# STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

## DRAFT PROCEEDINGS

## Loews Royal Pacific at Universal Orlando®
## Orlando, Florida, USA

**Saturday, September 25, 2010**
**11:00AM – 6:00PM EDT**

## Stakeholders in Attendance

Martin Alewijn, Wageningen University
Jayashree Arcot, University Of New South Wales
John Austad Covance Laboratories
Stan Bacler, CFIA
Steve Baugh, Chromadex
Mike Benoit, PBM Products
Sneh Bhandari, Silliker
Christopher J. Blake, Nestle Research Center
Esther Campos-Gimenez, Nestle Research Center
Scott Christiansen, PBM Nutritionals
Xiaogang Chu, Chinese Academy of Inspection & Quarantine
Neal E. Craft, Craft Technologies, Inc.
Marcel De Vreeze, NEN
Jonathan DeVries, Medallion Laboratories/General Mills
Julie Eble, Critical Path Services, LLC
Wayne C. Ellefson, Covance Laboratories
Sarwar Gilani, Health Canada
Brendon D. Gill, Fonterra Co-operative Group Ltd.
Donald L. Gilliland, Abbott Nutrition
James Harnly, USDA
Michael Hauer, ThermoFisher
Steve Holroyd, Fonterra
Chung M. Hyun, Nutrilite (Access Business Group)
Harvey E. Indyk, Fonterra Co-operative Group Ltd.
Wesley Alan Jacobs, Abbott Nutrition
Greg Jaudzems, Nestlé
Jindou Jiang, China Dairy Test Center
Erik J. M. Konings, Nestlé Research Center
Gayle Lancette
HanXia Liu, Test Technology Center Of CAIQ
Xiumei Lie, China CDC
Bob McMahon, Mead Johnson
Mardi Mountford, International Formula Council (IFC)
Richard Myers, Kemin Industries, Inc.
Melissa Meaney Phillips, NIST
Hilde Skår Norli, NMKL
Jacques Prodolliet, Nestlé
Daniel Quinn, ThermoFisher
Guenther Raffler, CLF (Danone)
Gyan Rai, Mead Johnson Nutritionals
Robert Rankin, IFC
Lars Reimann, Eurofins
Rama Rengarajan, Kellogg Company
Catherine A. Rimmer, NIST
Joe Romano, Waters Corporation
Karen Schimpf, Abbott Laboratories
Dan Schmitz, Abbott Nutrition
Katherine E. Sharpless, NIST .
Angela Song, Dumex Baby Food Co., Ltd
John Sorensen, Arla Foods
Karla Steele, Mead Johnson Nutrition
Darryl Sullivan, Covance Laboratories
John Szpylka, Medallion Labs
Joseph J. Thompson, Abbott Laboratories
Linda Thompson, Abbott Nutrition
Yoshiyuki Tokiwa, U.S. Pharmacopeia
Socrates Trujillo, FDA
Rosemary Walzem, Texas A & M University
Norman White, Abbott Laboratories
Charlie Winstead, Mead Johnson
Wayne Wolf, USDA
Dave Woollard, NZ Lab Services
Dajing Yang, Institute of Nutrition & Food Safety
Jinchuan Yang, Waters Corporation
Shi-an Yin, NIN & Food Safety-China
Ren Yiping, Zhejiang, Provincial Center
Seong-Jae Yoo, Martek Bioscience
Rui Zhang, JSCIQ of AQSIQ
Zhi-Xu Zhang, Agilent

**SPIFAN CHAIR**

Darryl Sullivan, Covance Laboratories

**Nutrient Working Group Chairs*:***

| | |
|---|---|
| Esther Campos-Gimenez, Nestlé Research Center | Vitamin $B_{12}$ |
| Jonathan W. DeVries Sr., Medallion Laboratories | Vitamin A |
| Donald L. Gilliland, Abbott Nutrition | Vitamin D |
| Harvey E. Indyk, Fonterra Co-operative Group Ltd. | Inositol |
| Erik J. M. Konings, Nestlé Research Center | Folic Acid |
| Karen Schimpf, Abbott Laboratories | Inositol |

