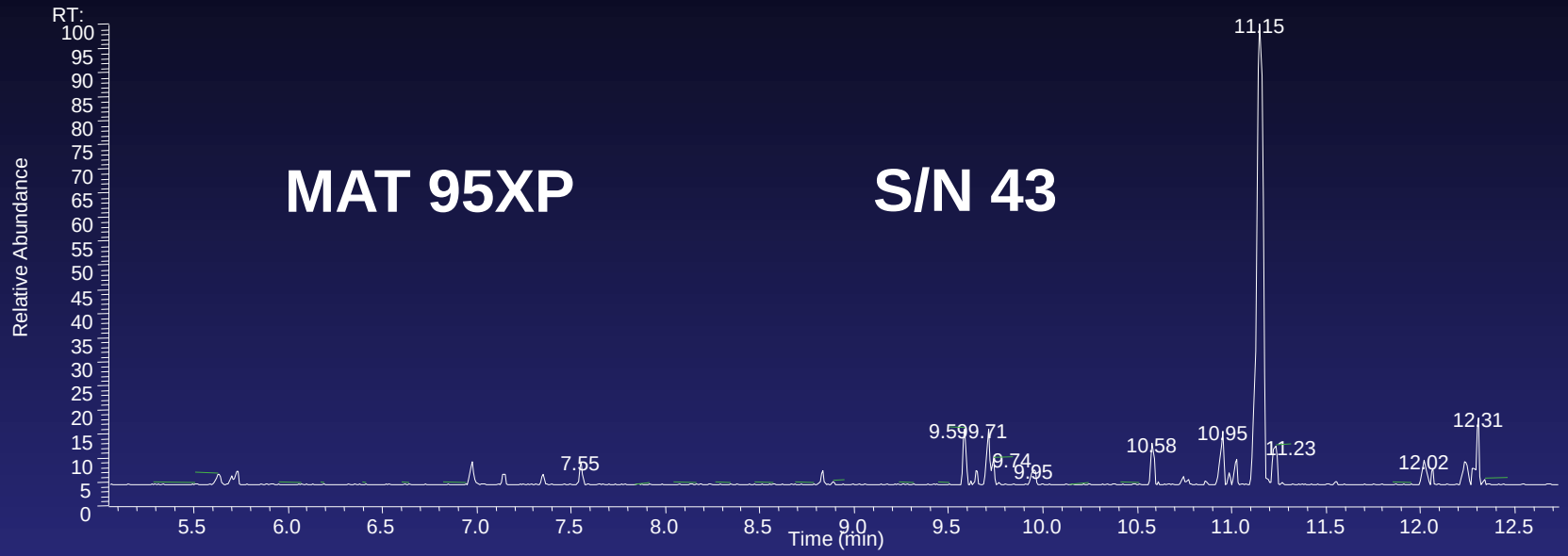
Mass Spectrometer Hardware Improvements

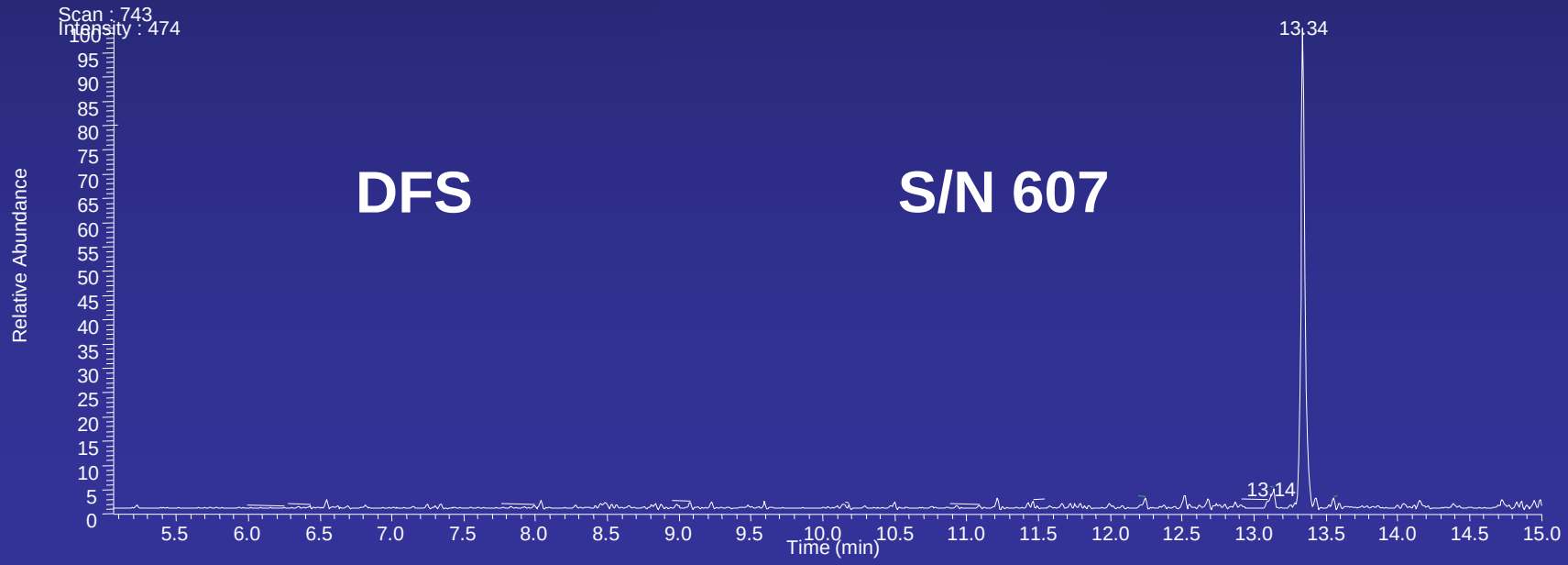
- **MAT 95XP HRMS (10,000 RP, Six MID Groups)**
- **New Thermo Electron DFS HRMS (13,000 RP, Six MID Groups)**
- **100 Background Level Serum Sample Extracts Measured for 21 PCDD/PCDF/cPCB Congeners**

Results for New DFS HRMS

- **Instrument DLs for 21 PCDD/PCDF/cPCB Congeners**
  - **5 Fold lower IDLs than MAT 95XP**
- **100 Serum sample extracts measured on MAT 95XP and the DFS HRMS**
  - **3 Fold improvement in MDL for all congeners**
  - **Reason for difference from 5 fold to 3 fold is chemical noise and matrix effects**

20 fg TCDD Standard by GC-IDHRMS






Seveso
Agent Orange
Calcasieu Parish, LA
Boehringer Plant Workers
Vietnam Ground Troops
NIOSH Workers
NHANES 1999-2010
VA Vietnam Chemical Corps
> 300 Individual Studies and Counting……..
Alaska Native Villages
Chapaevsk and Ufa, Russia
World Trade Center
Yucheng, Taiwan
National Children's Study
Sierra Vista, AZ
Great Lakes
Times Beach, MO
Vietnam Veterans Ranch Hand - 1982, - 1987, - 1992 - 1997, - 2002
New Zealand Population
New Zealand and Australian Sprayers


Seveso, Italy
Agent Orange
Calcasieu Parish, LA
NHANES 1999-2010
Vietnam Ground Troops
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>1,305,676 Congener Analyses
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IRAQ-US Army
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Great Lakes
Vietnam Ranch Hand - 1982, - 1987, - 1992 - 1997, - 2002
Times Beach, MO
New Zealand Population
E-Waste Workers, Vietnam
New Zealand and Australian Sprayers

Why so many high quality measurements over ~30 years?

