INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

# HIGH-THROUGHPUT GC-MS/MS ANALYSIS OF BFRs (INCLUDING EMERGING COMPOUNDS) IN FISH/SEAFOOD

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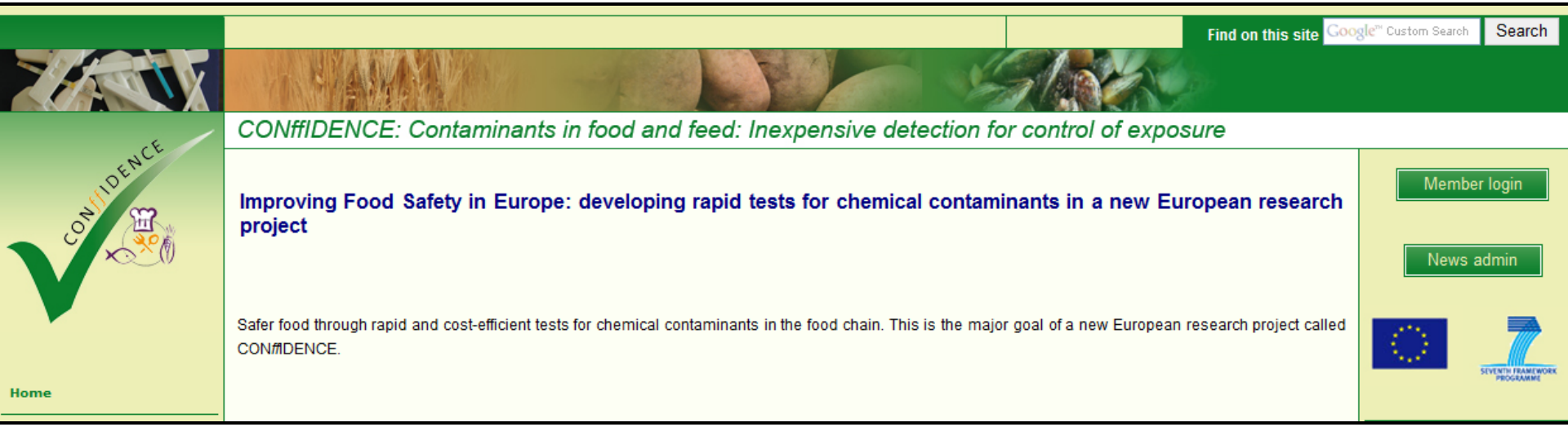
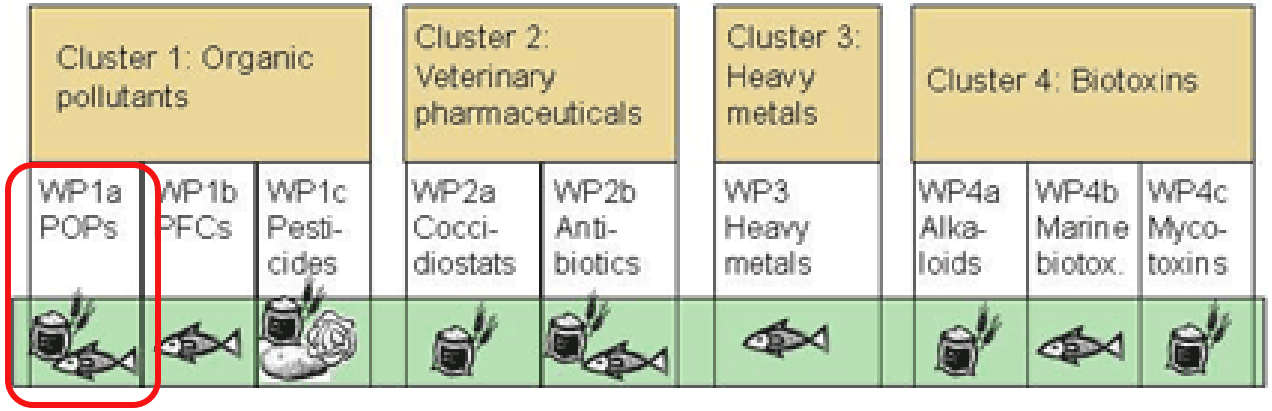
**Department of food chemistry and analysis**

**5th International Symposium on RECENT ADVANCES IN FOOD ANALYSIS**
November 1–4, 2011 Prague, Czech Republic

# CONffIDENCE: CONtaminants in Food and Feed: Inexpensive DEtectioN for Control of Exposure.




ICT PRAGUE

http://www.conffidence.eu/

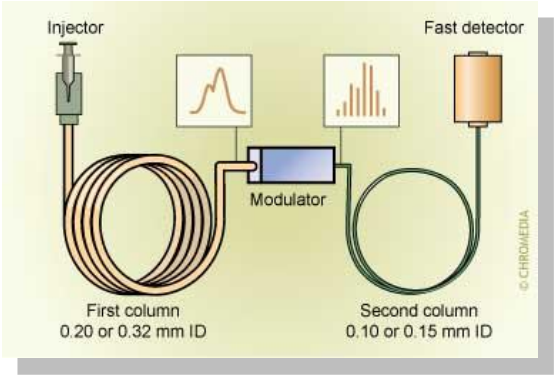
# CONffIDENCE – Project objectives

- To develop a simplified sample preparation strategy for the simultaneous determination of wide range of contaminants in food and feed focused on fish and cereal based baby food.