**SPIFAN Voting Members**

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|---|---|
| Arcot, Jayashree | University of New South Wales |
| Trujillo, Socrates | FDA, CFSAN |
| Sharpless, Kathy | NIST |
| Wolf, Wayne | USDA |
| Bacler, Stan | CFIA |
| De Vreeze, Marcel | NEN |
| Zhang, Rui | JSCIQ of AQSIQ |
| Jiang, JinDou | National Dairy Test Center |
| Austad, John | Covance |
| Szpylka, John | Medallion Labs |
| Bhandari, Sneh | Silliker |
| Woollard, Dave | NZ Laboratory Services |
| Myers, Rick | Kemin |
| Baugh, Steve | Chromadex |
| Rengarajan, Rama | Kellogg |
| Romano, Joe | Waters |
| Tokiwa, Yoshiyuki | USP |
| Mardi Mountford | IFC |
| Schmitz, Dan | Abbott |
| Rai, Gyan | Mead Johnson |
| Holroyd, Steve | Fonterra |
| Benoit, Mike | PBM Products |
| Raffler, Guenther | CLF (Danone) |
| Konings, Erik | Nestle |

**AOAC Staff & Consultants**

E. James Bradford
Delia Boyd
Scott Coates
Shane Flynn
Arlene Fox
Dawn Frazier
Zerlinde Johnson
Nora Marshall
Krystyna McIver
Deborah McKenzie
Alicia Meiklejohn
Tien Milor
Anita Mishra
Albert Pohland
Robert Rathbone
Garlon Riegler
Virginia Trainor

## I. Welcome & Introductions

The meeting was called to order at 11:06 a.m. by AOAC Executive Director, E. James Bradford, with introductions of the working group chairs and a presentation on achieving global acceptance. Bradford then introduced Darryl Sullivan of Covance Laboratories as the chair of SPIFAN and the meeting.

Sullivan reviewed the AOAC INTERNATIONAL Antitrust Policy and then briefed the stakeholders on the selection and composition of the voting members and their role in the consensus building process for the meeting. Voting members introduced themselves. The agenda was reviewed without questions and Sullivan encouraged people to recommend potential stakeholders and to sign up if interested in participating in working group(s).

Sullivan strongly encouraged stakeholders to participate in the standard method performance requirements (SMPRs) educational session being presented on Sunday, September 26, 2010, by AOAC Chief Scientific Officer, Scott Coates. As this session will provide the guidance for the working groups to develop SMPRs, Sullivan stated that attendance at this session is mandatory for anyone participating in the working groups.

## II. Purpose & Scope of the Project

Sullivan provided an overview of the current methodology for infant formula nutrients pointing out that there are gaps in the methods used to analyze infant formula, which is a highly regulated commodity globally. He further stated that there is a challenge and opportunity for global recognition of the methods developed from this project.

Sullivan gave a presentation that discussed the contract between AOAC and the International Formula Council. He reviewed the first Advisory Panel Meeting held on June 30, 2010, and other key events. There will be five stakeholder meetings. Thirteen different working group meetings will be held to draft SMPRs and identify candidate method(s) and laboratory(ies). The first deliverable is due in April 2011. Sullivan explained how the Advisory Panel selected the first five nutrients and recommended chairs for each nutrient working group. All recommended chairs accepted the role.

Sullivan also briefly discussed the AOAC standards development process and asked everyone to review this section of the meeting book. Sullivan said that the process will be open and transparent.

Sullivan also reiterated that the SMPR education session will be held on Sunday, September 26, 2010, in the Pacifica II room at 11:45 a.m. and that prior to that training session, at 11:15 a.m., Marcel de Vreeze will make a presentation on the global acceptance of analytical standards. Sullivan then recognized Anita Mishra, AOAC Executive for Scientific Business Development, who reviewed the project timeline and reminded stakeholders to sign up for working groups and to attend the SMPR education session.

