Carry over of PAs to milk and other animal derived products

Patrick Mulder

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Pyrrolizidinalkaloids in Lebens- und Futtermitteln – eine unterschätzte Gefahr?
Human exposure to PAs: food sources

- Cereals and other arable crops
- Honey and related products
- (Herbal) teas and supplements
- Animal derived products
  - Milk and milk products (yoghurt, cheese, pudding)
  - Eggs
  - Meat, liver and meat products
## PAs – Animal feed monitoring 2006-2012

### Occurrence of PAs in feedstuffs. Classification according to EU regulation 575/2011/EC

<table>
<thead>
<tr>
<th>Cat.</th>
<th>Commodity</th>
<th>No of samples</th>
<th>% Positive</th>
<th>Average concentration of main PA types (µg/kg)</th>
<th>Maximum (µg/kg)</th>
<th>Percentage Tertiary amine N-oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cereal grains</td>
<td>14</td>
<td>7.1</td>
<td>Senecionine 0.0, Echimidine 2.1, Heliotrine 0.0, Crotaline 0.0, Total 2.1</td>
<td>29.6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Oil seeds and fruits (soya)</td>
<td>71</td>
<td>26.8</td>
<td>3.4, 7.8, 0.0, 0.3, Total 11.5</td>
<td>342.7</td>
<td>37.9, 62.1</td>
</tr>
<tr>
<td>3</td>
<td>Legume seeds</td>
<td>13</td>
<td>15.4</td>
<td>0.0, 0.0, 9.8, 0.0, Total 9.8</td>
<td>112.6</td>
<td>14.4, 85.6</td>
</tr>
<tr>
<td>4</td>
<td>Tubers and roots</td>
<td>1</td>
<td>0</td>
<td>0.0, 0.0, 0.0, 0.0, Total 0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>Other seeds and fruits</td>
<td>5</td>
<td>40.0</td>
<td>0.0, 7.3, 8.6, 0.0, Total 15.9</td>
<td>43.1</td>
<td>36.1, 63.9</td>
</tr>
<tr>
<td>6</td>
<td>Forages and roughage (silage, hay, alfalfa)</td>
<td>302</td>
<td>58.9</td>
<td>232.2, 37.8, 0.0, 0.0, Total 269.9</td>
<td>22753</td>
<td>57.1, 42.9</td>
</tr>
<tr>
<td>7</td>
<td>Other plants (herbal supplements)</td>
<td>32</td>
<td>62.5</td>
<td>26.7, 69.4, 219.8, 0.0, Total 315.9</td>
<td>3209</td>
<td>8.2, 91.8</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>438</td>
<td>51.4</td>
<td>162.5, 32.5, 16.4, 0.0, Total 211.5</td>
<td>22753</td>
<td>51.6, 48.4</td>
</tr>
</tbody>
</table>

- Forages present largest risk, followed by herbal supplements
- Both PA free bases and PANOs are relevant
### Animal feedstuffs: Alfalfa (lucerne)

<table>
<thead>
<tr>
<th>Year</th>
<th>No of samples</th>
<th>Positive (%)</th>
<th>Average content (µg/kg)</th>
<th>Max (µg/kg)</th>
<th>Samples &gt;1000 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>6</td>
<td>83%</td>
<td>1440</td>
<td>3439</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>2007</td>
<td>13</td>
<td>85%</td>
<td>225</td>
<td>1409</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>2008</td>
<td>12</td>
<td>83%</td>
<td>716</td>
<td>6219</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>2009</td>
<td>17</td>
<td>88%</td>
<td>621</td>
<td>4507</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>2010</td>
<td>51</td>
<td>92%</td>
<td>225</td>
<td>2418</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>2011</td>
<td>50</td>
<td>86%</td>
<td>265</td>
<td>2027</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>2012</td>
<td>51</td>
<td>90%</td>
<td>356</td>
<td>4169</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>2013</td>
<td>48</td>
<td>92%</td>
<td>1007</td>
<td>15751</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>2014</td>
<td>50</td>
<td>68%</td>
<td>157</td>
<td>1498</td>
<td>9 (19%)</td>
</tr>
</tbody>
</table>

- Lucerne is prone to contamination with PAs
- 85% of PAs is of senecionine type, mostly originating from *Senecio vulgaris*
Animal transfer study

S jac

S inq

S vul

E vul

Rumen content

Feed

Urine, faeces

Milk

Yoghurt, cheese

jacobine

Senkirkine

echimidine N-oxide
Effect of the rumen

- Levels decrease quickly in the rumen
  - Efficient extraction of PA-plant material
  - Fast transfer to the intestinal tract
- Fast reduction (or transfer/degradation) of PANOs (<1% 2.5 h after administration)

Dosing

[Graphs showing time vs. concentration for different plants and cows, indicating dosing time points.]
PAs in milk – Ragwort (*Jacobaea vulgaris*)