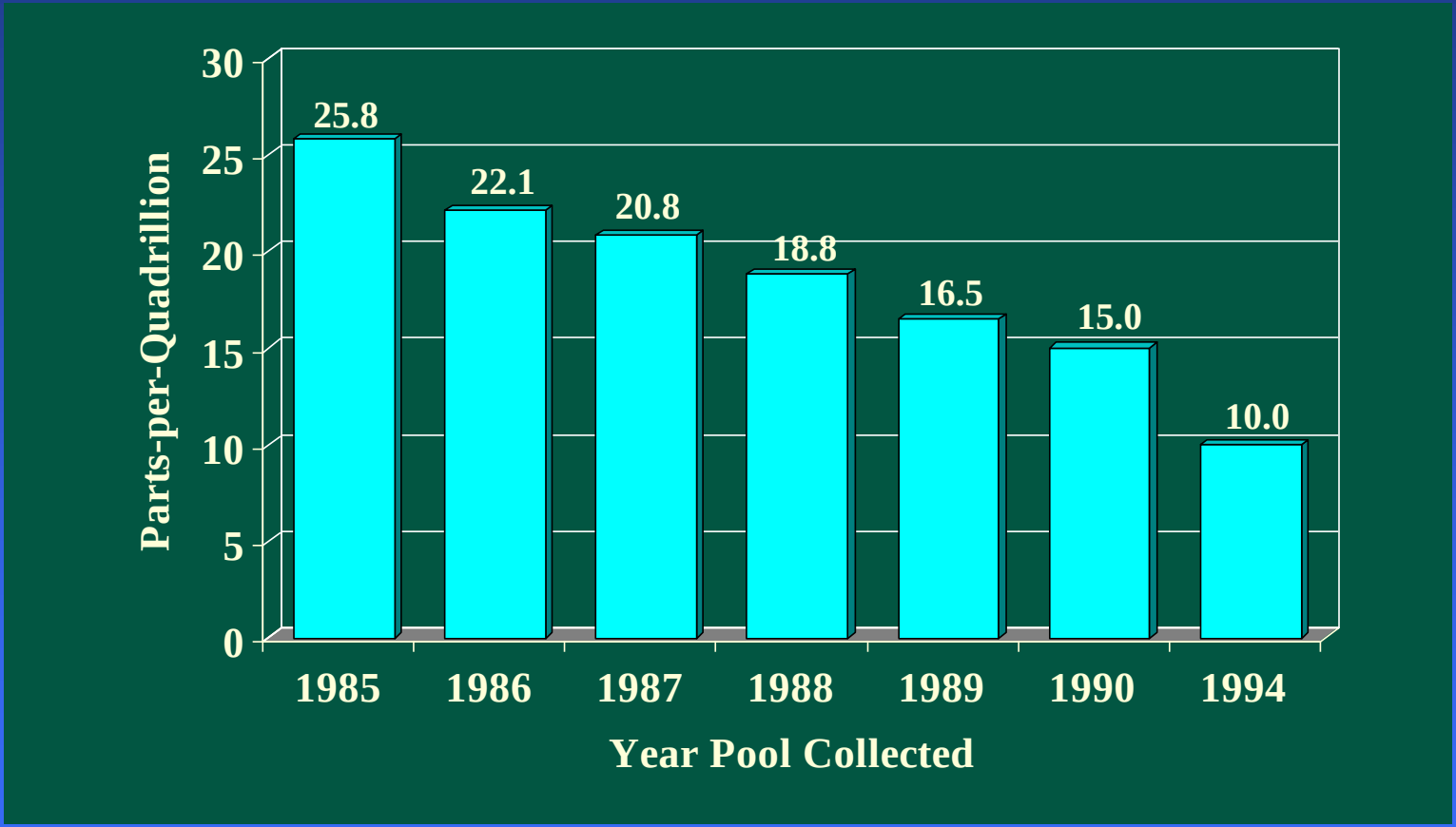
- Ruggedness and high sensitivity of the MAT 95XPs and over the past ~10 years the DFS HRMS
- Ruggedness and flexibility of the FMS system due to its design
  - Each sample prepared in a totally separate module
  - Each module uses its own separate disposable columns
  - No ‘cross talk’ (carryover) between modules

We need better (lower) sensitivity

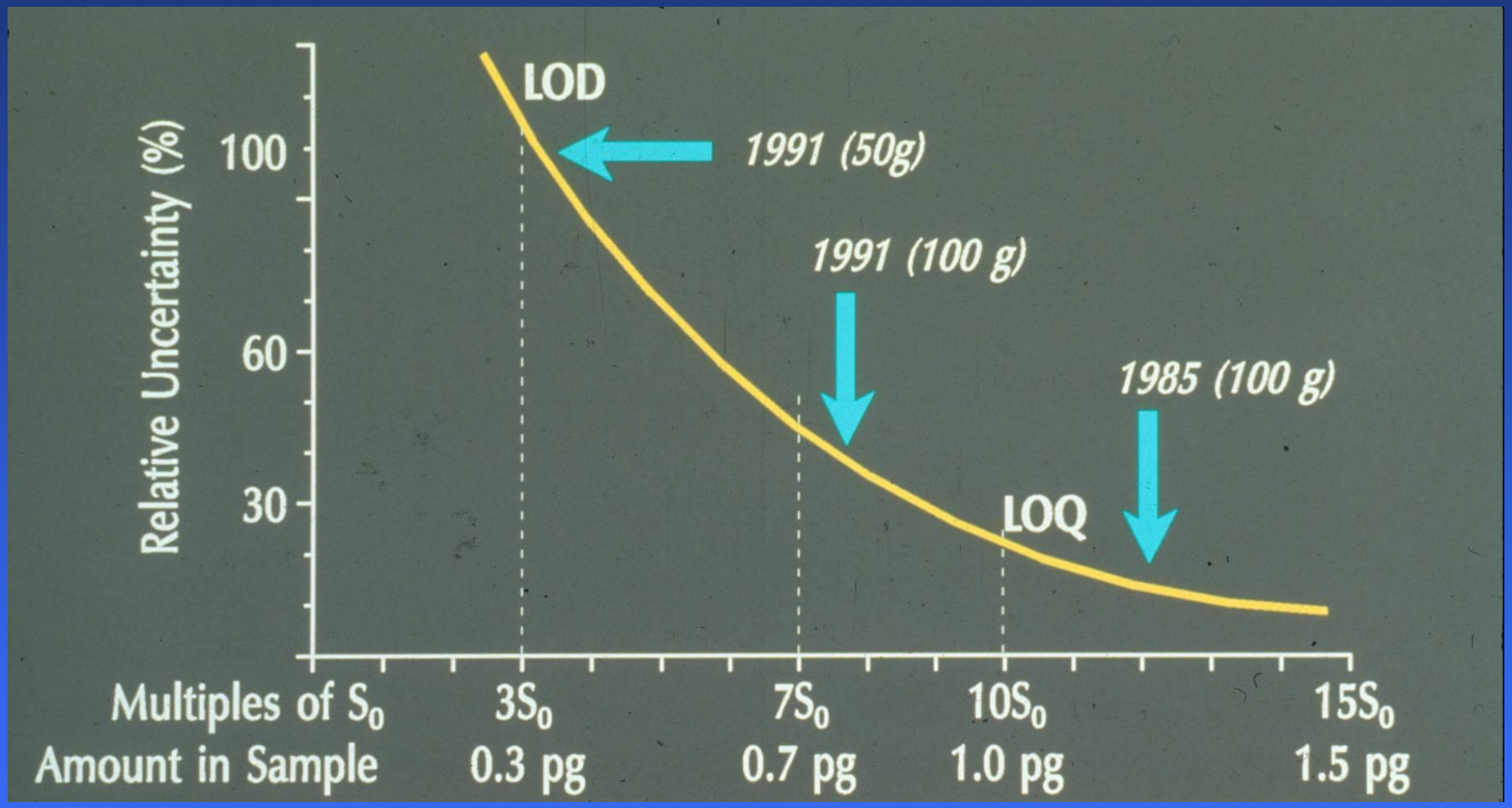
What Drives the Quest for Lower and Lower Detection Limits?

- Just what analytical scientists do!
- Declining environmental / human levels
  - Higher false positive – false negative rates
- Better "statistical power" for epidemiological studies
  - Higher "power" for same number of participants
  - Same "power" for smaller number of participants
    - Cost savings

2,3,7,8-TCDD Levels in Pooled Human Serum (Atlanta, GA)



