



Advances in Instrumental Techniques

J.-F. Focant et al.



EURL 11/2016

1957: American Chicken Gate

- Chick Edema Disease, Eastern USA
- >1.10⁶ broiler chicken suddenly died
- Feed contamination
- Oleic acid component
- PCP-treated beef hides
- PCDDs at ppm levels in PCP... (1962)



Acta Cryst. (1969). B25, 150

The Identification and Crystal Structure of a Hydropericardium-Producing Factor: 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin

By J. S. CANTRELL,* N. C. WEBB AND A. J. MABIS†

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239, U.S.A.

(Received 4 December 1967)

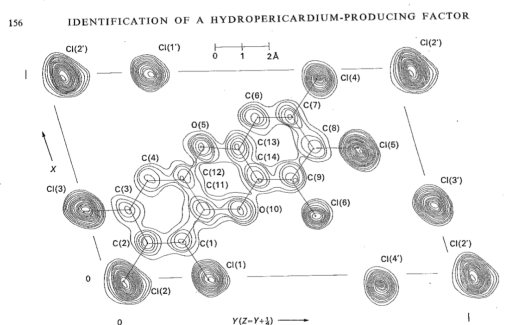
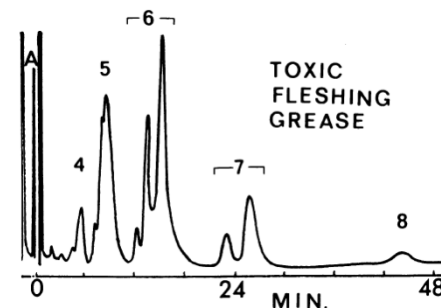


Fig. 4. Electron density in the (044) plane containing the molecule. Contours are at 1 e.Å⁻³ starting at 2 e.Å⁻³. The \times marks are projections onto (044) from the electron density maxima, which in most cases are a short distance from (044).

HxCDD structure
elucidated from
crystals isolated from
the HPTF

GC-ECD of a fleshing grease extract



Metcalf, L. D. Proposed source of chick edema factor. *J. Assoc. Offic. Anal. Chem.* 55 (1972) 542.

Prevention of Dioxin Contamination of Foods and Feeds

Since commercial chlorophenols are widely used as termite control and antimicrobial agents, there are numerous opportunities for direct and indirect contamination of food and food fats. Dioxin contamination of fats and fatty acids destined for use in foods or feeds can be minimized by control of sources of direct contamination with chlorophenols. These sources include the use of tri- and pentachlorophenol for hide preservation and other pretanning operations and in glue emulsions for dry rendering of fats. Care must also be taken in the use of chlorophenols industrially (as antimicrobials) or on the farm (as wood preservatives for fences, barns, etc.).

Firestone, D. Etiology of chick edema disease. *Environ. Health Perspect.* 5 (1973) 59.

Determination of Chlorinated Dibenzo-p-dioxins and Dibenzofurans in Various Materials

by W.B. Crummett* and R.H. Stehl*

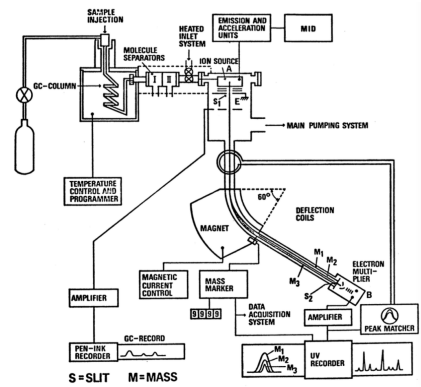


FIGURE 2. Schematic diagram of LKB 9000 gas chromatograph-mass spectrometer.

Crummett, W.B. and Stehl, R.H. Determination of Chlorinated Dibenzo-p-dioxins and Dibenzofurans in Various Materials. Environ. Health Perspect. 5 (1973) 15.

An Analytical Method for Detecting TCDD (Dioxin): Levels of TCDD in Samples from Vietnam

by Robert Baughman* and Matthew Meselson*

The gas chromatograph was a Bendix Model 2200 equipped with a thermal conductivity detector. The column was 5% SE-30 on 60/80 Chromosorb W, 2 m x 2 mm (id) stainless steel. The trap for preparative gas chromatography was a 150 mm x 1.5 mm (id) glass tube packed with 30 mm of glass wool.

An Associated Electrical Industries MS-9 focusing mass spectrometer and a 1024 time-averaging computer interface with the MS-9 as described earlier are used.

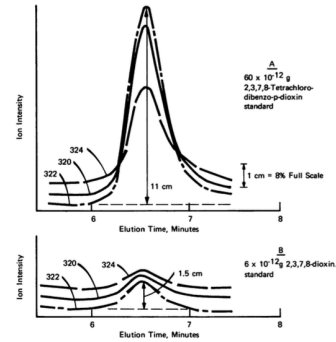


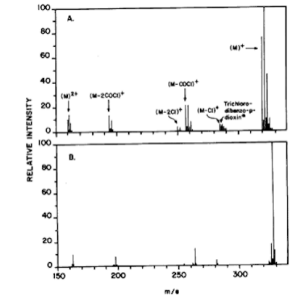
FIGURE 3. Gas chromatography-mass spectrometry for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin at maximum sensitivity. Conditions: LKB 9000 GC-MS; column, 3% OV-210 on Chromosorb W-H.P.; resolution, ca. 400; sample, 6 µl of standard solutions.

Table 3. Confirmation Procedures

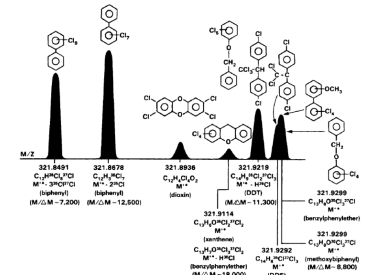
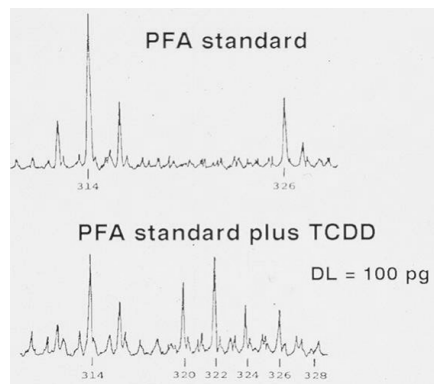
- A. Routine**
 - Follows ¹⁴C TCDD through highly specific cleanup
 - Has expected mass (± 3 mmu) at m/e 320 and 322
 - Has expected ratio of isotopic isomers at m/e 320 and 322
- B. Additional***
 - M⁻-COCl⁺ fragmentation peak has expected mass and isotopic isomer ratio
 - Percent recovery after partial photolytic decomposition equals that of ¹⁴C TCDD (7, 8)
 - Partition coefficient between dichloromethane-hexane and acetonitrile equals that of ¹⁴C TCDD (7).

