AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

THE FIFTEENTH MEETING

of the

AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals

Meeting at:
Gaithersburg Marriott Washingtonian Center
9751 Washingtonian Boulevard
Gaithersburg MD 20878, USA

Wednesday, March 15, 2017

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AOAC INTERNATIONAL
BYLAWS

As Amended September 26, 2010

ARTICLE I
Name

The name by which this Association shall be known is "AOAC INTERNATIONAL" (hereinafter referred to as the "Association").[1]

ARTICLE II
Purpose

The primary purpose of the Association is to promote methods validation and quality measurements in the analytical sciences.

ARTICLE III
Membership

Section 1. Types of Membership

There shall be three (3) types of membership in the Association: Individual Members, Sustaining Member Organizations, and Organizational Affiliates.

A. Individual Members

There shall be four (4) categories of Individual Members in the Association: Members, Retired Members, Student Members, and Honorary Members.

B. Sustaining Member Organizations

There shall be one (1) category of Sustaining Member Organizations.

C. Organizational Affiliate

There shall be one (1) category of Organizational Affiliate.

Section 2. Qualifications for Membership

A. Individual Members

[1] Members

Qualifications for Members shall be a degree in science, or equivalent as approved by the Board of Directors, and interest in supporting and furthering the purpose and goals of the Association. Such scientists shall be eligible for membership provided they are engaged, or have been engaged, directly or indirectly, in a field relevant to the purpose of the Association.

[2] Retired Members

[1] AOAC INTERNATIONAL was incorporated in the District of Columbia on January 20, 1932, as the Association of Official Agricultural Chemists. On November 10, 1965, the name of the corporation was changed to the Association of Official Analytical Chemists, and on September 12, 1991, the current name was adopted.

A current Member who is no longer actively engaged, directly or indirectly, in a field relevant to the purpose of the Association but who has served the Association as a Member for at least ten (10) years shall be eligible for Retired Member status upon written request and payment of the annual Retired Member dues. Any special benefits accorded Retired Members shall be determined by the Executive Director.

[3] Student Members

Any full-time student working toward an undergraduate or graduate degree in the areas of chemistry, microbiology, food science or other related science shall be eligible for Student Membership in AOAC INTERNATIONAL.

[4] Honorary Members

Honorary Members shall be persons recognized for their substantial contribution toward the achievement of the objectives of the Association. They shall be nominated by the Board of Directors and may be elected by a two-thirds vote of the Individual Members voting.

B. Sustaining Member Organizations

A Sustaining Member Organization shall be any agency of a local, state, provincial, national, or international government; a university, college, or academic department; or any firm, business, or organization with an interest in supporting and furthering the purpose of the Association. Every Sustaining Member Organization must have a designated representative(s). All such Sustaining Member Organization representatives must meet the qualifications for Members and become Individual Members with all the rights and privileges thereof.

C. Organizational Affiliate

An Organizational Affiliate Organization shall be any agency of a local, state, provincial, national, or international government; a university, college, or academic department; or any firm, business, or organization with an interest in supporting and furthering the purpose of the Association. Every Organizational Affiliate must have a designated representative(s). All such Organizational Affiliate representatives must meet the qualifications for Members and become Individual Members with all the rights and privileges thereof.

Section 3. Application for Membership

Applications or requests for membership shall be submitted to the Association's headquarters office. Membership shall become effective upon approval of the application or request, payment of any required membership dues, entry on the membership rolls, and assignment of a member number.

Section 4. Expulsion

The Board of Directors, at any duly called meeting of the Board, by a two-thirds vote of those holding office, may terminate the membership of any member who in its judgment has violated the Bylaws or has been guilty of conduct detrimental to the best interests of the Association. Any member convicted of a felony is subject to immediate expulsion from the Association. Expulsion of a member by the Board of Directors shall be final and shall cancel all rights, interest, or privileges of such member in the services or resources of the Association. Any member, for whom expulsion is proposed, for reasons other than conviction of a felony, shall be entitled to not less than 60 days advance notice of the charges, the date upon which a hearing will be scheduled, and the right to present evidence in defense. The date and place of any such hearing, if held other than at the headquarters or annual meeting site of the Association, must be reasonable with respect to the location of any individual so charged.

Section 5. Dues, Membership Year, and Waivers

A. Annual dues for membership in the Association shall be fixed by the Board of Directors, subject to approval by the majority of the Individual Members voting by ballot by any of the following means (whichever is deemed appropriate by the Board at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission.