- To implement a GC×GC–TOFMS for the determination of wide range of contaminants in food and feed.

- Fasten and simplified sample preparation method
- Decrease the consumption of chlorinated solvents
- Decrease the financial cost of analysis

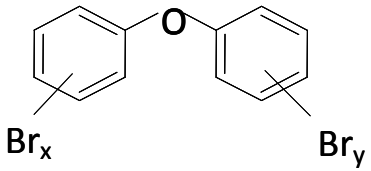
- Obtain high chromatographic resolution and low limits of quantification (LOQ)



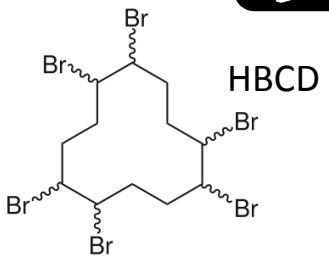
# Target analytes – CONffIDENCE project

- PBDE # 28, 47, 99, 100, 153, 154, 183
- HBCD, PBB # 153

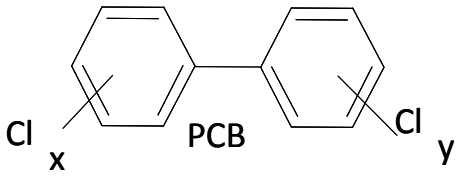
**9 BFRs**




x + y = 1 to 10
PBDE




HBCD



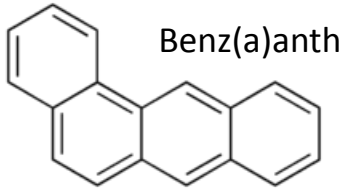

x + y = 1 to 10

- Non-*ortho* PCB # 77, 81, 126, 169
- Mono-*ortho* PCB # 105, 114, 118, 123, 156, 157, 167, 189
- Major PCB # 28, 52, 101, 138, 153, 180

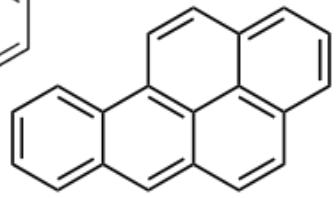
**18 PCBs**

Benzo(c)fluorene
Benzo(k)fluoranthene
Cyklopenta(c,d)pyrene
Dibenzo(a,e)pyrene
Dibenzo(a,h)pyrene
Dibenzo(a,i)pyrene
Dibenzo(a,l)pyrene
5-Methylchrysene
Benz(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(j)fluoranthene
Benzo(g,h,i)perylene
Chrysene
Dibenz(a,h)anthracene
Indeno(1,2,3-cd)pyrene