* Steps 2 and 3 of the additional procedures were carried out on the dichloromethane-hexane eluant from the alumina chromatography prior to preparative GLC.

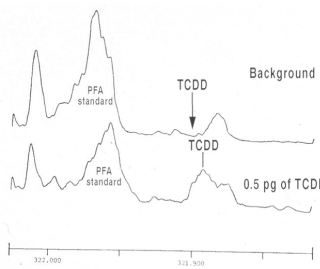
FIGURE 3. Linearity of response for presence of beef liver residue. The TCDD values are the amounts introduced into individual runs on the MS-9.



Baughman, R. and Meselson, M. An Analytical Method for Detecting TCDD (dioxin): Levels of TCDD in Samples from Vietnam. Environ. Health Perspect. 5 (1973) 27.



Tondeur et al., Bio. Med. Environ. Mass Spectrom. (1987) 14, 449.



- Direct Probe
- MS-9 HRMS
- Resolution 10,000 (10%)
- Time Averaging
- Repetitive Scanning

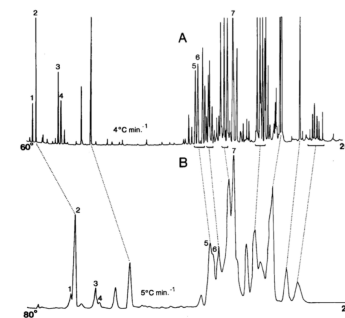


Fig. 3. Chromatograms of Calmus oil on (A) a 50 m capillary column and (B) a 4 m packed column with the same stationary phase.

Bartle, K.D. and Myers, P. History of Gas Chromatography. TRAC 21(2002) 547.

- Better separation
- Less material to be injected
- Need special introduction methods
- Need sensitive detectors

Anal. Chem. 1986, 58, 705-713
 High-Resolution Gas Chromatographic/High-Resolution Mass Spectrometric Analysis of Human Adipose Tissue for 2,3,7,8-Tetrachlorodibenzo-p-dioxin

Anal. Chem. 1987, 59, 2000-2005
 High-Resolution Gas Chromatographic/High-Resolution Mass Spectrometric Analysis of Human Serum on a Whole-Weight and Lipid Basis for 2,3,7,8-Tetrachlorodibenzo-p-dioxin

LABORATORY QUALITY ASSESSMENT MEASUREMENTS: U.S. AIR FORCE RAN VIETNAM VETERAN, NATIONAL INSTT CHEMICAL WORKERS

W. E. TURNER, D. G. PATTERSON

Division of Environmental Health Laboratory Sciences, Centers for Disease Control, Building 17 (Received in Germany 17)

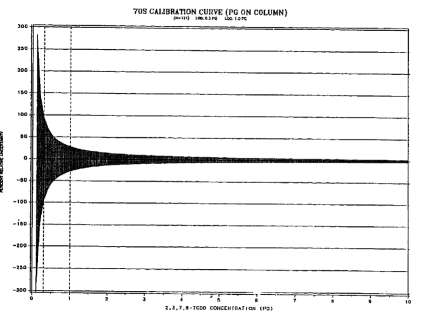


FIGURE 14. Percent Uncertainty: 70S Calibration Curve

- CVs 10-15%
- Ppt-ppq LODs
- Isotope Dilution
- QA/QC basics

EPA United States Environmental Protection Agency
 Office of Water Regulations and Standards
 Industrial Technology Division
 Office of Water April 1992

Method 1613: Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

EN 1948-1
 EN 1948-2
 EN 1948-3

NORME EUROPÉENNE
 EUROPÄISCHE NORM
 EUROPEAN STANDARD

EN 16215-2012

EUROPEAN STANDARD
 NORME EUROPÉENNE
 EUROPÄISCHE NORM

EN 16215

English Version
 Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Autres des animaux - Dosage des dioxines, des PCB de type dioxine et des PCB indicateurs par GC/HRMS
 Futtermittel - Bestimmung von Dioxinen und dioxinähnlichen PCBs mittels GC/HRMS und von Indikator-PCBs mittels GC/HRMS

The European Standard was approved by CEN on 9 March 2012.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
 COMITÉ EUROPÉEN DE NORMALISATION
 EUROPÄISCHER KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

© 2012 CEN. All rights of exploitation in any form and by any means reserved worldwide for CEN national Members. Ref. No. EN 16215:2012. E

EU Commission Documents

- Council Regulation 2001/102/EC
- Council Regulation (EC) No 2375/2001
- Commission Directive 2002/69/EC
- Commission Directive 2002/70/EC
- Commission Directive 2006/13/EC
- Commission Recommendation 2006/88/EC
- Commission Regulation (EC) No 199/2006
- Commission Regulation (EC) No 1881/2006
- Commission Regulation (EC) No 1883/2006
- Commission Regulation (EC) No 152/2009
- SANCO/11248/2011
- Commission Recommendation 2011/516/EU
- Commission Regulation (EC) No 252/2012
- Commission Regulation (EC) No 277/2012
- Commission Regulation (EC) No 278/2012
- Commission Regulation (EC) No 589/2014
- ...



Focant and De Pauw, Les Dioxines. La Recherche 323 (1999) 84.

The Gold Std is Thus...

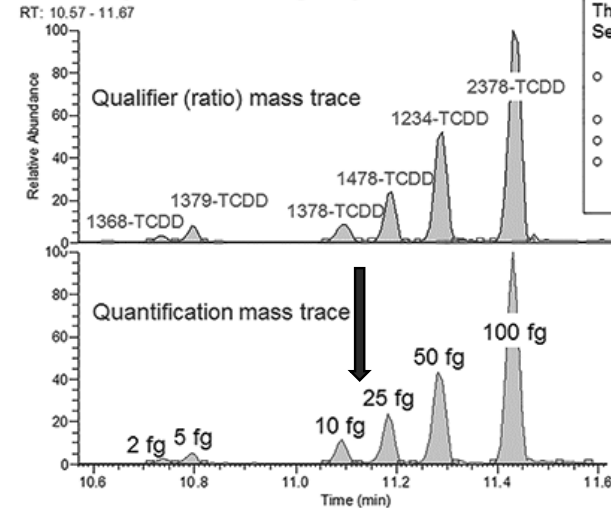
- Capillary GC, t_R dev. < 0.25%
- DF Sector MS
- EI 70eV, >10,000 R
- SIM, ^{13}C ID, IRs <20% RSDs, Reco. 40-130%
- LODs, LOQs (S/N)...



EU No 252/2012



Chromatogram of ONE single injection:



Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS

- World-Wide Method compliance (e.g., EPA 1613)
- Best Dioxin installation spec
- Highest available sensitivity
- Robust-by-size with large volume ion source



Sector 2 fg (SIM)

From 24 Karats Gold to...

