State-of-the-art in POPs Analysis: Outcomes of the DIFFERENCE and DIAC projects

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WAGENINGEN UR

RIVO

Dioxins in Food and Feed-Reference Methods and New Certified Reference Materials "*DIFFERENCE*"

Dioxin Analysis by Comprehensive Multi-Dimensional Gas Chromatography (GCxGC) "*DIAC*"

COMPETITIVE AND SUSTAINABLE GROWTH (GROWTH) PROGRAMME

DIFFERENCE

DIAC

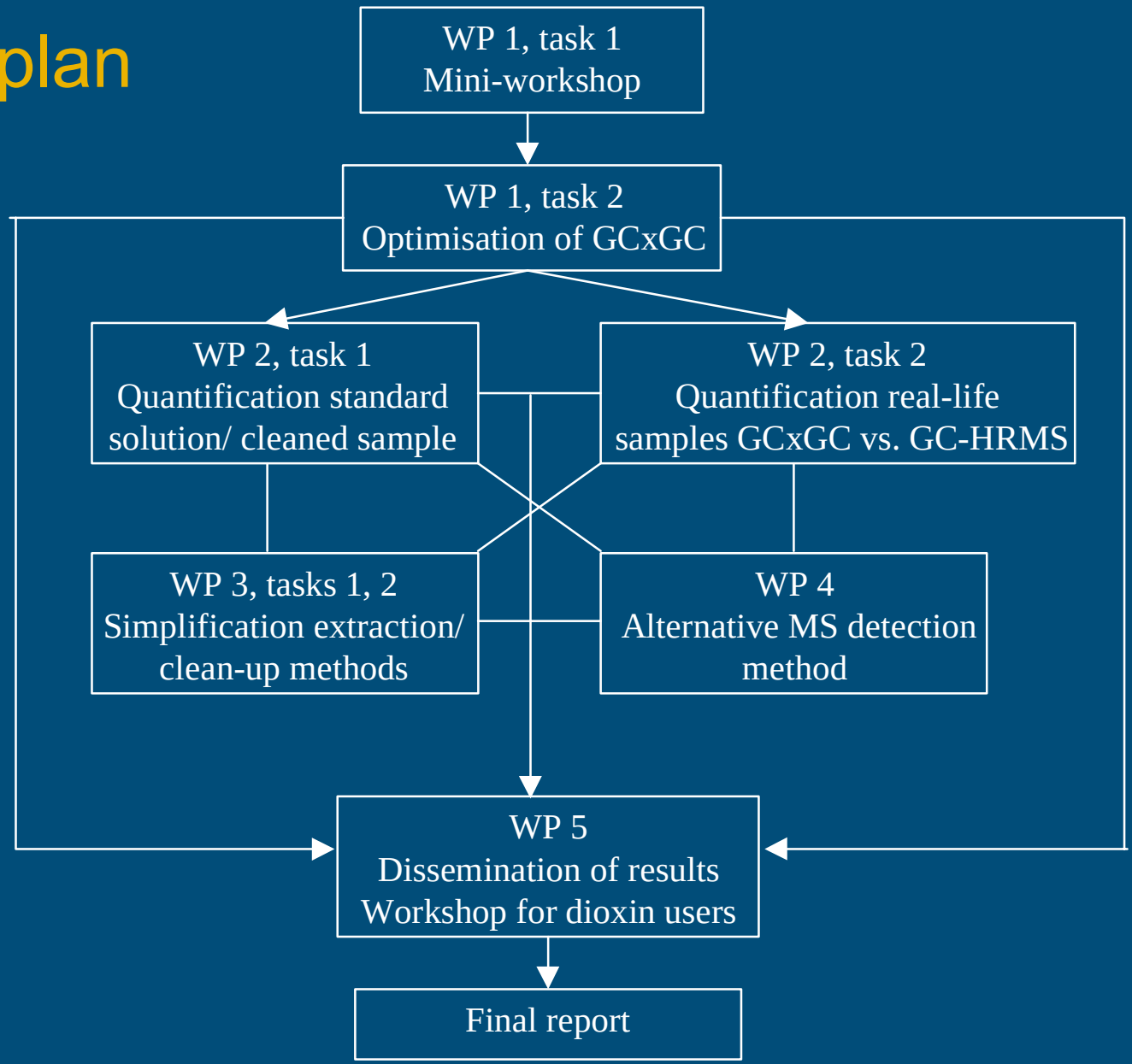
Background and Societal Needs

- Belgian chicken incident 1999
- Need for dioxin analysis capacity
- Need for cheap and reliable screening and confirmatory methods
- New EU MRLs, 1 July 2002

Objectives DIAC

- Optimisation of GCxGC-ECD system for dioxin analysis
- Selection of ‘best’ modulator
- Optimisation of quantification comparison with HRMS
- Test of alternative detection method: ToF-MS
- Simplification of extraction and clean-up

DIAC Workplan



DIFFERENCE

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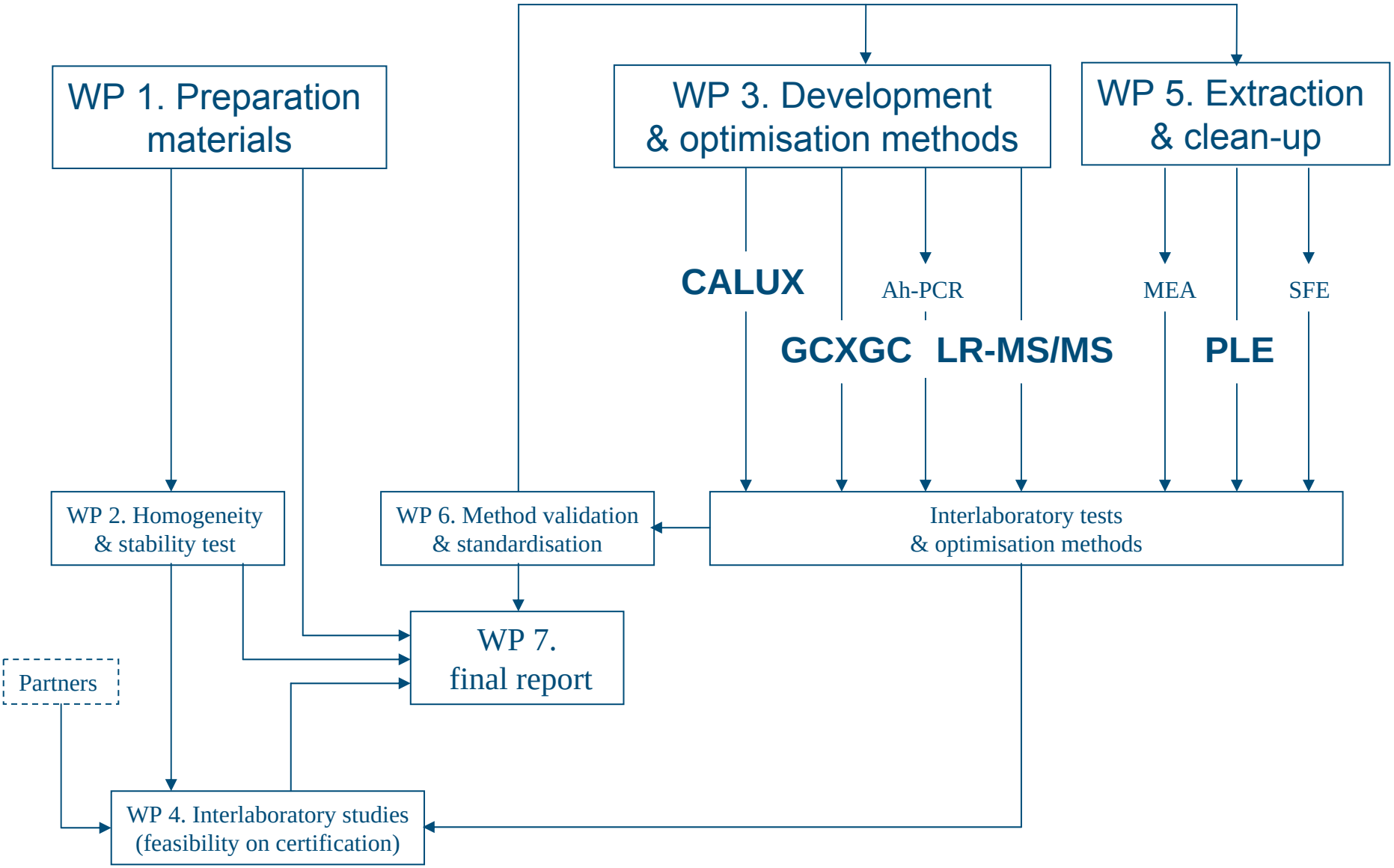
DIFFERENCE Objectives

- Selection of relevant food and feed matrices
- Preparation of candidate CRMs
- Feasibility of certification
- Optimisation of bio-analytical and chemical methods for dioxin analysis
- Validation and standardisation
- Optimisation of extraction and clean-up
- Standardised protocols for use in Europe

DIFFERENCE Workplan



WHO Dioxins, Furans and dioxin-like PCBs

PCDDs	PCDFs	Dioxin-like PCBs (+IUPAC nos.)
2,3,7,8-TCDD	2,3,7,8-TCDF	3,3',4,4'-TCB (77)
1,2,3,7,8-PcCDD	1,2,3,7,8-PcCDF	3,4,4',5-TCB (81)
1,2,3,4,7,8-HxCDD	2,3,4,7,8-PcCDF	3,3',4,4',5 -PeCB (126)
1,2,3,6,7,8-HxCDD	1,2,3,4,7,8-HxCDF	3,3',4,4',5,5'-HxCB (169)
1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDF	2,3,3',4,4'-PeCB (105)
1,2,3,4,6,7,8-HpCDD	2,3,4,6,7,8-HxCDF	2,3,4,4',5-PeCB (114)
OCDD	1,2,3,7,8,9-HxCDF	2,3',4,4',5-PeCB (118)
	1,2,3,4,6,7,8-HpCDF	2',3,4,4',5-PeCB (123)
	1,2,3,4,7,8,9-HpCDF	2,3,3'4,4',5-HxCB (156)
	OCDF	2,3,3',4,4',5'-HxCB (157)
		2,3',4,4',5,5'-HxCB (167)
		2,3,3',4,4',5,5'-HpCB (189)

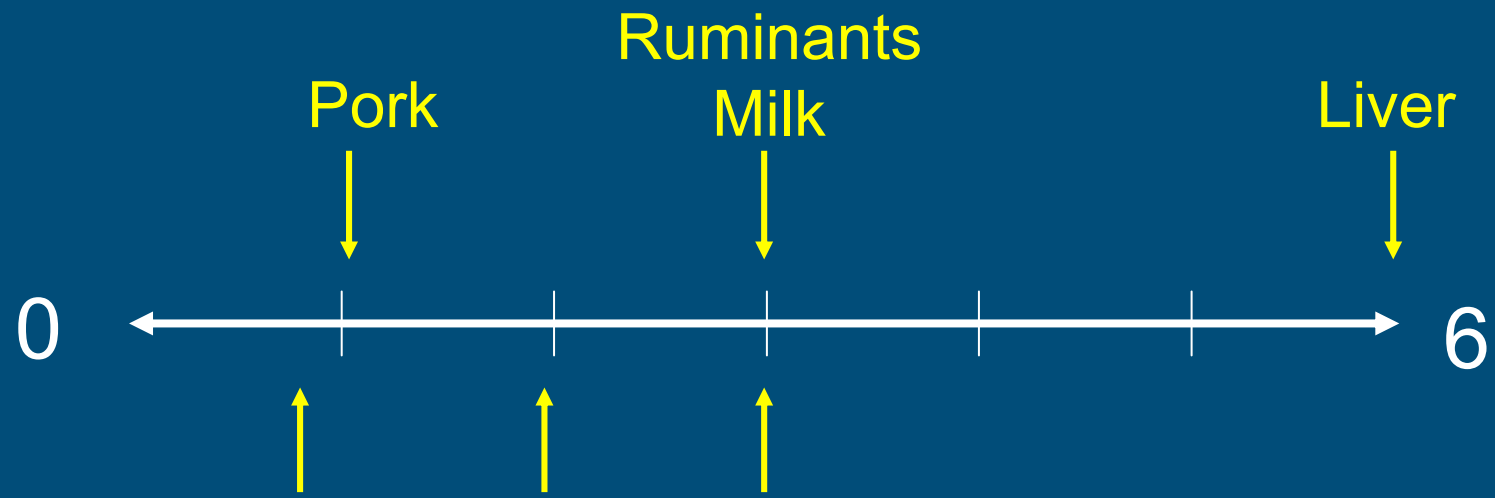
EU Requirements for Dioxin and dl- PCB Analysis

	Screening Methods	Confirmatory Methods
False Negative Rate	<1%	
Trueness		-20 to +20%
CV	<30%	<15%

Quality Criteria (2002/69/EC)

Performance of a method	1 – 8 pg TEQ
LOQ (confirmatory method): range 1/5 level of interest	LOQ at 1pgTEQ/g fat
High sensitivity and low limits of detection	Up to 1pgTEQ
High selectivity (specificity)	Interferences PCN, PCB, PCDE
High accuracy (trueness and precision)	r&R

EU: sensitivity requirements for food (pg diox./g fat)



Fish: 4 pg dioxins/g product

GCxGC studies

- GCxGC-ECD
- GCxGC-ToF-MS
- Various first and second column combinations
- Comparison five “modulator” types

RIVO, The Netherlands
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Conny Danielsson, Peter Haglund, Mikael Harju, Karin Wiberg

Free University, The Netherlands
Udo Brinkman, Maria Kristenson, René Vreuls

University of Bordeaux, France
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IQS, Barcelona, Spain
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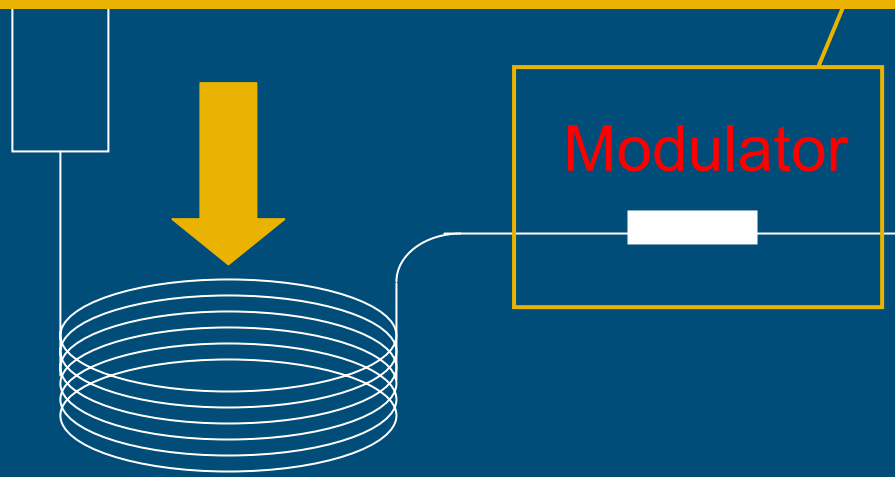
Principles of GCxGC

Selection of proper column combination



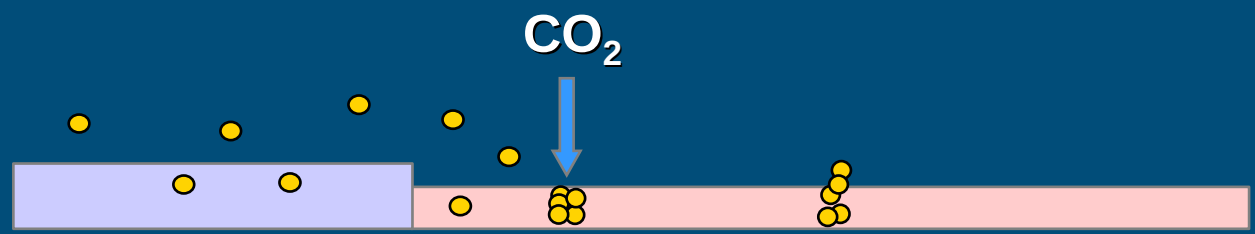
Selection of modulator

- 5 different modulators:
  - SV [illegible]EPER
  - LM[illegible]S
  - Quad N_2(l) jet
  - Dual CO_2 jet
  - Loop CO_2

