CCQM Activities and the Impact in Food Safety and Nutrition

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Chemical Metrology for Food Safety and Nutrition – Prominent Issues

- Nutrients (vitamins, carotenoids, fatty acids, cholesterol, elements)
- Proximates (fat, protein, carbohydrates), fiber
- Trans fats
- Phytochemicals
- Allergens
- Additives and Contaminants
  - Colors
  - Flavors
  - Hormones
  - Veterinary Drug Residues
  - Genetically-Engineered Foods
  - Mycotoxins and Phycotoxins
  - Preservatives
  - Toxic Metals, MeHg, As species
  - PCBs, Pesticides, PAHs, etc.
Nominal Concentrations of Measurands in Foods

- 1 g/g: Proximates, dietary fiber, minerals, GMOs
- 1 mg/g: Trans fatty acids, caffeine, nitrates
- 1 μg/g: Vitamins, allergens, toxic elements (lead, mercury)
- 1 ng/g: Pesticides, marine biotoxins (okadaic acid, yessotoxins), veterinary drug residues
- 1 pg/g: Mycotoxins (aflatoxin, ochratoxin), polycyclic aromatic hydrocarbons (PAHs), dioxins and dioxin-like PCBs
How has the CCQM Addressed Chemical Metrology for Food Safety and Nutrition?

What are the motivations for a particular CCQM study related to chemical measurements in food?

- Measurement services delivered by the National Metrology Institute (NMI) (current and planned)
- National and international food safety and nutrition regulations

Most of the CCQM studies to date have addressed food safety rather than nutritional assessment
Inorganic Analysis in Food

- Food analyses were early matrices used in CCQM IAWG comparisons
  - Some food matrices were convenient biological matrices (e.g., shellfish, bovine liver, algae) and not necessarily chosen as foods
- Primarily comparisons have been related to food safety (i.e., toxic metals)
- Nutritional element comparisons more recent
- Elemental speciation has been a focus (e.g., Hg, As, and Se speciation)
## CCQM IAWG Food Key Comparisons

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>CCQM-K31</td>
<td>As in fish or shellfish (2002)</td>
<td>CCQM-K75</td>
<td>Toxic metals in algae (2009)</td>
</tr>
<tr>
<td>CCQM-K45</td>
<td>Toxic metals in food (tin in tomato paste) (2005)</td>
<td>CCQM-K108</td>
<td>As species, total As, and Cd in brown rice flour (2013)</td>
</tr>
<tr>
<td>CCQM-K49</td>
<td>Toxic and essential elements in bovine liver (2006)</td>
<td>CCQM-K125</td>
<td>Iodine and other elements in infant formula</td>
</tr>
</tbody>
</table>

Most CCQM IAWG key comparisons have a parallel pilot study.
### Additional CCQM IAWG Food Pilot Studies

<table>
<thead>
<tr>
<th>CCQM-P11</th>
<th>As in shellfish</th>
<th>CCQM-P64</th>
<th>Trace elements in soybean powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCQM-P12</td>
<td>Pb in wine</td>
<td>CCQM-P96</td>
<td>As and arsenobetaine content in marine fish</td>
</tr>
<tr>
<td>CCQM-P13</td>
<td>Metals in synthetic food digest</td>
<td>CCQM-P97</td>
<td>Cd and Pb in Herbs</td>
</tr>
<tr>
<td>CCQM-P39</td>
<td>As, Se, Hg, Pb, methyl-Hg in tuna fish</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CCQM-P39 and IMEP-20: Pb in Tuna Fish

International Measurement Evaluation Program (IMEP)

Since 1988 IMEP sheds light on the actual state of practice in chemical measurement performance. IMEP aims to build up confidence where trade or border crossing problems exist.