- Com. Reg. (EU) 589/2014 (PBMS)
- GC-HRMS & GC-MS/MS as confirmatory...
- 24 karats GC-sHRMS (low level studies)
- 20 karats GC-MS/MS (food-feed control)

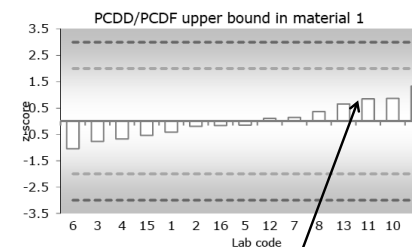


QQQ 50fg (MS/MS)

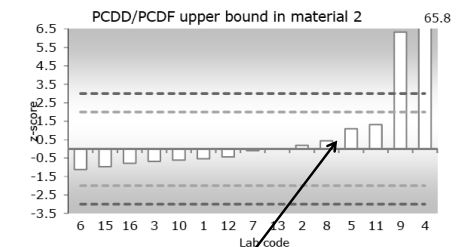
Accuracy & Reproducibility

Proficiency test (PT)

	Reported pg/g TEQ	Target Value pg/g TEQ	Accuracy
Material 1			
PCDD/Fs	1.10±0.20	1.01	8,8%
DL-PCBs	0.80±0.19	0.89	-10,3%
Total TEQ	1.90±0.36	1.90	-0,1%
Material 2			
PCDD/Fs	0.55±0.11	0.48	15,6%
DL-PCBs	0.82±0.21	0.85	-3,0%
Total TEQ	1.38±0.26	1.33	3,7%



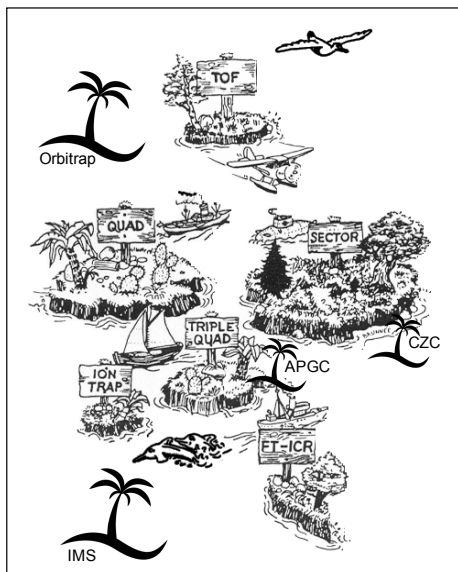
Z score = 0.80



Z score = 0.59

Wim Traag, Rikilt, NL

Other Gold Fineness?



Modified from: Brunnée, Int. J. Mass Spectrom. (1987) 76 (2) 125.

GC-QISTMS/MS

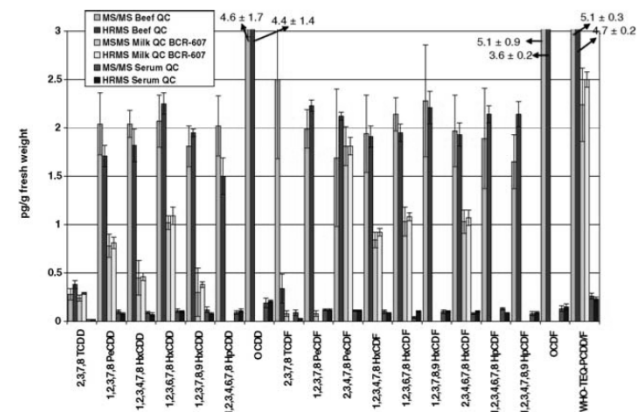


Fig. 3. Comparison of PTV-LV-GC-QISTMS/MS and GC-HRMS for the measurement of PCDD/Fs at the low pictogram level in biological matrices.

Focant et al., J. Chromatogr. A (2005) 1067, 265.

GC-FTICRMS

- GC-TQFTICRMS
- 50-100 k mass resolution
- 0.5-1.0 ppm mass accuracy
- 1pg IDLs
- Kendrick plot analyses

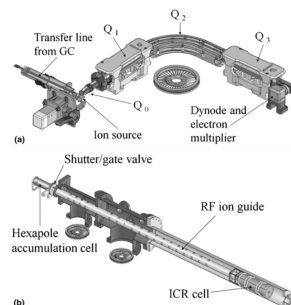


Figure 1. (a) Schematic of the GC transfer line and Varian 320-MS (diagram courtesy Varian Inc.). (b) Schematic of the hexapole accumulation cell, ion guide, and ICR cell (diagram courtesy Varian Inc.).

APPLICATION NOTE

Dioxin Analysis by Gas Chromatography-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (GC-FTICRMS)

Vince Y. Taguchi,^a Robert J. Nieczarz,^b Ray E. Clement,^a Stefan Krolik,^c and Robert Williams^d

GC-FTICRMS

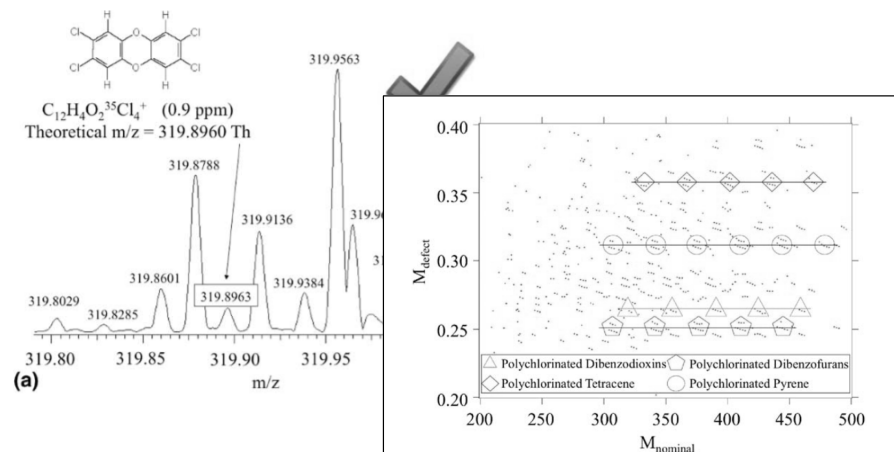
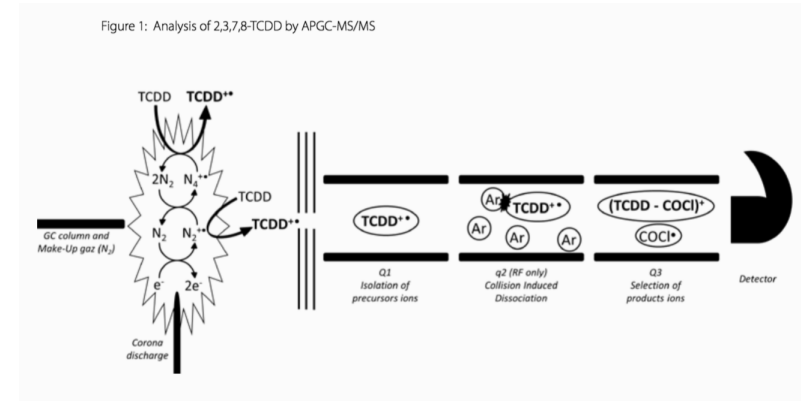


Figure 3. Use of a Kendrick plot for the identification of unknown series of polychlorinated compounds. The mass defect, M_{defect} , is plotted as a function of nominal mass, M_{nominal} , in a mass scale defined by the mass difference resulting from a substitution of chlorine for hydrogen.