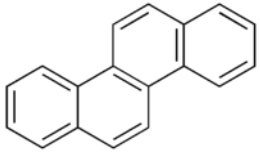
**15+1 EU PAHs**



Benz(a)anthracene

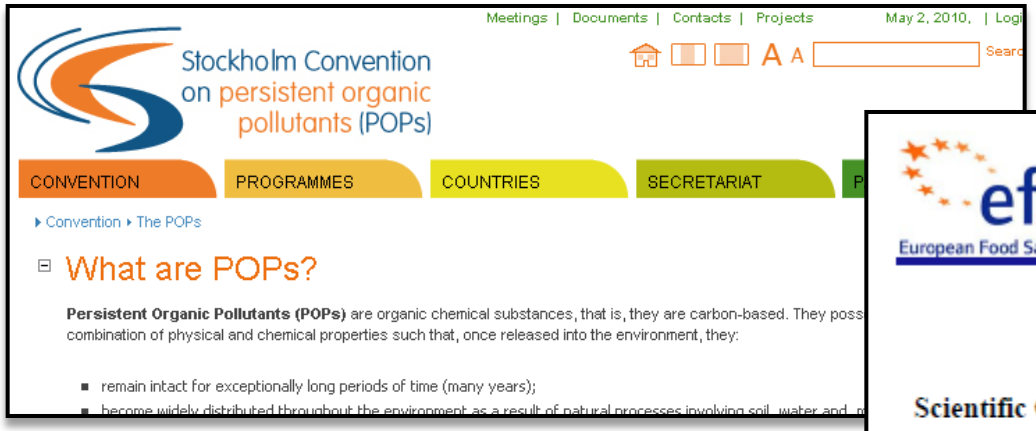


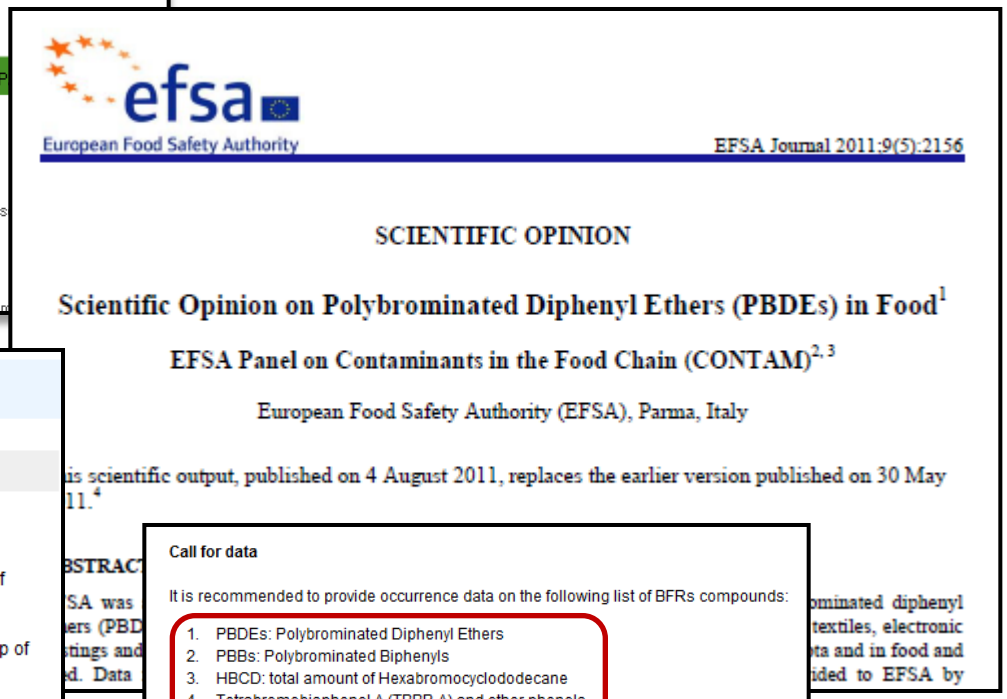
Chrysene

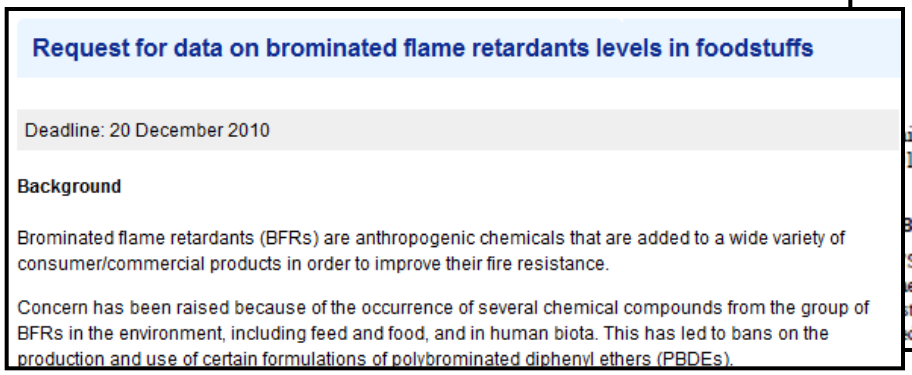


Benzo(a)pyrene

# Target analytes & Legislation




- PBDE # 28, 47, 99, 100, 153, 154, 183
- HBCD, PBB # 153

**9 BFRs**

+

- PBDE # 49, 66, 196,197,203, 206, 207, 209
- PBT, PBEB, HBB, BTBPE, OBIND, DBDPE

**14 BFRs**

# ACREDITATED SAMPLE PREPARATION

Time consuming, laborious, high consumption of chlorinated solvents….

