



# Sensitive Determination of dl- and ndl-PCBs in Foodstuffs and Animal Feed using the 7890A / 7000B GC-MS/MS System



The Chemical and Veterinary Analytical Institute  
Münsterland-Emscher-Lippe ,CVUA-MEL  
Münster, Germany

*in collaboration with*

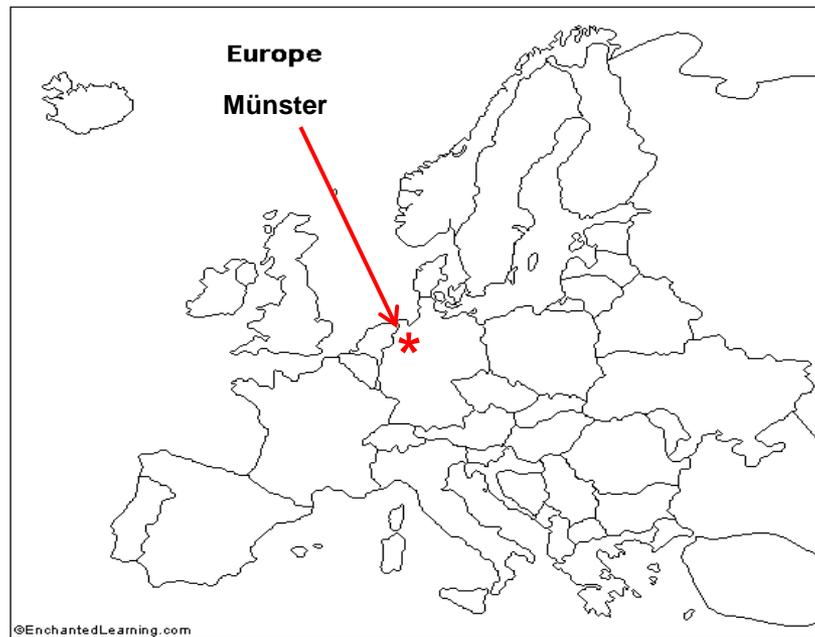


Agilent Technologies

Agilent Technologies  
Chris Sandy

EMEA Food Team GC-MS Applications Chemist





Chemical and Veterinary Analytical Institute  
Münsterland-Emscher-Lippe, CVUA-MEL  
Joseph-König-Str. 40,  
48147 Münster, Germany

Prof. Dr. Peter Fürst [peter.fuerst@cvua-mel.de](mailto:peter.fuerst@cvua-mel.de)  
Dr Thorsten Bernsmann, Dominik Baumeister



# Objectives of the Collaboration

**To assess the performance of GC-MS/MS in EI mode for the analysis of Dioxins and dl-PCBs in food and feedstuffs**

- Dioxins and dl-PCBs

(i) GC-MS/MS can be used as a sensitive screening method  
*and*

(ii) Possible use as a reliable and cost-effective alternative to GC-HRMS for *confirmatory* purposes in official food and feedstuff control

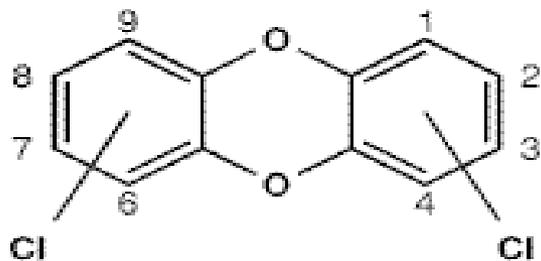
- To investigate whether GC-MS/MS is capable of determining ndl-PCBs at the level of interest discussed in the framework of setting harmonized MRL-values for ndl-PCBs

# Outline of Presentation

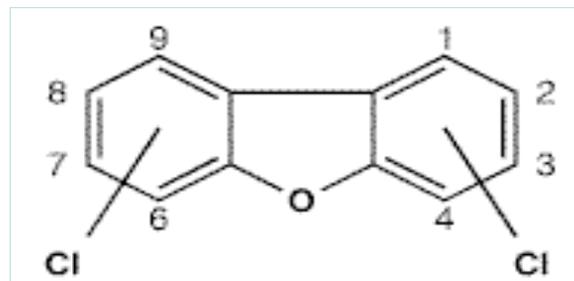
- 1. Introduction / Background**
  - Sources, Toxicology, Human exposure
- 2. EU-Legislation**
  - Scope, Maximum levels, action levels, consideration of LOQ
- 3. Comparison GC-HRMS vs. GC-MS/MS**
  - Analysis of foodstuff and feed samples
- 4. Summary**



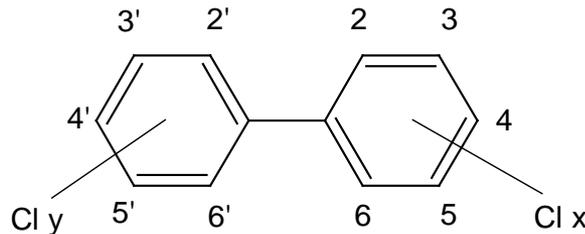
# 1. Introduction / Background



**PCDDs**



**PCDFs**



**PCBs**



# Why are Dioxins and PCBs a problem?

## Dioxins and PCBs ...

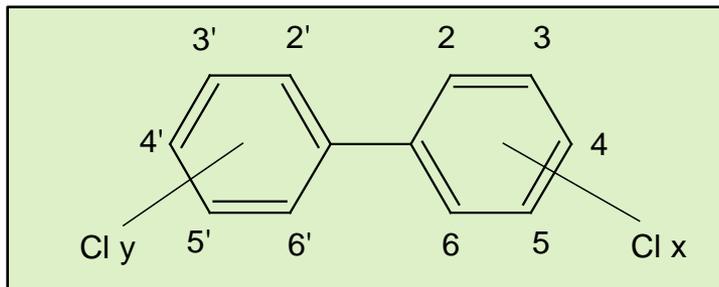
- are only little water-, but highly lipid soluble (lipophilic)
- show global distribution
- are accumulated in the food chain  
(especially congeners with 2,3,7,8-chlorine substitution)
- possess very long biological half-lives (highly persistent)
- are toxic in animal experiments and in humans

# Human exposure to Dioxins and PCBs

- **Occupational**
- **Accidental**
- **Environmental (background)**
  - **Direct route of exposure**
    - Inhalation of air and particulates
    - Ingestion of contaminated soil
    - Dermal absorption
  - **Indirect route of exposure**
    - Food consumption

**Food consumption contributes to more than 90% of total human Dioxin and PCB exposure**

# Polychlorinated Biphenyls (PCBs)



**Industrial production since 1929 (> 1.5 M tons)**

***Properties:***

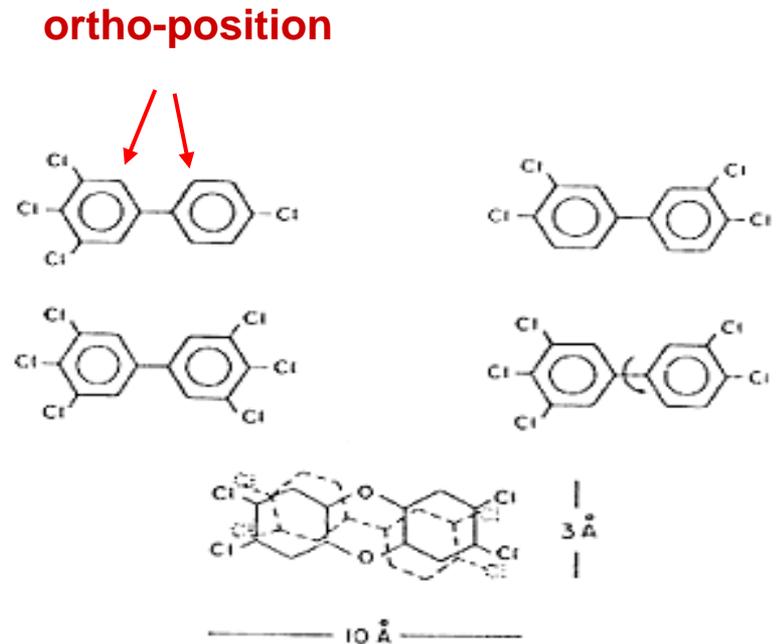
- lipophilic
- low flamability
- chemical and physical stability
- limited biological degradation

# Cl atoms	Congeners
1	3
2	12
3	24
4	42
5	46
6	42
7	24
8	12
9	3
10	1
<b>Total</b>	<b>209</b>

***Brand names: Clophen, Arochlor, Kanechlor, Phenochlor, Fenchlor, Pyralen***

# Dioxin-like (dl-) PCBs

According to their free rotation around the central axis, non-*ortho* and mono-*ortho* substituted PCBs may adopt a similar spatial structure to 2,3,7,8-TCDD and therefore exhibit comparable toxic effects as dioxins



# Important PCB congeners

(Nomenclature according to IUPAC)