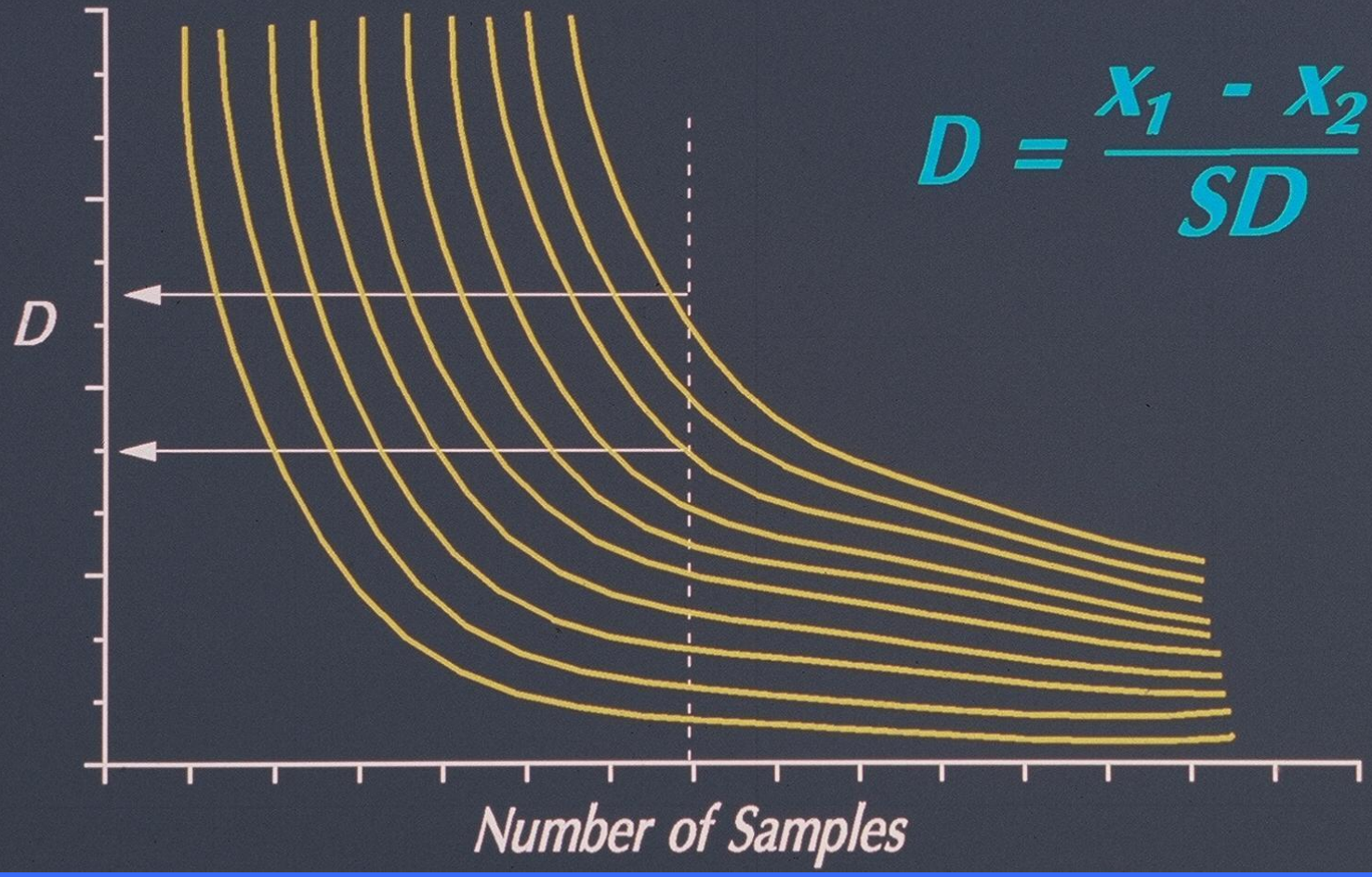
Measurement Uncertainty



Normal Human Levels

POWER CONTOURS

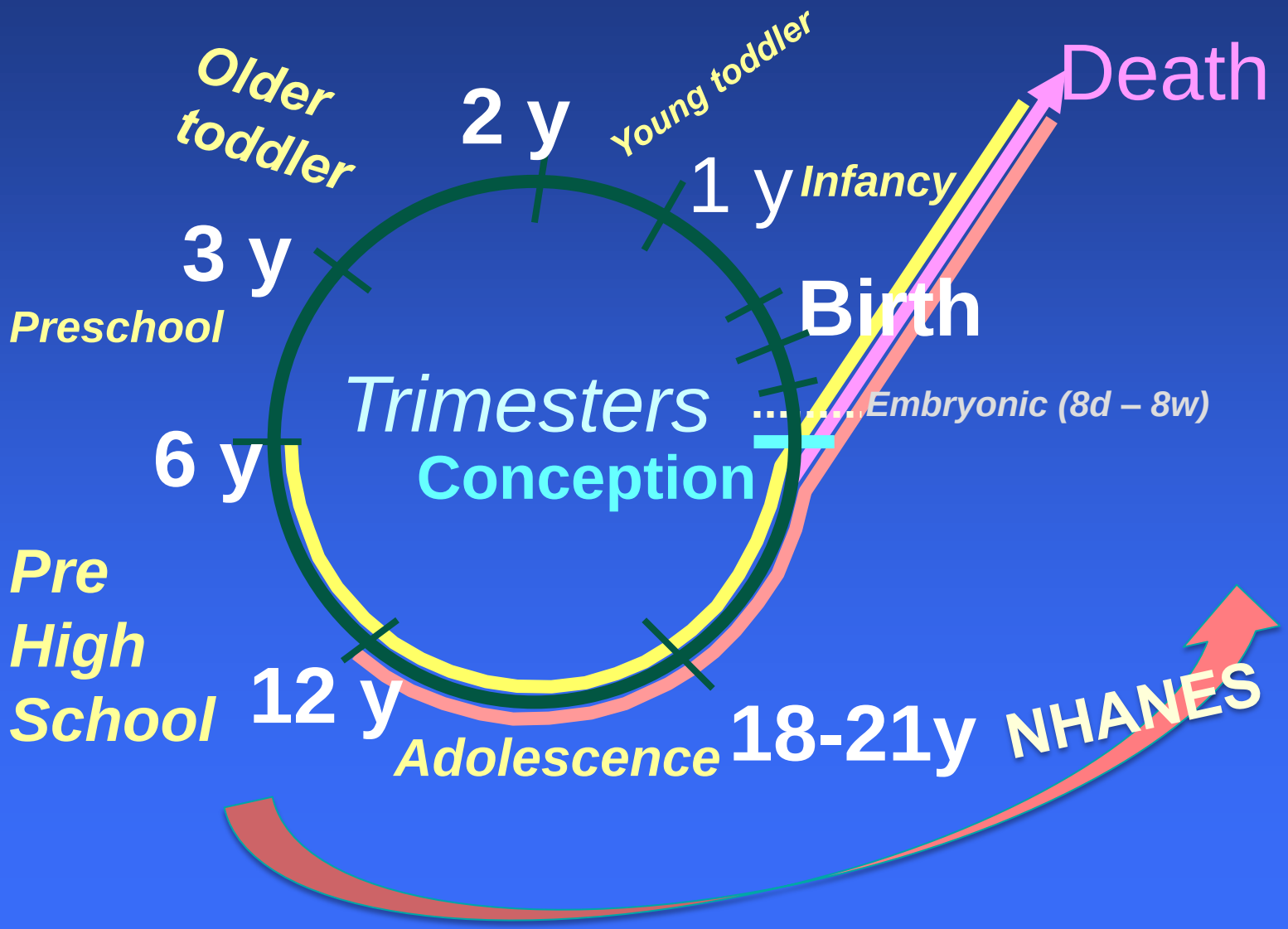
$$D = \frac{X_1 - X_2}{SD}$$



What Drives the Quest for Lower and Lower Detection Limits?

- **Question: Is there a threshold for human toxicity?**
  - **How many molecules are important?**

Human Life Stages



Need for Sensitivity Improvement

Very young children and babies

- **“Finger Stick” Blood drops**
- **Dried Blood Spots**
- **Urine**
- **Meconium**

Elderly people

Sick people

MEASUREMENT OF POPs IN DRIED BLOOD SPOTS

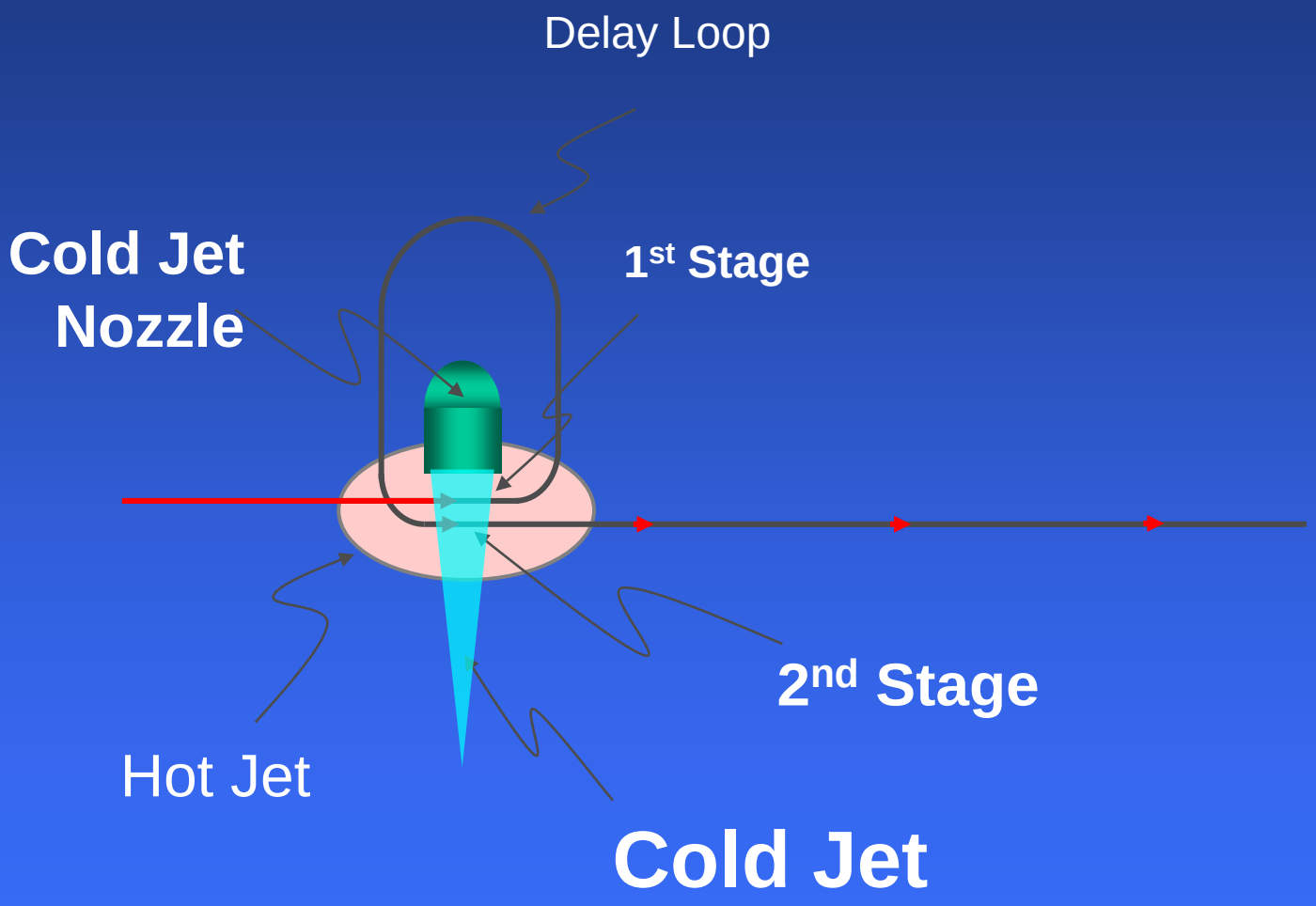
- Routinely Collected From Newborns in all States in the U. S.
  - Genetic Testing – eg. PKU
  - Stored by State Health Departments for Decades