B. Honorary Members of the Association shall be exempt from payment of dues and annual meeting registration fees.

C. The membership year and the delinquency date shall be determined by the Board of Directors.

D. The authority to grant waivers of membership dues rests with Executive Director.

E. Student Member dues shall be one-third of regular Member dues, rounded up to the nearest $5.00 increment.

Section 6. Members in Good Standing; Rights and Privileges

All Individual Members who maintain their membership by payment of dues as required under these Bylaws and who otherwise qualify shall be considered in good standing and entitled to full privileges of membership.

ARTICLE IV
Officers

Section 1. Elected Officers

The elected officers of the Association shall be Individual Members and shall consist of a President, President-Elect, Secretary, Treasurer, and Immediate Past President.

A. President

The President shall be the principal elected officer of the Association, shall preside at meetings of the Association and of the Board of Directors and of the Executive Committee, and shall be a member ex-officio, with right to vote, of all committees except the Nominating Committee. He or she shall also, at the annual meeting of the Association and at such other times as he or she shall deem proper, communicate to the Association or the Board of Directors such matters and make such suggestions as may in his or her opinion tend to promote the welfare and further the purpose of the Association and shall perform such other

duties as are necessarily incident to the office of President or as may be prescribed by the Board of Directors.

B. President-Elect

In the absence of the President, or in the event of the President's inability or refusal to act, the President-Elect shall perform the duties of the President, and, when so acting, shall have all the powers of and be subject to all the restrictions upon the President. The President-Elect shall perform such other duties as from time to time may be assigned to him or her by the President or by the Board of Directors.

C. Secretary

The Secretary shall give notice of all meetings of the Association, keep a record of all proceedings, attest documents, and, in general, perform such other duties as are usual of the office of Secretary and such other duties as may be assigned by the President or by the Board of Directors.

D. Treasurer

The Treasurer shall be responsible for the funds and securities of the Association; serve as financial officer of the organization and as Chairperson of the Finance Committee; manage the Board of Director's review of and action related to the Board of Director's financial responsibilities; serve as the chief Board liaison in overseeing and reviewing the annual audit, and in general, perform such other duties as are usual of the office of Treasurer and such other duties as may be assigned by the President or by the Board of Directors.

E. Immediate Past President

The Immediate Past President shall serve as advisor to the President and Directors and perform such other duties as may be assigned from time to time by the President or by the Board of Directors.

Section 2. Appointed Officers

The appointed officers shall include the Executive Director and such other appointed officers as may be designated by the Board of Directors from time to time.

A. Executive Director

The day-to-day administration and management of the Association's offices shall be vested in a salaried manager employed or appointed by, and directly responsible to, the Board of Directors. This manager shall have the title of Executive Director with responsibility for the management and direction of all operations, programs, activities, and affairs of the Association, as approved or delegated by the Board of Directors. The Executive Director shall have direct responsibility for employment and termination of employment and the determination of compensation for staff members within the budgetary framework determined by the Board of Directors. The Executive Director functions as the chief operating officer of the Association within the guidelines established by the policies and procedures of the Board of Directors and, as necessary, with the concurrence of the President. The Executive Director shall have such other duties as may be prescribed by the Board.

B. Other Appointed Officers

Other appointed officers shall have such duties as may be prescribed by the Board.

ARTICLE V
Nominations, Elections, Terms, and Appointments to the Board of Directors

Section 1. Nominating Committee

The Nominating Committee shall annually recommend to the Board of Directors a slate of Individual Members as potential nominees for the elected positions where vacancies will occur. The Nominating Committee shall consist of five (5) members who shall be three (3) immediate Past Presidents, as available, and two (2) Individual Members-at-Large of the Association. If three Past Presidents are not available to serve, other Individual Members-at-Large shall be appointed by the President to the extent necessary to form the five (5)-member committee.

Section 2. Elections and Terms of Office

The President-Elect, the Secretary, Treasurer, and the Directors of the Board of Directors shall be elected by a majority of Individual Members voting, from a slate of nominees recommended annually by the Board of Directors.

Terms of office for all Officers and Directors shall begin with the adjournment of the annual meeting following their election and shall end with the adjournment of the annual meeting occurring nearest the expiration of their term. The six (6) Directors shall be elected to staggered three-year terms with two Directors elected to full three-year terms each year, but not to more than two (2), consecutive, three-year terms. Appointment or election to fill an unexpired term shall not affect the eligibility of a person to subsequently be elected to two (2) full terms. The Secretary shall be elected to a one-year term and may be re-elected to successive one-year terms. The Treasurer shall be elected for a one-year term and may be re-elected to successive one-year terms. The President-Elect shall be elected to a one-year term; whereupon the current President-Elect shall become President and the current President shall become the Immediate Past President, each serving a one-year term.

