GCMSMS as a tool for analysis of POPs in food

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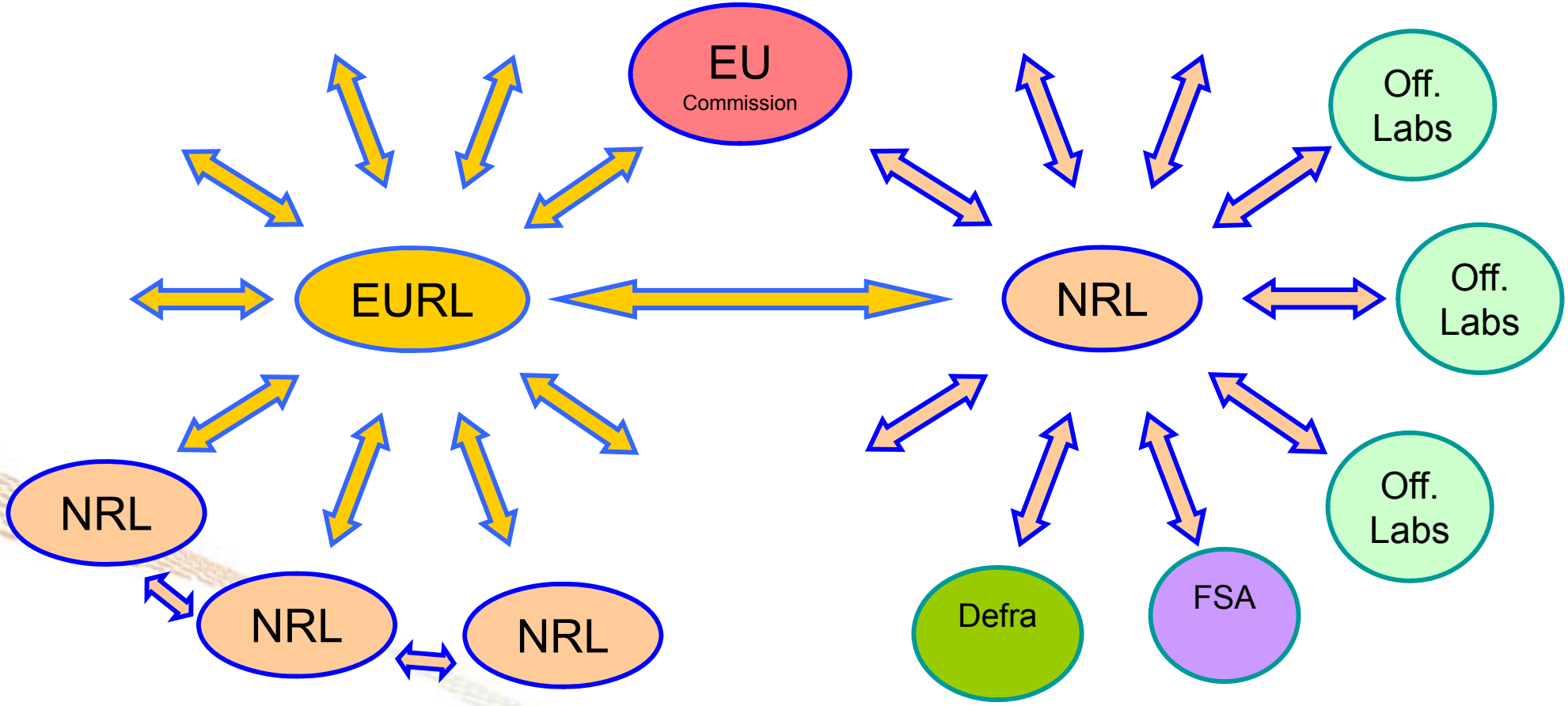
EU-RLs and NRLs Role

- Analytical laboratories designated by EU regulations
- Part of European risk management system.
  * EU-wide standards
  * organising comparative tests
  * training
  * scientific and technical assistance to the European Commission, especially if a Member State contest results of analyses or trans-boundary disputes
  * Coordinating National Reference Laboratories
  * international standards and practices

The network


EU
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The challenge
Dioxins and PCBs

- MRLs
  - Strict but feasible
  - Regulatory control
  - Uniform application (within EU)
- Action Levels
  - An ‘early warning’ tool - Lower than maximum levels
  - trigger action to identify sources and pathways of contamination
  - A pro-active approach to reduce the presence of dioxins in food and feed
  - interact with environmental and other control measures
- Target levels
  - indicate the levels to be achieved over time (feed and food) in order to ultimately bring human exposure for the majority of the EU population down to or below the TWI.
  - Target levels were to be established before the end of 2004, but are still not set.

Maximum Limits

- PCDD/Fs typically 0.75 - 4.5 pg/g fat (wet weight for fish)
- PCDD/Fs + PCBs 1.0 – 10.0 pg/g fat
- ∑ ICES 6 PCBs 40 – 200 ng/g fat

- Animal feed 0.75 - 1.0 ng WHO-PCDD/F TEQ/kg (12 % moisture)

Official Control Laboratories (OCLs)

- Operate at local level
- Responsible for formal enforcement action
- Generalist laboratories
  - forensic work; toy safety; labelling; food law;
- Chemical analysis of feed or food samples
  - pesticides; veterinary medicines; mycotoxins; GMOs; allergens; authenticity etc.

UK situation

- NRL function for dioxins resides in Fera
- UK OCL labs have diverse range of responsibilities
- Cash starved for decades
- None have dioxins capability (HRMS or CALUX)

  [Some owned by Eurofins and therefore have access to non-UK capability]
- Fera acts as NRL for Malta due to lack of home-based capability

HRMS

- Sensitive
- Conventional ‘gold standard’ confirmatory method for dioxins
- Expensive (~ € ¼M)
- Highly skilled operators required

GCMSMS

- Lacks sensitivity

Or does it?

But.....

- Cheaper
- Versatile
- Easier to use

Thermo TSQ Quantum XLS Ultra

- Method validation study
- Based on installation + 3 weeks (interrupted) work

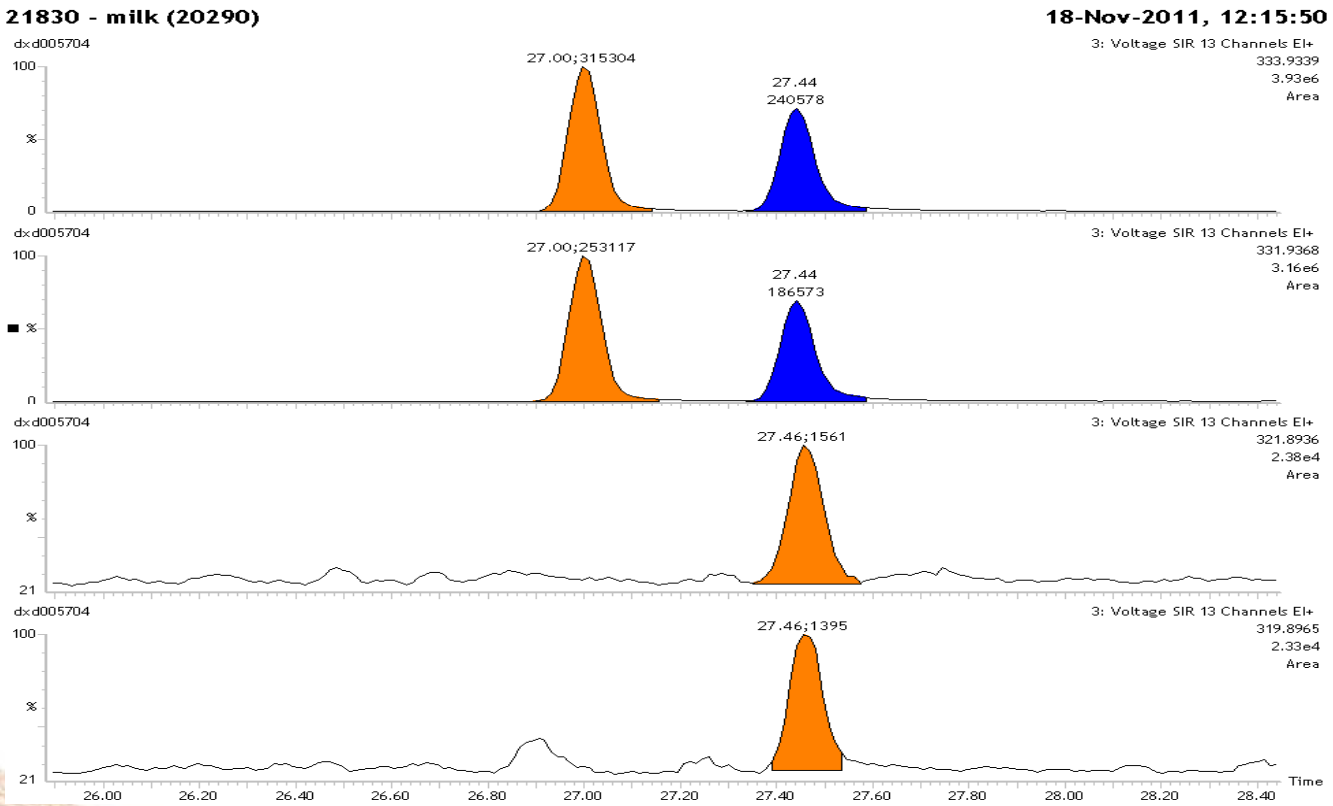
	Milk		Fish feed		Salmon	
	GC-MS/MS	High Res. MS	GC-MS/MS	High Res. MS	GC-MS/MS	High Res. MS
Results rounded to 2 d.p pg/g whole weight						
2378TCDD	<0.01	<0.01	0.06	0.05	0.05	0.04
12378PeCDD	0.01	0.01	<0.13	0.09	0.10	0.10
123478HxCDD	<0.01	<0.01	<0.03	0.03	<0.02	0.02
123678HxCDD	0.01	0.01	<0.07	0.06	<0.05	0.05
123789HxCDD	<0.01	<0.01	<0.02	0.02	<0.02	0.02
1234678HpCDD	<0.01	<0.01	0.09	0.13	0.03	0.03
OCDD	<0.01	<0.01	0.53	0.67	0.08	0.06
2378TCDF	<0.01	<0.01	1.07	1.09	0.39	0.42
12378PeCDF	<0.01	<0.01	0.14	0.10	0.04	0.07
23478PeCDF	0.03	0.03	0.41	0.39	0.13	0.14
123478HxCDF	0.01	0.01	<0.04	0.05	<0.01	0.02
123678HxCDF	0.01	<0.01	<0.04	0.04	<0.01	0.01
123789HxCDF	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
234678HxCDF	0.01	<0.01	0.09	<0.01	0.02	<0.01
1234678HpCDF	<0.01	<0.01	<0.05	0.06	0.02	<0.01
1234789HpCDF	<0.01	<0.01	<0.03	<0.01	<0.01	<0.01
OCDF	0.01	<0.01	<0.11	0.04	0.05	<0.01
PCB 77	0.07	0.05	29.68	34.90	20.18	24.69
PCB 81	0.03	0.03	1.60	1.75	0.84	1.01
PCB 126	0.20	0.22	7.16	7.43	4.21	4.65
PCB 169	0.07	0.06	1.29	1.32	0.85	0.89
TEQ pg/g whole weight						
2,3,7,8-TCDD	-0.010	-0.010	0.060	0.050	0.050	0.040
1,2,3,7,8-PeCDD	0.010	0.010	-0.130	0.090	0.100	0.100
1,2,3,4,7,8-HxCDD	-0.001	-0.001	-0.003	0.003	-0.002	0.002
1,2,3,6,7,8-HxCDD	0.001	0.001	-0.007	0.006	-0.005	0.005
1,2,3,7,8,9-HxCDD	-0.001	-0.001	-0.002	0.002	-0.002	0.002
1,2,3,4,6,7,8-HpCDD	0.000	0.000	0.001	0.001	0.000	0.000
OCDD	0.000	0.000	0.000	0.000	0.000	0.000
2,3,7,8-TCDF	-0.001	-0.001	0.107	0.109	0.039	0.042
1,2,3,7,8-PeCDF	-0.001	-0.001	0.007	0.005	0.002	0.004
2,3,4,7,8-PeCDF	0.015	0.015	0.205	0.195	0.065	0.070
1,2,3,4,7,8-HxCDF	0.001	0.001	-0.004	0.005	-0.001	0.002
1,2,3,6,7,8-HxCDF	0.001	-0.001	-0.004	0.004	-0.001	0.001
1,2,3,7,8,9-HxCDF	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001
2,3,4,6,7,8-HxCDF	0.001	-0.001	0.009	-0.001	0.002	-0.001
1,2,3,4,6,7,8-HpCDF	0.000	0.000	-0.001	0.001	0.000	0.000
1,2,3,4,7,8,9-HpCDF	0.000	0.000	0.000	0.000	0.000	0.000
OCDF	0.000	0.000	0.000	0.000	0.000	0.000
TEQ lower, ng/kg whole	0.029	0.030	0.389	0.470	0.259	0.270
TEQ upper, ng/kg whole	0.044	0.040	0.541	0.470	0.271	0.270
TEQ pg/g whole weight						
PCB 77	0.00	0.00	0.00	0.00	0.00	0.00
PCB 81	0.00	0.00	0.00	0.00	0.00	0.00
PCB 126	0.02	0.02	0.72	0.74	0.42	0.47
PCB 169	0.00	0.00	0.01	0.01	0.01	0.01
TEQ lower, ng/kg whole	0.02	0.020	0.73	0.760	0.43	0.480
TEQ upper, ng/kg whole	0.02	0.020	0.73	0.760	0.43	0.480
Total TEQ lower, ng/kg whole	**0.05**	**0.05**	**1.12**	**1.23**	**0.69**	**0.75**
Total TEQ upper, ng/kg whole	**0.06**	**0.06**	**1.27**	**1.23**	**0.70**	**0.75**