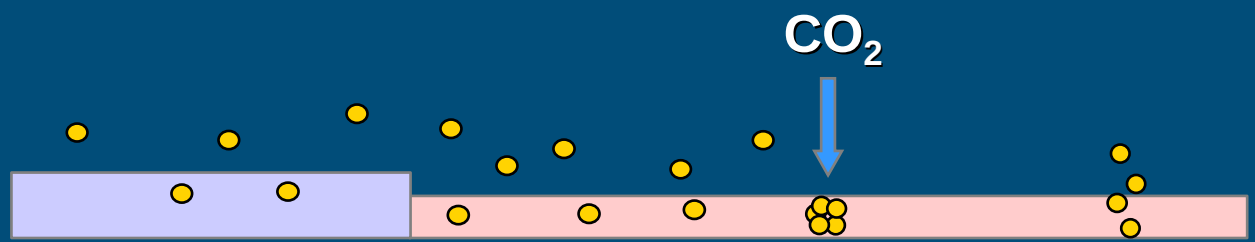
Cryogenic modulation

1. Trapping

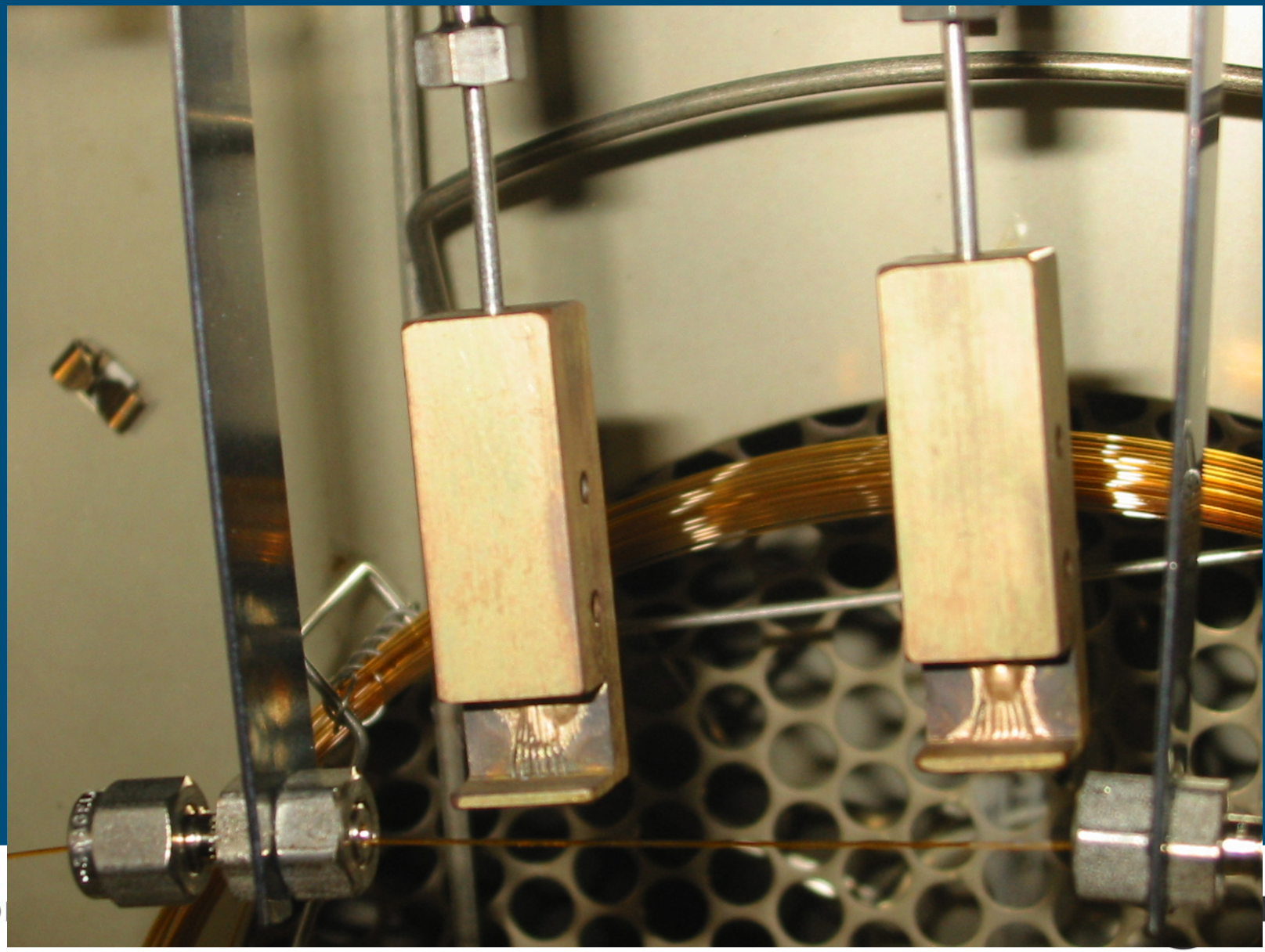
2. Release



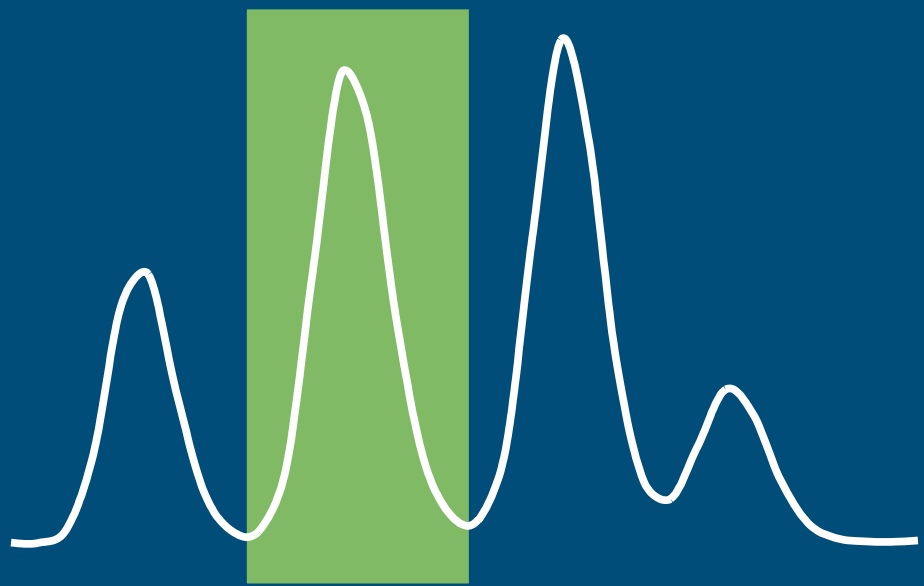
3. Trapping and Separation



Cryogenic modulator



How does GCxGC work ?



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How does GCxGC work ?

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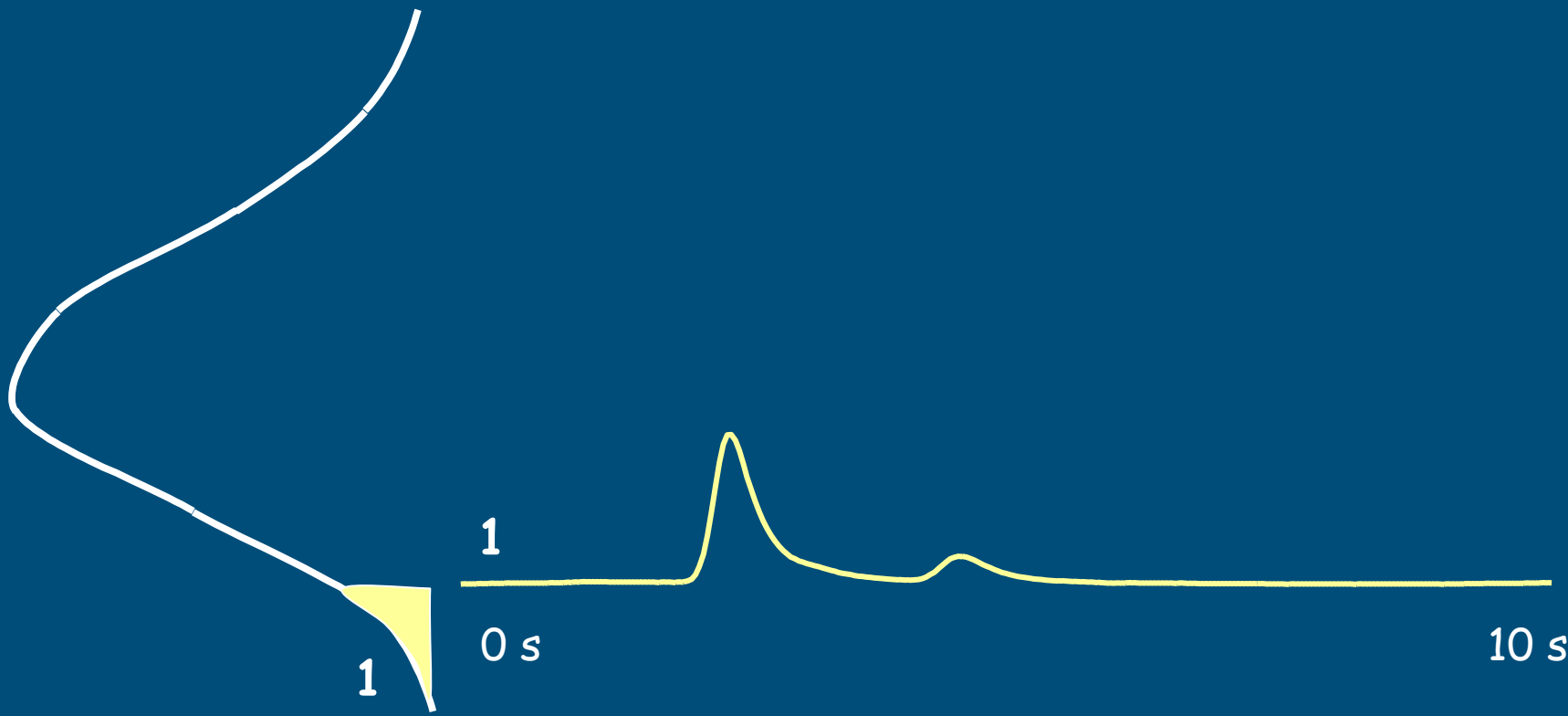

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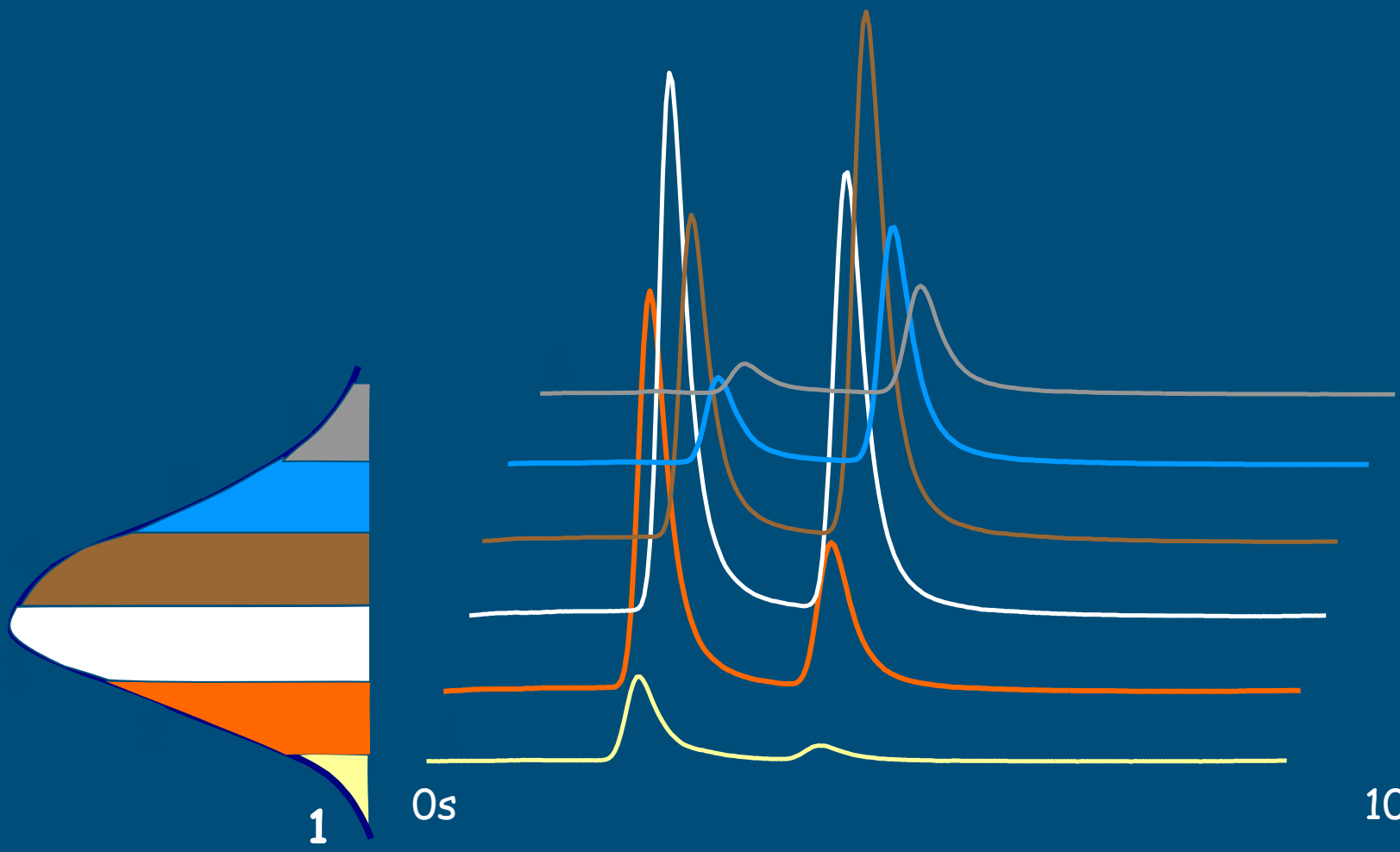
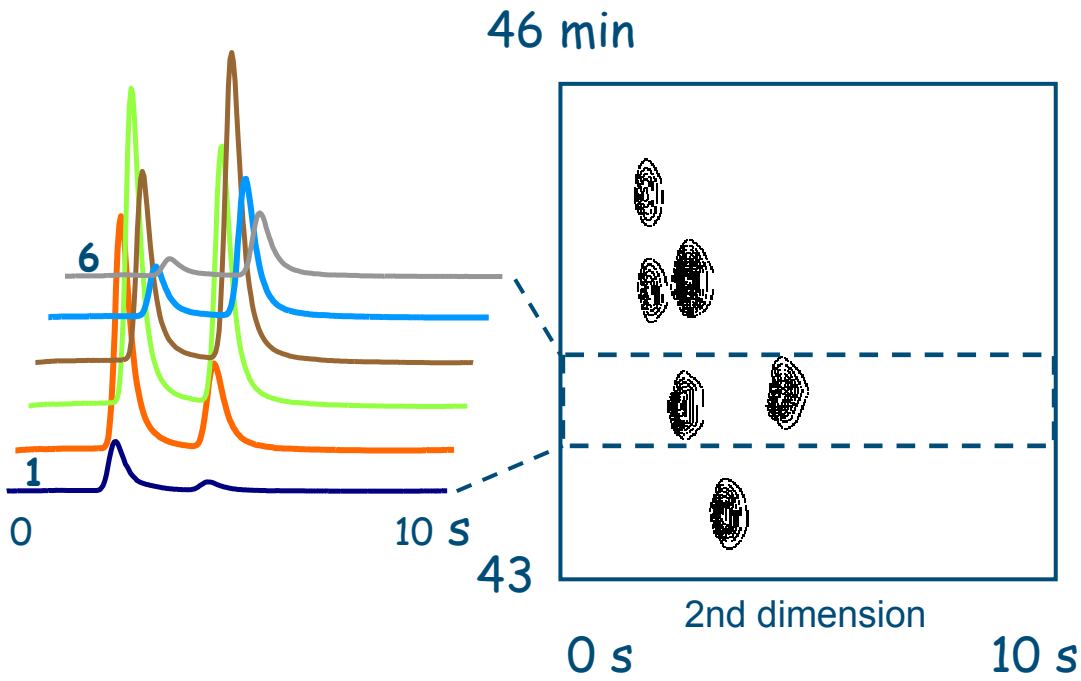
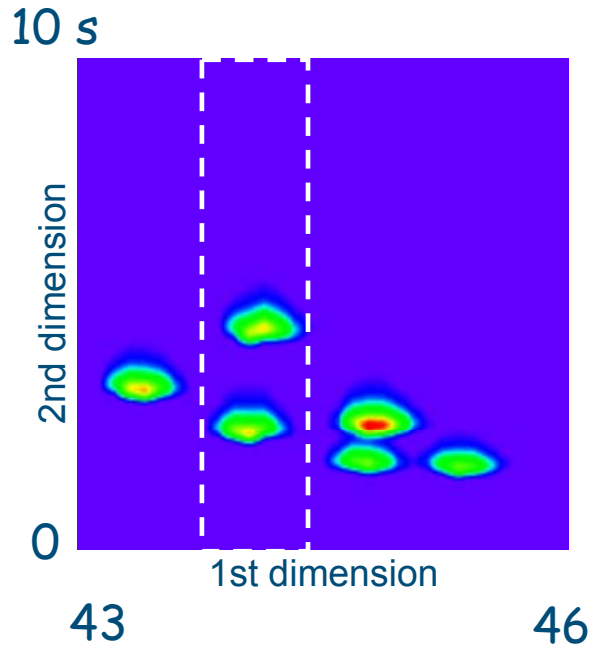
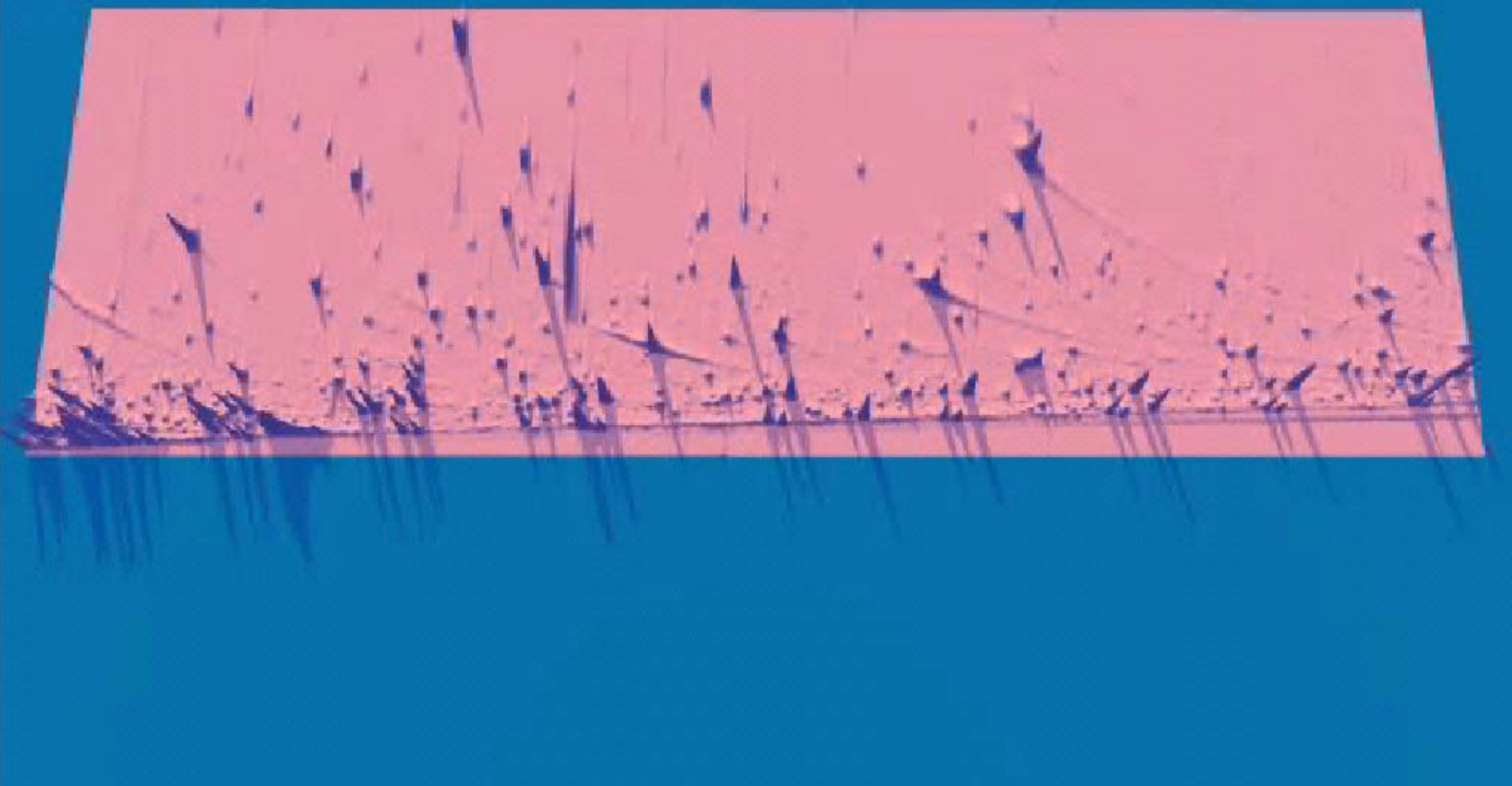