Analytical Background

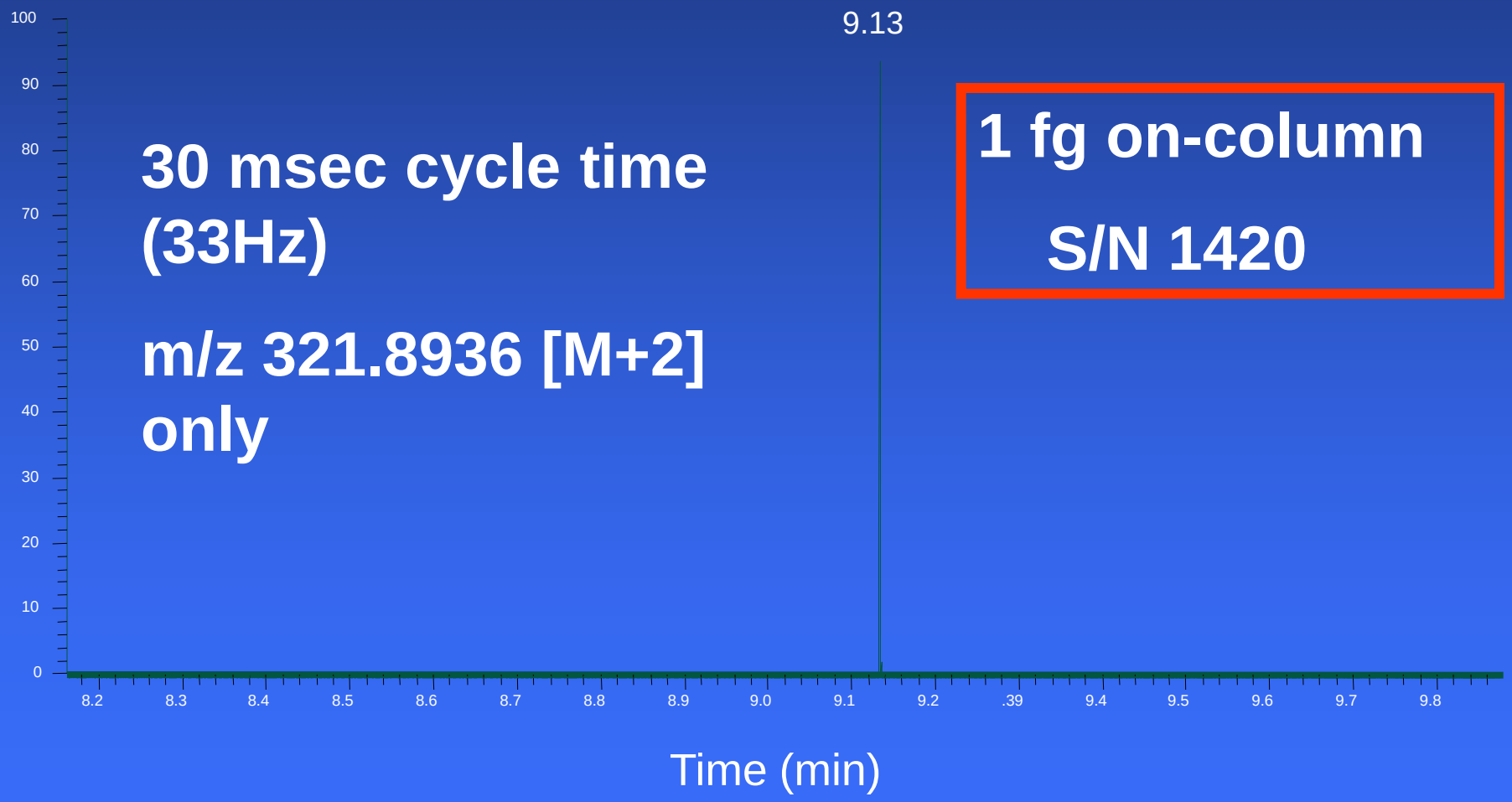
- **Dried Blood Spot analysis for POPs**
- **Small Sample** (≤ 100 ul Serum)
- **Limited sample size**
- **Extremly low analyte amounts**

Cryogenic Zone Compression of GC Analyte Peaks Prior to MS Ionization

Loop Modulator



^{12}C-2378-TCDD Standard GCxGC-HRMS, Loop Modulator



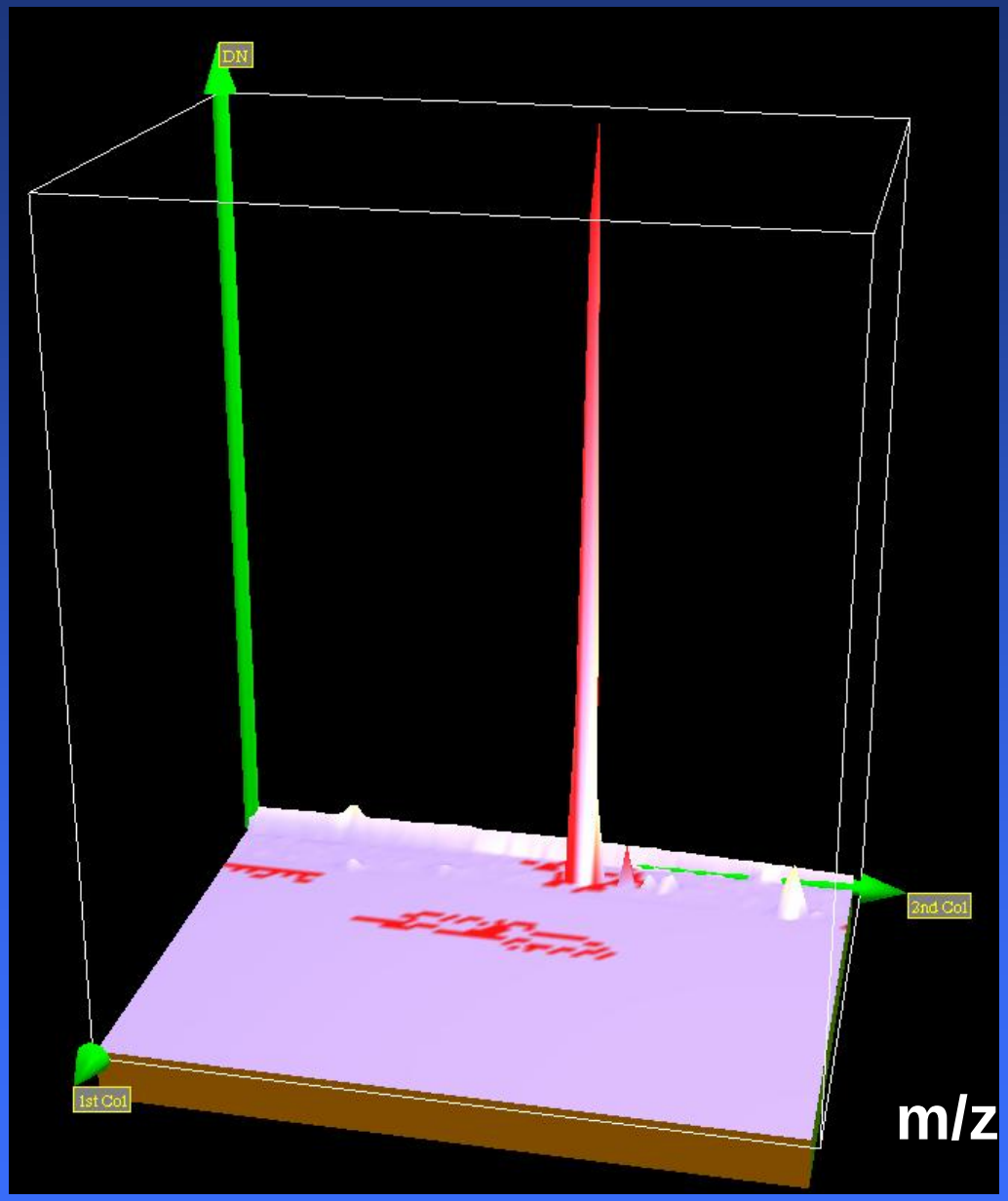
30 msec cycle time (33Hz)