APGC

- APGC as the missing link between LC and GC
- Soft ionisation process (molecular ion)
- N₂ make up gas, less source cleaning
- Exact mass (<5ppm) filtration
- Charge transfer vs protonation (water, IPA, ...)
- Need for dedicated MS libraries...
- Ideal for Brominated analytes...



[APPLICATION NOTE]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

An Inter-Laboratory Evaluation of a Con and Environmental Samples Using APGC

Bert van Bavel,¹ Dawei Geng,² Laura Cherta,² Jaime Nacher-Mestre,³ Esteban Abad,² Jody Dunstan,² Rhys Jones,² Alexander Kotz,² Hel Hagberg,² Ingrid Ericson Jogsten,² Joaquim Beltran,² Félix Hernán

The authors conclude that the use of APGC in combination with the Xevo TQ-S for the analysis of dioxins has the same potential, in terms of sensitivity and selectivity, as the traditional HRMS instrumentation used for this analysis, and that it is compliant with Regulation 589/2014/EU. The APGC-MS/MS benchtop system, however, is far easier to use, maintain, and it can be quickly converted for liquid chromatography analysis.

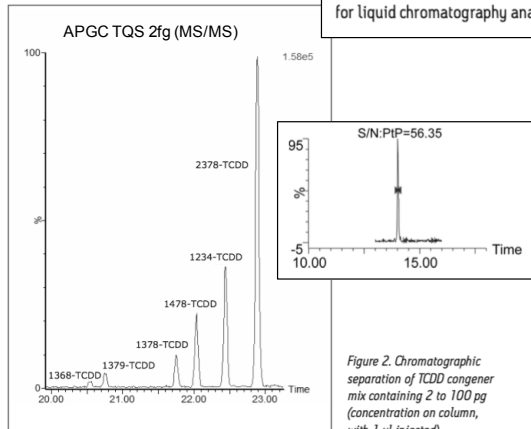


Figure 2. Chromatographic separation of TCDD congener mix containing 2 to 100 pg (concentration on column, with 1 µl injected).

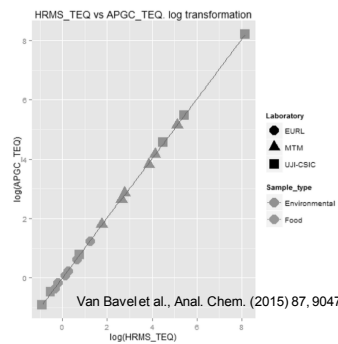


Figure 6. Comparison of APGC results and HRMS for different samples in three different laboratories, as described in the Materials and Methods section.

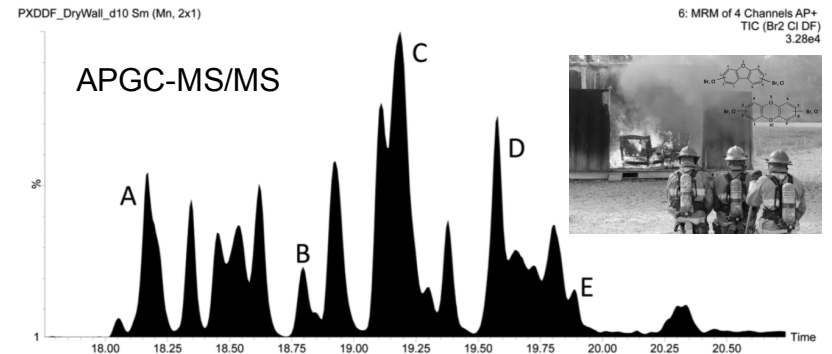


Figure 2. Total ion chromatogram of the Br₂Cl dibenzofuran homologue group in an electronics fire burnt dry wall sample. The letters represent the peaks for which peak-to-peak signal/noise (S/N) values were calculated. S/N values are as follows (A) 46, (B) 20, (C) 89, (D) 62, and (E) 12.

Organtini et al., Anal. Chem. (2015) 87, 10368.

- Used to detect what less sensitive GCxGC TOFMS highlighted

APGC

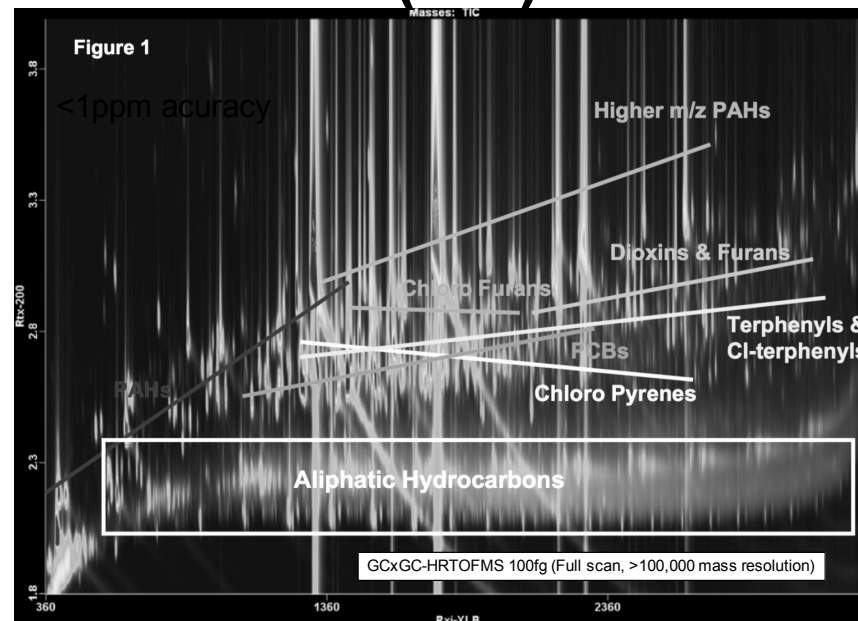
- 'New' Xevo TQ-**XS** (better MDLs than sector...)
- New pump, new ion guide, ... (ion transmission)
- Linear dynamic range of 6 decades...

