

CCQM Activities and the Impact in Food Safety and Nutrition

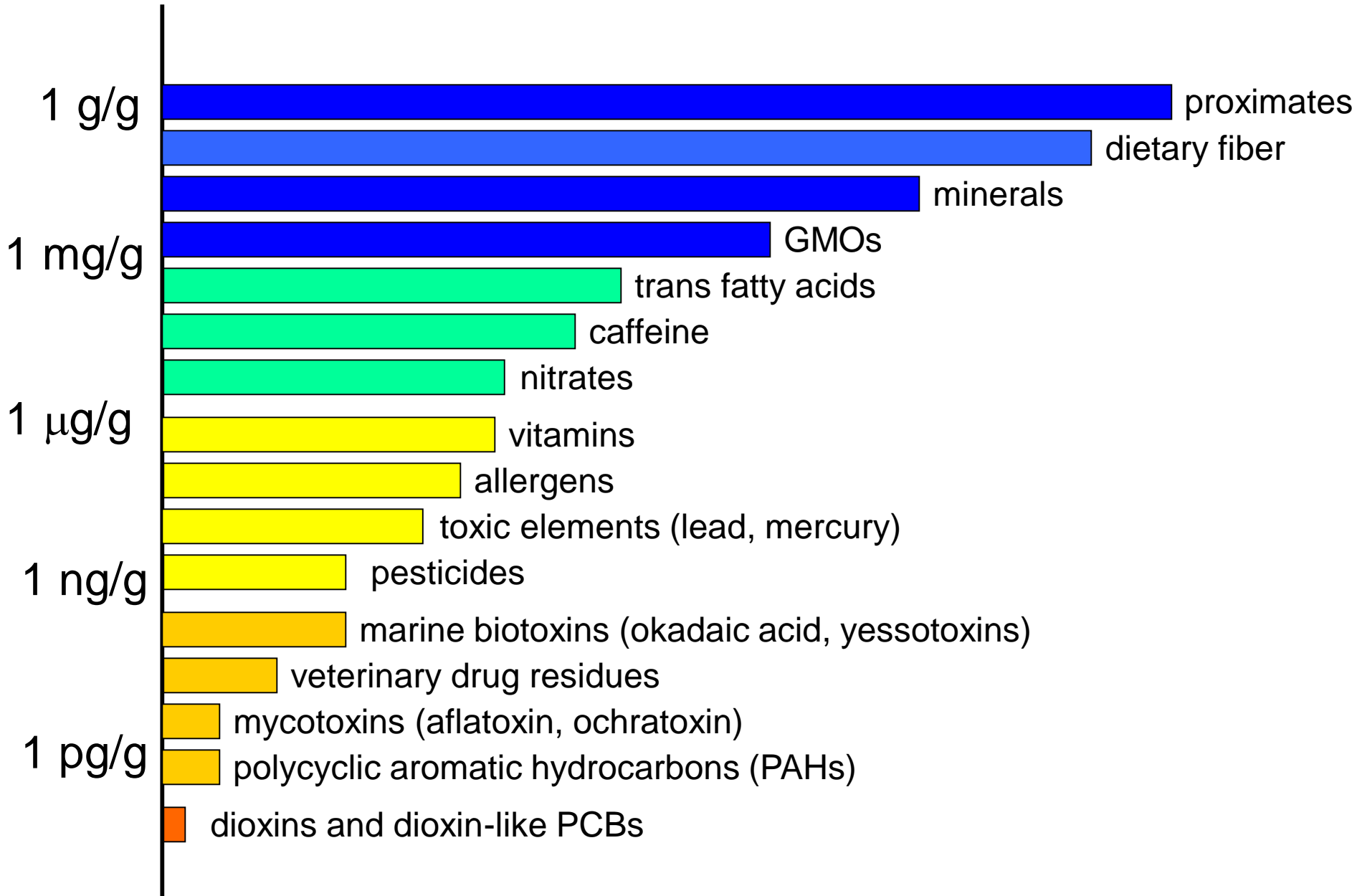
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Chemical Metrology for Food Safety and Nutrition – Prominent Issues

- 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



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

CCQM-K24	Cd in rice (2001)	CCQM-K56	Trace elements in soybean (2007)
CCQM-K30	Pb in wine (2006)	CCQM-K60	Total Se and Se speciation analysis of Se-rich wheat flour (2008)
CCQM-K31	As in fish or shellfish (2002)	CCQM-K75	Toxic metals in algae (2009)
CCQM-K43	Methyl-mercury in salmon fish (2005)	CCQM-K97	Arsenobetaine in fish (2011)
CCQM-K45	Toxic metals in food (tin in tomato paste) (2005)	CCQM-K108	As species, total As, and Cd in brown rice flour (2013)
CCQM-K49	Toxic and essential elements in bovine liver (2006)	CCQM-K125	Iodine and other elements in infant formula

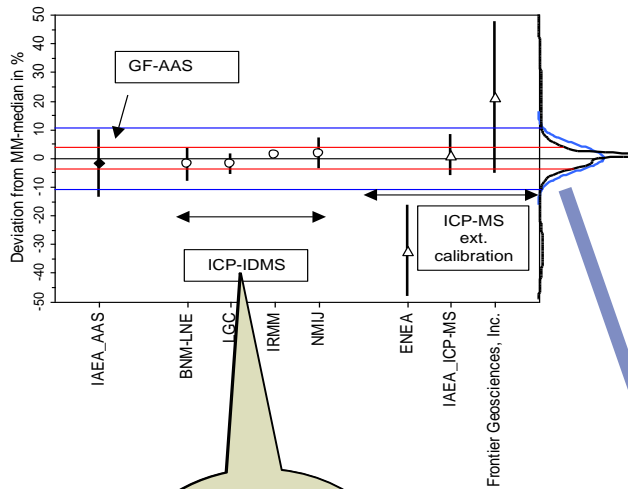
Most CCQM IAWG key comparisons have a parallel pilot study

Additional CCQM IAWG Food Pilot Studies

CCQM-P11	As in shellfish	CCQM-P64	Trace elements in soybean powder
CCQM-P12	Pb in wine	CCQM-P96	As and arsenobetaine content in marine fish
CCQM-P13	Metals in synthetic food digest	CCQM-P97	Cd and Pb in Herbs
CCQM-P39	As, Se, Hg, Pb, methyl-Hg in tuna fish		

CCQM-P39 and IMEP-20: Pb in Tuna Fish

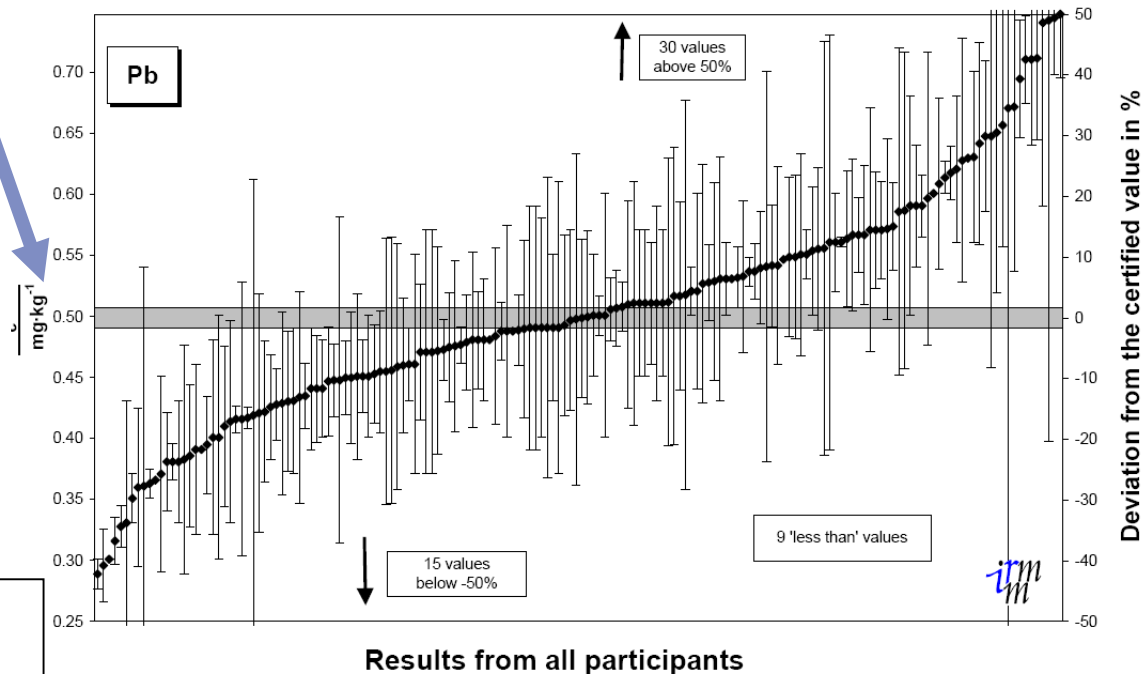
CCQM-P39



Note use of ICP-IDMS and agreement of results

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.



