The distribution of environmental contaminants and pharmaceuticals among skim milk, milk fat, curd, whey, and milk protein fractions through milk processing

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Heldur Hakk
David J. Smith
Milk

Top 10 cow milk production nations in 2014

- Milk consumption > 150 kg/capita/year in Europe and North America
- More than 6 billion people worldwide consume milk and milk products
- Milk products provide 11-14% of dietary energy in Europe and North America

Image: https://www.agclassroom.org/teacher/matrix/lessonplan_print.cfm?lpid=246
Data source: Food and Agriculture Organization of the United Nations
Milk Constituents

- Milk is a complex emulsion or colloid within a water-based fluid
  - Proteins (~3%, casein micelles, soluble whey)
  - Lipid (~3.5%, fat globules)
  - Sugars (lactose)
  - Minerals, vitamins, enzymes, etc.....

Ref: Dalgleish, D. G., P. Spagnuolo and H. D. Goff. 2004
US FDA Risk Assessment for Milk

- National Conference on Interstate Milk Shipments
  - Every tanker truck tested for β-lactam antibiotics
    - ~0.05% test positive from > 3 million tankers per year
    - July 1, 2017 Pilot study- one out of every 15 tanker trucks tested for tetracyclines
- Multi-criteria based ranking system on over 500 animal drugs
  - Likelihood of a drug’s use in lactating dairy cows
  - Likelihood of its presence in milk
  - Human exposure to drug residues through consumption of milk products
  - Potential to be a human health hazard
- Identified data gap
Compound Selection Criteria

- Drugs (or close analogs) were evaluated in the FDA 2015 ranking model
- Widespread environmental contaminants with potential to be present in cow milk
- Selected chemicals ensure the sample set spans a wide range of lipophilicity
- Commercial or in-house availability of radiolabeled test compounds
Drugs

Oxytetracycline
Penicillin G
Erythromycin A

Sulfadimethoxine
Ketoprofen
Thiabendazole
Ivermectin B1a
Drugs

Ciprofloxacin

Clarithromycin

Phenylbutazone

Thiamphenicol

Acetaminophen

Flunixin

Salicylic acid

Praziquantel
Environmental Contaminants

- **β-HBCD**
- **TBBPA**
- **BDE-99**
- **1278-TCDD**
- **2-OH 1378-TCDD**
- **PCB 118**
- **3’MeSO2 PCB 101**
Environmental Contaminants

Imidacloprid

Glyphosate

Triclocarban

Estrone

Bisphenol A
Compound Polarity

logP

logD
Milk Processing

3 concentrations
3 replicates/concentration
(20 – 2000 nM)
TLC Chromatograms

- Silica gel
- 1:1 Hex:CH$_2$Cl$_2$
- Imaging Scanner

14C β-HBCD Milk fat extract
1278-TCDD Milk fat extract

14C β-HBCD Standard
1278-TCDD Standard
## Compositional Analysis of Milk

<table>
<thead>
<tr>
<th>Milk Fraction Compositional Data</th>
<th>Total Solids %</th>
<th>Lipid %</th>
<th>Protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>%RSD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Milk</td>
<td>12.29 ± 0.54</td>
<td>4.4</td>
<td>3.86 ± 0.20</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>8.86 ± 0.44</td>
<td>5.0</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>Milk Fat</td>
<td>83.59 ± 3.58</td>
<td>4.3</td>
<td>82.10 ± 2.61</td>
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<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whey</td>
<td>6.46 ± 0.18</td>
<td>2.8</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>Curd</td>
<td>29.84 ± 2.39</td>
<td>8.0</td>
<td>1.95 ± 0.90</td>
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<tr>
<td><strong>Phase 3</strong></td>
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<tr>
<td>Retentate</td>
<td>8.56 ± 0.54</td>
<td>6.3</td>
<td>0.33 ± 0.33</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.71 ± 0.4</td>
<td>7.0</td>
<td>0.19 ± 0.19</td>
</tr>
</tbody>
</table>
Milk Processing

Phase 1

Fortification with [14C] or [3H] drugs

Milk Fat Separation

Whole milk
Milk Fat
Skim milk
Distribution between Skim Milk and Milk Fat

log D  -7.2  to  8.2
Distribution between Skim Milk and Milk Fat

log D  -7.2 to 8.2
Correlation of log P or log D vs log [Milk Fat]/[Skim Milk]

- For log P:
  - $y = 0.382x - 0.501$
  - $r^2 = 0.689$
  - $p = 9E-8$

- For log D (pH 6.8):
  - $y = 0.294x + 0.073$
  - $r^2 = 0.729$
  - $p = 1E-8$
Milk Processing

