PLE for Extraction of Dioxins in Animal Feed and Ingredients

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Belgian Chicken Gate 1999

✓ Animal feedingstuffs
Concentration Factor
EU Guidelines

COMMISSION DIRECTIVE 2006/13/EC
of 3 February 2006
(Text with EEA relevance)

REGULATIONS

COMMISSION REGULATION (EC) No 152/2009
of 27 January 2009
laying down the methods of sampling and analysis for the official control of feed
(Text with EEA relevance)
Key Points of EU Guidelines

✓ ISO/CEI 17025
✓ LOQs < 20% target level
✓ Blanks, QCs, CRMs, matrix-specific intercal.
✓ CVs < 15%, justness ±20%, recovery rates %…
✓ GC-IDHRMS
✓ Upperbound ≠ lowerbound < 20-40%
✓ Compliant EPA 1613b or other equivalent…
Interlaboratory study on harmonization of extraction procedures for determination of PCDD/F and PCB in feedingstuff


1Community Reference Laboratory (CRL) for Dioxins and PCBs in Feed and Food, State Laboratory for Chemical and Veterinary Analysis (CVUA), Freiburg, Germany

Figure 3: Comparison of WHO-PCDD/F-TEQ results on pg WHO-PCDD/F-TEQ/kg product. B1: Soxhlet extraction with toluene + polar solvent. B2: Soxhlet extraction with toluene. C1: PLE with toluene + polar solvent. D1: HCl-digestion (3 M) and Soxhlet extraction with toluene. D2: HCl-digestion (3 M) and Soxhlet extraction with toluene + polar solvent. Coefficient of variation (CV) in % in light grey
Need for Harmonization…

✓ Should it be a single method?
✓ Should labs be ‘free’ to use in-house methods?
✓ Manual vs automated?
✓ What in case of conflict?
✓ Methods inter-compatibility, ‘transportability’?
✓ Standard method (performance-based)?
✓ CEN method to come…
✓ Intercalibration exercises (matrix-specific)?
Automated Approach

✓ Automated extraction
✓ Automated clean-up
✓ Automated fractionation
✓ Automated GC-MS injection
Sequential PLE (ASE)
Extraction Cell

- Spiking
- Homogenization
- Drying (lyoph, …)
- 120°C, 1500 psi
- Hexane, toluene, EtOH, …
- Static and/or dynamic

10-30 min/sample
Parallel PLE

Extraction, solvent reduction and exchange
Parallel Clean-up and Fractionation
Focus on Extraction

- ASE (Dionex) : Sequential extractions
- PLE (FMS) : Parallel (6) extractions

- CEN recommended conditions (PT):
  - 100°C, 1500 psi, 15 min static
  - Flush with solvent, purge with nitrogen
  - 3 cycles (C1 = Tol, C2-3 = Tol/EtOH 90:10)
Mineral Clay PCDD/F Profile

ng/kg product

- ASE
- PLE
- CEN Consensus
Mineral Clay TEQ

- PCDDFs TEQ
- DL PCBs TEQ
- PCDDF+ DL PCBs TEQ

- ASE
- PLE
- CEN Concensus

ng TEQ/kg product

0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8
Bovine Feed TEQ

- PCDDFs TEQ
- DL PCBs TEQ
- PCDDF+ DL PCBs TEQ

ASE, PLE, CEN Consensus

ng TEQ/kg product
Fish Meal TEQ

- PCDDFs TEQ
- DL PCBs TEQ
- PCDDF+ DL PCBs TEQ

Ng TEQ/kg product:
- ASE
- PLE
- CEN Concensus
### Z-Scoring PT (TEQ)

<table>
<thead>
<tr>
<th></th>
<th>Z-scores</th>
<th>Concensus Level (ng/kg)</th>
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<tbody>
<tr>
<td></td>
<td>ASE</td>
<td>PLE</td>
</tr>
<tr>
<td><strong>Mineral clay</strong></td>
<td>PCDD/Fs</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>PCBs</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>PCDD/Fs+PCBs</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Bovine feed</strong></td>
<td>PCDD/Fs</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>PCBs</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>PCDD/Fs+PCBs</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Fish meal</strong></td>
<td>PCDD/Fs</td>
<td>1.4</td>
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<tr>
<td></td>
<td>PCBs</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>PCDD/Fs+PCBs</td>
<td>1.0</td>
</tr>
</tbody>
</table>

- ✓ ASE positive bias?
- ✓ PLE negative bias?

*Concensus value based on mainly Soxhlet…*
Extraction Condition Study

✓ CEN recommended conditions (PT):  
  100°C, 1500 psi, 15 min static  
  Flush with solvent, purge with nitrogen  
  3 cycles (C1 = Tol, C2-3 = Tol/EtOH 90:10)

✓ CART conditions  
  150°C, 1500 psi, 15 min static  
  Flush with solvent, purge with nitrogen  
  3 cycles (C1-2-3 = Tol/EtOH 90:10)
Animal Feed QC Belgium

✓ In-house animal feed QC for testing
✓ From Belgian 1999 crisis
✓ ‘Naturally’ contaminated
✓ At level close to the EU regulatory value
✓ To be certified later???
✓ Used to test the future CEN method
✓ Comparison between CEN and CART methods
✓ Comparison between ASE and PLE
✓ Preliminary data
Is 100°C enough?
Temperature Control Study

Is there T control-transfer differences?

✓ No ASE T data because no measure possible…
  
  Closed vessel type

✓ PLE T data possible because ‘open’ design
  
  PLE: T probe in EtGly/water mix inside the extraction cell
Temperature Study on PLE

PLE temperature monitoring (100°C)

Temperature (°C) vs. Time (min)

- Green line: PLE software reading
- Red line: Cell temp

±10% accuracy range
Temperature Study on PLE

PLE temperature monitoring (150°C)

Time (min)

Temperature (°C)

- PLE software reading
- Cell temp
Heat Transfer Aspect
Efficient Heat Transfer

PLE temperature monitoring (150°C)

+10%

5’ pre-heat

15’ extraction cycle

Temperature (°C)

Time (min)

PLE software reading
Cell temp
Cell temp corrected
Cell temp corrected & contact
Take Home Message

✓ Extraction of feed is complex…
✓ Automated extraction tools ok for animal feed
✓ Careful study of the extraction temperature is needed
✓ Is 100°C enough?
✓ Is what the software says really what the T is?
✓ A pre-heat step is needed