Absolute amount TCDD	S/N
100 fg	6000
10 fg	600
1 fg	70
250 ag	10
LOD 50 ag...	
IDLs 80 ag	



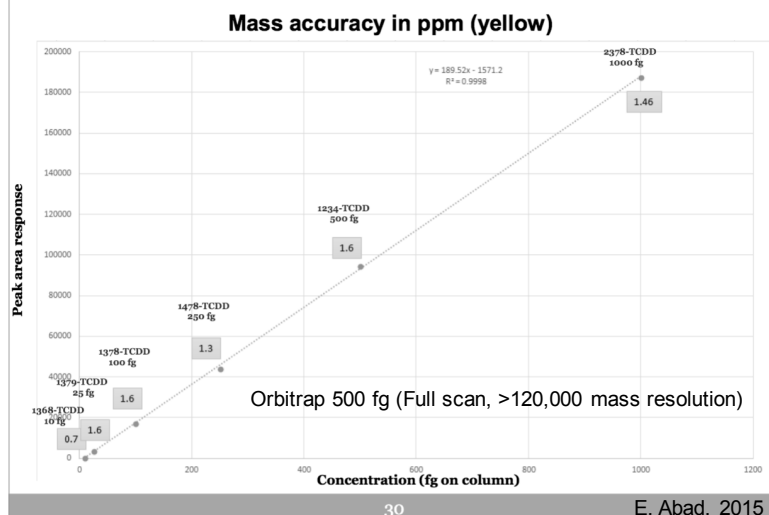
Jones, R., personal communication

GCxGC-(HR)TOFMS



GC-Orbitrap

Results: TCDD response across 10-1000 fg on column



GC-Orbitrap

- Q Exactive
- TIC @ 120 k Resolution...
- <1ppm mass accuracy
- Elemental composition
- Fast untarget HR screening
- Lower needs for sample clean-up...



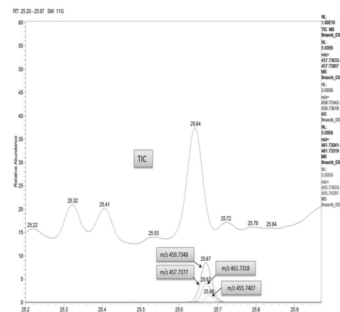
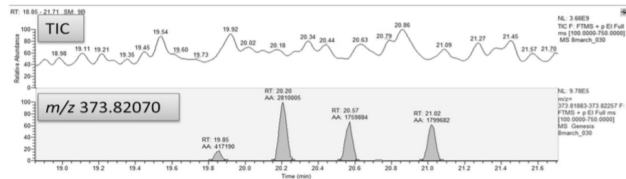


Figure 6. OCDD in bonito fish sample. Extracted ion chromatograms of the four most intense ions for OCDD and the total ion chromatogram (TIC) are shown.

C. Cojocariu et al., 2015



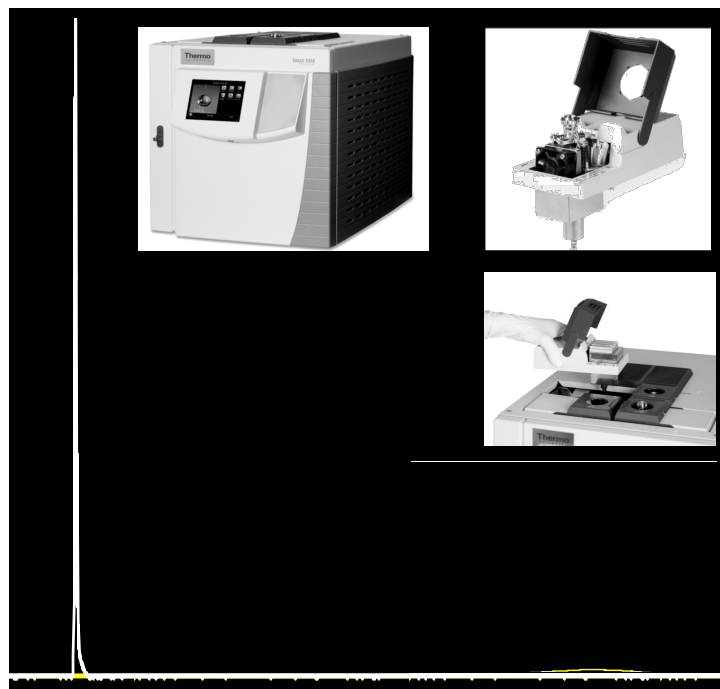
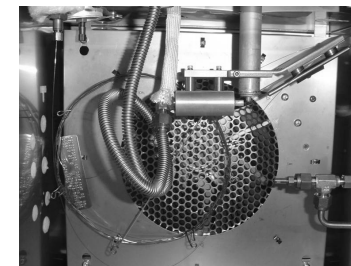
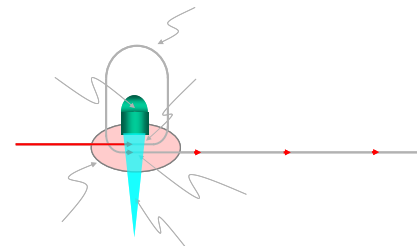
Cryogenic zone compression for the measurement of dioxins in human serum by isotope dilution at the attogram level using modulated gas chromatography coupled to high resolution magnetic sector mass spectrometry

Donald G. Patterson Jr.^{a,*}, Susan M. Welch^b, Wayman E. Turner^b, Andreas Sjödin^b, Jean-Francois Focant^c

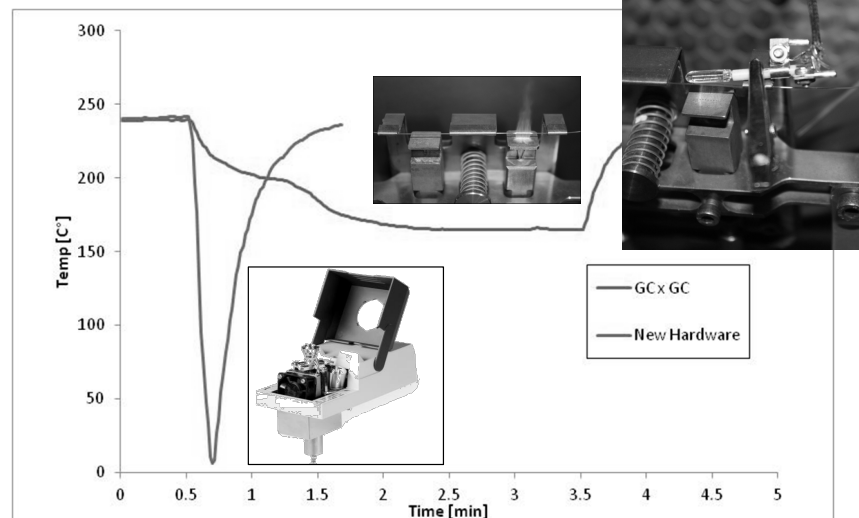
^a EnviroSolutions Consulting, Inc., 172 Camelot Way, #209 98, Jasper, GA 30843, USA