- **Dioxin-like PCBs**

**(Food and Feed: Regulated EU wide)**

**Non-ortho PCBs**

**PCB 77, 81, 126, 169**

**Mono-ortho PCBs**

**PCB 105, 114, 118, 123, 156, 157, 167, 189**

- **Non dioxin-like (Indicator) PCBs**

**(Food and Feed: EU Regulation EC 152/2009 in preparation)**

**PCB 28, 52, 101, (118), 138, 153, 180**

**Concentration of dl-PCBs (especially of non-ortho PCBs) in food is generally 1-2 orders of magnitude lower than Indicator-PCBs**

# Major Food Dioxin / PCB incidents

- 1998 Brazilian citrus pulp pellets
- 1999 Belgian PCB / dioxin incident
- 1999 Kaolinitic clay as anti-caking agent
- 1999 Drying of grass meal
- 2000 Pentachlorophenol in Choline chloride
- 2002 “Carbosan Copper” as feed additive
- 2004 Kaolinitic clay in Potato pulp
- 2005 Hydrochloric acid for gelatine production
- 2007 Guar gum from India
- 2008 Italian buffalo mozzarella
- 2008 Zinc oxide in Chilean pork
- 2008 Irish pork
- 2010 Organic maize from Ukraine
- 2011 German animal feed (Chicken / eggs)



# Dioxin Incident, Ireland December 2008

**FARMAGEDDON**



**Source**

**Extent**

**Risk Assessment**

## 2. Dioxin and PCB Legislation



# Dioxin and PCB Legislation

-  • US EPA 1613 (1994) (Dioxins)
-  • US EPA 1668 (PCBs)
-  • EU Legislation EC 1883/2006 (Dioxins and dl-PCBs)

Dioxin & dl-PCB confirmation by HRGC- High Resolution MS

-  • EU Legislation Amendment EC 152/2009 (ndl-PCBs)  
[Jan 1<sup>st</sup> 2012]



# Dioxin Legislation EU 1883/2006

## ANNEX II

### SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN OFFICAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS

#### 1. FIELD OF APPLICATION

The requirements set out in this Annex shall be applied where foodstuffs are analysed for the official control of the levels of dioxins (polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF)) and dioxin-like PCBs.

Monitoring for the presence of dioxins in foodstuffs may be performed by a strategy involving a screening method in order to select those samples with levels of dioxins and dioxin-like PCBs that are less than 25 % below or exceed the maximum level. The concentration of dioxins and sum of dioxins and dioxin-like PCBs in those samples with significant levels needs to be determined/confirmed by a confirmatory method.

Screening methods are methods that are used to detect the presence of dioxins and dioxin-like PCBs at the level of interest. These methods shall have a capacity for a high sample throughput and are used to sift large numbers of samples for potential positives. They shall be specifically designed to avoid false negatives.

Confirmatory methods are methods that provide full or complementary information enabling the dioxins and dioxin-like PCBs to be identified and quantified unequivocally at the level of interest.

# Dioxins and dl-PCBs specified in EU Food Legislation

Congener	TEF value	Congener	TEF value
<b>Dibenzo-p-dioxins (PCDDs) (n=7)</b>		<b>Dioxin-like PCBs: Non-ortho PCBs + Mono-ortho PCBs (n=12)</b>	
2,3,7,8-TCDD	1	<i>Non-ortho PCBs</i>	
1,2,3,7,8-PeCDD	1	PCB 77	0,0001
1,2,3,4,7,8-HxCDD	0,1	PCB 81	0,0001
1,2,3,6,7,8-HxCDD	0,1	PCB 126	0,1
1,2,3,7,8,9-HxCDD	0,1	PCB 169	0,01
1,2,3,4,6,7,8-HpCDD	0,01		
OCDD	0,0001		
<b>Dibenzofurans (PCDFs) (n=10)</b>		<i>Mono-ortho PCBs</i>	
2,3,7,8-TCDF	0,1	PCB 105	0,0001
1,2,3,7,8-PeCDF	0,05	PCB 114	0,0005
2,3,4,7,8-PeCDF	0,5	PCB 118	0,0001
1,2,3,4,7,8-HxCDF	0,1	PCB 123	0,0001
1,2,3,6,7,8-HxCDF	0,1	PCB 156	0,0005
1,2,3,7,8,9-HxCDF	0,1	PCB 157	0,0005
2,3,4,6,7,8-HxCDF	0,1	PCB 167	0,00001
1,2,3,4,6,7,8-HpCDF	0,01	PCB 189	0,0001
1,2,3,4,7,8,9-HpCDF	0,01		
OCDF	0,0001		

## EC 1883/2006

TEF = Toxic Equivalency Factor (1 = most toxic)

# TEF\* Values – WHO<sub>98</sub> / WHO<sub>05</sub>

Congener	TEF Value WHO 1998	TEF Value WHO 2005		TEF Value WHO 1998	TEF Value WHO 2005
Dibenzo-p-dioxins (PCDDs)			Dioxin-like PCBs		
2378-TCDD	1	1	Non-ortho PCBs		
12378-PCDD	1	1	PCB 77	0.0001	0.0001
123478-HxCDD	0.1	0.1	PCB 81	0.0001	<b>0.0003</b>
123678-HxCDD	0.1	0.1	PCB 126	0.1	0.1
123789-HxCDD	0.1	0.1	PCB 169	0.01	<b>0.03</b>
1234678-HpCDD	0.01	0.01			
OCDD	0.0001	<b>0.0003</b>			
Dibenzofurans (PCDFs)			Mono-ortho PCBs		
2378-TCDF	0.1	0.1	PCB 105	0.0001	<b>0.00003</b>
12378-PCDF	0.05	<b>0.03</b>	PCB 114	0.0005	<b>0.00003</b>
23478-PCDF	0.5	<b>0.3</b>	PCB 118	0.0001	<b>0.00003</b>
123478-HxCDF	0.1	0.1	PCB 123	0.0001	<b>0.00003</b>
123678-HxCDF	0.1	0.1	PCB 156	0.0005	<b>0.00003</b>
123789-HxCDF	0.1	0.1	PCB 157	0.0005	<b>0.00003</b>
234678-HxCDF	0.1	0.1	PCB 167	0.00001	<b>0.00003</b>
1234678-HpCDF	0.01	0.01	PCB 189	0.0001	<b>0.00003</b>
1234789-HpCDF	0.01	0.01			
OCDF	0.0001	<b>0.0003</b>			

**WHO<sub>05</sub> TEF Values come into effect Jan 1<sup>st</sup> 2012**

# The Toxic Equivalent (TEQ) System

- Each PCDD/PCDF and dl-PCB has its individual toxicity expressed as a **TEF** (Toxic Equivalency Factor)
- The **TEQ** value represents the total PCDD/PCDF and dl-PCB concentration of a sample, taking into account the *individual toxicity* of the 17 individual 2,3,7,8-substituted dioxins and the 12 individual dl-PCBs

# Consideration of LOQ for TEQ calculation

- In case of single congeners which are not quantifiable, three different concepts exist for calculating the TEQ:



The **concept of “upperbound”** requires using the limit of quantification for the contribution of each non-quantified congener to the TEQ

The **concept of “mediumbound”** requires using the half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ

The **concept of “lowerbound”** requires using zero for the contribution of each non-quantified congener to the TEQ.

# EU – legislation for Dioxins and dl-PCBs in food and feed

## Three pillars

Maximum levels

Action levels

Target levels



# EU – legislation for Dioxins and dl-PCBs in food and feed

- **Maximum levels**
  - Regulation (EC) No. 1881/2006 (Food)
  - Directive 2002/32/EC (Feed)  
(Maximum levels for PCDD/F and the sum of PCDD/F and dioxin-like PCBs)
- **Action levels (Early warning tool)**
  - Recommendation 2006/88/EC (Food)
  - Directive 2002/32/EC (Feed)  
(Separately for PCDD/F and dioxin-like PCBs)
- **Target levels**

Should have been set by the end of 2008

# Maximum levels (upperbound) for Dioxins and dl-PCBs in foodstuffs (pg WHO-TEQ/g fat)

Food	$\Sigma$ PCDD/F	$\Sigma$ PCDD/F + dl-PCB
Meat and meat products		
- ruminants (bovine, sheep)	3.0	4.5
- poultry and farmed game	2.0	4.0
- pigs	1.0	1.5
- liver and derived products of terrestrial animals	6.0	12.0
Muscle meat of fish and fishery products, except eel	4.0*	8.0*
Muscle meat of eel ( <i>Anguilla anguilla</i> ) and products thereof	4.0*	12.0*
Milk and milk products, including butter fat	3.0	6.0

