AOAC INTERNATIONAL

Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)

STAKEHOLDER MEETING

Meeting at Hilton Washington DC/Rockville
Hotel & Meeting Conference Center
Rockville, Maryland USA

Monday, April 4, 2011

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Wednesday, April 6, 2011

STAKEHOLDER CONSENSUS/TASK FORCES

SPIFAN Reaches Consensus on Draft Fitness-for-Purpose for First Five Priority Nutrients

(seated) Erik Konings, Jonathan DeVries, and Harvey Indyk; (standing) Esther Campos-Gimenez

On September 25, 2010, at the AOAC Annual Meeting in Orlando, Florida, USA, the first Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN), chaired by **Darryl Sullivan** of Covance, reached consensus on draft fitness-for-purpose statements for the first five (of at least 20) priority nutrients: vitamin A, vitamin D, vitamin B_{12}, folic acid, and inositol. AOAC issued a call for methods and conducted literature searches in October 2010, and more than 150 methods were collected for evaluation.

In support of a contract with the International Formula Council (IFC), AOAC is facilitating establishment of voluntary consensus standard method performance requirements (SMPRs) for infant formulas and adult/pediatric nutritional formulas. Stakeholders expressed an urgent need for methodology having global acceptance and the potential Codex adoption. This will help resolve potential disputes about various methods in use for the analysis of nutrients in infant formula and adult/pediatric nutritional formulas.

"The high-profile initiative calls for AOAC to fully engage key international stakeholders from government, industry, and major laboratories, so that these new and modern methods become the consensus reference methods for the entire industry around the world," said **James Bradford,** AOAC executive director.

Added Sullivan, who has been a champion in previous AOAC stakeholder efforts on nutrients in infant formulas and adult nutritionals, "The project is significant on many levels. Infant formulas and adult/pediatric nutritional formulas are a highly regulated and extensively tested industry because of the vulnerable populations they serve. Methods can help ensure safety and compliance. The multiplicity of forms of these nutrients is challenging, and to try and apply universal criteria will be a big effort that could have major impact."

Draft fitness-for-purpose statements were developed from the stakeholders' meeting, at which consensus was reached by some 80 key experts from government, formula manufacturers, contract research organizations, technology providers, academia, trade associations, standards developing organizations, raw ingredient suppliers, retailers, and consumer groups, among others. International representation is critical in ensuring that methods are accepted worldwide and meet regula-

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(front) Shane Flynn, Scott Coates, and Al Pohland; (back) Anita Mishra, Darryl Sullivan, and James Bradford

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tory testing requirements. As such, stakeholders came from Europe, Russia, Canada, Latin America, Asia, Australia/New Zealand, South Africa, The Netherlands, and the United States. The initiative is supported by a contract between AOAC and IFC, with Abbott Nutrition, Nestlé, Mead Johnson Nutrition, Fonterra, Danone, and PBM as funding partners.

"The infant formula and adult/pediatric nutritional formulas project truly requires global participation," said **Anita Mishra,** AOAC executive for scientific business development.

Defining "Infant Formula" and "Adult Nutritionals"

Before tackling draft fitness-for-purpose statements, the panel reached consensus on consistent terminology for infant formulas and adult/pediatric nutritional formulas that can be uniformly applied to all SMPRs. Three definitions for infant formula, which are essentially the same, were identified: Codex Standard 72-1981, Food Standards Australia New Zealand (FSANZ) 2.9.1, and FDA.

For the AOAC/IFC project, stakeholders recommended to incorporate the definition in Section 2.1.1 of Codex Standard 72-1981 because it is an international standard, and, therefore, more generally recognized. They stated that adoption of AOAC validated methods by Codex is one of the project's aims. The Codex definition is essentially the same as the United States (FDA) and New Zealand definitions.

SPIFAN approved the use of the Codex definition for infant formula with the addition of follow-on formula (0–36 months). 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.

Some stakeholders recommended that follow-on infant formula should be considered for inclusion because these products, which are formulated for babies up to 36 months, are available on the market. They stated that a difference exists in newborn infant formula versus follow-on infant formula, and that the difference could affect analytical testing.

It was determined that other dietary supplements not be included as they are out of scope of the current project.