Milk

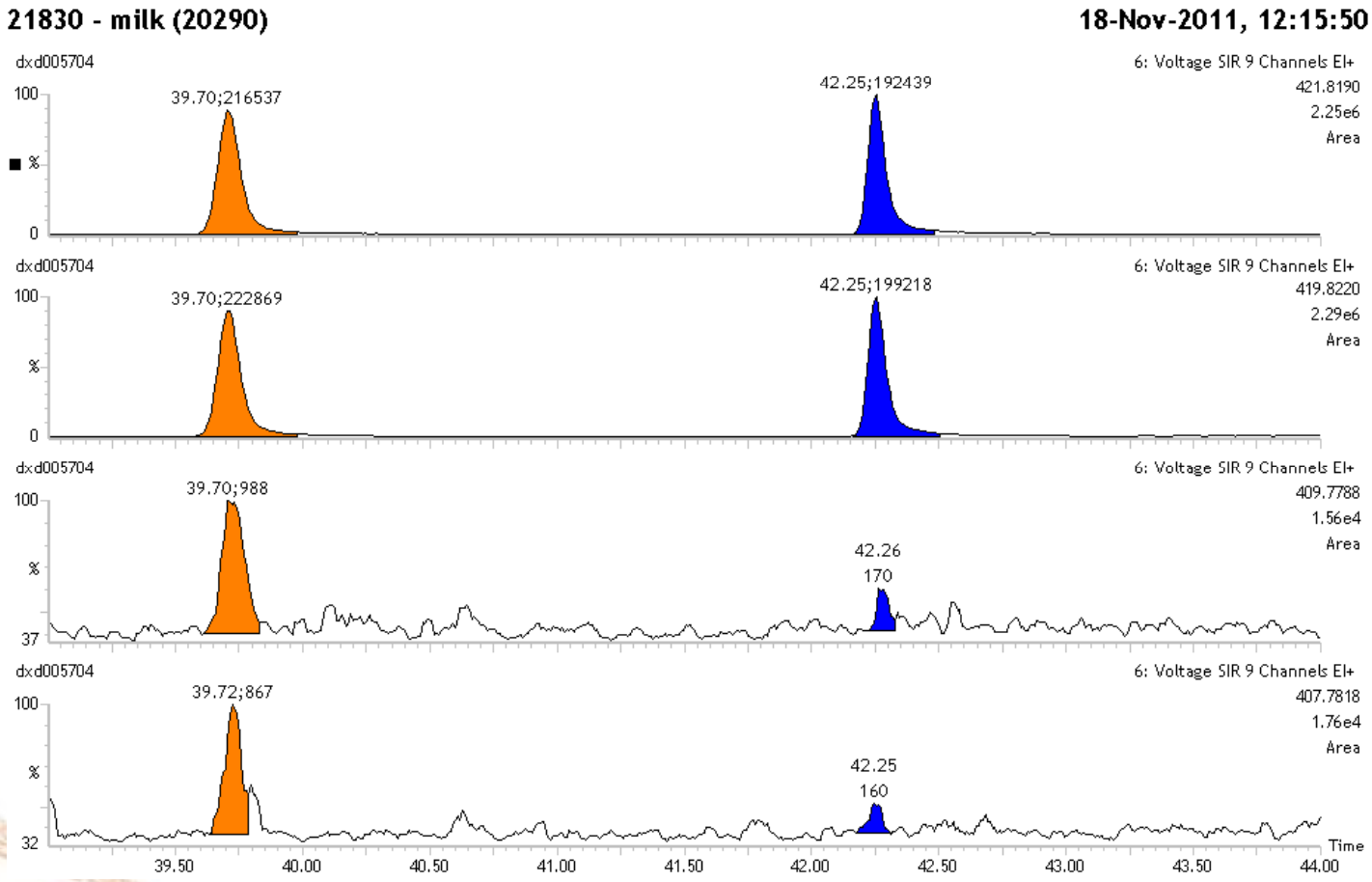
	GC-MS/MS	High Res. MS
Total TEQ lower, ng/kg fat	0.662	0.750
Total TEQ upper, ng/kg fat	0.780	0.750

ML = 2.5 pg/g fat PCDD/Fs only [5.5 pg/g fat inc. PCBs]

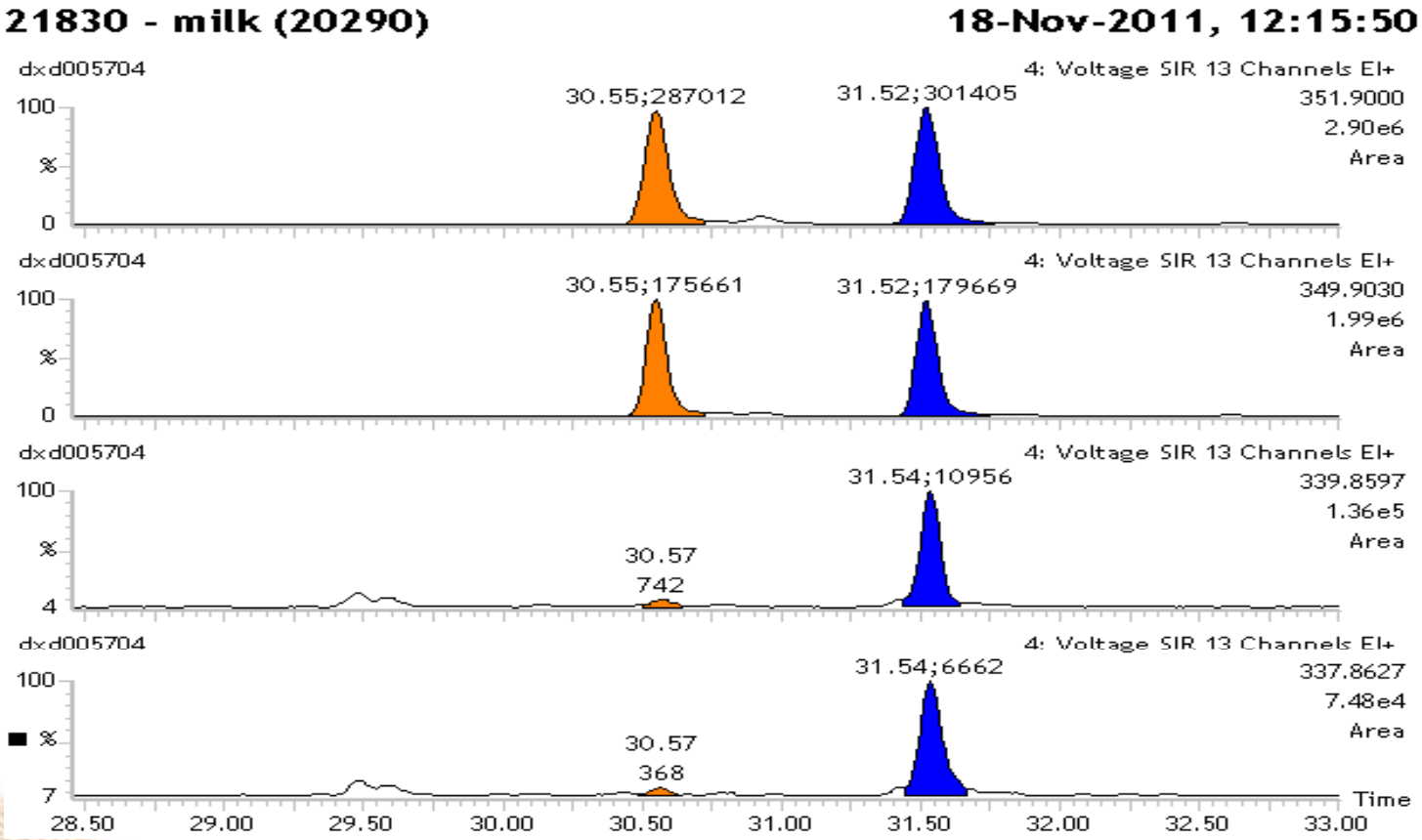
HRMS



2378-TCDD in milk - approx. 0.78pg/extract by HRMS-MS (<0.01pg/g whole weight, 0.11pg/g fat weight)