\* Whole fish

Regulation (EC) No 1881/2006

# Action levels (upperbound) for Dioxins and dl-like PCBs in foodstuffs (pg WHO-TEQ/g fat)

Food	$\Sigma$ PCDD/F	dl-PCB
Meat and meat products <ul style="list-style-type: none"> <li>- ruminants (bovine, sheep)</li> <li>- poultry and farmed game</li> <li>- pigs</li> <li>- liver and derived products of terrestrial animals</li> </ul>	1.5 1.5 0.6 4.0	1.0 1.5 0.5 4.0
Muscle meat of fish and fishery products, except eel	3.0*	3.0*
Muscle meat of eel ( <i>Anguilla anguilla</i> ) and products thereof	3.0*	6.0*
Milk and milk products, including butter fat	2.0	2.0

\* Whole fish

Commission Recommendation 2006/88/EC

# Specific EU requirements for Dioxin and dl-PCB testing

- The performance criteria that analytical methods have to fulfil are established in **Commission Regulation (EC) No 1883/2006** :  
*Laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs*
- The Regulation contains strict requirements (based on **EPA 1613 B**) concerning sensitivity, use of isotope labelled ( $^{13}\text{C}$ ) standards, GC peak separation, recoveries, trueness, precision, tolerances between lowerbound and upperbound results etc

# The 27 European Union Member Countries



<b>Austria</b>	<b>Latvia</b>
<b>Belgium</b>	<b>Lithuania</b>
<b>Bulgaria</b>	<b>Luxembourg</b>
<b>Cyprus</b>	<b>Malta</b>
<b>Czech Republic</b>	<b>Netherlands</b>
<b>Denmark</b>	<b>Poland</b>
<b>Estonia</b>	<b>Portugal</b>
<b>Finland</b>	<b>Romania</b>
<b>France</b>	<b>Slovakia</b>
<b>Germany</b>	<b>Slovenia</b>
<b>Greece</b>	<b>Spain</b>
<b>Hungary</b>	<b>Sweden</b>
<b>Ireland</b>	<b>United Kingdom</b>
<b>Italy</b>	

**Candidate Countries : Croatia, FYR Macedonia, Iceland, Turkey**

# EU Regulations for ndl-PCBs Amendment EC 152/2009

- **EFSA Risk assessment completed in 2005**
- **Legislation will be based on  $\Sigma$  6 ndl-PCBs, enforced January 1<sup>st</sup> 2012**
- **ndl-PCBs have no TEF values**
- **MRLs will be different for various food classes**
- **MRLs for Eggs, Meat, Fat, Liver and Fish oil etc will be in the ng/g range**

# 3. GC-MS/MS analysis of PCBs



# Requirements for analysis

- Exhaustive extraction of analytes  
(often at low femtogram levels in complex matrix)
- Meticulous clean-up  
(Separation from food and feed constituents and other contaminants present)
- Congener-specific GC separation of toxic from non-toxic congeners
- Sensitive analytical determination (down to low pg / fg levels)
- Comprehensive quality control

# Application Note dl- and ndl-PCBs

**Agilent Ref :  
5990-6950EN**



## Determination of Dioxin-Like and Non-Dioxin-Like Polychlorinated Biphenyl Congeners in Foodstuffs and Animal Feed Using the Agilent 7000 Triple Quadrupole GC/MS System

Application Note

Food Safety

### Authors

Prof. Dr. Peter Fürst  
Dr. Thorsten Bernsmann  
Dominik Baumeister  
Chemical and Veterinary Analytical  
Institute  
Münsterland-Emscher-Lippe  
(Chemisches und Veterinär-  
untersuchungsamt, CVUA-MEL)  
Joseph-König-Str. 40  
48147 Münster  
Germany

Chris Sandy  
Agilent Technologies UK Ltd  
610 Wharfedale Road  
Winnersh Triangle  
Wokingham  
Berkshire  
RG41 5TP  
UK

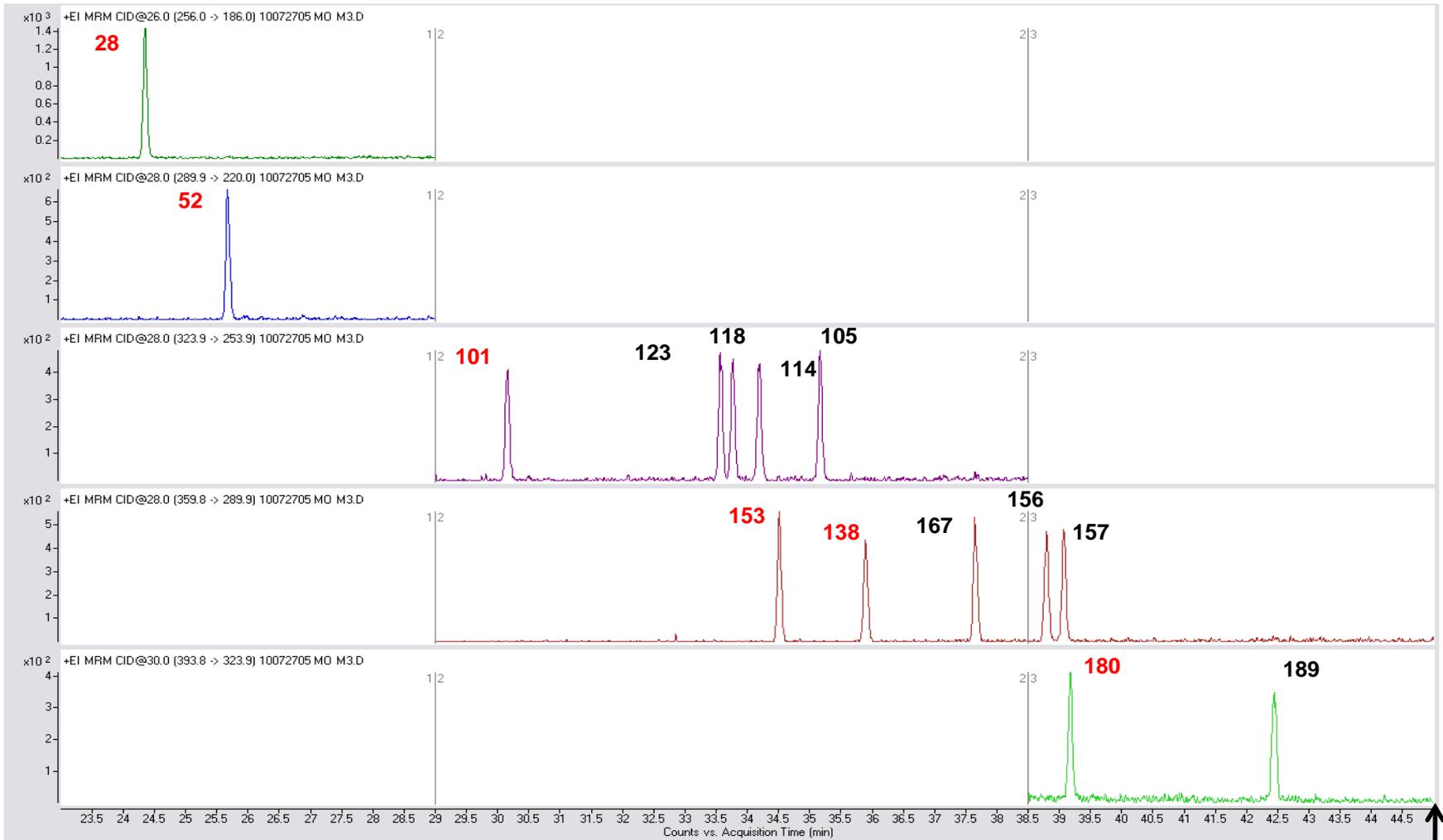
### Abstract

Two methods have been developed on the Agilent 7000 Triple Quadrupole GC/MS system for the analysis of polychlorinated biphenyl (PCB) congeners in foodstuffs and animal feed. The methods were shown to give linear response over the required concentration ranges. In addition, quantitative results for dioxin-like PCB (dl-PCB) congeners down to low pg TEQ/g levels and non-dioxin-like PCB (ndl-PCB) congeners at levels below 1 ng/g product were in good agreement with values obtained using a GC-High Resolution mass spectrometer. This application note demonstrates the determination of the 12 dl-PCB comprising eight mono-ortho PCB congeners (# 105, 114, 118, 123, 156, 157, 167 and 189) and four non-ortho PCB congeners (# 77, 81, 126 and 169) as well as the six ndl-PCB congeners (# 28, 52, 101, 138, 153 and 180) that are also known as "Indicator PCB" congeners.