## III. Definitions of Infant Formula and Adult Nutritionals

Sullivan recognized Scott Coates to present and facilitate the discussion on the definitions for infant formula and adult nutritionals. Coates reviewed three definitions for infant formula:

- Codex: Codex Standard 72 – 1981:
    - 2.1.1: Infant formula is defined as a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.
    - 2.1.2: The product is so processed by physical means only and so packaged as to prevent spoilage and contamination under all normal conditions of handling, storage and distribution in the country where the product is sold.
- New Zealand: New Zealand (Standard 2.9.1)**:**
    - Infant formula product is defined as a product based on milk or other edible food constituents of animal or plant origin, which is nutritionally adequate to serve as the principal liquid source of nourishment for infants.
    - Infant formula is defined as an infant formula product represented as a breast milk substitute for infants and which satisfies the nutritional requirements of infants aged up to 4 to 6 months.
- U.S. Food and Drug Administration:
    - "A food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk."

After comparing the three definitions, Coates recommended that SPIFAN use the Codex definition in 2.1.1 because it is an international standard and, therefore, more generally recognized. Adoption by Codex is one of the projects aims. The Codex definitions are essentially the same as those for New Zealand and the United States.

The chair recognized stakeholders as they discussed the age range for infant formula and the form in which infant formula is consumed (e.g., liquid and powder). Sullivan recognized Danone, who recommended that the age range should be 0-36 months. Sullivan expressed that the definitions were draft and that the working groups may offer changes, when the groups deliberate.

**Motion** by Baugh (Chromadex) for SPIFAN to accept the Codex definition 2.1.1 that "infant formula is defined as a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding," with the addition of follow-on formula (0-36 months). Second by Raffler (Danone).
**18 in favor, 0 opposed, 1 abstain. Motion passed.**

The chair recognized Coates again who reviewed two definitions for adult nutritionals.

Definition 1:

- . . . are generally complete and balanced nutritional supplements, which may be used as an adjunct to a diet or even as a sole source of nutrition.
- Ingredients typically include macronutrients such as proteins, carbohydrates, and lipids, and micronutrients, including vitamins and minerals.
- There are special derivatives of these products, i.e., medical foods, which may be used in the management of specific diseases.

Definition 2 – Dietary foods for special medical purposes:

- . . . a category of foods for particular nutritional uses specially processed or formulated and intended for the dietary management of patients and intended to be used under medical supervision.
- Nutritionally complete foods with a standard nutrient formulation which, used in accordance with the manufacturer's instructions, may constitute the sole source of nourishment for the persons for whom they are intended

After comparing the two definitions, Coates recommended that SPIFAN use the term 'adult/pediatric nutritional formula' instead of 'adult nutritionals.' The definition should be as follows: "nutritionally complete, specially formulated foods, which may constitute the sole source of nourishment."

The chair entertained discussion by the stakeholders and voting members as they considered the definition including both liquid and powder forms. Raffler (Danone) recommended removing "special medical purposes" and not including "dietary supplements." Sullivan expressed that the definitions were draft and that the working groups may offer changes, when the groups deliberate.

Sullivan entertained a motion to accept the recommended adult nutritional formula definition including renaming "adult nutritionals" to "adult/pediatric nutritional formula"; liquid form was added and medical purposes and dietary supplements were removed from the definition.

**Motion** by Bhandari (Silliker) for SPIFAN to accept the definition for adult/pediatric nutritional formula. The definition should be as follows: "nutritionally complete, specially formulated foods, consumed in liquid form, which may constitute the sole source of nourishment." Second by Schmitz.
**18 in favor, 0 opposed, 1 abstain. Motion passed.**

## IV. Presentations of the First Five (5) Nutrients

A. The chair recognized Jon DeVries, working group chair for vitamin A. DeVries presented historical and global regulatory perspectives for vitamin A analysis, including the definition of vitamin A, its chemistry, and the current methodology and challenges. Vitamin A's sensitivity to light, heat, and oxygen contribute to sample preparation challenges.

Sullivan entertained questions. A stakeholder asked if German methods were in place for vitamins A and E. DeVries replied that there is a listing of such methods that he would review and the working group could consider.

B. The chair recognized Don Gilliland, working group chair for vitamin D. Gilliland presented historical and global regulatory perspectives for vitamin D analysis, including the chemical structures and biological functions of vitamin D. He presented challenges with the current methodology. Matrixes are becoming increasingly complex, and vitamin D is challenging to measure. Existing methods use UV detection and rely on extensive sample preparation and chromatography. Methods are not evaluated/validated for current product matrixes, and are written more as guidance. In addition, measurement of pre-vitamin D is estimated and inconsistent.