Two definitions for adult nutritionals were provided: adult/pediatric nutritional formulas and dietary foods for special medical purposes. Stakeholders recommended use of the term "adult/pediatric nutritional formula" and accepted a simplified draft definition as "nutritionally complete, specially formulated foods, consumed in liquid form, which may constitute the sole source of nourishment."

Vitamin A

Armed with draft fitness-for-purpose statements developed for each of the five selected nutrients in preparation for the stakeholder meeting, each working group chair provided a historical background and examined analytical challenges, regulatory requirements, and existing methodology for each nutrient in an effort to reach consensus on what would be expected (performance) of each candidate method.

Jonathan DeVries, General Mills/Medallion Laboratories and chair of the Working Group on Vitamin A, reported that the term "vitamin A" was suggested in 1920 and is used in reference to the vitamin's biological activity, and applies to more than one vitamin A active substance. Vitamin A is essential for vision, bone growth, and reproductive functions in adults. Vitamin A deficiencies may cause xerophthalmia (dry eyes, no tears, eventual blindness), night blindness, loss of appetite, and muscular weakness, among others. Primary vitamin A forms in the diet include retinol, retinal, retinoic acid, and the carotenoids. The four forms of vitamin A available for fortification are retinol, retinyl acetate, retinyl palmitate, and the carotenoids.

DeVries reported the upper limits recommended by the

Institute of Medicine (IOM) and regulatory requirements (minimum and maximum limits) for vitamin A from FSANZ (14–43 µg/100 kJ), China (14–43 µg/100 kJ), Codex (14–43 µg/100 kJ), European Union (EU; 14–43 µg/100 kJ), Indonesia (75–150 µg/100 kcal), and the United States (250–750 IU/100 kcal). In addition, he highlighted official/approved methods from AOAC and the American Association for Clinical Chemistry (AACC) for vitamin A, using techniques such as spectrophotometry (AOAC **960.45, 970.64, 938.04, 975.23,** AACC 86-01-02), colorimetry (AOAC **974.29,** AACC 86-05-01, 86-02-01, 86-03-01), and mechanized chromatography (AOAC **992.04, 992.06, 2001.13, 2002.06, 2005.07,** AACC 86-06-01).

Analytical challenges include the sensitivity of retinol and the carotenes to oxidation (light, heat/oxygen), the occurrence of isomerization during analysis (laboratory lighting and low-actinic glassware), the need to conduct saponifications in the presence of antioxidants and under nitrogen, and the problems presented by standards that are rarely pure, sample preparation, and column temperature control.

Draft Fitness-for-Purpose Statement Approved by SPIFAN

For vitamin A, analytical method(s) must determine retinol and retinyl esters at the concentration level (as a minimum requirement) of 383 µg/100 mL of liquid and reconstituted solid infant formula, milk- or soy-based, and adult nutritionals. The limit of quantitation (LOQ) is ≤7.00 µg/100 mL. Time-to-signal is not necessarily significant.

Initially part of the draft fitness-for-purpose statement, carotenes were removed as some stakeholders recommended handling them as a separate analyte because of their complexity and instability. Stakeholders also recommended that candidate methods should apply to rice-based formulas.

Vitamin D

Presented by **Donald Gilliland,** Abbott Nutrition and chair of the working group, forms of vitamin D include calciferol, ergocalciferol (D_2), and cholecalciferol (D_3). The first scientific description of vitamin D deficiency (rickets) was found in the 1600s. In the 1920s, UV light exposure, irradiated foods, and cod-liver oil were linked to rickets prevention in animals. Vitamin D helps regulate calcium and phosphorus blood levels, enables normal bone mineralization, modulates neuromuscular and immune function, and plays a role in reducing inflammation and in regulating cell proliferation, differentiation, and apoptosis.

Vitamin D is heavily regulated, especially in infant formula. Current methodologies include AOAC **992.26** and **995.05,** EN 12821, and ISO 14892:2002. However, 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.