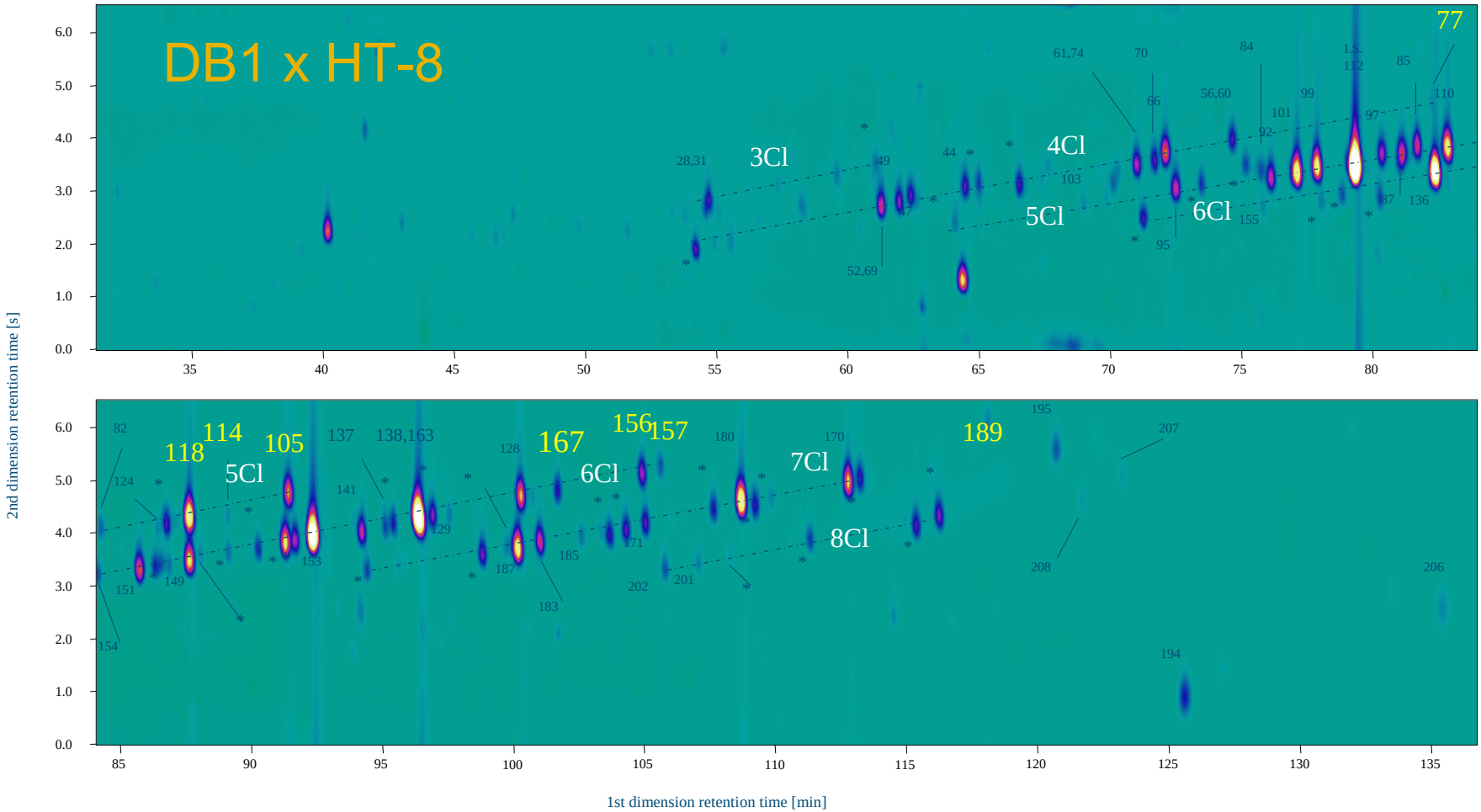
2D plots


46 min
6
1
0
10 s
43
2nd dimension
0 s
10 s

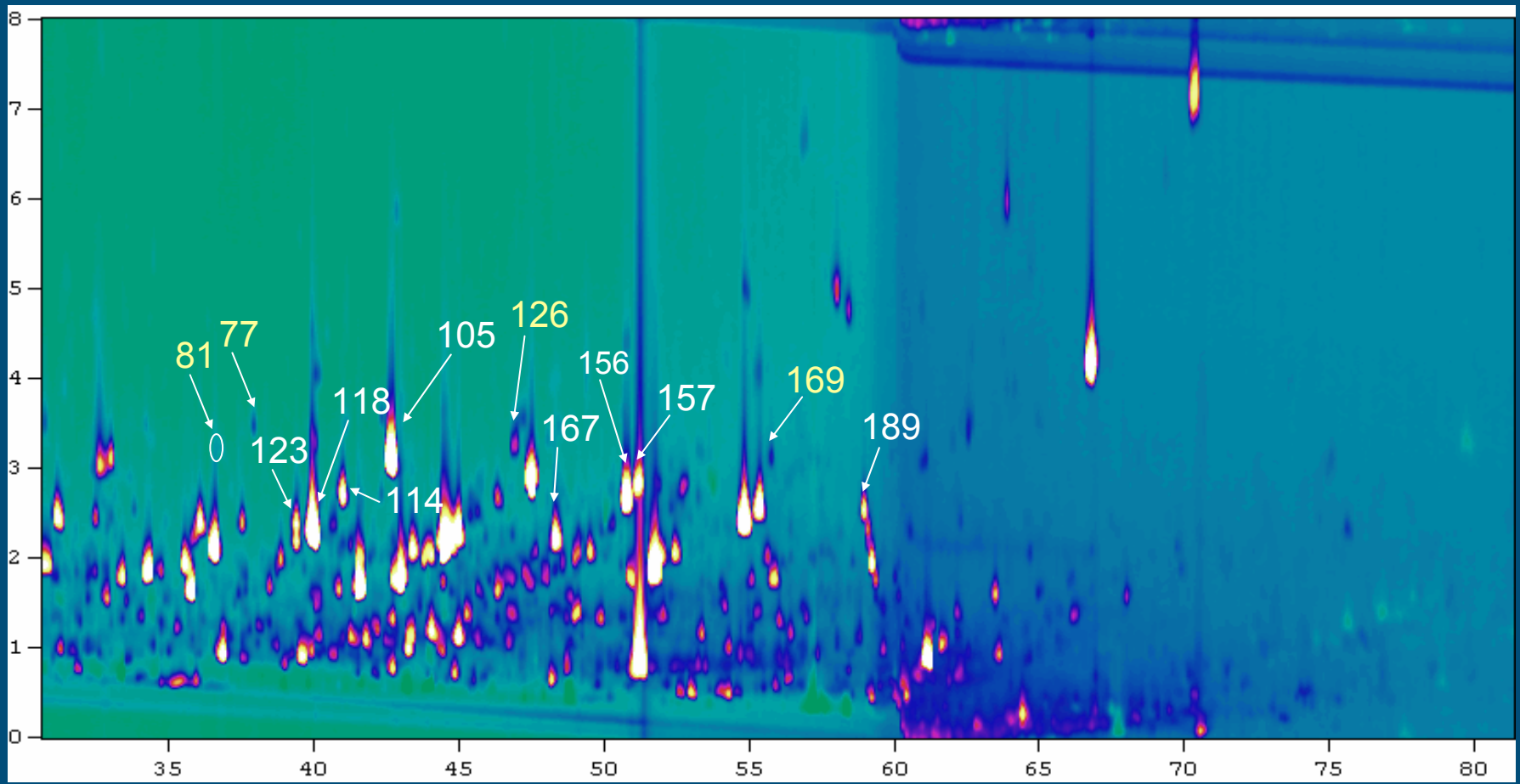

10 s
2nd dimension
0
1st dimension
43
46



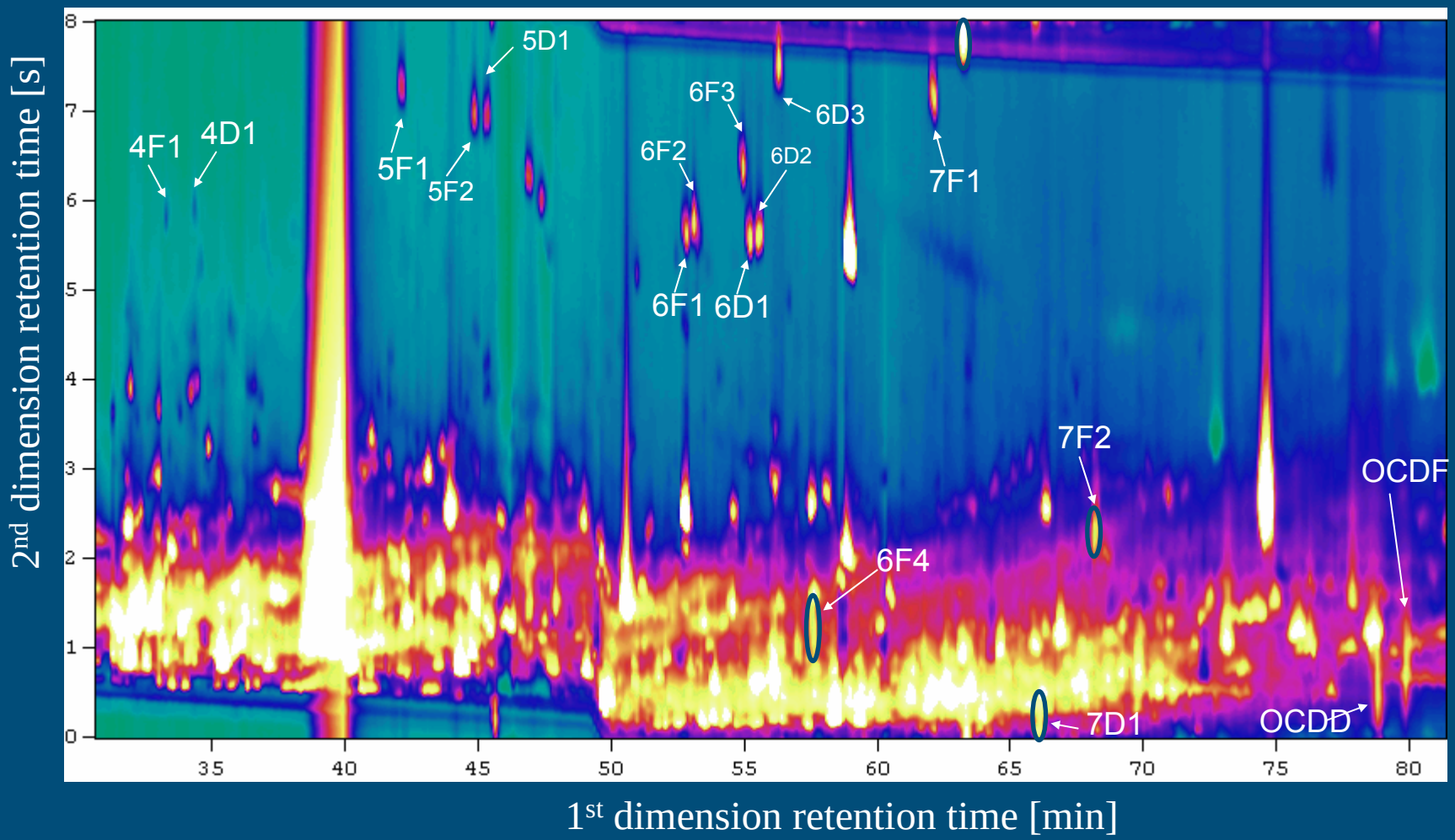
Cod liver: WHO-PCB separation



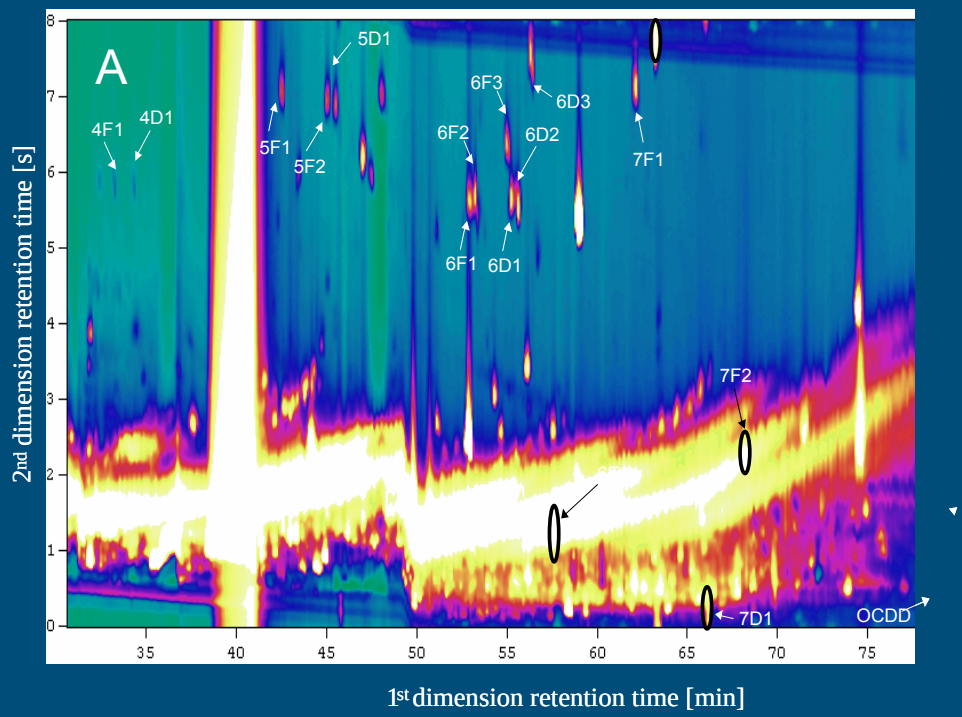
Milk: PCB fraction (DB-XLB x LC-50)