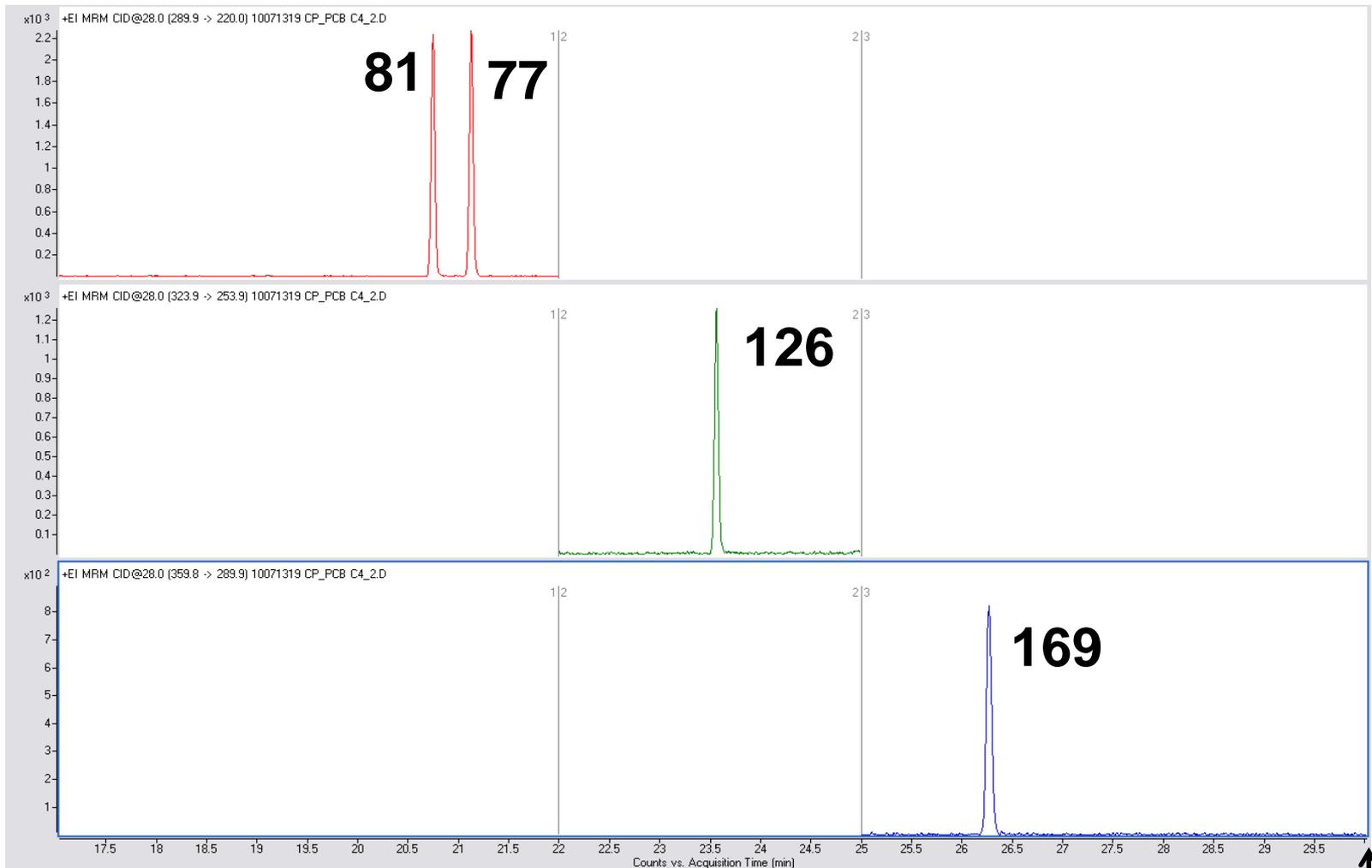
 **Agilent Technologies**

# Separation of mono - *ortho* PCBs (6 ndl-PCBs in red)



45 minutes

# Separation of non - *ortho* PCBs



30 minutes

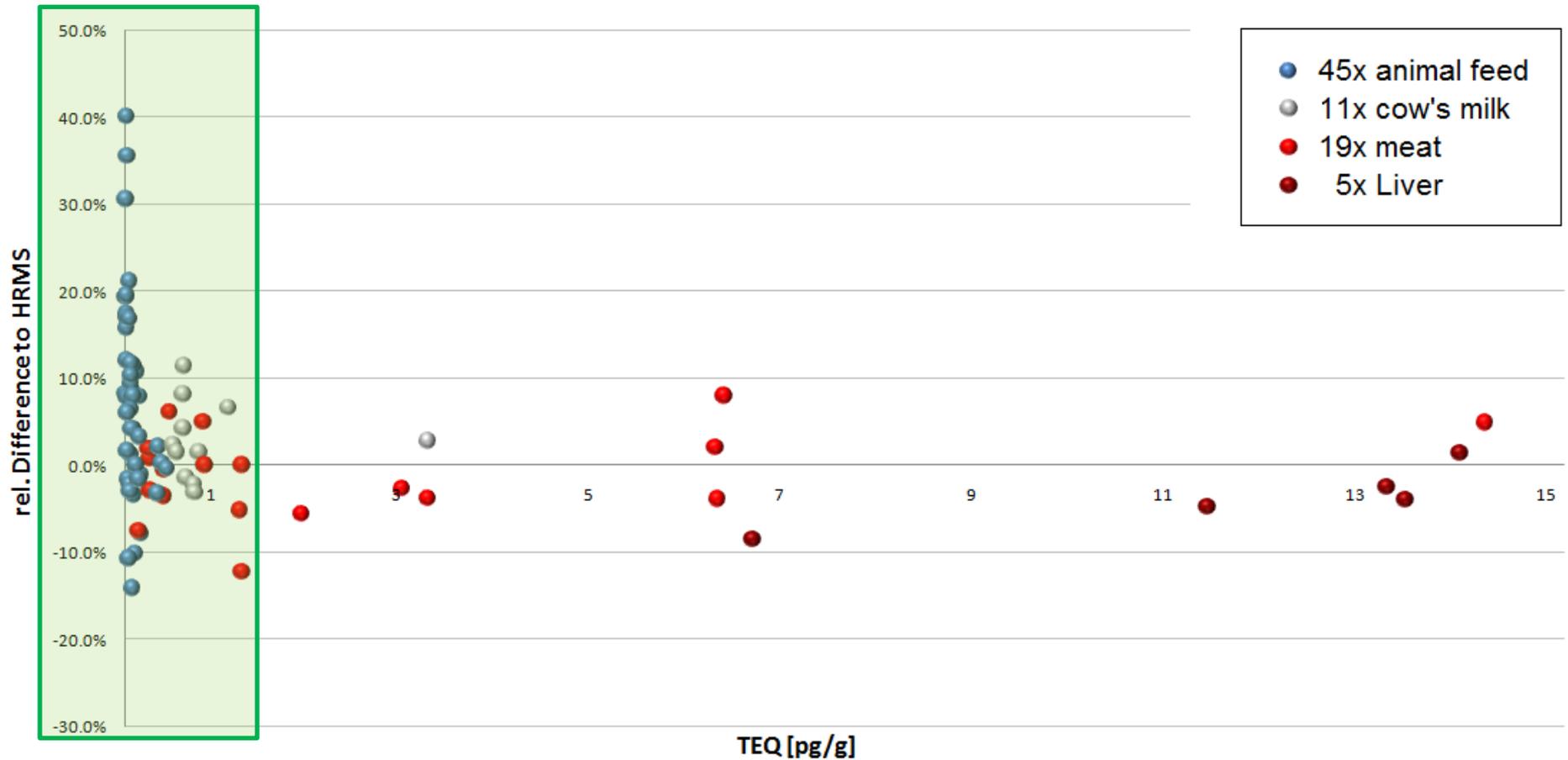
# Performance of the 7000 GC-MS/MS System for the Determination of **dl- and ndl-PCBs** in Food and Feed