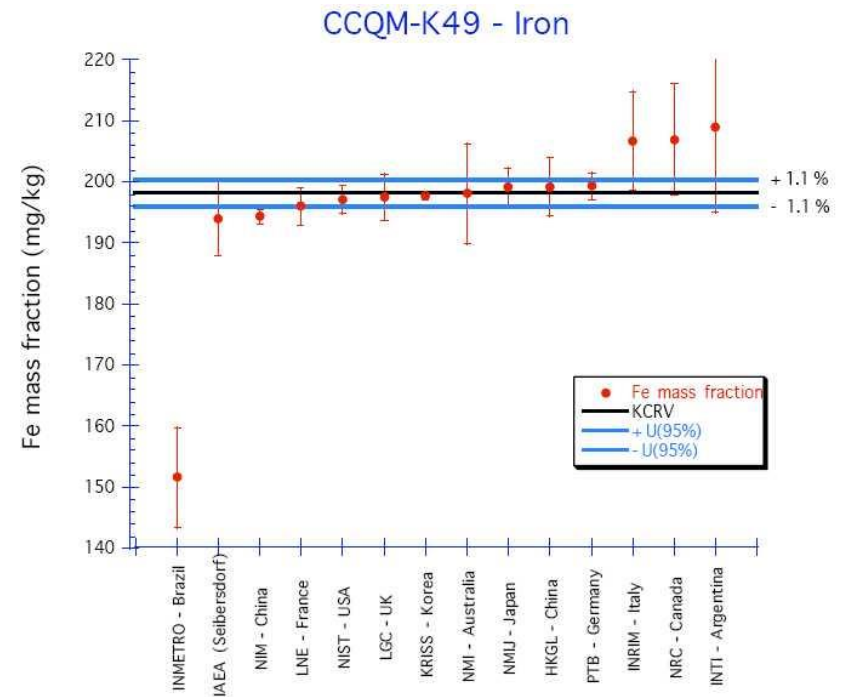
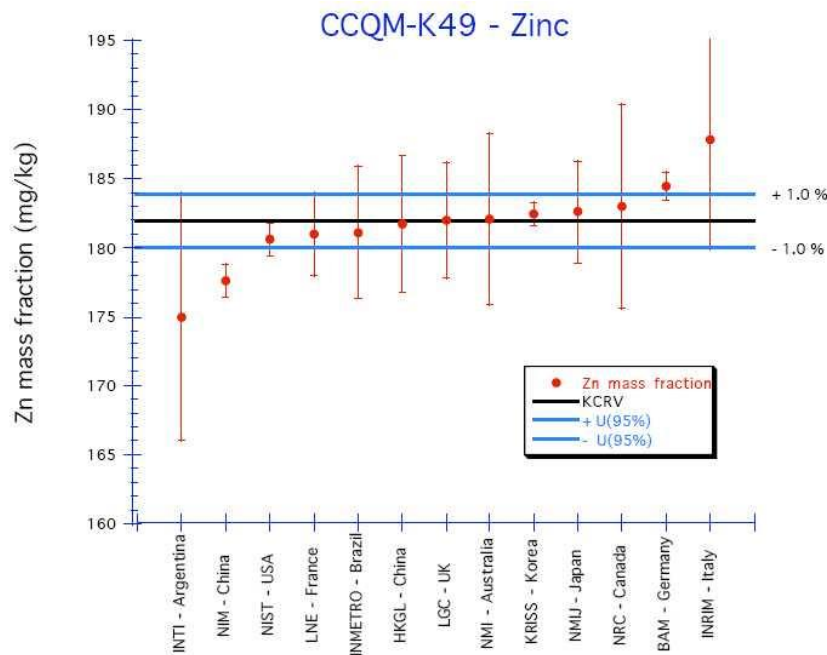
IMEP-20 Trace Elements in Tuna Fish (As, Pb, Hg, MeHg, Se)
 Conducted in 2003
 235 laboratories participated

CCQM-K49: Toxic and Essential Elements in Bovine Liver

- 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

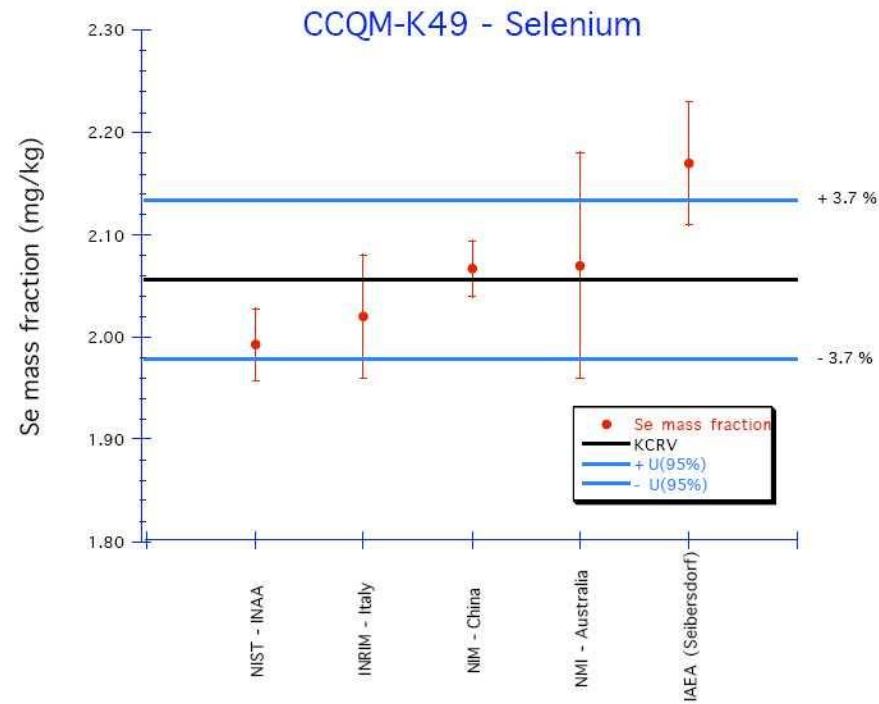
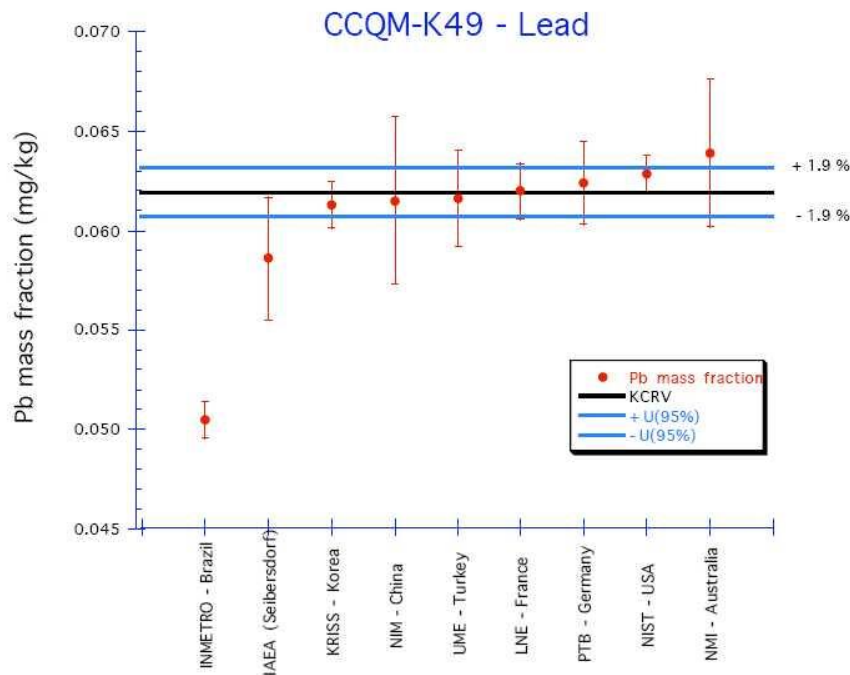
CCQM-K49: Toxic and Essential Elements in Bovine Liver

