



BioDetection Systems

## 6<sup>th</sup> BioDetectors Meeting 2012

# Dioxin/PCB crisis in feed/food – A decade of DR CALUX testing

**Peter A. Behnisch**  
**BioDetection Systems**  
**Science Park, Amsterdam**

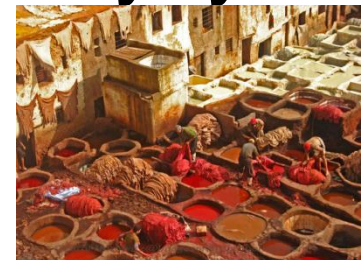




# Outline of Presentation

- **Current situation in Europe**
- **German Dioxin Crisis 2011**
- **Chile Food/Feed monitoring 2008**
- **Dutch Clay/Potato peeling crisis 2004**
- **Sydney harbor fish screening 2005**
- **Slovak Republic Food/Feed monitoring**
- **Norway Fish monitoring school project**
- **Taiwan feed and food monitoring**
- **BDS Client survey 2005**
- **Other food monitoring programs using DR CALUX**

- **2010 – Maize from Ukraine (Netherlands)**
- **2011 – eggs (Belgium/Netherlands)**
- **2011 – eggs and milk (Italy)**
- **2011 – Biodiesel used as feed led to 25% polluted market eggs (Germany)**
- **2011 – Sugar molasses (Germany)**
- **2012 – Red colorants (The Netherlands)**
- **2012- Nearly weekly egg recalls in Germany by KAT**





## **New EC guidelines 252/2012, 277/2012 and 278/2012 for screening for dioxins/PCBs by DR CALUX**

- **Cell based screening tests can now report samples for dioxins and dioxin-like PCBs as compliant or suspected to be non-compliant (suspected)**
- **New WHO-TEFs are more close to DR CALUX-REPs, lowering the amount of false negative for DR CALUX in case of high PCB contaminated samples**
- **False-compliant rate with respect to maximum levels is below 5% (before it was 1%)**
- **Repeatability RSD below 20%**
- **20 confirmed results per matrix group demanded for evaluation of false negative rate**
- **Suitable cut-off level at 2/3rd of maximum level**



## New EC 277 and 278/2012 guidelines: Cut-off levels for DR CALUX

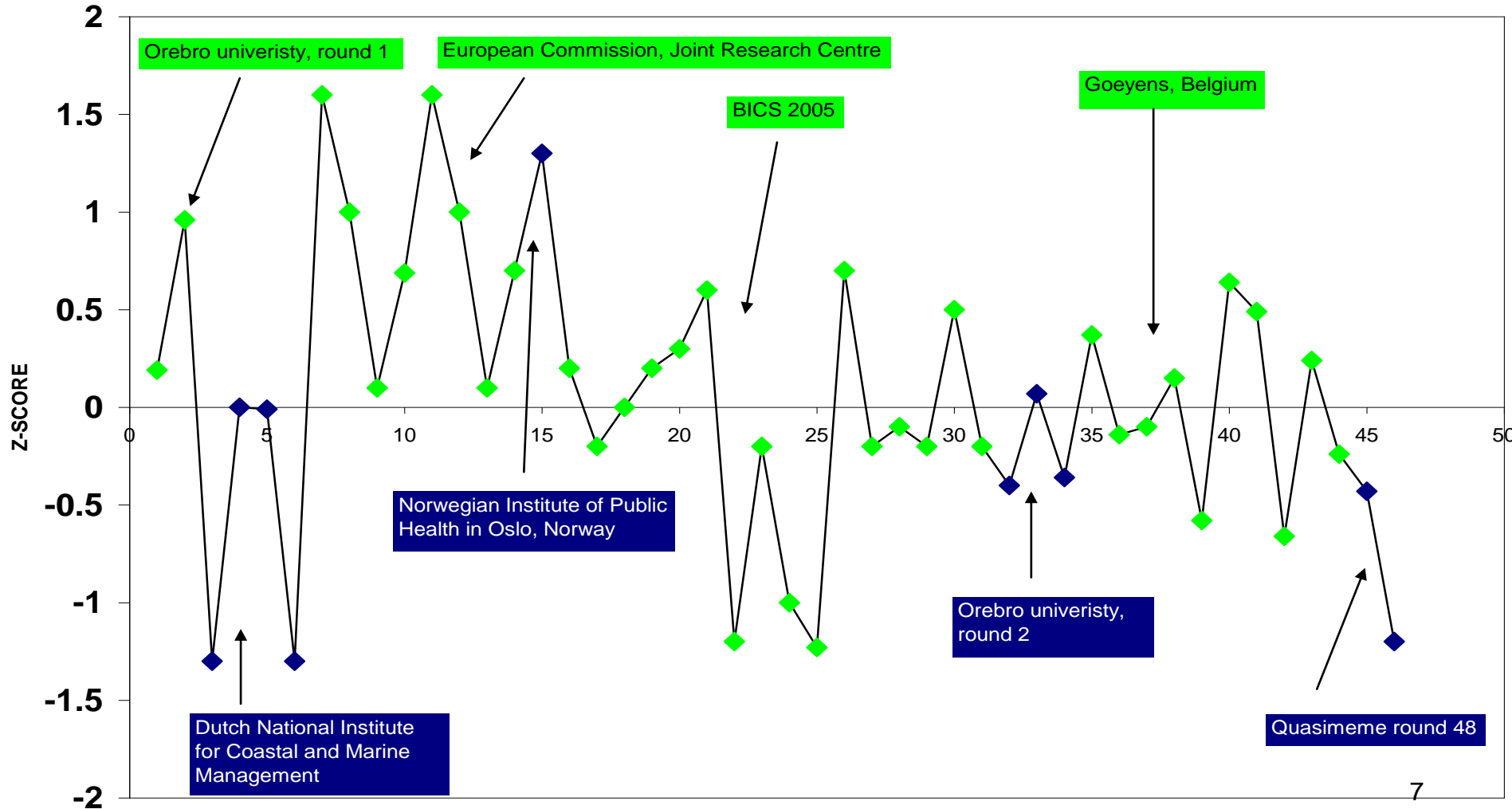
<b>Matrix</b>	<b>Old Total-TEQ</b>	<b>Old cut-off</b>	<b>New Dioxin</b>	<b>New Total-TEQ</b>	<b>New Cut-Off</b>
<b>Poultry</b>	<b>4</b>	<b>1.5</b>	<b>1.75</b>	<b>3</b>	<b>1.2</b>
<b>Bovine</b>	<b>4.5</b>	<b>2.25</b>	<b>2.5</b>	<b>4</b>	<b>1.7</b>
<b>Fish</b>	<b>8</b>	<b>3</b>	<b>3.5</b>	<b>6.5</b>	<b>2.3</b>
<b>Milk</b>	<b>6</b>	<b>2.25</b>	<b>2.5</b>	<b>5.5</b>	<b>1.7</b>
<b>Eggs</b>	<b>6</b>	<b>2.25</b>	<b>2.5</b>	<b>5</b>	<b>1.7</b>
<b>Plant oils</b>	<b>1.5</b>	<b>0.56</b>	<b>0.75</b>	<b>1.25</b>	<b>0.5</b>
<b>Babyfood</b>	<b>no</b>	<b>no</b>	<b>0.1</b>	<b>0.2</b>	<b>0.07</b>



**BDS DR CALUX vs HRGC/HRMS –  
170 comparisons: < 1% false negativ and < 8% false positive**

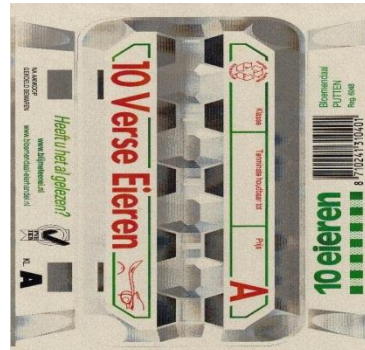
Matrix	N	False negative	False positive
egg	18	0	3
fish	14	0	0
Poultry meat	9	0	1
Ruminant meat	6	0	0
Pig meat	14	0	1
fish meal	8	0	0
Fish oil	15	0	1
milk	33	0	3
Feed, plant origin	18	0	4
Feed, vegetable oil	7	0	1

# Proficiency test performance BDS (2004-2011)



## Acceptance by International Quality systems

- **MVO Code of dioxins: 100% of batches for coconut, thermic treated oils and oils direct used as animal feed have to be tested by dioxins/PCBs**
- **Private laboratories have also to report in case of higher dioxin/PCB levels**
- **Increase quality product labels on EU market such as IKB (eggs), QS (feed) German Meat Association VDF since Jan 2011, or GMP Plus**





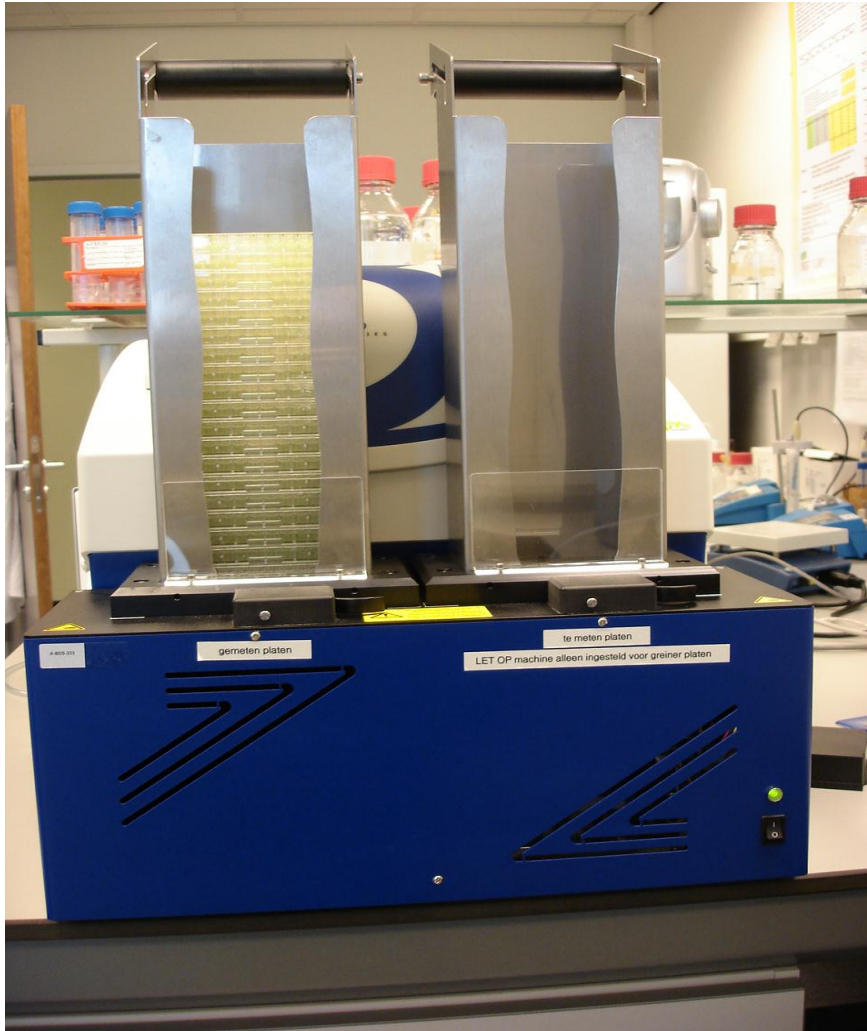


# Modernisation by High-Through Put Robot (HTPS)



**40 samples/hour  
dosing on 96 well  
plates**

# High-Through Put Robot (HTPS)



**40 samples/hour  
dioxin and separate  
PCB-TEQ  
measurement**



# Outline of Presentation

- Compliance with EU regulations
- **German Dioxin Crisis 2011**
- Chile Food/Feed monitoring 2008
- Dutch Clay/Potato peeling crisis 2004
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## Toxic Trade

The players in the dioxin scandal

**Petrotech AG** (Emden, Germany) manufactures biofuel from spent cooking fats. The process also produces mixed fats.



**Olivet** (Rotterdam, the Netherlands) buys the fats and sells them on.



**Harles and Jentzsch** (Uetersen and Bösel, Germany) uses the fats in the production of feed fats. It is still not clear at what point the dioxin got into the fats.



**Feed producers** mix the feed fats in with their animal feed.



**Farmers** buy the animal feed and use it to feed their hens, turkeys and pigs.



- **4700 farms at first closed – issue taken up by international media**
- **70% of Germans doubted, if their breakfast eggs are dioxin-free**
- **Ca. 70% lower revenues for farmers/industry – rough calculations talked from 20 Mio Euro/week damage**
- **Most local testing needed > 10 days = lack of local testing capacity**
- **Screening via DR CALUX offers time and cost saving**
- **More than 95% of the ca 1800 samples from Germany tested as express analysis in 2-3 days**

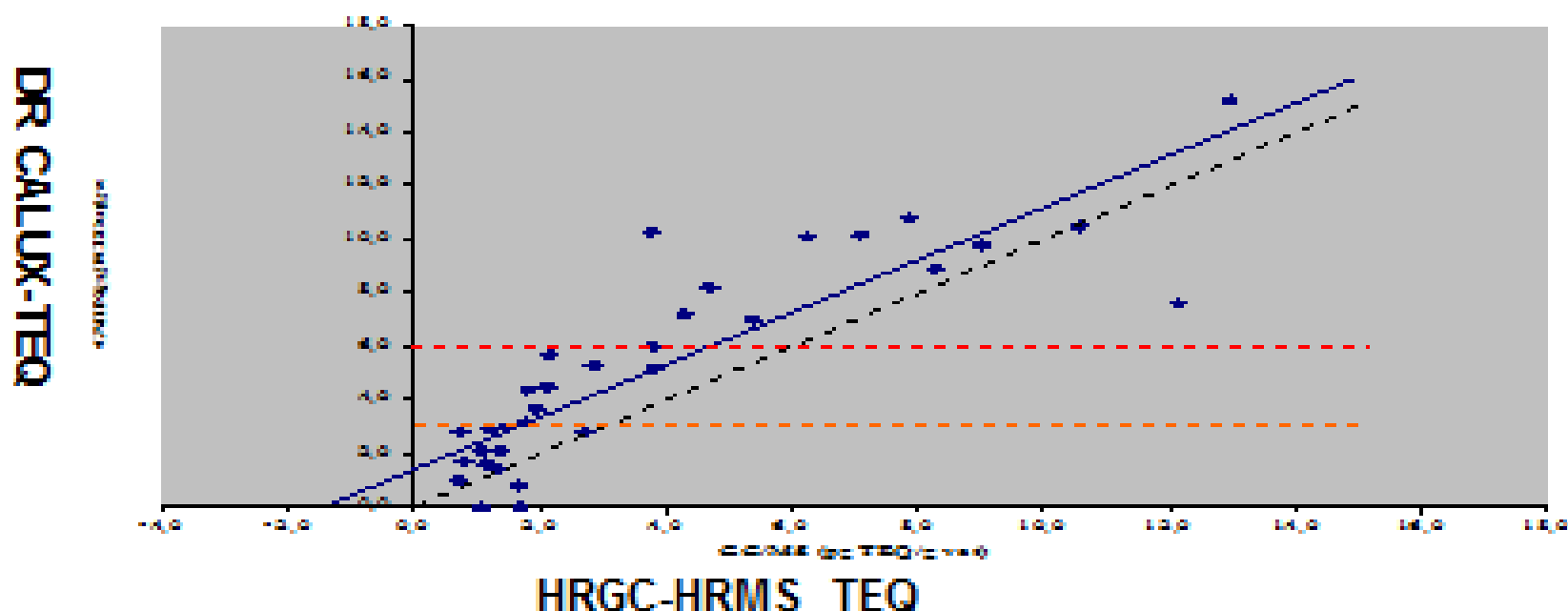


## Situation in January/February 2011

- **More than 15 countries banned German meat**
- **German Meat Association (VDF) in cooperation with German Authorities (BMELV) and the Russian Import Authorities accepted more than 40 labs for the German meat exports – only 4 non-German laboratories**
- **BDS only accepted non-German lab using screening technology DR CALUX**
- **Regarding acceptance of ISO 17025 accredited labs please notice that according EC/764/2008 all reports of such accredited labs have to be accepted in all EC member states**
- **BDS ISO 17025 accredited since 2005 and new approved in mid 2011 until 2014**

## Earlier Dutch reports about dioxins in eggs analysed by DR CALUX and HRGC/HRMS

- Controlling egg dioxin levels from laying hens with outdoor run by A. Kijlstra, W. Traag and R. Hoogenboom (2008)



- Bio-eggs in Dutch farms by J. de Vries (2002)

At 2002 from 68 “biological” eggs 9 (13%) eggs were suspected in the DR-CALUX. HRGC/MS showed, that total TEQ varied between 2.6 and 14.9  $\mu\text{g TEQ/g}$  per gram of fat.



