

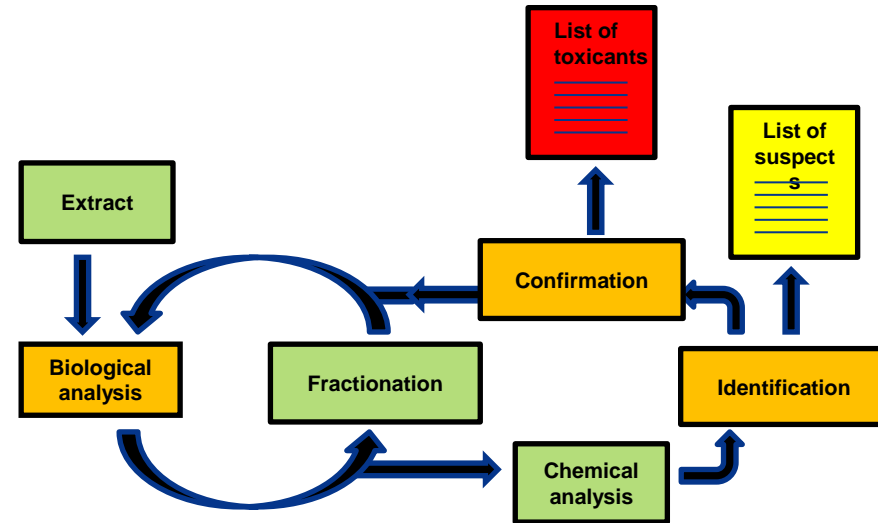


Identification of thyroid hormone-disrupting compounds in polar bear plasma by effect-directed analysis (EDA)

Eszter Simon, Timo Hamers, Elisabeth Lie, Katharina Løken and Marja Lamoree

Aim of our EDA study

- EDA → biota
→ new toxic endpoints
- Identify known/unknown TH-disruptors
- Identification strategy



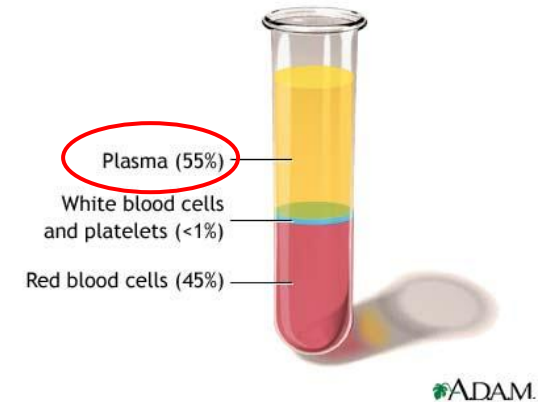
Polar bear plasma



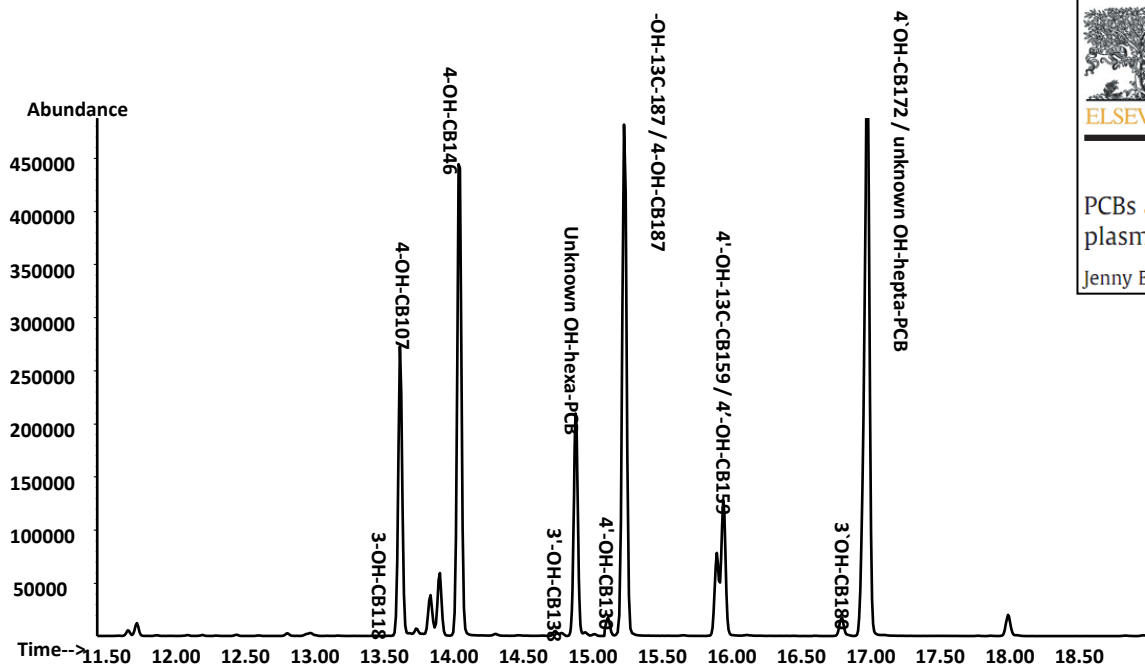
- Collaboration with Norwegian partners
- BearHealth project
- $n = 31$
- 1998 and 2008



Norwegian University of
Science and Technology




Target analyzed compounds



OH-CBs, PFASs

Contents lists available at SciVerse ScienceDirect



Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

PCBs and OH-PCBs in polar bear mother-cub pairs: A comparative study based on plasma levels in 1998 and 2008

Jenny Bytingsvik ^{a,*}, Elisabeth Lie ^b, Jon Aars ^c, Andrew E. Derocher ^d, Øystein Wiig ^e, Bjørn M. Jenssen ^{a,**}

Environment International 49 (2012) 92–99



Environment International

journal homepage: www.elsevier.com/locate/envint

Perfluoroalkyl substances in polar bear mother-cub pairs: A comparative study based on plasma levels from 1998 and 2008

