



Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)
Meeting at
HILTON WASHINGTON DC/ROCKVILLE HOTEL &
EXECUTIVE MEETING CTR
Rockville, MD

Stakeholder Meeting
DRAFT MEETING PROCEEDINGS
June 18 - 22, 2012

MEETING ATTENDEES:

John Austad	Covance	Adrienne McMahon	Pfizer Nutrition Ireland
Lei Bao	AQSIQ	William Mindak	FDA - CFSAN
Brad Barrett	AB Sciex	Anita R. Mishra	AOAC INTERNATIONAL
Michael Benoit	Perrigo	Mardi Mountford	International Formula Council (IFC)
Sneh Bhandari	Silliker Inc.	Li Na	Chinese Academy of Inspection & Quarantine Comprehensive
Charles Black	Abbott Nutrition	Angela Nugroho	Eurofins Scientific
Christopher Blake	Nestec Ltd. - Nestlé Research Center	Kassandra Oates	Thermo Fisher Scientific
Anita Burgher	Pfizer Nutrition	Edwin Phifer	FDA
Nick Cellar	Abbott Nutrition	Shang-Jing Pan (Jean)	Abbott Nutrition
Esther Campos-Gimenez	Nestlé Research Center	Melissa Meaney Phillips	NIST
Scott Christiansen	Perrigo	Shay Phillips	Thermo-Johnson
Qinhua Chu	Shanghai Entry-exit Inspection & Quarantine Bureau	Dan Quinn	Thermo Fisher Scientific
Fred Claussen	EPL Bio-Analytical Services, Inc.	Jeanne Rader	FDA
Kevin Cockell	Health Canada	Guenther Raffler	Central Laboratories Friedrichsdorf GmbH
Neal Craft	Craft Technologies, Inc.	Robert Rankin	International Formula Council/IFC
Jonathan DeVries	Medallion Laboratories/General Mills	Mitzi Rettinger	Cerilliant Corporation
Aurelie DuBois	International Dairy Federation (IDF)	Catherine Rimmer	NIST
Jaap Evers	Fonterra Cooperative/International Dairy Federation (IDF)	Shane Rutherford	Massey University – Riddet Institute
Nana Farkye	California Polytechnic State University	Maria Ines R.M. Santoro	University of Sao Paulo
Ping Feng	Pfizer Nutrition	Karen Schimpf	Abbott Nutrition
Fengxia Zhang	Chinese Academy of Inspection & Quarantine Comprehensive	Daniel Schmitz	Abbott Nutrition
Ton Gerssen	NEN/International Organization for Standardization (ISO)	Kathy Sharpless	NIST
Sarwar Gilani	Health Consultant	Matthew Sliva	Perrigo
Brendon Gill	Fonterra Cooperative	Darryl Sullivan	Covance Laboratories
Qian Graves	FDA - CFSAN	Joseph J. Thompson	Abbott Laboratories
Keith Griswold	Pepsico	Marina Torres-Rodriguez	LATU
Dehua Guo	Shanghai Entry-exit Inspection & Quarantine Bureau	Victor Vandell	Biotage
Jim Harnly	USDA	Wayne Wargo	Abbott Nutrition
Michael Hauer	Thermo Fisher Scientific	Norman White	Abbott Nutrition
Harvey Indyk	Fonterra Cooperative	Wayne Wolf	USDA (Retired)
Ramesh Jampala	Eurofins Analytical Services India Pvt. Ltd.	Laura Wood	NIST
Greg Jaudzems	Nestle	David Woollard	New Zealand Lab Services Ltd.
Guifeng Jiang	Thermo Fisher Scientific	Chao Wu	Hilmar Ingredients
George Joseph	AsureQuality	Deng Xiaojun	Shanghai Entry-exit Inspection & Quarantine Bureau
Fergus Keenan	Thermo Fisher Scientific	Guo Xindong	Guangzhou Quality and Test Institute
Khalil Kerdahi	FDA, DOS	Jinchuan Yang	Waters Corp.
Estela Kneeteman	National Institute of Industrial Technology	Shan Yi	National Dairy Test Center
Erik Konings	Nestlé Research Center	Tracey Yi	Pfizer Nutrition
Mihir Kumar Kundu	FSSAI	Chen Yiguang	Guangzhou Quality & Test Institute
Petra Lutter	Nestlé Research Center	Ren Yiping	Zhejiang Provincial CDC
		Qiu mei Zhang	National Dairy Test Centre
		Yu Zhang	Zhejiang University
		Linda Zhao	Abbott Nutrition
		Yue Zhenfeng	Shenzhen Entry-exit Inspection & Quarantine Bureau