Gilliland reported the recommended daily amounts from IOM and FD&C Act/Codex Standard 72–1981. Regulatory limits for infant formula from FDA's Infant Formula Act are 40–100 IU/100 kcal, Codex Standard 72–1981 (1–2.5 µg/100 kcal), and FSANZ Standard 2.9.1 (0.25–0.63 µg/100 kj). Recommended limits for adult nutritionals from WHO/FAO, FDA, and IOM are >5, 10, and >5 µg/day, respectively. In addition, he addressed U.S.- and Codex-expected concentrations for infant formulas (<6 months, 6–12 months). For adult nutritionals, expected concentrations are product label-dependent.

Draft Fitness-for-Purpose Statement Approved by SPIFAN

Analytical method(s) must determine vitamin D, which includes D_2 and D_3, at the concentration level (as a minimum requirement) of 5.10 µg/100 mL. LOQ is ≤0.119 µg/100 mL.

Folic Acid

Folic acid is the most common, most stable synthetic form used for food fortification. Folate is the naturally occurring form, present in the majority of foods (especially rich sources are liver, yeast extracts, green leafy vegetables, legumes, and some fruits). In 1931, Lucy Wills et al. demonstrated that an autolyzed yeast preparation (Marmite) was effective against nutritional megaloblastic anemia in pregnant women. In 1941, Mitchell et al. processed 4 tons of spinach to obtain a purified substance with acidic properties, which was an active growth factor for rats and *Lactobacillus casei.* They named the factor "folic acid." Folic acid was synthesized and its structure was determined in 1946 by Angier et al.

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Erik Konings, Nestlé Research Center and chair of the Working Group on Folic Acid, reported that the safe upper level is 1000 µg folic acid/day (IOM). Children and adults both require folic acid in order to produce healthy red blood cells and prevent anemia. A lack of folic acid can result in health problems, such as neural tube defects in babies, pernicious anemia, cognitive decline, impaired immune function, and interference with methotrexate therapy.

Current analytical techniques include a microbiological method (AOAC **992.05,** EN 14131, which is a turbidimetric method and is used as the reference method with limitations); HPLC using affinity chromatography purification, HPLC separation, and electrochemical detection; UPLC/MS/MS; and biospecific methods. According to Konings, analytical methods should include calibration and measurement of the biologically active vitamers.

Konings presented minimum and maximum levels for infant formula (0–6 months: 10–50 µg/100 kcal for Codex and EU), follow-on formula (6–12 months: 4–NS µg/100 kcal for Codex and 10–50 µg/100 kcal for EU), and foods for special medical purposes (<1 year: 10–50 µg/100 kcal for Codex and 4–25 µg/100 kcal for EU; >1 year: 10-50 µg/100 kcal for EU). Different countries' tolerance limits were also reported, with the tightest range of 90–120%.

Draft Fitness-for-Purpose Statement Approved by SPIFAN

Analytical method(s) must determine folate, which includes folic acid (common, most stable, synthetic form, used to fortify food) and 5-methyl-tetrahydrofolate (the most abundant natural form in the human diet, usually comprises polyglutamate), at the concentration level (as a minimum requirement) of 85.0 µg/100 mL. LOQ is ≤0.478 µg/100 mL.

Wayne Wolf, Socrates Trujillo, and Rick Myers

Vitamin B_{12}

Esther Campos-Gimenez, Nestlé Research Center and chair of the Working Group on Vitamin B_{12}, reported that in 1934, Whipple, Murphy, and Minot shared the Nobel Prize in Physiology or Medicine for their life saving discovery found in liver. The disorder was referred to as "pernicious anemia" because before this discovery, death was inevitable. In 1948, the substance in liver was isolated and named cobalamin (vitamin B_{12}). The molecule was so complex that its structure could only be worked out through the aid of advanced technology. In 1956 English physicist Dorothy Hodgkin completed the mapping of B_{12}'s chemical structure by using x-ray crystallography, for which she received the 1964 Nobel Prize in chemistry. The last vitamin to be discovered, vitamin B_{12} was finally synthesized by Robert Burns Woodward in 1971.