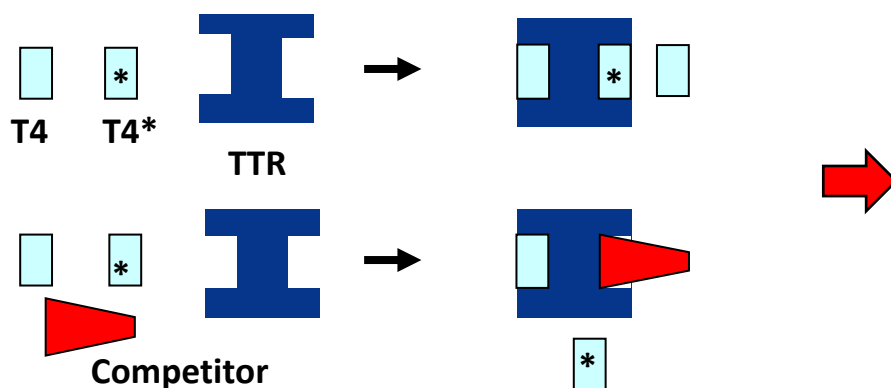
Jenny Bytingsvik ^{a,*}, Stefan P.J. van Leeuwen ^{b,1}, Timo Hamers ^b, Kees Swart ^b, Jon Aars ^c, Elisabeth Lie ^d, Else Mari Espseth Nilsen ^a, Øystein Wiig ^e, Andrew E. Derocher ^f, Bjørn M. Jenssen ^{a,*}

T₄*-TTR binding assay

Solid-phase Extraction (SPE_{MCX})



¹²⁵I-T₄-TTR binding assay

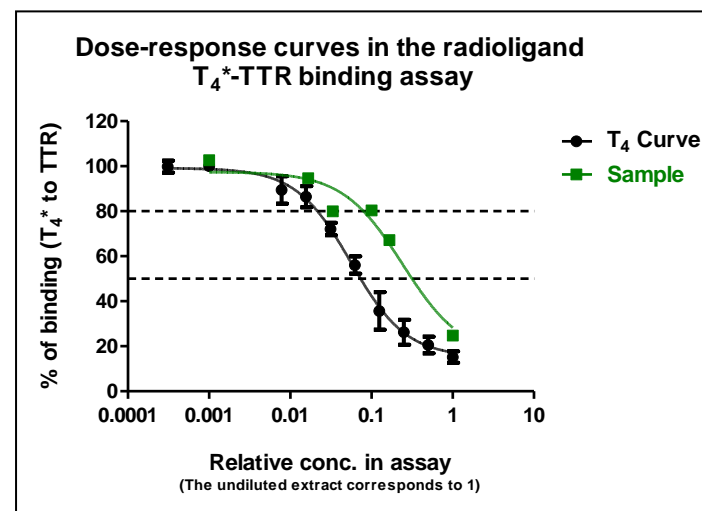


ENVIRONMENTAL
Science & Technology

ARTICLE
pubs.acs.org/est

Blood Plasma Sample Preparation Method for the Assessment of Thyroid Hormone-Disrupting Potency in Effect-Directed Analysis

Eszter Simon,[†] Jenny Bytingsvik,[‡] Willem Jonker,[†] Pim E. G. Leonards,[†] Jacob de Boer,[†] Bjørn M. Jenssen,[‡] Elisabeth Lie,[§] Jon Aars,^{||} Timo Hamers,[†] and Marja H. Lamoree^{*,†}



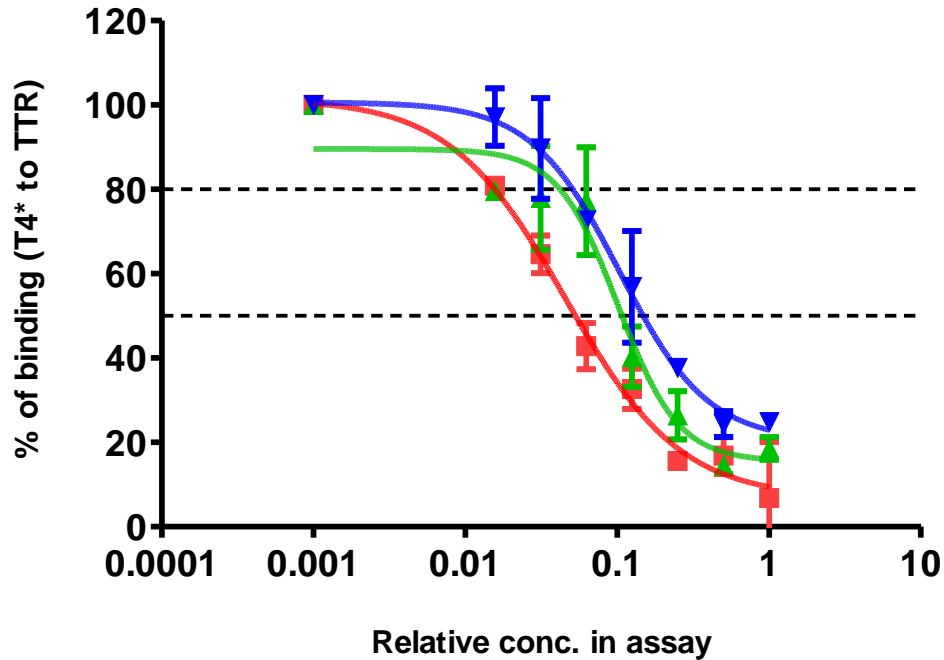
Sample selection for EDA



■ Polar bear1 (1998)

▲ Polar bear 2 (2008)

▼ Polar bear 3 (2008)