IMEP-20 Trace Elements in Tuna Fish (As, Pb, Hg, MeHg, Se)
Conducted in 2003
235 laboratories participated
Focus on toxic (As, Cd, Pb, Cr) and essential elements (Fe, Se, Zn)

Listed the analytical difficulty as: easy (Fe, Zn), moderate (Se, Pb, Cd), and difficult (As, Cr)

Study coordinated by NIST

Sample was candidate SRM 1577b Bovine Liver

16 participants using different techniques including ID-ICP-MS, ICP-MS, ICP-OES, ID-TIMS, INAA, RNAA, and FAAS
CCQM-K49: Toxic and Essential Elements in Bovine Liver

“Easy Elements” – Mass Fraction 200 mg/kg
KCRV Uncertainty (%) = 1.0% and 1.1% respectively

CCQM-K49 - Zinc

CCQM-K49 - Iron
CCQM-K49: Toxic and Essential Elements in Bovine Liver

“Moderate Difficulty” – Mass Fraction 60 µg/kg to 2000 µg/kg

KCRV Uncertainty (%) = 1.9% and 3.7% respectively
CCQM-K49: Toxic and Essential Elements in Bovine Liver

“Difficult Elements” – Mass Fraction 20 µg/kg to 50 µg/kg
KCRV Uncertainty (%) = 6.7% and 6.4% respectively
CCQM-P39: MeHg in Tuna Fish

- CCQM IAWG invites expert laboratories to participate in pilot studies of emerging measurement applications.

**CCQM-P39: methylmercury in tuna fish**

Mixture Model-median: \(1.967 \pm 0.054 \times 10^{-5} \text{ mol (CH}_3\text{Hg)} \cdot \text{Kg}^{-1}\); \([ \mu \pm \sigma \times t_s / \sqrt{n} ]\)

Invited expert laboratories

Deviation from the MM-median in %
CCQM-K97 and P133: Arsenobetaine in fish tissue

Each bar indicates the expanded unc.

Shape
●: ICPMS
▲: MS
■: AFS

Colour
●: LC/ext cal
●: LC/ID
●: LC/std add
Total Selenium and Selenium Methionine in Wheat Flour (CCQM-K60 and P-86.1)

Key Comparison Pilot Study

Includes guest expert laboratories with expertise in a new area of work
Organic Analysis in Food

- First food comparison (pilot) in 2006 and Key comparisons in 2010
- Primarily comparisons have been related to food safety
- Only one key comparison study related to nutrition
CCQM Pilot Comparisons for Food Safety Measurements

  - Coordinated by NIMC
  - 9 Participants

- CCQM-P109 Acrylamide in Food (Potato Chips) (2008)
  - Coordinated by KRISS
  - 8 Participants
CCQM Key Comparisons for Food Safety Measurements (Organic)

- CCQM-K85 Malachite Green in Fish Tissue (2010)
  - *Malachite green is a banned antifungal*
  - Coordinated by LGC
  - 6 participants
  - CCQM-P88 coordinated by LGC in 2007

- CCQM-K81/P122 Chloramphenicol in Pig Muscle (2010)
  - *Chloramphenicol is a banned antibiotic*
  - Coordinated by PTB and BVL
  - 6 Participants
  - CCQM-P90 Chloramphenicol in Milk

- CCQM-K95 Pesticides in Tea (2011)
  - *Intended to represent pesticide contaminants in food matrix*
  - Coordinated by NIMC and HKGL (First OAWG Core Study)
  - 18 participants

- CCQM-K103/P145 Melamine in Milk Powder (2012)
  - *Prompted by the adulteration of milk*
  - Coordinated by NIMC and HKGL
  - 7 participants
CCQM Key Comparisons for Food Safety (Organic)

Blue: Data used for KCRV; Red: Data excluded from KCRV
CCQM-K95 Pesticides in Tea

CCQM-K95: Beta-endosulfan results

KCRV = 727 µg/kg (Median)

u(KCRV) = 14 µg/kg
CCQM-K95 Pesticides in Tea

Lessons Learned:

Incomplete recovery due to not pre-wetting tea prior to solvent extraction
CCQM Key Comparisons for Organic Nutrients in Food

- CCQM-K62 Nutrients in Infant/Adult Formula (2008)
  - Coordinated by NIST
  - 3 Participants (NIST, KRISS, NIMC)
  - Measurements for niacin (vitamin B₃), folic acid (vitamin B₉), and retinol (vitamin A)
  - Follow up to CCQM-P78 (2006)
    - Same three analytes (NIST, KRISS, and NIMC + 2 non-NMIs)
CCQM-K62 Nutrients in Infant Formula

Folic Acid (Vitamin B₉)  Niacin (Vitamin B₃)  Retinol (Vitamin A)
How Can We Assess the Impact of CCQM Activities Related to Food Measurements?

- Are the measurement services of the National Metrology Institutes (NMIs) improved and/or expanded?
- Are Certified Reference Materials (CRMs) produced to improve and assure the quality of chemical measurements for food safety and nutrition?
  - CRMs used worldwide to improve and assure the quality of chemical measurements
  - New CRMs developed to meet emerging chemical measurement needs
Certified Reference Materials (CRMs)

- **Reference Material (RM):** Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

- **Certified Reference Material (CRM):** Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.

  *International Vocabulary of Basic and General Terms in Metrology, International Organization for Standardization (ISO), 2012 (VIM)*

- **Standard Reference Materials (SRMs) are Certified Reference Materials (CRMs) issued by the National Institute of Standards and Technology (NIST)**

- Homogeneous, stable materials well-characterized for one or more chemical and/or physical properties

- Assist laboratories worldwide in validating analytical measurements of chemical composition
CRM Development for Organic Nutrients

- In 2006 for CCQM-P78 and 2008 for CCQM-K62, only NIST had a CRM for infant formula (SRM 1846)
- NIST had recently developed ID-LC-MS method for niacin and folic acid (and other vitamins)
- KRISS and NIMC were developing capabilities for these measurements at the same time
U.S. Food Regulations

- Infant Formula Act of 1980
- Nutrition Labeling and Education Act of 1990 (NLEA)
- Dietary Supplement Health and Education Act of 1994

**Nutrition Facts**

<table>
<thead>
<tr>
<th>Amount</th>
<th>Per Serving</th>
<th>Cereal with 1/2 cup Skim Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>130</td>
<td>170</td>
</tr>
<tr>
<td>% Daily Value**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Fat</td>
<td>0g*</td>
<td>0% 0%</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>0g</td>
<td>0% 0%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0mg</td>
<td>0% 0%</td>
</tr>
<tr>
<td>Sodium</td>
<td>200mg</td>
<td>8% 11%</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>30mg</td>
<td>10% 12%</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>4g</td>
<td>16% 16%</td>
</tr>
<tr>
<td>Sugars</td>
<td>18g</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>3g</td>
<td></td>
</tr>
</tbody>
</table>

* Amount in Cereal. One half cup skim milk contains an additional 40 calories, 65 mg sodium, 6 g total carbohydrates (6 g sugars), and 4 g protein.

**Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

Ingredients: Cod liver oil, gelatin, water, and glycerin.
Infant Formula Act of 1980

“To amend the Federal Food, Drug and Cosmetic Act to strengthen the authority under the Act to assure the safety and nutrition of infant formulas, and for other purposes.”

Infant formula is now one of the most regulated foods in U.S.