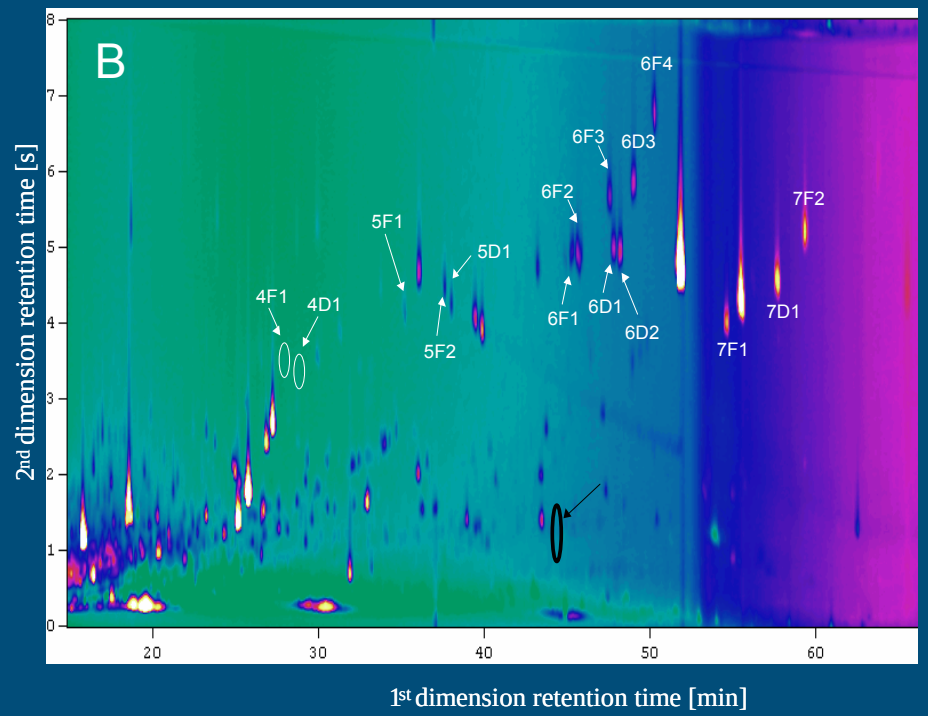


Milk: Dioxins (DB-XLB x LC-50)

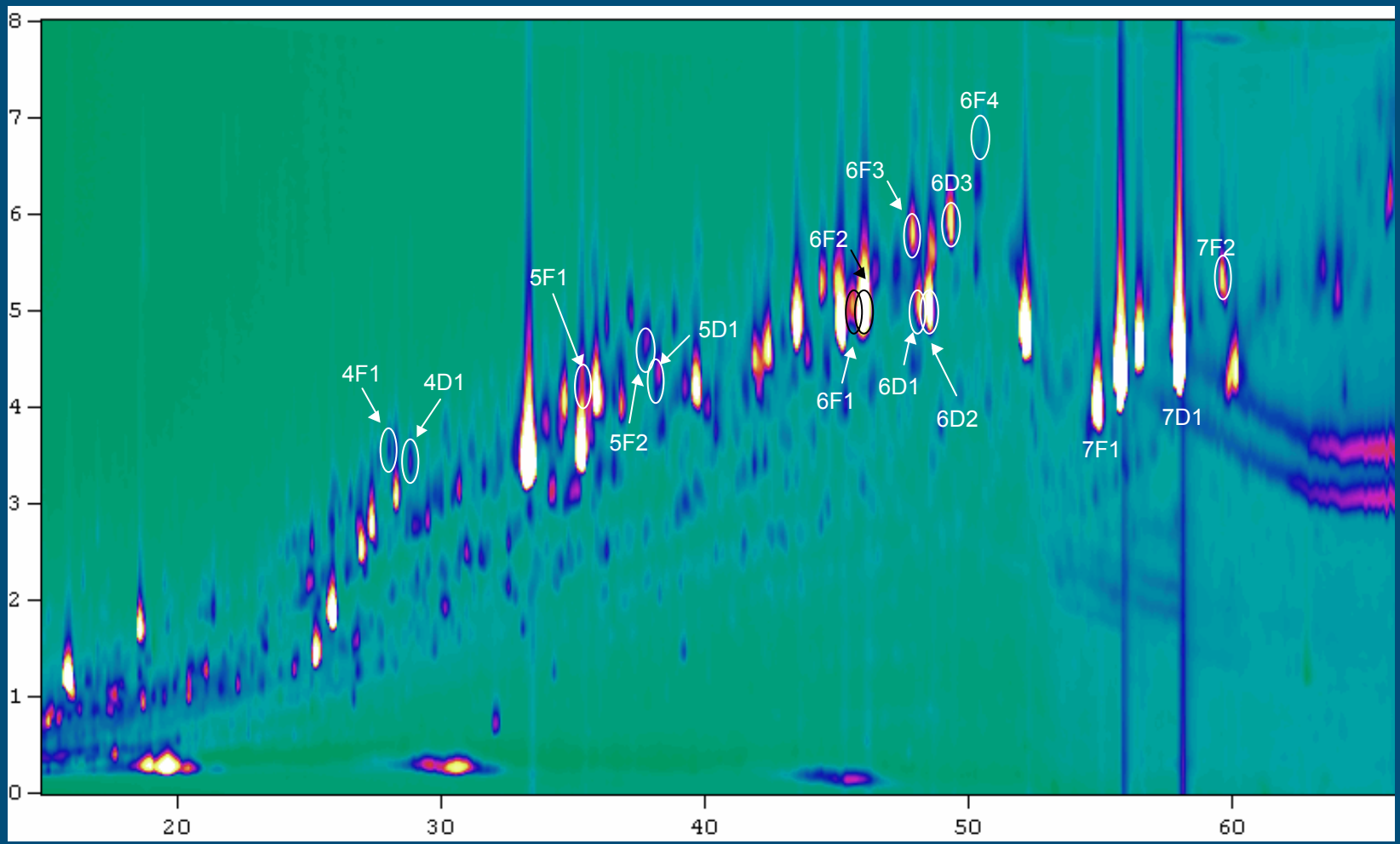


Improved clean-up and solvent grade





Sewage sludge with improved clean-up

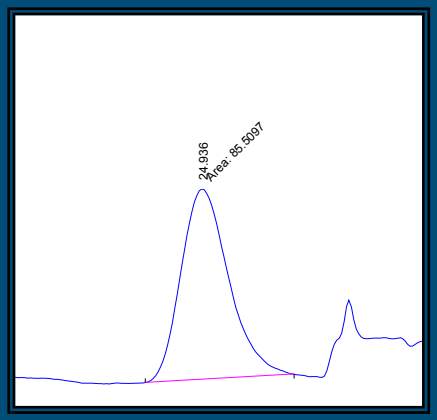


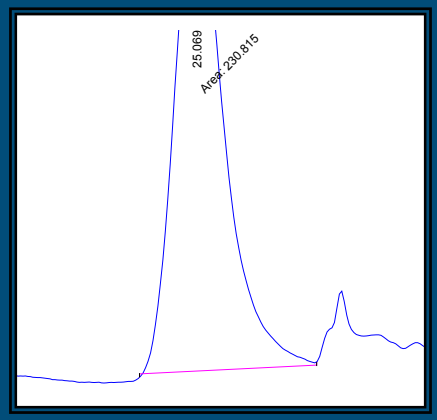
Modulator comparison and column selection

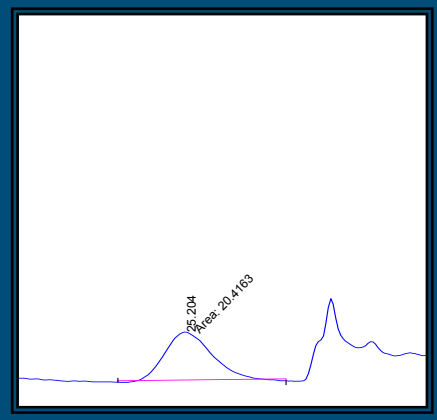
- Cryogenic modulation with CO_2 had best performance
- Most suitable column combinations:
  - DB-XLB x LC50
  - DB-1 x 90% cyanopropyl
  - HT5 x BPX 50
- Other phases less suitable because of:
  - high background levels (bleeding of column)
  - not all critical congener pairs (with different TEF values) could be separated from each other

Integration and identification example: 23478-PeCDF

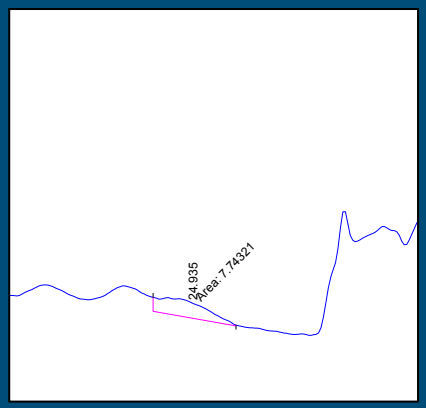
Standard

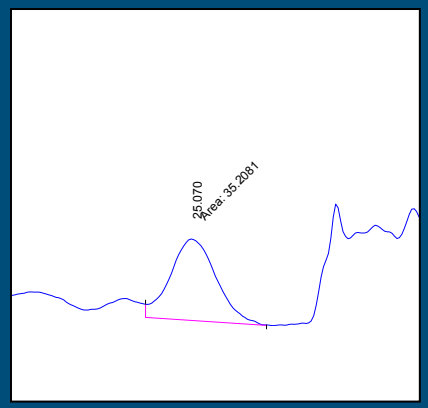


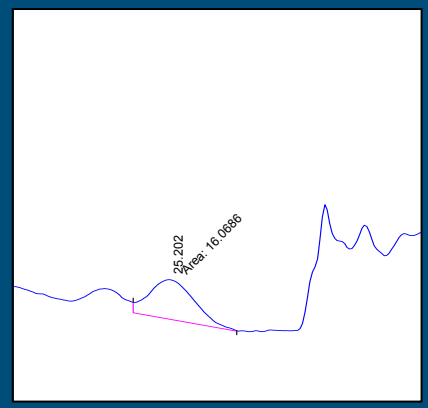




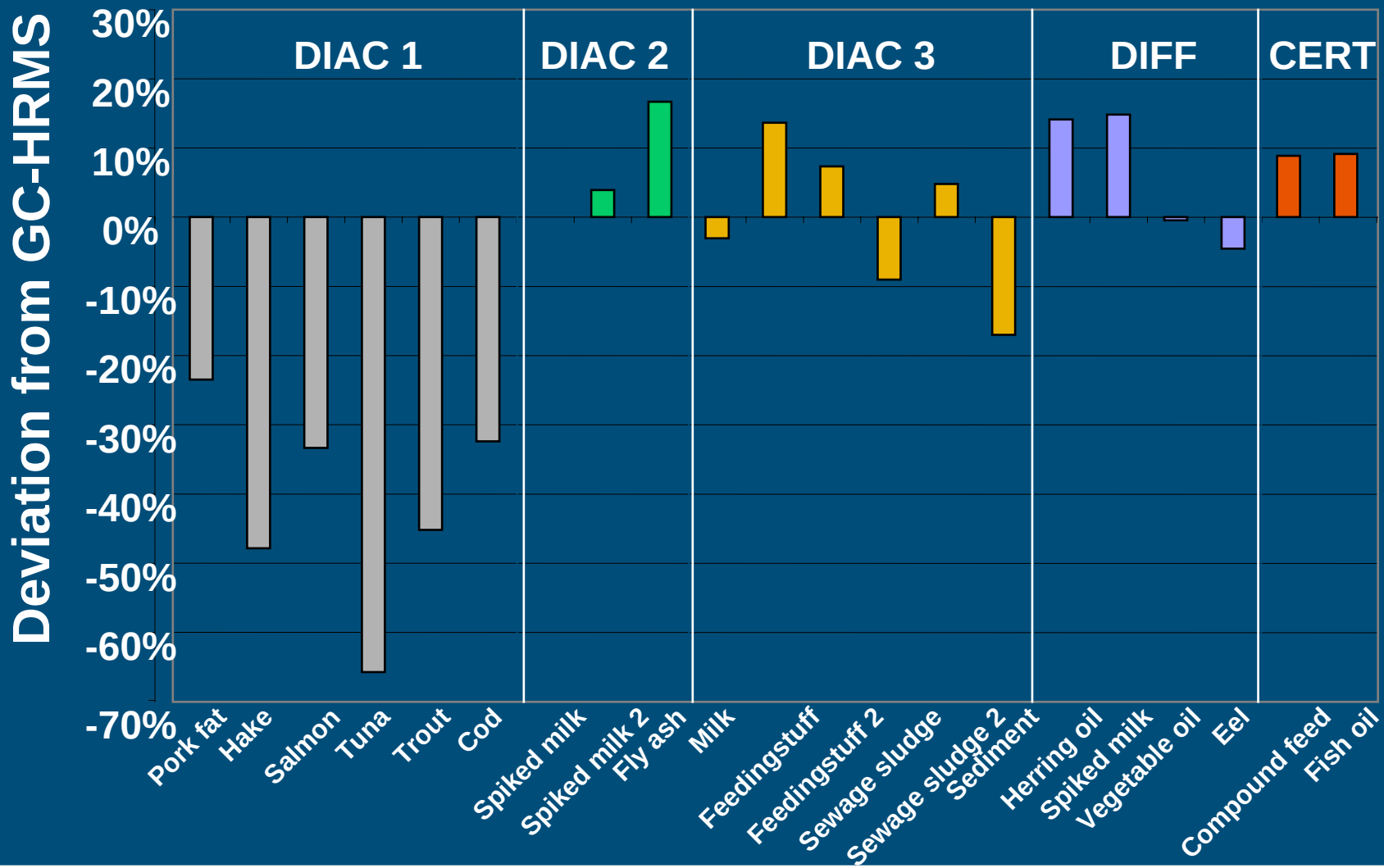
Sample







Accuracy



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Conclusions GCxGC-ECD

- High selectivity
- High sensitivity but:
  - Multi clean-up/fractionation steps are needed
- Integration of peaks is time-consuming
  - various retention time markers in GCxGC plane
- Improved software requirement

GC-LRMS/MS

- GC-ITMS/MS (GCQ/Polaris)
- MS/MS mode
- Electron impact (EI)

