Speciation issues in food control
– are we ready?

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Rikke V. Hedegaard, Kåre Julshamn, Xenia T. Trier and Erik H. Larsen

National Food Institute (DTU Food)
Technical University of Denmark
Agenda

• Speciation and food safety – where are we now??
• Haven’t we heard about this before??
• Legislation and standardisation issues
• Selected examples
  - Arsenic speciation analysis
  - Organotin speciation analysis
• Are we ready??
COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006

setting maximum levels for certain contaminants in foodstuffs

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>Cadmium</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Meat (excluding offal) of bovine animals, sheep, pig and poultry (⁹)</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Horsemeat, excluding offal (⁹)</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Liver of bovine animals, sheep, pig, poultry and horse (⁹)</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Kidney of bovine animals, sheep, pig, poultry and horse (⁹)</td>
</tr>
</tbody>
</table>

etc

- only maximum levels for total amounts of Pb, Cd, Hg and Sn
- no regulation on trace element species
Speciation and regulation - some historical viewpoints

**1998**

*Speciation analysis: where is it going? An attempt at a forecast*

Bernhard Welz
Department of Applied Research, Weizmann Institute, Rehovot, Israel

*SPECTROCHIMICA ACTA PART B* 53 (1998) 109–175

© Springer-Verlag 1999

**1999**

*Conference Contribution*

Torsten Berg · Erik H. Larsen

*Speciation and legislation – Where are we today and what do we need for tomorrow?*


© Springer-Verlag 1999

**2007**

*Toxic metal species and food regulations — making a healthy choice*

Kevin A. Francesconi

Section: Forum

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Vicious circle of progress

Toxicological evaluation
(Risk assessment)

Analytical data

Legislation
(Risk management)

Method development
Examples

- Arsenic speciation analysis
  - HPLC-ICPMS

- Organotin speciation analysis
  - GC-ICPMS
Arsenic compounds in the marine environment

More than 50 different arsenic species have been found in the marine environment – incl lipid-soluble As compounds.
Speciation analysis of arsenic of scallop kidney

Cation-exchange with gradient elution – extraction with aqueous methanol
Column: Chrompack Ionosphere 5C; Mobile phase: Pyridine; pH = 2.7

- seven compounds identified by coelution with available standards
- **16** non-identified peaks

Sloth et al, JAAS, 2003, 18, 452-459
**Arsenic – chronic toxicity**

Long term exposure => skin diseases
- Keratosis, gangrene, melatosis
- Skin cancer
  … and also
- Lung, kidney, liver, bladder cancers
- Cancer slope factor: 1.5 (mg kg$^{-1}$ day$^{-1}$)$^{-1}$ (for inorganic As)
  (US EPA 2005)

**WHO PTWI for inorganic arsenic: 15 µg/kg bw/week**
*(Provisional Tolerable W*eekly *I*ntake)*
For a 70 kg person => 150 µg / day
Analysis of inorganic arsenic in seafood samples

Microwave assisted alkaline hydrolysis

Subsample + extractant

Microwave treatment 20 min, 90°C

I: Solubilisation of sample matrix
II: Conversion of As(III) to As(V)

Determination of total inorganic arsenic as As(V) by anion-exchange HPLC-ICP-MS

➢ No conversion of other arsenic compounds to inorganic arsenic

Analysis of inorganic arsenic by anion exchange HPLC-ICPMS

Inorg As = sum As(III)+As(V) - measured as As(V)

Blue mussel sample BCR278R

### Inorganic Arsenic in Fish and Marine Mammals

<table>
<thead>
<tr>
<th>Sample identification</th>
<th>Inorganic arsenic (μg/kg)</th>
<th>Total arsenic (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon (<em>Salmo salar</em>)</td>
<td>&lt; 0.0006</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Cod (<em>Gadus morhua</em>)</td>
<td>&lt; 0.0006</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Wolfish (<em>Anarhichas lupus</em>)</td>
<td>&lt; 0.0006</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>Wolffish (<em>Anarhichas lupus</em>)</td>
<td>&lt; 0.0006</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Anglerfish (<em>Lophius piscatorius</em>)</td>
<td>&lt; 0.0006</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Anglerfish (<em>Lophius piscatorius</em>)</td>
<td>&lt; 0.0006</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>Atlantic halibut (<em>Hippoglossus hippoglossus</em>)</td>
<td>&lt; 0.0006</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Mackerel (<em>Scomber scombrus</em>)</td>
<td>&lt; 0.0006</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Mackerel (<em>Scomber scombrus</em>)</td>
<td>&lt; 0.0006</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Herring (<em>Clupea harengus</em>)</td>
<td>&lt; 0.0006</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Herring (<em>Clupea harengus</em>)</td>
<td>&lt; 0.0006</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Tuna fish (<em>Thunnus alalunga</em>)</td>
<td>0.008 ± 0.001</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

For all marine mammals, inorganic arsenic constitutes less than 1% of total arsenic.