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



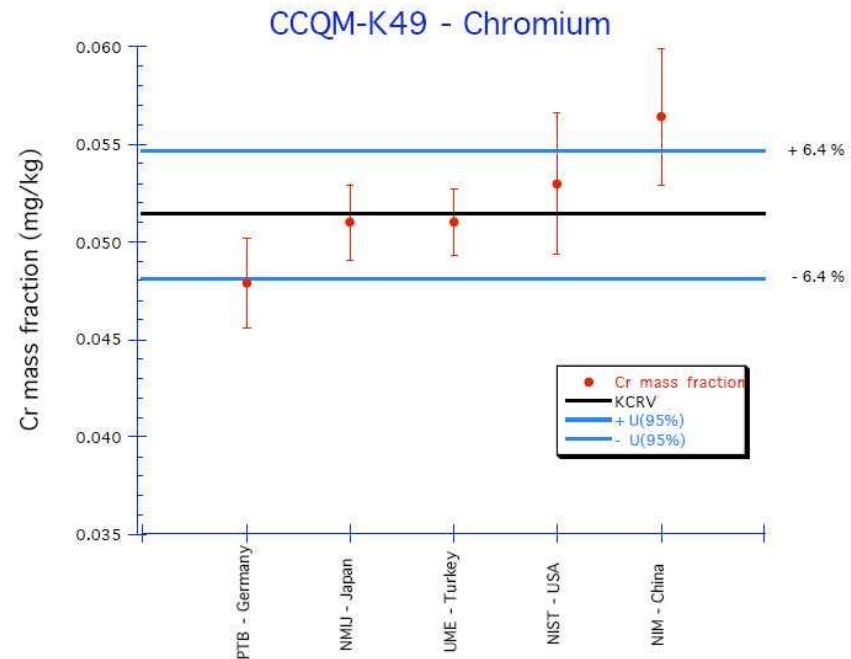
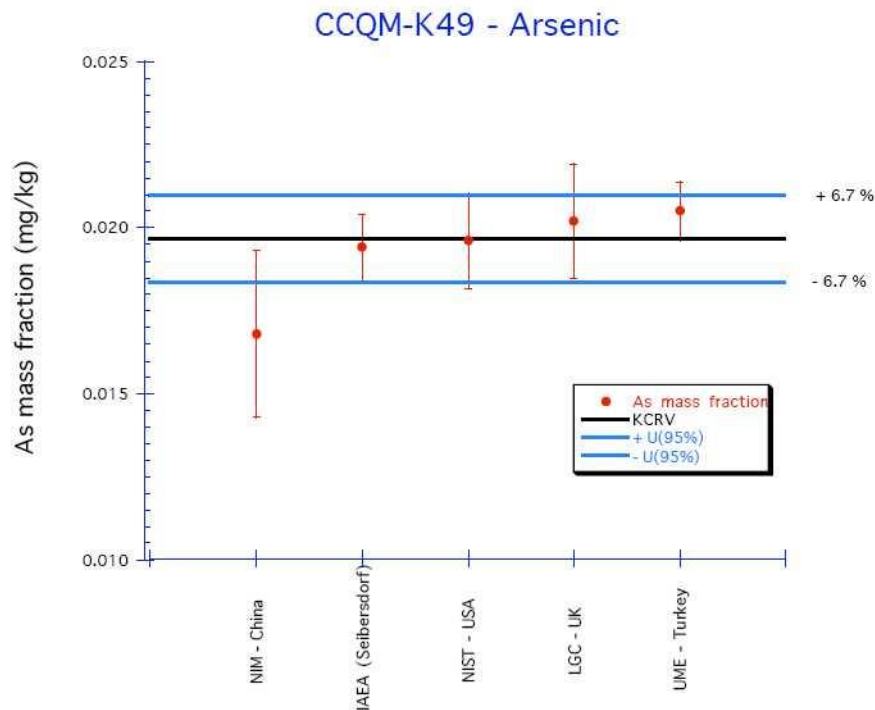
CCQM-K49: Toxic and Essential Elements in Bovine Liver

“Moderate Difficulty” – Mass Fraction 60 $\mu\text{g}/\text{kg}$ to 2000 $\mu\text{g}/\text{kg}$
KCRV Uncertainty (%) = 1.9% and 3.7% respectively



CCQM-K49: Toxic and Essential Elements in Bovine Liver

“Difficult Elements” – Mass Fraction 20 $\mu\text{g}/\text{kg}$ to 50 $\mu\text{g}/\text{kg}$
KCRV Uncertainty (%) = 6.7% and 6.4% respectively

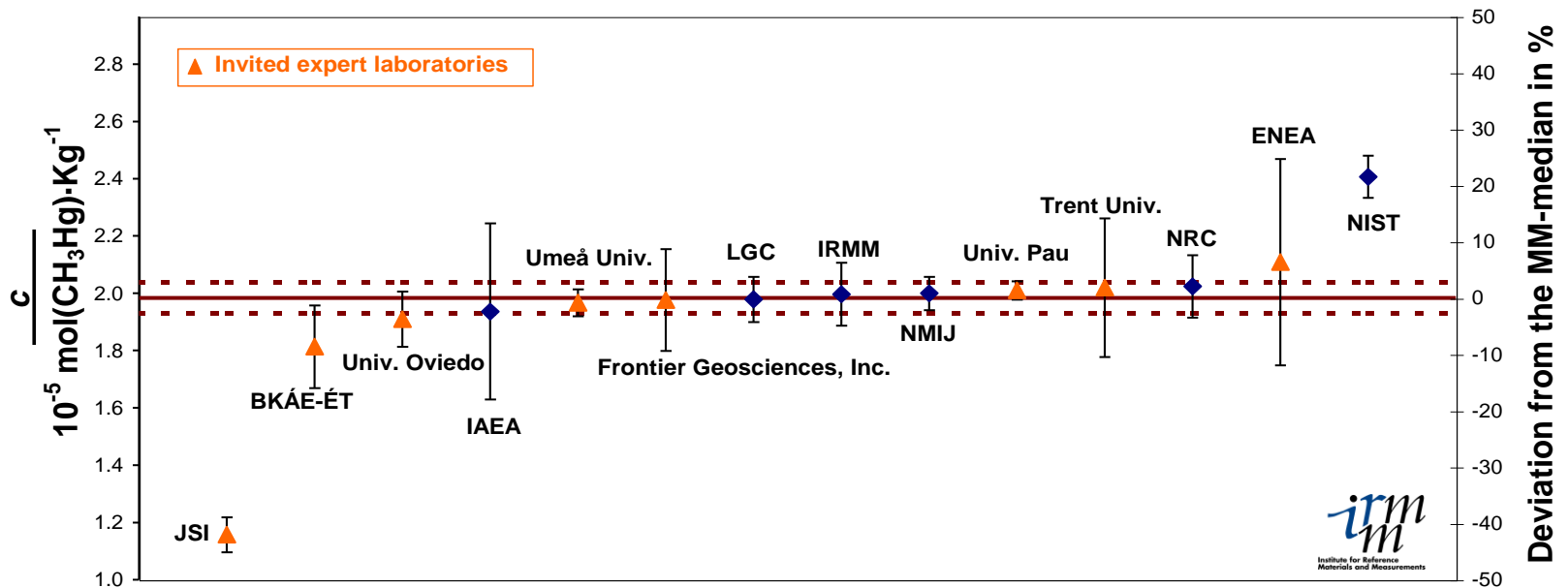


CCQM-P39: MeHg in Tuna Fish

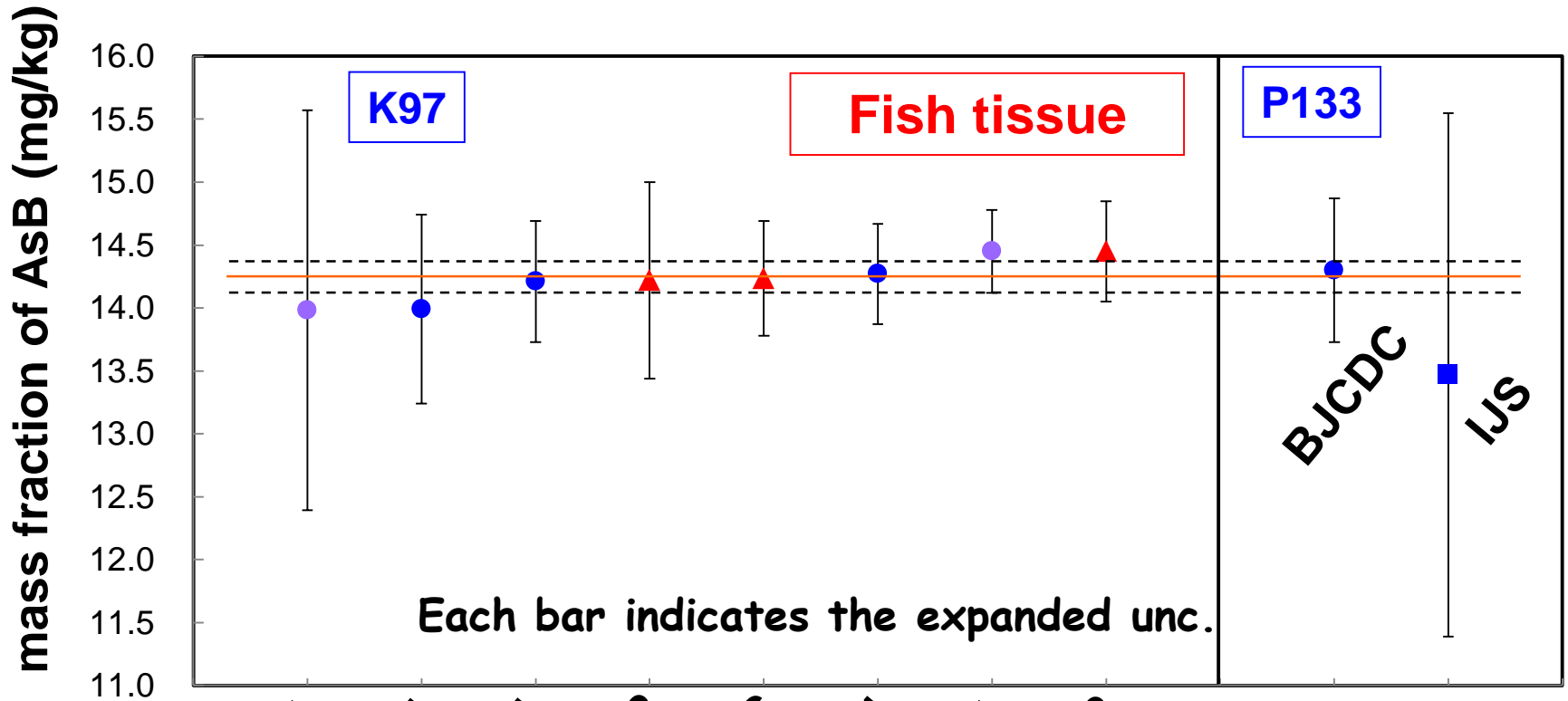
- CCQM IAWG invites expert laboratories to participate in pilot studies of emerging measurement applications