## Comparative results vs GC-HRMS



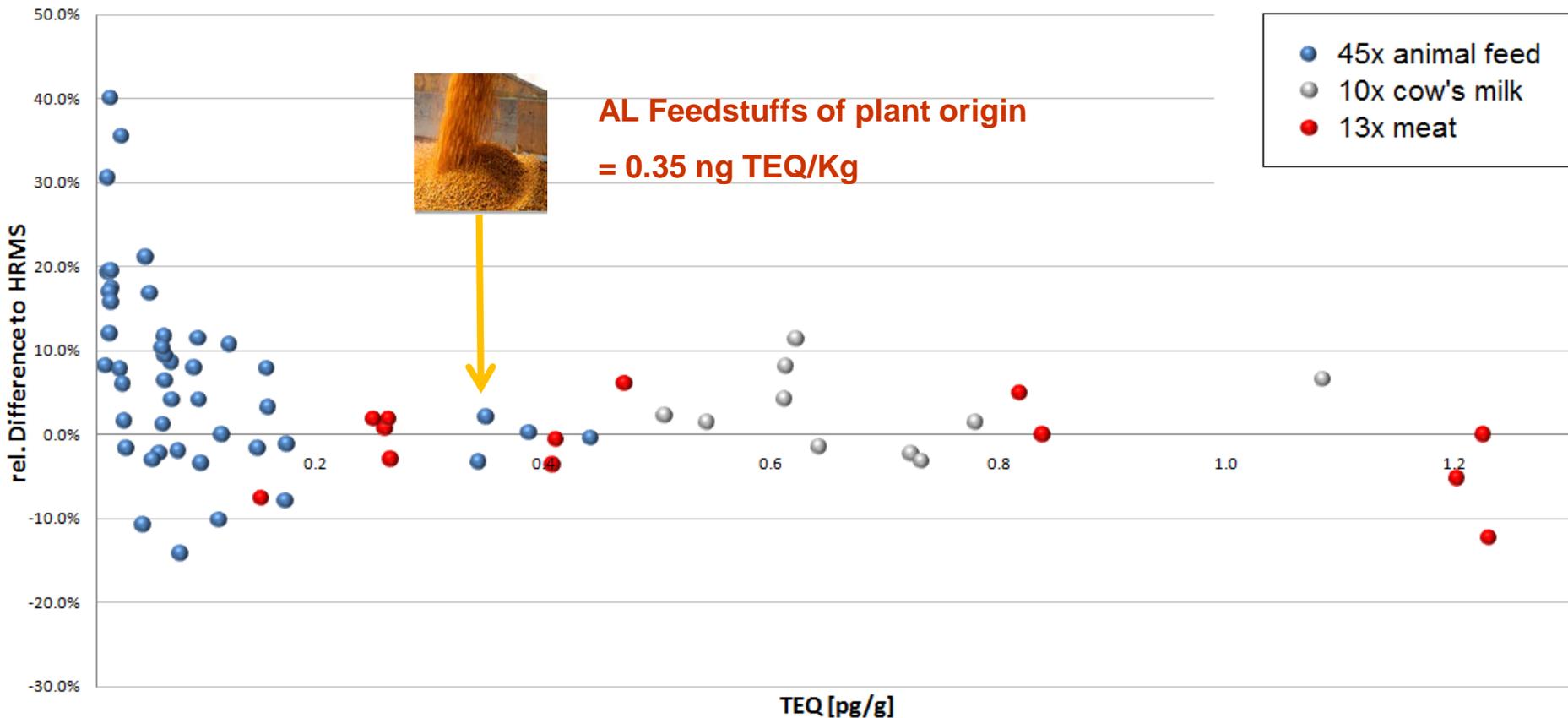
# GC-HRMS vs. GC-MS/MS Results (Upperbound dl-PCB-TEQ<sub>WHO98</sub>)

(n = 80)



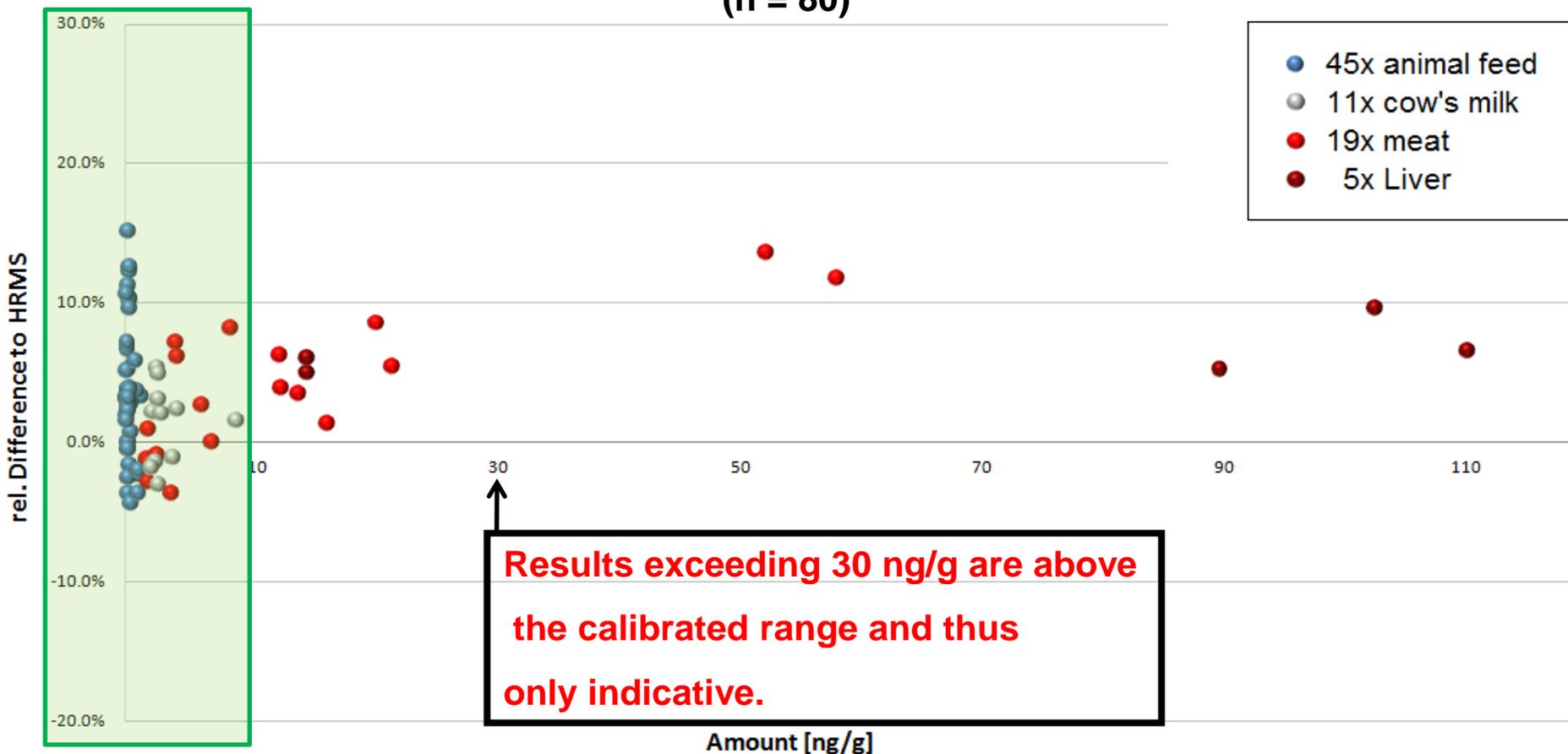
# GC-HRMS vs. GC-MS/MS Results (Upperbound dl-PCB-TEQ<sub>WHO98</sub>)

(n = 68)



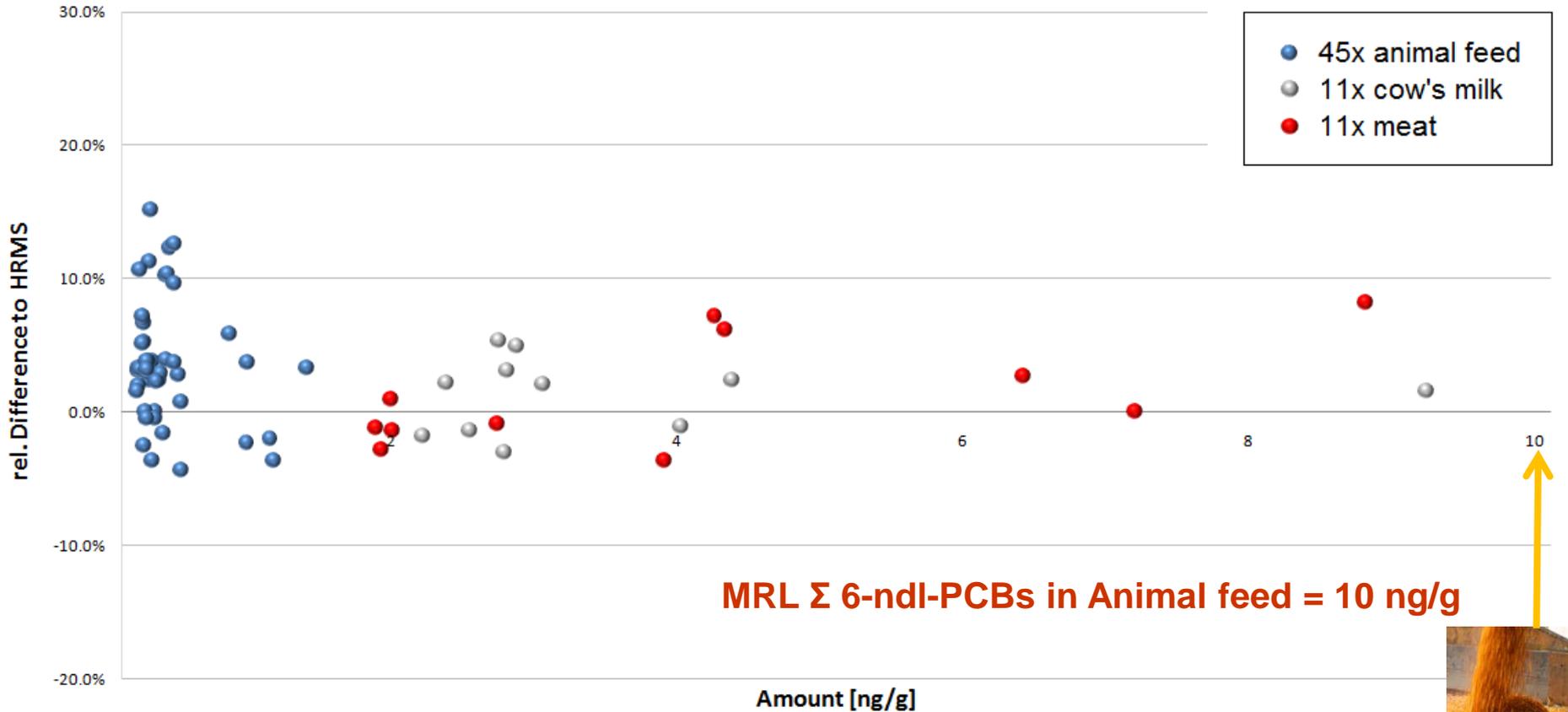
# GC-HRMS vs. GC-MS/MS Results (Sum of 6 Indicator – ndl-PCBs)