- 3 Cows fed by gavage
  - 4 Days 200 g dried material (1% of feed intake) in the morning
  - Morning and evening milk analysed

**Plant material**

Avg content: 12.1 µg/L 0.05%
PAs in milk – Common groundsel (*Senecio vulgaris*)

- 4 Days: 200 g dried material (1% of feed intake) in the morning
- Morning and evening milk analysed

Avg content: 
1.5 µg/L 
0.01%
PAs in milk – Vipers bugloss (*Echium vulgare*)

- 4 Days: 200 g dried material (1% of feed intake) in the morning
- Morning and evening milk analysed

Avg content: 5.1 µg/L 0.05%

Plant material
Mass balance PAs

Only small part of PAs is recovered

Excretion is fast: in urine max conc <5 h

Hydroxylated compounds formed

Milk and faeces: only PA FBs, urine: 80% PANOs

<table>
<thead>
<tr>
<th>Treatment (n=3)</th>
<th>PAs consumed (mg)</th>
<th>PAs excreted (mg)</th>
<th>Excreted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk</td>
<td>Urine</td>
<td>Faeces</td>
</tr>
<tr>
<td>Ragwort</td>
<td>753±78</td>
<td>0.37±0.11</td>
<td>21.3±3.9</td>
</tr>
<tr>
<td>Common groundsel</td>
<td>558±33</td>
<td>0.05±0.05</td>
<td>12.5±2.0</td>
</tr>
<tr>
<td>Vipers bugloss</td>
<td>335±39</td>
<td>0.16±0.09</td>
<td>6.6±2.0</td>
</tr>
</tbody>
</table>
Effect of processing: Dairy production

- NIZO pilot facilities, Ede, the Netherlands
- Ragwort contaminated milk
- Samples from intermediate and end products

**Milk:**
- Standardised milk A: 1.5% fat, 4 kg
- Pasteurisation: 76°C; 15 sec
- Sterilisation: 140°C; 4 sec

**Yoghurt:**
- Standardised milk B: 3% fat, 5 kg
- High pasteurisation: 93°C; 5 min

**Cheese:**
- Standardised milk C: 3.5% fat, 12 kg
- High pasteurisation: 72°C; 15 sec

* Processing performed by using a laboratory scale continuous flow heating system

**Thermised milk**
- Thermisation: 68°C; 13 sec
- Cream: 31% fat
- Stored: 4°C; 18 hours
- 7.5 kg
- Skimmed milk: 0.1% fat
- Stored: 4°C; 18 hours
- 44 kg

**Centrifuge**
- 45°C

**Cream and Skimmed milk combined to standardised milk A, B and C**
- Raw milk: 55 kg
- Thermisation: 68°C; 13 sec
- Centrifuge: 45°C
- Cream: 31% fat
- Stored: 4°C; 18 hours
- 7.5 kg

**Semi skimmed milk:**
- Standardised milk A: 1.5% fat (Past.)
- 2 kg

**Semi skimmed milk:**
- Standardised milk B: 3% fat
- 5 kg
- Yoghurt milk: 3% fat
- 5 kg
- Yoghurt culture: 42°C; 6 hours to pH6
- Cooling 4°C
- Yoghurt: 3.0% fat
- 5 kg

**First whey:**
- Aprr. 8 kg, incl. 2.1 L wash water

**Second Whey:**
- 4 kg

**Minicheeser:**
- Cheese culture; rennet
- 31°C; 56 min
- Gouda cheese: 4 x 250 g

**Yoghurt culture**
- 42°C; 6 hours to pH6

**Centrifuge**
- 45°C

**Standardised milk A**
- 1.5% fat
- 4 kg

**Standardised milk B**
- 3% fat
- 5 kg

**Standardised milk C**
- 3.5% fat
- 12 kg

**High pasteurisation**
- 72°C; 15 sec

**Minicheeser:**
- Cheese culture; rennet
- 31°C; 56 min

**Cooling 4°C**
- 4°C

**Thermisation**
- 68°C; 13 sec

**Sterilisation**
- 140°C; 4 sec

**Pasteurisation**
- 76°C; 15 sec

**Cooling 4°C**
- 4°C
Ragwort PAs: transfer to cheese and yoghurt

- PAs stable during pasteurisation/UHT process
- Cheese and yogurt production: ca. 50% reduction of PAs
Conclusions transfer studies

- Carry-over rate to milk
  - 0.05% for ragwort, vipers bugloss
  - 0.01% for common groundsel

- PAs containing hydroxyl groups are preferably transferred
  - Jacoline: 5% based on presence in ragwort
  - Only PA FBs, no PANOs

- PAs are relatively stable during dairy processing and cheese production
EXTERNAL SCIENTIFIC REPORT