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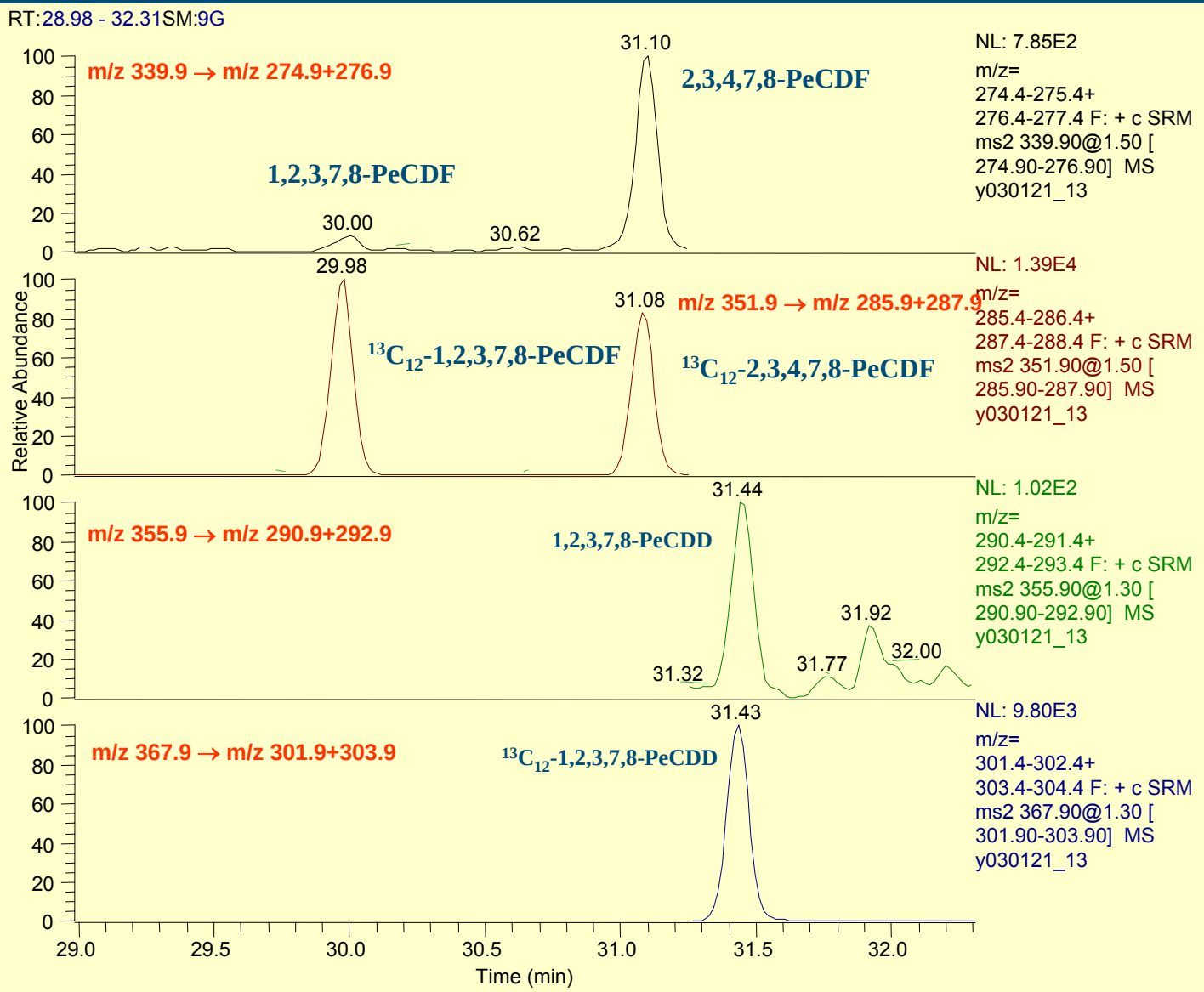
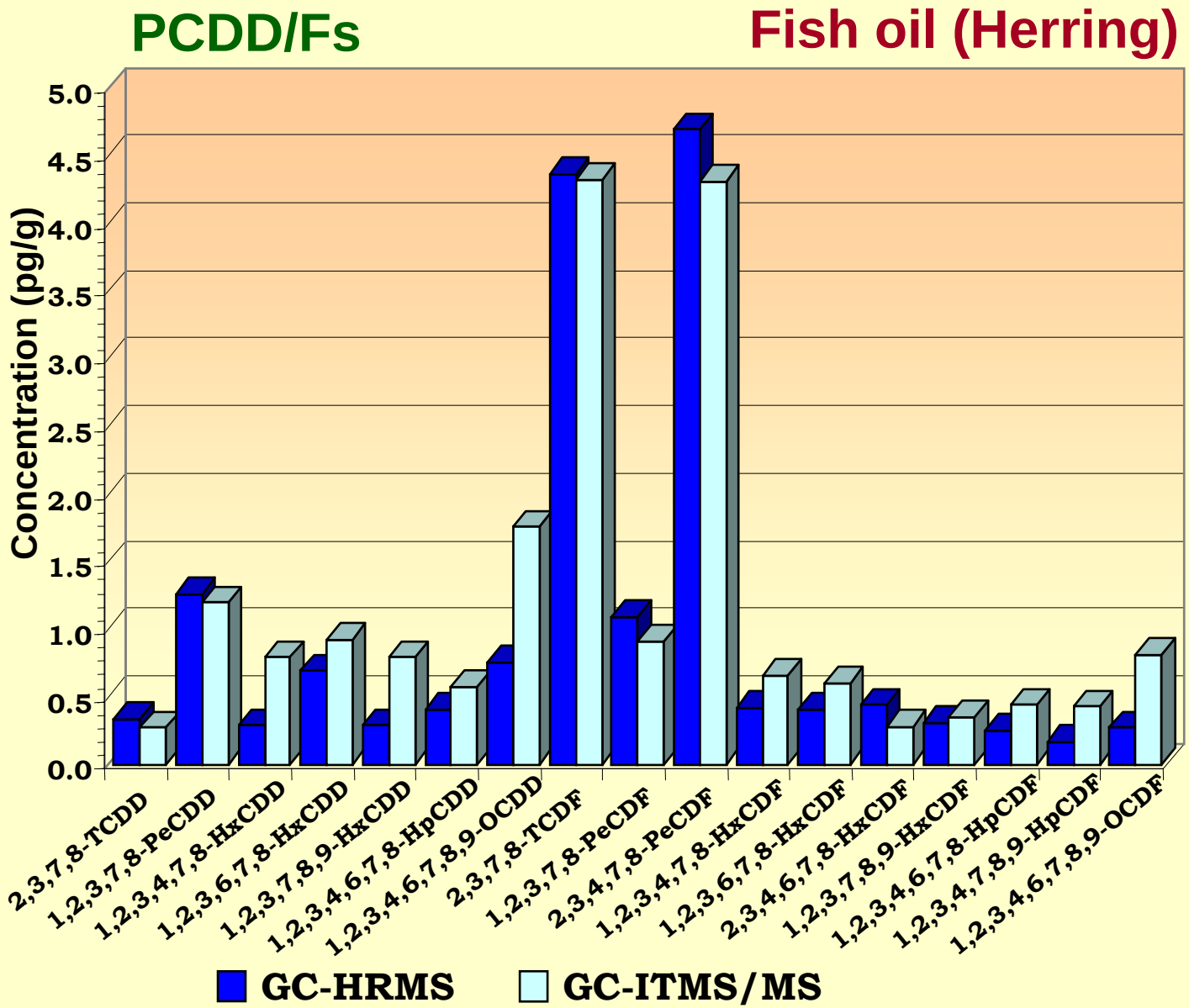
Clean Fish Extract: Dioxins

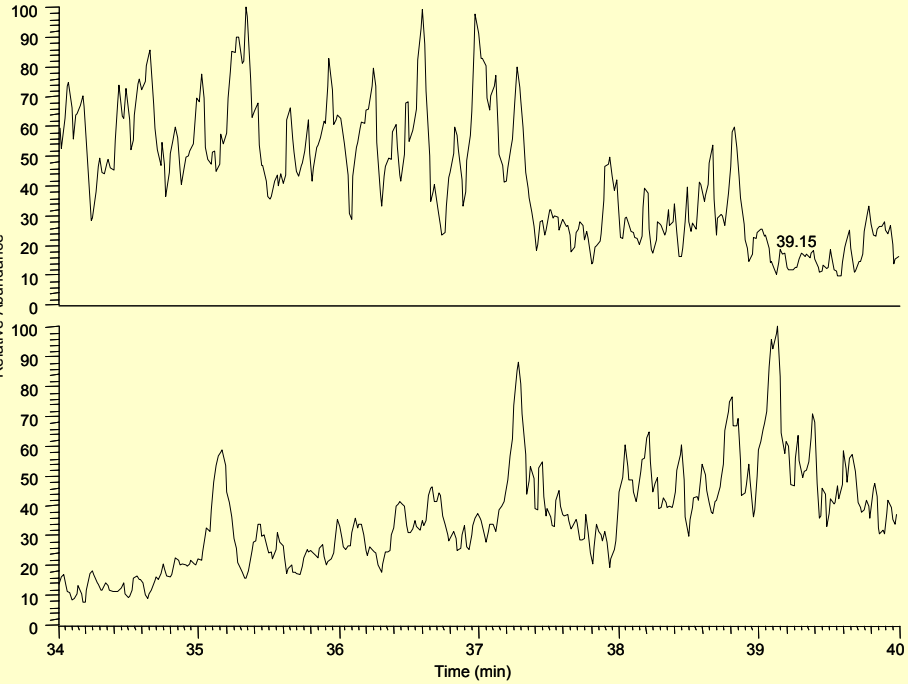


Fish oil: HRMS vs. LRMS/MS

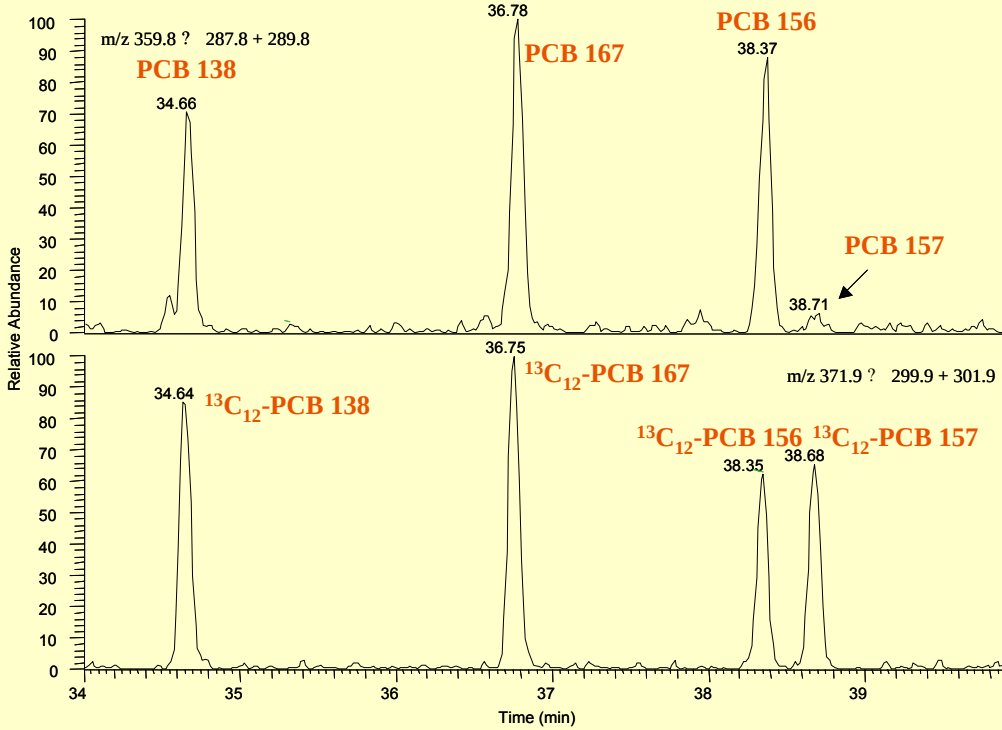


Problems with clean-up

Insufficient clean-up



Suitable clean-up



Hexa-mono-ortho-PCBs

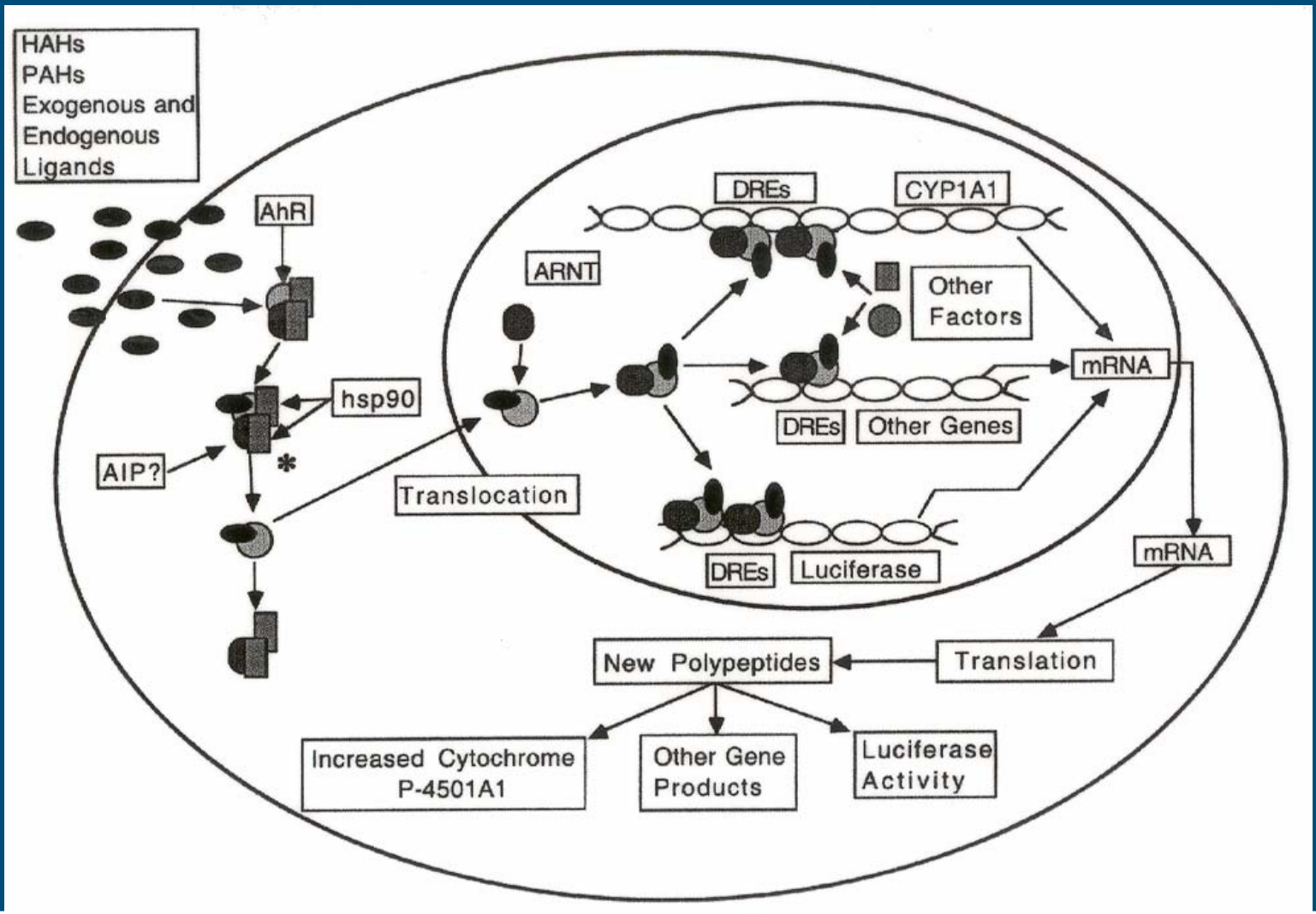
Conclusions GC-ITMS/MS

- Low detection limits and high selectivity
- Appropriate clean-up and fractionation method needed
- Further studies needed in order to prove the general applicability of the GC-ITMS/MS for the analysis of PCDD/Fs and dioxin-like PCBs

DR-CALUX studies

- *Scientific Institute of Public Health,* Beernaert H., Carbonnelle S., Hanot V., Hellebosch L., Roos P., Van Loco J., Van Overmeire I., Van Wouwe N. and I. Windal
- *Vrije Universiteit Brussel,* Baeyens W., Sanctorum H. and C. Schroijen
- *Université de Liège,* De Pauw E., Eppe G. and M. Scippo
- *Federal Agency for Safety of the Food Chain,* Behets S., Fontaine A. and H. Vanderperren
- *Flemish Institute for Technological Research* Koppen G., Schoeters G. and R. Van Cleuvenbergen
- *RIKILT – Institute of Food Safety,* Bovee T., Hoogenboom R. and W. Traag
- *Xenobiotic Detection Systems,* Brown D., Chu M., Clark G. and Gordon J.