(n = 80)



# GC-HRMS vs. GC-MS/MS Results (Sum of 6 Indicator – ndl-PCBs)

(n = 67)





# dl- and ndl-PCBs by GC-MS/MS

## Summary - 1

**The Agilent 7000 GC-MS/MS system provides :**

- **Chromatographic results that meet legislated screening requirements for “EU methods” for dl-PCBs**
- **Good linearity and response reproducibility for dl- and ndl-PCBs in food and feed over the range of interest**





# dl- and ndl-PCBs by GC-MS/MS

## Summary - 2

- dl-PCB detection down to low pg WHO-TEQ/g
- dl-PCB TEQ values of GC-HRMS and GC-MS/MS are comparable and generally in a range of +/- 10%
- At lower dl-PCB TEQ values (Upperbound concs.) the difference between HRMS and MS/MS is due to the 2-3 times lower LOD of the HRMS system





# dl- and ndl-PCBs by GC-MS/MS

## Summary - 3

- The results of the comparison between GC-MS/MS and GC-HRMS indicate that GC-MS/MS has the *potential* as an alternative confirmatory method for the determination of dl-PCBs in official food and feed control at the current Maximum Residue Levels
- Individual ndl-PCB analysis  $< 1$  ng/g - meets new EU Regulated MRLs



# Acknowledgements



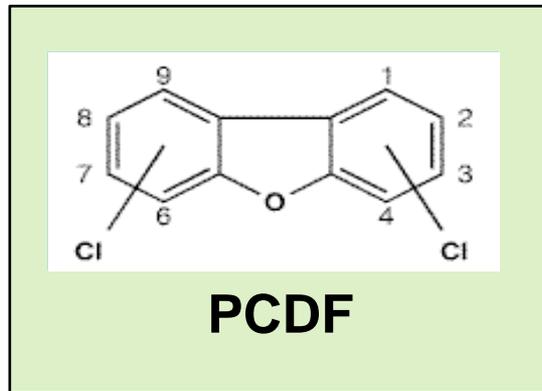
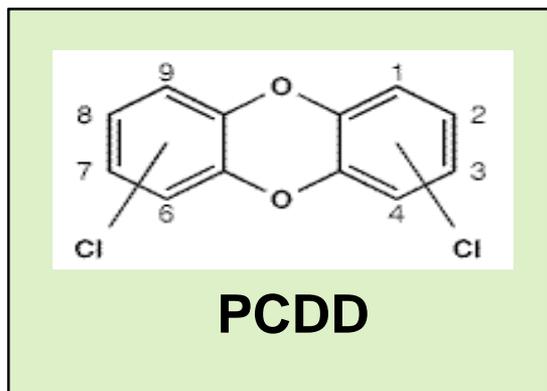
**Prof. Dr. Peter Fürst, Dr Thorsten Bernsmann,  
Dominik Baumeister**



**CVUA-MEL, Münster, Germany**

# Additional Slides

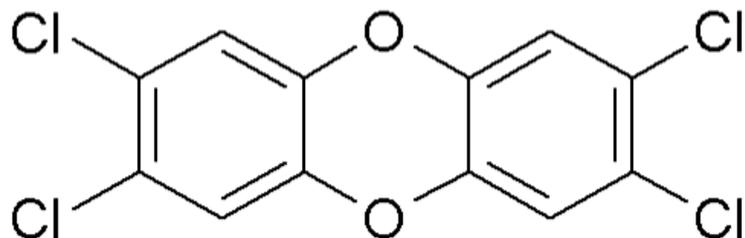
# Polychlorinated dibenzo-p-dioxins (PCDD) and Polychlorinated dibenzofurans (PCDF)



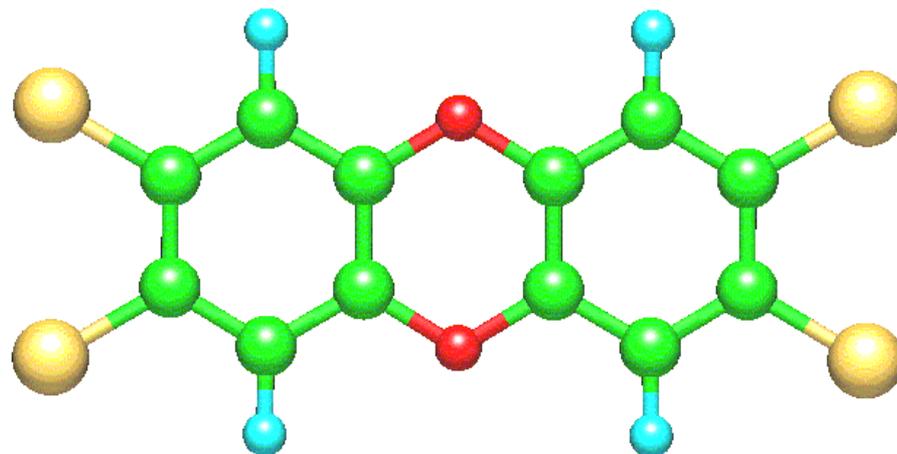
	# Isomers	# Isomers
# Cl Atoms	PCDD	PCDF
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
<b>Total</b>	<b>75</b>	<b>135</b>

- ‘Dioxins’ = PCDD and PCDF
- 210 different congeners with 1 - 8 chlorine atoms
- No use nor technical and industrial production
- Formation as unwanted by-products during thermal and industrial processes
- Increase of environmental levels in parallel to the beginning of industrial chlorine chemistry

# The best-known Dioxin



## 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2378-TCDD)



# Tolerable intake for Dioxins and dioxin-like PCBs

## World Health Organization 1990

10 pg 2,3,7,8-TCDD/kg bodyweight/day

## World Health Organization 1998

1 – 4 pg TEQ/kg b.w./day

## Scientific Committee for Food 2001

14 pg TEQ/kg b.w./week

## Joint Expert Committee for Food Additives 2001

70 pg TEQ/kg b.w./month

**1 pg = 0.000 000 000 001 gram**

# Expression of Dioxins and dl-PCBs as Toxic Equivalents (TEQ)

## Assumption

- Similar qualitative mechanism of action (binding to the Ah receptor)
- Different quantitative binding affinity

Estimation of toxic equivalency factors (TEF)

- Toxicity of 2,3,7,8-TCDD = 1
- TEF = toxicity (congener / 2,3,7,8-TCDD)

## Calculation

$$\text{TEQ} = \sum (\text{congener concentration} * \text{TEF})$$

**Based on increased knowledge TEF values are reviewed and updated from time to time**

# TEF\* Values – WHO 1998 / WHO 2005

Congener	TEF Value WHO 1998	TEF Value WHO 2005		TEF Value WHO 1998	TEF Value WHO 2005
Dibenzo-p-dioxins (PCDDs)			Dioxin-like PCBs		
2378-TCDD	1	1	Non-ortho PCBs		
12378-PCDD	1	1	PCB 77	0.0001	0.0001
123478-HxCDD	0.1	0.1	PCB 81	0.0001	<b>0.0003</b>
123678-HxCDD	0.1	0.1	PCB 126	0.1	0.1
123789-HxCDD	0.1	0.1	PCB 169	0.01	<b>0.03</b>
1234678-HpCDD	0.01	0.01			
OCDD	0.0001	<b>0.0003</b>			
Dibenzofurans (PCDFs)			Mono-ortho PCBs		
2378-TCDF	0.1	0.1	PCB 105	0.0001	<b>0.00003</b>
12378-PCDF	0.05	<b>0.03</b>	PCB 114	0.0005	<b>0.00003</b>
23478-PCDF	0.5	<b>0.3</b>	PCB 118	0.0001	<b>0.00003</b>
123478-HxCDF	0.1	0.1	PCB 123	0.0001	<b>0.00003</b>
123678-HxCDF	0.1	0.1	PCB 156	0.0005	<b>0.00003</b>
123789-HxCDF	0.1	0.1	PCB 157	0.0005	<b>0.00003</b>
234678-HxCDF	0.1	0.1	PCB 167	0.00001	<b>0.00003</b>
1234678-HpCDF	0.01	0.01	PCB 189	0.0001	<b>0.00003</b>
1234789-HpCDF	0.01	0.01			
OCDF	0.0001	<b>0.0003</b>			