^b National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

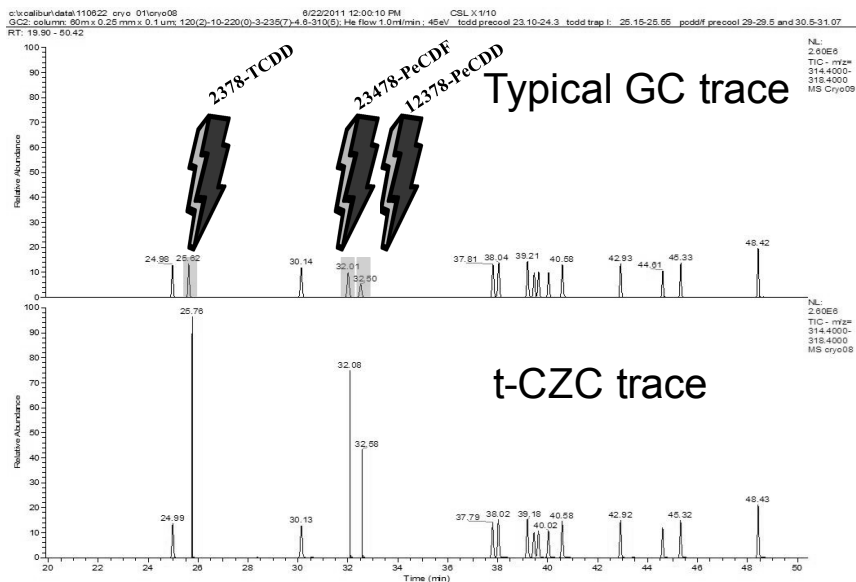
^c CARI, Organic and Biological Analytical Chemistry, Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-tilman, S-4000 Liège, Belgium



Hardware Evolution

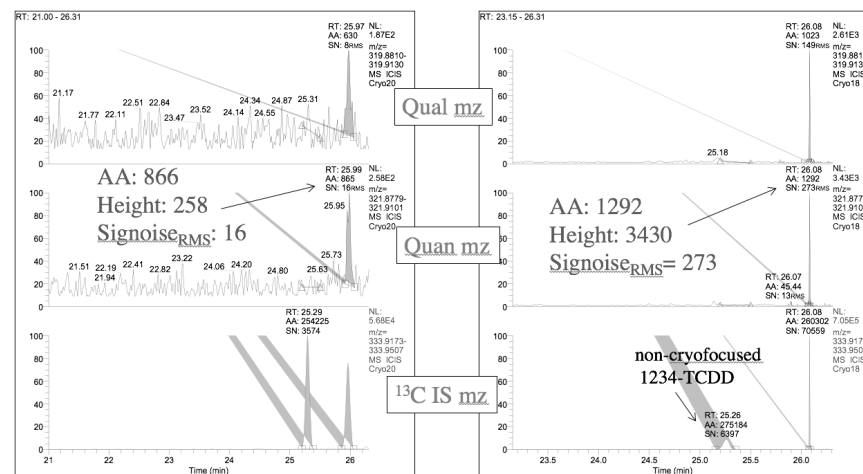


timed-CZC

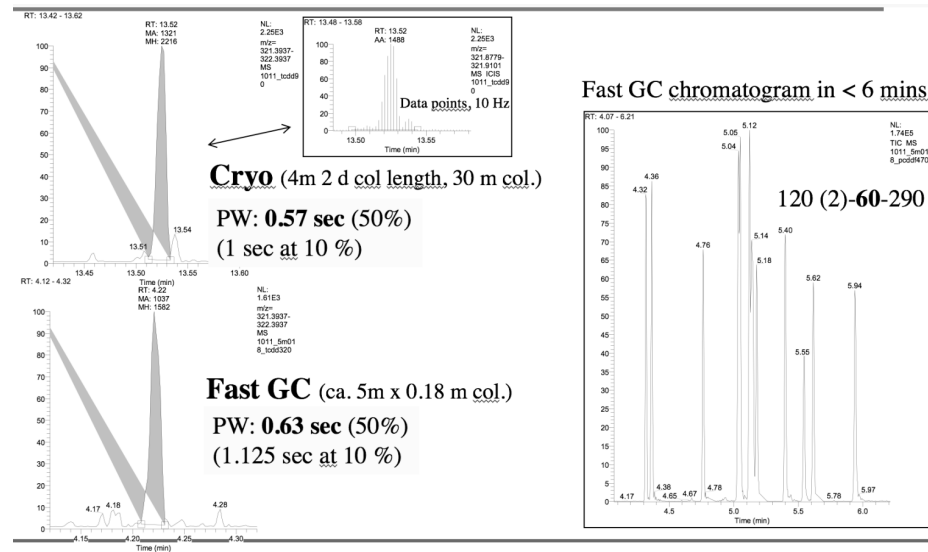
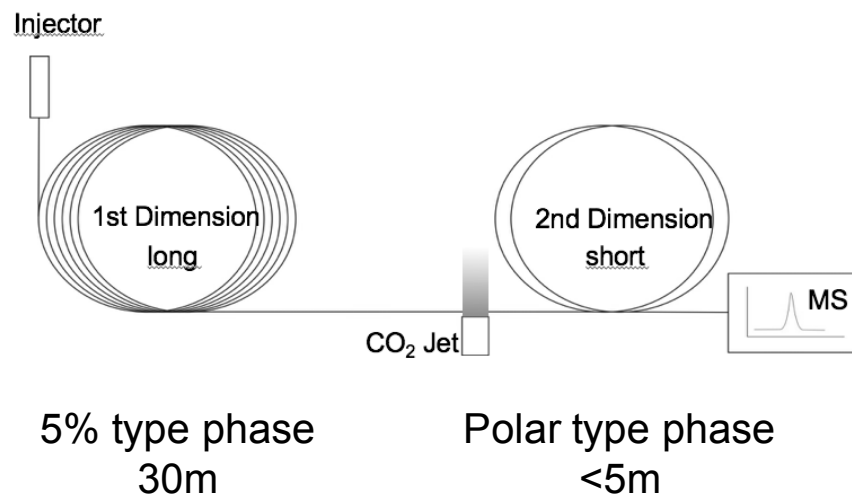


timed-CZC

standard GC-HRMS vs. CZC GC-HRMS
 pooled blood sample on DFS (HRMS) – 2378-TCDD ca. 10 fg

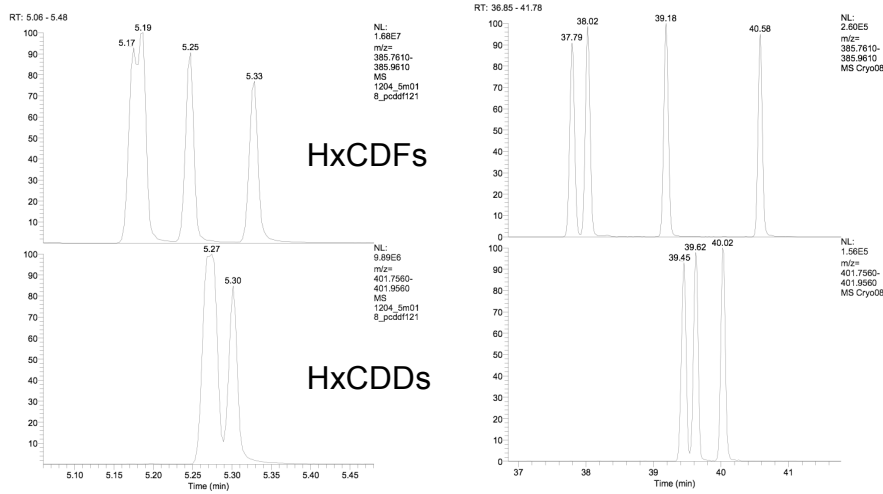


²D is Still There...