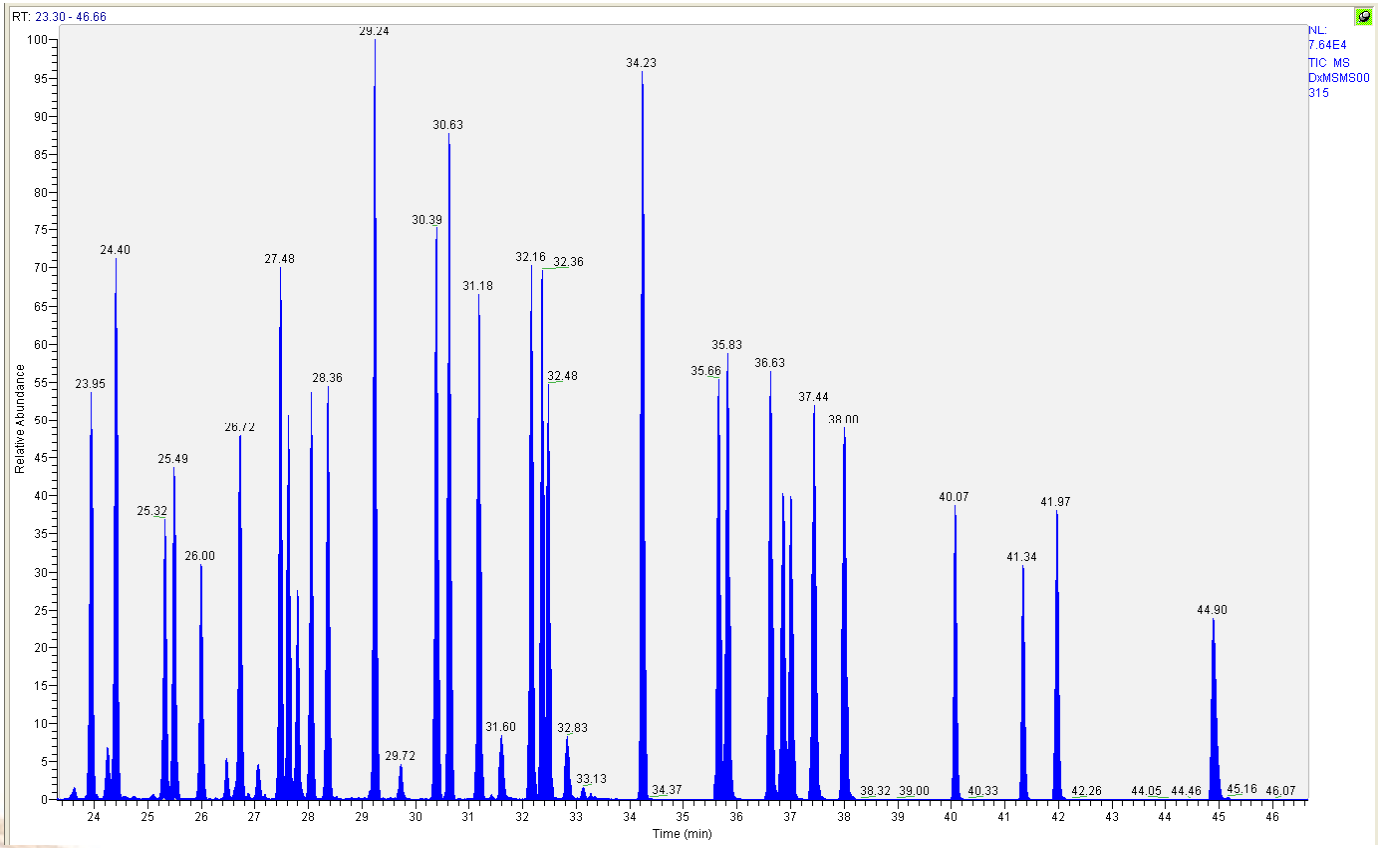


Heptafurans - ion ratios all in range. 0.06 and 0.01 pg/g fat

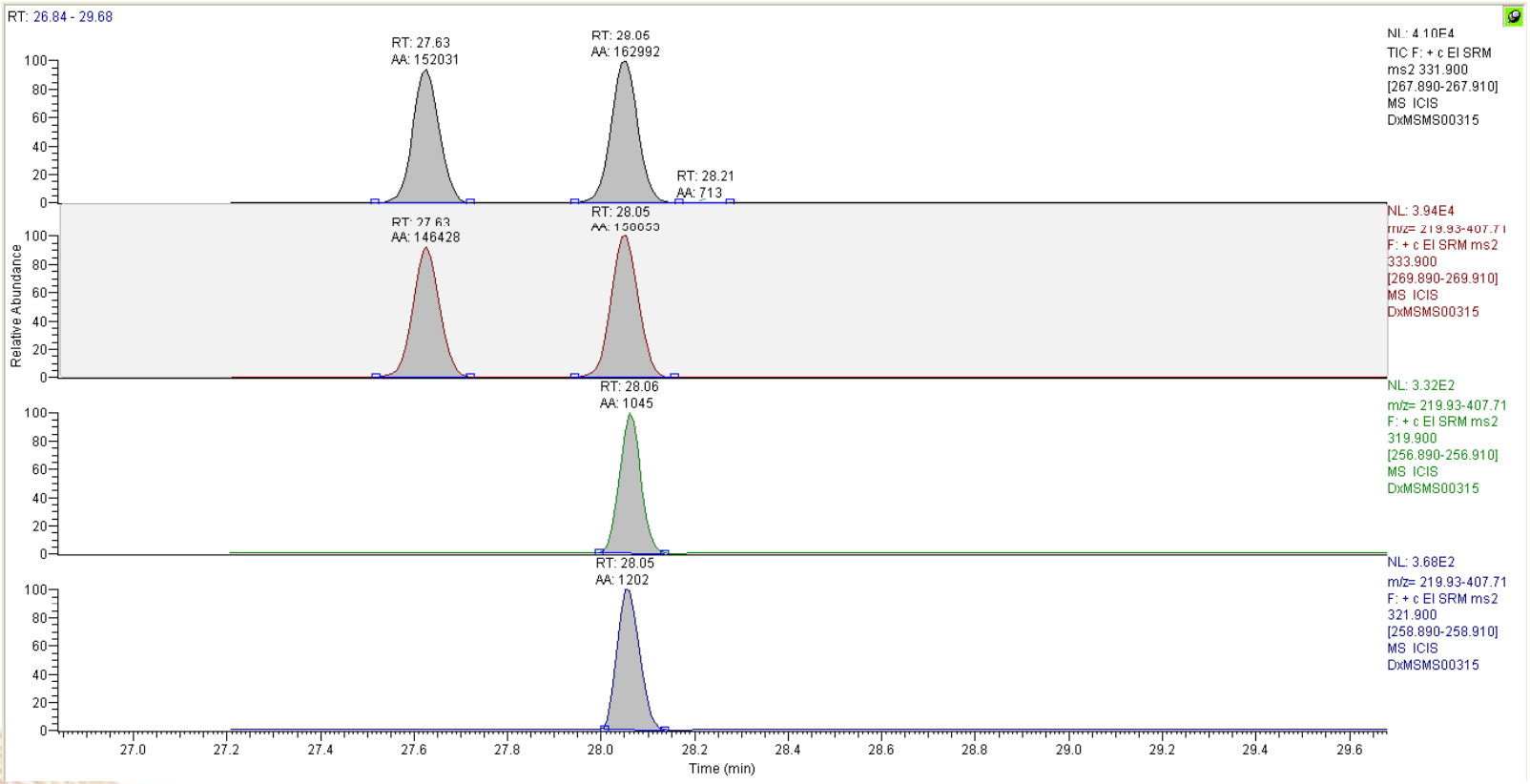


Pentafurans @ 0.26 and 4.52 pg/extract respectively (12378 and 23478) equates to <0.01pg/g whole weight and 0.03pg/g whole weight or 0.04 and 0.63 pg/g fat weight respectively