German **pig meat** > 97,6% negative;  
less than 2.5 % false positive by DR CALUX

	PCB - TEQ	PCDD/F -TEQ	Total- TEQ	Sample compliant PCDD/F - 25%	Sample compliant t TOTAL - 25%	Sample compliant PCDD/F - 50%	Sample compliant TOTAL - 50%
<b>Pig Meat; N</b>	496	496	502	99,8%	97,6	94%	89%
<b>Pig Meat; Mean</b>	0,23	0,21	0,43				
<b>Pig Meat; Range</b>	0,1- 1,1	0,1- 0,77	0,1- 1,47				

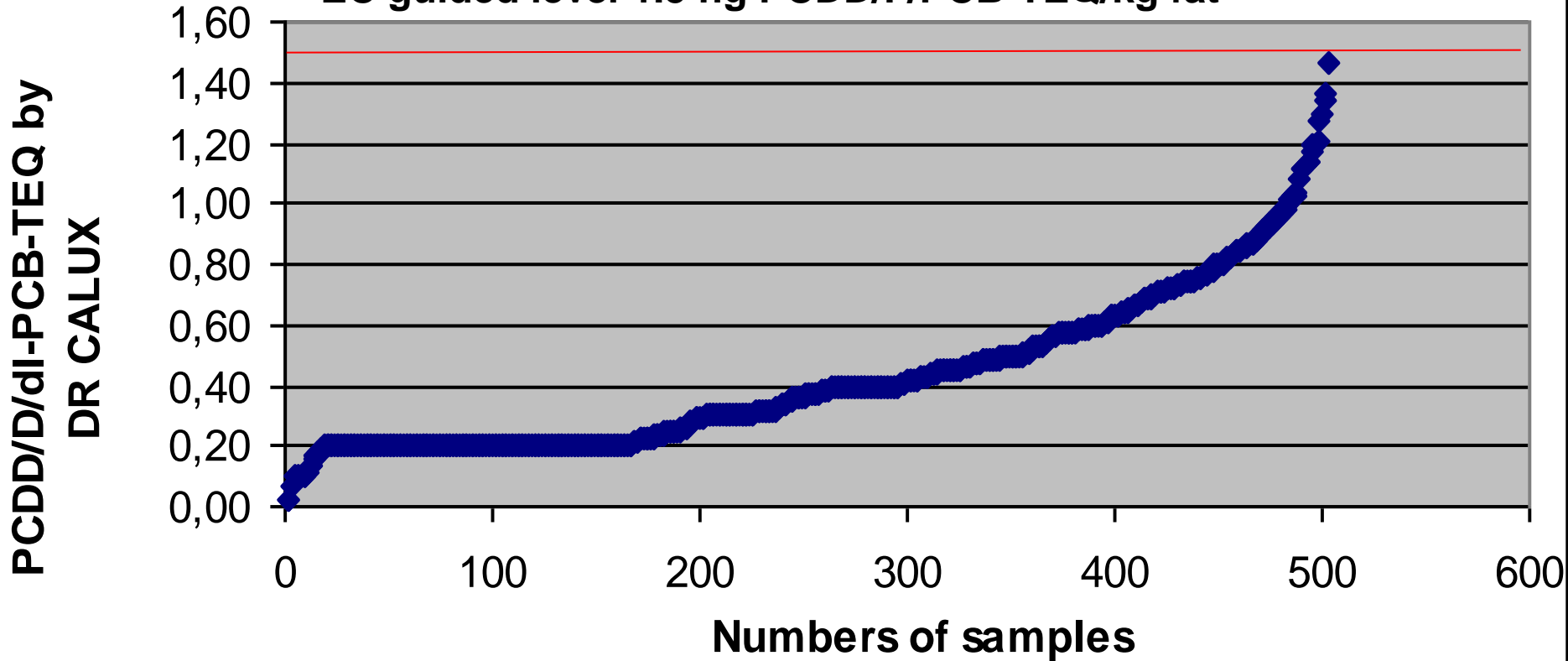




# DR CALUX analysis for **PCB + PCDD/F-TEQ** (# 502 samples): German **pig meat** Distribution

**Total-PCDD/F/dl-PCB-TEQ by DR CALUX for German pig meat in Jan/Feb 2011 in pg PCDD/F/dl-PCB-TEQ/g fat**

**EC guided level 1.5 ng PCDD/F/PCB-TEQ/kg fat**

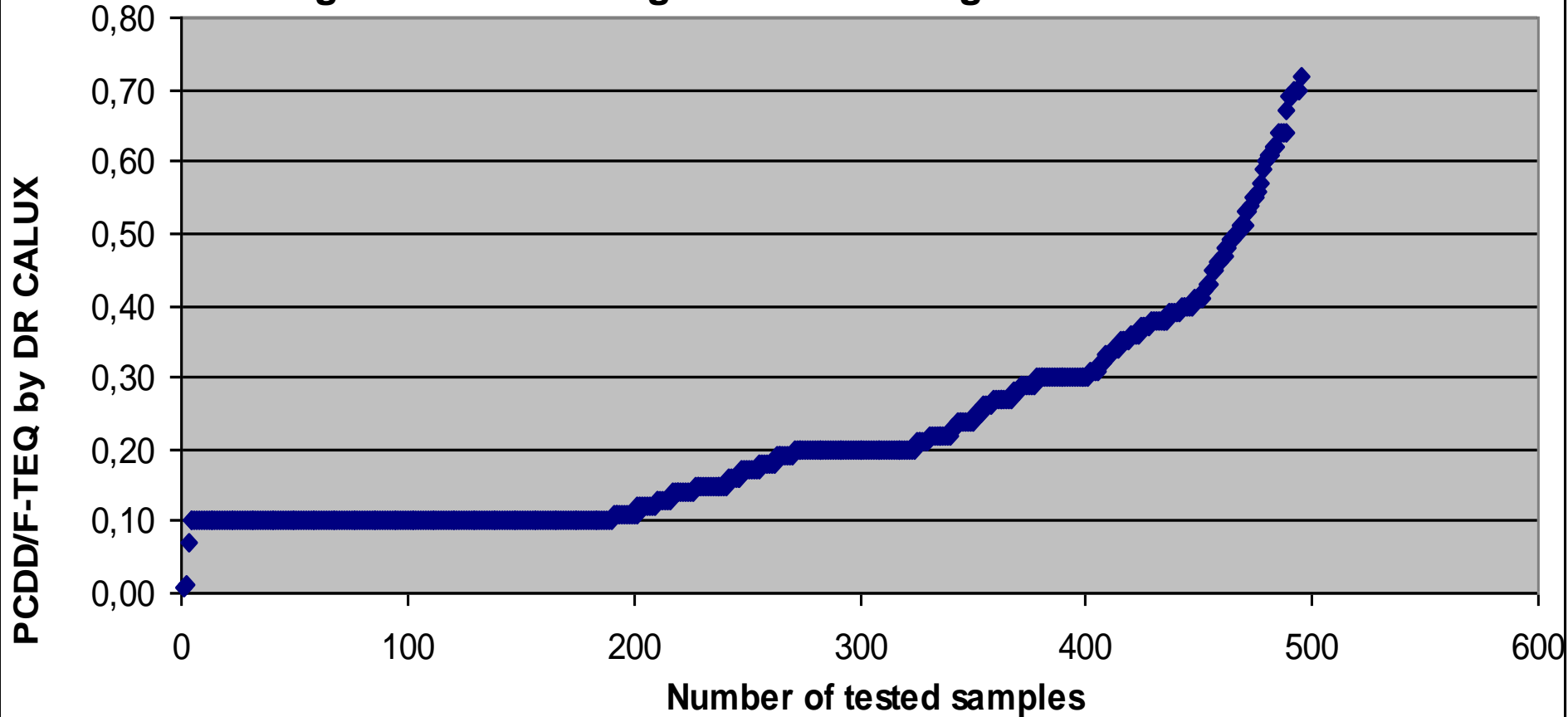




# DR CALUX analysis for PCDD/Fs (# 496 samples): German pig meat Distribution

Distribution PCDD/F-TEQ by DR CALUX for German pig meat in Jan/Feb 2011

EC guided level 1.0 ng PCDD/F-TEQ/kg fat





German **pig liver** > 97% negative;  
less than 3 % false positive by DR CALUX

	PCB- TEQ	PCDD/F -TEQ	Total- TEQ	Sample compliant PCDD/F - 25%	Sample compliant TOTAL - 25%	Sample compliant PCDD/F - 50%	Sample compliant TOTAL - 50%
<b>Pig Liver; N</b>	32	37	34	99%	97%	93%	90%
<b>Pig Liver; Mean</b>	0,80	0,78	1,57				
<b>Pig Liver; Range</b>	0,1- 2,1	0,1- 3,4	0,1- 5,2				



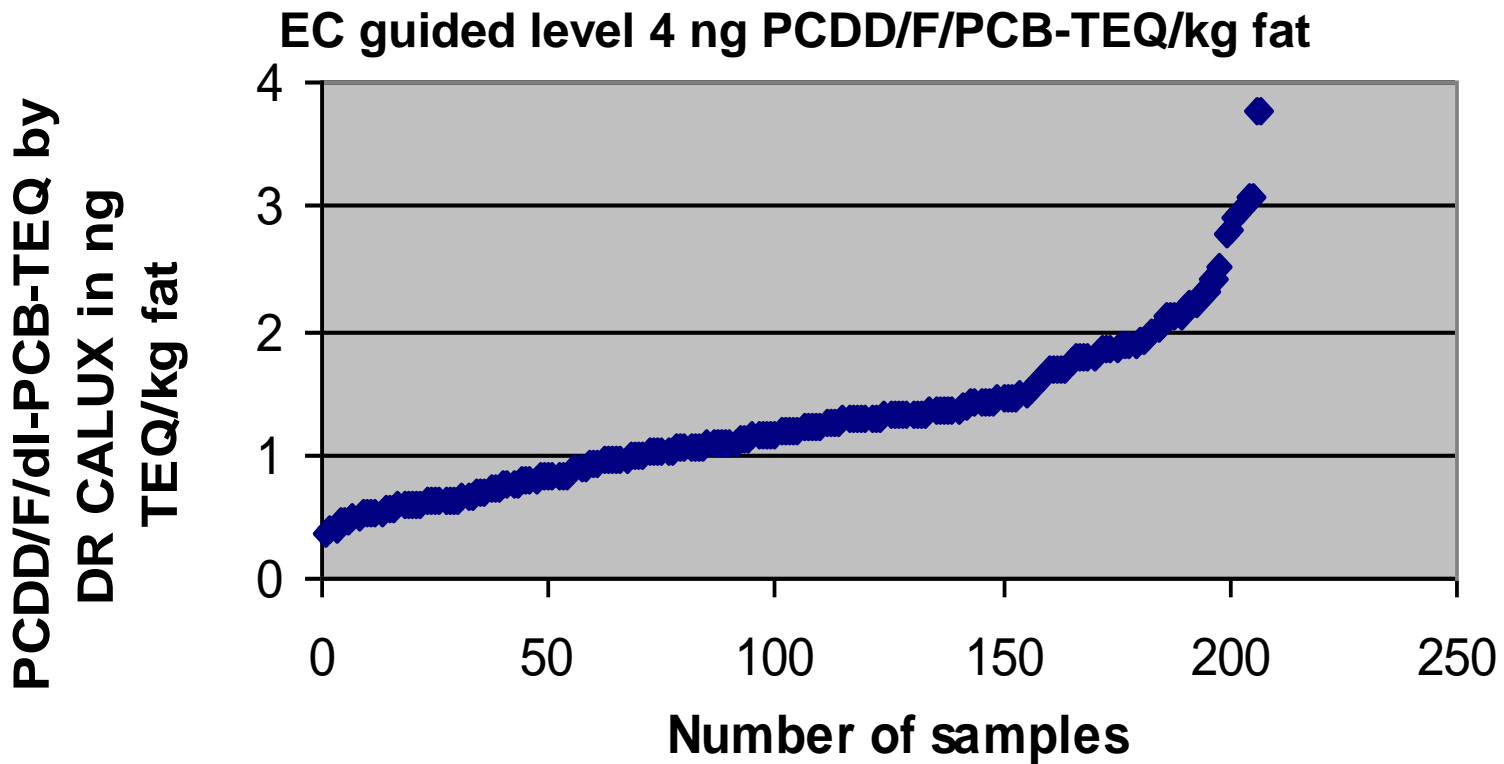
German **poultry meat** > 98% negative;  
less than 2 % false positive by DR CALUX

	PCB -TEQ	PCDD/F -TEQ	Total- TEQ	Sample compliant PCDD/F - 25%	Sample compliant TOTAL - 25%	Sample compliant PCDD/F - 50%	Sample compliant TOTAL - 50%
Chicken Meat; <b>N</b>	201	201	207	99,5%	98%	93%	88%
Chicken Meat; <b>Mean</b>	0,74	0,56	1,3				
Chicken Meat; <b>Range</b>	0,3- 3,3	0,3 – 1,7	0,6- 3,8				



DR CALUX analysis for **PCDD/F/PCB-TEQ** (# 207 samples):  
German **poultry meat** Distribution