Possibly a lot of time saved here...

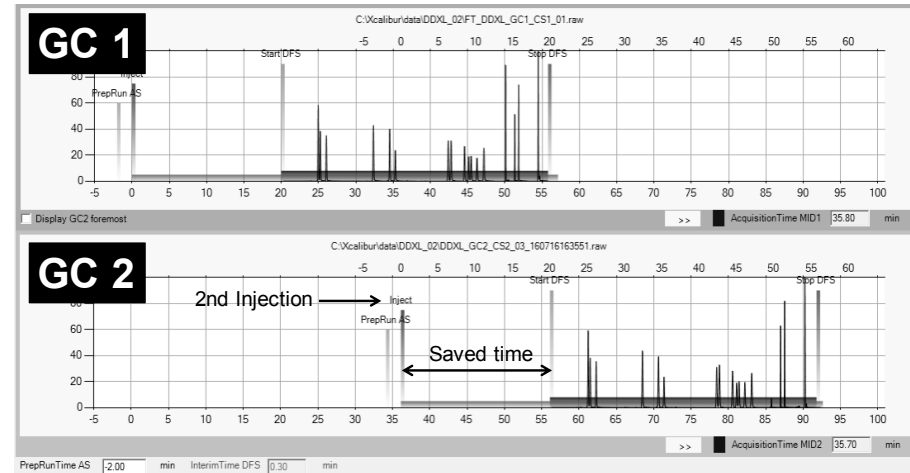
FGC But...



5m FGC

60m CZC

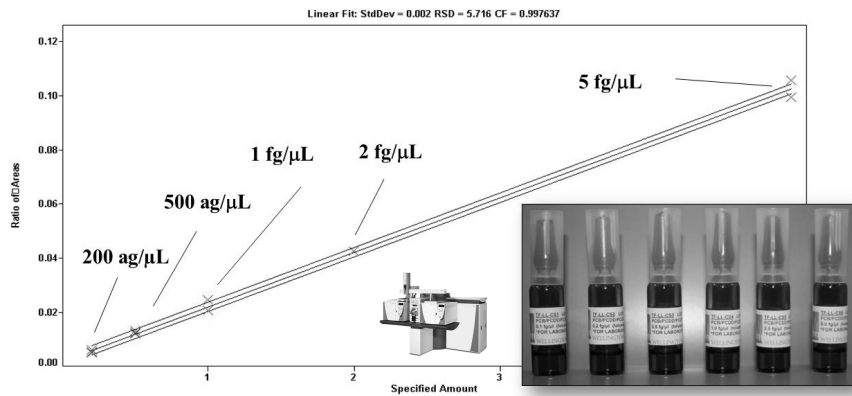
Unless We Do Dual Data...



92 min vs 2x57 (+10) min for 2 runs (16% saved)

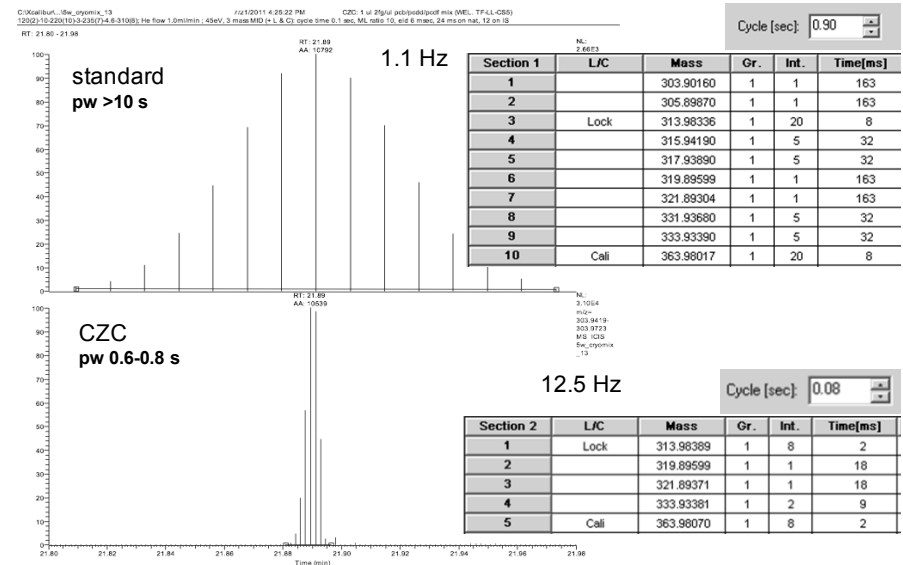
timed-CZC

Triplicate five point calibration curve of TCDD (4uL injected) using t-CZC.



*Special certified Standards from Wellington Labs.

Get the Sector MS Speeded Up



Ion Mobility...

- New orthogonal separation
- Potential enhanced peak capacity...
- Shape & charge selective
- Collision cross sections (<0.5% RSD)
- Overpass isobaric issues and t_R issues...
- GC(xGC)-IMS-QTOF ???
- Chromatogram / Mobilogram / Fragmentogram...



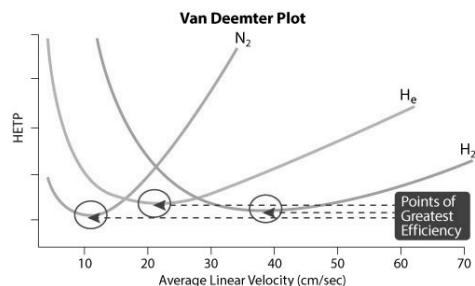
Sample Prep...

- ASE, PLE, MAE, SPE, Soxhlet, ...
- Si (GPC) / Al / C
- Si / C / Al
- Si / Flo / C(1) & C(2)
- Si(1) & Si(2) / C / Al
- Si / C
- High throughput
- Low carry over
- Lower costs than before...