m/z 321.8936 [M+2] only

1 fg on-column

S/N 1420

^{12}C-2378-TCDD Standard



313 ag ^{12}C-2378-TCDD

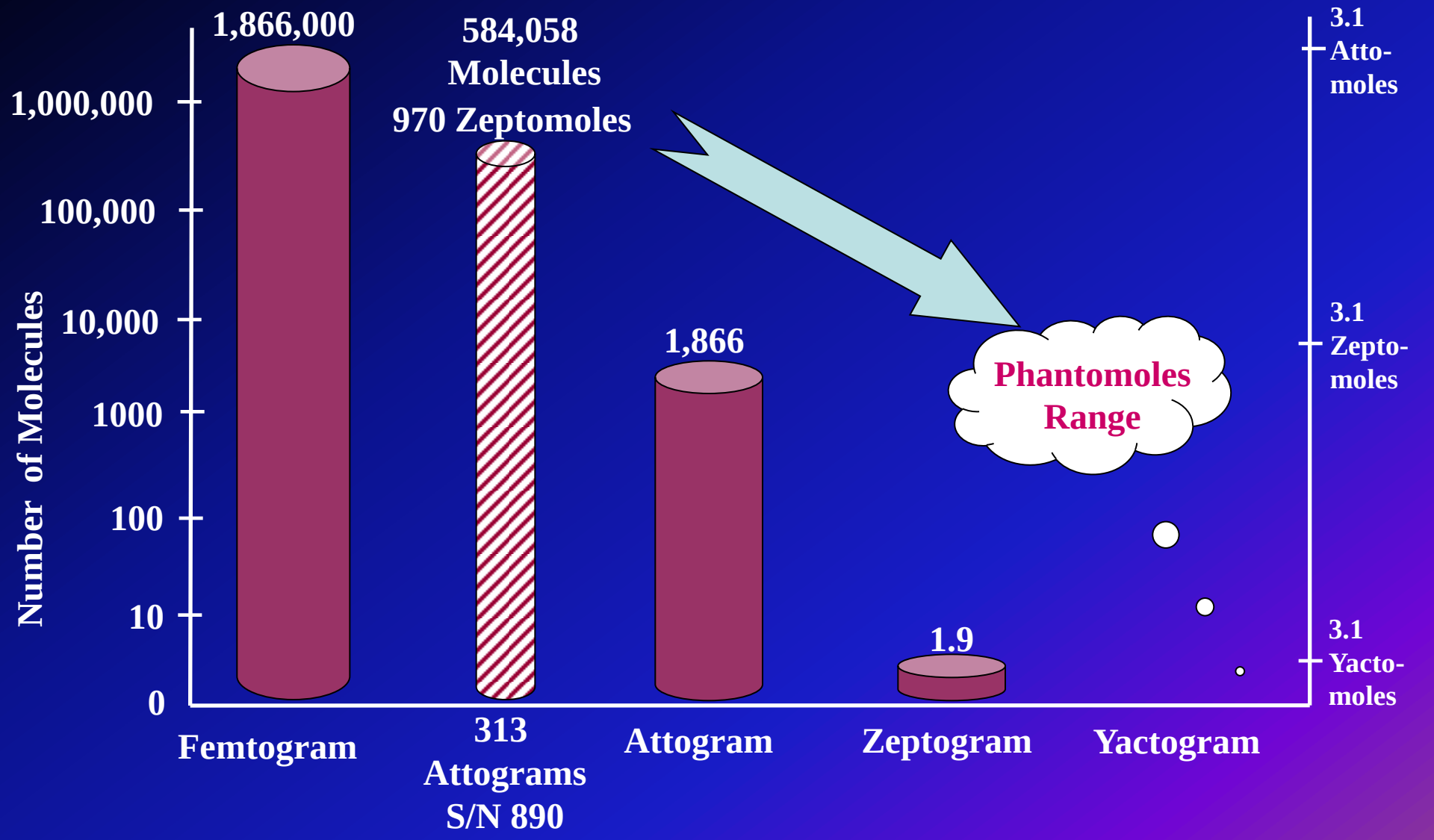
(S/N>400, 4 Sigma)

Maximal sensitivity

Linear calibration : 0.313, 0.625, 1.25, 2.5, 10, 20 fg/µl

m/z 321.8936 [M+2] only

2,3,7,8-TCDD by CZC-HRMS



WHAT DOES THE FUTURE LOOK LIKE?

New approach on the DFS:

Time-Controlled Cryogenic Zone Compression (T-CZC) GC-HRMS

Paper P-0012

ACKNOWLEDGEMENTS
Timed Cryogenic Zone Compression Team

Heinz Mehlmann--Thermo

Dirk Krumwiede--Thermo

Jef Focant--University of Liege

Andreas Sjodin--CDC

Wayman Turner—CDC Retired

Donald G. Patterson Jr.--Consultant

History

- R&D work at CDC Atlanta
- HRMS with GCxGC
- Short column
- Long modulation times
- The entire peak is cryo-trapped in one single event.

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Cryogenic zone compression for the measurement of dioxins in human serum by isotope dilution at the attogram level using modulated gas chromatography coupled to high resolution magnetic sector mass spectrometry

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Dioxins
DDE
BB153
Human serum
Dried-blood spot (DBS)

ABSTRACT

A liquid nitrogen jet-cooled thermal modulator dedicated to comprehensive two-dimensional gas chromatography has been mounted in a GC oven coupled to a high resolution magnetic sector mass spectrometry instrument. The data acquisition parameters of the slow double-focusing magnetic sector MS instrument have been optimized to accommodate the description of the narrow modulated GC peaks. Acquisition rates were increased to 20 Hz, while maintaining high mass resolution. Selected ion monitoring (SIM) descriptors, typically including several ions for both native and labeled analytes, were thus reduced to one or two to ensure enough MS cycle time. For maximization of the sensitivity enhancement due to cryogenic zone compression (CZC), the entire GC peak of interest was trapped and remobilized in one event. Optimization of the method resulted in the ability to detect low attogram (ag) amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (313 ag gives a S/N of 400:1), a level that had not yet been attained using classical GC–HRMS. An isotope-dilution calibration curve was constructed using $^{13}C_{12}$-2,3,7,8-TCDD as the internal standard over the range of 500 ag/μL to 35,000 ag/μL (R^2 = 0.9953). Analyses of a standard natural human reference serum-matrix NIST SRM 1589a containing 223 ag of 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD) (70% recovery rate assumed) resulted in a peak with a S/N of 188:1 (4 sigma, m/z = 355.8546). Measurement of 2,2-bis (4-chlorophenyl-1,1,1-trichloroethane) (DDE) and 2,2′,4,4′,5,5′-hexabromobiphenyl (BB-153) in human dried-blood spot (DBS) samples is also reported to illustrate the usefulness of such a sensitive technique. Finally, some of the challenges related to sample preparation, blank levels, and to the fact of measuring of such a limited number of molecules (less than 600,000 TCDD molecules) are discussed.

t-CZC paper at: http://pops.thermo-bremen.com

As featured in Chromatography Today February / March 2012

Time Controlled Cryogenic Zone Compression (T-CZC): A Novel Gas Chromatographic Tool for Increasing Sensitivity

by Dirk Krumwiede, Heinz Mehlmann, Kyle D'Silva
POPs Center of Excellence, Thermo Fisher Scientific (Bremen) GmbH, Hanna-Kunath-Strasse 11, 28355 Bremen, Germany
dirk.krumwiede@thermofisher.com

The analysis of semi-volatile trace contaminants represent a unique challenge to the analytical chemist. Modern techniques of gas chromatography and mass spectrometry have reduced instrumental limits of detection from the nanogram range in the 1970s, using packed chromatography columns and quadrupole mass spectrometry; to the low femtogram range during the twenty-first century, using capillary columns coupled to high resolution or tandem quadrupole mass spectrometers [1].

Some compounds which require such low levels of detection are chlorinated dioxins and furans; a group of structurally similar halo-organic compounds, which are of great interest because some of these congeners are extremely toxic, persistent and bio-accumulative [2].

For such compounds low limits of detection can now be achieved in many sample types with the combination of sensitive mass spectral detection and careful chromatography. Generally, when even lower limits of detection are required, the analytical chemist has the possibility of combining increasingly selective sample preparation together with significantly increased sample size.

However, when sample sizes are small, and residue levels are low, a unique analytical challenge is presented, such as with dried blood spot (DBS) analysis. Typical DBS sample sizes can be small, only 50-150 µL [3]. Residue levels are very low, especially for lipophilic compounds like dioxins, and there is no opportunity to scale up sample size to achieve low limits of detection. Usually this challenge would preclude the analysis of dioxins and furans in such samples. However, large archives of dried blood spot samples exist in hospitals globally. These are routinely sampled from children at birth in many countries [3]. These samples present an unprecedented sample resource for epidemiological and toxicological studies of population background exposure to dioxins and furans, providing these significant analytical challenges can be overcome.