AOAC STAFF:

Delia Boyd	Standards Development
Jim Bradford	Executive Director
Lauren Chelf	Director, Meetings & Expositions
Scott Coates	Chief Scientific Officer
Arlene Fox	Sr. Director, Proficiency Testing
Dawn Frazier	Executive, Scientific Business Development
May Jones	Program Manager, Association Activities
Liz Cribbin Korte	Program Manager, Association Activities
Nora Marshall	Research Institute/Standards Development
Alicia Meiklejohn	Executive Office, Strategic Business Development
Krystyna McIver	Executive, Scientific Business Development
Deborah McKenzie	Sr. Director, Standards Development/Research Institute
Tien Milor	Managing Editor, ILM
Anita Mishra	Executive for Scientific Business Development
LaKia Phillips	Standards Development/Research Institute
Robert Rathbone	Sr. Director, Publications
Gar Riegler	Sr. Director, HR, Administration & Administration
Joyce Schumacher	Chief Financial Officer

DAY I – Monday, June 18, 2012

I. WELCOME AND INTRODUCTION TO STAKEHOLDER MEETING

Darryl Sullivan (SPIFAN Chair) called the meeting to order and welcomed the participants to stakeholder panel for infant formula and adult nutritionals (SPIFAN) Whey Protein: Casein Ratio (WPC) meeting. All the participants introduced themselves beginning with the voting panel. Sullivan provided the stakeholder panel background information on the WPC project along with an overview of the standards development and general SPIFAN processes which included working groups and expert review panels (ERP).

II. PRESENTATION ON GLOBAL REGULATORY ENVIRONMENT IN CHINA FOR WPC

Xiao Jing (Department of Food Safety Standard and Inspection, National Institute of Nutrition and Food Safety; China CDC, Secretary of National Food Safety Standard Review Committee) presented on the global regulatory environment in China on WPC as well as understanding the recent changes. China has a national standard for milk-based infant formula that requires the ratio of whey and casein protein to be 60:40 percent. The National Food Safety Standard Review Committee collected methods for WPC and took action on this issue because it was determined that the certain products did not meet the 60:40 percent ratios required in China. The China GB method 10765-2010 also states that whey protein should not exceed 60%. China has proposed a new GB method that would be in compliance with government requirements; the methods match with primary and product standards and are fit for emergency.

III. TECHNICAL PERSPECTIVE: GENERAL OVERVIEW

The WPC Working Group Co-Chairs discussed the technical perspective and challenges regarding analytical methods for infant formula and adult nutritionals for WPC. Regulatory requirements and fitness for purpose were also discussed. Discussions included different methods giving different results, the underestimation of percent of whey protein, and providing methods to support current and future standards. A summary of the May 3, 2012 and May 23-24, 2012 preparatory meetings was provided including available technology and methods as well as strengths and weaknesses.

DAY 2 – June 21, 2012

VII. DISCUSS TECHNICAL STANDARD METHOD PERFORMANCE REQUIREMENTS (SMPRs)

Darryl Sullivan provided an overview and guidance on developing SMPRs; how they have evolved, identifying the different types and uses for SMPRs. The Working Group Co-Chairs led discussions on the rationale for disputes, conducted a preliminary review of methods, and identified additional methods for consideration.

The working group reached general agreement after an extensive discussion on the SMPR and the following applicability statement:

Determination of total whey proteins, including hydrolyzed forms, as a percent of protein content¹. To be applicable to milk based infant formula products (including those from bovine milk and, if possible, milk of other species and products containing hydrolyzed casein). ¹Protein content as defined by appropriate regulatory agencies.

The working group reached general agreement on the method performance requirements and consensus on the SMPR for WPC:

Analytical range	20 - 100*	
Limit of quantitation (LOQ)	≤ 10*	
Repeatability (RSD)	20 - 100*	≤ 3%
Recovery	95% to 105% of theoretical	
Reproducibility (RSD)	20 - 100*	≤6%

* g /100 g protein (unless otherwise specified in regulation).