**\*Red figures = changed TEF value**

# Calculation of PCDD/F WHO-TEQ

Fish oil, pg/g, lipid based



Compound		Result	WHO-TEFs	Single TEQ
2.3.7.8-	Tetra-CDD	0,31	1	0,31
1.2.3.7.8-	Penta-CDD	0,64	1	0,64
1.2.3.4.7.8-	Hexa-CDD	0,16	0,1	0,016
1.2.3.6.7.8-	Hexa-CDD	0,5	0,1	0,054
1.2.3.7.8.9-	Hexa-CDD	0,33	0,1	0,033
1.2.3.4.6.7.8-	Hepta-CDD	4,7	0,01	0,0469
1.2.3.4.6.7.8.9-	Octa-CDD	25	0,0001	0,002488
2.3.7.8-	Tetra-CDF	2,22	0,1	0,222
1.2.3.7.8-	Penta-CDF	0,34	0,05	0,017
2.3.4.7.8-	Penta-CDF	0,34	0,5	0,17
1.2.3.4.7.8-	Hexa-CDF	0,12	0,1	0,012
1.2.3.6.7.8-	Hexa-CDF	0,07	0,1	0,007
1.2.3.7.8.9-	Hexa-CDF	n.d.	0,1	-
2.3.4.6.7.8-	Hexa-CDF	0,11	0,1	0,011
1.2.3.4.6.7.8-	Hepta-CDF	0,28	0,01	0,0028
1.2.3.4.7.8.9-	Hepta-CDF	n.d.	0,01	-
1.2.3.4.6.7.8.9-	Octa-CDF	1,92	0,0001	0,000192
<b>WHO-TEQ</b>				<b>1,5</b>

Step 1:

**Multiplication of 17 individual results with corresponding TEF**

Step 2:

**Sum of 17 individual results = Total TEQ**

# EU Regulations for ndl-PCBs

Draft

COMMISSION REGULATION (EU)

of

amending Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down methods of sampling and analysis for the official control in feed as regards dioxins, dioxin-like PCBs and **non dioxin-like PCBs**

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules<sup>1</sup>, in particular Article 11 (4) thereof,

Whereas:

- (1) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed<sup>2</sup> provides for maximum and action levels for dioxins and furans, for the sum of dioxins, furans and dioxin-like PCBs and non-dioxin-like PCBs and for action levels for dioxins and furans and for dioxin-like PCBs in feed.
- (2) Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed<sup>3</sup> lays down provisions for the determination of dioxins (PCDD/PCDF) and dioxin-like PCBs.
- (3) **The application of new maximum levels for non-dioxin like PCBs and a very significant update of the criteria for screening methods requires significant amendments to the Annex V, section B of Regulation (EC) 152/2009. For reasons of clarity, it is appropriate to replace the whole section.**

**Due for enforcement**

**January 1<sup>st</sup> 2012**

**GC-HRMS or**

**GC-MS/MS acceptable**

**techniques**

# Dioxins / PCBs Sample Prep

## Sample extraction

Eg. Soxhlet or ASE with toluene/acetone (7/3) or hexane  
Addition of <sup>13</sup>C-isotope labelled internal standards



## Removal of fat

Sulphuric acid coated silica column  
Elution of PCDD/F/PCB analytes with hexane



## Separation of PCDD/F and PCB on Florisil

PCB elution with hexane (1)  
PCDD/F elution with toluene (2)



### 1. PCBs

#### Clean up and separation with active carbon

- 1a.) mono-*ortho* PCB / ndl-PCB eluted with Dichloromethane/cyclohexane (1/1)
- 1b.) non-*ortho* PCB eluted with toluene



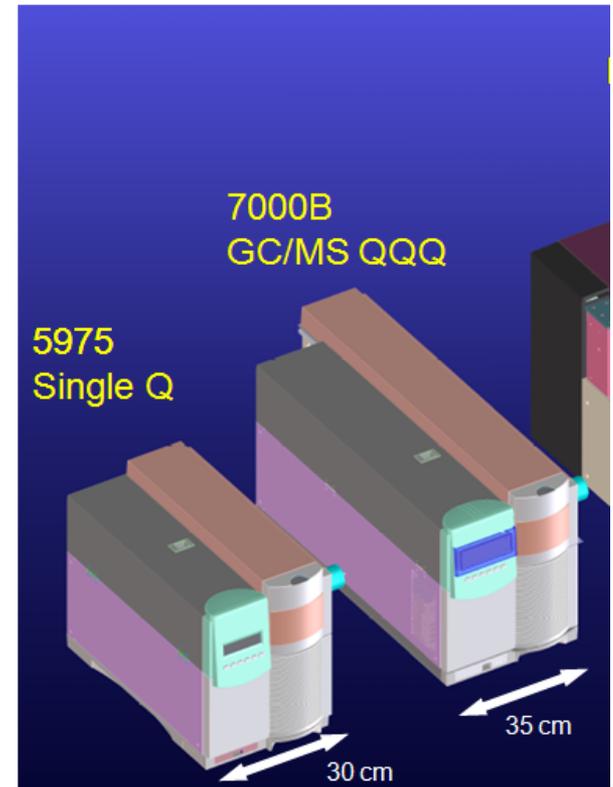
### 2. PCDD/F

#### Clean up with active carbon

# 7890 / 7000B GC-MS/MS System



← 1.03 m →



# GC-HRMS System

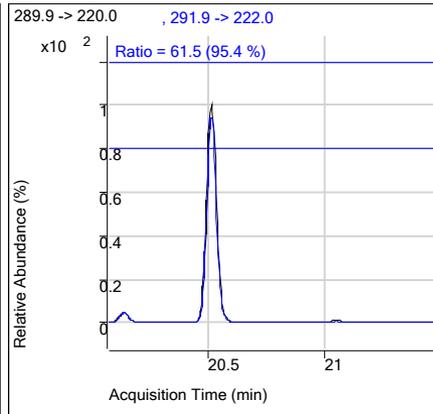
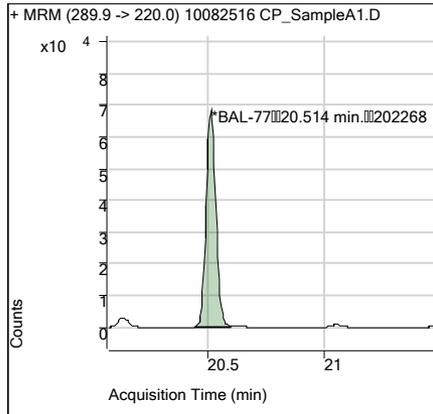


← 3.2 m →

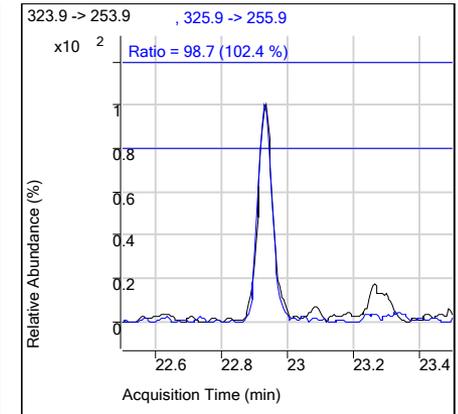
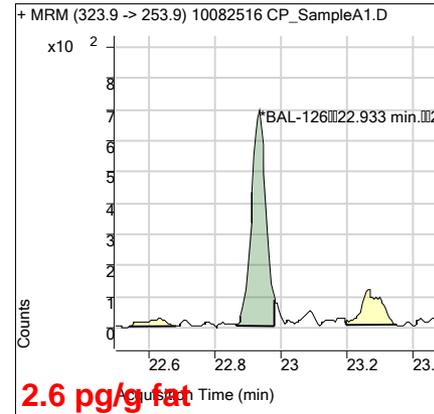
# 2010 Proficiency test – Pork Fat

## Non-ortho dl-PCBs

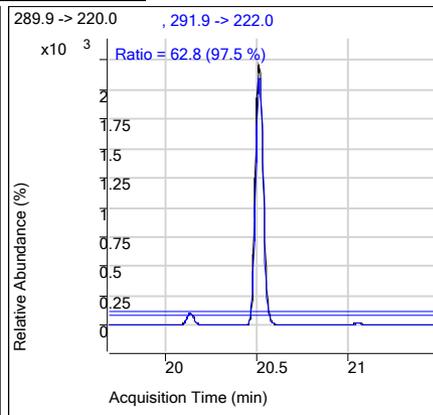
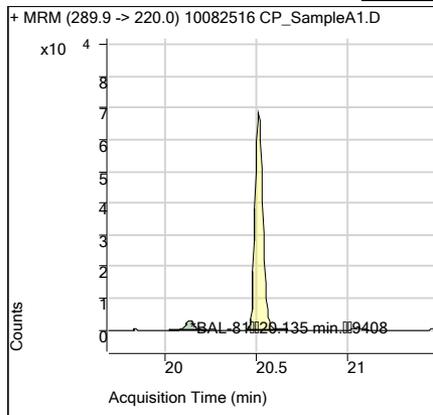
### PCB 77