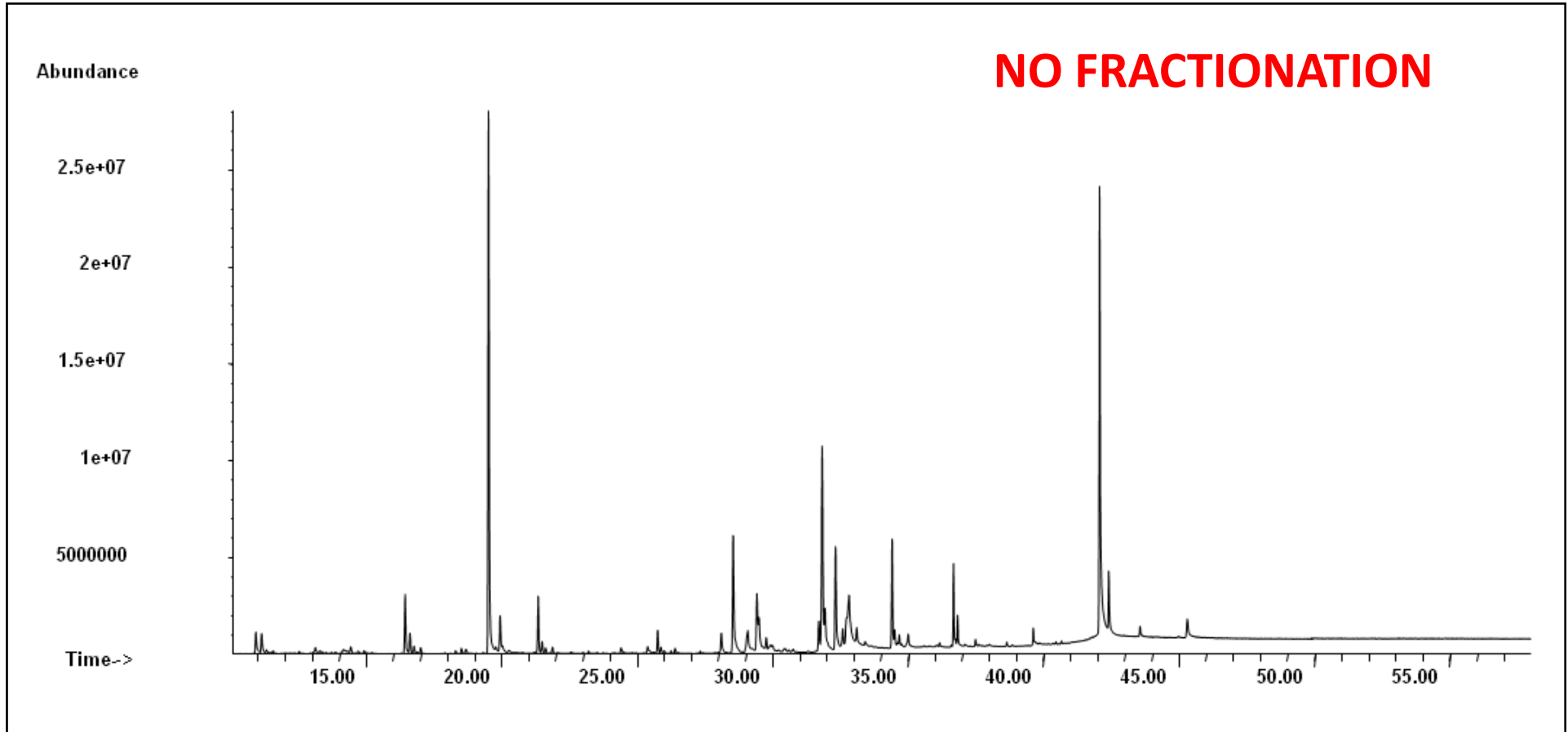


Selected cubs (n=3)	T ₄ -Eq (nM)
Calculated TTR-binding activity	~870
Measured TTR-binding activity	~2100