Current Manufacturing Regulations and Guidelines include:

- Infant Formula Quality Control Procedures
- Infant Formula Labeling Requirements
- Nutrient Requirements for Infant Formula
Infant Formula Regulations

Infant Formula Quality Control Procedures

- “During manufacture, a manufacturer shall test each production aggregate for nutrients as follows:
  - “Each nutrient premix used in the manufacture of an infant formula shall be tested for each nutrient….to ensure that the premix is in compliance with the manufacturer’s specifications.”
  - During manufacturing process,…each production aggregate shall be tested for at least one indicator nutrient for each of the premixes….to confirm that nutrients….are present, in the proper concentrations…."
  - “At the final product stage……each production aggregate shall be tested for vitamins A, C, E, and thiamin.”
SRM 1846 Infant Formula vs. SRM 1849a Infant/Adult Nutritional Formula

- Proximates
- Fatty acids
- Vitamins C, B<sub>2</sub>, B<sub>6</sub>, and niacin
- Other water-soluble vitamins, tocopherols, and vitamins D and K
- Ca, P, Mg, Fe, Zn, Cu, Na, K, I, Cl
- **5 Certified; 38 Reference** (issued 1996)

**bold = certified values**

**normal typeface = reference values**
Certified Values for Vitamins in SRM 1849a Infant/Adult Nutritional Formula

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Mass Fraction (mg/kg)</th>
<th>Manufacturer</th>
<th>NIST LC/abc</th>
<th>Collaborating Laboratories</th>
<th>NIST ID-LC/MS</th>
<th>NIST LCS</th>
<th>NIST LC/FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>784 ± 65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine</td>
<td>12.57 ± 0.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>20.37 ± 0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacinamide</td>
<td>109 ± 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>68.2 ± 1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>13.46 ± 0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic Acid</td>
<td>2.293 ± 0.062</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>1.99 ± 0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline Ion</td>
<td>1090 ± 110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Retinol** 7.68 ± 0.23
**Retinyl Palmitate** 14.30 ± 0.20
**Tocopheryl Acetate** 158 ± 18
**Total Tocopherol** 219 ± 16
**Cholecalciferol** 0.111 ± 0.017
**Phylloquinone** 1.06 ± 0.17

Improved ID-LC-MS/MS for the Determination of B Vitamins

SRM 2384 Baking Chocolate

1: B₆ Pyridoxamine
2: B₃ Niacin
3: B₁ Thiamine
4: B₆ Pyridoxal
5: B₆ Pyridoxine
6: B₃ Niacinamide
7: B₅ Panthothenic acid
8: B₂ Riboflavin

## Infant Formula CRMs Available

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>KRISS (Korea) 108-02-001</th>
<th>NIM (China) GBW(E) 100227(^b)</th>
<th>NIM (China) GBW 10037</th>
<th>NIST (USA) SRM 1849a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issue Date</td>
<td>2014</td>
<td>2010</td>
<td>2012</td>
<td></td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>B(_1)</td>
<td>6.60 ± 0.46</td>
<td>12.57 ± 0.98</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>B(_2)</td>
<td>20.3 ± 0.31</td>
<td>20.2 ± 1.2</td>
<td>20.37 ± 0.52</td>
</tr>
<tr>
<td>Niacin</td>
<td>B(_3)</td>
<td>80.5 ± 3.2</td>
<td>39.8 ± 1.2</td>
<td>65.3 ± 3.7</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>B(_5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>B(_6)</td>
<td>4.71 ± 0.3</td>
<td>13.46 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>Folic Acid</td>
<td>B(_9)</td>
<td>1.58 ± 0.09</td>
<td></td>
<td>2.29 ± 0.06</td>
</tr>
</tbody>
</table>

Current NIST Food-Matrix SRMs

Most of these SRMs have values assigned for 40 to 60 nutrients using higher order methods based in ID-MS methods

1. SRM 3274 Fatty Acids in Botanical Oils
   SRM 3275 Fatty Acids in Fish Oils
   SRM 3276 Carrot Extract in Oil
   SRM 3278 Tocopherols in Edible Oils