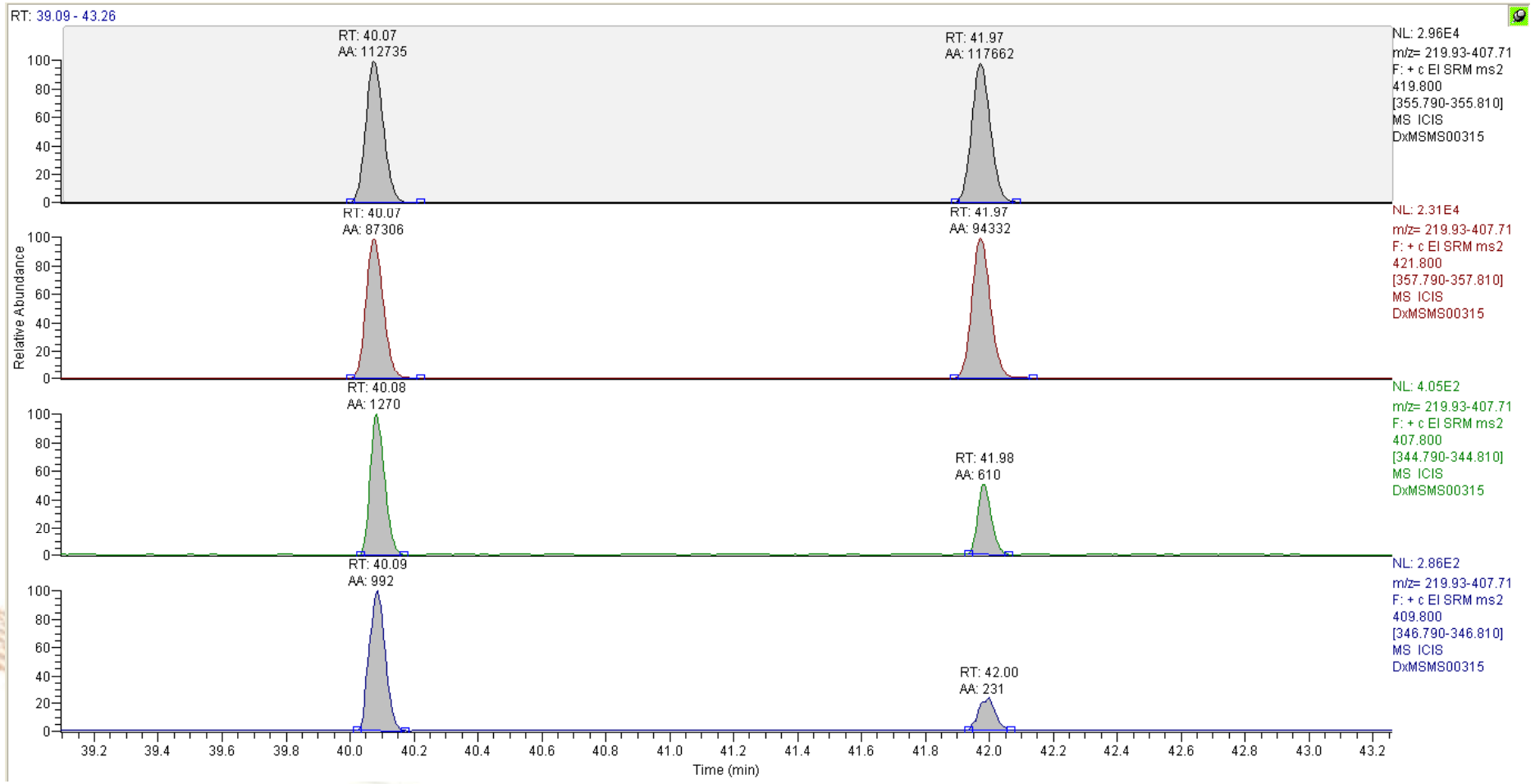
TSQ



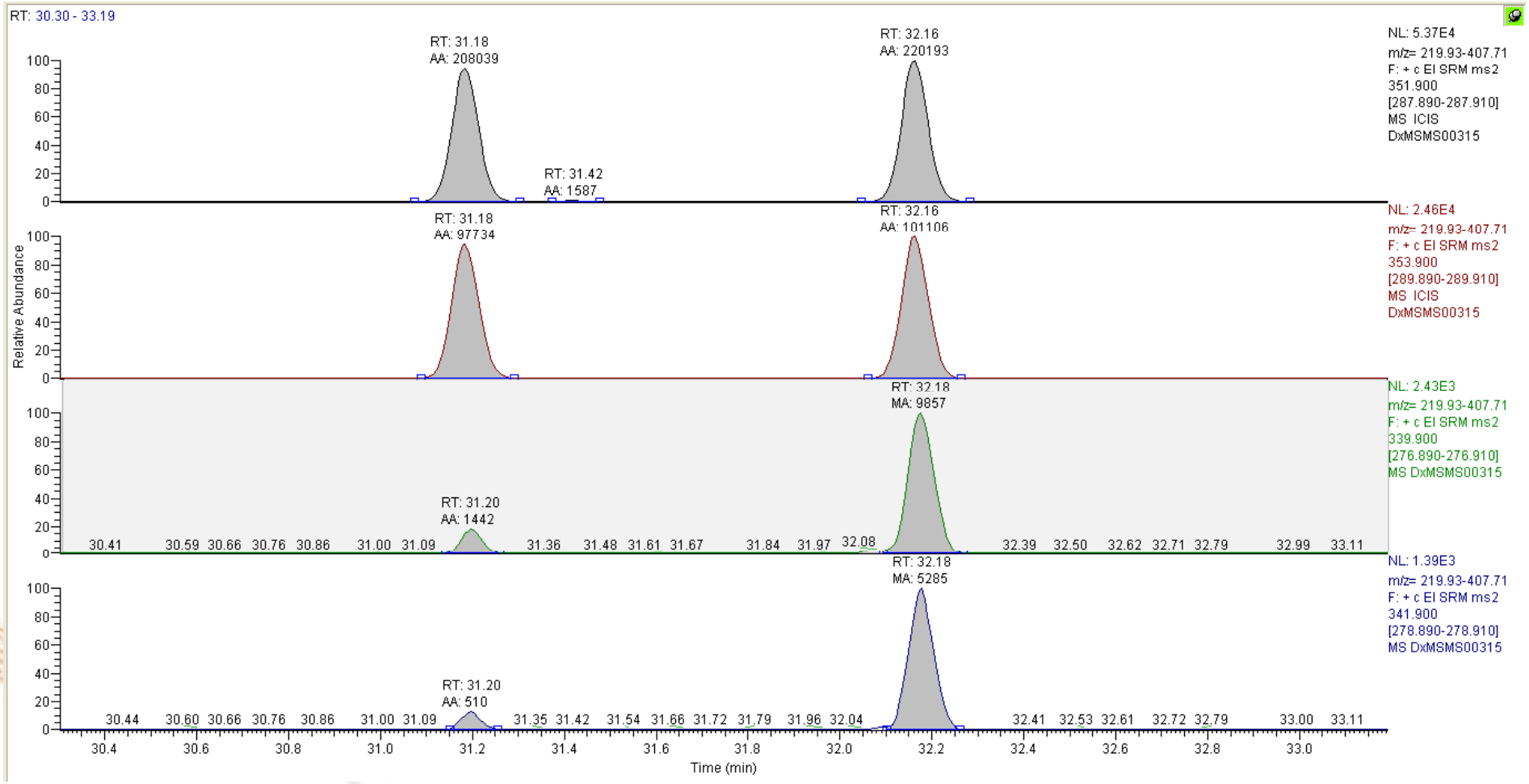
Milk sample – all transitions in one chromatogram



^{13}C 2378TCDD (top two ions); 2378TCDD (lower 2 ions)– approx. 0.65 pg/extract (< 0.01pg/g whole weight, 0.11pg/g fat weight)



Heptafurans in milk – 2nd native congener ion ratio is out 0.06 and 0.01 pg/g fat



Pentafurans in milk

12378 Pentafuran native ion ratio out of range – approximately 0.7 pg/extract

23478 Pentafuran native ion ratio in range – approximately 4.5 pg/extract

<0.01pg/g whole weight and 0.03pg/g whole weight or 0.04 and 0.63 pg/g fat weight respectively

Fish feed

	GC-MS/MS	High Res. MS
TEQ pg/g whole weight		
Total TEQ lower, ng/kg whole	0.389	0.470
Total TEQ upper, ng/kg whole	0.541	0.470

ML = 1.25 pg/g PCDD/Fs only [whole weight, based on 12% moisture; 4.0 pg/g inc. PCBs]

Salmon

	GC-MS/MS	High Res. MS
TEQ pg/g whole weight		
Total TEQ lower, ng/kg whole	0.259	0.270
Total TEQ upper, ng/kg whole	0.271	0.270

ML = 3.5 pg/g wet wt PCDD/Fs only
[6.5 pg/g wet weight inc PCBs]

Conclusions

- Very sensitive – within one order of magnitude of HRMS? – but unable to estimate noise and verify LOD
- Can measure dioxins for compliance with regulatory limits
- ∑ ICES 6 PCBs – no problem!
- At lower levels ion ratios out of tolerance - due to lack of noise?
- Screening method? Confirmatory standard?
- Good clean-up and expertise still needed!

- Need to undertake ‘repeatability’ study on real samples to evaluate consistency

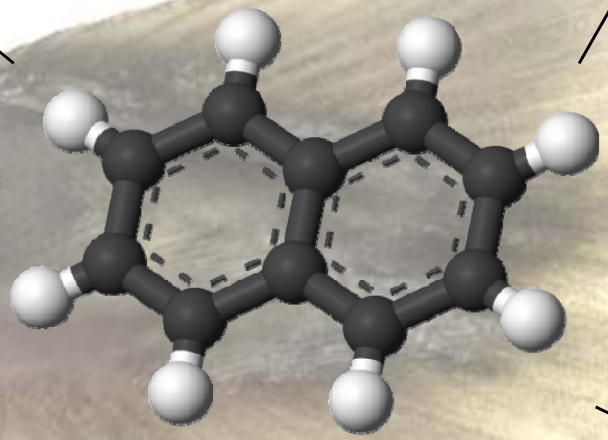
PCNs

Low viscosity oils to high melting point solids

Halowax, Nibren waxes, Seekay waxes, Cerifal Materials, N-Oil

MP = 260 ºC – 440 ºC

BP = -2.3 ºC – 192 ºC



Uses:

Insulating coatings for electrical wires,

wood preservatives,

rubber and plastic additives,

capacitor dielectrics,

lubricants.

75 Congeners

Bioaccumulation

Long-range atmospheric transport

Persistence = PCNs still being detected in several matrices even after production has been ceased

Toxicity and ecotoxicity – Dioxin like evidence

PCNs

- ‘Legacy’ contaminants
- Used widely until 1950s; similar to PCBs
- Can be formed from *de novo* synthesis during combustion

- In 1990s PCB usage perceived as more recent than PCN usage – PCBs received more attention

Now both are about half a century legacy problem

PCNs in food

- Ubiquitous, most prevalent in fish
- PCNs 52, 66/67, and 73 most often found
- Few 10s of ng/kg (∑ 11 congeners) common in fish

Toxicity of PCNs

- Dioxin-like toxicity typically 1-2 orders of magnitude lower than for dioxins
- Exhibit other forms of toxicity

Risk assessment

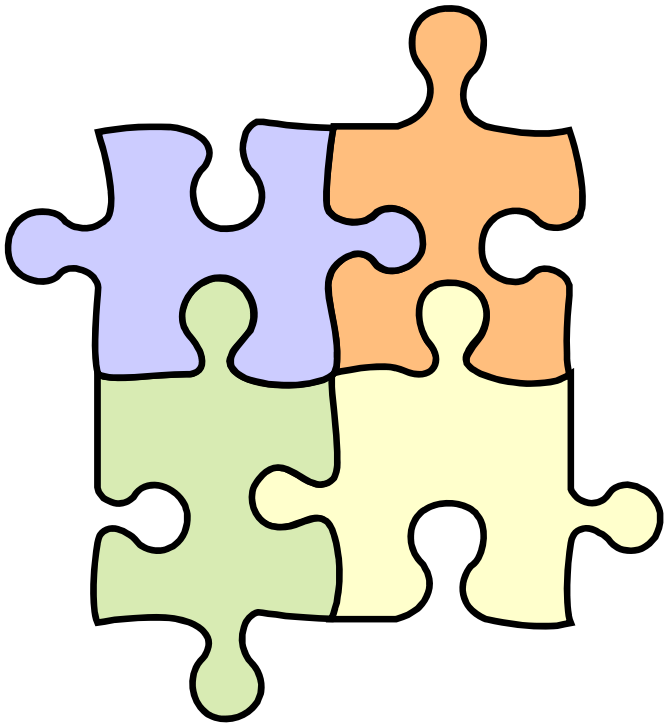
- TDI, TWI and TMI values are based on 2378-TCDD and extrapolated to dioxin-like compounds
- Originally applied to PCDD/Fs with the use of TEFs
- Later extended to dl-PCBs

Should PCNs be included?

Completing the jigsaw

- picture we are getting for dioxins in food has been clearing over the last years with more and more data
- far from complete
- Dioxin-like PCBs generally included in the overall picture

many more compounds have a similar mode of toxic action



Exposure and risk assessment

- Lower concern for PCNs based on limited background data

But.....

- Adds to total TEQ?
- Other modes of toxic action
- Very little monitoring undertaken
- Number of specific contamination incidents (where exposure might become important) cannot be reliably estimated

HRMS

- Sensitive
- Conventional ‘gold standard’ confirmatory method for dioxins
- Expensive (~ € ¼M)
- Highly skilled operators required

GCMSMS

- Lacks sensitivity

Or does it?

But.....