DR-CALUX assay



CALUX activity

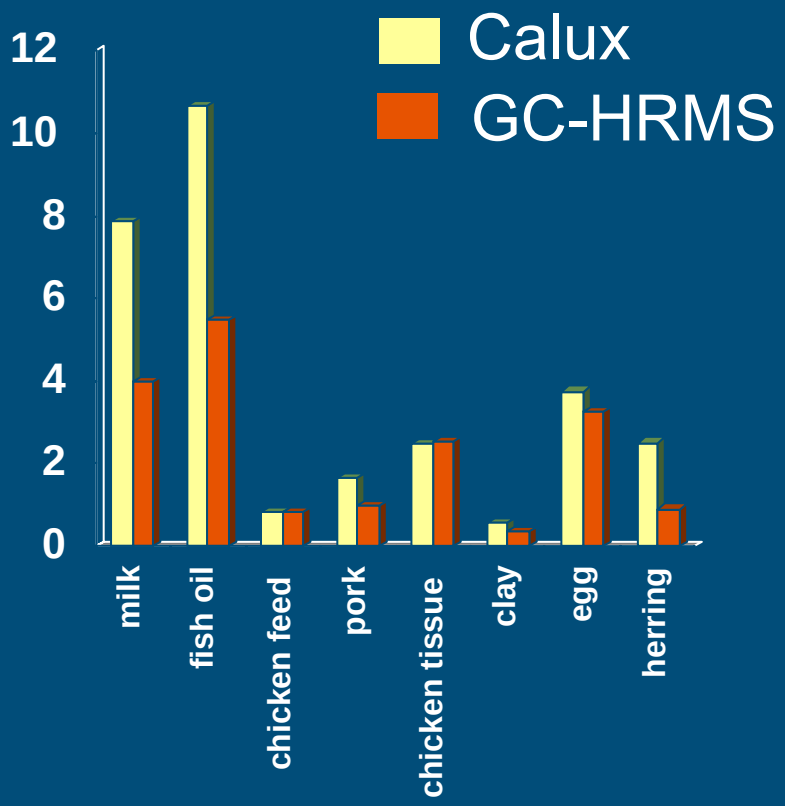
- Cells respond to all compounds of the sample extract that activate the AhR (dioxin-like activity)

both the solvent and the sample contaminants

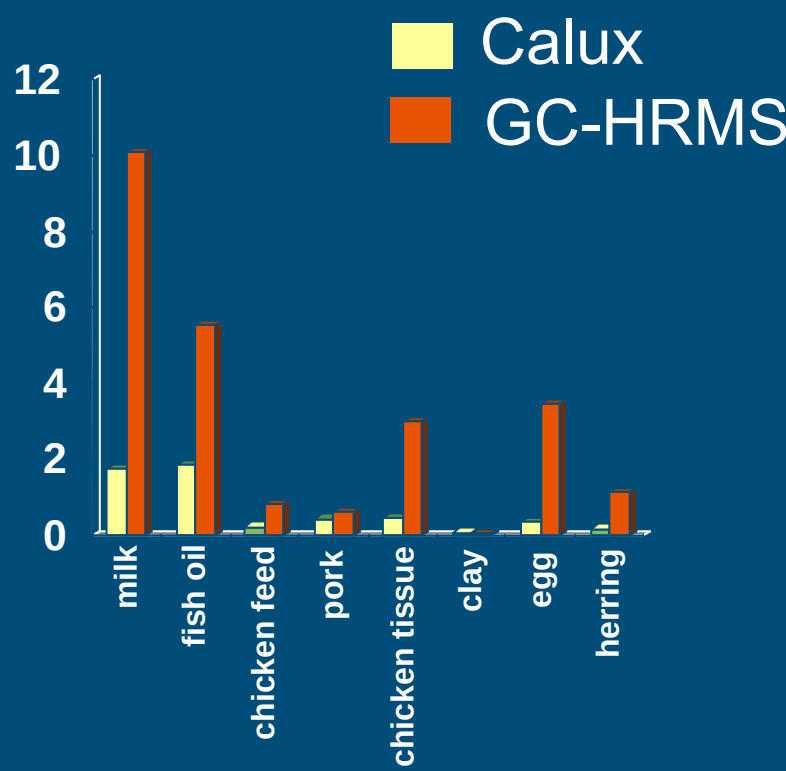
- Total dioxin-like activity (Total TEQ of a sample)
- Separation of dioxins and PCBs from many other compounds

Accuracy of CALUX results

DIOXIN fraction



PCB fraction



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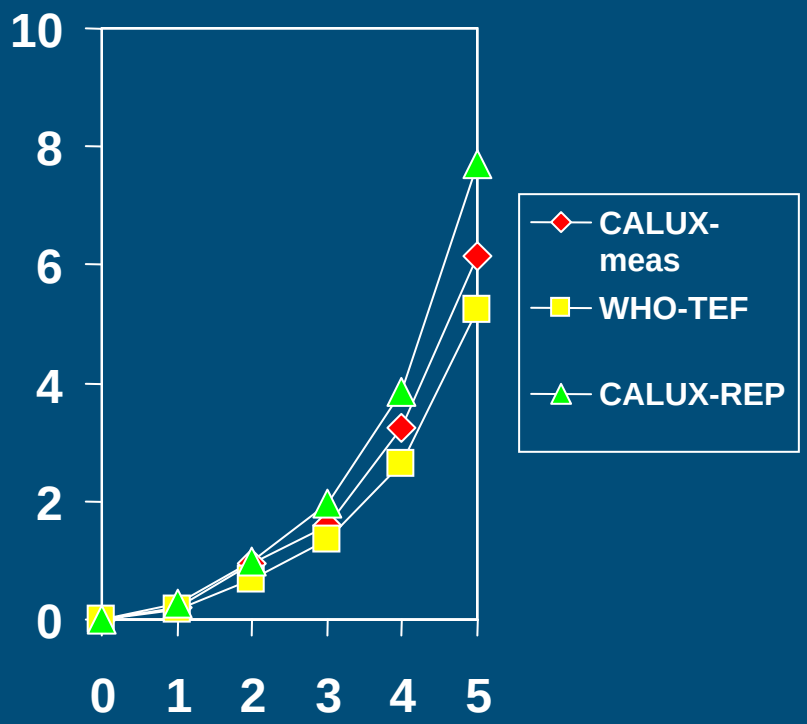
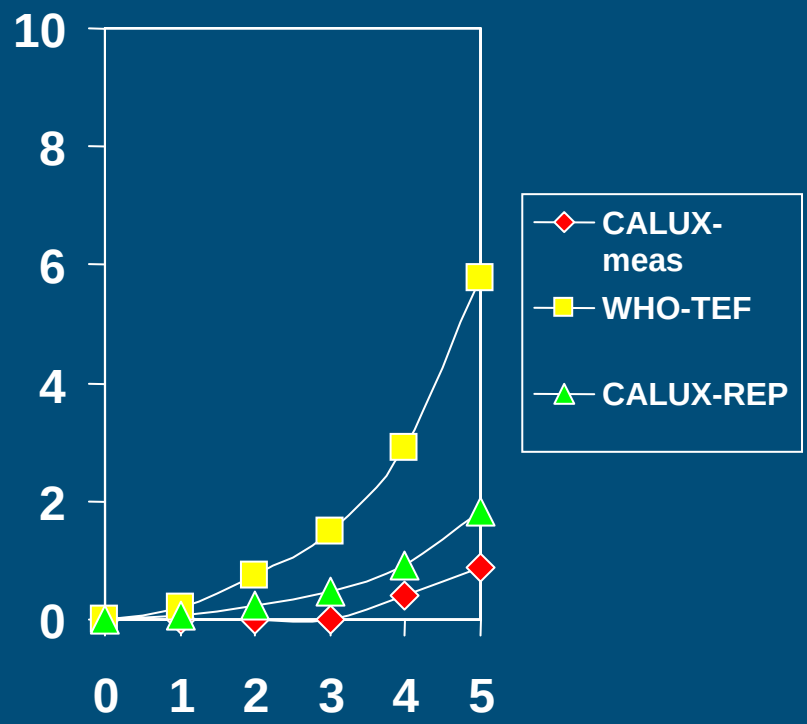
REP ≠ TEF

DIOXIN fraction



PCB fraction



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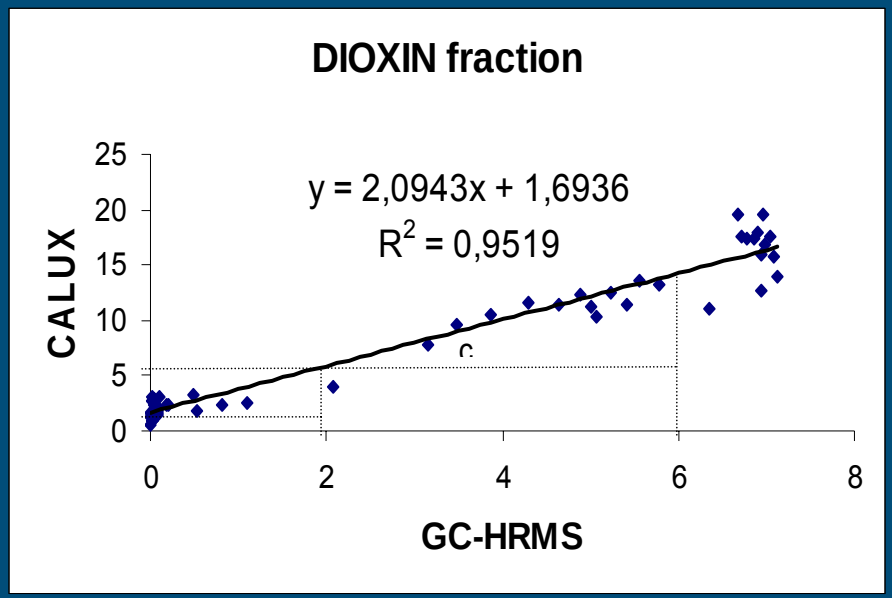
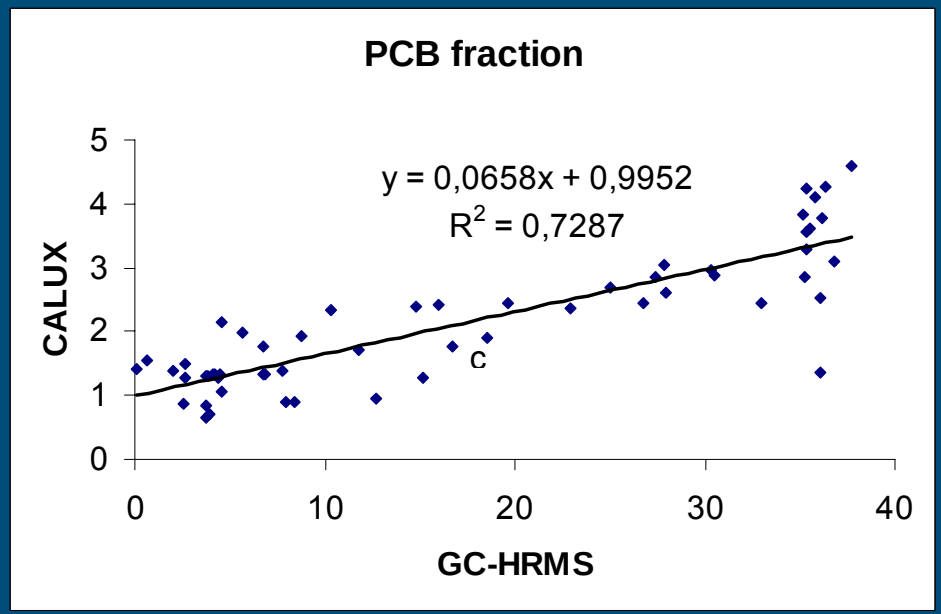
DR-Calux vs GC-HRMS in fish oil





Conclusions DR-CALUX

- High sensitivity
- Accuracy is lower
- Fast and cheap method
- Screening method

Pressurized liquid extraction (PLE)

- PLE extraction: Dionex ASE 200 and ASE 300
- Non-selective ASE with external clean-up
- Selective ASE with on-line clean-up
  - Sulphuric acid silica
  - Integrated carbon fractionation

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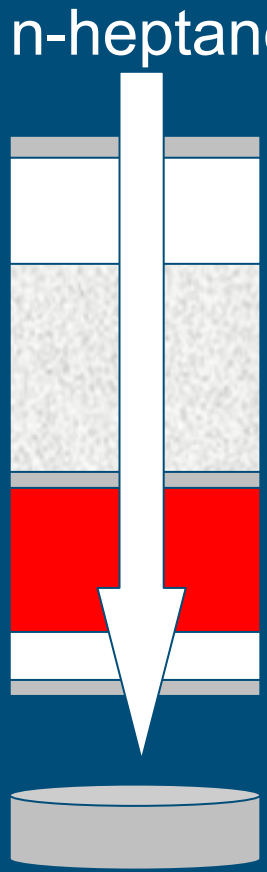
Geel
Belgium

Peter Haglund,
Umeå University
Umeå
Sweden

Selective PLE fat retainer



Björklund, Müller, von Holst, Anal. Chem. 2001, 73, 4050
*Sporring, Björklund, J. Chrom. A **2004**, 1040, 155*

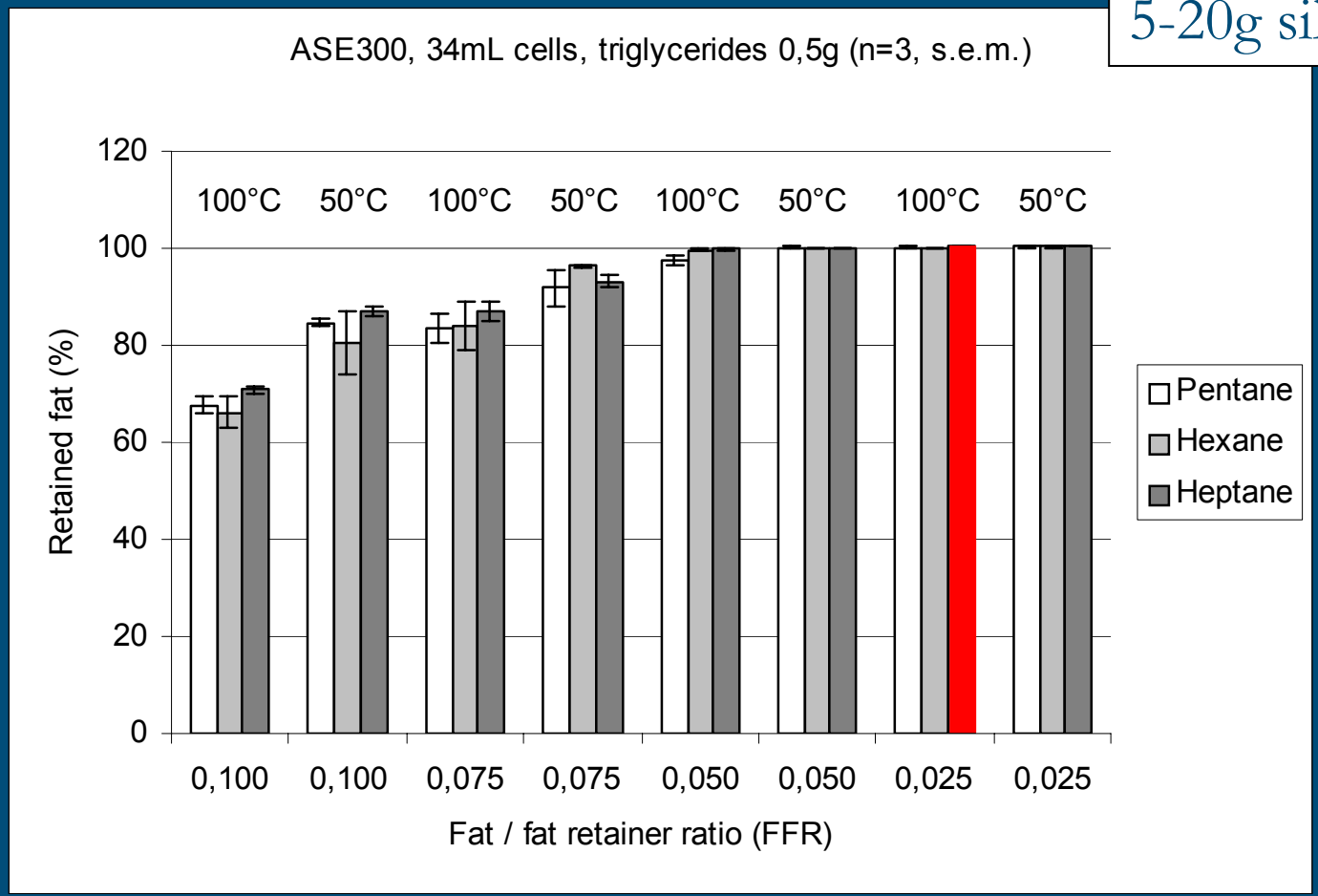
Fat retainers

- Ratio fat/fat retainer: 0.025
- Recovery about 100%
- Coextracted fat: 500 mg fat, 1-3 mg fat left
- Colour: clear florisil and sulphuric acid silica only
- Reaction with H2SO4: No reaction with sulphuric acid silica