### PCDD/F/dl-PCB-TEQ by DR CALUX for German poultry meat in Jan/Feb 2011

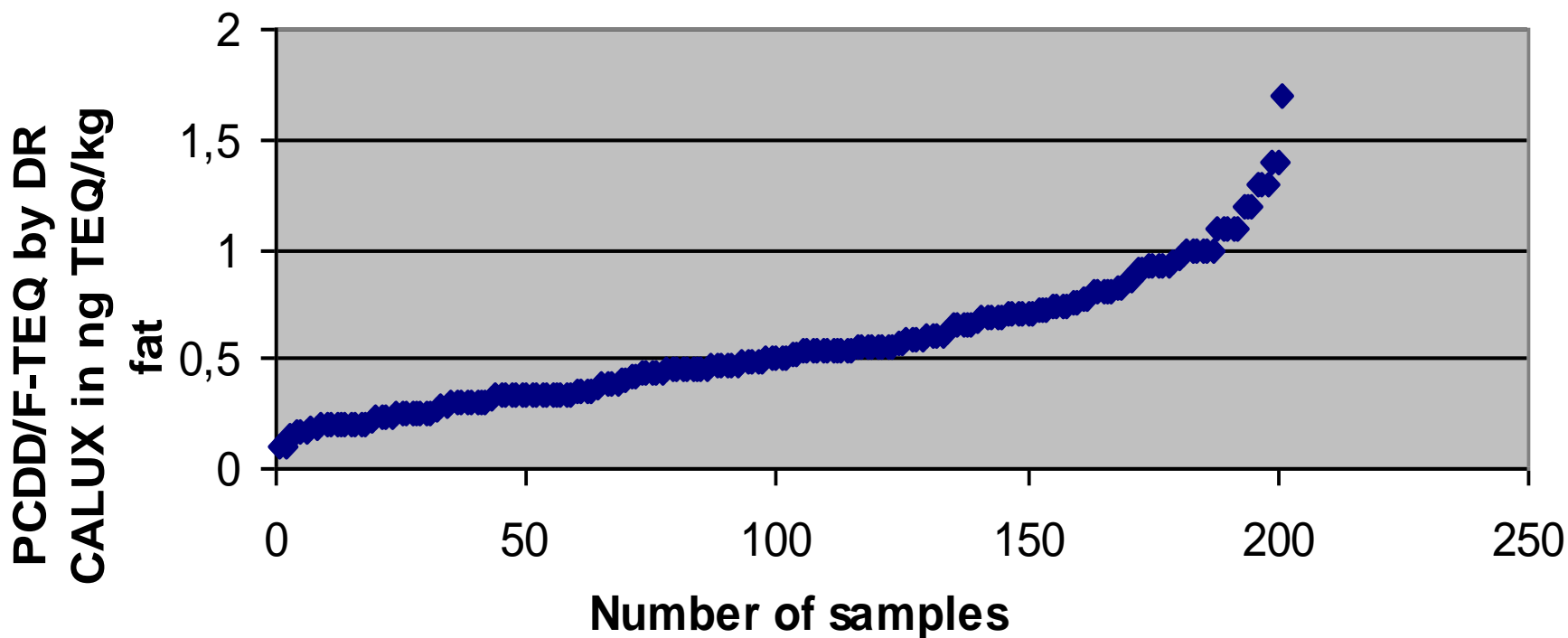




# DR CALUX analysis for PCDD/F-TEQ (# 201 samples): German **poultry meat** Distribution

**PCDD/F-TEQ by DR CALUX of German poultry in  
Jan/Feb 2011 (cut-off 1,4 pg PCDD/F-TEQ/gr fat)**

EC guided level 2 ng PCDD/F-TEQ/kg fat

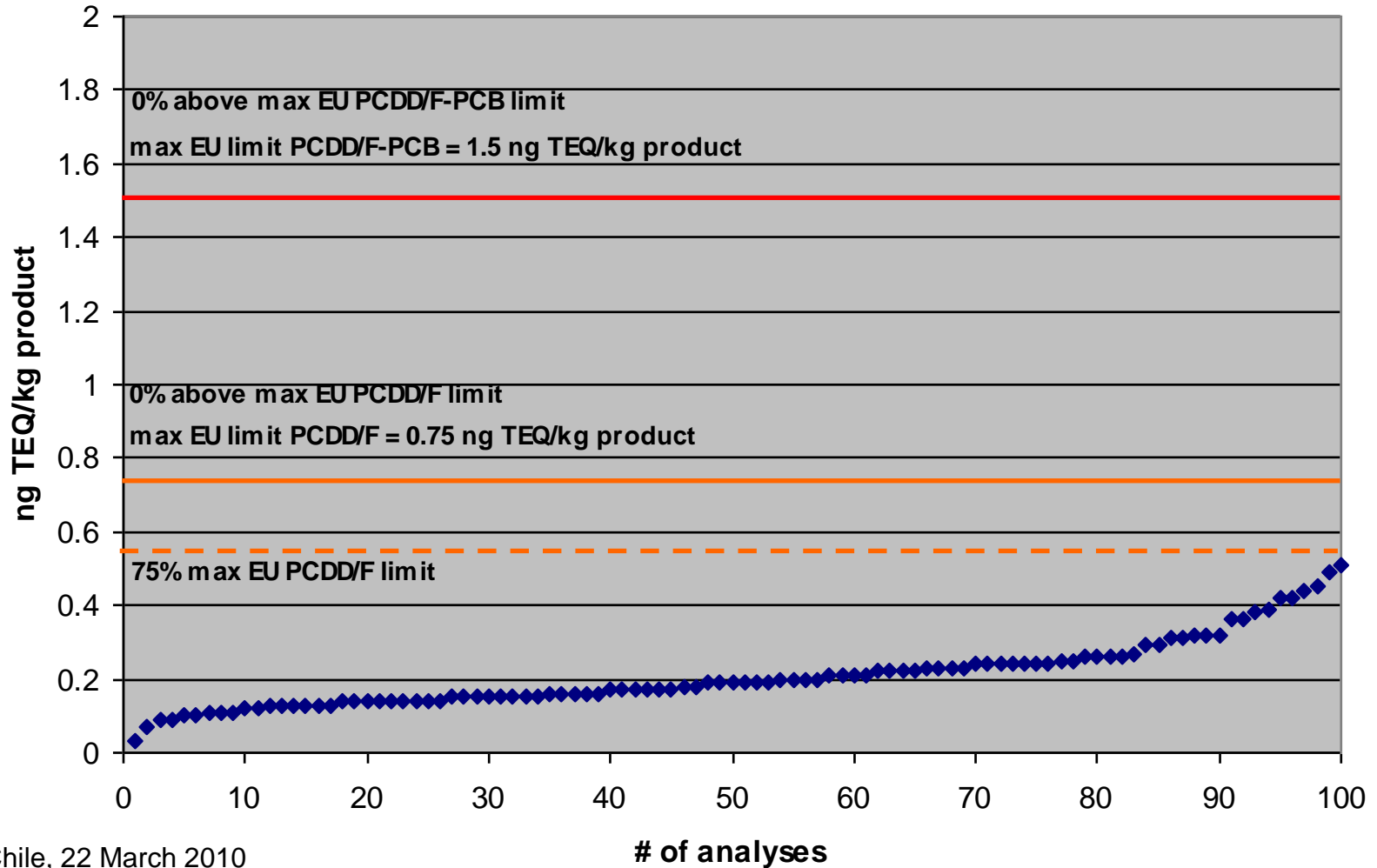


- Compliance with EU regulations
- German Dioxin Crisis 2011
- Kuwait Monitoring of imported feed/food 2009
- **Chile Food/Feed monitoring 2008**
- Dutch Clay/Potato peeling crisis 2004
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- Norway Fish monitoring school project
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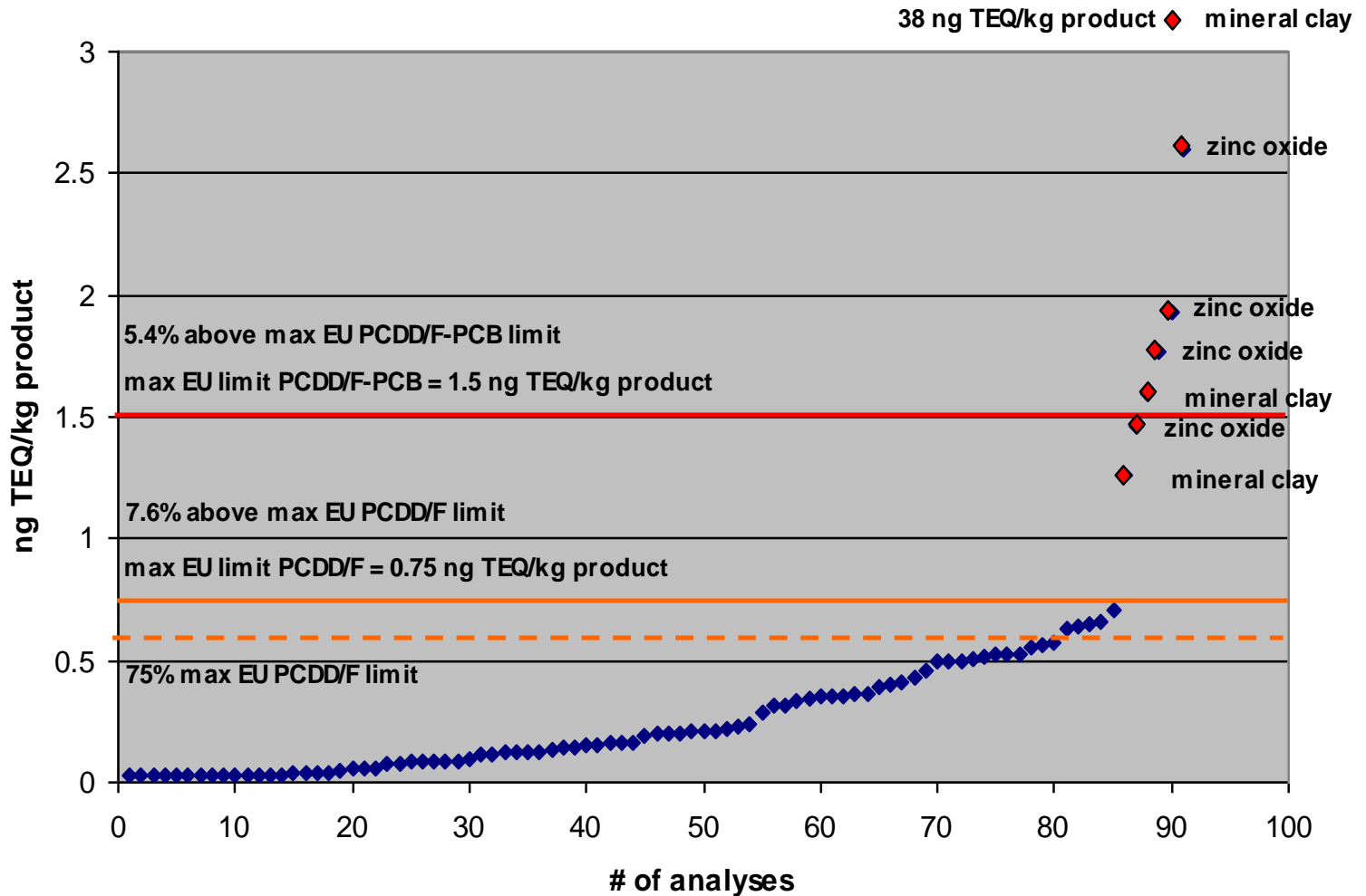
# Total DR CALUX<sup>®</sup> distribution for FEED samples, 2009 Asprocer Program, Chile