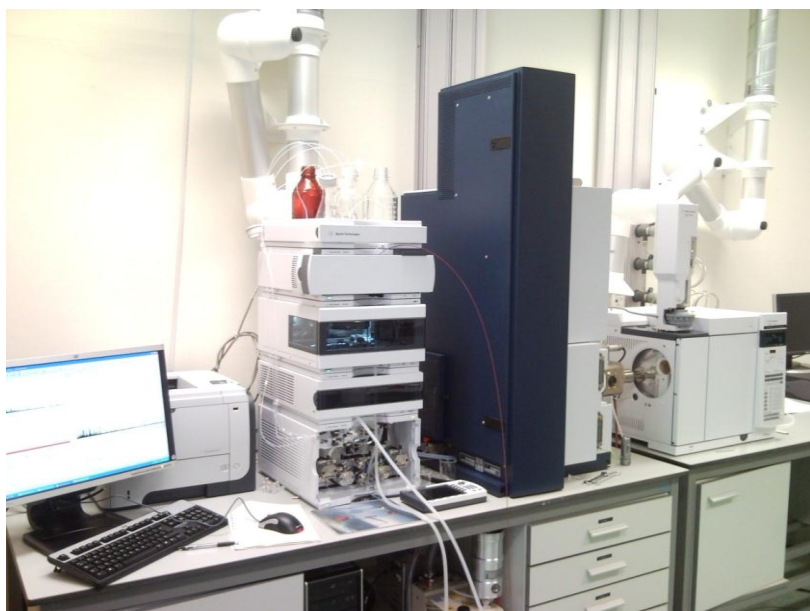


54-60 % unexplained activity

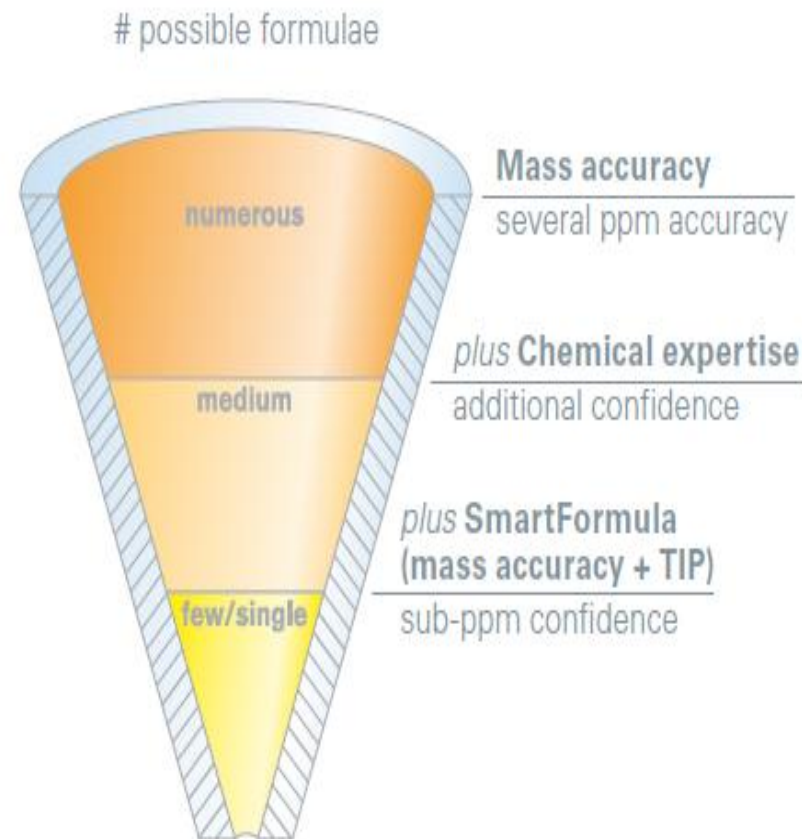
GC-EI-MS – plasma extract



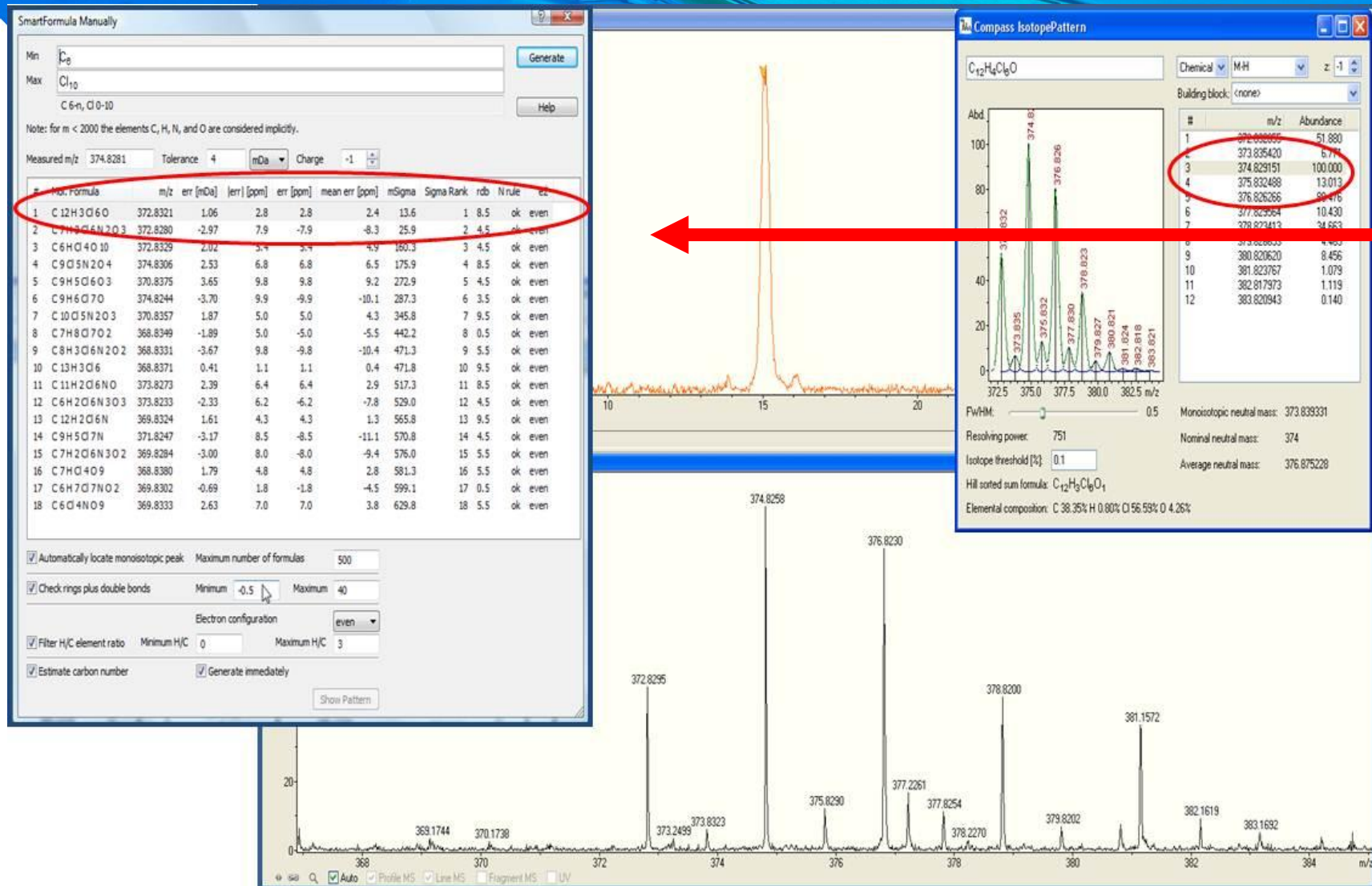
Chemical analysis and confirmation



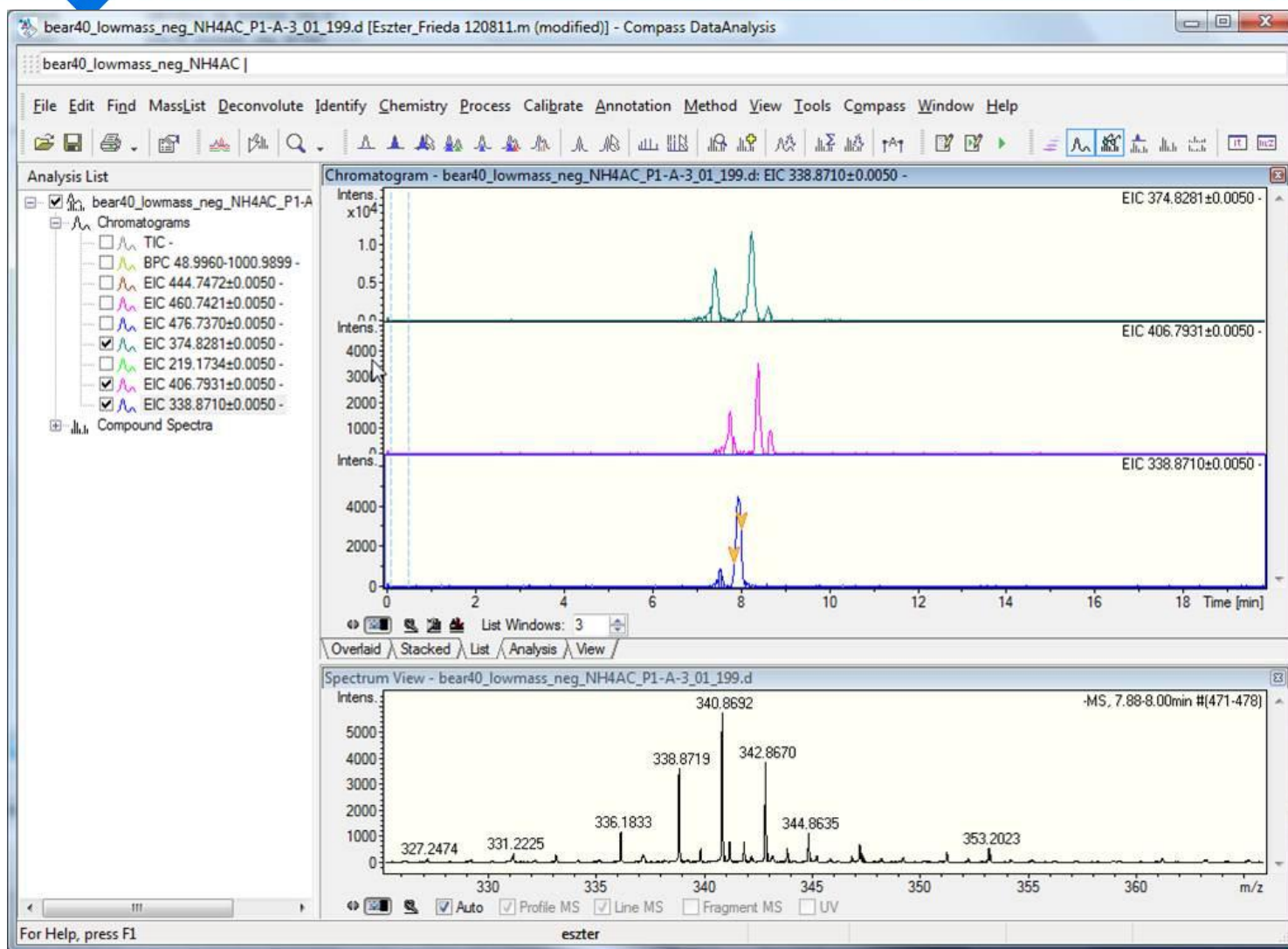
LC-Time-of-Flight-MS



Confirmation of "knowns" – LC-ToF-MS



Confirmation of "knowns" – LC-ToF-MS

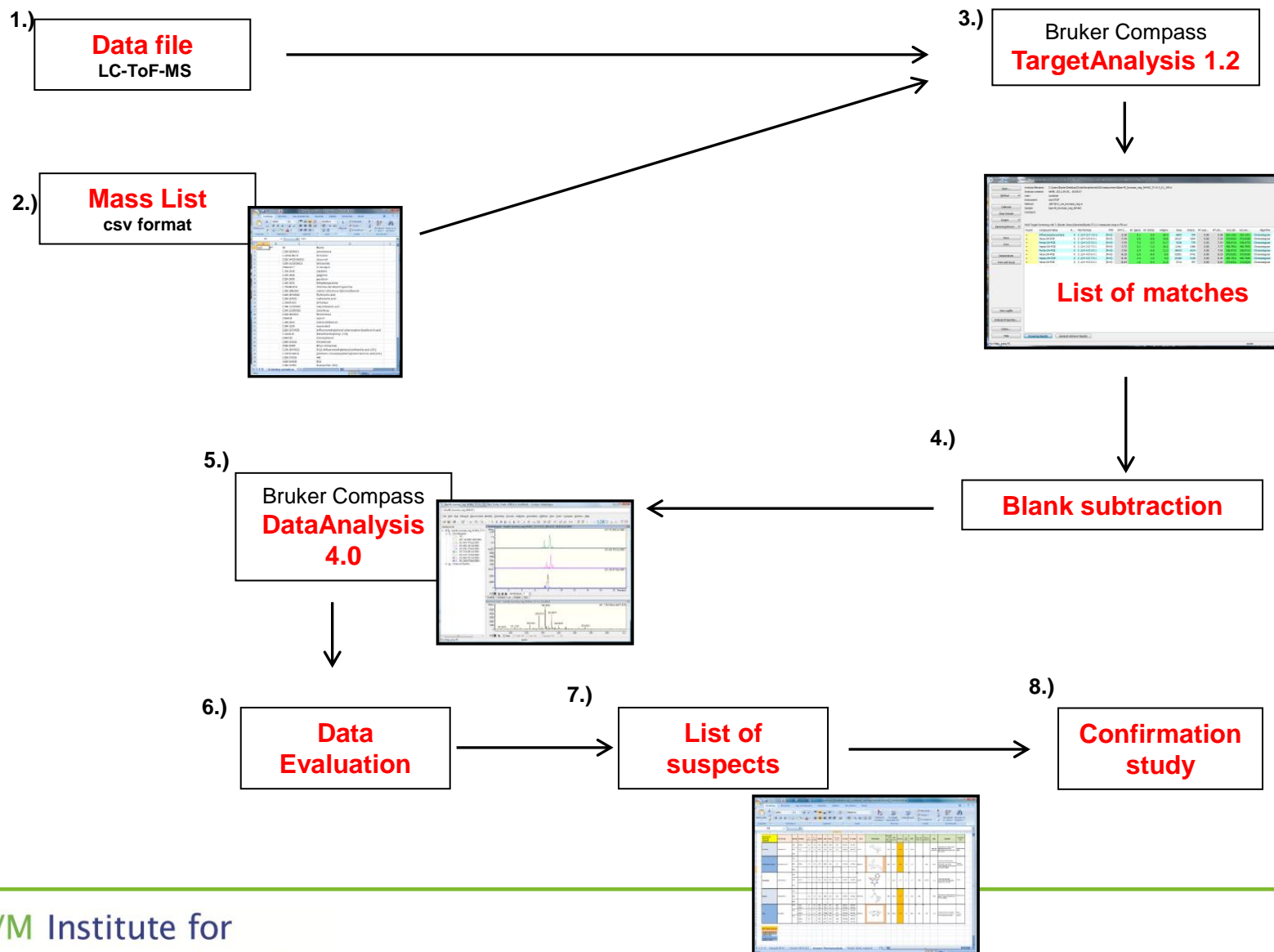


OH-hexa-CBs

OH-hepta-CBs

OH-penta-CBs

Identification strategy – Mass libraries



MASS LIBRARIES

	Library	Nr. of compounds in library	Nr. of matches	Potential suspects