**Isolation**
**7h / 10 samples**

**Clean up**
**1 h / 1 sample**

**Identification & quantification**
**1 h / 1 sample**

**PBDE + HBCD** | **PCB** | **PAH**

**Soxhlet extraction**
hexane – dichloromethane
(1:1, *v/v*)

**Gel permeation chromatography**
cyclohexane-ethyl acetate

**Gel permeation chromatography**
chlorophorm

**GC/MS (NCI)**

**GC/MS (EI)**

**GC/MS (EI)**
**LC/FLD**

**Non-*ortho* PCB**

**Soxhlet extraction**
hexane – dichloromethane
(1:1, *v/v*)

**Clean up on silicagel column**

**Fractionation on pyrenyl column**

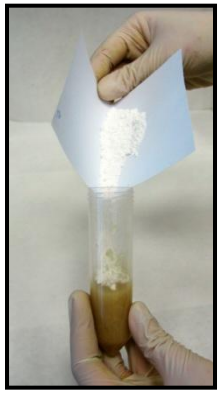
**GC/MS (NCI)**

# INTEGRATED SAMPLE PREPARATION

Weight 10 g of fish tissue

Add 5 mL $H_2O$ & 10 mL EtOAc

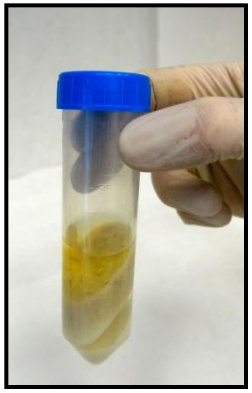
Shake 1 min



Add 2 g NaCl & 4 g $MgSO_4$

Shake 1 min

Centrifuge



Take aliquot of 5 mL from organic layer

Evaporate

Clean-up silica minicolumn

Evaporate and eliminate the residues under the stream of $N_2$


Re-dissolve in 0.5 mL of isooctane and transfer into the vial

# INTEGRATED SAMPLE PREPARATION - SUMMARY

BFR | PCB | PAH | Non-*ortho* PCB

Isolation
10 min

**Extraction**
Shaking ($H_2O$ + ethyl acetate)

**Partition** (transfer into organic phase)
$MgSO_4$ + NaCl

Clean up
30 min

**Clean up**
Silica SPE minicolumn

Identification & quantification
1 h

**Identification & quantification**
**GC×(GC)-TOFMS (EI)**

**TIME SAVING**
- One sample = < 1hour
- Six samples at one time

**SOLVENT VOLUME REDUCTION**

**COST SAVING**

# INTEGRATED SAMPLE PREPARATION

Analytica Chimica Acta 707 (2011) 84–91

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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


## Simplified and rapid determination of polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in fish and shrimps integrated into a single method

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ARTICLE INFO

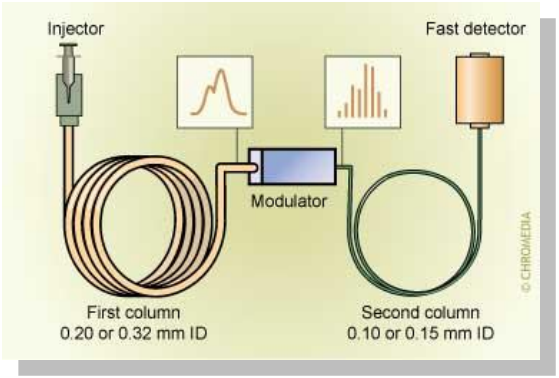
*Article history:*
Received 18 July 2011
Received in revised form
13 September 2011