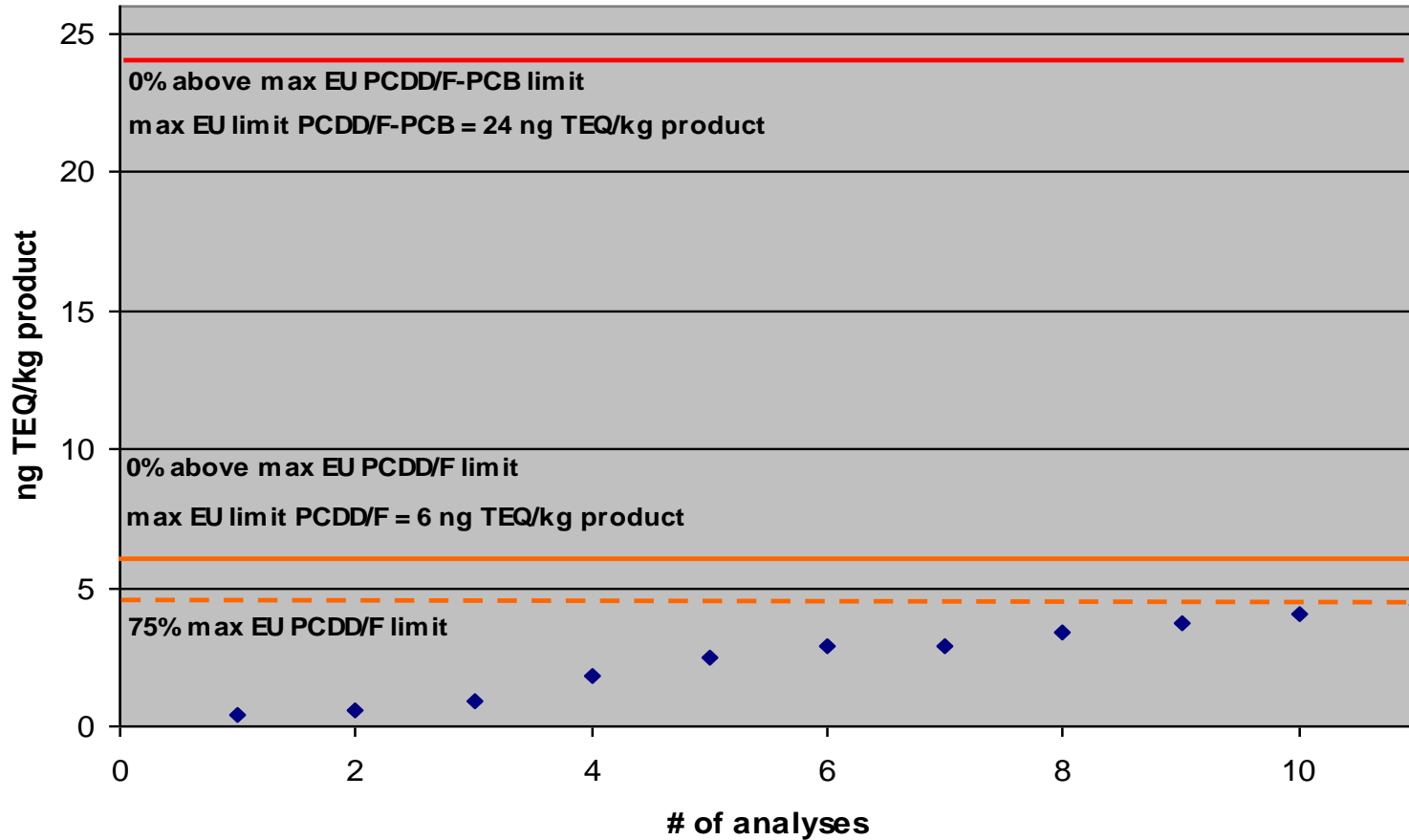


# Total DR CALUX<sup>®</sup> distribution for MINERAL samples, 2009 Asprocer Program, Chile



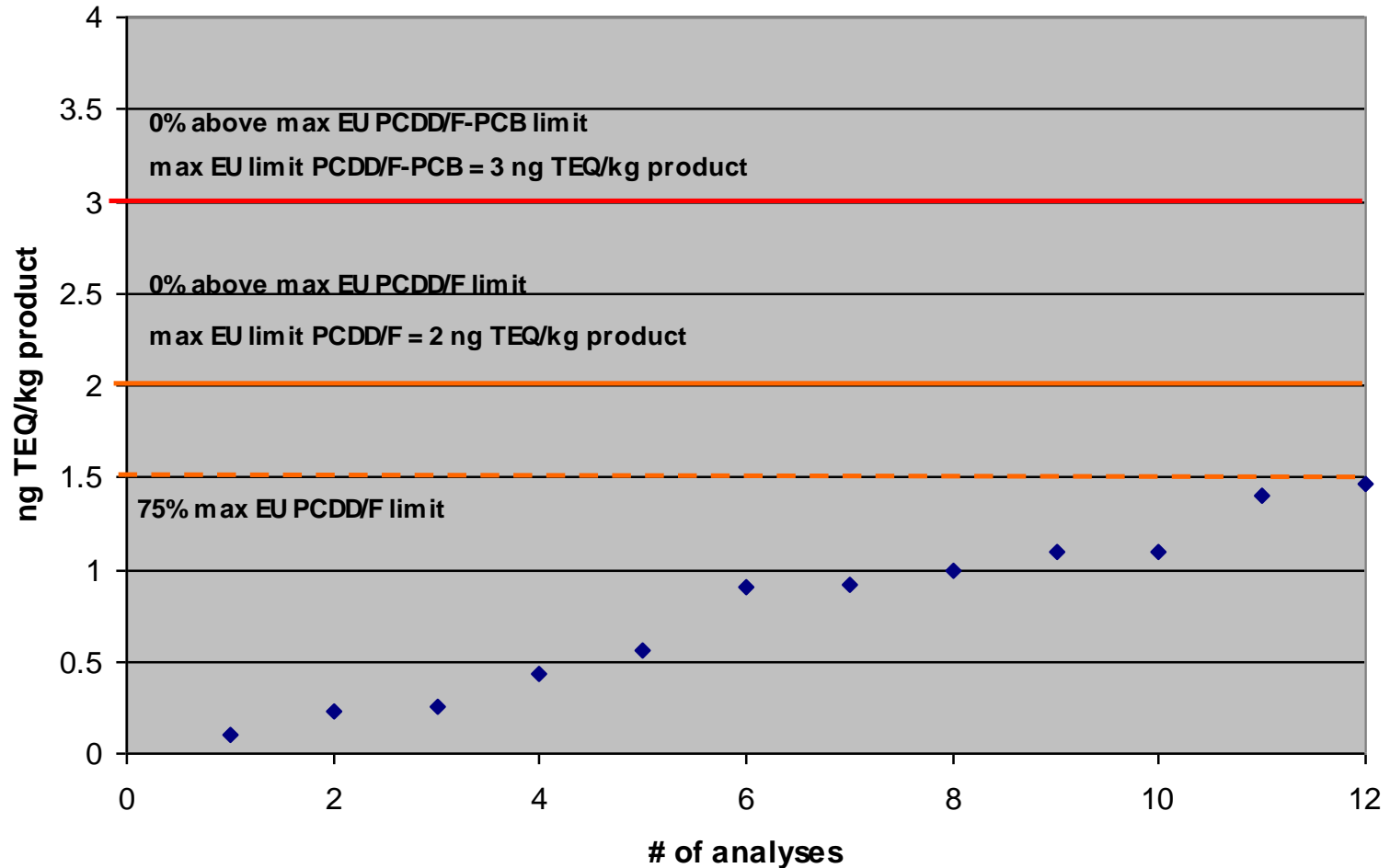


# Total DR CALUX<sup>®</sup> distribution for FISH OIL samples, 2009 Asprocer Program, Chile



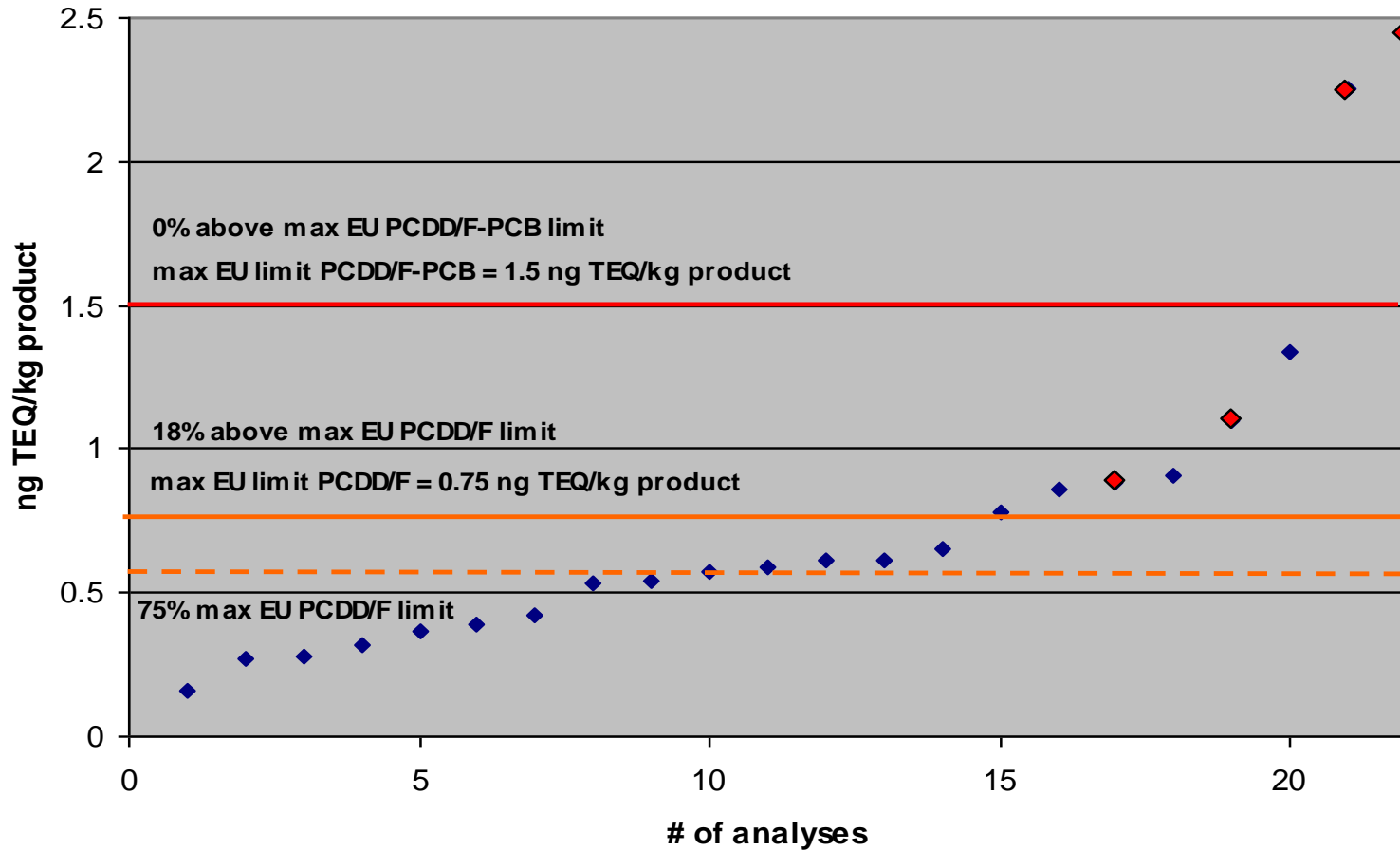


# Total DR CALUX<sup>®</sup> distribution for ANIMAL OIL samples, 2009 Asprocer Program, Chile





# Total DR CALUX<sup>®</sup> distribution for VEGATABLE OIL samples, 2009 Asprocer Program, Chile



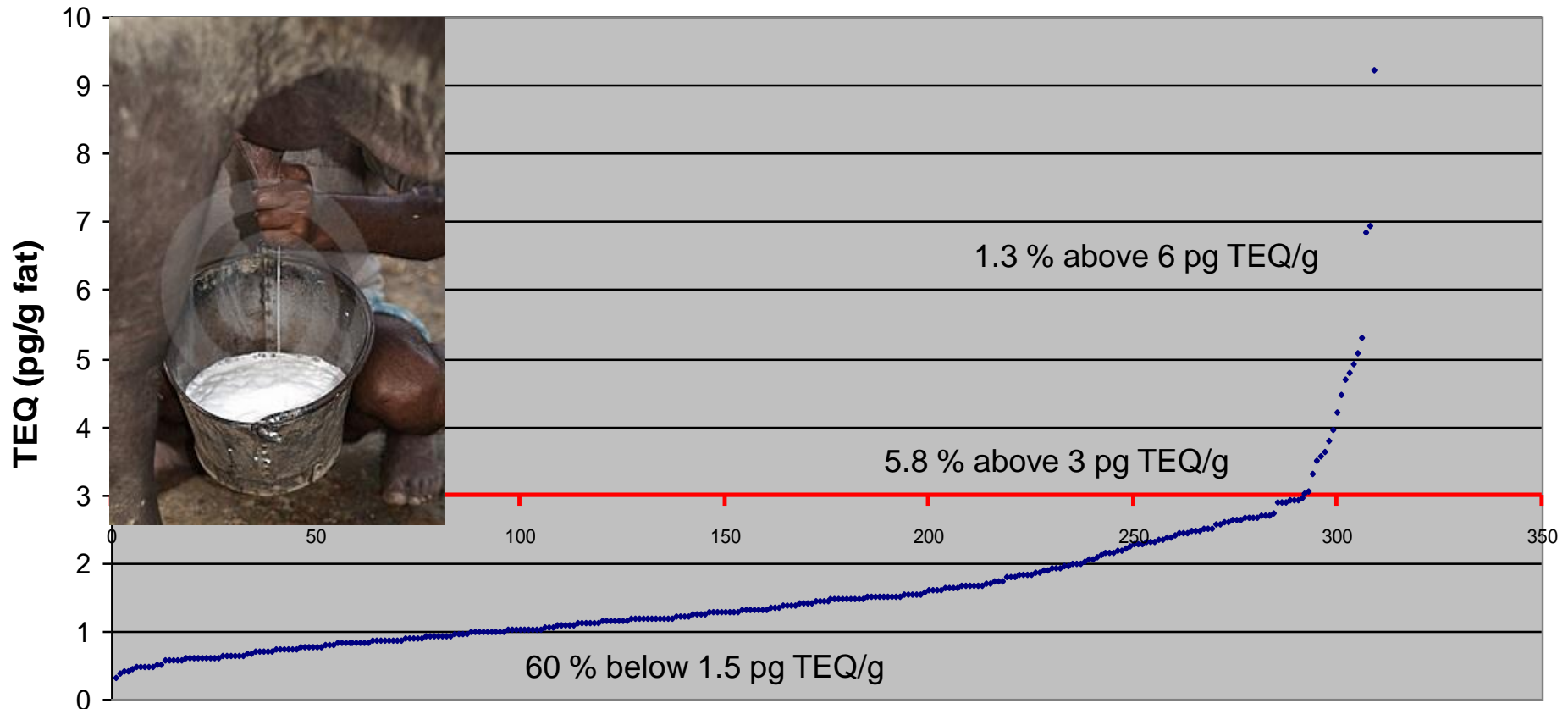
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## Clay effecting potato peeling used a animal feed.

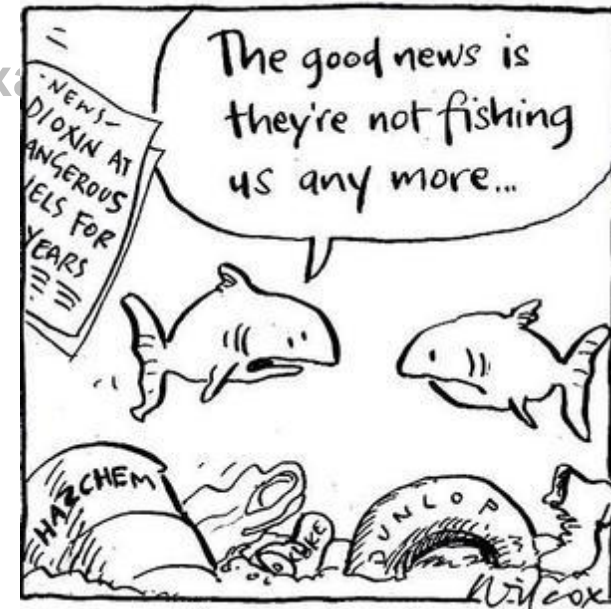
total DR CALUX TEQ distribution for milk samples, 2004 Dutch clay crisis



• reported TEQ (sorted from lowest to highest)

# Outline of Presentation

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- Chemical TEQ vs. DR CALUX TEQ – a few examples
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Soils Recycled Organics & Remediation Technologies Unit,  
Sydney, Australia