Cryogenic peak modulation is a well established technique used for comprehensive GCxGC applications since 1991 [4]. In GCxGC a cryogenic modulator continuously and rapidly traps and releases the eluent from a first dimension column onto another short second dimension column in a very narrow band. The combination of two different column phases for the first and second dimension results in a substantial increase of chromatographic separation power. This is due to combined chromatographic selectivity, cryogenic peak focusing and fast second dimension chromatography. Each first dimension chromatographic peak is modulated and several second dimension chromatograms are obtained (Figure 1); dedicated software tools allow to construct two-dimensional chromatograms for data evaluation. This

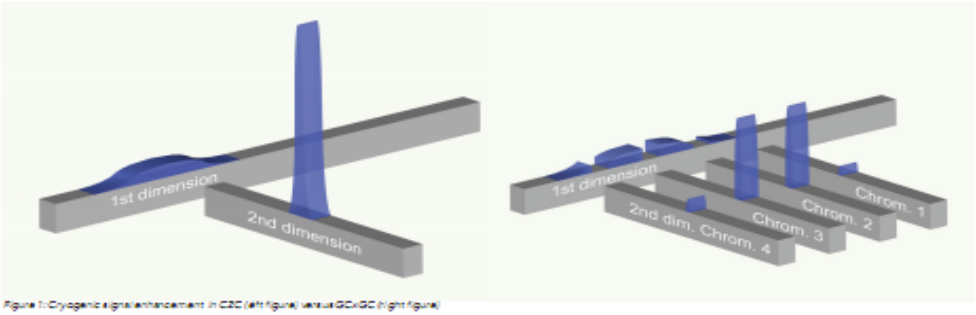


Figure 1: Cryogenic signal enhancement in CZC (left figure) versus GCxGC (right figure)

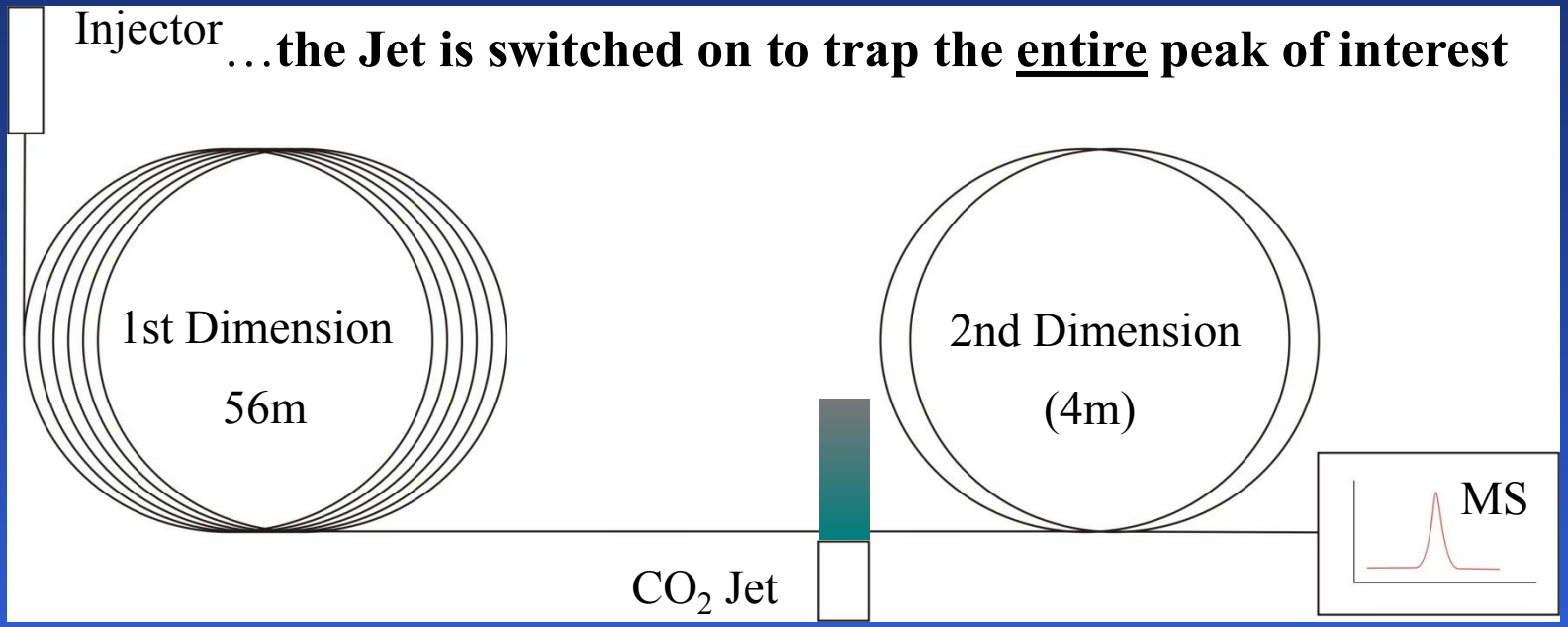
In Google:

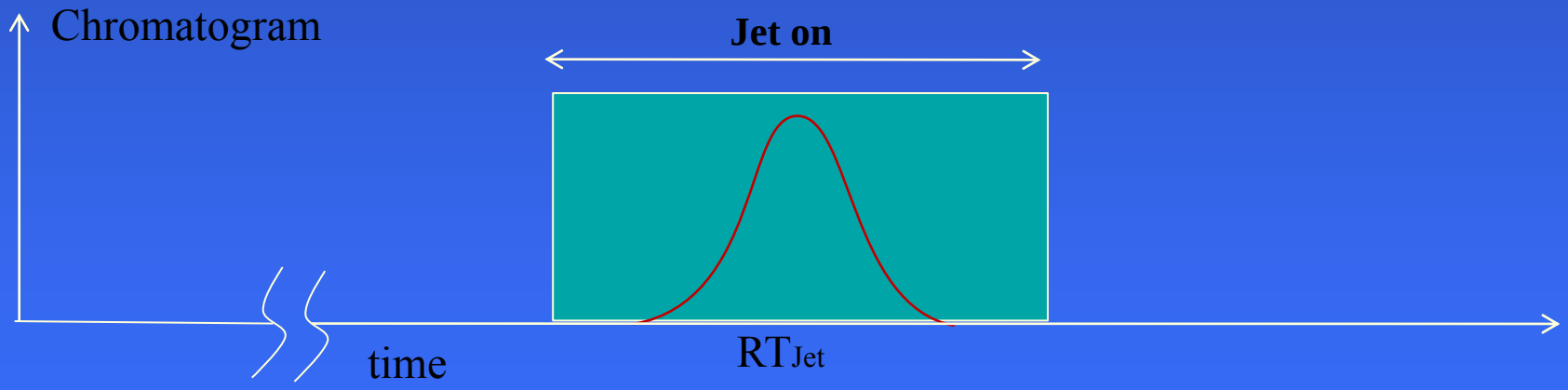
- type „POPs“ & „Thermo“

- first hit is this page

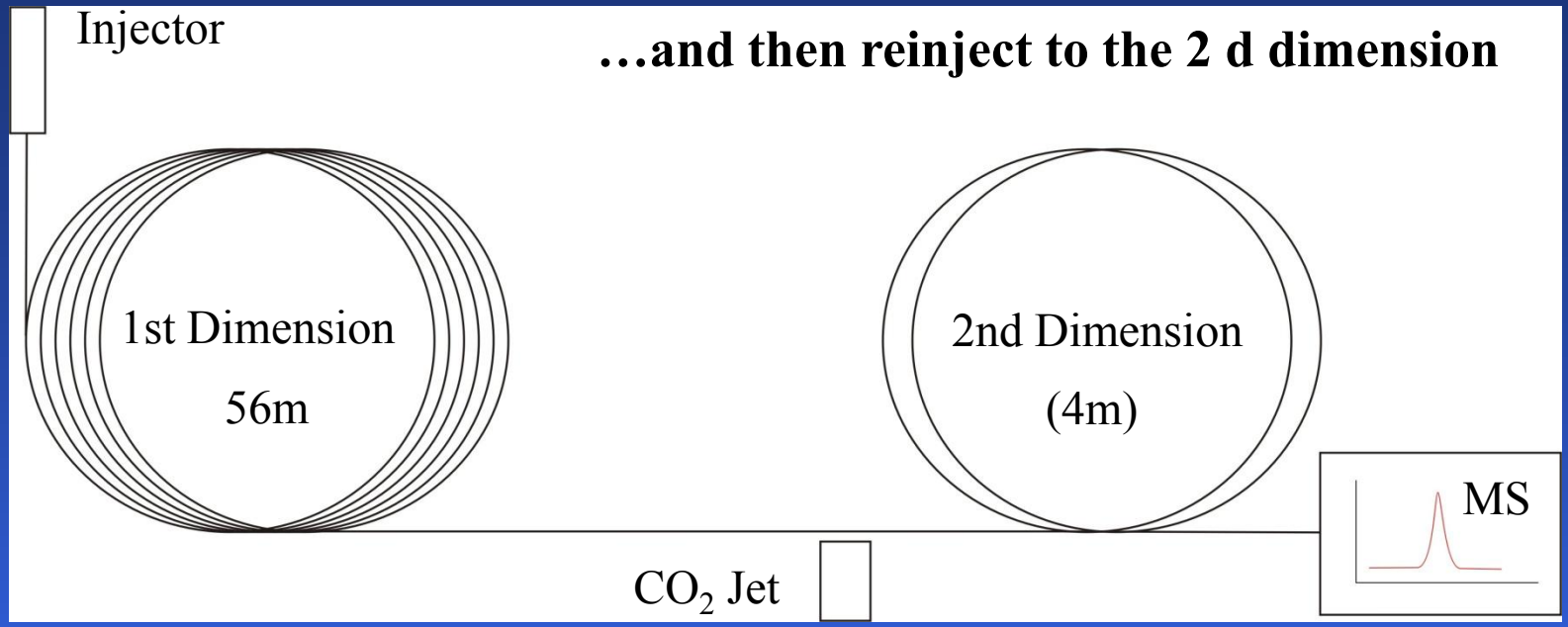
Chromatography Today
February / March 2012