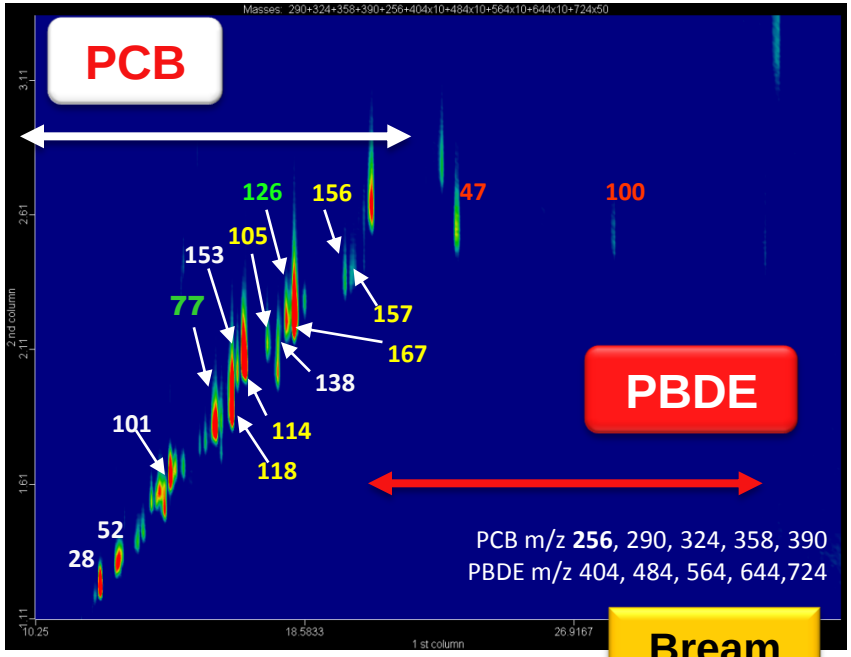
ABSTRACT

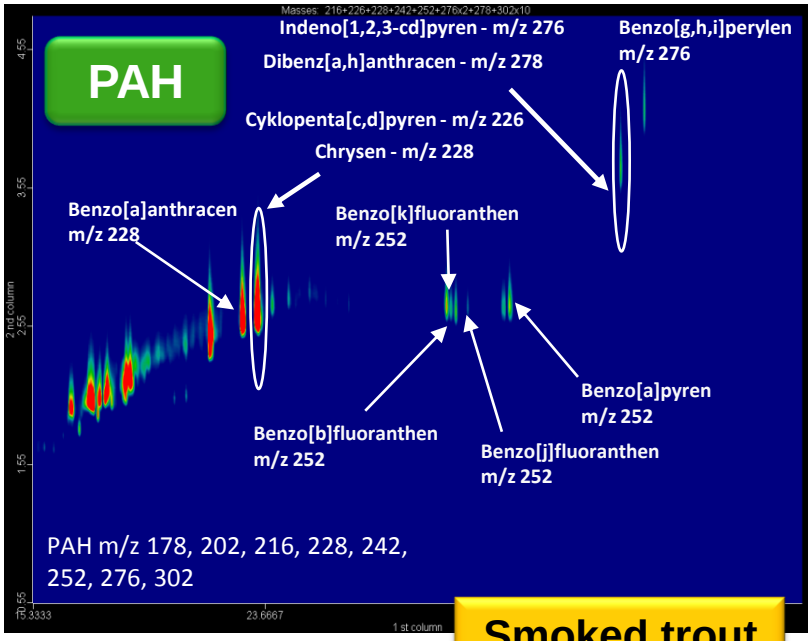
In this study, a new rapid and flexible method for the simultaneous determination of 18 key representatives of polychlorinated biphenyls (PCBs), 7 polybrominated diphenyl ethers (PBDEs), and 32 polycyclic aromatic hydrocarbons (PAHs) in fish and shrimps by gas chromatography coupled to mass spectrometry

# GC×GC–TOFMS (EI) – fish muscle tissue

*Agilent 6890N Pegasus III, LECO Corp.*




| Analytes | Desired LOQ CONfIDENCE | Achieved LOQ GC×GC-TOFMS (8 µL) |
|---|---|---|
| PBDEs | ≤ 0.2 µg/kg | 0.025 – 1 µg/kg** |
| HBCD | ≤ 0.2 µg/kg | 1 µg/kg |
| PBB 153 | ≤ 0.2 µg/kg | 0.25 µg/kg |
| Σ dl-PCBs | 2 ng WHO-PCB-TEQ/kg | 1.27 ng WHO-PCB-TEQ/kg |
| B[a]P | ≤ 2 µg/kg | 0.05 µg/kg |
| Other PAHs | N/A * | 0.01 – 0.5 µg/kg |