The Working Group Co-Chairs also reviewed several methods submitted to the working group. The working group endorsed methods WPC-17/18/26/28 be advanced. Below are the comments on each method.

METHOD	TITLE	COMMENTS
WPC-16	Part I: Determination of α-lactalbumin, β-lactoglobulin A and β-lactoglobulin B in whey protein powder, colostrum, raw milk, yogurt, UHT milk and infant formula by capillary electrophoresis	Will not capture hydrolyzed whey; good for intact whey protein. Will not capture denatured protein.
WPC-17	Determination of bovine α-lactalbumin in baby food and infant formula LC-MS/MS Coupled with Stable Isotope Dilution Method	May not capture hydrolyzed whey; good method for some whey proteins; needs development for SMPR
WPC-18	Determination of Whey Protein in Milk-Based Infant Formula	Variability of the raw materials to determine performance. Incorporate free amino acids. Can we find or determine a standard whey protein?
WPC-26	Determination of Whey Protein Content in Milk Based Infant Formula Finished Products Using Amino Acids Calculation Method	Variability of the raw materials to determine performance. Can we find or determine a standard whey protein?

WPC-28	Development and Validation of a Method for the Quantification of Milk Proteins in Food Products Based on Liquid Chromatography with Mass Spectrometric Detection	May not capture hydrolyzed whey; good method for some whey proteins; needs development for SMPR
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DAY 3: June 22, 2012

VIII. STAKEHOLDER PANEL: FINAL SMPR DOCUMENTS AND METHOD AUTHOR PRESENTATIONS

Sullivan welcomed & introduced the stakeholder panel meeting including the voting members. The panel reviewed and provided recommendation and subsequent approval for the WPC SMPR developed by the working group.

❖ *There was a motion to accept the WPC SMPR:*

Sharpless moved/Woollard seconded to approve the SMPR for WPC as amended. The motion did not pass.

8 – Approve
 11 – Reject
 4 – Abstain

A discussion ensued with the reason for no votes as:

- Free amino acids (5) / Hydrolyzed forms (1)
- Whey protein definition needed (3)

After a thorough discussion on the topics in question, the panel was polled again for the above stated concerns, the motion carried as follows:

❖ *Motion to approve the applicability as per version 1.1 and define whey protein (include definition for whey protein)*

18 – Approve
 5 – Reject
 2 – Abstain

❖ *Motion to accept the SMPR as amended – final draft*

17 – Approve
 5 – Reject
 2 – Abstain

EXPERT REVIEW PANEL (ERP):

The ERP was convened to review all methods submitted that fulfilled the requirements to be granted First Action *Official Methods*SM status. In addition, the ERP considered additional information/documentation submitted for candidate dispute resolution methods with First Action *Official Methods*SM status along with the rationale for the disputes. The following nutrients were among those that were reviewed:

- Folate
- Inositol
- Nucleotides/sides
- Vitamin E
- Whey protein: casein ratio

Review of WPC Methods

WPC-17

<ul style="list-style-type: none">▪ Not all SMPR requirements are met at this time▪ Specific sequences▪ More data need to be collected▪ Method has potential	<ul style="list-style-type: none">▪ Do not limit the availability of test methods▪ Availability of reagents▪ Steer away from single source▪ Commercially available
<ul style="list-style-type: none">▪ Confident▪ Potential to look at peptides▪ Not clear if it captures denatured whey protein▪ Question on whether it will pick up hydrolyzed protein▪ Doesn't hit the target as it pertains to hydrolyzed protein▪ Products that have intact whey risk in applying to hydrolyzed whey	<ul style="list-style-type: none">▪ Lyseine in the peptide?▪ Signature is unique▪ Keeping the research in progress▪ For mass spec▪ Meets SMPR requirements
<ul style="list-style-type: none">▪ Has a good specificity & selectivity▪ Spectrometry could detect▪ Suitable to quantitate▪ Recommendation: move forward	<ul style="list-style-type: none">▪ Only 3 data points▪ Need additional information on<ul style="list-style-type: none">○ Ruggedness○ Test materials
<ul style="list-style-type: none">▪ Hydrolyzed form of protein▪ Not one particular to another particular	<ul style="list-style-type: none">▪ Method is on target▪ Used as an indication▪ Needs additional work