   Sullivan entertained questions. Two stakeholders agreed that use of LC/MS/MS could be a future improvement to the method. Another stakeholder commented that there are methods for vitamin D in other matrices, including meat, that the working group may want to consider. However, a stakeholder responded with information on the regulatory requirements: 1) there are different regulatory requirements for vitamin D for different matrices; and 2) the regulatory requirements are not consistent with levels for pre-vitamin D forms (vitamins $D_2$ and $D_3$). There was a question as to the current type of extraction used. Gilliland responded that saponification is currently used, but that changes occur when heating the sample. Hydroxylated forms are problematic.

C. The chair recognized Esther Campos-Giménez, working group chair for vitamin $B_{12}$. Campos-Giménez presented the historical and global regulatory perspectives and the current methodology. She explained that the current Codex method is an outdated (over 20 years old) microbiological method. It is lengthy, requires 20-24 hours, and has poor precision. Campos-Giménez discussed the advantages of chromatographic and biosensor methodology in meeting regulatory requirements. However, these techniques would require adaptation for liquids to meet lower regulatory limits.

   Sullivan entertained questions. A voting member asked if biosensor-based methodology was frequently used. The response was that there is a laboratory with experience using such methodology. A followup response was that additional data on accuracy and precision for this methodology would be needed. Campos-Giménez said that input from the group will be needed with respect to the performance of chromatographic methodology. A stakeholder replied that some preliminary work has been done.

D. The chair recognized Erik Konings, working group chair for folic acid. Konings presented the historical and global regulatory perspectives of folic acid and terminology. He also described the types of current methodology and their challenges. The current microbiological method has poor precision and specificity, and LC methods tend to give 20-30% lower values for unfortified foods when compared to the microbiological method. Konings further explained that analysis for folic acid is complicated by endogenous folic acid in foods. Konings recommended that for testing fortified foods, an UPLC/MS/MS method should be evaluated as a possible reference method. He discussed the current method for routine folate analysis by HPLC. He said that additional calibration is needed. Konings also compared the units of

measurement by CODEX and Europe for folic acid in fortified foods. He recommended UPLC/MS/MS for folic acid and 5-methyl-tetrahydrofolate (5MTHF) as an option for a future reference method.

Sullivan entertained stakeholder questions. One stakeholder commented that the extraction technique should be discussed by all of the working groups.

E. The chair recognized Harvey Indyk and Karen Schimpf, working group co-chairs for inositol. Indyk presented the historical and regulatory perspectives and identified inositol as an essential nutrient, rather than a vitamin. Although there are various forms of inositol, only myo-inositol is biologically active. Indyk explained that Codex has no requirements for inositol other than a guidance upper limit (GUL). He also described the challenges with the current methodology: 1) the microbiological method has a long incubation period and has poor precision; and 2) the chromatographic methods require derivatization. Indyk recommended that the chosen method should be able to analyze free and bound myo-inositol that possesses inositol activity.

Sullivan entertained stakeholder comments, all of which supported inositol presentation.

## V. Fitness for Purpose

A. The chair recognized DeVries to present the draft fitness-for-purpose statement for vitamin A.

1. An Analytical method(s) to determine Retinol, Retinyl Esters and Carotenes in the Concentration Ranges (as a minimum requirement) from 35 µg RE/100 mL to 169 µg RE/100 mL of liquid and reconstituted solid infant formula, milk- or soy-based, and adult nutritionals. Time to signal is not necessarily significant. Vitamin A levels meeting regulatory requirements will be covered by the concentration ranges of this quantitative reference assay conducted in a laboratory by a trained chemist.