CCQM-P39: methylmercury in tuna fish

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



CCQM-K97 and P133: Arsenobetaine in fish tissue



Shape

- : ICPMS
- ▲: MS
- : AFS

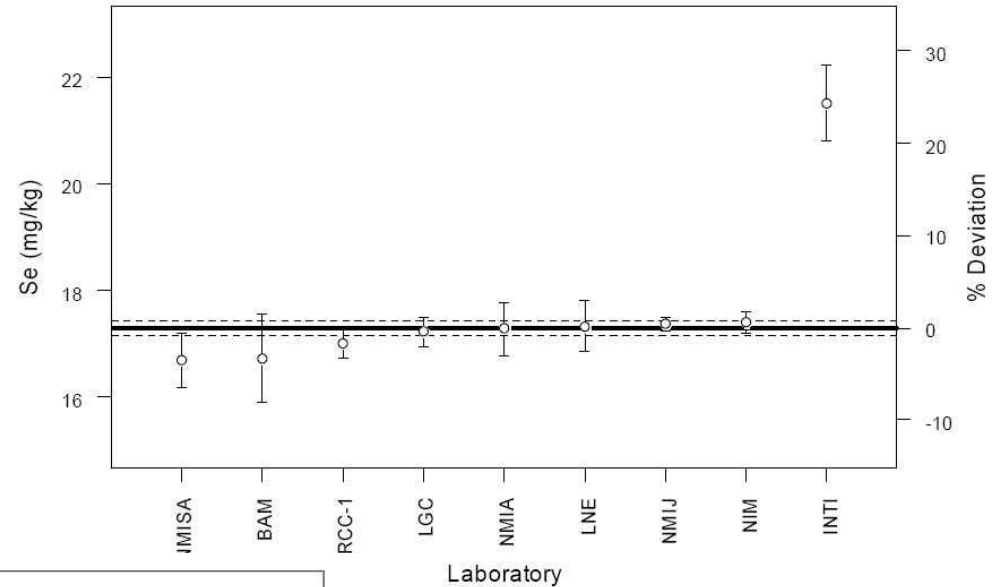


Colour

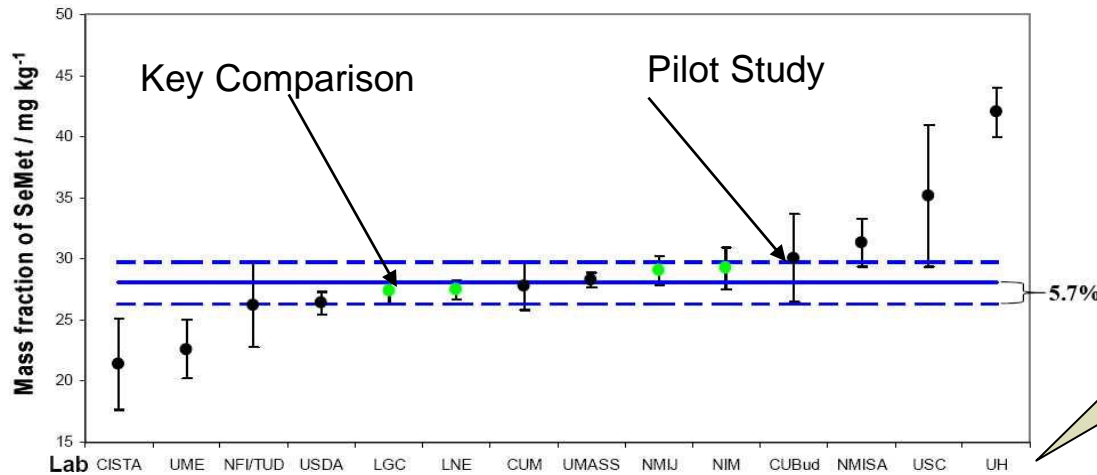
- : LC/ext cal
- : LC/ID
- : LC/std add

Total Selenium and Selenium Methionine in Wheat Flour (CCQM-K60 and P-86.1)

Total selenium



Selenium methionine



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

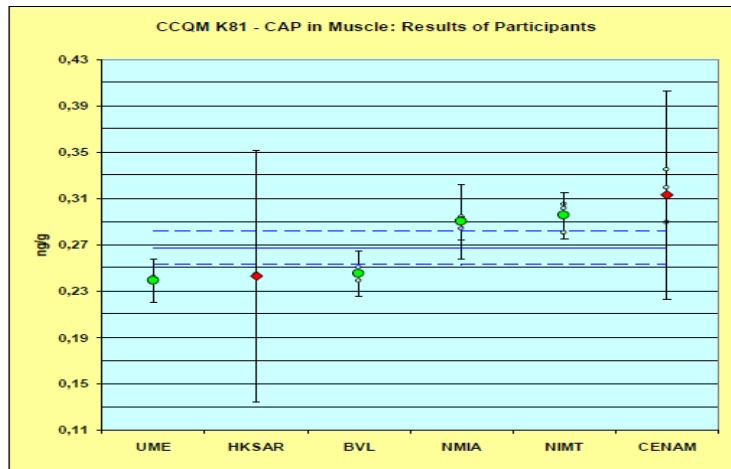
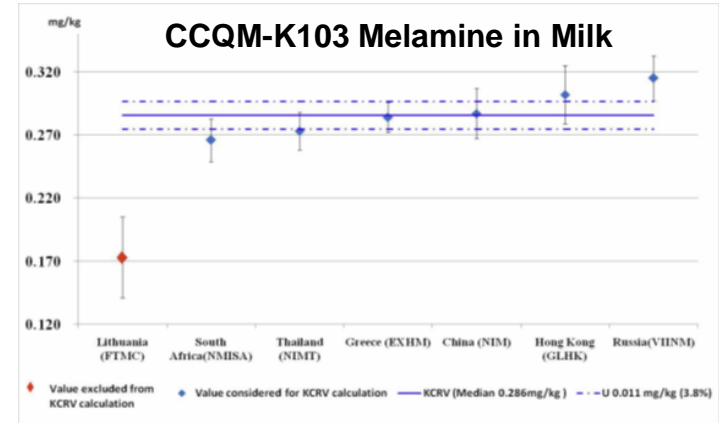
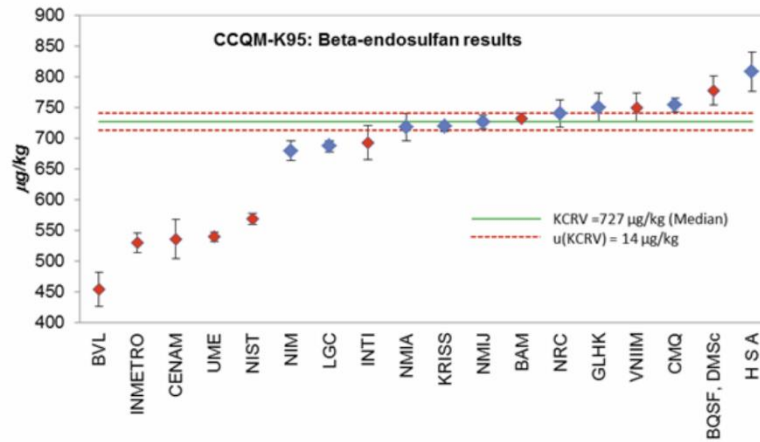
- CCQM-P91 Pesticides in Food: Pyrethroids in Apple Juice (2008)
 - Coordinated by NIMC
 - 9 Participants
- CCQM-P109 Acrylamide in Food (Potato Chips) (2008)
 - Coordinated by KRISS
 - 8 Participants

CCQM Key Comparisons for Food Safety Measurements (Organic)