Occurrence of Pyrrolizidine Alkaloids in food

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\textsuperscript{c} Institute for Research and Technology in Food and Agriculture (IRTA), Monells, Spain
Sampling of animal-derived products

- Total: 746 samples
- 16% organic
Requirements

- **Very low LOQs**
  - 0,05-0,1 µg/L in milk
  - 0,25-0,5 µg/kg in meat, egg, yoghurt, cheese
  - 0,25-1 µg/kg in liver

- **In-house validated methods**
  - Broad scope: 35 PAs
  - MMS calibration
  - Cheese and liver samples were done with std addition
### Survey results: Animal derived products

<table>
<thead>
<tr>
<th>Category</th>
<th>Samples analysed</th>
<th>Samples &gt; LOQ</th>
<th>% &gt; LOQ</th>
<th>Highest conc. (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All animal-derived food products</td>
<td>746</td>
<td>13</td>
<td>1.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>268</td>
<td>11</td>
<td>4.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Pasteurised and UHT milk</td>
<td>182</td>
<td>11</td>
<td>6.0</td>
<td>0.17</td>
</tr>
<tr>
<td>Fermented milk products</td>
<td>27</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>34</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk powder (infant formula)</td>
<td>25</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh eggs</td>
<td>205</td>
<td>2</td>
<td>1.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Meat and meat products</td>
<td>273</td>
<td>0</td>
<td>0.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Beef meat</td>
<td>80</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork meat</td>
<td>79</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry meat (chicken breast)</td>
<td>83</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (beef, pork, chicken)</td>
<td>31</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Only limited exposure to PAs
# Survey results: Animal derived products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Origin</th>
<th>Description</th>
<th>Organic/Non-organic</th>
<th>Pyrrolizidine alkaloid</th>
<th>Conc. (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB14/0204</td>
<td>Germany</td>
<td>Semi-skimmed milk, past.</td>
<td>Non-organic</td>
<td>Senkirkine</td>
<td>0.05</td>
</tr>
<tr>
<td>FB14/0210</td>
<td>Germany</td>
<td>Skimmed milk, past.</td>
<td>Non-organic</td>
<td>Otosenine</td>
<td>0.08</td>
</tr>
<tr>
<td>FB14/0211</td>
<td>Germany</td>
<td>Semi-skimmed milk, past.</td>
<td>Organic</td>
<td>Otosenine</td>
<td>0.06</td>
</tr>
<tr>
<td>FB14/0235</td>
<td>Germany</td>
<td>Semi-skimmed milk, past.</td>
<td>Organic</td>
<td>Otosenine</td>
<td>0.11</td>
</tr>
<tr>
<td>IRTA 510</td>
<td>Greece</td>
<td>Skimmed milk, UHT</td>
<td>Non-organic</td>
<td>Senkirkine</td>
<td>0.16</td>
</tr>
<tr>
<td>IRTA 514</td>
<td>Greece</td>
<td>Whole milk, UHT</td>
<td>Non-organic</td>
<td>Senkirkine</td>
<td>0.06</td>
</tr>
<tr>
<td>IRTA 652</td>
<td>Spain</td>
<td>Goat milk, UHT</td>
<td>Non-organic</td>
<td>Retrorsine</td>
<td>0.11</td>
</tr>
<tr>
<td>IRTA 153</td>
<td>Spain</td>
<td>Semi-skimmed milk, UHT</td>
<td>Organic</td>
<td>Jacoline</td>
<td>0.06</td>
</tr>
<tr>
<td>RIK M21</td>
<td>Netherlands</td>
<td>Semi-skimmed milk, past.</td>
<td>Organic</td>
<td>Jacoline</td>
<td>0.05</td>
</tr>
<tr>
<td>RIK M20</td>
<td>Netherlands</td>
<td>Whole milk, past.</td>
<td>Non-organic</td>
<td>Lycopsamine</td>
<td>0.12</td>
</tr>
<tr>
<td>IRTA 639</td>
<td>Spain</td>
<td>Whole milk, past.</td>
<td>Organic</td>
<td>Lycopsamine Echimidine</td>
<td>0.11 0.06</td>
</tr>
</tbody>
</table>

Possible PA plant sources

- *Senkirkine*
- *Otosenine*
- *Retrorsine*
- *Jacoline*
- *Lycopsamine Echimidine*

Floral images:

- *Senkirkine* (S ing)
- *Jacoline* (S jac)
- *Retrorsine* (E vul)
- *Lycopsamine Echimidine* (S off)
- *Lycopsamine* (S ag)
Conclusions

- Compared to other food sources animal derived products do not strongly attribute to the overall exposure to PAs in food products (compared to consumption of tea and herbal supplements).

- Nevertheless efforts should be made to reduce the contamination of milk products by PAs:
  - Good Agricultural Practices
  - Better product control
Questions?

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