Conclusion sulphuric acid silica preferred

Clean-up of fat using selective ASE

5-20g silica



Sporring, Björklund, J. Chrom. A 2004, 1040, 155

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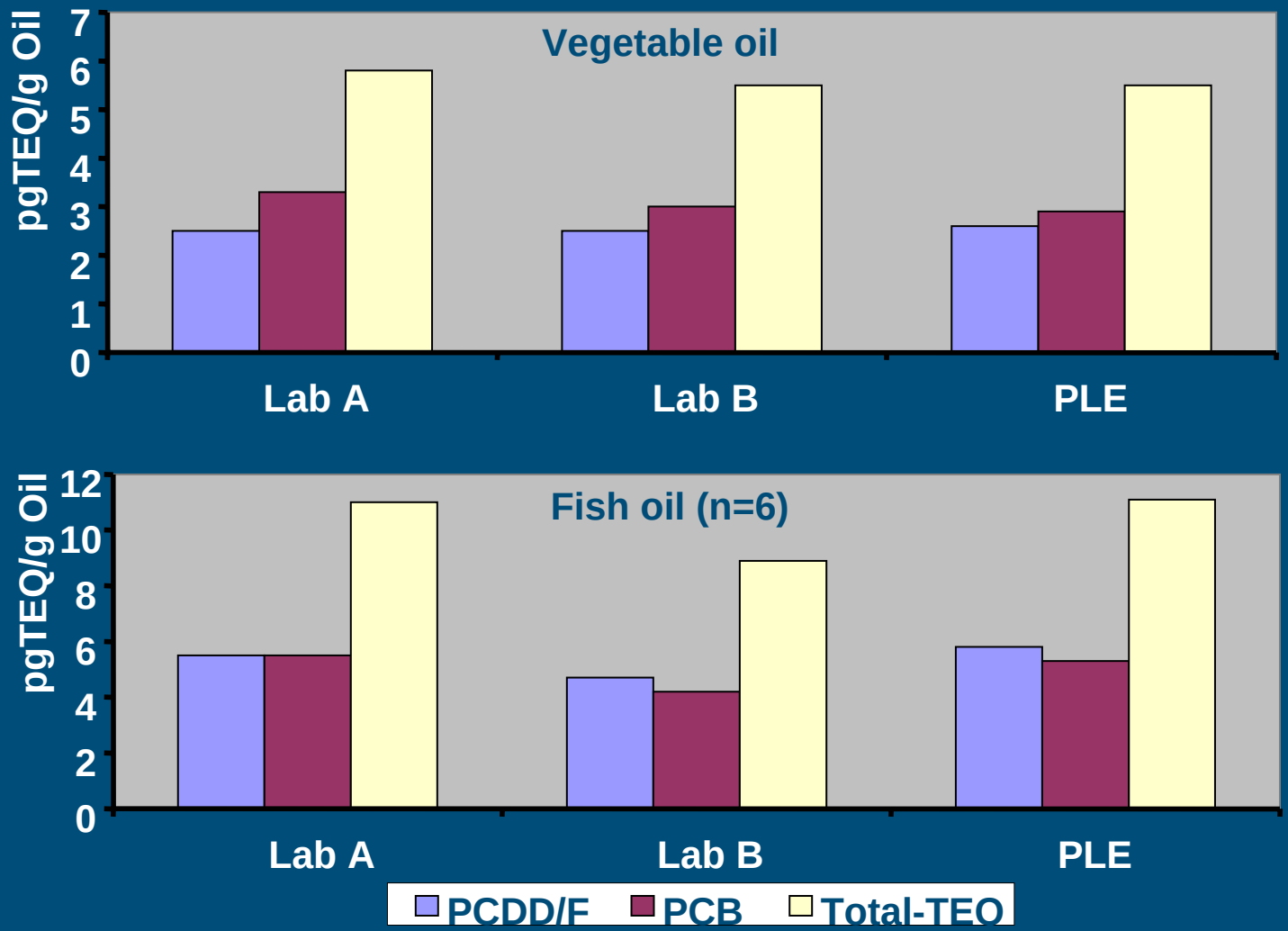
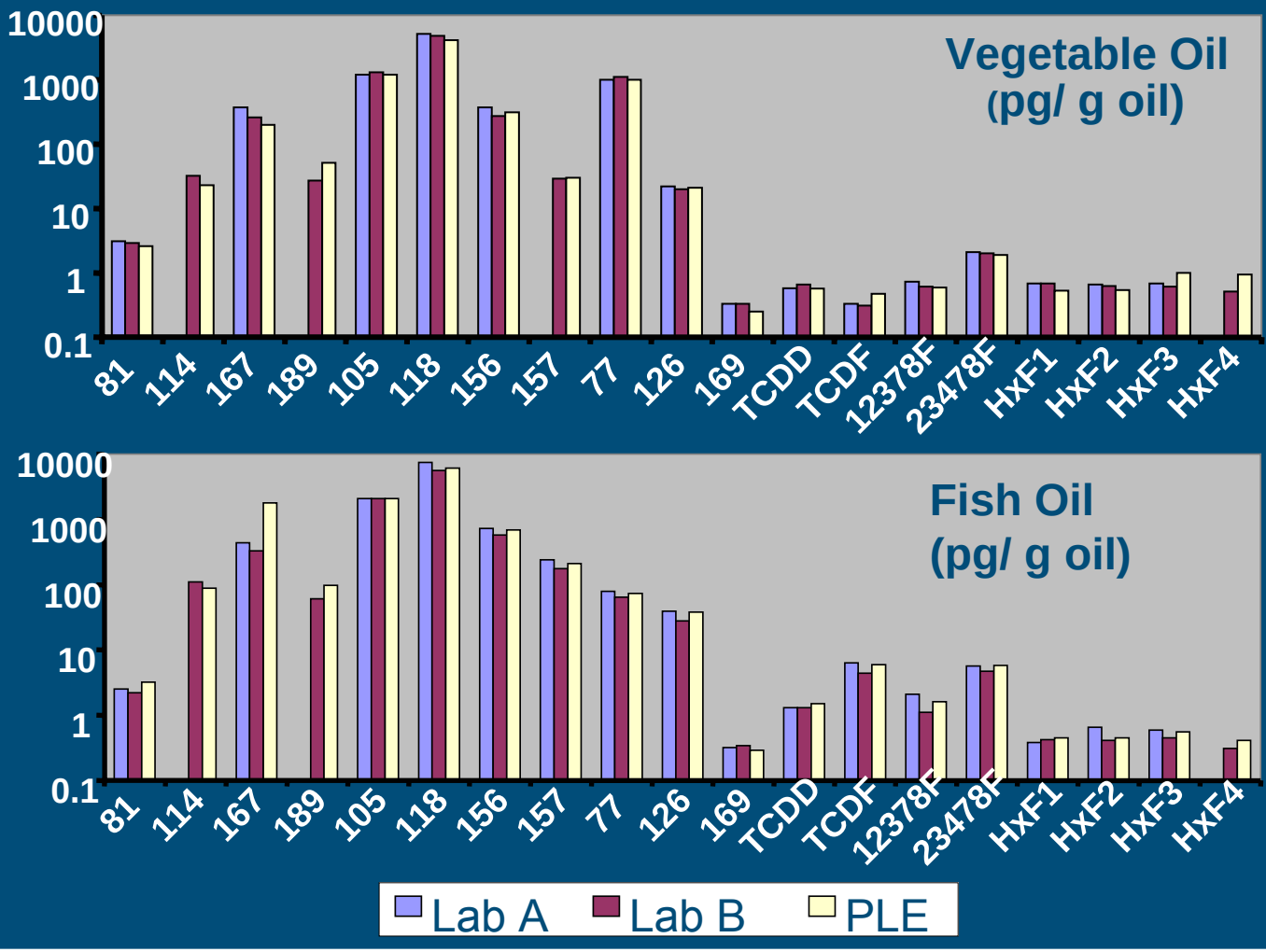
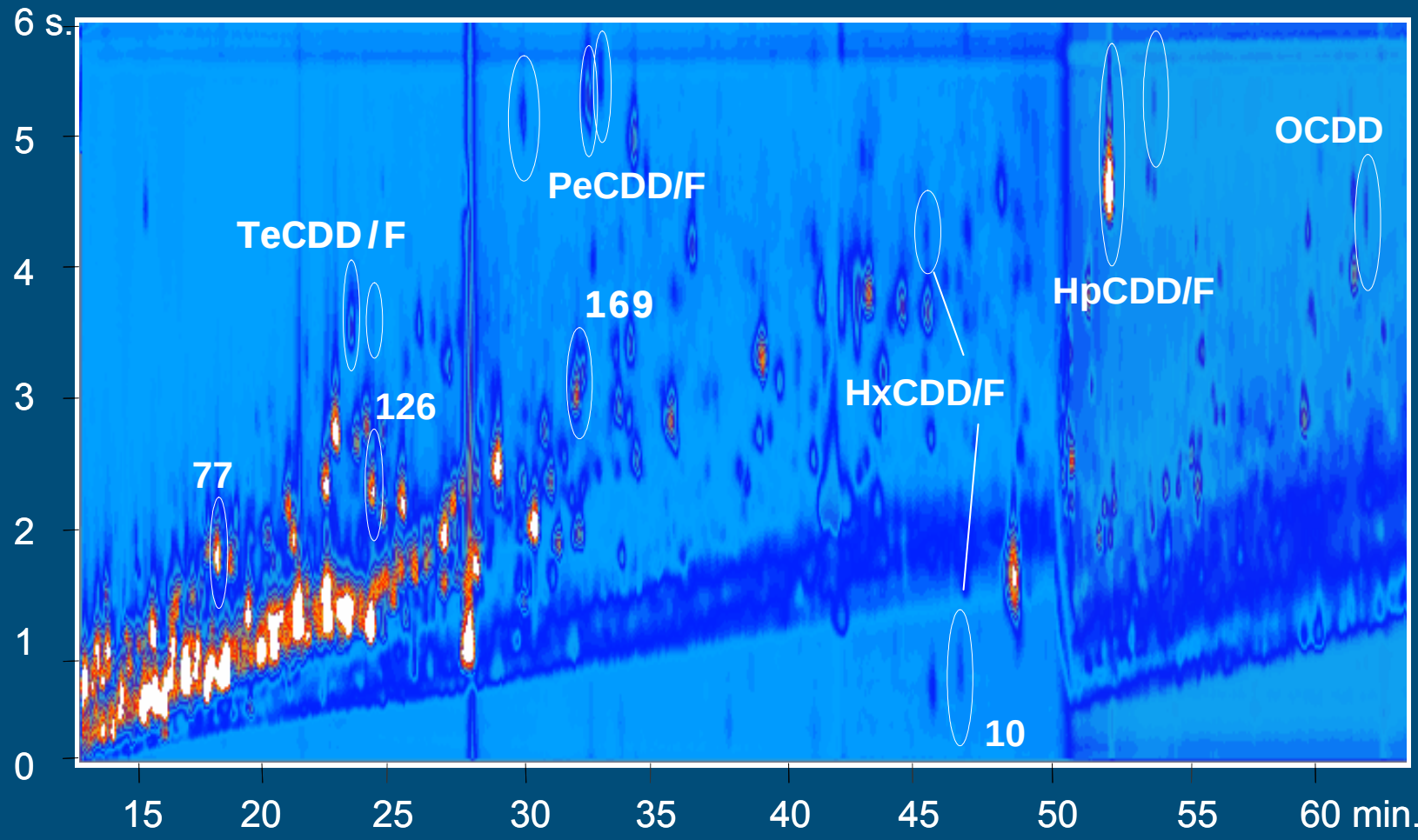
Traditional extraction/clean-up vs. PLE



Individual congeners

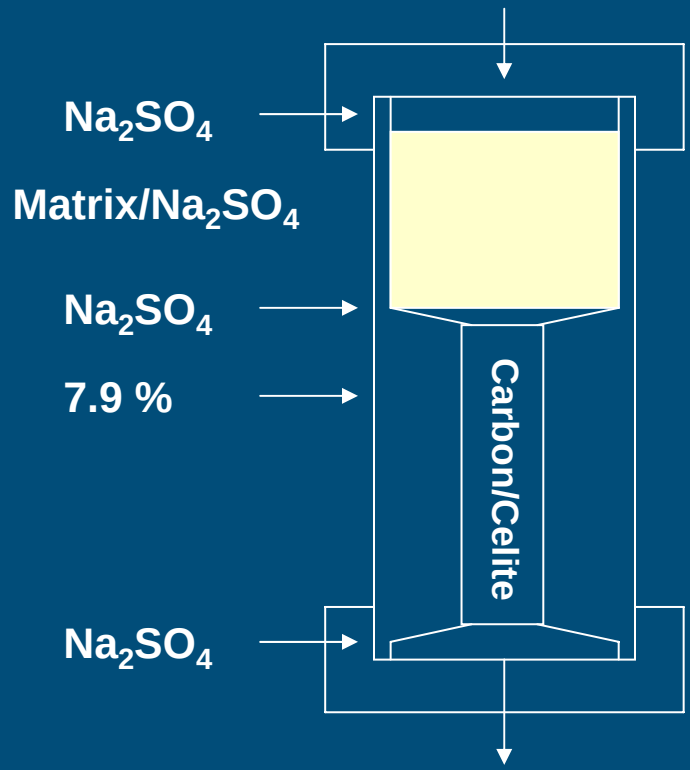


Fish Oil: Non-ortho PCBs and PCDD/Fs

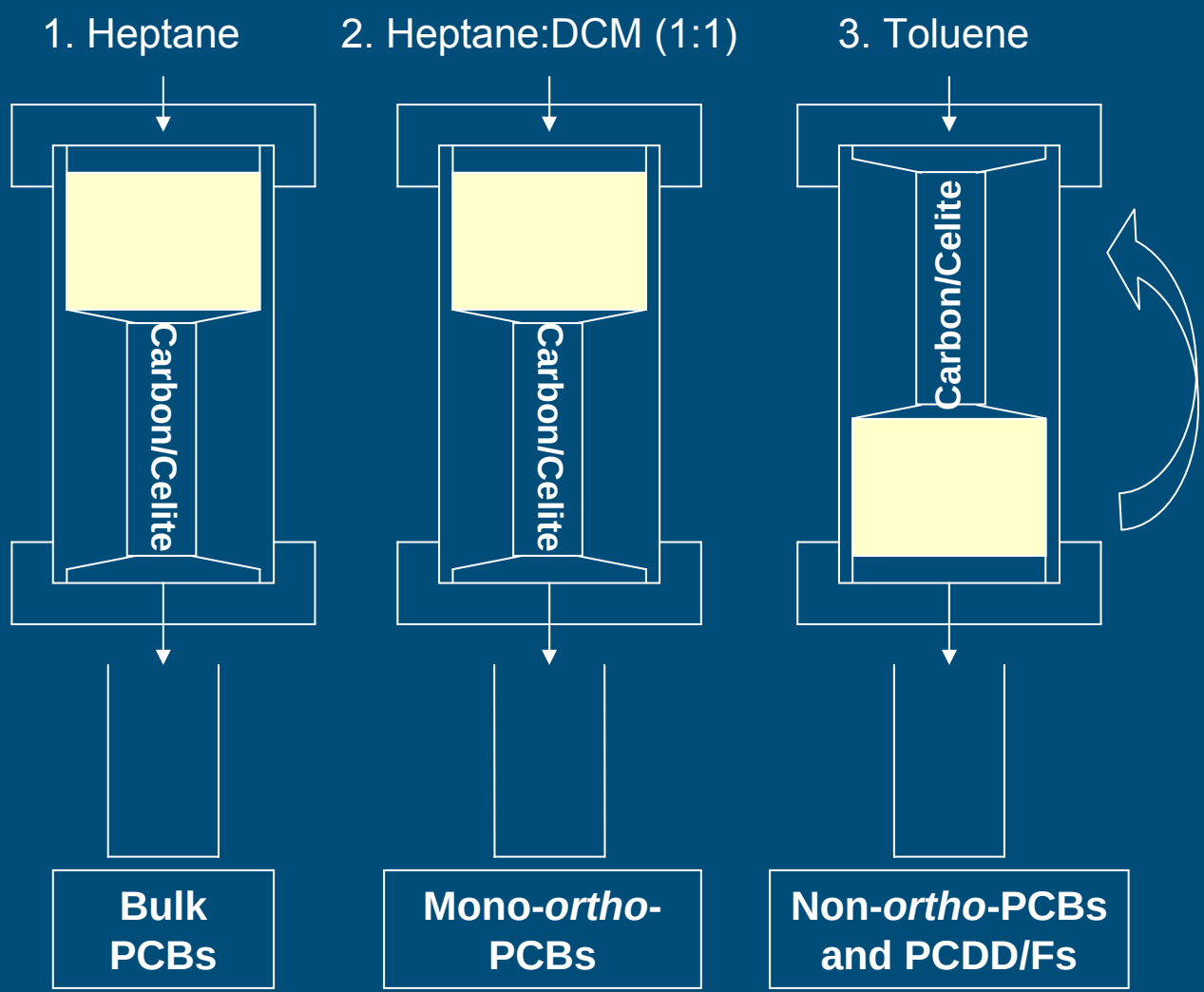


Selective PLE with carbon

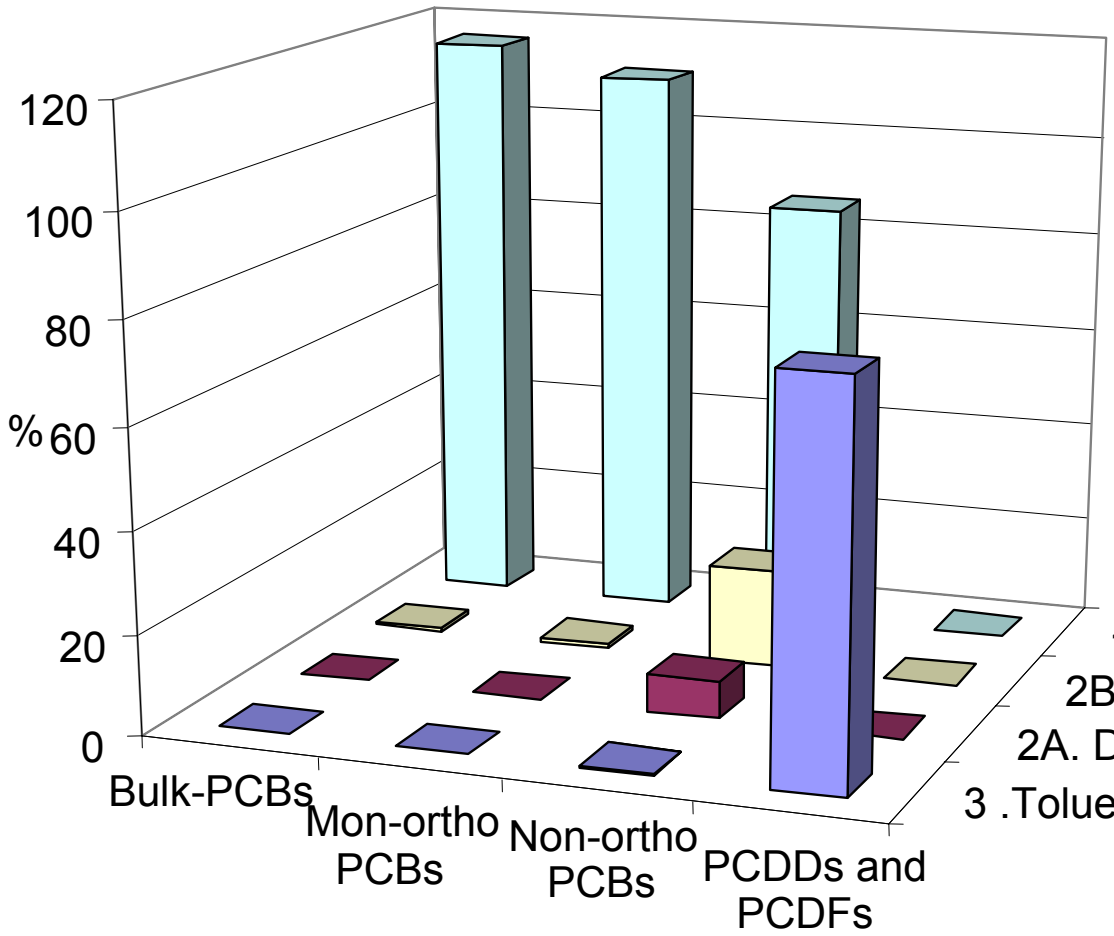
- Dionex ASE200; 33 mL cell
- 3g Fish oil mixed in Na_2SO_4
- “Normal” PLE parameters
- Three consecutive extractions (fractions)