   Sullivan entertained stakeholder questions. A stakeholder suggested that carotenoids be removed from the statement. Another stakeholder suggested that retinol be used in the fitness for purpose for methods appropriate to infant formula, but that carotenoids be used in a fitness-for-purpose statement for adult/pediatric formula. While Sullivan observed no stakeholder agreement on this point, he explained that the statement was a draft and Coates indicated that recommendation was a starting point and that additional ideas could be addressed within the working group. The draft fitness-for-purpose statement was then revised to:

   > An Analytical method(s) to determine Retinol and Retinyl Esters in the Concentration Ranges (as a minimum requirement) from 35 µg RE/100 mL to 169 µg RE/100 mL of liquid and reconstituted solid infant formula, milk- or soy-based, and adult nutritionals. Time to signal is not necessarily significant. Vitamin A levels meeting regulatory requirements will be covered by the concentration ranges of this quantitative reference assay conducted in a laboratory by a trained chemist.

   Sullivan then entertained the following motion:

**Motion** by Baugh for SPIFAN to accept the revised draft fitness-for-purpose statement for vitamin A that includes the changes as discussed. Second by Schmitz.
**19 in favor, 0 opposed, 1 abstain. The motion passed.**

B. The chair recognized Gilliland to present the draft fitness-for-purpose statement for vitamin D.
    1. An analytical method to determine cholecalciferol – vitamin $D_3$, ergocalciferol – vitamin $D_2$, and pre-vitamin D content with the analytical range of 20 – 200 ng/mL and an MDL of 2 ng/mL for infant formula (0-12 months) with an energy content of 1 - 3 mg/100 kcal and concentration range of 0.6 – 2.10 mg/100 mL using reporting units of mg vitamin D/100 mL in infant formula (milk-based, soy-based, and hydrolysate) and in adult nutritionals for elemental diets with high and low fat content in standard versus diabetic formulations; protein hydrolysates and in NIST SRM 1849. Time to result should be less than 24 hours and the method should be performed by a trained analyst and in a laboratory with proficiency testing and proficiency verification.

Sullivan entertained questions. A stakeholder asked if the $a_n a_l$yte was pre-vitamin D, and it was confirmed that pre-vitamin D was included. Another stakeholder said that LOQ would be high; however, it was stated that it would be 50% for infant formula and 200% for adult nutritionals. Sullivan asked the stakeholders if there was an endorsement of Gilliland's recommended fitness-for-purpose statement.

**Motion** by Bhandari to accept the fitness-for-purpose statement (as presented). Second by Baugh.
**19 in favor, 0 opposed, 1 abstain. The motion passed.**

C. The chair recognized Campos-Giménez to present the draft fitness-for-purpose statement for vitamin $B_{12}$.
    1. Vitamin B12 is a generic term that includes cobalt-containing corrinoids with the biological activity of cyanocobalamin. Endogenous forms are hydroxycobalamin, methylcobalamin, adenosylcobalamin and aquocobalamin, while cyanocobalamin is the main synthetic form used in fortification (hydroxycobalamin can also be used but it is generally dedicated to supplements). Regulatory requirements need the inclusion of natural amounts in the ingredients as well as the added cyanocobalamin (or hydroxycobalamin). Infant formula and adult nutritionals are generally fortified. The analytical range which should be covered for infant formula and adult nutritionals is at least 0.07-7.5 µg/100 g or 100 mL (might be higher due to non-specified GUL). Matrices include milk- and soy-based infant and follow-on formulae (ready to feed, concentrate and powders), hydrolyzed formulas, as well as medical foods/adult nutritionals. According to regulatory requirements (tightest tolerances 90-120%), the method should be able to provide accurate and precise results in the range of 0.07 – 7.5 µg/100 g. The method of choice is intended to be used as a reference method for dispute resolution. A maximum time-to-signal should be not more than 1 day. The analysis will be performed by trained analysts in accredited laboratories.

Sullivan entertained stakeholder questions. A stakeholder suggested that the "type of method" does not have to be included. It was stated that "type of method" will be resolved in the Call for Methods that AOAC will issue. Sullivan then entertained a motion.