## **Dioxins: A fishy business; the analysis of dioxins in seafood from Sydney Harbour**



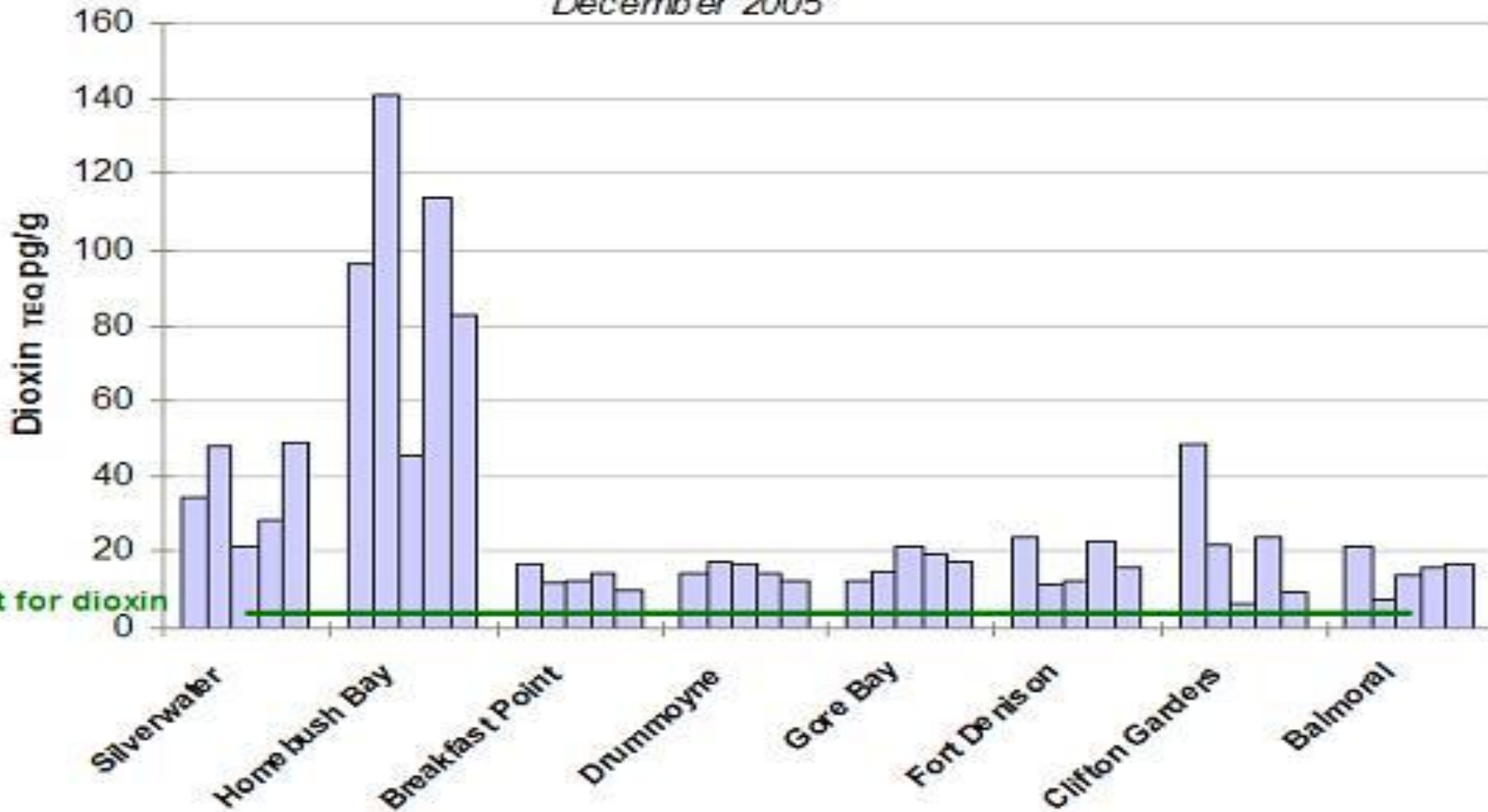




# Sydney Harbour; preliminary results

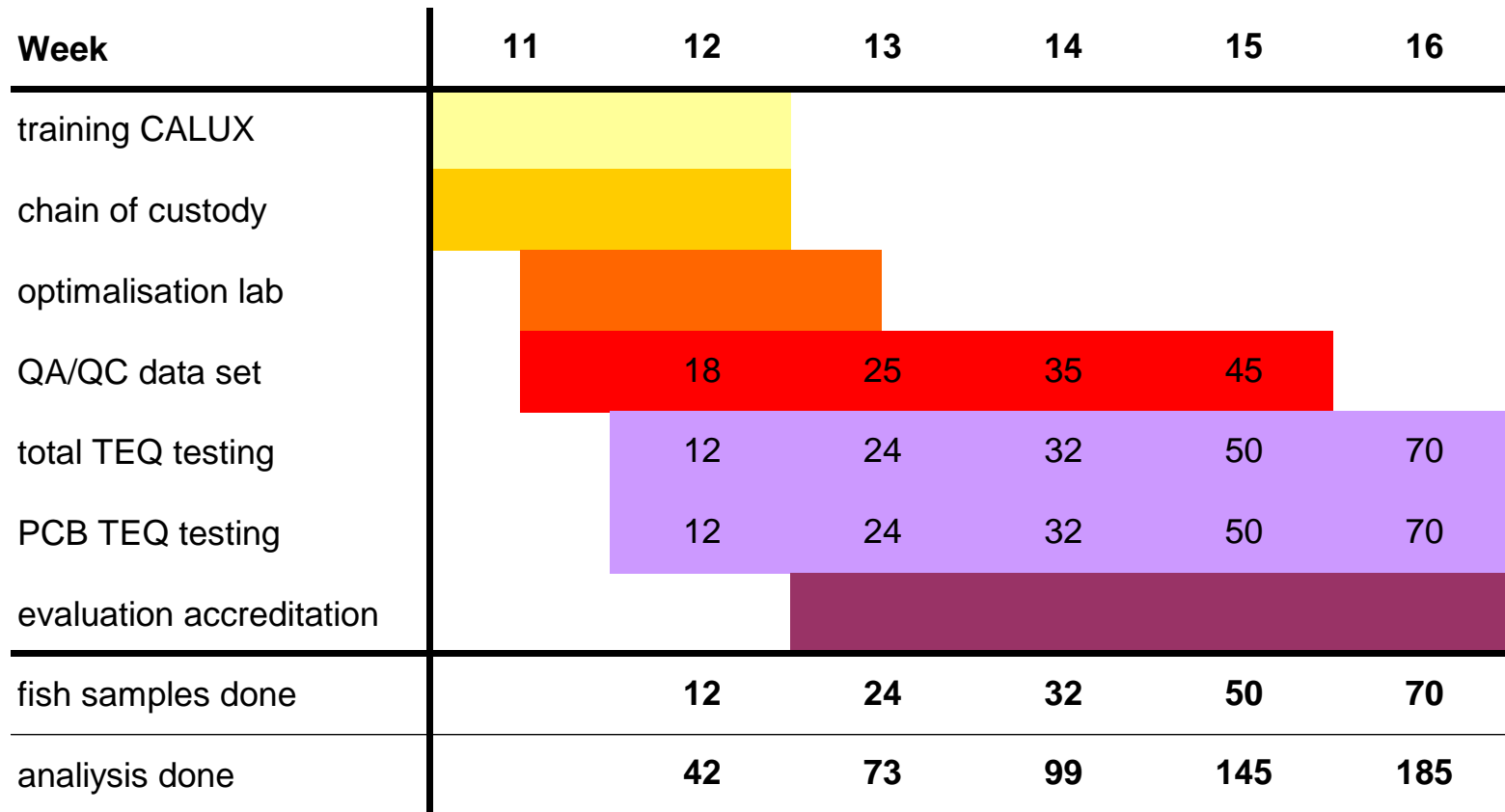
## Port Jackson Bream Dioxin Results

December 2005



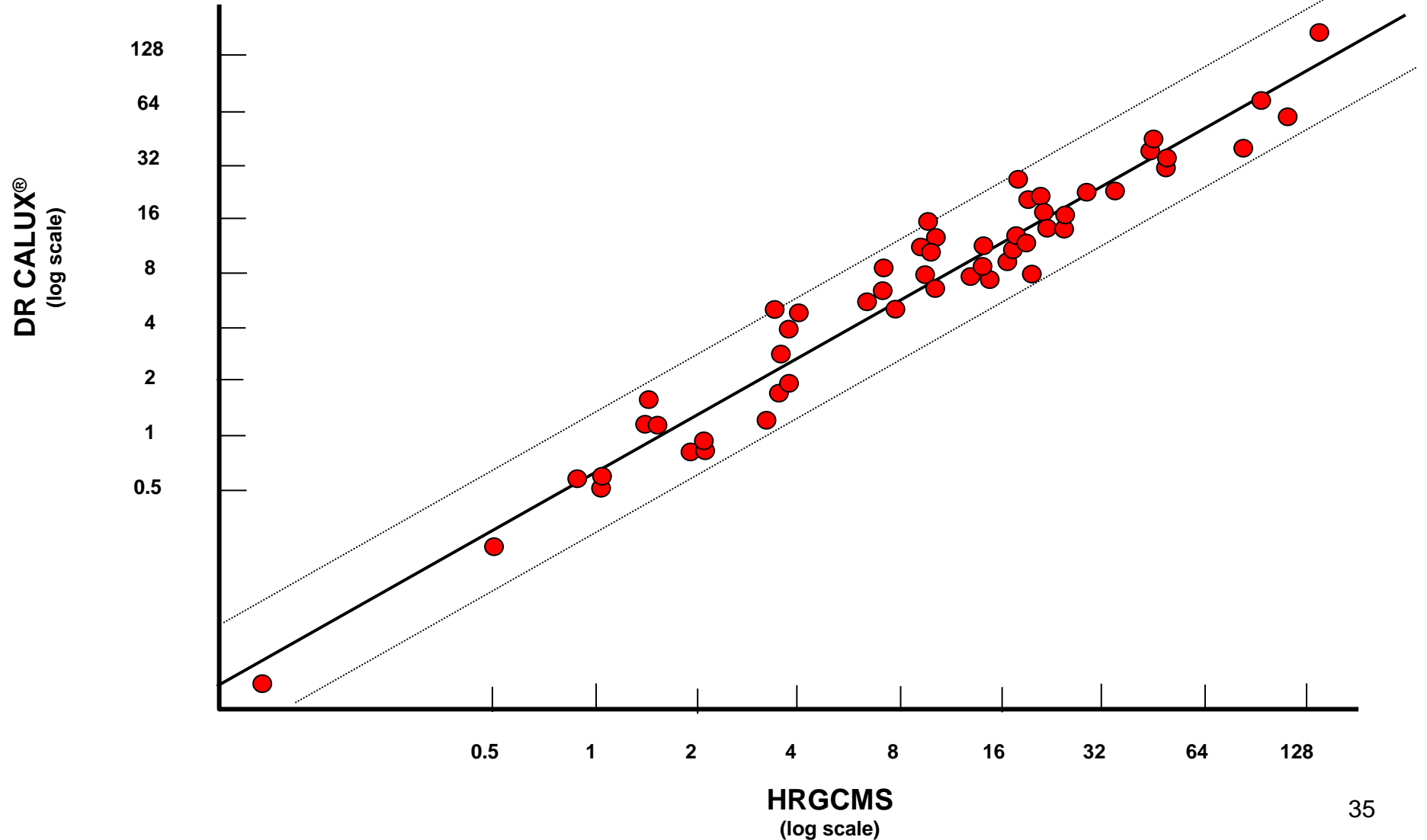


# Time line rapid implementation





# Sydney harbour: Comparison of DR CALUX<sup>®</sup> and HR-GCMS

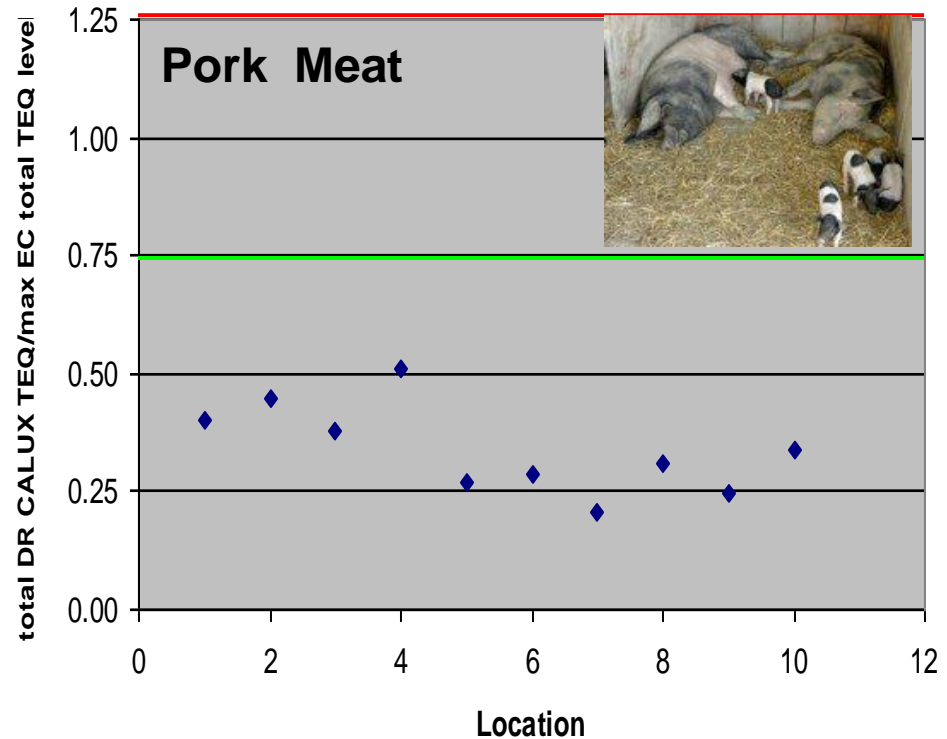
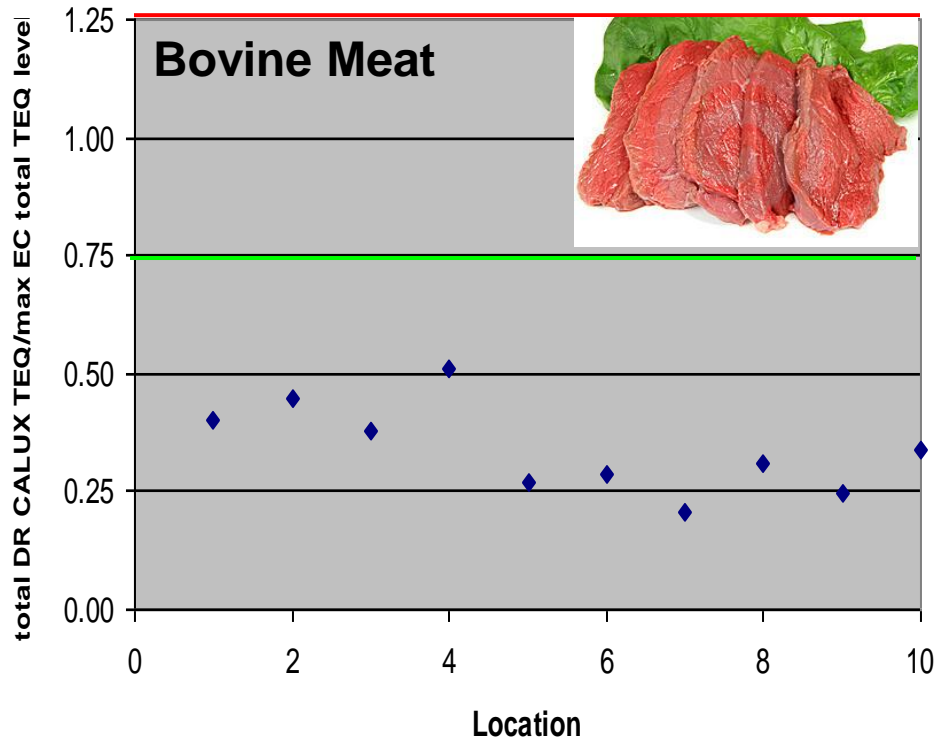