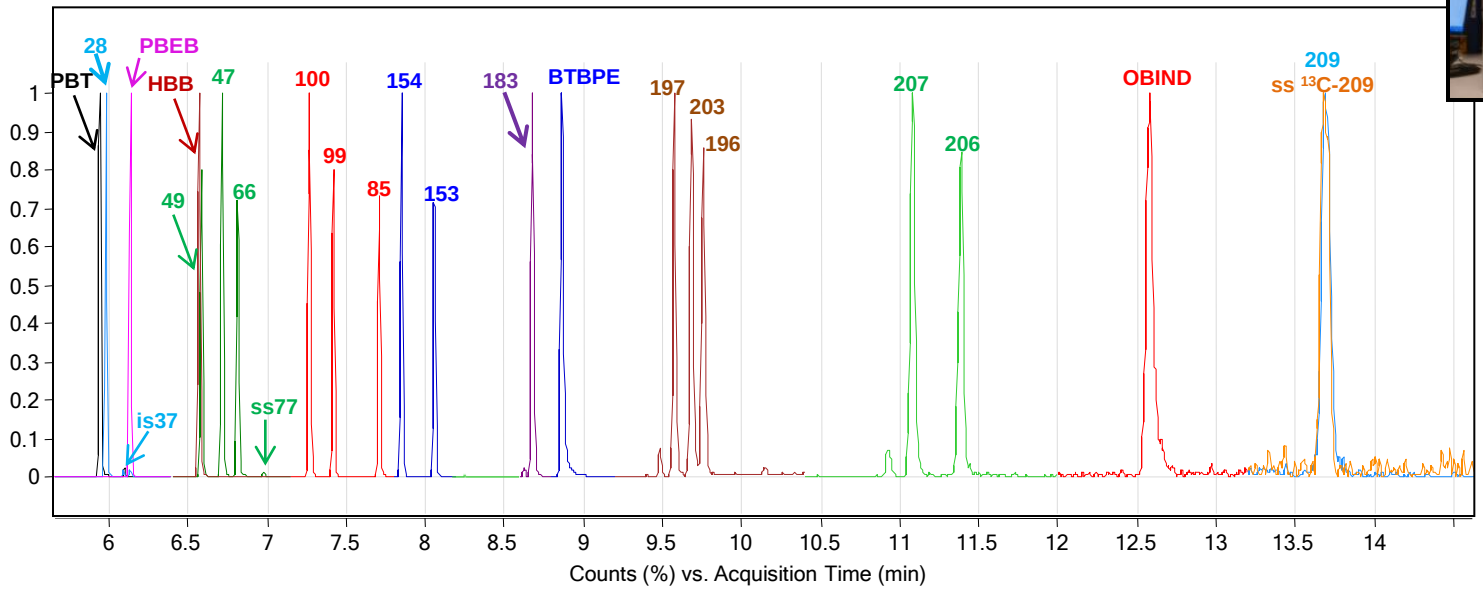
* *N/A – not available*

** *BDE28, 47–0.025 µg/kg; BDE100–0.1 µg/kg; BDE99, 153–0.25 µg/kg; BDE154, 183–1 µg/kg.*

# GC–MS/MS (EI)

*Agilent GC 7890A*
*Agilent 7000B GC/MS*
*Triple Quad*

***Fish muscle tissue spiked at 5 µg/kg (injected equivalent of matrix – 20 mg).***




## MS/MS transitions

+ MRM (407.8000 -> 248.1000)
+ MRM (485.7000 -> 326.0000)
+ MRM (565.7000 -> 405.8000)
+ MRM (643.4000 -> 483.8000)
+ MRM (721.6000 -> 561.8000)
+ MRM (801.7000 -> 641.5000)
+ MRM (879.8000 -> 719.6000)
+ MRM (799.4000 -> 639.5000)
+ MRM (811.8000 -> 651.4000)
+ MRM (551.7000 -> 472.5000)
+ MRM (406.7000 -> 246.8000)
+ MRM (499.7000 -> 484.6000)
+ MRM (852.7000 -> 771.7000)
+ MRM (356.8000 -> 277.8000)

**Column: Rxt-1614 (15m × 0.25mm × 0.1µm)**

Injection mode: pulsed splitless (20 psi, 1.5 min)
Injection temperature: 80°C (0.02 min), @ 600°C/min to 300°C(1 min), @ 100°C/min to 280°C
Injection volume: 2 µL

**Oven temperature: 110°C (1.5 min), @ 30°C/min to 320°C (10 min)**
**Post run – back flush: 280°C (2 min, 50 psi)**
Carrier gas: helium (constant pressure)

Source temperature: 280°C
Collision gas: nitrogen (1.5 mL/min)

| Analytes | Achieved LOQ |
|---|---|
| PBDE 28-100; PBT; PBEB; HBB; BTBPE | 0.005 µg/kg |
| PBDE 153-183; PBB 153 | 0.01 µg/kg |
| PBDE 196-203 | 0.05 µg/kg |
| PBDE 206-207 | 0.1 µg/kg |
| PBDE 209 | 0.5 µg/kg |
| OBIND | 1 µg/kg |
| DBDPE | N/A * |

CONffIDENCE
≤ 0.2 µg/kg

Could be even better

BDE 206
BDE 207
BDE 209
OBIND

** Note: DPDPE - the MS/MS transitions were not found.*

**Chemical ionization**

# GC–MS/MS (EI) – validation

*Agilent GC 7890A*
*Agilent 7000B GC/MS Triple Quad*

**Procedure blank**

- With each batch of six samples, the procedure blank was prepared.

**Recovery (%) and repeatability RSD (%)**

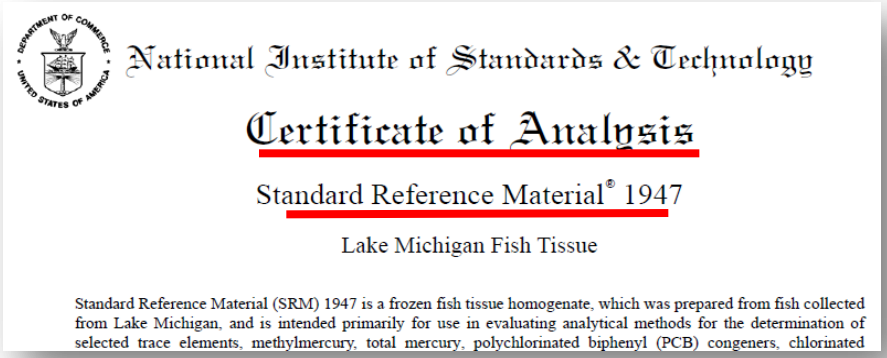
- Fish muscle tissue (trout and salmon – 2 and 14% of fat) spiked with all target analytes at two concentration levels (n=6).
- Level 1 and 2 = 1 and 5 µg/kg