Phase 2

Milk Fat Separation

Fortification with [14C] or [3H] drugs

Curding
Distribution between Curd and Whey

\[ \log D = -7.2 \]
Distribution between Curd and Whey

log D -7.2 8.2
Correlation of log P or log D vs log [curd]/[whey]

\[ y = 0.192x + 0.679 \]
\[ r^2 = 0.634 \]
\[ p = 7E-7 \]

\[ y = 0.151x + 0.957 \]
\[ r^2 = 0.709 \]
\[ p = 4E-8 \]
Distribution between Whey Retentate and Permeate

<table>
<thead>
<tr>
<th></th>
<th>Average % Permeate</th>
<th>Average % Retentate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>1.0 ± 0.00</td>
<td>1.3 ± 0.01</td>
</tr>
<tr>
<td>CIPR</td>
<td>0.9 ± 0.01</td>
<td>1.0 ± 0.02</td>
</tr>
<tr>
<td>TAP</td>
<td>1.0 ± 0.03</td>
<td>1.1 ± 0.01</td>
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<tr>
<td>TYL</td>
<td>1.0 ± 0.01</td>
<td>1.0 ± 0.02</td>
</tr>
<tr>
<td>CLA</td>
<td>0.6 ± 0.02</td>
<td>1.2 ± 0.02</td>
</tr>
<tr>
<td>PBZ</td>
<td>0.1 ± 0.01</td>
<td>2.7 ± 0.02</td>
</tr>
<tr>
<td>PZQ</td>
<td>0.7 ± 0.02</td>
<td>1.2 ± 0.02</td>
</tr>
<tr>
<td>FNX</td>
<td>0.3 ± 0.02</td>
<td>2.1 ± 0.09</td>
</tr>
</tbody>
</table>

Normalized Drug Distribution (%)
Distribution between Whey Retentate and Permeate

log D -7.2 to 8.2
Chemicals Associated with Whey

Bound + Free

Free
Percentage of Chemical in Whey Associated with Protein

![Graph showing the percentage of chemical associated with protein](image)

Log D -7.2 to 8.2
## Overall Distribution

<table>
<thead>
<tr>
<th>Product</th>
<th>GLY</th>
<th>OTET</th>
<th>ASP</th>
<th>PENG</th>
<th>IMI</th>
<th>CIPR</th>
<th>TAP</th>
<th>TYL</th>
<th>KETO</th>
<th>CLA</th>
<th>SDMX</th>
<th>ERY</th>
<th>PBZ</th>
<th>PZQ</th>
<th>THIA</th>
<th>FNX</th>
<th>BPA</th>
<th>E1</th>
<th>MeSO2-PCB</th>
<th>TCC</th>
<th>2-OH-1378-TCDD</th>
<th>PCB-118</th>
<th>IRV</th>
<th>β-HBCCD</th>
<th>1278-TCDD</th>
<th>TBBPA</th>
<th>BDE-99</th>
</tr>
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<tbody>
<tr>
<td>Whole Milk</td>
<td></td>
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<tr>
<td>High Fat Curd</td>
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<tr>
<td>Low Fat Curd</td>
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<td>Whey</td>
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</tbody>
</table>
Milk products and production relationships

Legend

- Types of cheese: Whey products, Sour milk products, Powdered products
- Butter products: Cream products, Types of milk, Fat Content

Figure adapted from https://en.wikipedia.org/wiki/Dairy_product#/media/File:Milkproducts_v2.svg
Conclusion

- Twenty-seven environmental contaminants and pharmaceuticals encompassing a wide range of physicochemical properties were utilized to determine the xenobiotic distribution among milk fractions.

- Log D values were better indicators than log P values for predicting the distribution of chemicals between fat and skim fractions and between insoluble (curd) and soluble (whey) proteins.

- Phenolic chemicals such as BPA, OH-TCDD and TBBPA are less predictable based on their lipophilicity to predict distribution into milk products.
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  ▫ Chi Yuen Yeung
Thank you
Organochlorine pesticide distribution in an organic production system for cow’s milk in Chiapas, Mexico

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aDepartment of Agricultural and Animal Production of the Autonomous Metropolitan University, Xochimilco, D.F., Mexico; bFaculty of Veterinary Medicine and Animal Husbandry of the National Autonomous University of Chiapas, Tuxtla Gutiérrez, Chiapas, Mexico

Abstract

The presence of organochlorine pesticides in samples of forage, milk production system for cow’s milk (samples of forage, milk, green manure). The organochlorine pesticides were extracted from forage, milk. The gas chromatography by electron capture detector (GC-ECD) was used to study the organochlorine pesticides and their decomposition products. The GC-ECD analysis was performed using a capillary column (30 m x 0.25 mm ID). The temperature program was 100°C for 1 min, then increased to 220°C at a rate of 5°C/min.