### Fish Muscle

<table>
<thead>
<tr>
<th>Sample identification</th>
<th>Inorganic arsenic (μg/kg)</th>
<th>Total arsenic (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobster, tail meat (<em>Homarus gammarus</em>)</td>
<td>&lt; 0.0006</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Lobster, head and thorax meat (<em>Homarus gammarus</em>)</td>
<td>0.037 ± 0.005</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Crab, white meat (<em>Cancer pagurus</em>)</td>
<td>0.060 ± 0.009</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>King crab, white meat (<em>Paralithodes cinctus</em>)</td>
<td>0.065 ± 0.001</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Norway lobster (<em>Nephrops norvegicus</em>)</td>
<td>0.020 ± 0.003</td>
<td>1 ± 3</td>
</tr>
<tr>
<td>Shrimp (<em>Pandalus borealis</em>)</td>
<td>&lt; 0.0006</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Shrimp (<em>Pandalus borealis</em>)</td>
<td>&lt; 0.0006</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>Shrimp (<em>Pandalus borealis</em>)</td>
<td>&lt; 0.0006</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>Horse mussel (<em>Modiolus modiolus</em>)</td>
<td>0.0012 ± 0.002</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Scallop muscle (<em>Pecten maximus</em>)</td>
<td>0.008 ± 0.001</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Oyster (<em>Ostrea edulis</em>)</td>
<td>0.014 ± 0.002</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Mink whale (<em>Balaenoptera Acutorostrata</em>)</td>
<td>&lt; 0.0006</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>Harp seal (<em>Pagophila groenlandicus</em>)</td>
<td>&lt; 0.0006</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Hooded seal (<em>Cystophora cristata</em>)</td>
<td>&lt; 0.0006</td>
<td>0.22 ± 0.03</td>
</tr>
</tbody>
</table>

### Crustaceans & Bivalves

For all samples, inorganic arsenic constitutes less than 1% of total arsenic.

### Fish Identification

- **Salmon**: *Salmo salar*
- **Cod**: *Gadus morhua*
- **Wolfish**: *Anarhichas lupus*
- **Anglerfish**: *Lophius piscatorius*
- **Atlantic halibut**: *Hippoglossus hippoglossus*
- **Mackerel**: *Scomber scombrus*
- **Herring**: *Clupea harengus*
- **Tuna fish**: *Thunnus alalunga*
- **Lobster, tail meat**: *Homarus gammarus*
- **Lobster, head and thorax meat**: *Homarus gammarus*
- **Crab, white meat**: *Cancer pagurus*
- **King crab, white meat**: *Paralithodes cinctus*
- **Norway lobster**: *Nephrops norvegicus*
- **Shrimp**: *Pandalus borealis*
- **Scallop muscle**: *Pecten maximus*
- **Oyster**: *Ostrea edulis*
- **Mink whale**: *Balaenoptera Acutorostrata*
- **Harp seal**: *Pagophila groenlandicus*
- **Hooded seal**: *Cystophora cristata*
Data from 175 blue mussel (Mytilus edulis) samples collected along the Norwegian Coastline.

Arsenic in rice – an emerging health issue?

Exposure to inorganic arsenic from rice: A global health issue?☆

Yong-Guan Zhu a,b,*, Paul N Williams d,c, Andrew A Meharg c,**

a Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, 18 Shuangjing Road, Beijing 100085, China
b Institute of Urban Environment, Chinese Academy of Sciences, Xian 710063, China
c School of Biological Sciences, University of Aberdeen, Cruckshank Building, St Machar Drive, Aberdeen AB24 3UU, UK

FOOD SAFETY

Arsenic and Paddy Rice: A Neglected Cancer Risk?

11 JULY 2008 VOL 321 SCIENCE www.sciencemag.org
Arsenic in rice – an emerging health issue?

Rapid communication

Inorganic arsenic levels in baby rice are of concern

Andrew A. Mehar a,*, Guoxin Sun b, Paul N. Williams a,b, Eureka Adomako a, Claire Deacon a, Yong-Guan Zhu a, Joerg Feldmann c, Andrea Raab c

* School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, UK
b Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Median consumption of organic arsenic levels for UK babies from baby rice is above threshold considered safe.

- 17 samples from supermarkets in Aberdeen
- Total arsenic levels: 0.12 – 0.47 mg/kg
- Inorganic arsenic levels in rice milk exceed EU and US drinking water standards

- 35% above Chinese max level of 0.15 mg/kg iAs
- 19 rice milk samples from supermarkets
- EU drinking water ML: 10 µg/L total As (100% of samples exceeded)
- US drinking water ML: 10 µg/L iAs (80% of samples exceeded)
# Commission Directive 2003/100/EC on animal feed