- Cheaper
- Versatile
- Easier to use

Reproducibility

Cod liver oil – 5 replicate extracts analysed by GC-HRMS and GC-MS/MS

HRMS - replicate extracts (in pg/g whole weight)

	1	2	3	4	5	n	Mean	CV%
PCN52	371.87	373.99	379.81	381.98	380.12	5	377.6	1.2
PCN53	6.50	7.07	7.23	6.20	6.75	5	6.8	6.2
PCN66/67	40.67	37.48	33.89	37.00	37.00	5	37.2	6.5
PCN64/68	7.85	8.31	8.41	8.71	7.83	5	8.2	4.6
PCN69	9.03	8.75	8.35	8.34	8.48	5	8.6	3.5
PCN71/72	7.52	6.85	7.17	7.22	7.01	5	7.2	3.5
PCN73	3.42	3.69	3.06	3.14	3.74	5	3.4	9.1
PCN74	0.65	0.64	0.72	0.73	0.76	5	0.7	7.5
PCN75	0.12	0.10	0.10	0.12	0.10	5	0.1	10.3

MS/MS - replicate extracts - replicate extracts (in pg/g whole weight)

	1	2	3	4	5	n	Mean	CV%
PCN52	366.42	378.35	368.16	368.76	364.84	5	369.3	1.4
PCN53*	*5.60*	*6.69*	*5.89*	*5.26*	*6.07*	*5*	5.9	*9.1*
PCN66/67	31.45	32.09	33.33	31.18	30.76	5	31.8	3.1
PCN64/68	8.41	8.95	8.13	9.13	8.72	5	8.7	4.7
PCN69	7.43	7.39	8.03	7.77	8.21	5	7.8	4.6
PCN71/72	5.85	6.43	7.07	6.88	6.65	5	6.6	7.2
PCN73	2.65	3.21	2.73	2.37	2.84	5	2.8	11.1
PCN74	*<0.33*	*<0.59*	0.70	0.66	0.62	3	0.7	5.6
PCN75	*<0.20*	*<0.10*	*<0.21*	*<0.21*	0.18	1	0.2	-

*PCN 53 could not be confirmed by MS/MS as second transition was masked by the syringe standard

Comparison of PCN results using HRMS and MS/MS

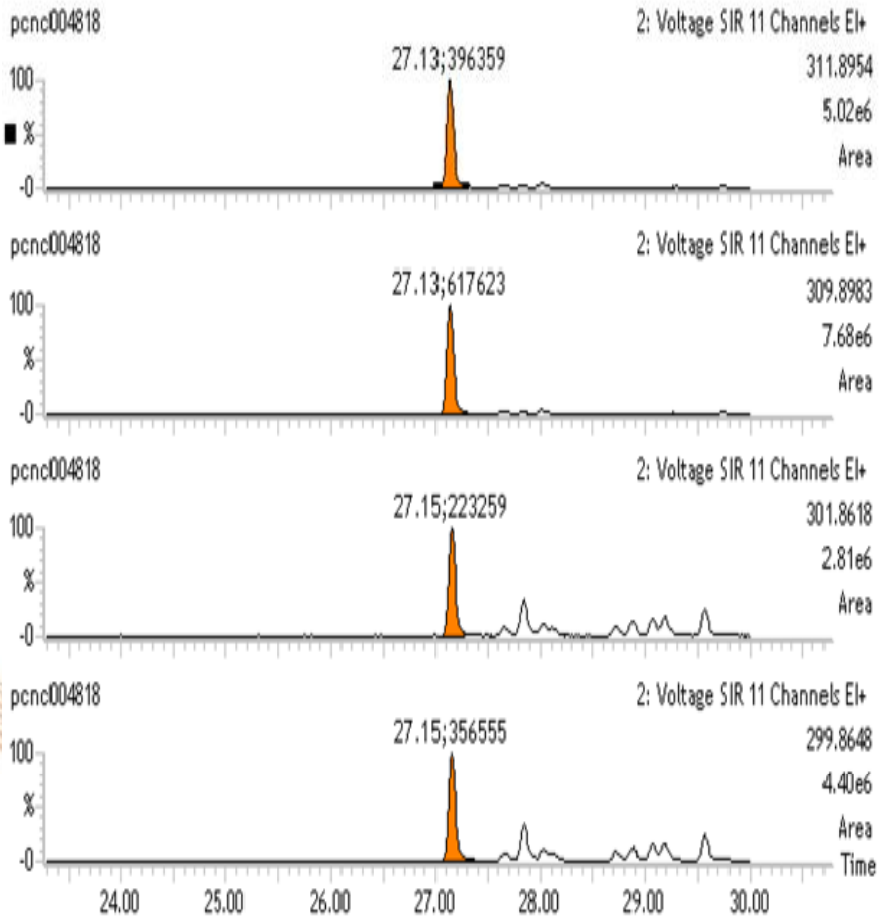
Results in pg/g fat	fish		eggs		fats & oils	
	HRMS	MS/MS	HRMS	MS/MS	HRMS	MS/MS
PCN52/60	47.52	49.74	5.40	5.98	0.53	<0.80
PCN53	7.55	7.61*	1.11	1.01*	0.44	0.47*
PCN66/67	6.11	5.90	0.85	0.93	0.23	<0.44
PCN64/68	2.00	2.32	0.62	0.65	<0.06	0.06
PCN69	2.78	2.31	0.88	1.03	<0.11	<0.18
PCN71/72	3.33	3.00	0.53	0.53	0.10	<0.11
PCN73	0.75	<0.93	0.48	0.32	0.08	<0.05
PCN74	0.25	<0.19	<0.056	<0.04	<0.06	<0.03
PCN75	<0.17	<0.32	<0.105	<0.13	<0.11	<0.09