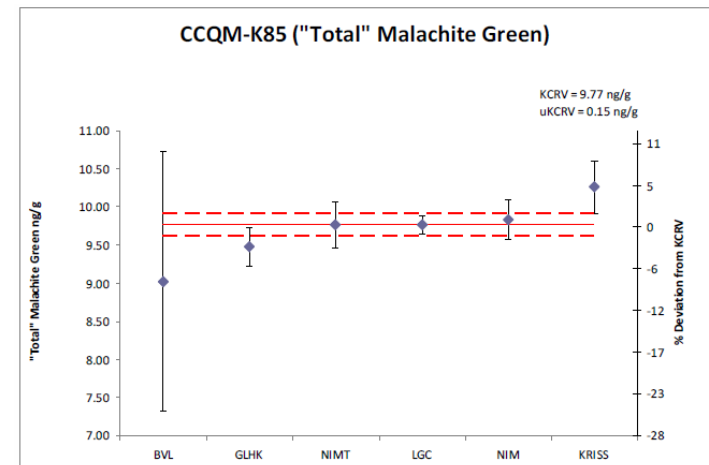
- CCQM-K85 Malachite Green in Fish Tissue (2010)
 - *Malachite green is a banned antifungal*
 - Coordinated by LGC
 - 6 participants
 - CCQM-P88 coordinated by LGC in 2007
- CCQM-K81/P122 Chloramphenicol in Pig Muscle (2010)
 - *Chloramphenicol is a banned antibiotic*
 - Coordinated by PTB and BVL
 - 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

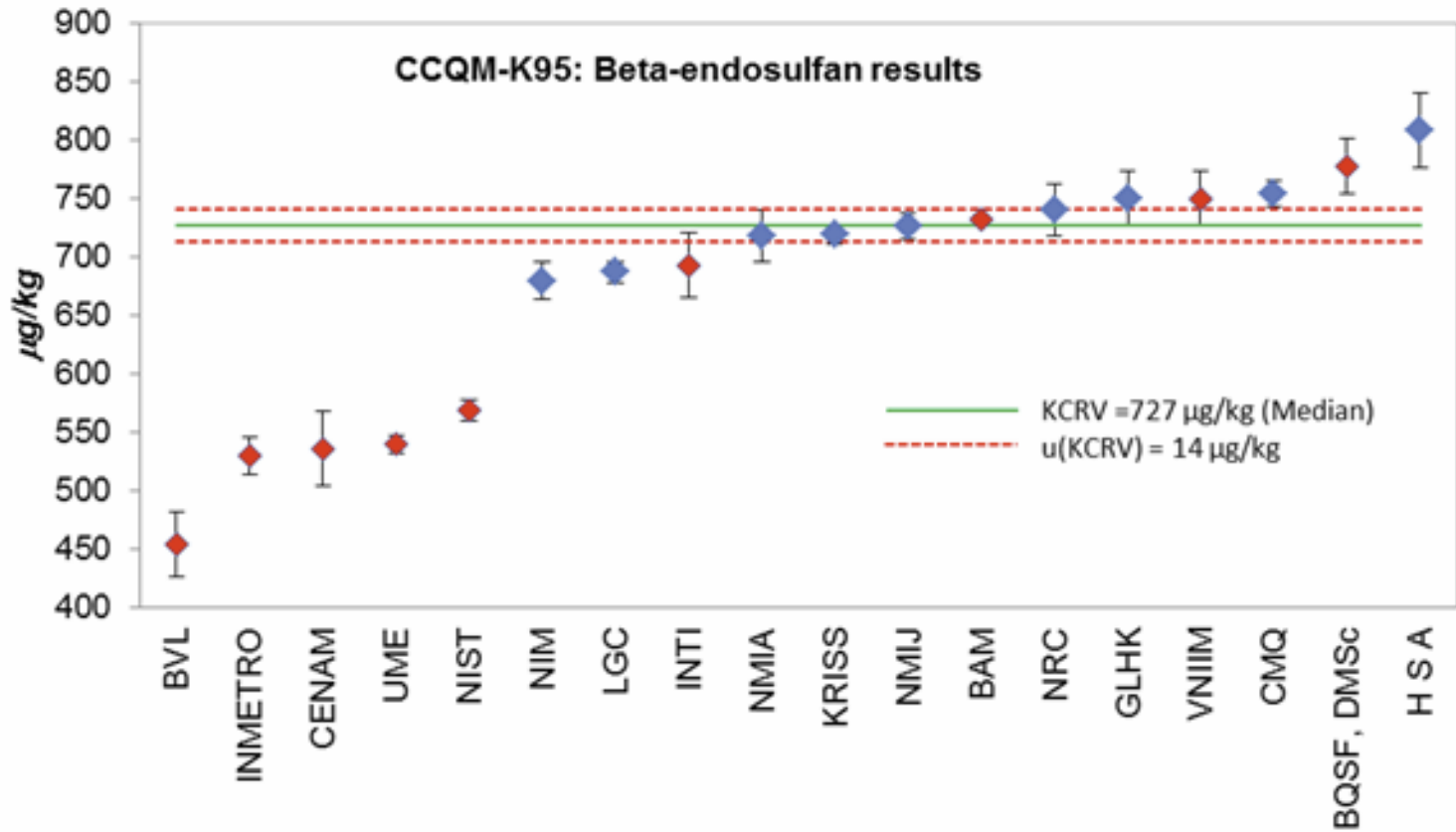


CCQM-K91 Chloramphenicol in Pig Muscle

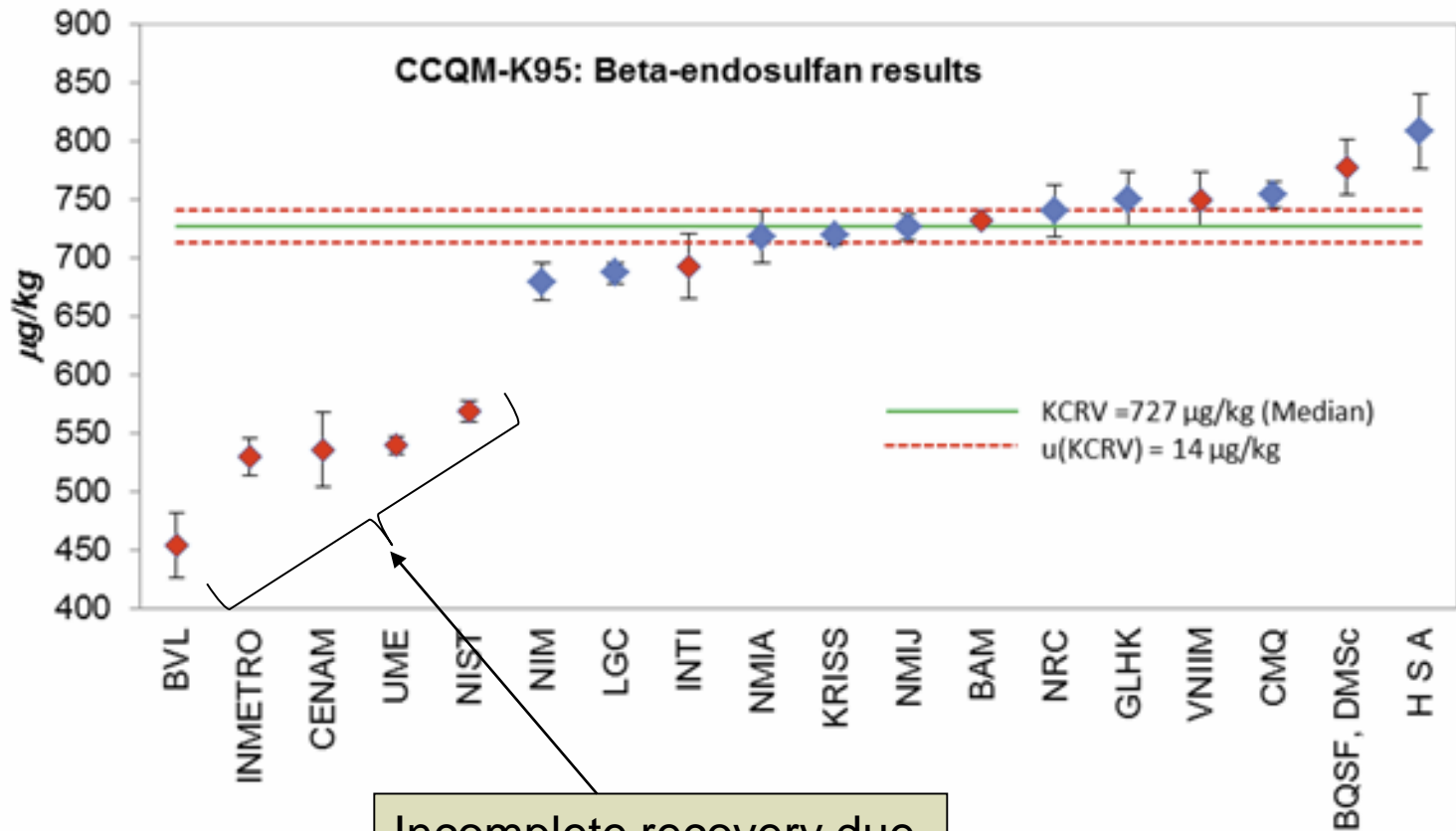


CCQM-K85 Malachite Green in Fish

CCQM-K95 Pesticides in Tea



CCQM-K95 Pesticides in Tea

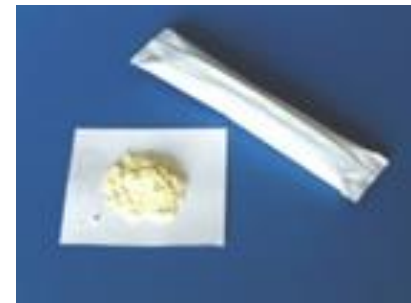


Lessons Learned:

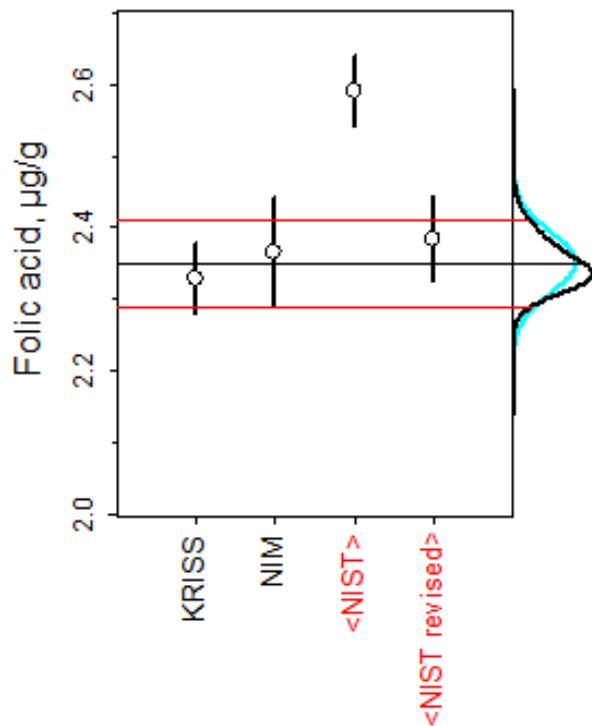
Incomplete recovery due to not pre-wetting tea prior to solvent extraction

CCQM Key Comparisons for Organic Nutrients in Food

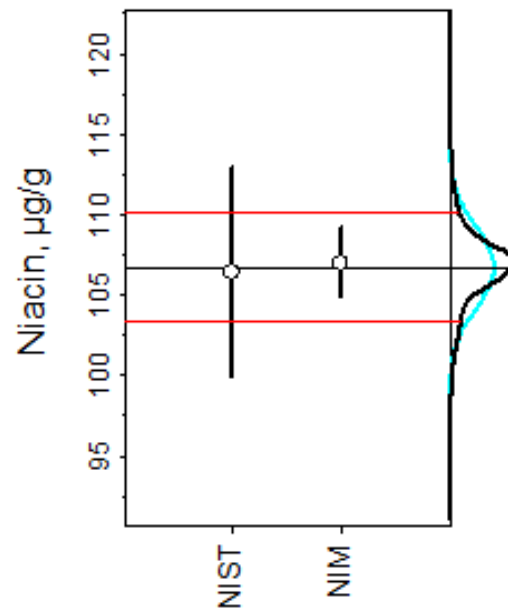
- CCQM-K62 Nutrients in Infant/Adult Formula (2008)
 - Coordinated by NIST
 - 3 Participants (NIST, KRISS, NIMC)
 - Measurements for niacin (vitamin B₃), folic acid (vitamin B₉), and retinol (vitamin A)
 - Follow up to CCQM-P78 (2006)
 - Same three analytes (NIST, KRISS, and NIMC + 2 non-NMIs)



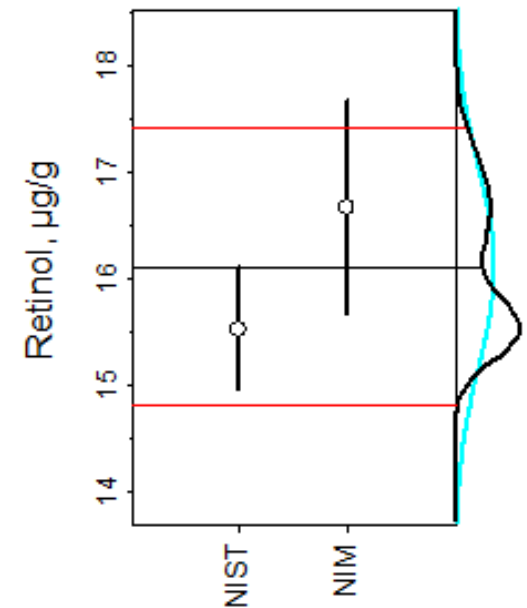
CCQM-K62 Nutrients in Infant Formula



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

Supplement Facts		
Serving Size 1 Capsule		
Amount Per Capsule		% Daily Value
Calories	20	
Calories from Fat	20	
Total Fat	2 g	3%*
Saturated Fat	0.5 g	3%*
Polyunsaturated Fat	1 g	↑
Monounsaturated Fat	0.5 g	↑
Vitamin A	4250 IU	85%
Vitamin D	425 IU	106%
Omega-3 fatty acids	0.5 g	↑

* Percent Daily Values are based on a 2,000 calorie diet.
 † Daily Value not established.

Ingredients: Cod liver oil, gelatin, water, and glycerin.

Nutrition Facts		
Serving Size 1 cup (35g)		
Servings Per Container 10		
Amount Per Serving	Cereal	Cereal with 1/2 cup Skim Milk
Calories	130	170
Calories from Fat	0	0
% Daily Value**		
Total Fat 0g*	0%	0%
Saturated Fat 0g	0%	0%
Cholesterol 0mg	0%	0%
Sodium 200mg	8%	11%
Total Carbohydrate 30mg	10%	12%
Dietary Fiber 4g	16%	16%
Sugars 18g		
Protein 3g		
Vitamin A	25%	25%
Vitamin C	25%	25%
Calcium	0%	15%
Iron	10%	10%

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

	Calories: 2,000	2,500
Total Fat	Less than 65g	80g
Sat Fat	Less than 20g	25g
Cholesterol	Less than 300mg	300mg
Sodium	Less than 2,400mg	2,400mg
Total Carbohydrate	300g	375g
Dietary Fiber	25g	30g

Calories per gram:
 Fat 9 • Carbohydrate 4 • Protein 4

Infant Formula Regulations

- 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.”

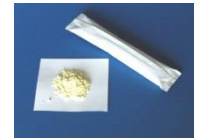
SRM 1846 Infant Formula vs. SRM 1849a Infant/Adult Nutritional Formula

- Proximates
- Fatty acids
- **Vitamins C, B₂, B₆, 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

- Proximates
- **Fatty acids, cholesterol**
- **Vitamins C, B₁, B₂, B₃, B₅, B₆, B₁₂, folic acid, biotin, choline, carnitine**
- **Vitamins A, D₃, E, and K₁**
- **Ca, P, Mg, Fe, Zn, Cu, Na, K, Mn, Cr, Mo, Se, I, Cl**
- *myo*-Inositol, 5-MTHF
- Amino acids
- Nucleotides
- **46 Certified; 46 Reference** (issued 2011)



