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

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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
Latest dioxin crisis

• 2010 – Maize from Ukraine (Nederlands)
• 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

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Old Total-TEQ</th>
<th>Old cut-off</th>
<th>New Dioxin</th>
<th>New Total-TEQ</th>
<th>New Cut-Off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry</td>
<td>4</td>
<td>1.5</td>
<td>1.75</td>
<td>3</td>
<td>1.2</td>
</tr>
<tr>
<td>Bovine</td>
<td>4.5</td>
<td>2.25</td>
<td>2.5</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>Fish</td>
<td>8</td>
<td>3</td>
<td>3.5</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Milk</td>
<td>6</td>
<td>2.25</td>
<td>2.5</td>
<td>5.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Eggs</td>
<td>6</td>
<td>2.25</td>
<td>2.5</td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td>Plant oils</td>
<td>1.5</td>
<td>0.56</td>
<td>0.75</td>
<td>1.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Babyfood</td>
<td>no</td>
<td>no</td>
<td>0.1</td>
<td>0.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>
### BDS DR CALUX vs HRGC/HRMS – 170 comparisons: < 1% false negative and < 8% false positive

<table>
<thead>
<tr>
<th>Matrix</th>
<th>N</th>
<th>False negative</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>egg</td>
<td>18</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>fish</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poultry meat</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ruminant meat</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pig meat</td>
<td>14</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fish meal</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>milk</td>
<td>33</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Feed, plant origin</td>
<td>18</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Feed, vegetable oil</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Proficiency test performance BDS (2004-2011)

- Orebro University, round 1
- European Commission, Joint Research Centre
- BICS 2005
- Goeyens, Belgium
- Norwegian Institute of Public Health in Oslo, Norway
- Orebro University, round 2
- Dutch National Institute for Coastal and Marine Management
- Quasimeme round 48
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.
Situation in January 2011

- 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 pg TEQ per gram of fat.
German **pig meat** > 97,6% negative; less than 2.5% false positive by DR CALUX

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

Number of tested samples vs. PCDD/F-TEQ by DR CALUX
**German pig liver** > 97% negative; less than 3 % false positive by DR CALUX

<table>
<thead>
<tr>
<th></th>
<th>PCB-TEQ</th>
<th>PCDD/F-TEQ</th>
<th>Total-TEQ</th>
<th>Sample compliant PCDD/F - 25%</th>
<th>Sample compliant TOTAL - 25%</th>
<th>Sample compliant PCDD/F - 50%</th>
<th>Sample compliant TOTAL - 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Liver; N</td>
<td>32</td>
<td>37</td>
<td>34</td>
<td>99%</td>
<td><strong>97%</strong></td>
<td>93%</td>
<td>90%</td>
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<tr>
<td>Pig Liver; Mean</td>
<td>0,80</td>
<td>0,78</td>
<td>1,57</td>
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<tr>
<td>Pig Liver; Range</td>
<td><strong>0,1-2,1</strong></td>
<td><strong>0,1-3,4</strong></td>
<td><strong>0,1-5,2</strong></td>
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<td></td>
<td></td>
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</tbody>
</table>
German *poultry meat* > 98% negative; less than 2 % false positive by DR CALUX

<table>
<thead>
<tr>
<th></th>
<th>PCB -TEQ</th>
<th>PCDD/F -TEQ</th>
<th>Total-TEQ</th>
<th>Sample compliant PCDD/F - 25%</th>
<th>Sample compliant TOTAL - 25%</th>
<th>Sample compliant PCDD/F - 50%</th>
<th>Sample compliant TOTAL - 50%</th>
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<tbody>
<tr>
<td>Chicken Meat; N</td>
<td>201</td>
<td>201</td>
<td>207</td>
<td>99,5%</td>
<td>98%</td>
<td>93%</td>
<td>88%</td>
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<tr>
<td>Chicken Meat; Mean</td>
<td>0.74</td>
<td>0.56</td>
<td>1.3</td>
<td></td>
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<td></td>
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<tr>
<td>Chicken Meat; Range</td>
<td>0.3-3.3</td>
<td>0.3 – 1.7</td>
<td>0.6-3.8</td>
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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

EC guided level 4 ng PCDD/F/PCB-TEQ/kg fat
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
Outline of Presentation

- 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
- Sydney harbor fish screening 2005
- Slovak Republic Food/Feed monitoring
- Norway Fish monitoring school project
- BDS Client survey 2005
Total DR CALUX® distribution for FEED samples, 2009 Asprocer Program, Chile

- 0% above max EU PCDD/F-PCB limit
- max EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product
- 0% above max EU PCDD/F limit
- max EU limit PCDD/F = 0.75 ng TEQ/kg product
- 75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for MINERAL samples, 2009 Asprocer Program, Chile

38 ng TEQ/kg product • mineral clay

5.4% above max EU PCDD/F-PCB limit
max EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product

7.6% above max EU PCDD/F limit
max EU limit PCDD/F = 0.75 ng TEQ/kg product

75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for FISH OIL samples, 2009 Asprocer Program, Chile

- Max EU limit PCDD/F-PCB = 24 ng TEQ/kg product
- Max EU limit PCDD/F = 6 ng TEQ/kg product
- 0% above max EU PCDD/F-PCB limit
- 0% above max EU PCDD/F limit
- 75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for ANIMAL OIL samples, 2009 Asprocer Program, Chile

- Max EU limit PCDD/F-PCB = 3 ng TEQ/kg product
- Max EU limit PCDD/F = 2 ng TEQ/kg product
- 0% above max EU PCDD/F-PCB limit
- 0% above max EU PCDD/F limit
- 75% max EU PCDD/F limit

Santiago, Chile, 22 March 2010
Total DR CALUX® distribution for VEGATABLE OIL samples, 2009 Asprocer Program, Chile

- 0% above max EU PCDD/F-PCB limit
- max EU limit PCDD/F-PCB = 1.5 ng TEQ/kg product
- 18% above max EU PCDD/F limit
- max EU limit PCDD/F = 0.75 ng TEQ/kg product
- 75% max EU PCDD/F limit
Outline of Presentation

- Compliance with EU regulations
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Dutch Dioxin crisis 2004:
Clay effecting potato peeling used in animal feed.