<table>
<thead>
<tr>
<th>Undesirable substances</th>
<th>Products intended for animal feed</th>
<th>Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>1. Arsenic (9)</td>
<td>Feed materials with the exception of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- fish meal made from grass, from dried lucerne and from dried clover, and dried sugar beet pulp and dried molasses sugar beet pulp</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>- palm kernel expeller</td>
<td>4 (9)</td>
</tr>
<tr>
<td></td>
<td>- phosphates and calcareous marine algae</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>- calcium carbonate</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>- magnesium oxide</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>- feedingstuffs obtained from the processing of fish or other marine animals</td>
<td>15 (9)</td>
</tr>
<tr>
<td></td>
<td>- seaweed meal and feed materials derived from seaweed</td>
<td>40 (9)</td>
</tr>
<tr>
<td></td>
<td>Complete feedingstuffs with the exception of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- complete feedingstuffs for fish and complete feedingstuffs for fur animals</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>- mineral feedingstuffs</td>
<td>12</td>
</tr>
</tbody>
</table>

(9) Upon request, inorganic.

Footnote in the Commission directive

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Max levels for total arsenic

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National Food Institute
Total arsenic in fish feed products

<table>
<thead>
<tr>
<th>Product</th>
<th>N</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete feedingstuffs</td>
<td></td>
<td>5.87</td>
<td>5.80</td>
<td>1.17</td>
<td>3.40 - 8.34</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10</td>
<td>7.93</td>
<td>7.70</td>
<td>4.00</td>
<td>3.62 - 18.2</td>
</tr>
<tr>
<td>Fish oils</td>
<td>6</td>
<td>11.30</td>
<td>11.17</td>
<td>1.81</td>
<td></td>
</tr>
</tbody>
</table>

Results in mg kg\(^{-1}\)

ML = 15 mg As/kg

Inorganic arsenic in fish feed products

<table>
<thead>
<tr>
<th>Product</th>
<th>N</th>
<th>Range</th>
<th>% of total As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Feedingstuffs</td>
<td>13</td>
<td>10 - 61</td>
<td>0.18 - 1.20</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10</td>
<td>All &lt; 7</td>
<td>-</td>
</tr>
</tbody>
</table>

Results in µg kg⁻¹

*Sloth et al, Aquaculture Nutr., 2005, 11, 61-66*
Arsenic and food/feed control – present status

- Food – no maximum levels established
- Feed – maximum levels for total arsenic
- EFSA opinion on arsenic in food – expected in 2009
- CEN (European Standardisation Organisation)
  - TC327 WG4 Feedingstuffs (Heavy metals and minerals)
  - TC275 WG10 Foodstuffs (Trace elements)
Organotin speciation by GC-ICPMS
Routes of exposure to organotin compounds

- Agriculture
- Antifoulings
- Industry
- PVC-Materials

Seafood

Water

Seafood

Sediment

Food, beverage

Tap water pipes

PVC-Materials

TDI: 0.25 µg/kg bw/day

∑TBT, DBT, TPhT and DOT
### Legislation on OTCs in Food Contact Materials

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Maximum level (µg Sn/kg foodstuff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sum$ DBT, TBT, TPhT and DOT</td>
<td>40 (6)</td>
</tr>
<tr>
<td>$\sum$ MMT, DMT</td>
<td>180</td>
</tr>
<tr>
<td>MOT</td>
<td>1200</td>
</tr>
<tr>
<td>MDDT</td>
<td>12000 (50)</td>
</tr>
<tr>
<td>DDDT</td>
<td>24000 (50)</td>
</tr>
</tbody>
</table>

- Max levels on organotin migrating from the packaging material
- Testing by the use of food simulators (water, acid, oil, alcohol etc)
- BUT no maximum levels on organotins in the foodstuff itself!!

**Assumptions:**
- 1 kg food per 6 dm²
- 100 mL in contact with 0.6 dm²

Reference: EFSA (2005); proposed EFSA values in parenthesis

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**National Food Institute**
Organotin migration from Food Contact Materials I

Chromatogram of 9 standards + IS
Organotin migration from Food Contact Materials II

Small scale survey on 33 FCMs
- Baking paper, PVC cling films, silicone baking forms, lids with PVC gaskets
- PUR-agglomerated cork wine stoppers

Output of DK survey:
- 33 samples
- 11 contained OT (mainly DBT)
- 3 exceeded EFSA guideline limit

Overlaid standard and sample
- DBT concentration: 15.8 µg/kg

> EFSA guideline value of 6 µg/kg
...so are we ready or what??

- speciation methods are more and more commonly used
- instrumentation is widely available
- legislation on species has started
- ...and more is expected in the future!

- standardised methods are not ready!
- ...but the need is known by authorities
- legislation is still behind!
- Lack of CRMs (e.g. for iAs)
...so are we ready or what?

Toxicological evaluation (Risk assessment)

Analytical data

Legislation (Risk management)

Method development

...has the circle been broken??
Acknowledgements

*Coworkers:*
Rikke V. Hedegaard, Birgitte Koch Herbst, Marianne Hansen
Xenia T. Trier and Erik H. Larsen
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Siri Barggaard and A.K. Undebye

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