AOAC Method **986.23** for cobalamin in milk-based infant formula is in need of modernization. Current techniques used are microbiological assays, biospecific methods, and chromatographic methods. Microbiological methods are inexpensive and sensitive, but time consuming, not specific, have poor precision, and require a high number of repeats. Biosensor-based analysis is expensive, semiautomated, provides real-time results, and sample preparation is rapid (Qflex Kit for Vitamin B_{12} is certified as a *Performance Tested Method*℠). Chromatographic methods are based on the chromatographic separation and chemical detection of native or derivatized vitamin B_{12}. Methods are relatively expensive, highly specific, provides acceptable precision results, and dedicated equipment is not required.

Campos-Gimenez presented minimum and maximum limits (ranges where methods need to perform satisfactorily) from Codex, EU, FSANZ, FDA, and China for infant formula, follow-on formula and foods for special medical purposes. The tightest tolerances are 90–120%.

Draft Fitness-for-Purpose Statement Approved by SPIFAN

Analytical method(s) must determine vitamin B_{12} including cobalt-containing corrinoids with the biological activity of cyanocobalamin (main synthetic form used in fortification) at the concentration level (as a minimum requirement) of 1.40 µg/100 mL. LOQ is ≤0.0120 µg/100 mL.

Inositol

Working group co-chairs **Harvey Indyk,** Fonterra, and **Karen Schimpf,** Abbott Nutrition, reported that myo-inositol was first discovered in 1850, but interest in its potential nutritional benefits did not begin until the 1940s. It is essential for the growth of most cells in culture, insulin signal transduction, cytoskeleton assembly, maintenance of cell membrane potential, and serotonin activity modulation, among others. Deficiencies in rat and gerbil showed hypolipidemia and fatty liver. Like other vitamins, inositol acts catalytically, but unlike other vitamins, inositol phosphates are energy-yielding compounds. Inositol occurs in foods in the form of free myo-inositol, phytic acid and inositol phosphates, and inositol-phospholipids.

There are no reported deficiencies of inositol in humans. There is no recom-

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AOAC Introduces SMPRs to Stakeholder Panel on Infant Formula and Adult Nutritionals

In an effort to provide guidance to the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN), AOAC held a training session on September 26, 2010 at the AOAC Annual Meeting to demonstrate the integral role standard method performance requirements (SMPRs) play in AOAC's third-party consensus-building process. Led by **Scott Coates,** chief scientific officer, members of SPIFAN examined the process and components of SMPRs, fitness-for-purpose versus SMPRs, and development of an AOAC SMPR guideline. In addition, Coates showed how the proposed SMPR guideline was successfully implemented in AOAC's initiative on endocrine disrupting compounds (EDCs) in freshwater.

Scott Coates provides an overview of AOAC's SMPR process to members of SPIFAN.

Background

SMPRs are increasingly gaining popularity as the Association executes activities in biothreat detection, veterinary drug residues, and EDCs. The infant formula initiative will produce voluntary consensus SMPRs for at least 20 priority nutrients.

Coates reported that in the 1990s, the AOAC *Performance Tested Methods*^{SM} program organized several multimethod evaluations (antibiotic residues in milk, aflatoxin in grain, peanut allergens) in which acceptance criteria were included in a common study protocol. In 2004, as a result of a project to evaluate hand-held assays (HHAs) in support of a contract with the U.S. Department of Homeland Security, AOAC developed a common study protocol, and for the first time developed and published a separate acceptance criteria/SMPR statement.

Since then, study protocols and SMPRs have been developed for various AOAC projects:

- Veterinary drug residues in animal tissues
- Drug residues in shrimp, catfish, tilapia, and salmon
- PCR-based assays for *Bacillus anthracis, Yersinia pestis,* and *Francisella tularensis*
- Antibody-based HHAs for ricin and *B. anthracis*
- Aerosolized PCR technology for *Burkholderia pseudomallei* and *Burkholderia mallei*
- Portable PCR technology for *Bacillus anthracis* in visible powders
- Estrone in freshwater

Development of an SMPR Guideline

To ensure consistency and uniformity in SMPRs developed by various stakeholder panels, Coates reviewed all SMPRs to date and has proposed a standard format and guidance, which "would help stakeholder panels from the onset, as well as help in the review of SMPRs," he said.

The proposed SMPR guideline, which has been reviewed by AOAC technical staff, Official Methods Board (OMB), and statistical advisors, specifies the following components: intended use, applicability, analytical technique, definitions, method performance requirements, system suitability tests and/or analytical quality control, reference method(s), reference material(s), and validation guidance.