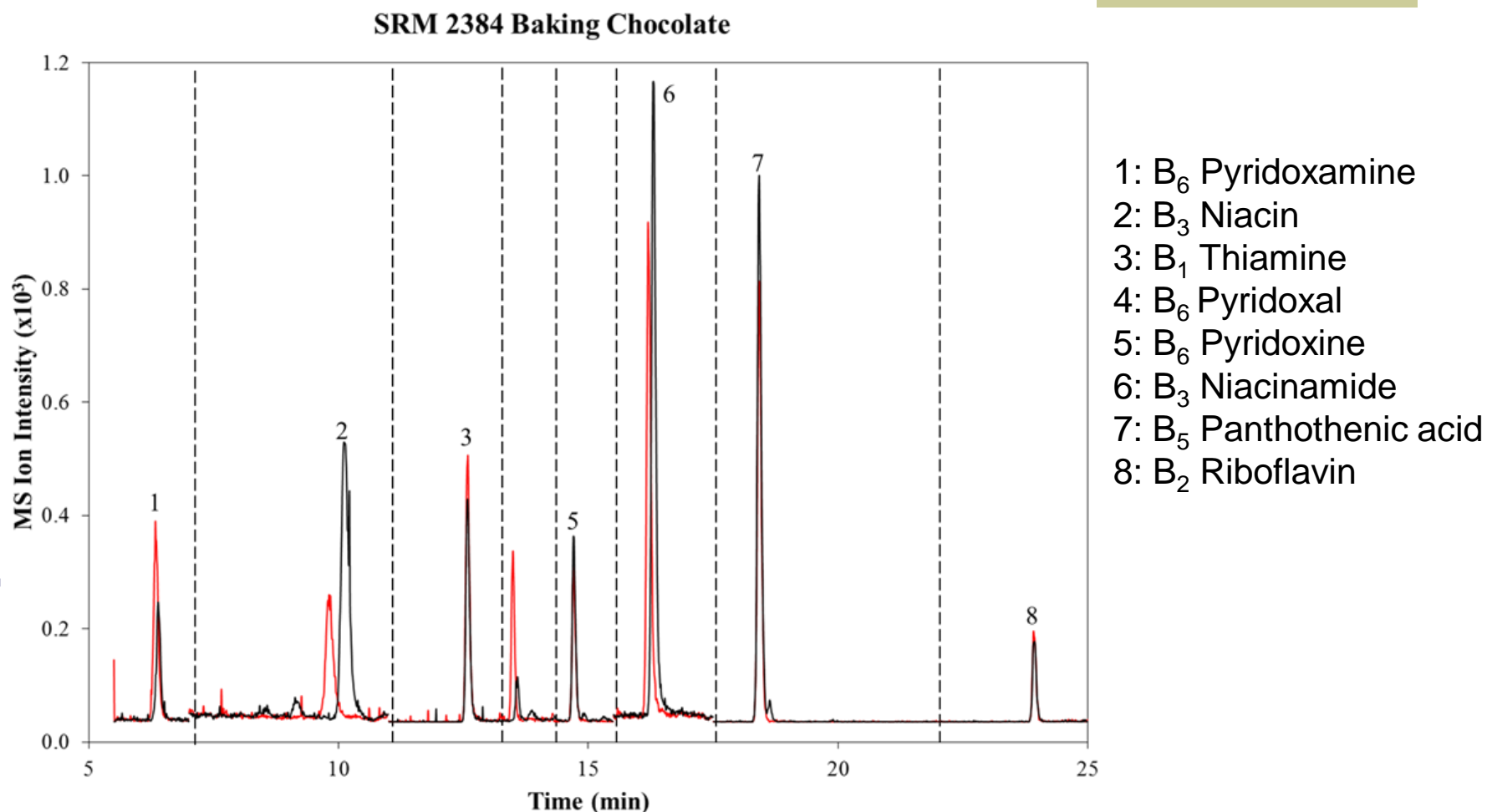
Certified Values for Vitamins in SRM 1849a Infant/Adult Nutritional Formula

	Mass Fraction (mg/kg)	Mass Fraction (mg/kg)	
Ascorbic Acid 	784 ± 65	Retinol 	7.68 ± 0.23
Thiamine 	12.57 ± 0.98	Retinyl Palmitate 	14.30 ± 0.20
Riboflavin 	20.37 ± 0.52	Tocopheryl Acetate 	158 ± 18
Niacinamide 	109 ± 10	Total Tocopherol 	219 ± 16
Pantothenic Acid 	68.2 ± 1.9	Cholecalciferol 	0.111 ± 0.017
Pyridoxine 	13.46 ± 0.93	Phylloquinone 	1.06 ± 0.17
Folic Acid 	2.293 ± 0.062		
Biotin 	1.99 ± 0.13		
Choline Ion 	1090 ± 110		

-  NIST LC/abc
-  Collaborating Laboratories
-  Manufacturer
-  NIST ID-LC/MS
-  NIST LC/FL
-  NIST ID-LC/MS/MS

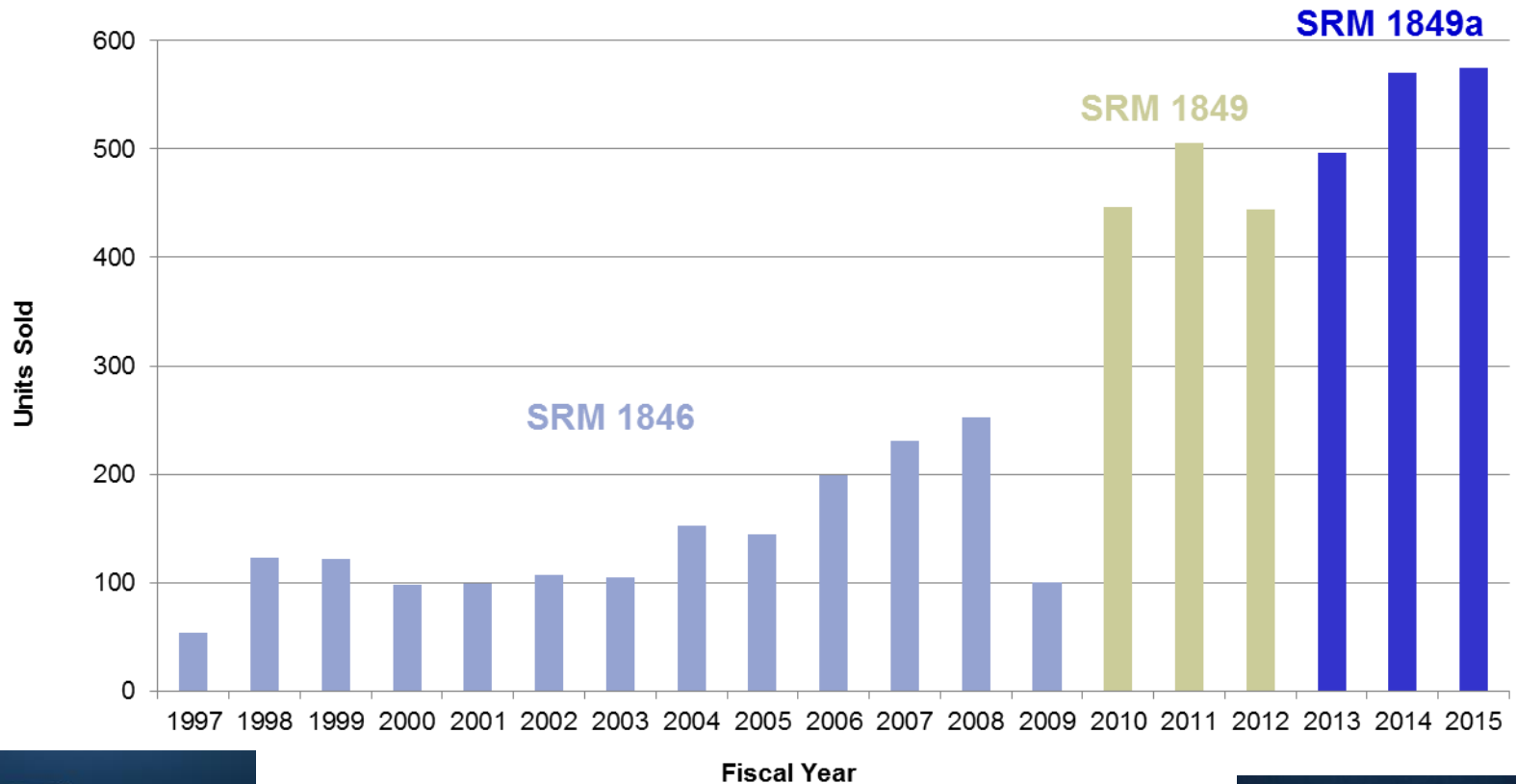
K.E. Sharpless *et al.*, *J. AOAC Int.* 93:1262-1274 (2010)
K.W. Phinney *et al.*, *Anal. Chem.* 83:92-98 (2011)

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



Melissa M. Phillips, Anal. Bioanal. Chem. 407 (11):2965-2974 (2015)