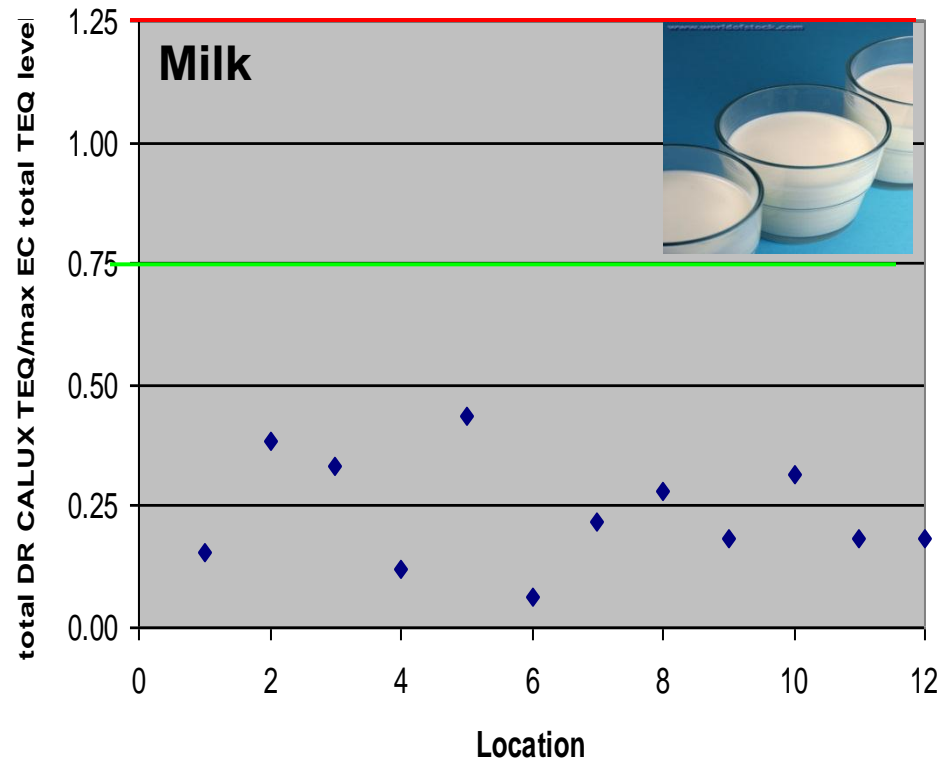
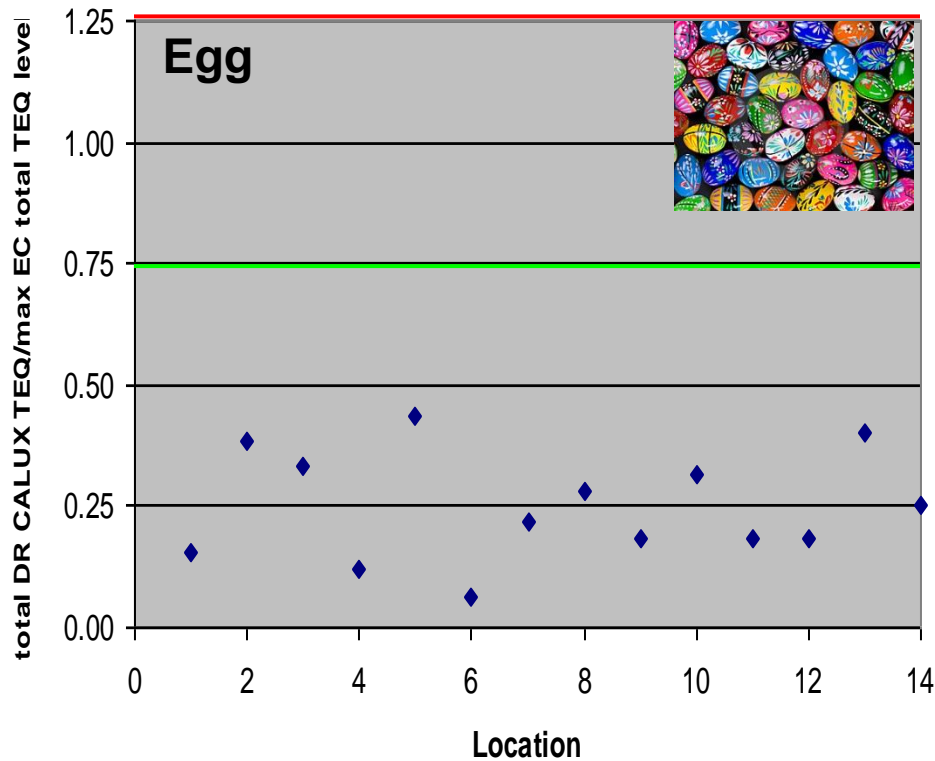
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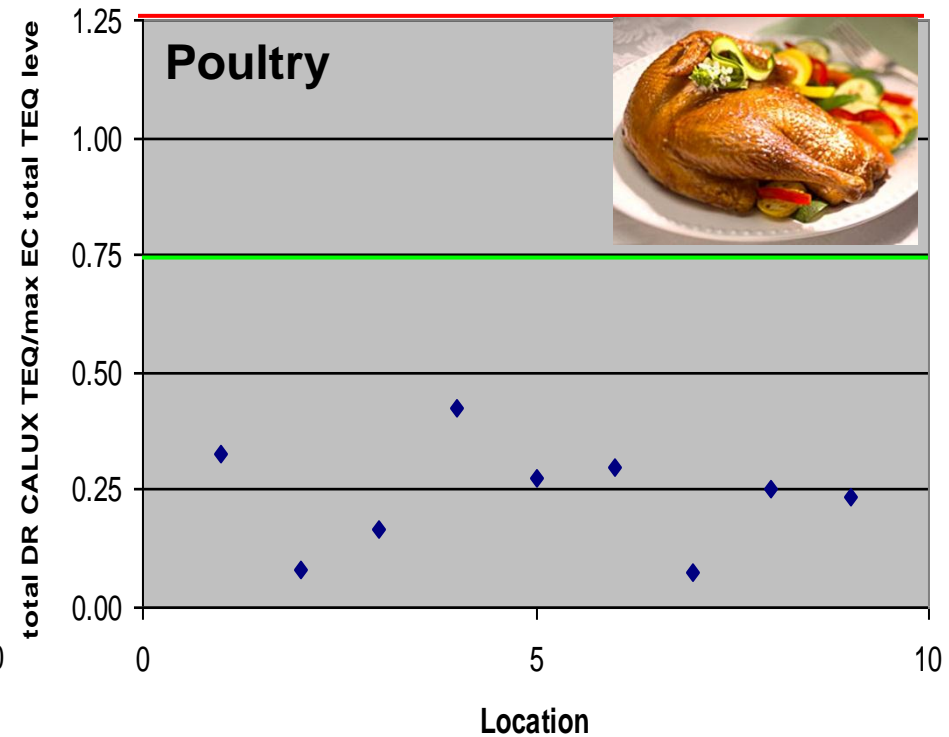
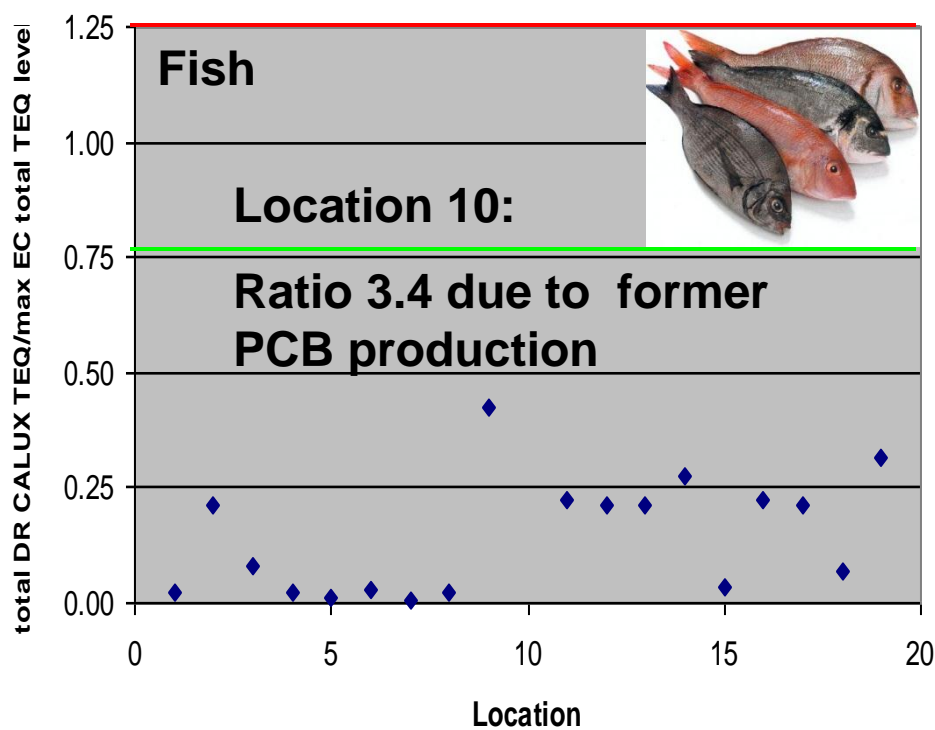
Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ



## Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ



## Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ





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## Dioxins in fish with BDS CALUX

Eldbjørg Sofie Heimstad<sup>1</sup>, Gaute Grønstøl<sup>2</sup>, Karl Torstein  
Hetland<sup>3</sup>, Javier Martinez Alarcon<sup>1</sup>, Charlotta Rylander<sup>1,4</sup>  
and Espen Mariussen<sup>1,5</sup>

<sup>1</sup>Norwegian Institute for Air Research (NILU)

<sup>2</sup>University of Bergen, Bergen.

<sup>3</sup>Norwegian Centre for Science Education

<sup>4</sup>Institute of Community Medicine, University of Tromsø

<sup>5</sup>Norwegian Defence Research Establishment (FFI)



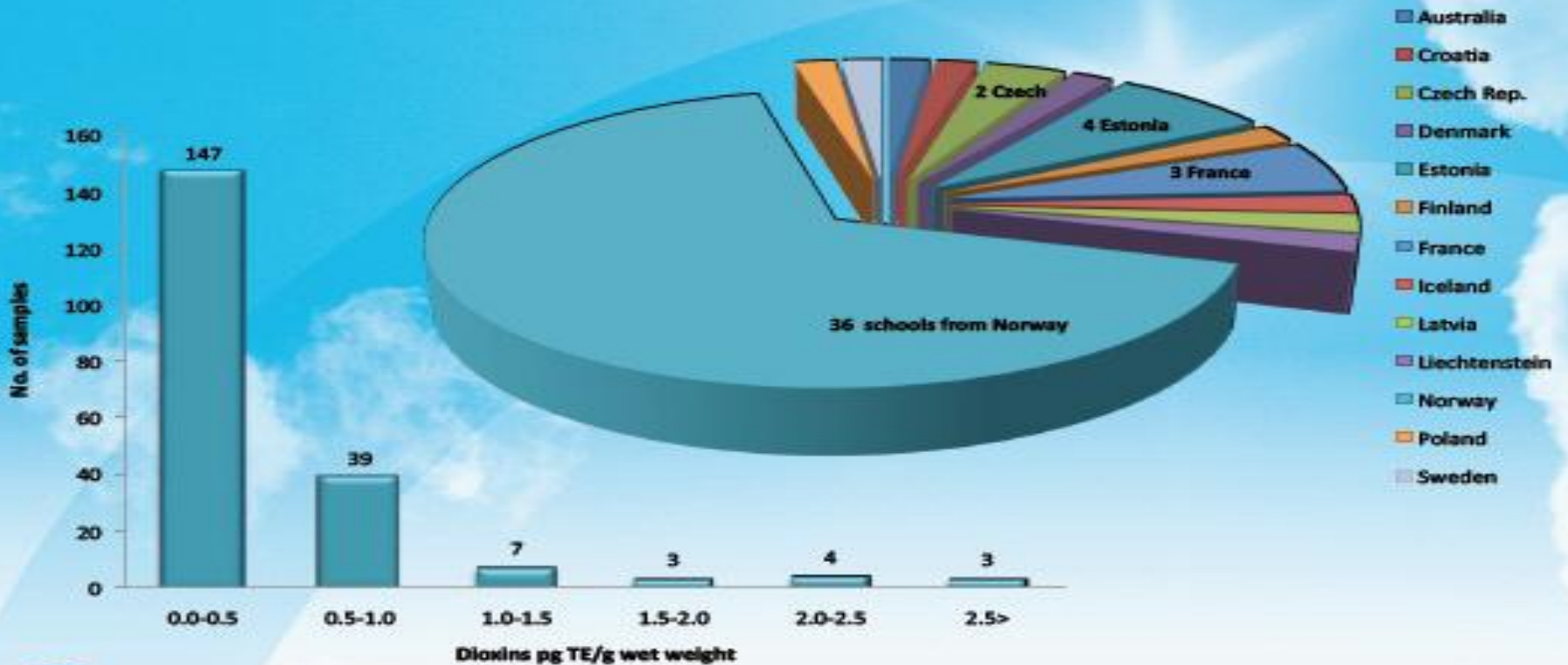
## Global POP – a school project

- Kids participate with field work and data publishing in a research project
- Investigate dioxins and dioxin-like compounds (CALUX) in fish common for consumption
- Schools from Arctic areas will be encouraged to participate. TEQ will be determined by NILU in fish that were caught by the students
- Investigate dioxins and dioxin-like compounds (CALUX) in fish common for consumption



## Results

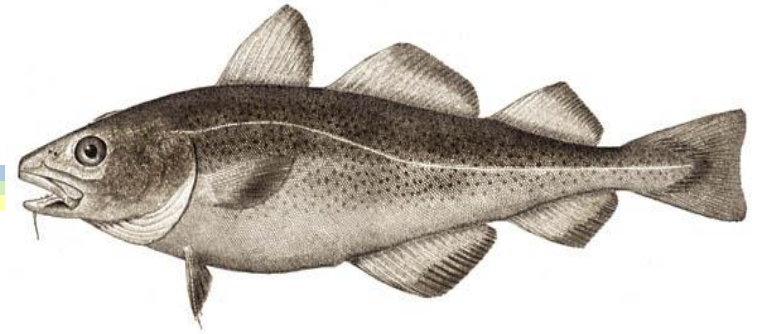
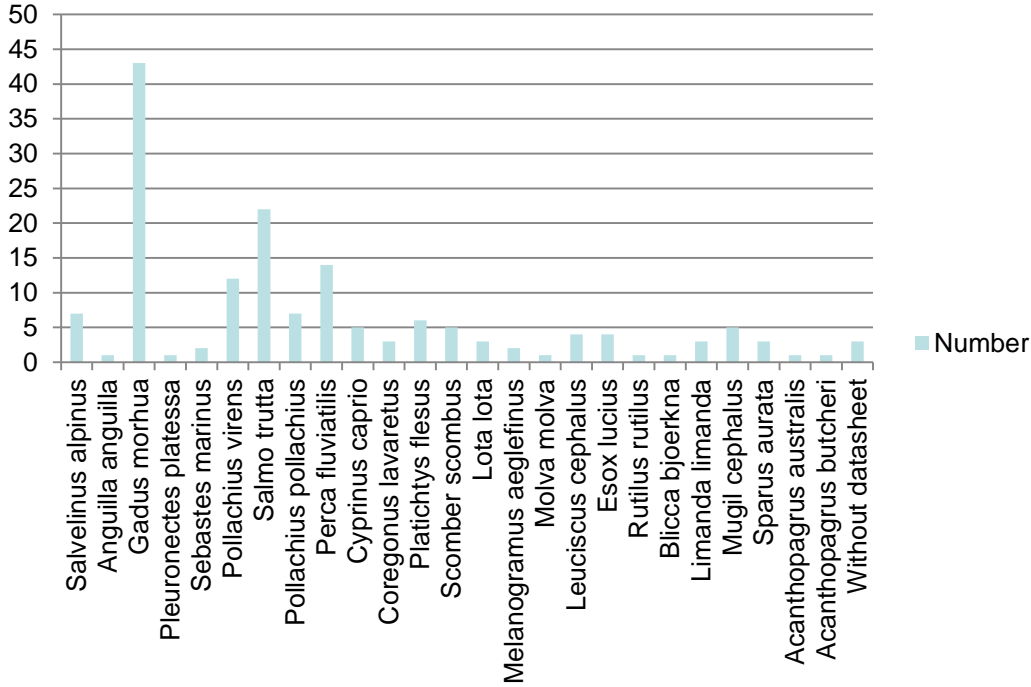
203 samples from 54 schools in 13 countries



No previous published data from Norway and very few international studies

# Global POP

Number



*Gadus morhua*, cod.



*Perca fluviatilis*, European perch.

*Salmo trutta*, trout.

