Dioxins analysis at RIKILT

Wim Traag and Ron Hoogenboom
- Introduction on dioxins
- Use of CALUX and GC-HRMS
- Recent affaires
- RIKILT approach and the next generation of GC-HRMS method
Dioxins and PCBs

PCDD

PCDF

PCB
Dioxin analysis

Sophisticated analytical methods required:

- Extraction of dioxins from fat (acid/base silica, GPC)
- Removal of possible interfering contaminants (Al$_2$O$_3$)
- Separation dioxins and PCBs on activated carbon column
- Separation and confirmation of different congeners (HRGC/HRMS) use of labelled standards for recovery control
- Different analysis of non-ortho and other dioxin-like PCBs
## Quantification

### Result of dioxin analysis in chicken (content in pg/g fat)

<table>
<thead>
<tr>
<th>Dioxin Compound</th>
<th>TEF</th>
<th>TEQ</th>
<th>pg TEQ/g fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDF</td>
<td>0.95</td>
<td>0.1000</td>
<td>0.09</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>&lt;0.10</td>
<td>0.0500</td>
<td>&lt;</td>
</tr>
<tr>
<td>2,3,4,7,8-PeCDF</td>
<td>0.30</td>
<td>0.5000</td>
<td>0.15</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>0.22</td>
<td>0.1000</td>
<td>0.02</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDF</td>
<td>0.14</td>
<td>0.1000</td>
<td>0.01</td>
</tr>
<tr>
<td>2,3,4,6,7,8-HxCDF</td>
<td>0.19</td>
<td>0.1000</td>
<td>0.02</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDF</td>
<td>&lt;0.10</td>
<td>0.1000</td>
<td>&lt;</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>0.98</td>
<td>0.0100</td>
<td>0.01</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-HpCDF</td>
<td>&lt;0.25</td>
<td>0.0100</td>
<td>&lt;</td>
</tr>
<tr>
<td>OCDF</td>
<td>2.00</td>
<td>0.0001</td>
<td>0.00</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>0.57</td>
<td>1.0000</td>
<td>0.57</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>0.70</td>
<td>1.0000</td>
<td>0.70</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDD</td>
<td>0.37</td>
<td>0.1000</td>
<td>0.04</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>0.79</td>
<td>0.1000</td>
<td>0.08</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD</td>
<td>0.99</td>
<td>0.1000</td>
<td>0.10</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDD</td>
<td>0.78</td>
<td>0.0100</td>
<td>0.05</td>
</tr>
<tr>
<td>OCDD</td>
<td>25.40</td>
<td>0.0001</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Total content in pg TEQ/g fat:** 1.84
<table>
<thead>
<tr>
<th>Congener</th>
<th>Content pg/g</th>
<th>TEF</th>
<th>Content pg TEQ/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
<td>0.57</td>
<td>1.0</td>
<td>0.57</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>0.37</td>
<td>0.1</td>
<td>0.037</td>
</tr>
<tr>
<td>OCDD</td>
<td>25.4</td>
<td>0.0001</td>
<td>0.0025</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>0.61</td>
</tr>
</tbody>
</table>
Screening method for dioxins

- Requirements:
  - quick and relatively cheap
  - high sample throughput
  - obeying the TEQ-principle
  - no false-negatives, few false-positives

- GC/MS reference method used for:
  - confirmation
  - source identification
CALUX-assay

- Chemical Activated LUciferase gene eXpression assay

- Developed by University in Wageningen in cooperation with University of Michigan (later UC Davis) and RIKILT
CALUX screening assay

Aarts et al. 1993

Luciferin + ATP
Clean-up of samples

- Fat extraction
- Acid silica clean-up (6-8 hrs)
- CALUX-assay (24 hrs incubation)
Quantitative approach

Response (RLUs) vs. Concentration TCDD (pM)
Use of reference samples

- Matrix-specific reference materials are required:
  - To correct for possible contaminants from solvents
  - To correct for possible recovery losses
  - To correct for the difference in “CALUX-TEFs” and WHO-TEFs
RIKILT has been involved in many incidents on:

- Dioxins
- PAH’s
- Hormones
- Etc
Dioxins in citrus pulp from Brazil (1998)
The Belgian dioxin crisis in 1999
Elevated dioxin content in milk in 2004
(Lelystad affaire)

- Mixed pooled (RMO) sample of September contains dioxins
  (= four RMO’s ≈ 20 farms) 1.5 pg TEQ/gram fat

- Individual RMO samples analysed using CALUX, one suspected

- Confirmation of suspected sample with GC-HRMS
  5.1 pg TEQ/gram fat (three farms)

- Samples of these farms analysed using CALUX
  two on background level, one suspected

- GC-HRMS 20 pg TEQ/gram fat
### Lelystad affaire

<table>
<thead>
<tr>
<th>Compound</th>
<th>TEQ [ng]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDF</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>2,3,4,7,8-PeCDF</td>
<td>0.64</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>0.82</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDF</td>
<td>0.35</td>
</tr>
<tr>
<td>2,3,4,6,7,8-HxCDF</td>
<td>0.23</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDF</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>0.3</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-HpCDF</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>OCDF</td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>8.6</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>8.62</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDD</td>
<td>3.74</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>5.97</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD</td>
<td>6.94</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDD</td>
<td>8.27</td>
</tr>
<tr>
<td>OCDD</td>
<td>3.59</td>
</tr>
<tr>
<td>Total content TEQ [lb]</td>
<td>19.43</td>
</tr>
<tr>
<td>Total content TEQ [ub]</td>
<td>19.45</td>
</tr>
</tbody>
</table>
Pattern in milk resembles kaolinic clay (1999)
Samples from contaminated farm

- All feeding stuffs sampled (≈ 25)
- No kaolinic clay present on farm
- Several samples suspected using CALUX, only potato peels highly contaminated, level in milk can be explained
- CALUX result confirmed using GC/HRMS; pattern comparable with milk
Potato peels
Hint from AID: Since the summer of 2004 McCain uses a different procedure for selection of the potatoes

- Clay instead of salt
- Samples clay taken
- Content 1600 ng TEQ/kg
- Similar pattern as potatoes
Contamination of farm in Lelystad in time

Release
16 December

Model RIVM
More affaires in 2004 ????????????
Samples from Lickebaert-area (near Rotterdam)

- A number of contaminated milk samples (4-5 pg TEQ/g fat)
- Pattern comparable with reference sample milk from the same area as in 1989
- Extended screening of milk samples with CALUX
- Last quarter of 2004 levels are decreasing
Dioxin pattern in milk samples from Lickebaert

Fraction of total TEQ (%)

- 133438
- 133439
- 133440
- 3019REF
- 132823
RIKILT APPROACH FOR DIOXIN ANALYSIS (1)

- Screening all samples using CALUX
- Compare CALUX response of samples with appropriate reference samples (tested with GC-HRMS)
- All suspected samples have to be confirmed with GC-HRMS
- 5-10% of negative samples have to be analysed with GC-HRMS
- New matrices always have to be analysed with both techniques
RIKILT APPROACH FOR DIOXIN ANALYSIS (2)

- GC-HRMS method is currently modified by:
  - Extraction using ASE
    - Animal feed with toluene after pre-wetting
    - Biological samples after freeze drying with hexane
  - Purification using power prep
    - Combination of four columns
      - Jumbo acidified silica column (capacity 3 gram fat)
      - Silica column
      - Al₂O₃ column
      - Carbon column
Next steps

After power prep clean-up two fractions are obtained
- Fraction A contains mono-ortho + indicator pcb’s
- Fraction B contains dioxins + non-ortho pcb’s
- Both fractions are automatically concentrated using turbovap (keeper is added) ; Endpoint + fixed time

Concentrated fractions are analysed on two GC-HRMS
- Fraction A 2 µl using splitless injection
- Fraction B 25-50 µl using LVI
RIKILT APPROACH FOR DIOXIN ANALYSIS (3)

- Next steps
- Positive samples are analysed by TOF-MS
- In 2005 GC X GC will be implemented