**LOQ (µg/kg), linearity ($R^2$), …**

**Final independent control - trueness**

| PBDEs & ABFRs | GC-EI-MS/MS (2 µL) |
|---|---|
| REC [%] | 78 - 115 |
| RSD [%] | 2 - 14 |
| LOQ [µg/kg] | 0.005 - 1 |
| Linearity ($R^2$) | 0.9925 - 0.9999 |


***Trout***



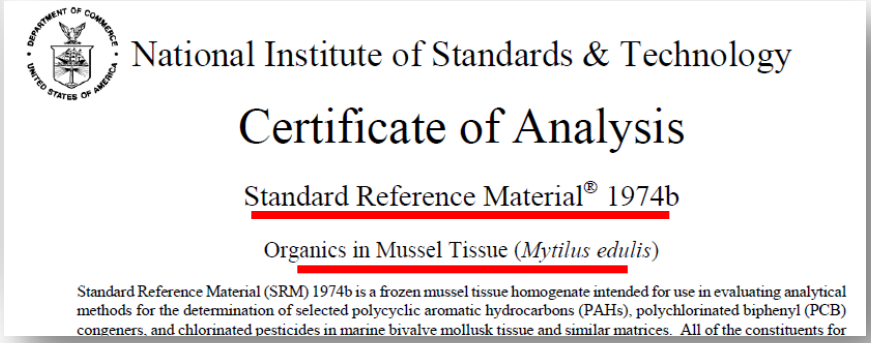
National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1947

Lake Michigan Fish Tissue

Standard Reference Material (SRM) 1947 is a frozen fish tissue homogenate, which was prepared from fish collected from Lake Michigan, and is intended primarily for use in evaluating analytical methods for the determination of selected trace elements, methylmercury, total mercury, polychlorinated biphenyl (PCB) congeners, chlorinated



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1974b

Organics in Mussel Tissue (*Mytilus edulis*)

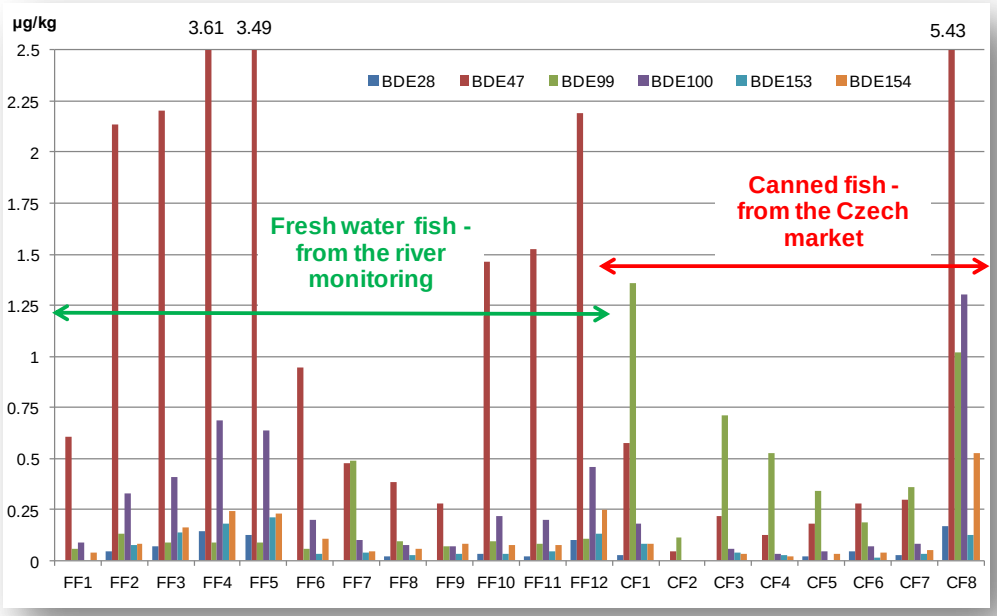
Standard Reference Material (SRM) 1974b is a frozen mussel tissue homogenate intended for use in evaluating analytical methods for the determination of selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, and chlorinated pesticides in marine bivalve mollusk tissue and similar matrices. All of the constituents for

# GC–MS/MS (EI) – real life samples





| | Fish | Fat [%] | Note |
|---|---|---|---|
| Canned fish | mackerel | 22.7 | vegetable oil |
| | mackerel | 14.3 | brine |
| | mackerel | 13.9 | brine |
| | mackerel | 11.5 | brine |
| | sardines | 5.4 | vegetable oil |
| | sardines | 11.2 | vegetable oil |
| | smoked sprats | 15.9 | vegetable oil |
| | cod liver | 28.5 | oil |
| Fresh fish | Crucian carp | 0.3 | Trmice - Bílina |
| | Roach | 2.4 | Trmice - Bílina |
| | Chub | 5.3 | Děčín |
| | Chub | 4.3 | Děčín |
| | Chub | 2.6 | Ústí nad Labem cofluence of Elbe & Bílina |
| | Bream | 0.7 | Obříství |
| | Chub | 2.4 | Obříství |
| | Chub | 1.4 | Valy - Hradec Králové - Elbe |
| | Chub | 0.7 | Elbe |
| | Bream | 2.9 | Debrná - Verdek - Elbe |
| | Bream | 2.4 | Hluboká nad Vltavou |
| | Roach | 0.9 | Podolí Vltava |