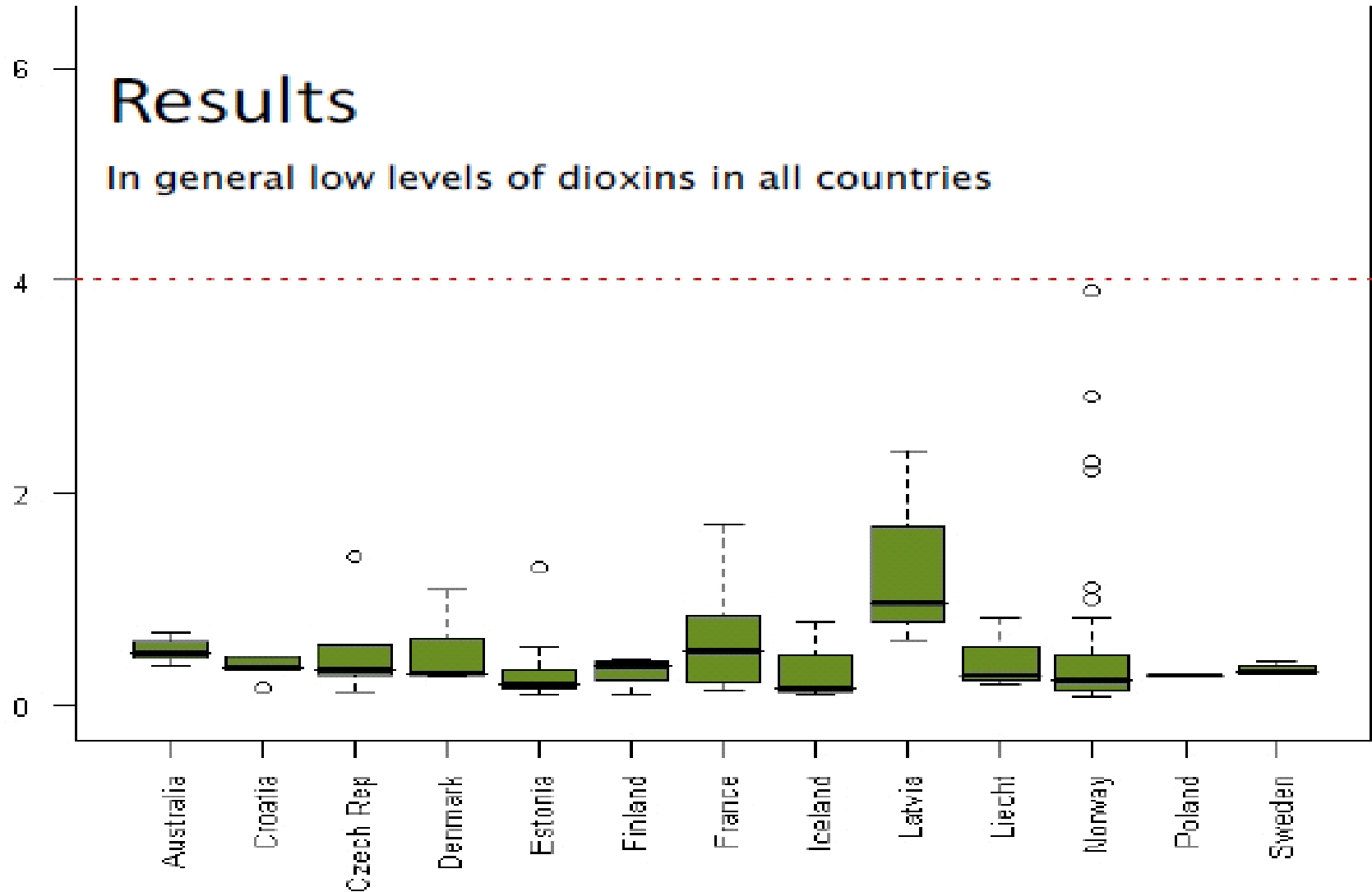


# EU wide Fish Monitoring from 54 schools

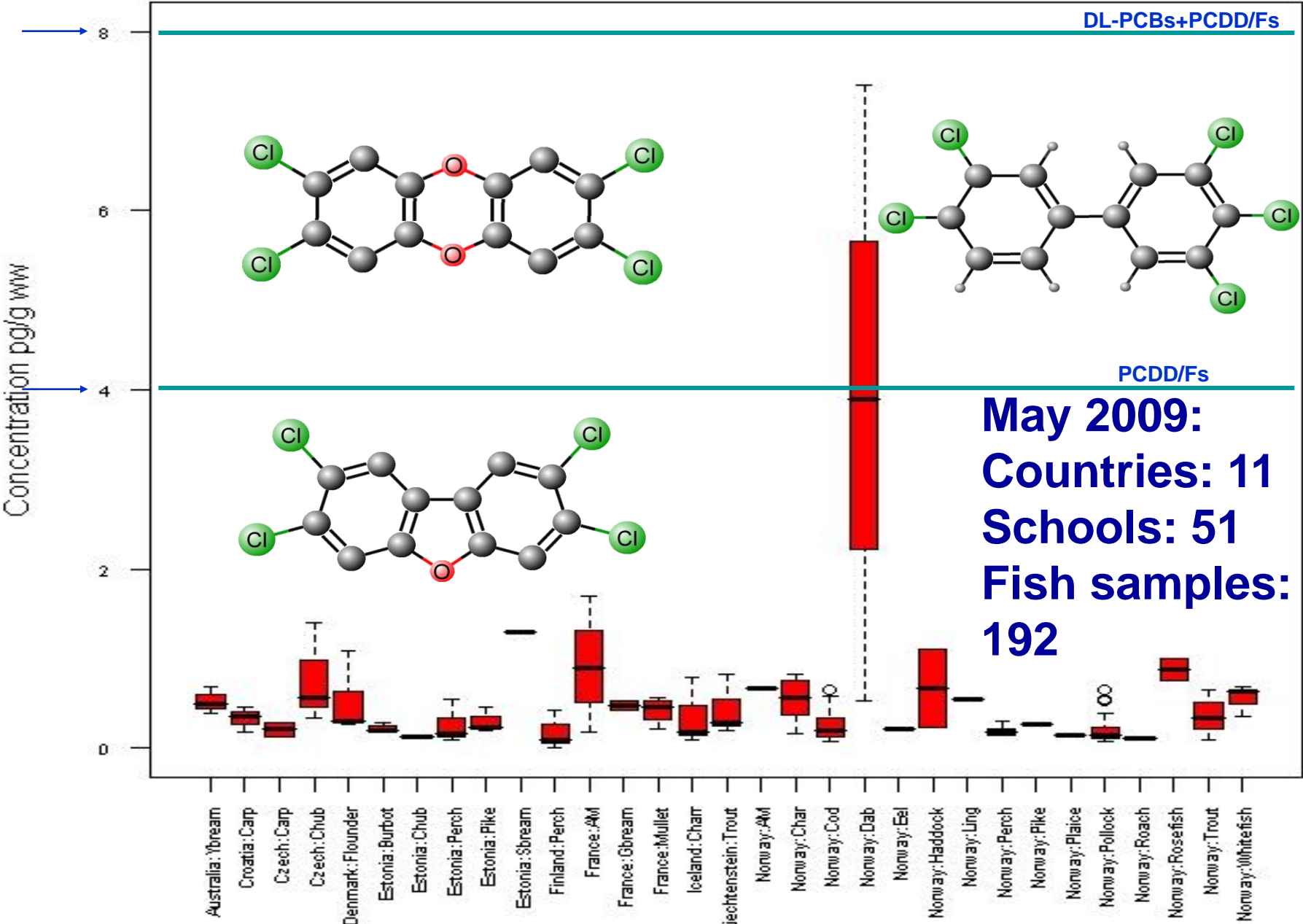
## Results

In general low levels of dioxins in all countries

Concentration (pg TEQ/g) wet weight

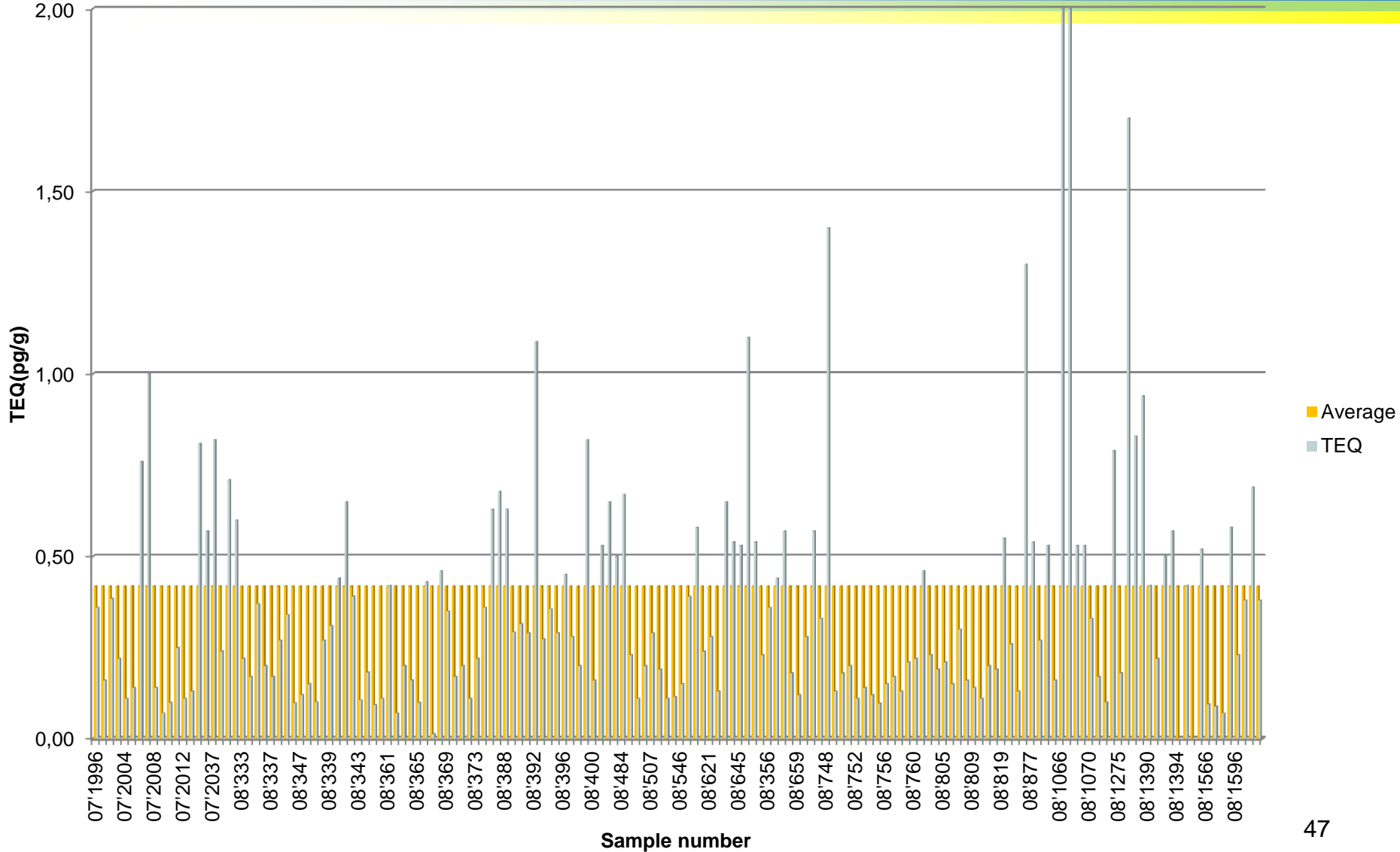


# Dioxins TEQ levels





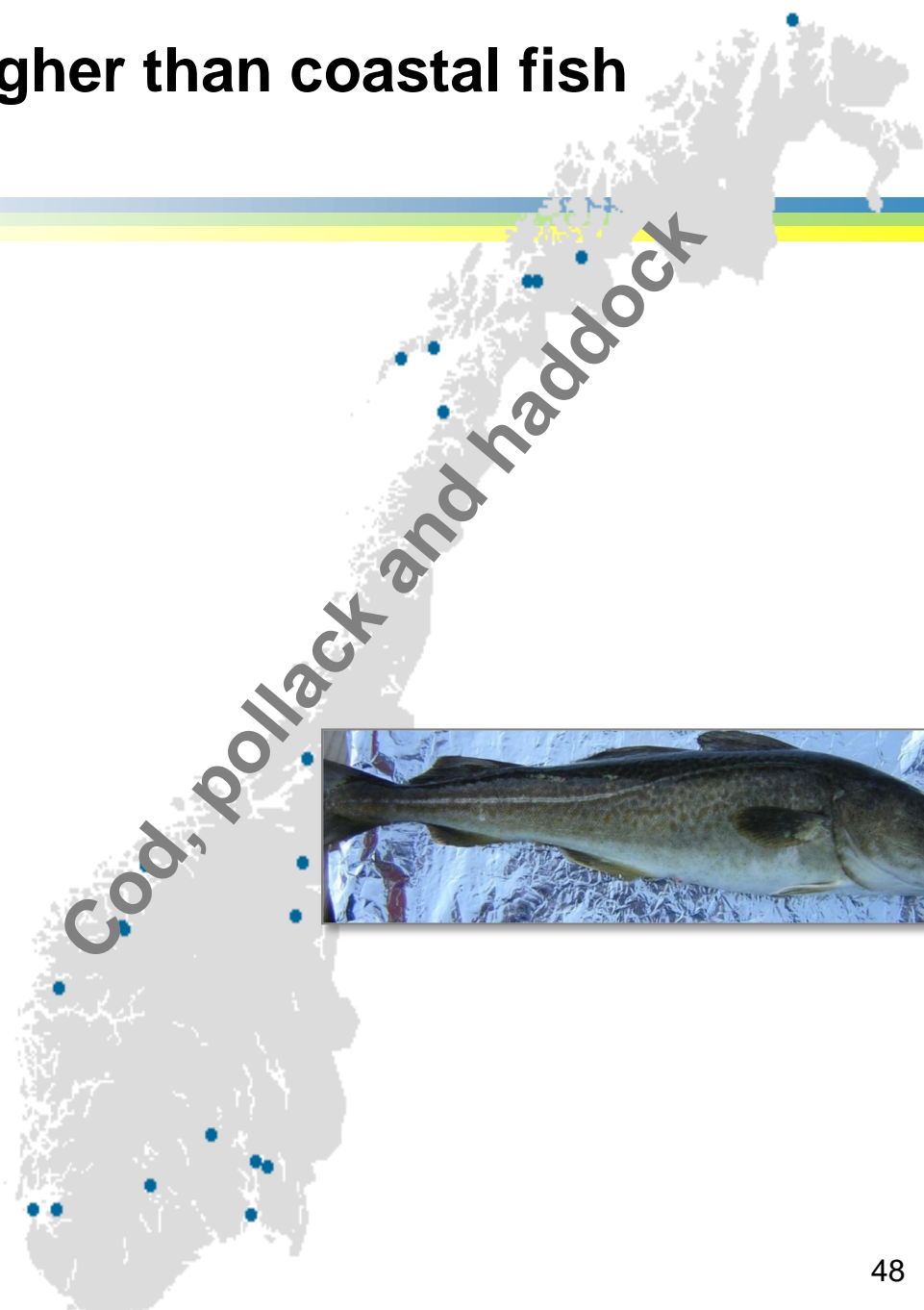
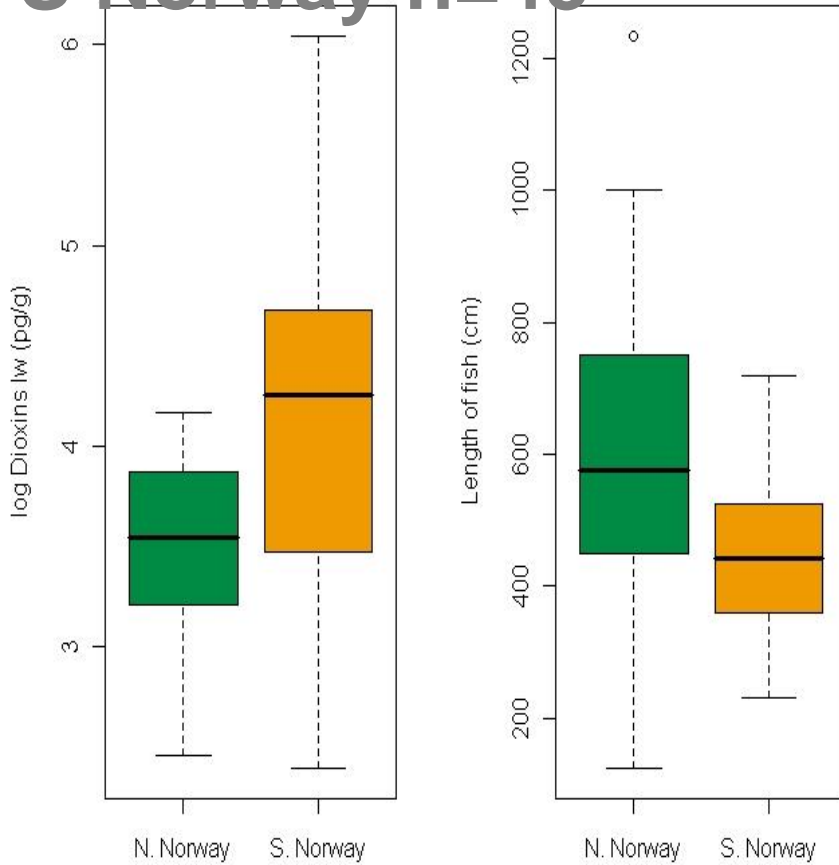
# Low average level of all samples: 0,42 (pg TEQ/g)