Distribution of Infant Formula SRMs

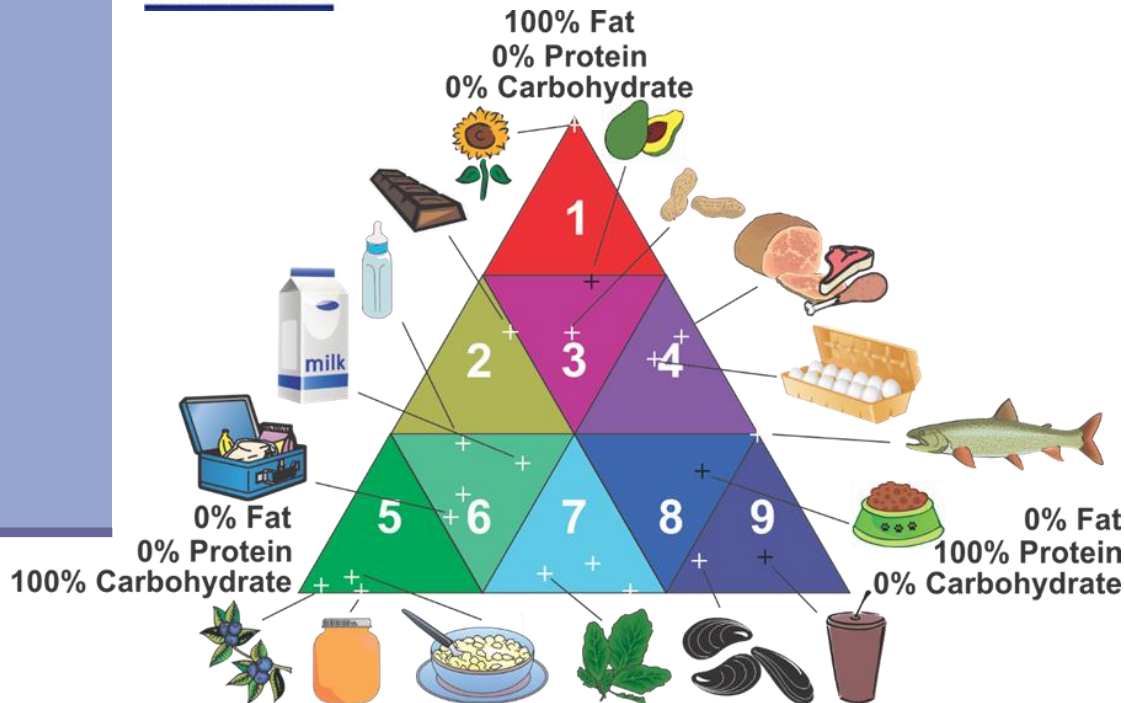


Infant Formula CRMs Available

		Infant Formula CRMs (Certified Mass Fraction in mg/kg)			
Vitamins		KRISS (Korea) 108-02-001	NIM (China) GBW(E) 100227 ^b	NIM (China) GBW 10037	NIST (USA) SRM 1849a
Issue Date		2014		2010	2012
Thiamine HCl	B ₁		6.60 ± 0.46		12.57 ± 0.98
Riboflavin	B ₂	20.3 ± 0.31	20.2 ± 1.2		20.37 ± 0.52
Niacin	B ₃	80.5 ± 3.2	39.8 ± 1.2	65.3 ± 3.7	109 ± 10
Pantothenic Acid	B ₅				
Pyridoxine HCl	B ₆		4.71 ± 0.3		13.46 ± 0.93
Folic Acid	B ₉	1.58 ± 0.09			2.29 ± 0.06

Both KRISS and NIMC developed ID-LC-MS methods and CRMs for vitamins in infant formula: KRISS - Shin *et al.*, **Food Chemistry** 138:1109-1115 (2013) and NIMC - Ting *et al.*, **J. Food Composition and Analysis**, 23:367-372 (2010)

Current NIST Food-Matrix SRMs



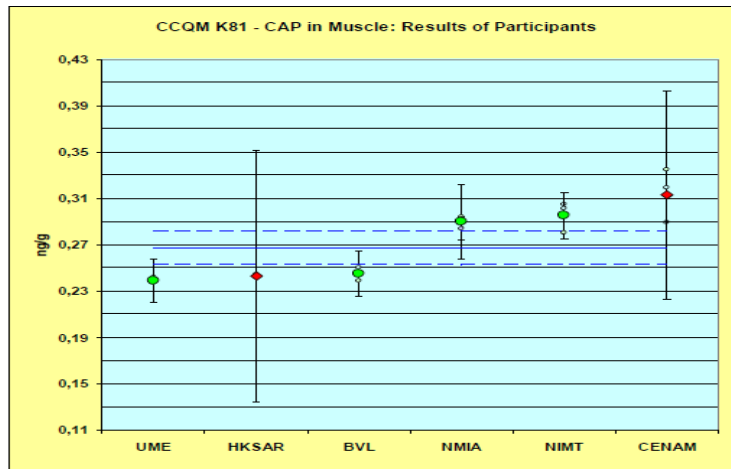
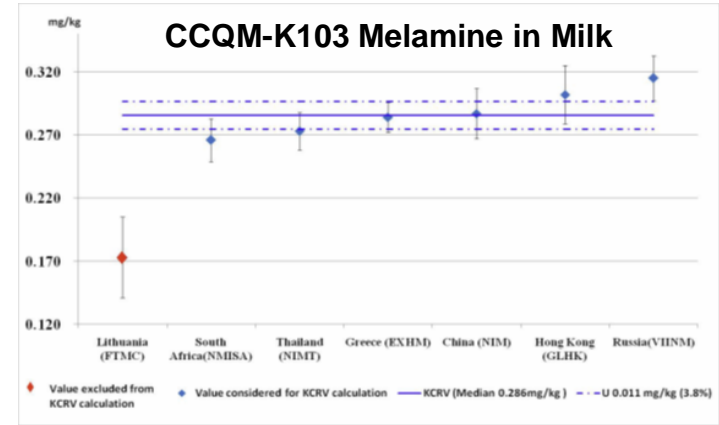
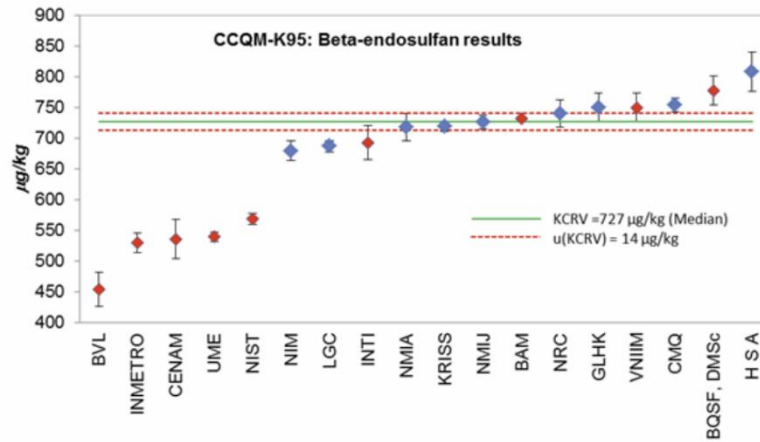
- 1 **SRM 3274 Fatty Acids in Botanical Oils**
SRM 3275 Fatty Acids in Fish Oils
SRM 3276 Carrot Extract in Oil
SRM 3278 Tocopherols in Edible Oils
- 2 **SRM 2384 Baking Chocolate**
- 3 **SRM 2387 Peanut Butter**
SRM 2386 Avocado Powder*
- 4 **SRM 1546a Meat Homogenate**
SRM 1845a Whole Egg Powder
- 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*

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

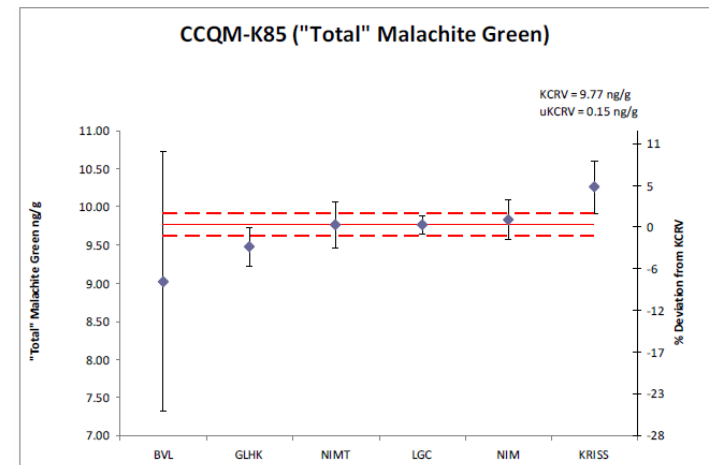
* *in preparation*

CCQM Key Comparisons for Food Safety (Organic)

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



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
 - B. Kim *et al.*, **Anal. Bioanal. Chem.** 398(2):1035-1042 (2010)
 - BAM ERM-BD272 Crispbread and ERM-BD274 Rusk
 - Koch *et al.*, **J. Agric. Food Chem.** 57(18):8202-07 (2009)
- Malachite Green in fish (**CCQM-K85**)
 - LGC produced CRM LGC 1706 Malachite Green Oxalate (for purity)
- Chloramphenicol in meat (**CCQM-K81**)
 - ERM-BB130 Chloramphenicol in Pork Meat (IRMM) (2010)
- Melamine in Milk (**CCQM-K103**)
 - GLHK-11-02 Melamine in Milk (2011)
 - P.Y. Hon *et al.*, **J. Chromatogr.** 121839):6907-6913 (2011)
- Pesticides in Tea (**CCQM-K95**)
 - GLHK-11-03 Organochlorine Pesticides in Tea (2015)
 - D.W.M. Sin *et al.*, **Anal. Bioanal. Chem.** 2015

Impact: Arsenic Species in Rice



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1568b

Rice Flour

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining elements in rice flour and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. This SRM is rice flour prepared from a single lot of river rice by a commercial manufacturer. A unit of SRM 1568b consists of a single bottle containing approximately 50 g of material sealed inside an aluminized pouch.

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

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

^(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)

NMIJ provided measurements to NIST to assign the certified values

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 fo 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

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