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

- 60% below 1.5 pg TEQ/g
- 5.8% above 3 pg TEQ/g
- 1.3% above 6 pg TEQ/g

Reported TEQ (sorted from lowest to highest)
Outline of Presentation

- International food/feed crisis
- Compliance with EU regulations
- Chemical TEQ vs. DR CALUX TEQ – a few examples
- Dutch Clay/Potato peeling crisis 2004
- Sydney harbor fish screening 2005
- Slovak Republic Food/Feed monitoring
- Norway Fish monitoring school project
- Other Monitoring Programs
Dioxins: A fishy business;
the analysis of dioxins in seafood from Sydney Harbour
Sydney Harbour; preliminary results
# Time line rapid implementation

<table>
<thead>
<tr>
<th>Week</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
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<tbody>
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<td>training CALUX</td>
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<td>chain of custody</td>
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<td></td>
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<td>25</td>
<td>35</td>
<td>45</td>
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<td>total TEQ testing</td>
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<td>24</td>
<td>32</td>
<td>50</td>
<td>70</td>
<td></td>
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<tr>
<td>PCB TEQ testing</td>
<td>12</td>
<td>24</td>
<td>32</td>
<td>50</td>
<td>70</td>
<td></td>
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<td>evaluation accreditation</td>
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<td></td>
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<tr>
<td>fish samples done</td>
<td>12</td>
<td>24</td>
<td>32</td>
<td>50</td>
<td>70</td>
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<tr>
<td>analysis done</td>
<td>42</td>
<td>73</td>
<td>99</td>
<td>145</td>
<td>185</td>
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</table>
Sydney harbour: Comparison of DR CALUX® and HR-GCMS
Outline of Presentation

- International food/feed crisis
- Compliance with EU regulations
- Chemical TEQ vs. DR CALUX TEQ – a few examples
- Dutch Clay/Potato peeling crisis 2004
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- Norway Fish monitoring school project
- Other food monitoring programs using DR CALUX
Ratio: Total DR CALUX TEQ vs. accepted European Total-TEQ

Bovine Meat

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

Fish
Location 10:
Ratio 3.4 due to former PCB production

Poultry
Outline of Presentation

- International food/feed crisis
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- Dutch Clay/Potato peeling crisis 2004
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- Czech Food/Feed monitoring
- **Norway Fish monitoring school project**
- Taiwan Feed/Food Monitoring
- Other food monitoring programs using DR CALUX
Dioxins in fish with BDS CALUX

Eldbjørg Sofie Heimstad¹, Gaute Grønstøl², Karl Torstein Hetland³, Javier Martinez Alarcon¹, Charlotta Rylander¹,⁴ and Espen Mariussen¹,⁵

¹Norwegian Institute for Air Research (NILU)
²University of Bergen, Bergen.
³Norwegian Centre for Science Education
⁴Institute of Community Medicine, University of Tromsø
⁵Norwegian 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
Norwegian Fish Monitoring Project; Polar Bear

Results

203 samples from 54 schools in 13 countries

36 schools from Norway

No previous published data from Norway and very few international studies
Global POP

Number

Salmo trutta, trout.

Gadus morhua, cod.

Perca fluviatilis, European perch.
Results

In general low levels of dioxins in all countries
May 2009:
Countries: 11
Schools: 51
Fish samples: 192
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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- **Taiwan Monitoring of feed and food**
- BDS Client survey 2005
- Other food monitoring programs using DR CALUX
# TACTRI/COA in Taiwan: Egg and Fish monitoring

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nr of samples</th>
<th>Fat %</th>
<th>Total-TEQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>31</td>
<td>10</td>
<td>2.0 (1.3-4.8)</td>
</tr>
<tr>
<td>Eel-Feed</td>
<td>6</td>
<td>9</td>
<td>0.4 (0.2-0.5)</td>
</tr>
<tr>
<td>Seabass-Feed</td>
<td>9</td>
<td>11</td>
<td>0.9 (0.3-1.7)</td>
</tr>
<tr>
<td>Tilapia-Feed</td>
<td>8</td>
<td>8</td>
<td>0.3 (0.2-0.5)</td>
</tr>
<tr>
<td>Duck eggs</td>
<td>8</td>
<td>15</td>
<td>1.8 (1.4-2.4)</td>
</tr>
<tr>
<td>Salted duck eggs</td>
<td>16</td>
<td>46</td>
<td>1.2 (0.6-2.8)</td>
</tr>
</tbody>
</table>
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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® TEQ in feed samples (n=100)
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Other Monitoring Programs using DR CALUX®

I. Ireland 2008 – used PCB electricity transformator 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)
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® 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!

Finland wants a special permission from EU to market dioxin salmon.