| Fresh fish (n=12) | BDE 47 | BDE 99 |
|---|---|---|
| Average [µg/kg] | 1.61 | 0.12 |
| Median [µg/kg] | 1.49 | 0.09 |
| Min - Max [µg/kg] | 0.25 - 3.61 | 0.06 - 0.49 |
| % > LOQ | 100 | 100 |

| Canned fish (n=8) | BDE 47 | BDE 99 |
|---|---|---|
| Average [µg/kg] | 0.89 | 0.58 |
| Median [µg/kg] | 0.25 | 0.44 |
| Min - Max [µg/kg] | 0.04 - 5.43 | 0.11 - 1.36 |
| % > LOQ | 100 | 100 |

| | |
|---|---|
| PBEB, HBB, BTBPE, OBIND, DBDPE | 0.01 - 1.47 µg/kg |

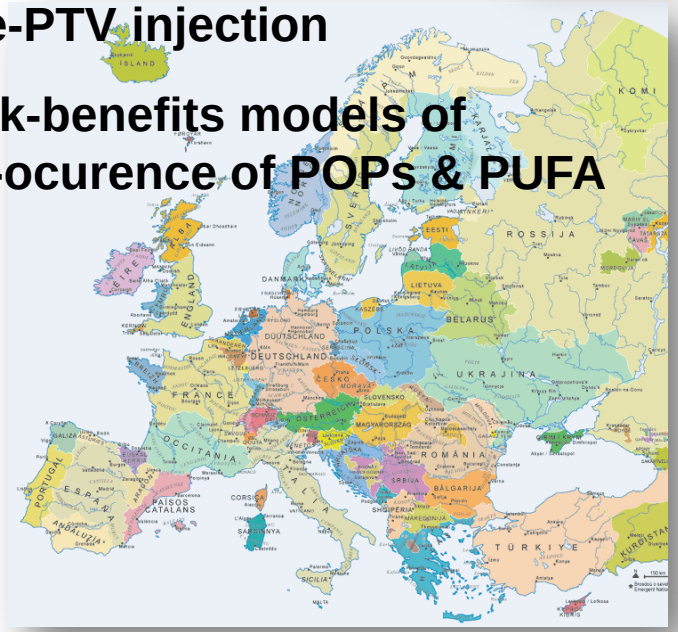
# Conclusions

- **A simple, fast and cheap method for the determination of 16 PBDEs and 6 ABFRs has been optimized and validated.**
- **6 fish muscle tissue samples can be processed within 1 hour.**
- **GC–MS/MS system equipped with QqQ mass analyzer in EI was shown to be an effective tool for identification and quantification of all target analytes.**
- **Low limits of quantification (0.005–1 μg/kg) needed for exposition studies which require quantification of target contaminants in food even at very low levels, were achieved using GC– MS/MS.**
- **The independent control of the whole analytical procedure was done using the standard reference materials.**
- **No "confirmation" method is needed; analytes can be conclusively identified, as well as quantified.**

**SEE MORE DURING THE CONffIDENCE OPEN DAY – November 3, 2011**

# Future plans

- Further decrease of LOQs - chemical ionisation
  - large volume-PTV injection
- Analysis of batch of ca 120 samples – risk-benefits models of co-ocurence of POPs & PUFA
  - Sampling sites - Baltic Sea
    - North Sea
    - Atlantic Ocean
    - Mediterranean Sea
    - Scandinavia
    - Netherlands
    - Czech republic
  - Fish species - Herring
    - Cod / Hake / Whiting
    - Trout / Salmon
    - Bivalves




# Acknowledgement


- *European research project CONffIDENCE "Contaminants in food and feed: Inexpensive detection for control of exposure" which is a part of Seventh framework program (call KBBE–2007–2–4–02)*


- *Financial Support from Specific University Research (MSMT NO. 21/2011)*


- *Agilent Technologies*


- *My supervisor Prof. Jana Hajslova and the whole team from the Department of Food Chemistry and Analysis (ICT, Prague)*

# Thank you for your kind attention….

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# Open Day CONffIDENCE

CONffIDENCE: Safer food through rapid and cost-efficient tests for chemical contaminants in the food chain

Open Day at RAFA 2011

3 November 2011

Stella Hall: 13:00 – 16:00

Posters (23)

Demonstrations (8)

RIKILT WAGENINGEN UR