1	Reported TTR-disrupting and blood accumulating compounds	248	20	1
2	P and/or B pharmaceuticals*	106	5	4
3	P and/or B halogenated chemicals **	594	25	25
4	Bruker library including dyes, pharmaceuticals, pesticides	225	20	1
5	EnviMass – Fresh water contaminants	143	3	-
	Total	1316	73	31

* Howard and Muir, ES&T 2010; ** Howard and Muir, ES&T2011

Match is only based on chemical formula and exact mass, not on t_R !

Analytical confirmation



Status	Nr. of compounds
Retention time (t_R)/accurate mass matches	1
Uncertain matches	2
No peak in the standards	9
No retention time (t_R) matches	12
Standards are not available	3
Standards are way too expensive	4
Total	31

C, H, N, O, (P, Si)

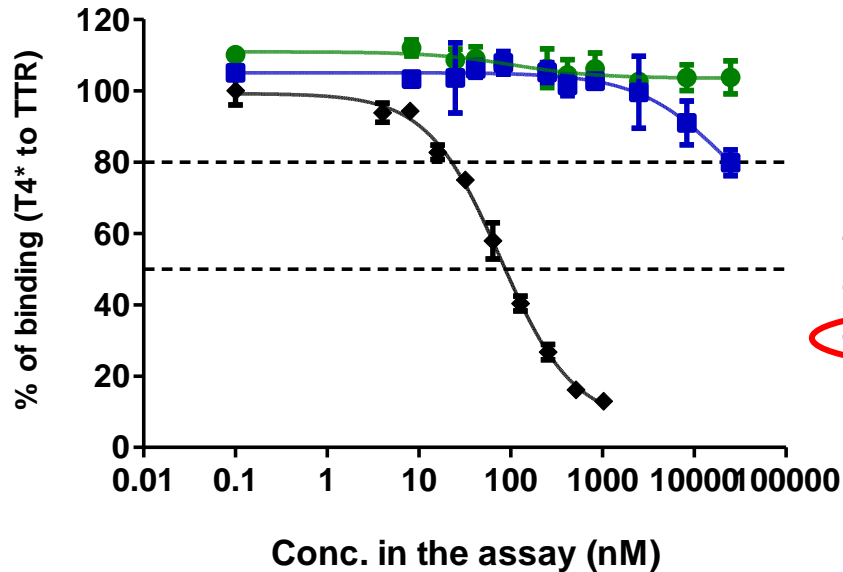


NONYLPHENOL



Toxicological confirmation - nonylphenol

Nonylphenols in the T4*-TTR binding assay



~ 16.3 ng/mL – Linear NP

~ 4.3 ng/mL – Branched NP

◆ T4 curve
● Linear NP
■ Branched NP

~ 0.005 %
of the total activity

Isotope Cluster Analysis

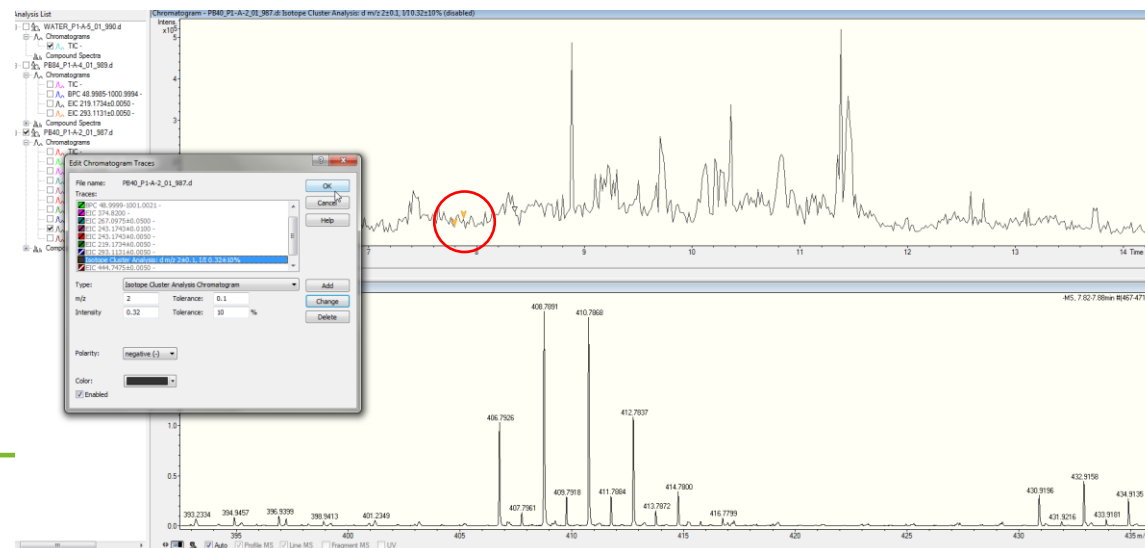
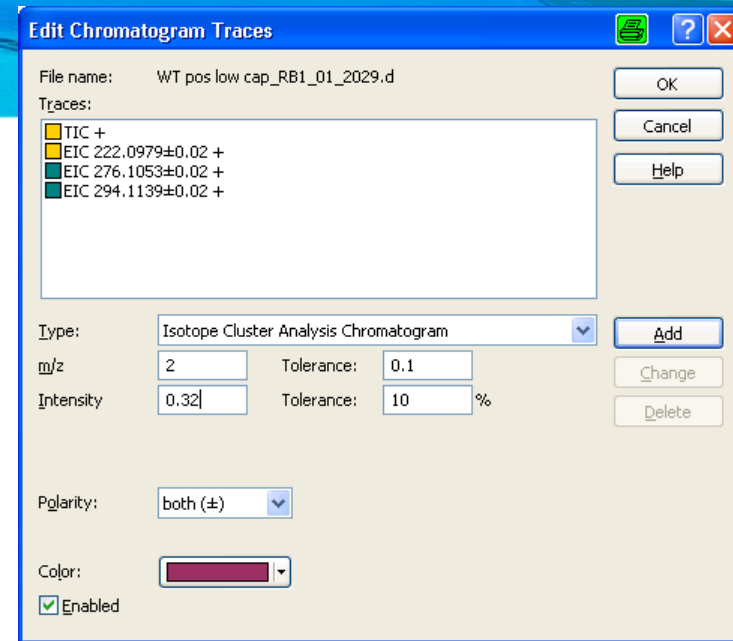
Data Analysis 4.0
Edit Chromatogram Traces