Integrated carbon fractionation of PCBs and Dioxins



Elution profile integrated carbon fractionation



Fish oil

Fat recovery (%)	
Fraction 1	98,7
Fraction 2	0,7
Fraction 3	0,1

Conclusions PLE

- Fast method
- Integrated carbon PLE cost efficient method
- Less labour intensive than traditional method
- Attractive alternative for traditional dioxin method

Validation studies

VITO (Flemish Institute for Technological Research) R. Van Cleuvenbergen

RIVO
S. Van Leeuwen, J. de Boer

Other partners from DIFFERENCE and participants outside DIFFERENCE

Method validation – Interlaboratory studies

- Verification of calibration curves
- Verification of analytical process
- Verification of matrix effects during quantification
- Repeatability, within & between lab reproducibility (ISO 5725)
- Detection Capability
- Selectivity
- Ruggedness

- Standards, quality control solution, clean fish extract
- Vegetable oils with spikes of PCBs, PCNs, PCDE's
- Fish oil, milk, vegetable oil, vegetable oils with spikes, cereal based feed, chicken, vegetable feed, egg, fish, pork

DIFFERENCE: (Candidate) CRMs for Dioxin Analysis

DIFFERENCE

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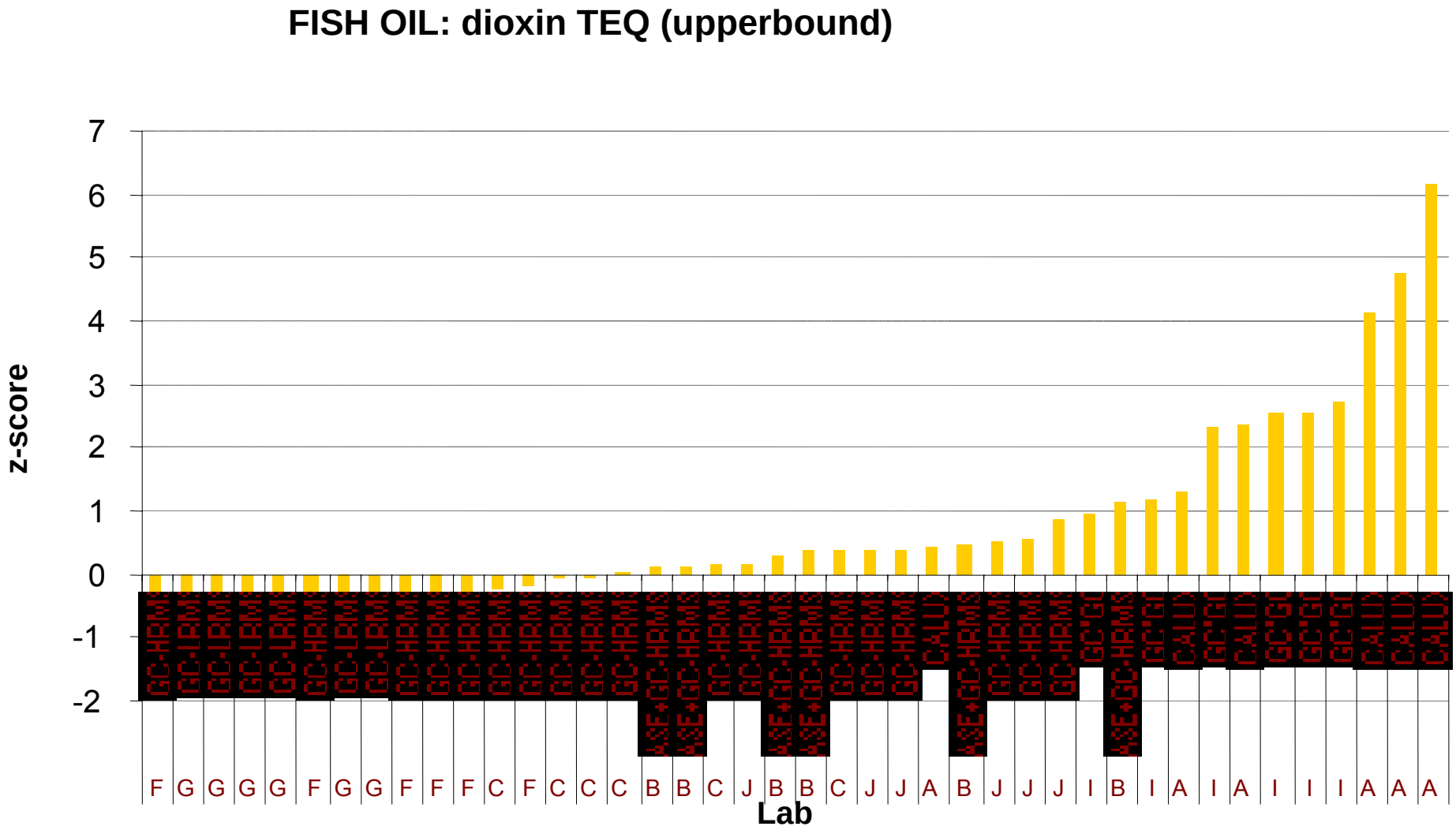
DIFFERENCE

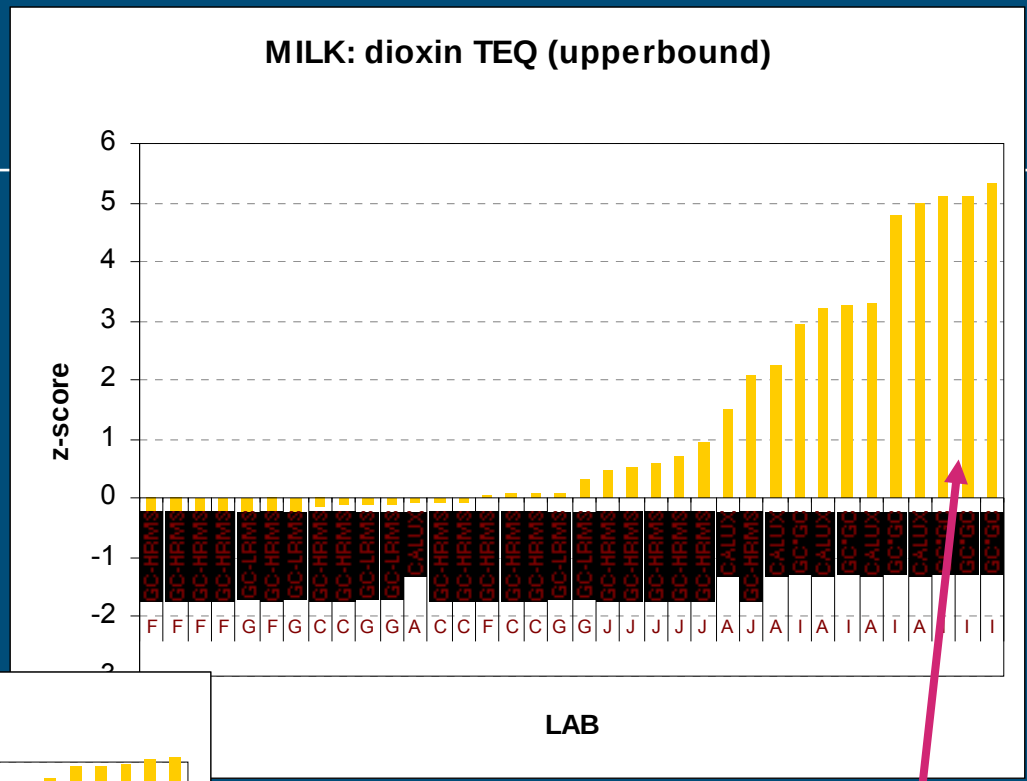
DIFFERENCE

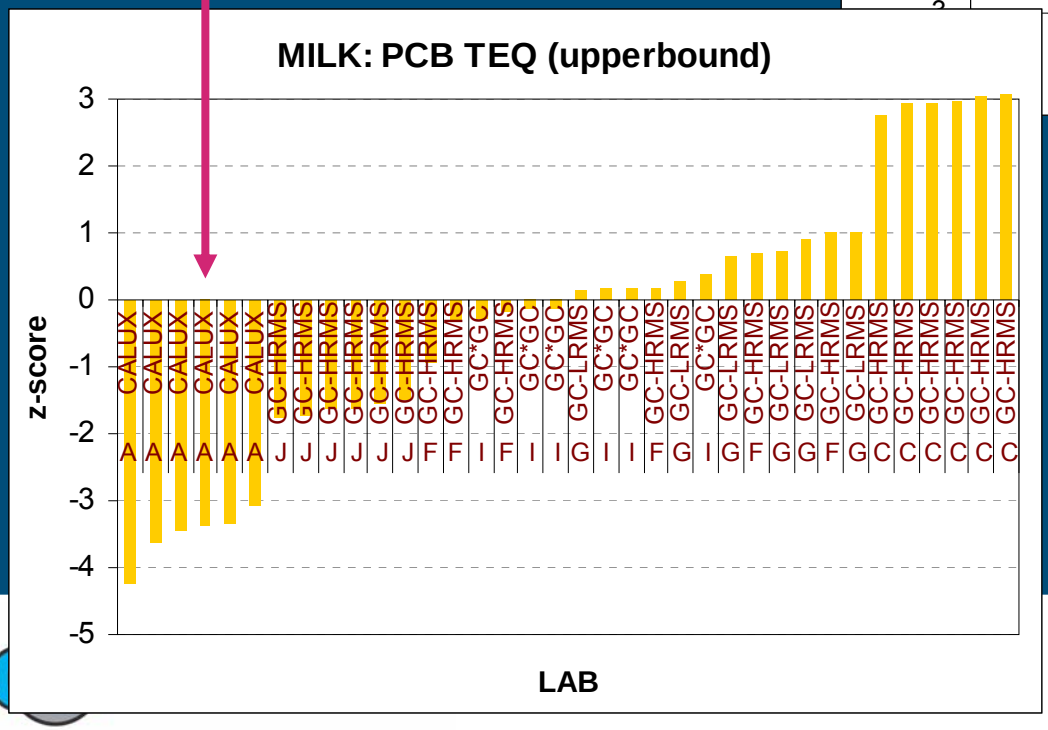
Fish Oil: z-scores



Spiked Milk

CALUX:
signal suppression due to high spike of mono-ortho CB-118?

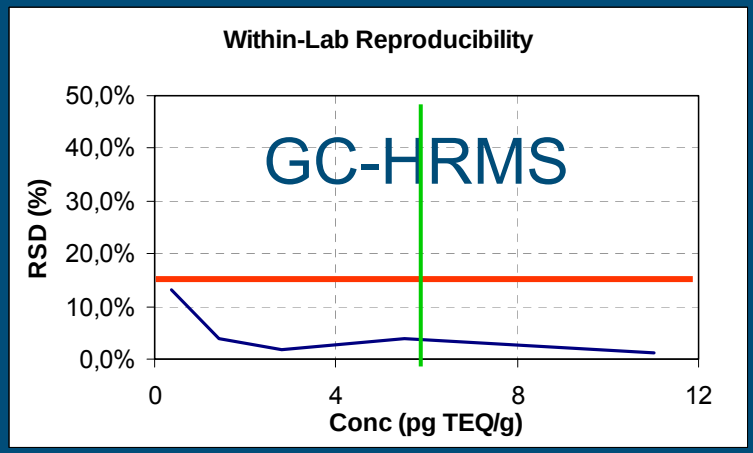


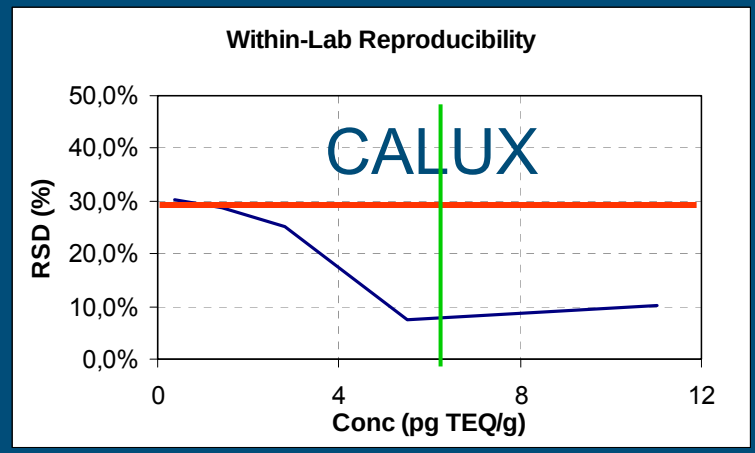


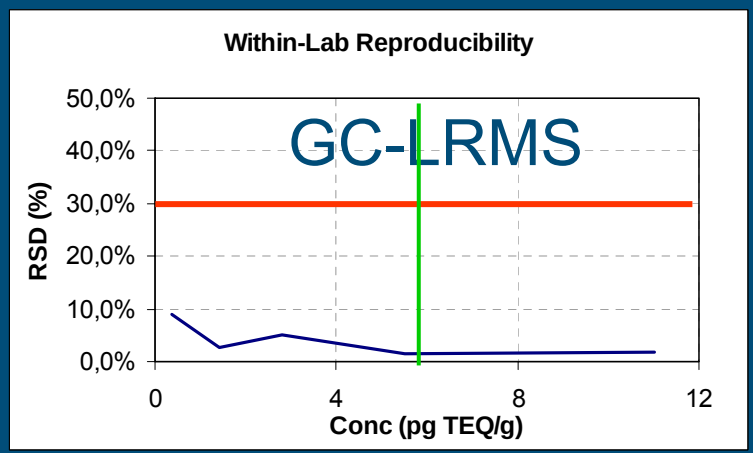
GCxGC:
overestimation due to combination LOQ and reporting upperbounds

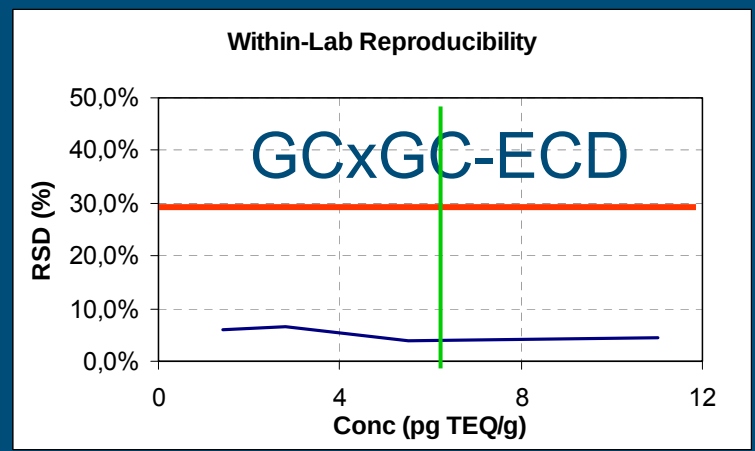
DIFFERENCE

Precision (%) for spiked vegetable oil









DIFFERENCE

Maximum residue level

Precision requirement

Discussion

Technique	Sens.	Accur.	Precis.	Remarks
GC-HRMS	+	+	+	Confirmatory method
GC-LRMS	+	+	+	Potential alternative for HRMS?
GCxGC-ECD	+/-	+/-	+	Improved software required
CALUX	+	+/-	+	Screening technique

Conclusions

- GCxGC-ECD (and GCxGC-ToF-MS), and GC-ion-trap MS/MS may serve as alternative (routine) methods for dioxin analysis
- CALUX is the alternative for times of crisis, but corrections for recovery are essential
- The use of PLE will significantly reduce the extraction and clean-up time

Acknowledgement

All DIFFERENCE and

DIAC partners

Thank you for your attention

www.dioxins.nl