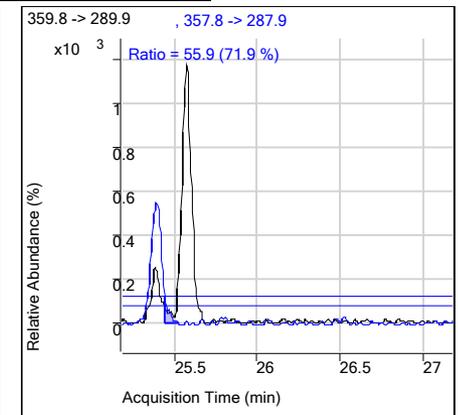
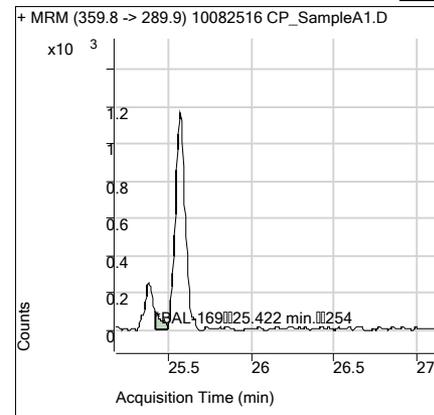
### PCB 126



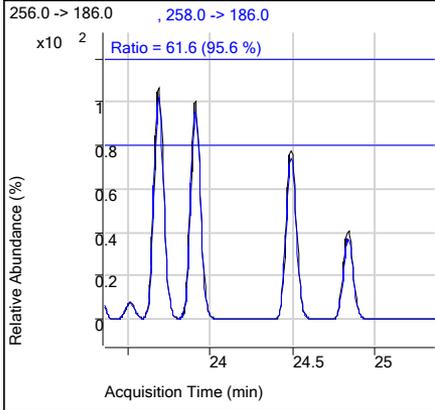
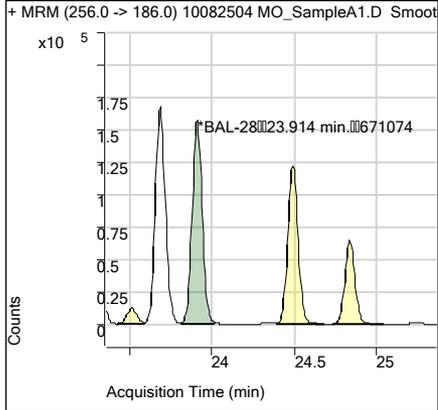
### PCB 81



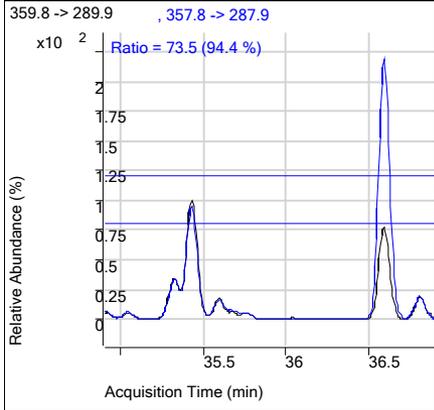
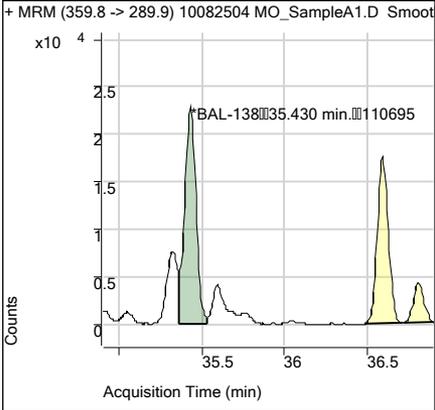
### PCB 169



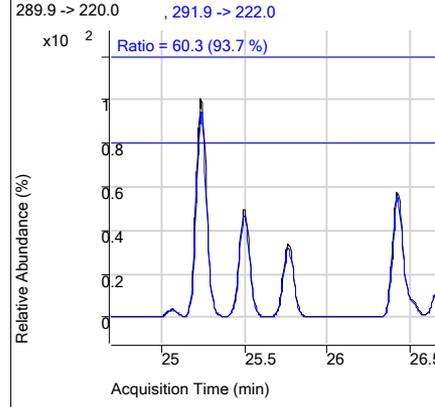
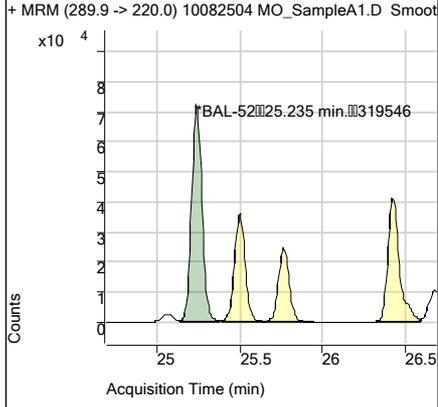
# PCB 28



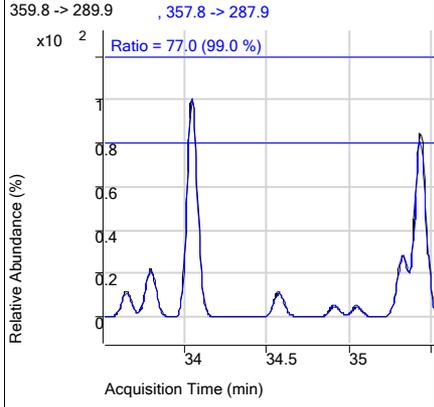
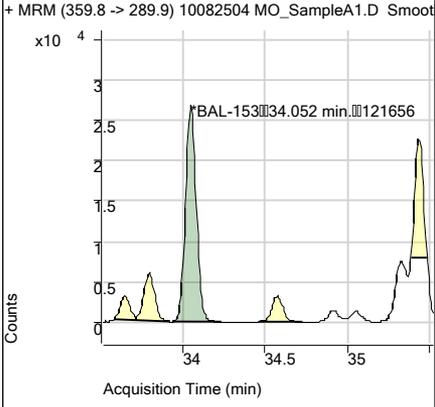
# PCB 138



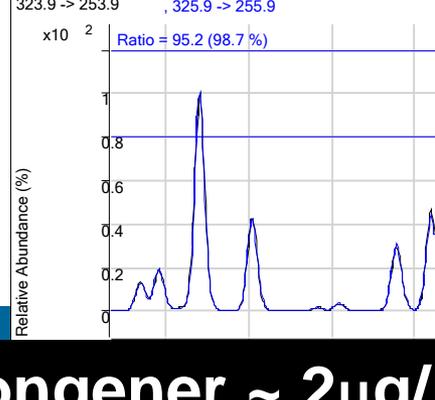
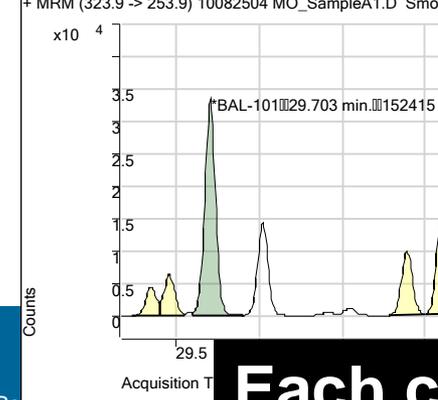
# PCB 52



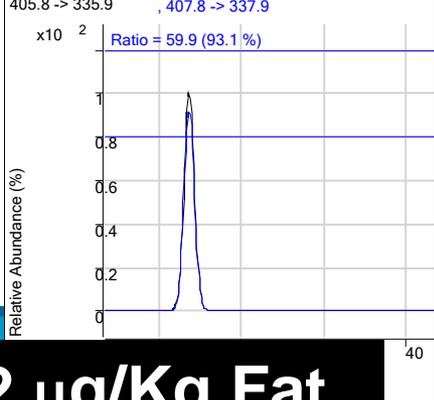
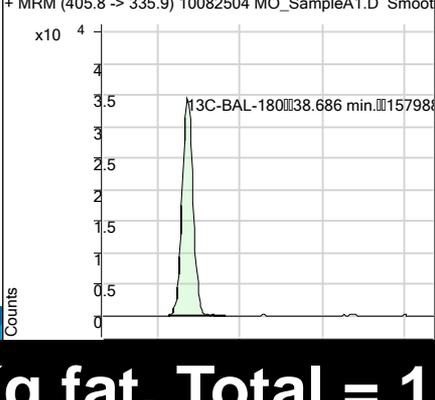
# PCB 153



# PCB 101



# PCB 180



**Each congener ~ 2 μg/Kg fat. Total = 12 μg/Kg Fat**