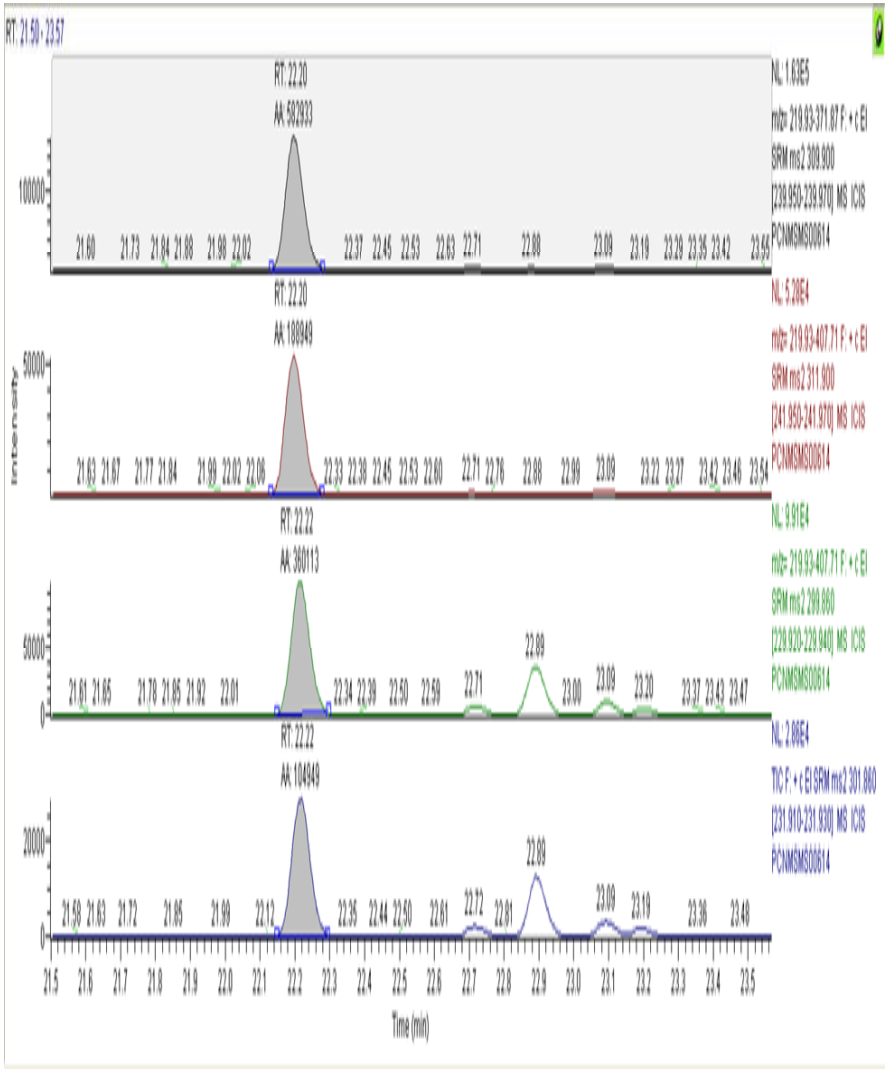
PCN 52/60 in fish

^{13}C PCN 52

^{13}C

^{13}C





HRMS

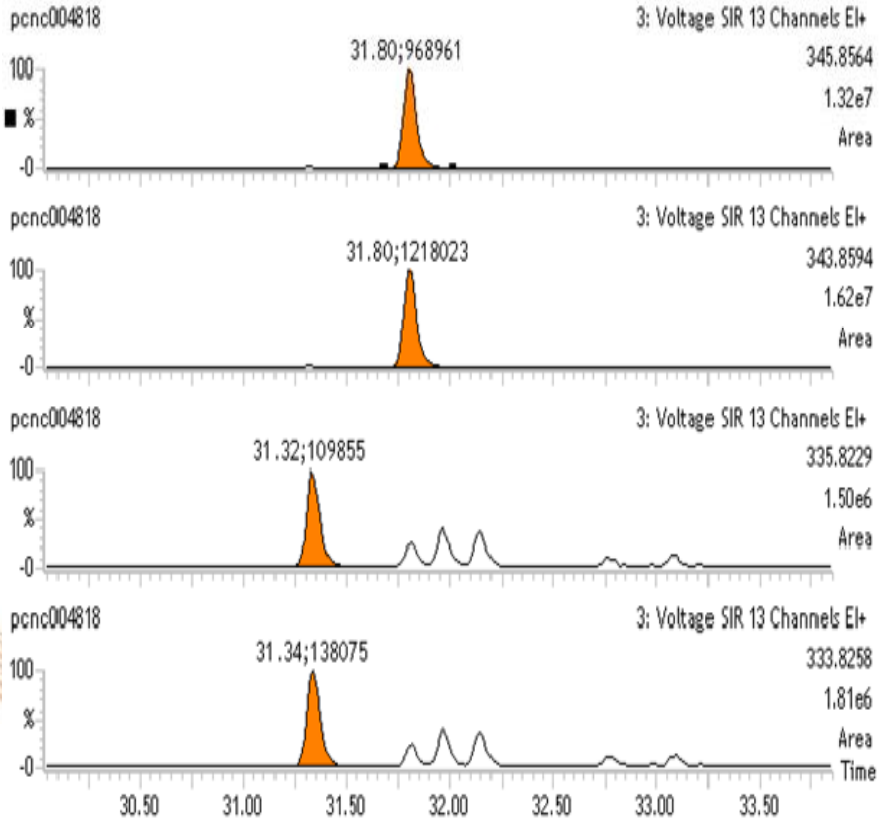
MS/MS

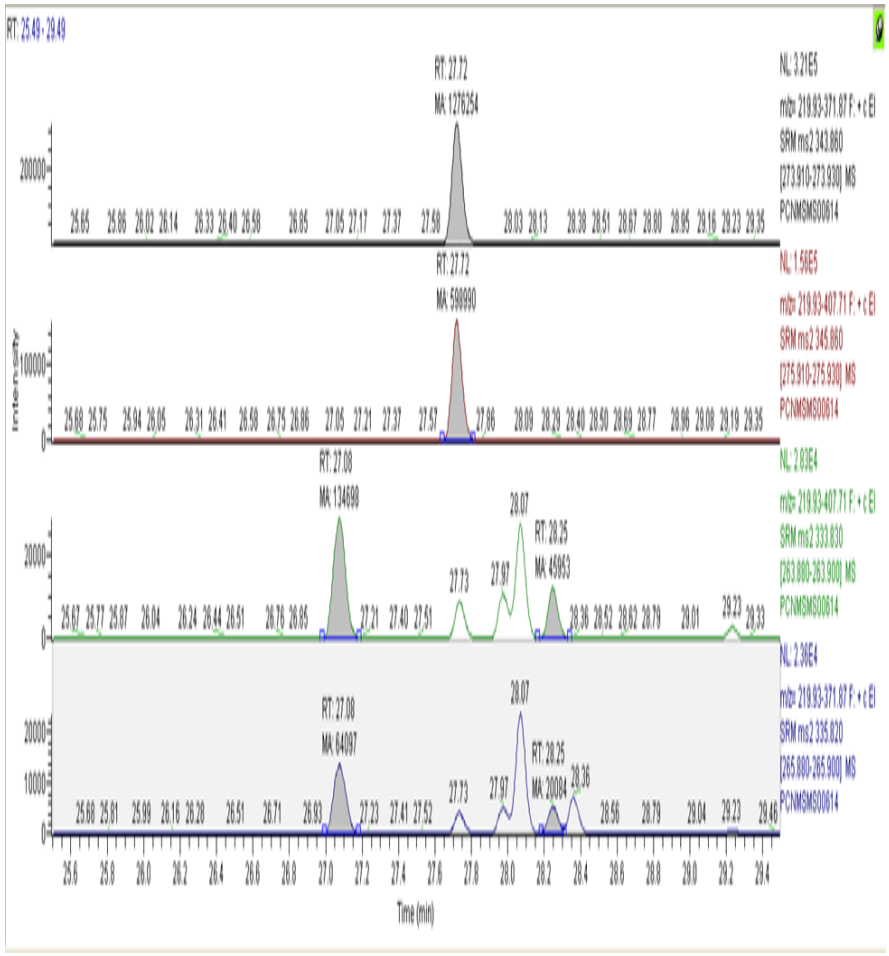
PCN 66/67 in fish

^{13}C PCN-64

^{13}C

^{13}C





HRMS

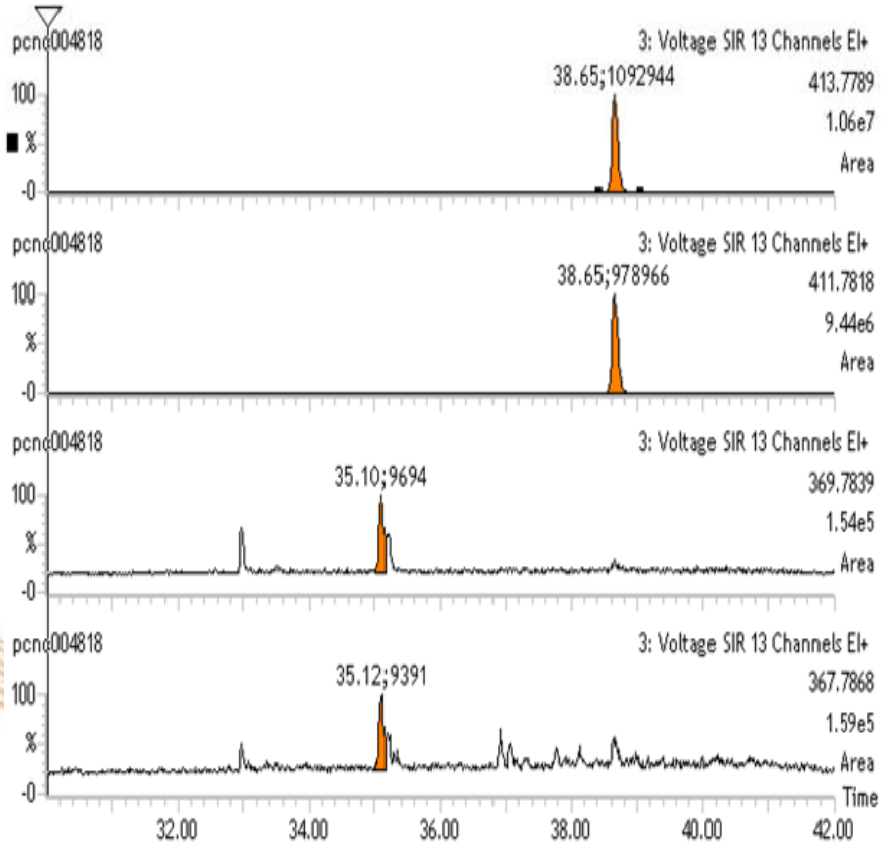
MS/MS

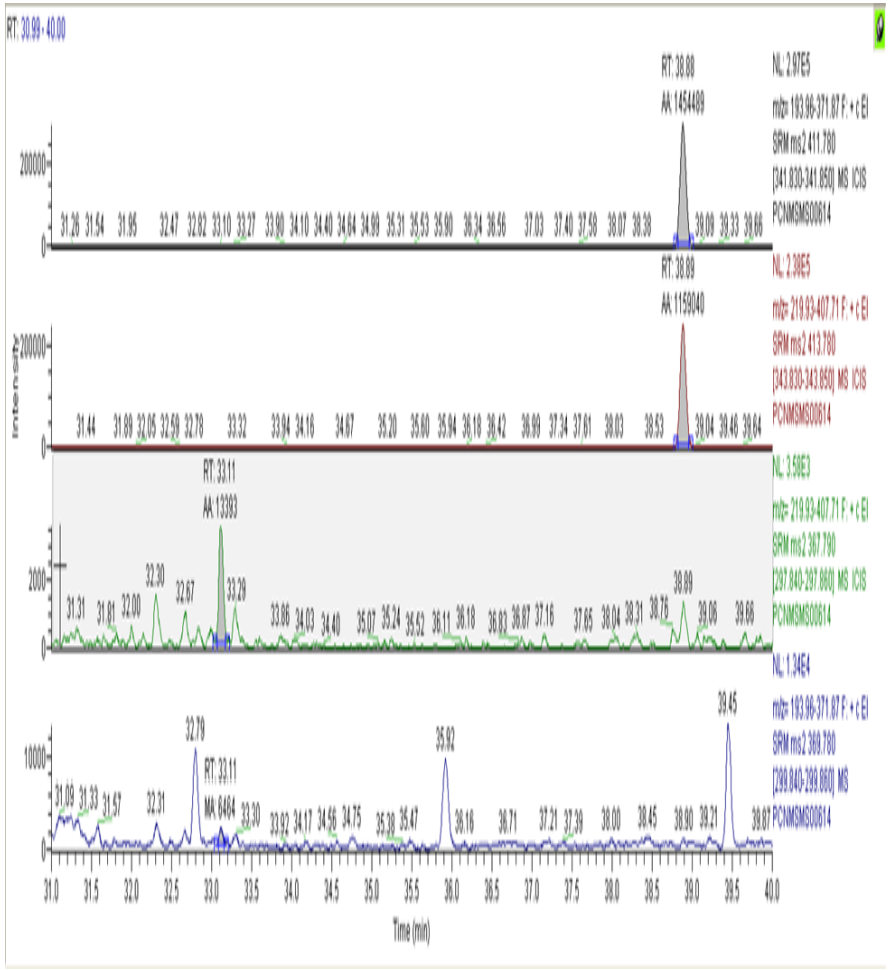
PCN 73 in fish (incomplete separation from PCN-74)

^{13}C PCN 75

^{13}C

^{13}C



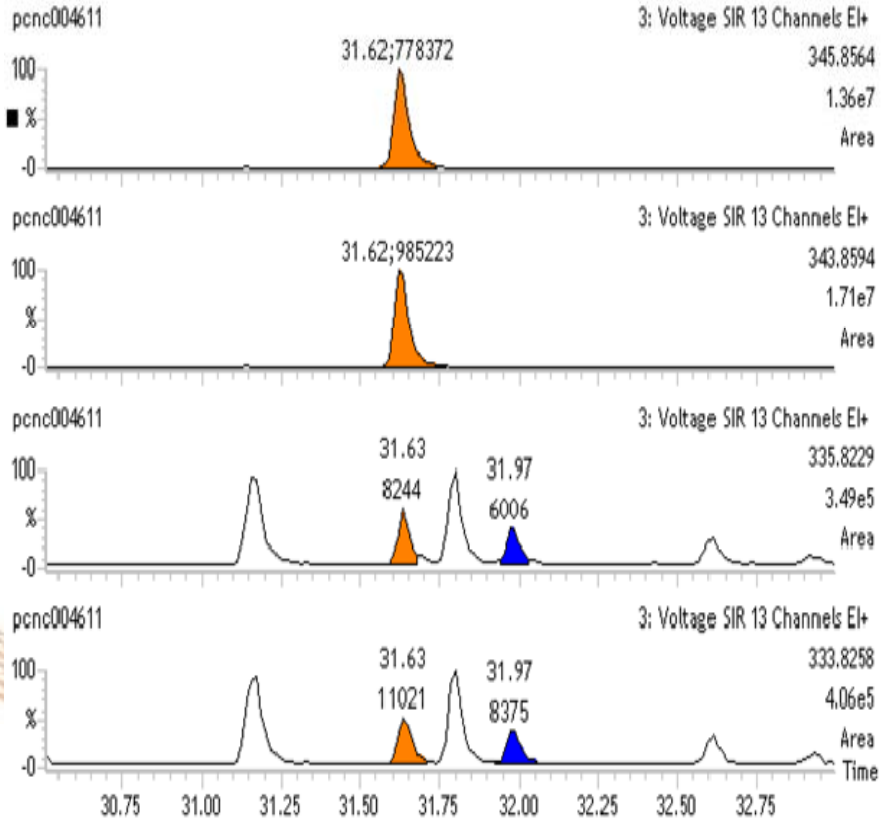


HRMS

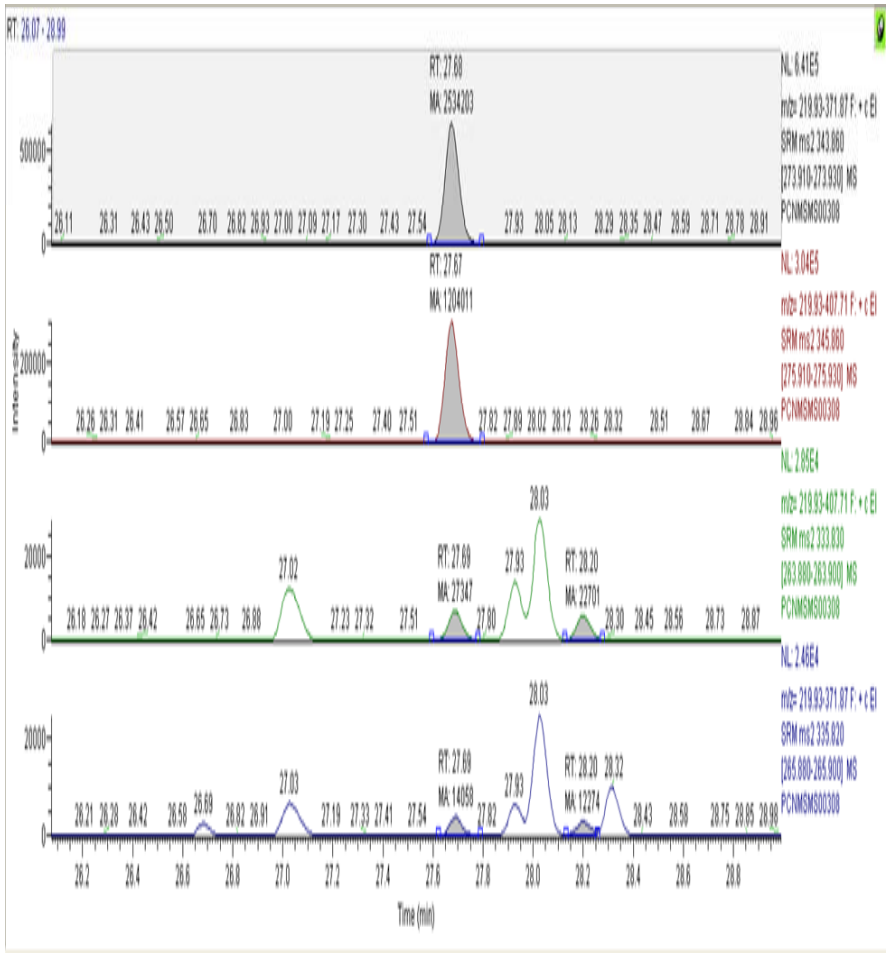
MS/MS – small peaks result in poor ion ratios for PCN 73 in fish