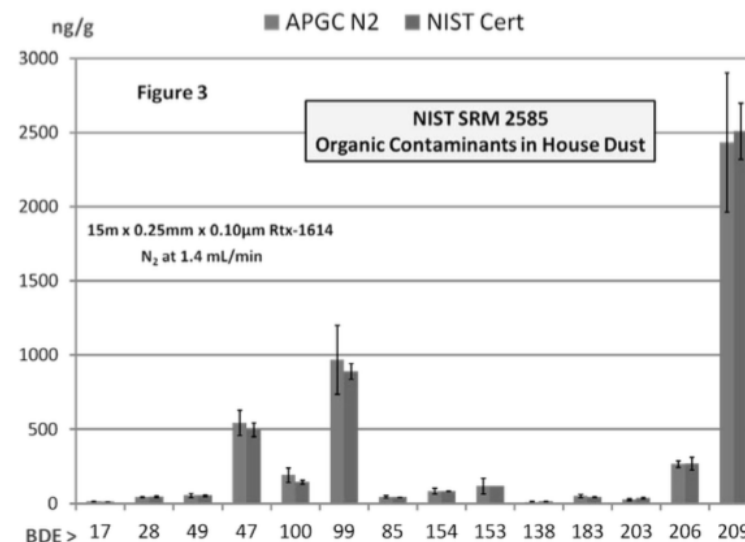


Miscellaneous Tips & Tricks

- Moving from He to N₂ (Phlogisticated air)



- APGC well suited for N₂ use as carrier gas...
- Slight peak broadening (8ml/min flow ok)



ChromaBLOGraphy

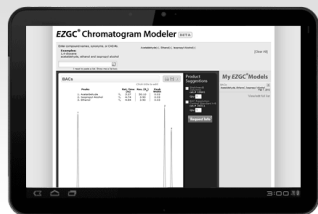
Topical and timely insights from top chromatographers.

EZGC[®] Chromatogram Modeler

The new, Web-based version of our popular Pro EZGC[®] application is now even easier! Create optimized methods, customized to your separation needs, in seconds.

Log In to Get Started!

Don't have an account yet? Click here to register for free!

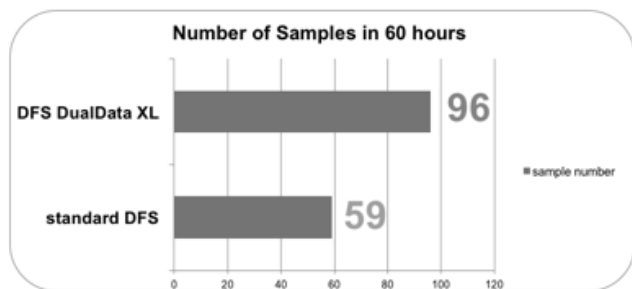


RESTEK

- Moving from He to H₂
- H₂ has faster optimal linear velocity
- Higher productivity (52%), same LOQs
- Reactive reducing gas...
- Possible dechloration (<5%) (effect on TEQ...)
- Reduction of analyte response (compensated by better ion source on the QQQ...)
- To be compared to He Dual Data...



60 h weekend sequence (Friday evening to Monday morning)



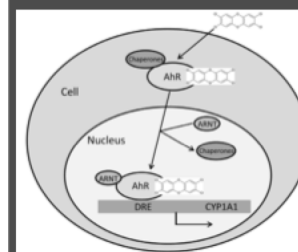
- Standard dual GC DFS GC-HRMS: Number of measured samples: 59 (Run Time: 60 h)
- DFS with Dual Data XL: Number of measured samples: 96 (Run Time: 59.5 h)
Additional measured samples: 37
Increase in efficiency 63%

Efficiency could be increased up to 88% (111 samples) by further method optimization

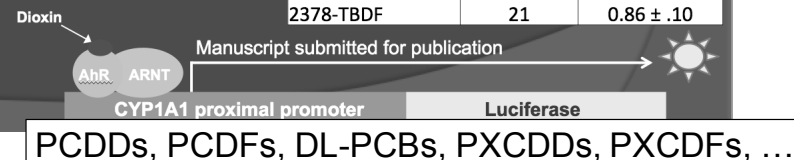
ThermoFisher
SCIENTIFIC

CALUX ??

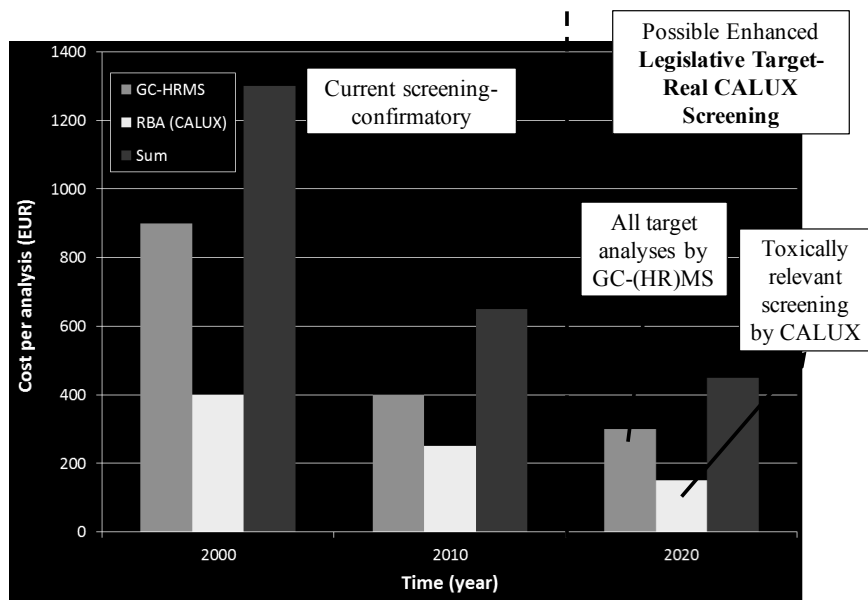
Potency studies in human liver cells



Compound	Number of replicates	Potency
2378-TCDD	21	1.00
2Br, 378Cl DD	18	0.99 ± .13
23Br, 78Cl DD	19	0.84 ± .10
2378-TBDD	17	0.77 ± .14
2378-TCDF	21	0.83 ± .07
3Br, 278Cl DF	20	0.97 ± .12
23Br, 78Cl DF	20	1.02 ± .15
2378-TBDF	21	0.86 ± .10




The Early 2000's Scenario is not True Anymore...



Aiming for the Platinum level

Gold Karats	Routine Dioxins	Exploratory Dioxins	Sensitivity	Mass Resolution	Comprehensiveness
Sector MS	24	24	22	20	16
QQQ MS/MS	20	18	20	14	16
APGC-TQXS	20	24	24	16	18
GCxGC-HRTOFMS	16	22	16	22	24
GC-Orbitrap	20	20	20	22	22
GC-TQFTICRMS	14	18	16	22	22



- ✓ One came a long way...
- ✓ Still lots to do
- ✓ The perfect MS analyser is yet to be found
- ✓ We can revisit our early 2000 approaches