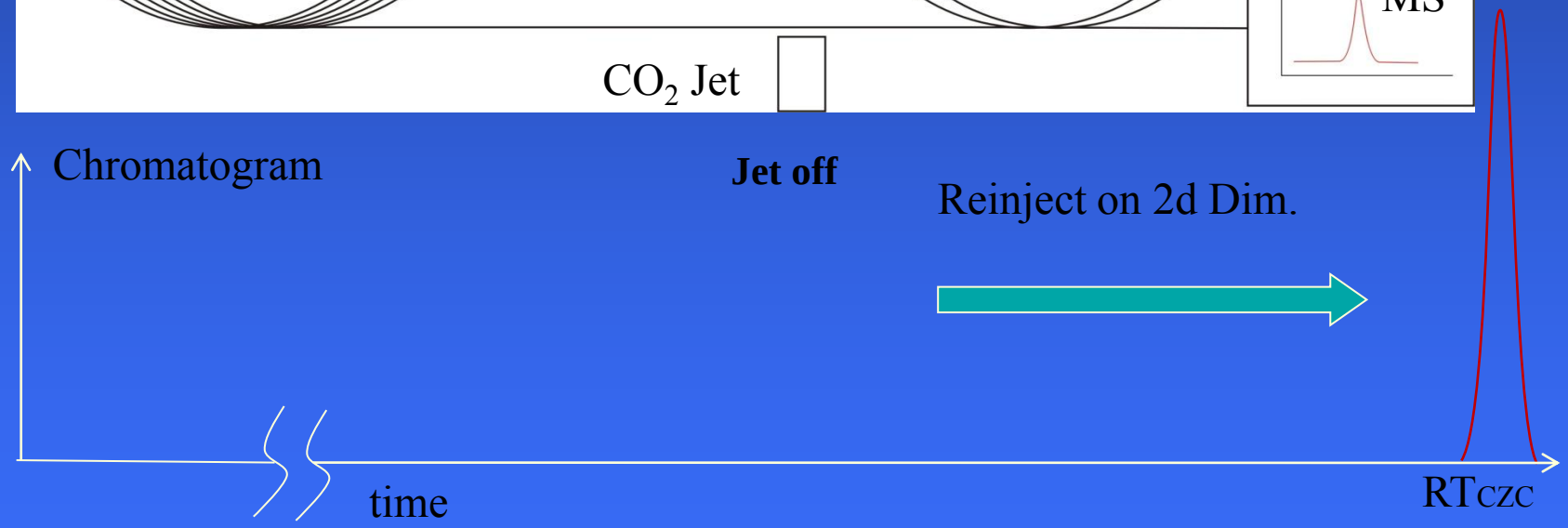
Timed CZC - Principle



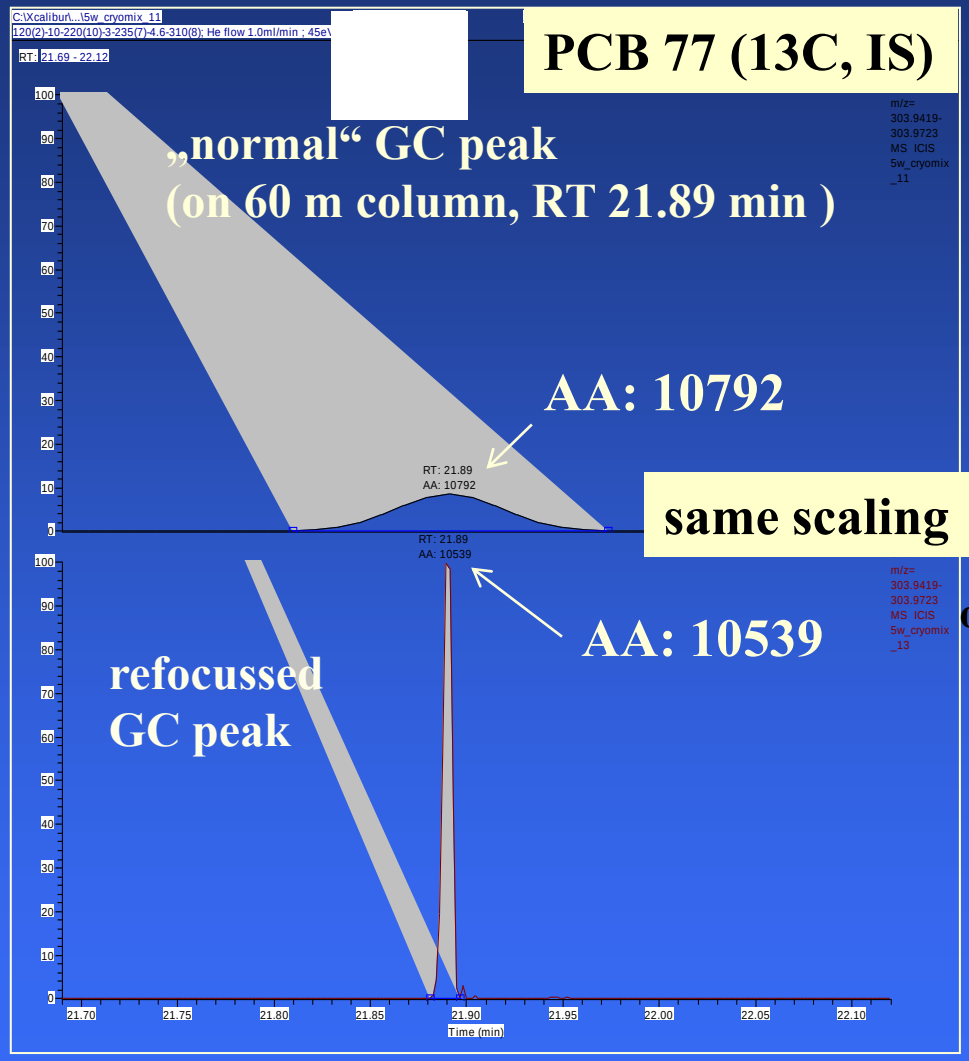


Timed CZC - Principle





CZC Peak Zone Compression – basic effect



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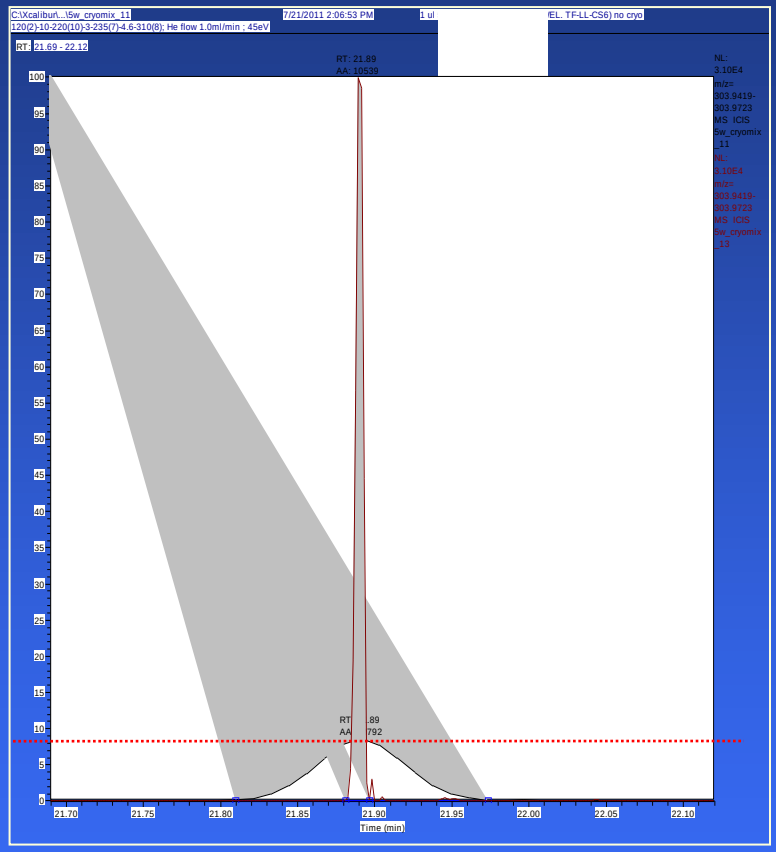
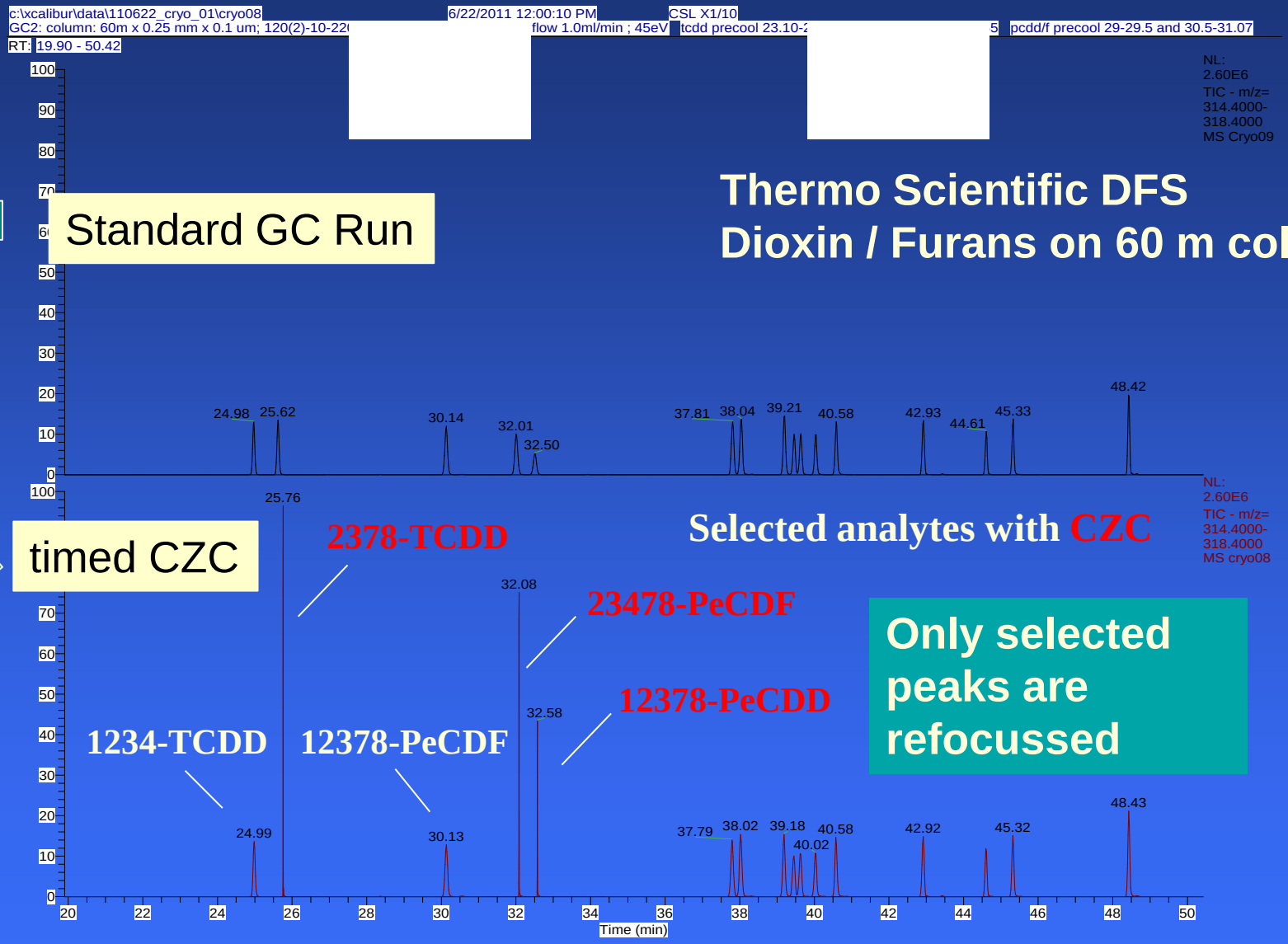


Thermo Fisher Scientific

Time controlled CZC: targeted cryofocusing



Thermo Fisher Scientific

LOWER & LOWER & LOWER ...THE LIMBO

5 fg/ul

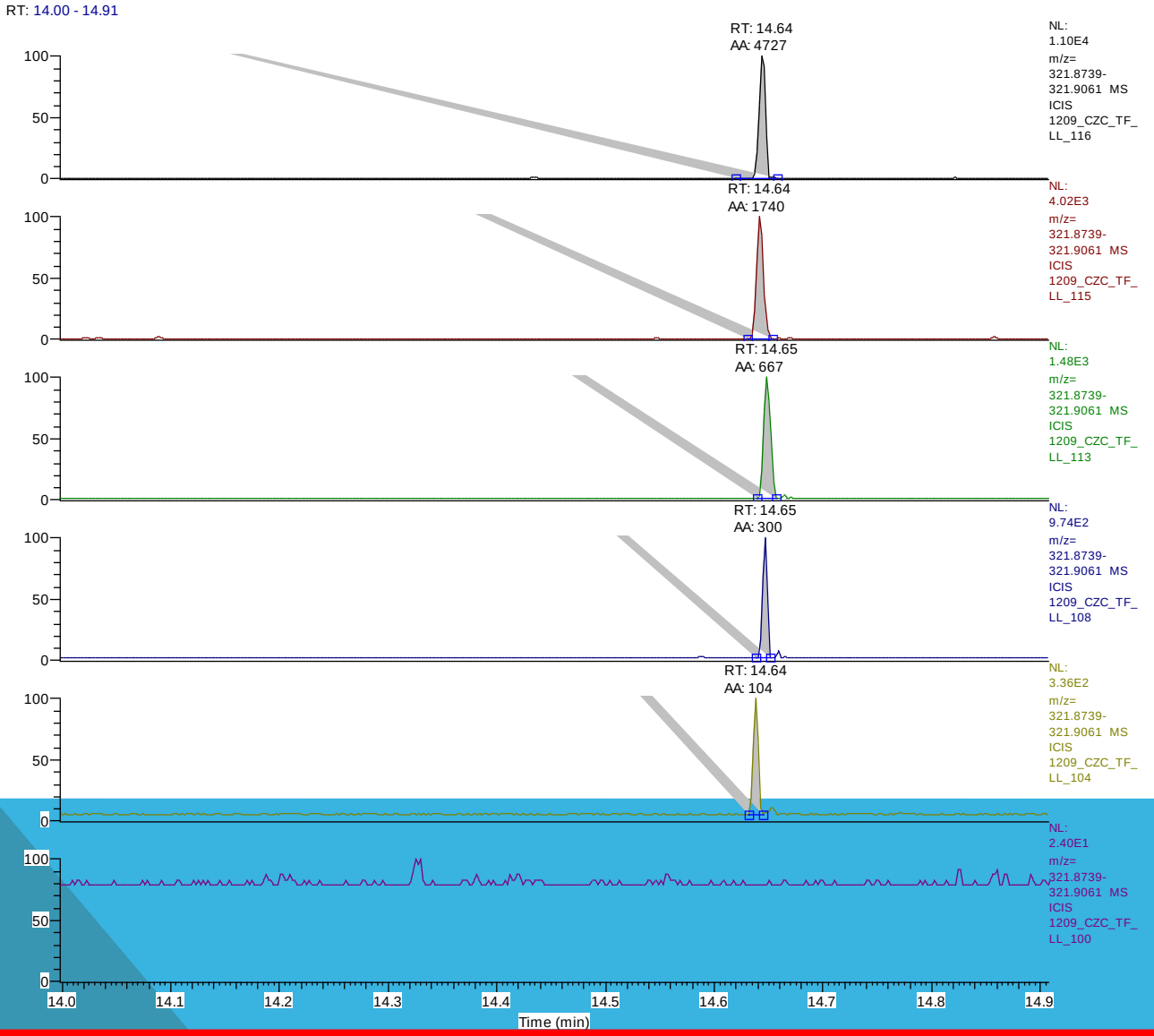
2 fg/ul

1 fg/ul

500 ag/ul

200 ag/ul

blank



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Conclusions t-CZC

- Clear gain in sensitivity.
- Combination of t-CZC and standard measurement possible.
- Free choice of analytes to be cryo-focused.
- Switching from normal to t-CZC without hardware change.
- No special software needed for chromatograms nor quantification.
- Very low CO_2 consumption compared to GCxGC.
- Use of standard columns.

Conclusions t-CZC

- **A lot of work yet to do**
  - **Ion statistics (isotope ratios)**
    - **Repeatability**
    - **LODs in matrix**
      - **Hardware**
      - **Software**
      - **Blanks**
  - **Ruggedness for large studies**
  - **Scan speeds & dwell times**
    - **Many more**

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