PCN 64/68 & PCN 71/72 in eggs

^{13}C PCN 64



HRMS: PCN 64/68 (orange) and PCN 71/72 (blue) in eggs



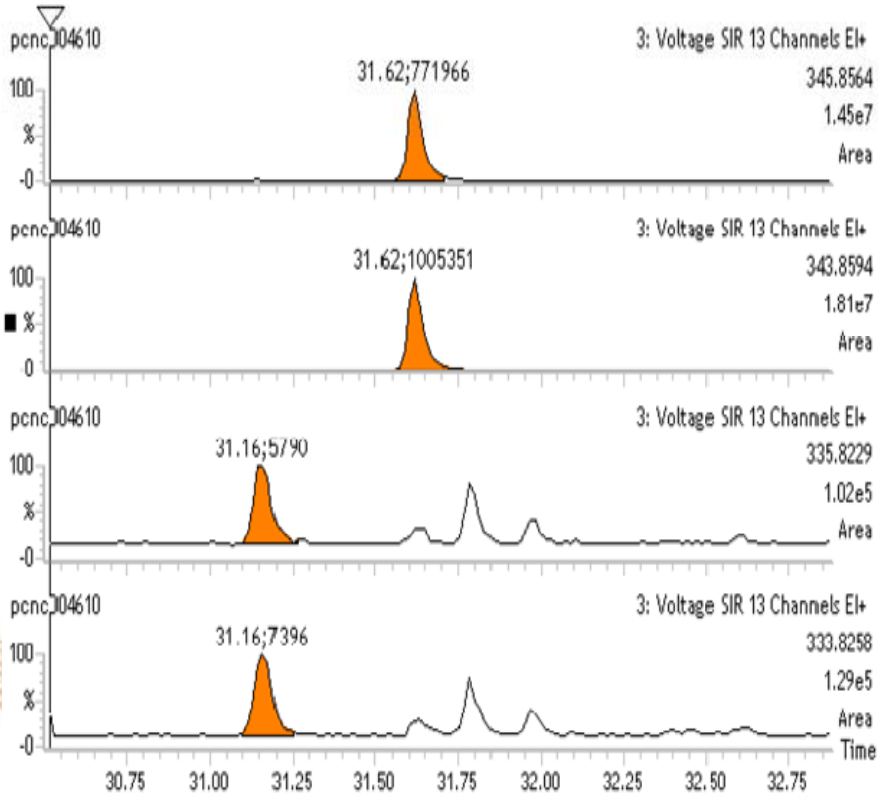
MS/MS: PCN 64/68 and PCN 71/72 in eggs

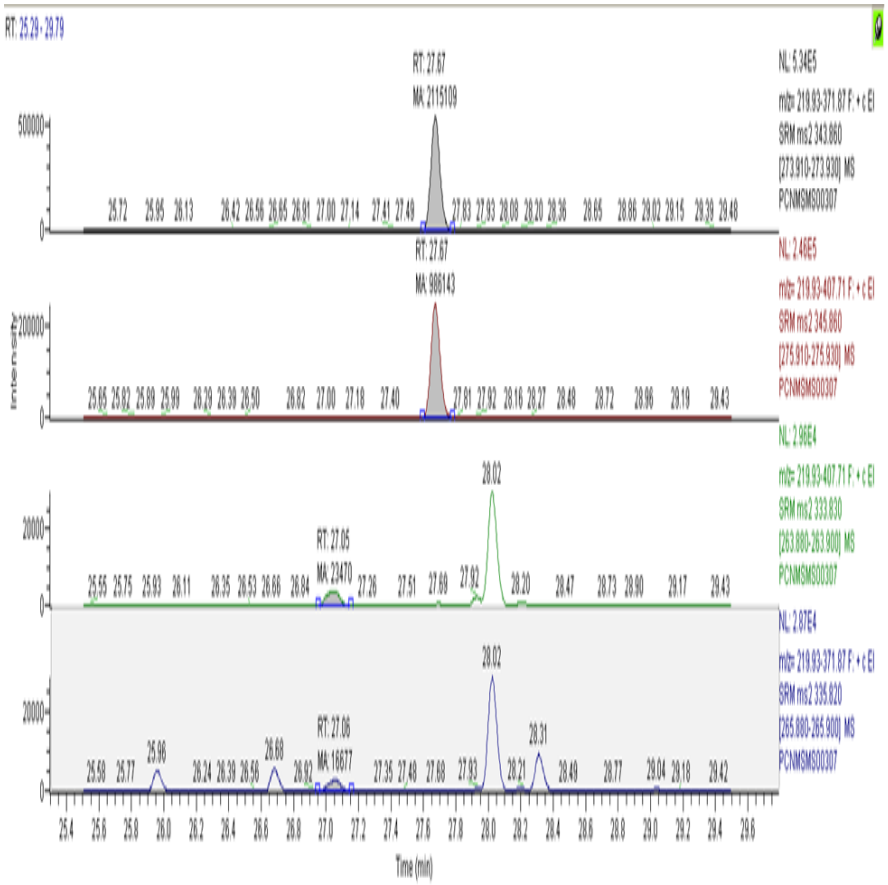
PCN 66/67 in fats & oils

^{13}C PCN 64

^{13}C

^{13}C



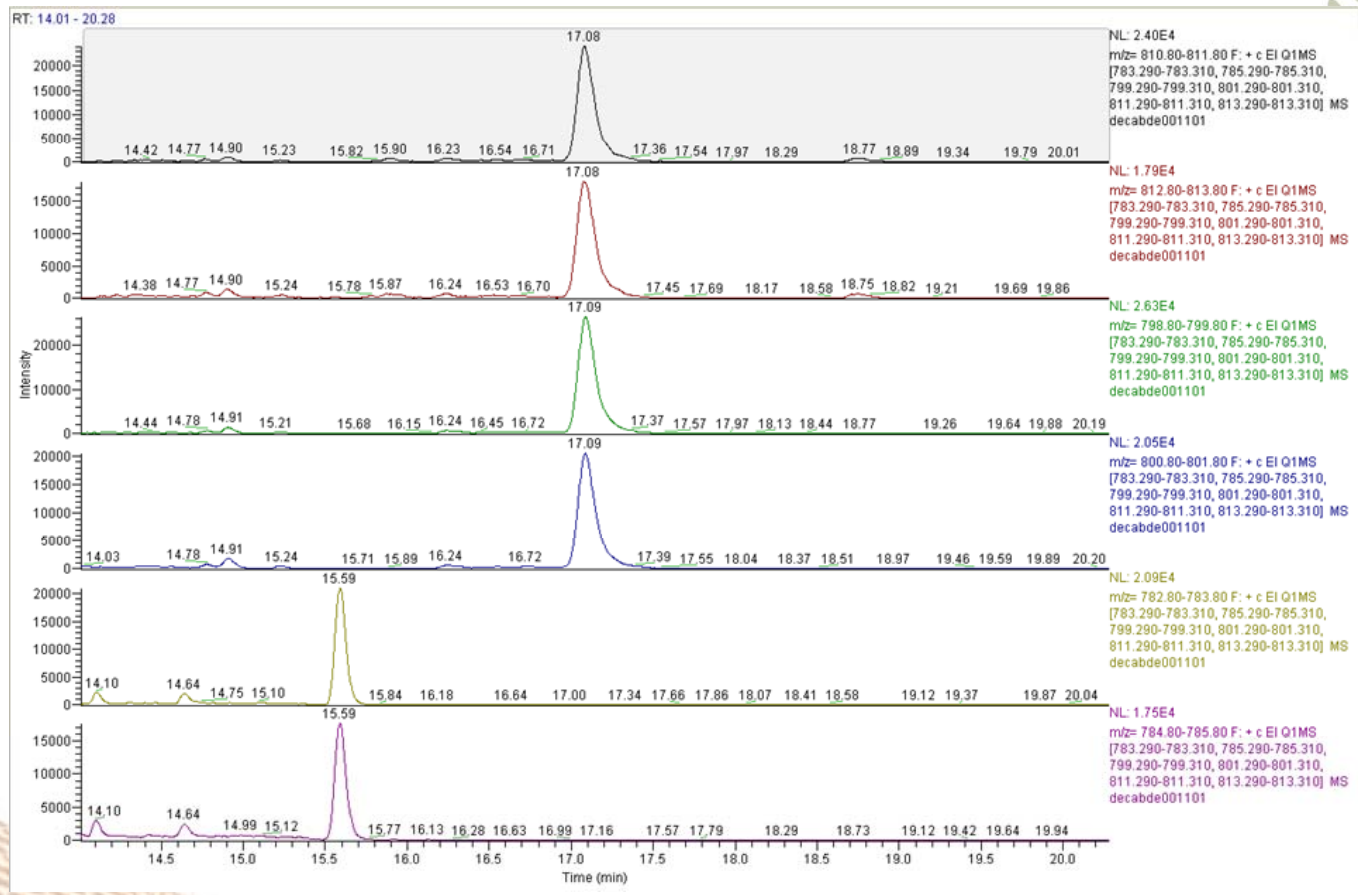


HRMS

MS/MS

Summary

- PCNs are ubiquitous in the environment and in food
- Contribute to dioxin-like toxicity, but not yet included in TEQ system or regulatory framework
- HRMS offers best analysis
- MS/MS is capable of measuring at levels found in food for major congeners