AOAC began implementing the standard format and guidance for SMPRs with stakeholder meetings on June 29, 2010, for analysis of seafood contaminants resulting from the Gulf oil spill and on July 8–9, 2010, for determining the concentration of EDCs in freshwater located near poultry, swine, and dairy animal feeding operations.

"A few improvements were identified, but overall, the SMPR guideline was very useful in establishing assay performance requirements for quantitative measurement of estrone," Coates said.

The guideline is designed

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to contain all of AOAC's validation requirements on chemistry and microbiology, quantitative and qualitative tests, definitions, evaluation recommendations, expected results, and informative sections.

Coates reported that "the draft SMPR guideline is on the OMB's agenda for formal adoption as an AOAC guideline." He views the guideline as a working document that will "continually be revised as AOAC executes additional projects and as new issues arise, for example, expected results at ppt concentration."

Fitness-for-Purpose vs SMPR

SMPRs are based on the fitness-for-purpose statement developed by stakeholders and establish the minimum performance that stakeholders expect of a method. Like fitness-for-purpose statements, SMPRs represent what is expected of a method but are more specific, detailed analytical requirements for a test method. SMPRs include acceptance requirements and are used to qualify methods for AOAC approval. SMPRs are acknowledged as AOAC consensus standards because of the process by which they are developed and adopted. This process, which includes (*1*) openness, (*2*) balance of interest, (*3*) due process, (*4*) an appeals process, (*5*) consensus, and (*6*) transparency, renders AOAC standards to be considered acceptable.

Fitness-for-purpose statements ensure that a method is appropriate for its intended use and identify analytical needs, but are not as explicit as SMPRs. Fitness-for-purpose is used to collect candidate methods and establish SMPRs.

SMPR Process

In developing SMPRs, typically AOAC establishes a stakeholder panel, from which smaller working groups may be created if needed, to identify priorities and address analytical issues such as analyte and application (matrix), current techniques, technical challenges, and regulatory requirements. Through consensus, a fitness-for-purpose statement is developed that "focuses and narrows the scope of a method search to a manageable number and allows AOAC to collect the most promising methods."

A call for methods is issued based on the fitness-for-purpose statement. All methods collected are initially reviewed by AOAC's chief scientific officer and/or working group chair. Methods purported to meet fitness-for-purpose are recommended for further evaluation by topic-specific working groups or expert review panels (ERPs).

SMPRs are developed based on fitness-for-purpose and provide detailed requirements, such as purpose and application of the method, analyte and matrix, method classification, performance parameters, LOQ, LOD, applicability range, reference method comparison, bias, precision, recovery, reference methods and materials, system suitability requirements, and time to signal. If working groups are used, recommendations such as SMPRs are provided to the stakeholder panel for approval to post for public comment.

All SMPRs are posted to the AOAC Web site for a 30-day comment period. Comments received are reviewed by AOAC's chief scientific officer and chair of the stakeholder panel. Comments and replies are submitted to the stakeholder body for review.

Once approved by the stakeholder panel, SMPRs are evaluated by an AOAC SMPR Review Team, approved by the OMB chair, to ensure that the documents (performance requirements and the accompanying manuscripts) follow AOAC policies, procedures, and format and reflect consensus of the stakeholders.

Once finalized, SMPRs are codified and, ultimately, published in the *Official Methods of Analysis*^{SM} (OMA; most likely in a separate section) and the *Journal of AOAC INTERNATIONAL.*

Conclusion

SMPRs provide minimum performance criteria that a method must meet or exceed and have become integral to AOAC's third-party consensus-building process. AOAC activities have resulted in SMPRs valuable to the analytical sciences community in addressing critical needs through consensus. Once SMPRs are adopted by stakeholders, any method can be used as long as it meets the established performance requirements.

For more information, contact Scott Coates, chief scientific officer, at scoates@aoac.org or **Deborah McKenzie,** senior director, methods development and approval processes, at dmckenzie@aoac.org. ■

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> **—SCOTT COATES, AOAC CHIEF SCIENTIFIC OFFICER**