WPC-18

<ul style="list-style-type: none">▪ Using standard AA hydrolyzed methods	<ul style="list-style-type: none">▪ Can analyze all forms of protein▪ Technology is widely available▪ Indirect method using AA▪ Matrices from other manufacturers
<ul style="list-style-type: none">▪ The method is conceptually sound▪ Appears to stand up nicely▪ Need documentation▪ Well characterized▪ Technique is not difficult▪ Not costly▪ Can apply to hydrolyzed▪ Products of other species	<ul style="list-style-type: none">▪ Looks robust rugged▪ Some concerns around bovine limitations▪ AA
<ul style="list-style-type: none">▪ Indirect method▪ Can add nitrofreon	<ul style="list-style-type: none">▪ Method calibration
<ul style="list-style-type: none">▪ Endorse the approach in theory▪ Need to see more data▪ Calibration▪ Different▪ AA profile▪ Will make significant changes to data	<ul style="list-style-type: none">▪ Made with the same lot? Yes▪ Calibration is based on the<ul style="list-style-type: none">○ Nitrogen by Khejhal○ Total protein not true
<ul style="list-style-type: none">▪ Regulatory requirements	<ul style="list-style-type: none">▪ Free AA▪ Does it have a big impact
<ul style="list-style-type: none">▪ Using particularly hydrolyzed (mildly hydrolyzed)	<ul style="list-style-type: none">▪ % of SMPR is higher, either change the SMPR or change the method

WPC-26

<ul style="list-style-type: none">▪ Speaks to variability<ul style="list-style-type: none">○ Apply to whey profile<ul style="list-style-type: none">▪ + W/4▪ Mix of other manufacturers products	<ul style="list-style-type: none">▪ Similar, but model is different▪ Recommend to move forward▪ Free AA content▪ Looks at Lysteine (difficult to analyze)<ul style="list-style-type: none">○ oxidation▪ use WPC-18 data▪ Corrects free AA▪ Need information on AA from hydrolyzed products▪ Modify the calculation/standard (combine the methods)
<ul style="list-style-type: none">▪ Not enough analytical composition▪ Ratio gives a greater sensitivity	<ul style="list-style-type: none">▪ Hydrolyzed whey▪ Whey patter

WPC-28

<ul style="list-style-type: none">▪ Comments:<ul style="list-style-type: none">○ Additional work needed○ Use other enzymes (hydrolyzed infant formula)<ul style="list-style-type: none">▪ Not suitable○ Could do a digestion<ul style="list-style-type: none">▪ To determine typical hydrolyzed whey same approach to the other mass spec	<ul style="list-style-type: none">○ Could provide data on reproducibility<ul style="list-style-type: none">▪ Used for food allergen▪ Reference standard (LGC)○ Validated peptide marker (hydrolyzed whey)
<ul style="list-style-type: none">▪ Similar to the other▪ Question on casein▪ If you can determine the whey content▪ Combine the 2 methods	<ul style="list-style-type: none">▪ Specific fragments known to come from the specific protein (not as indirect)
<ul style="list-style-type: none">▪ Direct method▪ Amount of peptide▪ Applaud the suggestion of looking at hydrolyzed (partially hydrolyzed)	<ul style="list-style-type: none">▪ Like the method▪ Provides proof▪ Add couple of peptides markers▪ Recommendation: specific types of whey

❖ *Motion to move all four (WPC-17/18/26/28 to first action:*
Gilliland moved/Christiansen seconded. The motion did not pass.

4 – Approve
7 – Reject
2 – Abstain

~~Additional method reviews and discussions to take place during the meetings in September 2012.

DISCUSSION ON PLANNED ACTIVITIES BEFORE FINAL ACTION STATUS

Sullivan led a discussion with the panel and others on the planned activities during the period prior to final action.

WRAP UP/NEXT STEPS:

SPIFAN will review the candidate methods for possible endorsement after which, an ERP will consider the submitted methods which meet the SMPR for First Action *Official MethodsSM* status during the September 2012 meeting.