**Motion** by Wolf to accept the fitness-for-purpose statement (as presented). Second by Raffler.
**19 in favor, 0 opposed, 1 abstain. The motion passed.**

D. The chair recognized Erik Konings to present the draft fitness-for-purpose statement for folic acid.
    1. Folic acid is the common, most stable, synthetic form used for food fortification. Natural folates comprise an extended family of mono- and polyglutamates (usually 5-7 glutamyl residues) of pteroic acid, which qualitatively exhibit the biological activity of folic acid. The most abundant natural form in human diet is 5-methyl-tetrahydrofolate (5-MTHF). Regulatory requirements need the inclusion of natural amounts in the ingredients as well the added nutrients in the products. Furthermore, besides the use of N-Pteroyl-L-glutamic acid as ingredient in infant formula, follow-up infant formula, and foods for special medical purposes, the use of calcium-L-methyl-folate is allowed in formulas for special medical purposes. According to regulatory requirements (tightest tolerances 90-120%), the method should be able to provide accurate and precise results in the range of 2-250 microgram/100g or 100ml for infant formula and adult nutritionals (The range might be higher due to unspecified maximum amounts in the regulation). Typical matrices to be covered are: ready-to-feed products, powders (milk- and soy-based), hydrolyzed infant formulas, medical foods/adult nutritional, and a Standard Reference Material. The future method of choice will intend to be used as a reference method for dispute resolution. A maximum time-to-signal should be 3 days. The analysis will be performed by trained analysts of accredited laboratories.

    Sullivan entertained stakeholder comments and questions. A stakeholder asked about the determination of the maximum time-to-signal. The response was that 3 day is a maximum time recommendation. The chair entertained a motion.

    **Motion** by Schmitz to accept the fitness for purpose. Second by Austad.
    **19 in favor, 0 opposed, 1 abstain. The motion passed.**

E. The chair acknowledged Harvey Indyk and Karen Schimpf as co-chairs for the Inositol Working Group and recognized Harvey Indyk to present the draft fitness-for- purpose statement for inositol.
    1. An official method for the determination of inositol is intended to be used as a reference method for dispute resolution of the inositol concentrations in infant, adult, and pediatric formulas as defined by AOAC. Inositol will include free myo-inositol and any bound forms of myo-inositol that possess inositol activity. The product matrices that this method will be applicable for use with include ready-to-feed liquids, concentrated liquids, and powders which can be formulated with any combination of

milk, soy, rice, whey, hydrolyzed protein, and amino acids with and without intact protein. The method should also be appropriate for use with the ingredients used to formulate the above mentioned products. The method should be able to accurately quantitate a minimum of 2 mg inositol per 100 mL of formula and a maximum of 200 mg inositol per 100 g of powder as defined by regulatory standards. This method should be rugged and simple enough that trained technicians and chemists at any site throughout the world can generate comparable data for the same sample. For the quantitation of free inositol in a sample, it should take less than 1 hour to prepare and analyze a sample with a previously calibrated system.

Sullivan entertained stakeholder comments and questions. Stakeholders asked questions on the forms of myo-inositol and Indyk stated that the working group should define the forms of free and bound myo-inositol to be analyzed. Stakeholders also discussed the use of the word "ingredients" in the statement. It was stated that the working group co-chairs will discuss deleting that term from the statement (at a later date). Sullivan then entertained a motion.

**Motion** by Schmitz to accept the fitness-for-purpose statement (as written). Second by Trujillo.
**19 in favor, 0 opposed, 1 abstain. The motion passed.**

F. The chair recognized Coates who explained that the stakeholder-approved fitness-for-purpose statements are first drafts and that changes may be made. He described the process for additional stakeholder engagement in the process: establishing working groups, issuing Call for Methods, drafting SMPRs and requesting public comment, recommending candidate methods, and identifying interested laboratories to characterize and conduct validation studies on the methods Coates thanked stakeholders for the work they completed during the discussions, but reminded everyone that much work is still ahead.

## VI. Next Steps

Mishra asked working group chairs to submit their methods to Virginia Trainor as part of the AOAC Call for Methods in preparation of the November 8 working group sessions to be held in Gaithersburg, Maryland. Logistical information for the meeting will be forthcoming. Mishra also reminded everyone about the session on Sunday, September 26, 2010, at 11:00 a.m. The agenda includes the presentation by Marcel de Vreeze followed by an SMPR education by Coates. Sullivan thanked everyone and adjourned the meeting.