A Survey of Organochlorine Pesticide Residues in Cheese Samples from Three Mexican Regions

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Leticia Alpuche, Cristina Bárcenas & Jaime Rendón

Instituto de Ecología, Xalapa, Ver., Mexico

Organochlorine pesticide residues were determined in cheese samples from three Mexican regions. All samples were found to be contaminated by these residues: p,p'-DDE was present in 100% of the samples. Other pesticides found with high frequencies were HCB, three of the BHC isomers, as well as heptachlor and its epoxide. Also found, although less frequently, were pesticides of p,p'-DDD, p,p'-DDT, aldrin, dieldrin and endrin. Samples from the region

Residual Content of Hexachlorobenzene in Spanish Cheeses

A. Bentabol, a M. D. Garrido, a M. Jodral

Table 1. Concentration of HCB in groups of Spanish cheeses

<table>
<thead>
<tr>
<th>Type of Cheese</th>
<th>Sample Size</th>
<th>HCB Concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrially-made</td>
<td>64</td>
<td>12.48</td>
</tr>
<tr>
<td>Farm-produced</td>
<td>82</td>
<td>6.76</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>9.26</td>
</tr>
</tbody>
</table>
Milk Processing

Phase 1
Pasteurized, whole milk
Fortify 20, 200, or 2000 nM POP
Incubate 30 min, 38°C
Centrifuge @ 4000xg, 45 min, 35°C
Separate milk fat/skim milk
48 g skim milk, 2 g milk fat
Assay fractions for radioactivity

Phase 2
Skim milk from (1)
Add rennet, 1 hr, 38°C
Centrifuge @ 3000xg, separate whey/curd
Assay fractions for radioactivity

Phase 3
Whey from (2)
Ultrafiltration @ 4000xg
Separation of retentate and permeate
Assay fraction for radioactivity
Milk Processing

**Phase 1**
- Whole milk
- Milkfat (low moisture)
- Skim milk

**Phase 2**
- Milkfat
- Skim milk
- Whey
- Curd

**Phase 3**
- Ultrafiltrate (1/3 vol)
- Permeate (2/3 vol)
Partition Coefficient, Distribution Coefficient

- \( \log D_{\text{acids}} = \log P + \log \left[ \frac{1}{1+10^{(pH-pKa)}} \right] \)
- \( \log D_{\text{bases}} = \log P + \log \left[ \frac{1}{1+10^{(pKa-pH)}} \right] \)
- acids \((pH - pKa) > 1\) \( \log D_{\text{acid}} = \log P + pKa - pH \)
- bases \((pKa - pH) > 1\) \( \log D_{\text{bases}} = \log P - pKa + pH \)
- Non-ionizables \( \log D \sim \log P \)
- Sources of \( \log P \), mainly Chemspider
- Sources of \( pKa \), mainly Drug Bank
# Polarities of Studied Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>log P</th>
<th>log D</th>
<th>Compound</th>
<th>log P</th>
<th>log D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>-2.36</td>
<td>-7.16</td>
<td>Clarithromycin</td>
<td>3.16</td>
<td>0.97</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>-1.5</td>
<td>-5.00</td>
<td>Bisphenol A</td>
<td>3.43</td>
<td>3.43</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>-0.43</td>
<td>-0.44</td>
<td>Estrone</td>
<td>3.69</td>
<td>3.69</td>
</tr>
<tr>
<td>Thiamphenicol</td>
<td>-0.27</td>
<td>-0.27</td>
<td>MeSO2-PCB</td>
<td>4.02</td>
<td>4.02</td>
</tr>
<tr>
<td>Tylenol</td>
<td>0.34</td>
<td>0.34</td>
<td>Flunixin</td>
<td>5.40</td>
<td>3.40</td>
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<tr>
<td>Ciprofloxacin</td>
<td>0.65</td>
<td>-0.43</td>
<td>Triclocarban</td>
<td>5.66</td>
<td>5.66</td>
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<tr>
<td>Aspirin</td>
<td>1.19</td>
<td>-2.20</td>
<td>OH-TCDD</td>
<td>6.28</td>
<td>6.28</td>
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<tr>
<td>Sulfadimethoxine</td>
<td>1.48</td>
<td>1.23</td>
<td>PCB-118</td>
<td>6.30</td>
<td>6.30</td>
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<tr>
<td>Penicillin G</td>
<td>1.67</td>
<td>-1.60</td>
<td>Ivermectin</td>
<td>6.61</td>
<td>6.61</td>
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<tr>
<td>Salicylic acid</td>
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<td>-1.95</td>
<td>HBCD</td>
<td>6.63</td>
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<tr>
<td>Praziquantel</td>
<td>2.44</td>
<td>2.44</td>
<td>1278-TCDD</td>
<td>6.79</td>
<td>6.79</td>
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<tr>
<td>Ketoprofen</td>
<td>2.81</td>
<td>0.46</td>
<td>TBBPA</td>
<td>7.29</td>
<td>7.29</td>
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<tr>
<td>Erythromycin</td>
<td>2.83</td>
<td>1.24</td>
<td>BDE-99</td>
<td>8.19</td>
<td>8.19</td>
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<tr>
<td>Thiabendazole</td>
<td>2.93</td>
<td>2.93</td>
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</table>
# Chemicals Associated with Whey