Isotope Cluster Analysis
plot

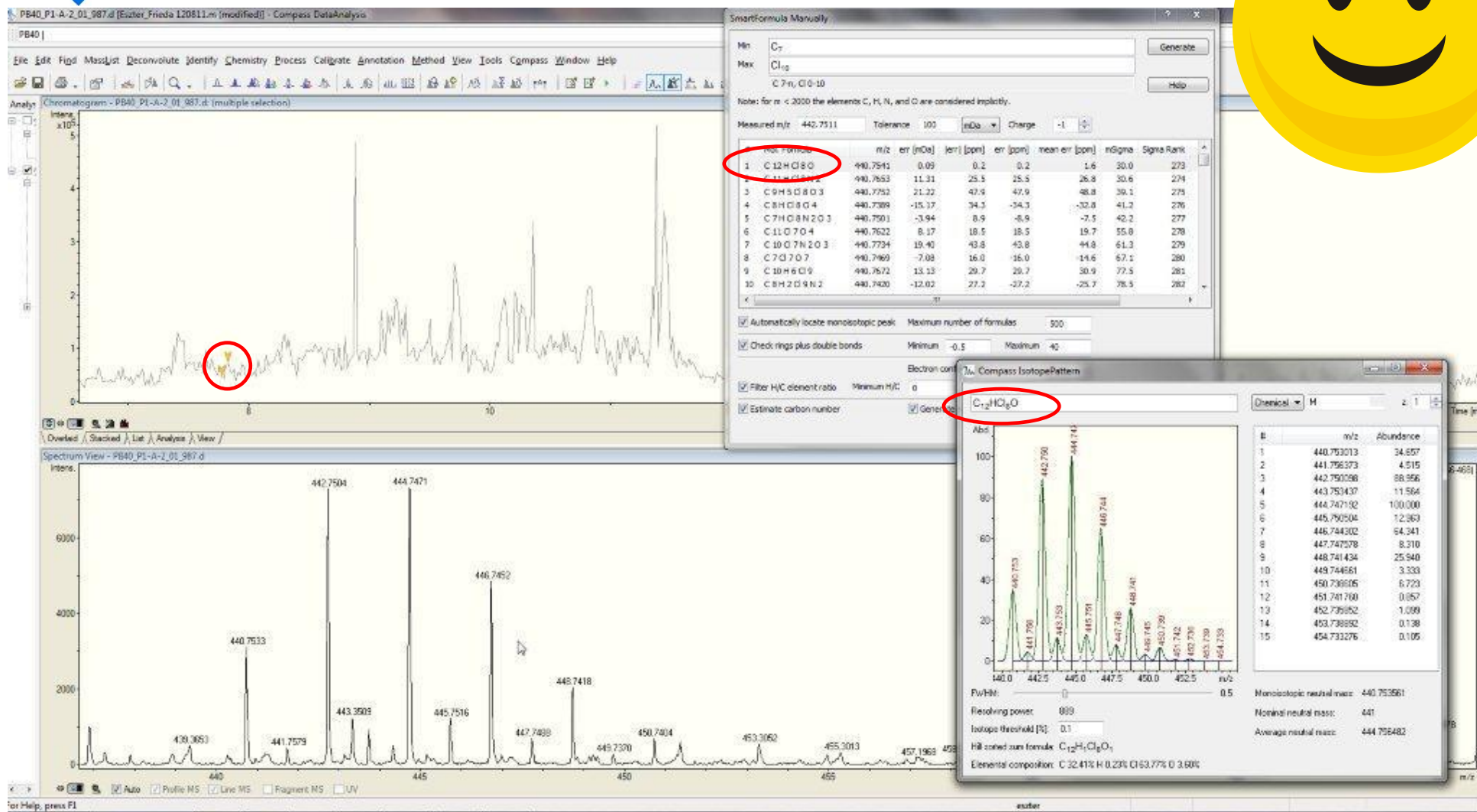
Manual Integration

Smart Formula Manually

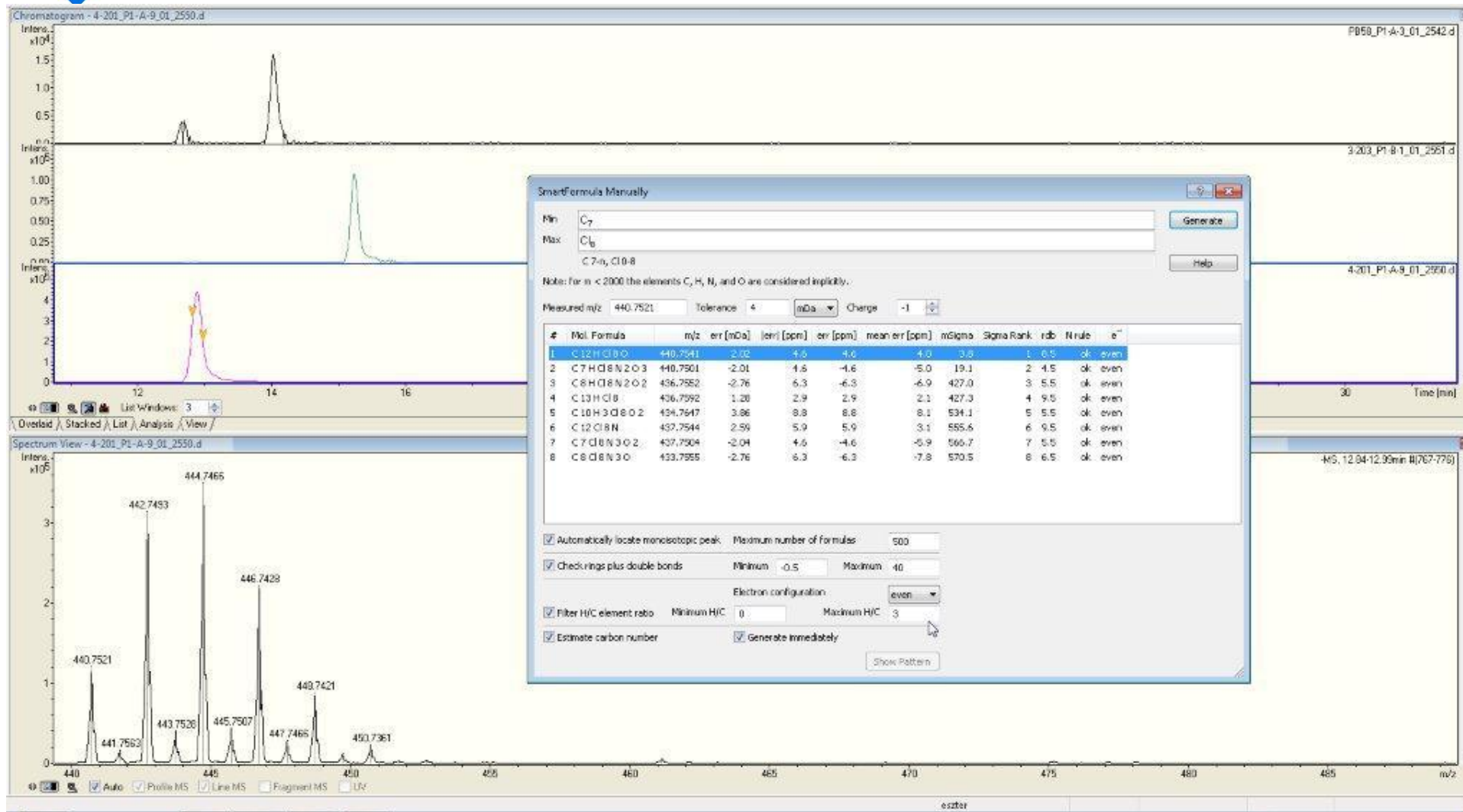
List of suspects



3 (di)OH-octaCBs

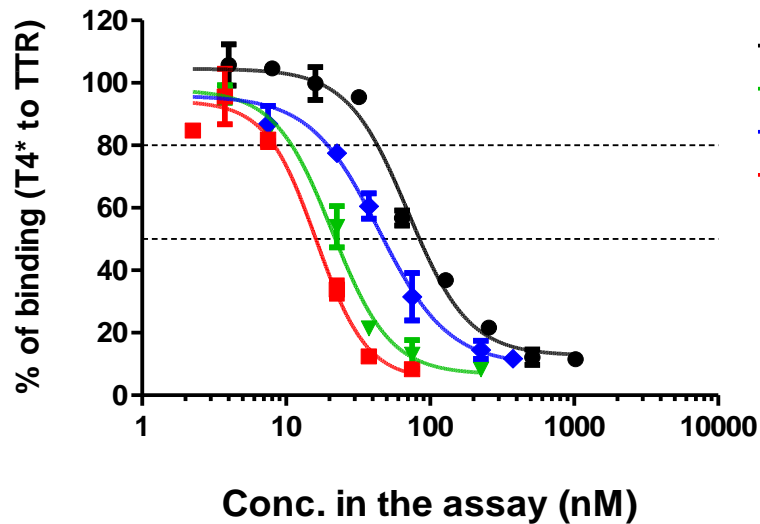


Analytical confirmation –OH-octaCBs



Confirmation study

TTR binding potency of hydroxy-octaCBs



● T4 Curve

▼ 4-OH-CB201

◆ 3-OH-CB203

■ 4,4-diOH-CB202

~ 1-17 ng/mL

~ 13-44 ng/mL

~ 3-69 ng/mL unknown congener

29-34%

of the total activity

Concluding remarks

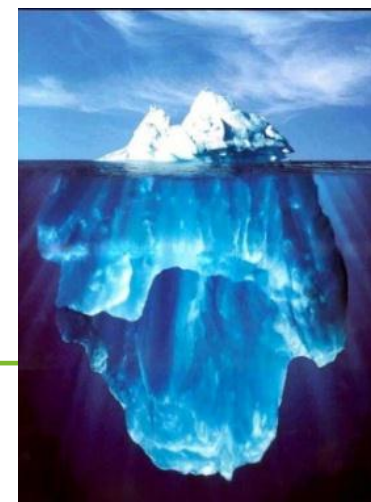
- Identification still very difficult
- Confirmation hampered by lack of standards
- Presence of elements with distinctive isotopes facilitates identification
- Creation of mass libraries to search for “known unknowns”



- MassBank initiative:

massbank.jp

massbank.normandata.eu/MassBank



Acknowledgement

Colleagues (IVM):

Martin van Velzen
Sicco Brandsma
Pim Leonards
Jana Weiss

Norwegian partners: Jenny Bytingsvik, Bjørn M. Jenssen

Bruker Daltonics



THANK YOU FOR YOUR ATTENTION!