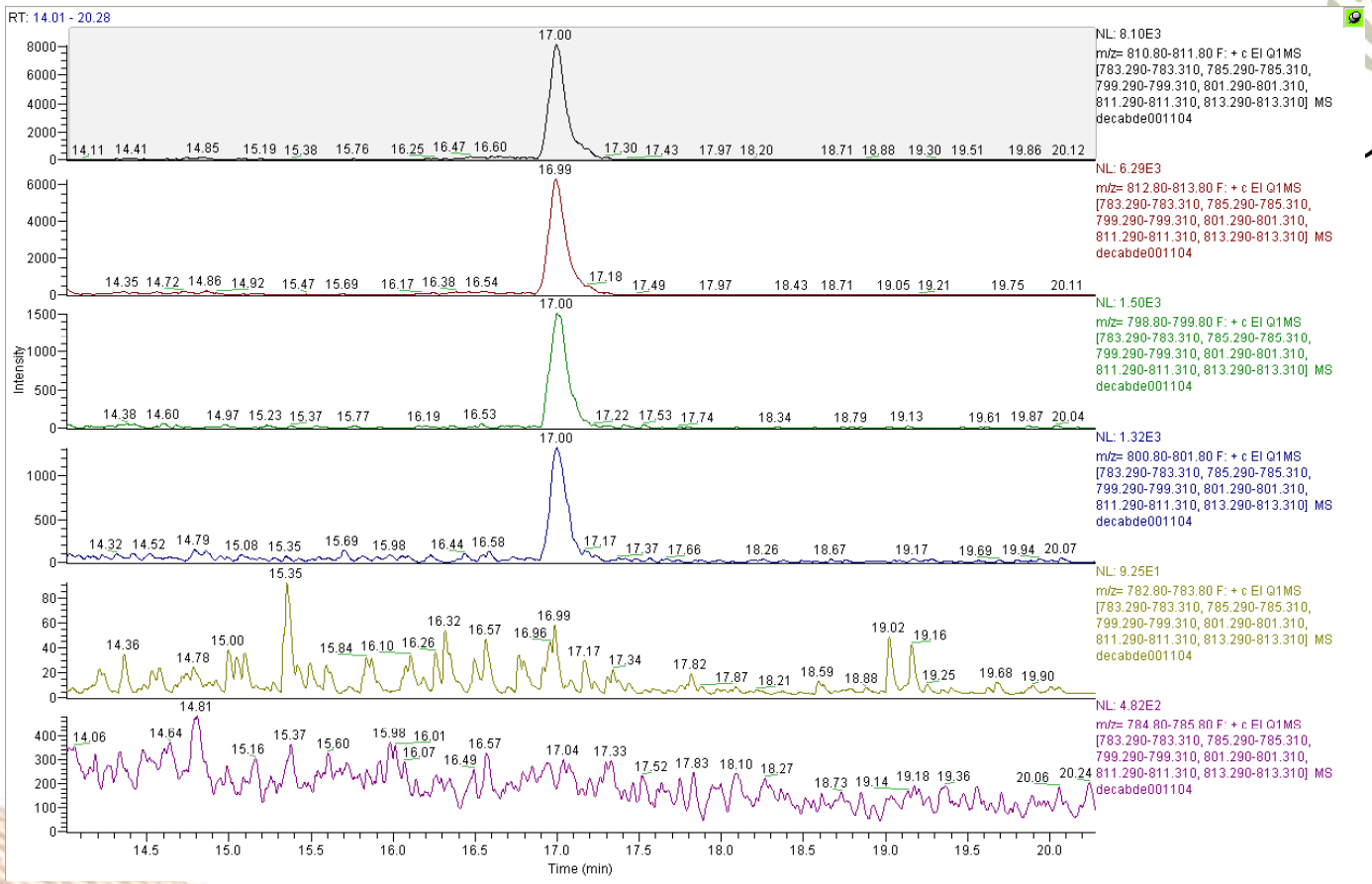


^{13}C-BDE-209

BDE-209

BB-209

RRF calibration std. – single quad. mode – 50pg/ul

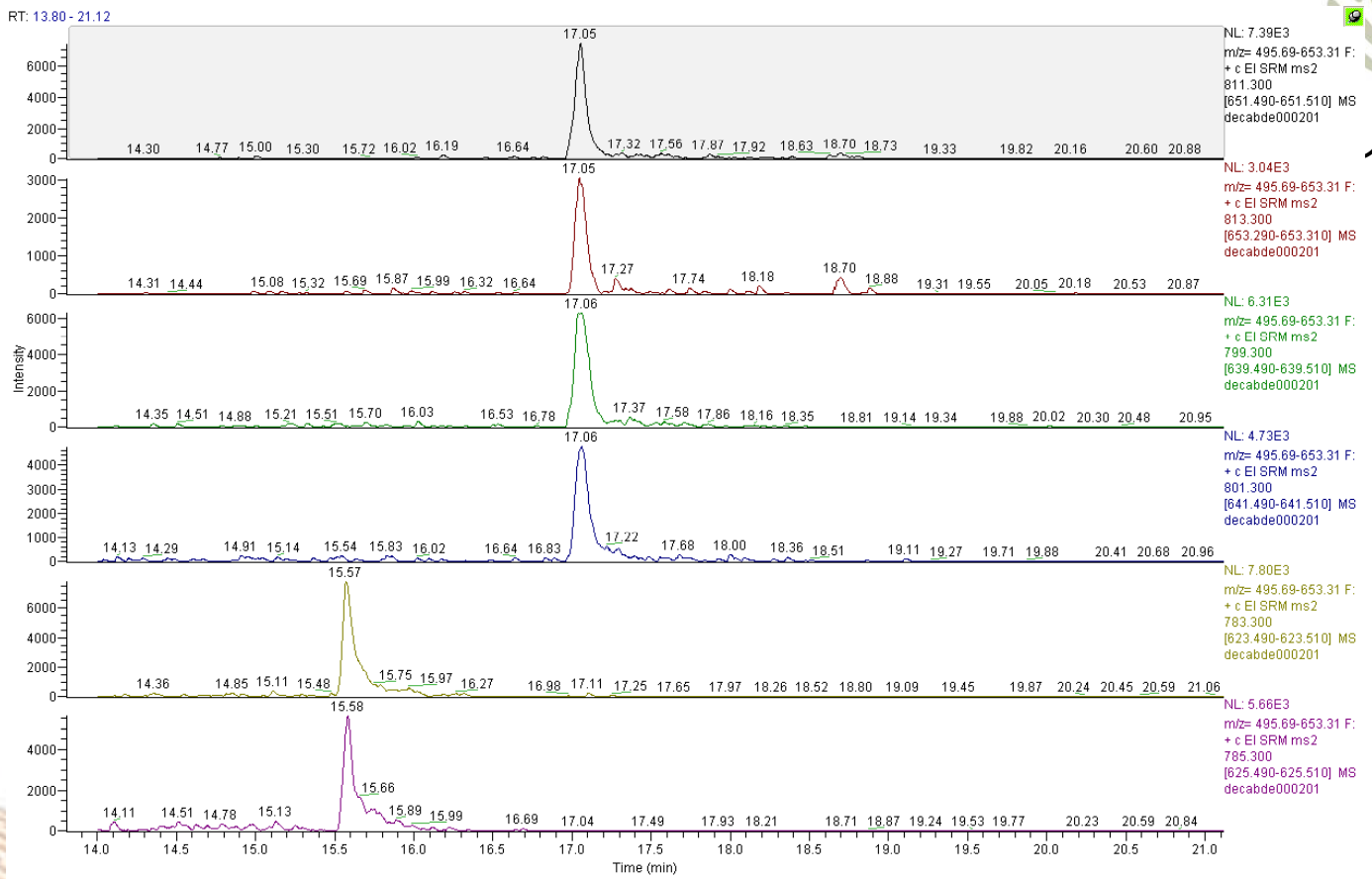


^{13}C-BDE-209

BDE-209

BB-209

‘Blank’ extract – single quad. Mode (some BDE-209 present)

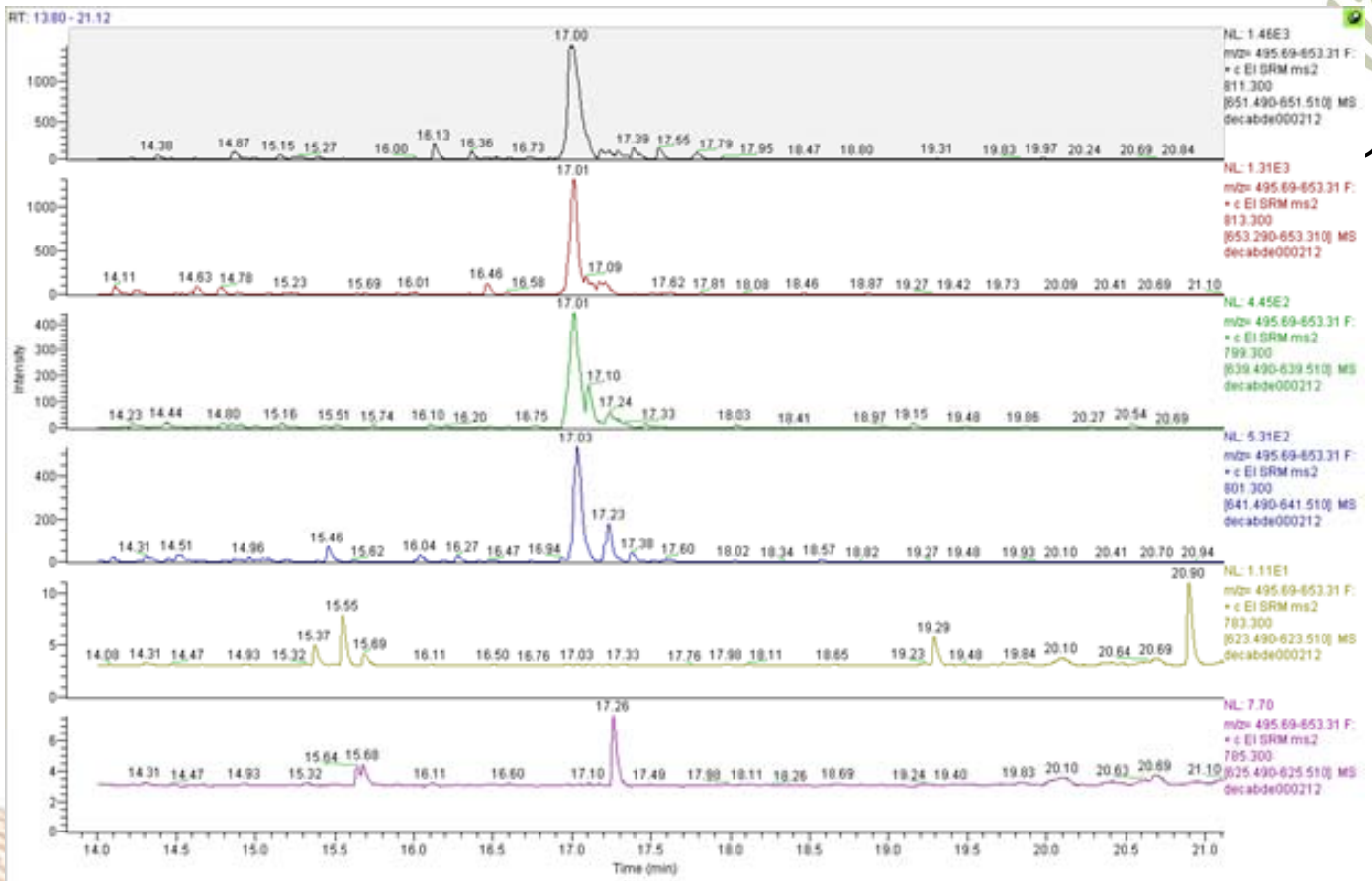


^{13}C-BDE-209

BDE-209

BB-209

RRF calibration std. – MS/MS mode – 50pg/ul



^{13}C-BDE-209

BDE-209

BB-209

‘Turbot’ extract – MS/MS mode

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fera