2. SRM 2384 Baking Chocolate
3. SRM 2387 Peanut Butter
   SRM 2386 Avocado Powder*
4. SRM 1546a Meat Homogenate
   SRM 1845a Whole Egg Powder
5. SRM 2383a Baby Food Composite
   SRM 3233 Fortified Breakfast Cereal
   SRM 3287 Blueberries
6. SRM 1849a Infant/Adult Nutritional Formula
   SRM 1548a Typical Diet
   SRM 1544 Fatty Acids in a Frozen Diet Composite
   SRM 1549a Whole Milk Powder
7. SRM 1566b Oyster Tissue
   SRM 2385 Slurried Spinach
   SRM 3234 Soy Flour
8. SRM 3290 Dry Cat Food*
9. SRM 1946 Lake Superior Fish Tissue
   SRM 1947 Lake Michigan Fish Tissue
   SRM 1974c Mussel Tissue
   SRM 3252 Protein Powder Drink Mix*

* in preparation
CCQM Key Comparisons for Food Safety (Organic)

Blue: Data used for KCRV; Red: Data excluded from KCRV

CCQM-K103 Melamine in Milk

CCQM-K91 Chloramphenicol in Pig Muscle

CCQM-K85 Malachite Green in Fish
## CRMs for Food Safety

- **Acrylamide in food (CCQM-P109)**
  - KRISS CRM 108-10-003 Acrylamide in Potato Chips
  - BAM ERM-BD272 Crispbread and ERM-BD274 Rusk

- **Malachite Green in fish (CCQM-K85)**
  - LGC produced CRM LGC 1706 Malachite Green Oxalate (for purity)

- **Chloramphenicol in meat (CCQM-K81)**

- **Melamine in Milk (CCQM-K103)**
  - GLHK-11-02 Melamine in Milk (2011)

- **Pesticides in Tea (CCQM-K95)**
Impact: Arsenic Species in Rice

Following CCQM-108 Arsenic Species, total As and Cd in Brown Rice Flour, coordinated by NMIJ, NIST added values for As species to SRM 1568b

Table 2. Certified Mass Fraction Values (Dry-Mass Basis) for Selected Arsenic Species in SRM 1568b

<table>
<thead>
<tr>
<th>Arsenic Species</th>
<th>Mass Fraction (mg/kg, as As)</th>
<th>Coverage Factor, k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylarsinic acid (DMA) (a,b,c)</td>
<td>0.180 ± 0.012</td>
<td>2.0</td>
</tr>
<tr>
<td>Monomethylarsonic acid (MMA) (a,c)</td>
<td>0.0116 ± 0.0035</td>
<td>2.0</td>
</tr>
<tr>
<td>Inorganic arsenic (iAs) (a,c,d)</td>
<td>0.092 ± 0.010</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NMIJ provided measurements to NIST to assign the certified values.

(a) NIST IC/ICP-MS
(b) NIST LC/MS/MS
(c) NMIJ IC/ICP-MS
(d) As (III) and As (V), reported as total inorganic arsenic (iAs)
CRMs for Elemental Speciation

- Arsenic species in rice
  - ERM-BC211 Rice from IRMM
  - SRM 1568b Rice (NIST)
  - NMIJ is producing a rice flour CRM for As species
- Arsenobetaine in fish
  - NMIJ has CRM for arsenobetaine in fish
- Selenomethionine and total Selenium in yeast
  - SELM-1 issued in 2006 by NRC Canada after coordinating a CCQM Pilot Study
  - ERM-BC210 Wheat Flour for Se and Selenomethionine
Impact of CCQM in Food Safety and Nutrition - Summary

- Improvement and expansion of capabilities for more NMIs worldwide
- Development of improved, higher order methods for organic and inorganic nutrients and contaminants in food matrices
- Development of new, improved CRMs to improve and assure the quality of chemical measurements for food safety and nutrition worldwide from numerous NMIs
- Closer collaboration among NMI in chemical metrology research and CRM development
Acknowledgements

- Mike Sargent (LGC Ltd.), Chair of CCQM IAWG
- Lindsey Mackay (NMIA), Chair of the CCQM OAWG
- Members of the CCQM OAWG and IAWG