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Association</th>
<th>log D</th>
<th>Compound</th>
<th>% Association</th>
<th>log D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>0.6</td>
<td>-7.16</td>
<td>Thiabendazole</td>
<td>12.0</td>
<td>2.93</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>7.6</td>
<td>-5.00</td>
<td>Flunixin</td>
<td>66.2</td>
<td>3.40</td>
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<tr>
<td>Aspirin</td>
<td>5.2</td>
<td>-2.20</td>
<td>Bisphenol A</td>
<td>81.9</td>
<td>3.43</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>6.6</td>
<td>-1.60</td>
<td>Estrone</td>
<td>81.9</td>
<td>3.69</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>4.4</td>
<td>-0.44</td>
<td>MeSO2-PCB</td>
<td>55.6</td>
<td>4.02</td>
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<tr>
<td>Ciprofloxacin</td>
<td>12.8</td>
<td>-0.43</td>
<td>Triclocarban</td>
<td>96.9</td>
<td>5.66</td>
</tr>
<tr>
<td>Thiamphenicol</td>
<td>3.6</td>
<td>-0.27</td>
<td>OH-TCDD</td>
<td>79.5</td>
<td>6.28</td>
</tr>
<tr>
<td>Tylenol</td>
<td>2.8</td>
<td>0.34</td>
<td>PCB-118</td>
<td>96.4</td>
<td>6.30</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>22.6</td>
<td>0.46</td>
<td>Ivermectin</td>
<td>102.2</td>
<td>6.61</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>22.8</td>
<td>0.97</td>
<td>HBCD</td>
<td>101.8</td>
<td>6.63</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>13.9</td>
<td>1.23</td>
<td>1278-TCDD</td>
<td>69.5</td>
<td>6.79</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>16.5</td>
<td>1.24</td>
<td>TBBPA</td>
<td>88.7</td>
<td>7.29</td>
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<td>Praziquantel</td>
<td>79.2</td>
<td>2.44</td>
<td>BDE-99</td>
<td>8.19</td>
<td>8.19</td>
</tr>
</tbody>
</table>
Distribution between skim milk and milk fat

**Drug Distribution (%)**

- **ASP**: 1.0 ± 0.02
- **CIPR**: 1.0 ± 0.00
- **TAP**: 1.0 ± 0.06
- **TYL**: 1.0 ± 0.01
- **CLA**: 0.5
- **PBZ**: 0.5 ± 0.01
- **PZQ**: 0.9 ± 0.01
- **FNX**: 2.6 ± 0.07

**logD (pH 6.8)**

- **ASP**: -2.2
- **CIPR**: -0.4
- **TAP**: -0.3
- **TYL**: 0.3
- **CLA**: 1.0
- **PBZ**: 1.5
- **PZQ**: 2.4
- **FNX**: 3.4

**Average % Skim**

**Average % Milk Fat**
Distribution between whey and curd

<table>
<thead>
<tr>
<th>Drug</th>
<th>Average % Whey</th>
<th>Average % Curd</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>1.0 ± 0.06</td>
<td>0.6 ± 0.02</td>
</tr>
<tr>
<td>CIPR</td>
<td>0.6 ± 0.02</td>
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</tr>
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<tr>
<td>CLA</td>
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<td>0.4 ± 0.01</td>
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<tr>
<td>PBZ</td>
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<tr>
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</tr>
<tr>
<td>FNX</td>
<td>3.6 ± 0.09</td>
<td>3.9 ± 0.10</td>
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