# River fish higher than coastal fish

N Norway: n=27,

S Norway n=43







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## TACTRI/COA in Taiwan: Egg and Fish monitoring

<b>Sample</b>	<b>Nr of samples</b>	<b>Fat %</b>	<b>Total-TEQ</b>
<b>Eggs</b>	<b>31</b>	<b>10</b>	<b>2.0 (1.3-4.8)</b>
<b>Eel-Feed</b>	<b>6</b>	<b>9</b>	<b>0,4 (0.2-0.5)</b>
<b>Seabass-Feed</b>	<b>9</b>	<b>11</b>	<b>0.9 (0.3-1.7)</b>
<b>Tilapia-Feed</b>	<b>8</b>	<b>8</b>	<b>0.3 (0.2-0.5)</b>
<b>Duck eggs</b>	<b>8</b>	<b>15</b>	<b>1.8 (1.4-2.4)</b>
<b>Salted duck eggs</b>	<b>16</b>	<b>46</b>	<b>1,2 (0.6-2.8)</b>

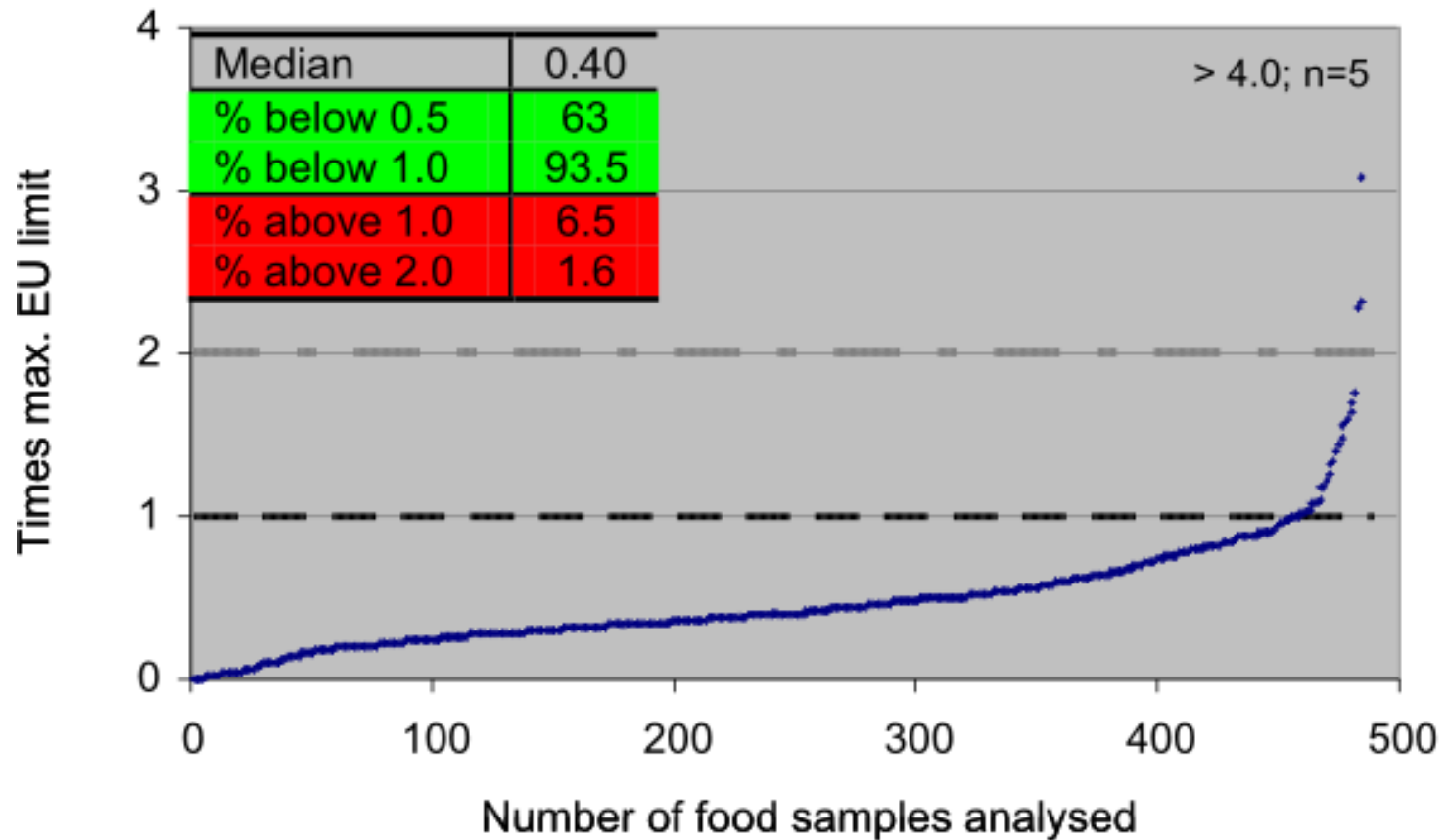


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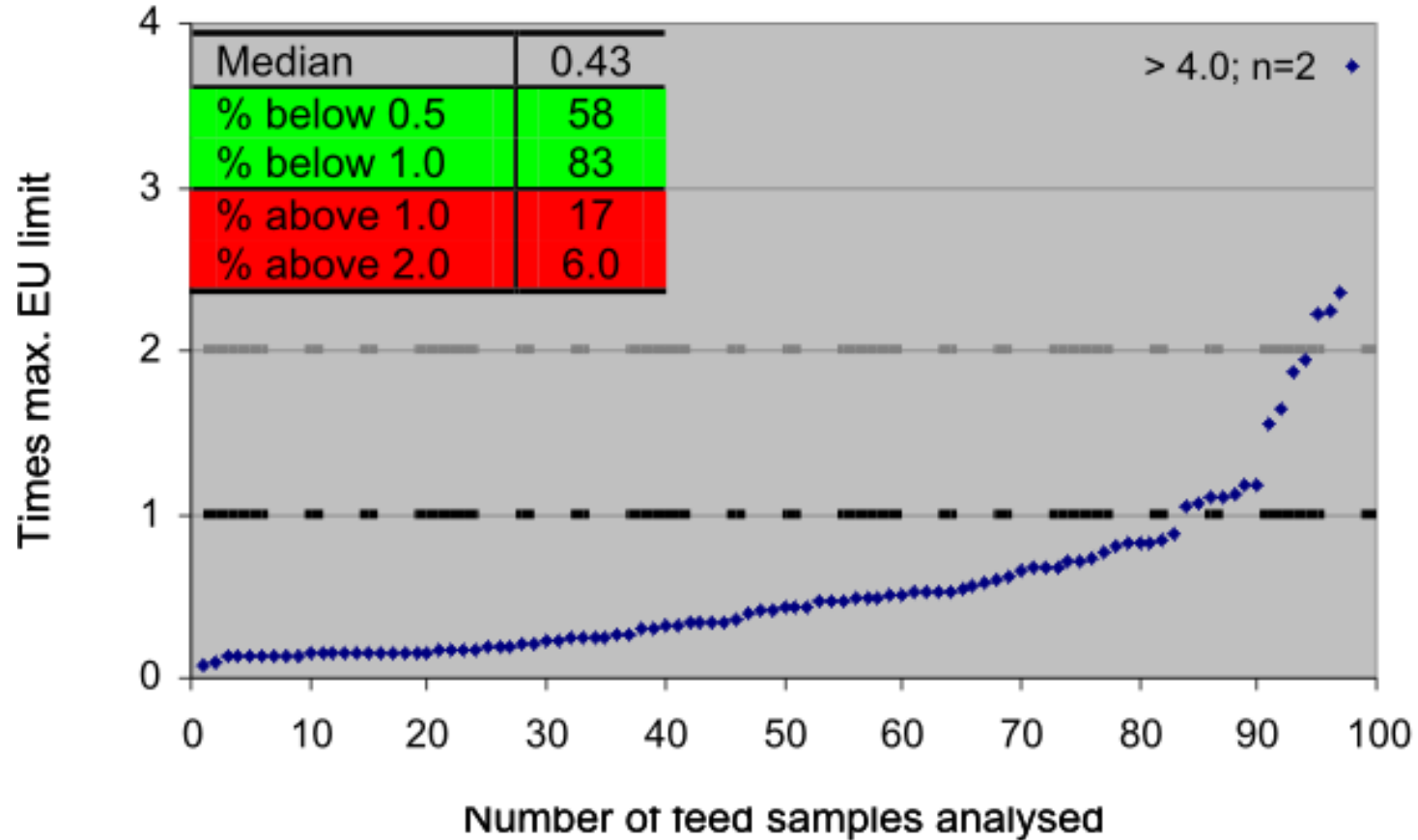


# BDS clients survey in 2006: Dioxin/PCB-TEQ by DR CALUX® TEQ in food samples (n=490)





# BDS clients survey in 2006: Dioxin/PCB-TEQ by DR CALUX<sup>®</sup> TEQ in feed samples (n=100)





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## Other Monitoring Programs using DR CALUX®

**I. Ireland 2008 – used PCB electricity transformer oil in food recycling contaminated more than 80% of pig meat export**

☞ **Intensive monitoring program incl. DR CALUX®.**

**II. Italian „Mozarella Crisis“ in 2008**

☞ **Intensive monitoring program of local farmer association incl. DR CALUX®**

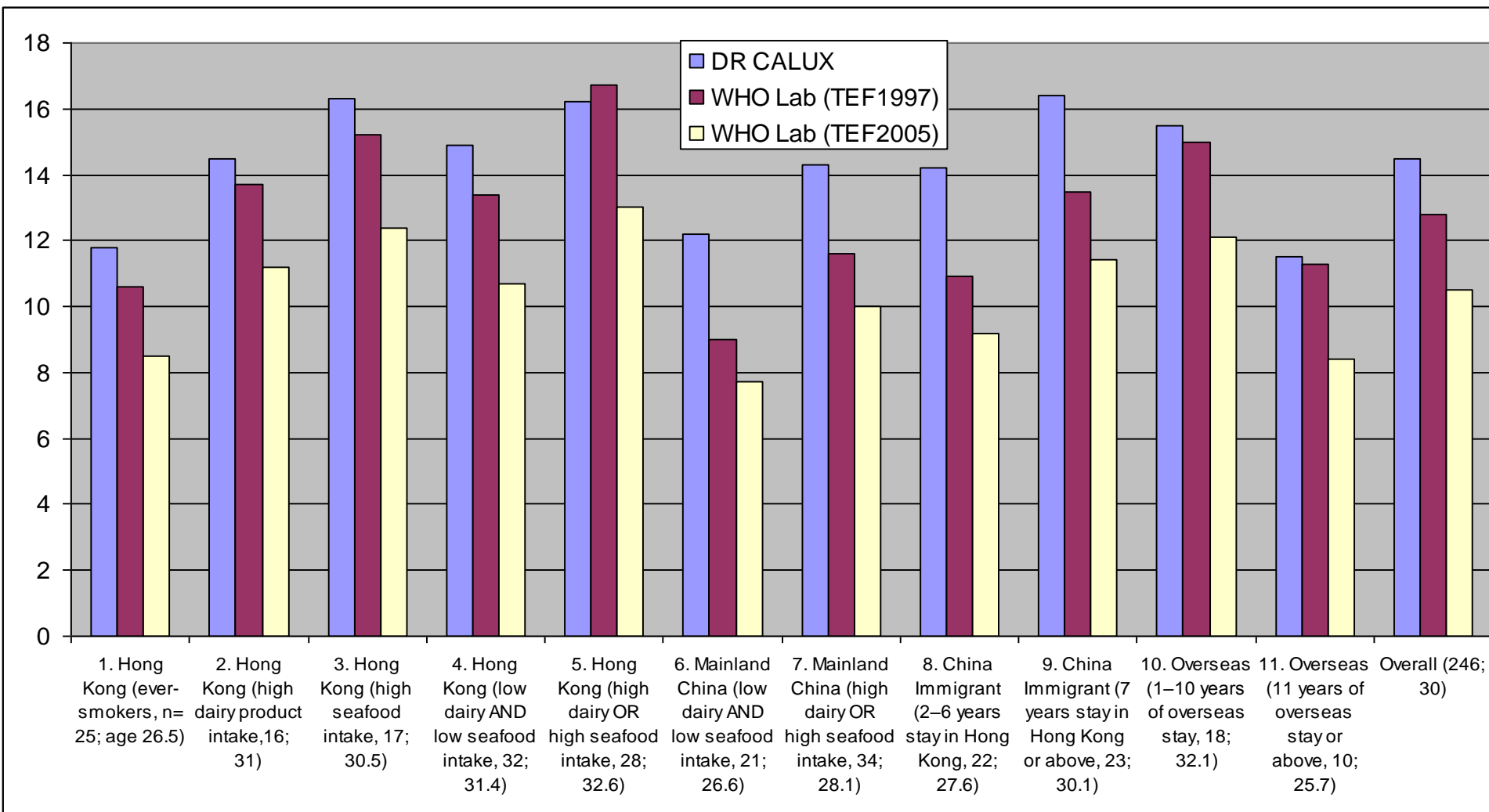
☞ **III. E.g. Israel, Mauretania, Chile, Thailand, China, Korea, Spain.... are currently running monitoring programs via DR CALUX**



# Mother milk in Hong Kong and China



Comparison DR CALUX-Total-TEQ (**BDS**) and HRGC/HRMS WHO-Total-TEQ (**WHO Reference lab**) for pooled breast milk samples (pg/g fat) from Hui et al. Chemosphere 69, 1287 (2007)







## Take home message

- The DR CALUX cell-based screening method is used for feed/food testing since **more than 15 years**....and evaluated in many laboratory in many countries
- The new EC guidelines open now via the qualitative screening approach an easier and faster approach for the reporting of **compliant or suspected samples**
- BDS is further focusing to **decrease the costs for the PCDD/F/PCB-TEQ analysis (under ISO 17025 mode) via easier clean-up methods and Robotics!**
- The results of these studies shows again that the DR CALUX<sup>®</sup> bioassay for screening of PCDD/Fs and dioxin-like PCBs in feed and food is an important device to identify the few percentage of the EU limit exceeding samples among the **bulk of the compliant samples....!!!**



# DIOXIN SCANDAL



IN GERMANY:

THERE IS A RISK  
THAT OUR CHILDREN END  
UP EATING DIOXIN-FOOD!



IN FINLAND:

THERE IS A RISK THAT  
OUR CHILDREN ARE NO MORE  
ABLE TO EAT DIOXIN-FOOD!