Section 3. Appointments

Directors-at-Large are appointed by the Board in accordance with Article VI, Section 2. Directors-at-Large are appointed for one (1) year terms, renewable at the discretion of the elected Board.

ARTICLE VI
Board of Directors

Section 1. Composition

The Board of Directors shall consist of eleven (11) elected members to include the President, President-Elect, Secretary, Treasurer, Immediate Past President, six (6) Directors, and up to three (3) appointed Directors-at-Large, all of whom shall be Individual Members of the Association. The elected Board shall reflect the makeup of the Association membership and shall not be dominated by any single interest.

Section 2. Powers and Duties

The Board of Directors shall provide supervision, control, and direction of the affairs of the Association, shall determine the Association's policies or changes therein within the limits of the Bylaws, shall actively prosecute

its purpose, and shall have discretion in the disbursement of its funds. It may adopt such rules and procedures for the conduct of its business as shall be deemed advisable, and may, in the execution of the powers granted, appoint such agents as it may consider necessary. The Board of Directors may appoint up to three (3) Directors-at-Large, if, in their opinion, such appointments advance the purpose of the Association. Directors-at-Large shall be accorded the same voting privileges as elected Directors.

Section 3. Meetings

Except that the Board shall have a regular meeting at the time and place of the annual meeting, the Board shall meet, in person or via telephone conference call, upon call of the President at such times and places as he or she may designate within the policies adopted by the Board, and shall be called to meet upon demand of a majority of its members. Notice of all meetings of the Board of Directors shall be sent by any of the following means (whichever is deemed appropriate by the President at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission to each member of the Board at his or her last recorded address or number at least fourteen (14) days in advance of in-person meetings or forty-eight (48) hours in advance of conference call meetings.

Section 4. Quorum

A quorum for any meeting of the Board is six (6) Board members elected in accordance with Article V (1). Any less number may: (1) set a time to adjourn, (2) adjourn, (3) recess, or (4) take measures to obtain a quorum.

Section 5. Absence

Any member of the Board of Directors unable to attend a meeting of the Board shall notify the President and state the reason for his or her absence. If a member of the Board is absent from two (2) consecutive meetings, he or she may be removed by a two-thirds vote of the Board Members then in office.

Section 6. Compensation

Members of the Board of Directors, as such, shall not receive any compensation for their services as Board members, but the Board may, by resolution under policies it may adopt, authorize reimbursement of expenses incurred in the performance of members' duties. Such authorization may prescribe conditions and procedures for approval and payment of such expenses. Nothing herein shall preclude a Board member from serving the Association in any other capacity and receiving compensation for such services, if compensation is customarily paid for such services.

Section 7. Resignation or Removal

Any member of the Board may resign at any time by giving written notice to the President, Secretary, Treasurer, or to the Board of Directors. Such resignation shall take effect at the time specified therein, or, if no time is specified, at the time of acceptance thereof as determined by the President or the Board.

Any member of the Board may be removed by a three-fourths vote of the Board members then in office and present at any regular or special meeting of the Board.

Section 8. Vacancies: Members of the Board

If a vacancy should occur in the membership of the elected Board of Directors, any Past President may be appointed by action of the remaining members of the Board to temporarily fill such vacancy until the next

regularly scheduled election. At the next regularly scheduled election nominations will be presented to fill the vacancy for the unexpired portion of the term remaining.

Section 9. Vacancies: President and Other Officers

If the office of the President shall become vacant, the President-Elect shall thereupon become President of the Association for the unexpired term, followed by his or her duly elected term. In the event the office of President becomes vacant at a time when the office of President-Elect is also vacant, the Presidency shall be filled for the remainder of the term by the action of the Board of Directors. If any other officer position shall become vacant, the office may be filled for the remainder of the term by action of the Board.

ARTICLE VII
Committees

Section 1. Committee Formation

The Board of Directors shall form and adopt terms of reference for such standing or special boards, committees, subcommittees, task forces, or task groups as may be required by these Bylaws or as the Board may determine necessary to carry out the affairs of the Association.

Section 2. Committee Appointments

Subject to the requirements of these Bylaws and the specific terms of reference adopted by the Board, the President shall make the appointments to fill the vacancies occurring in the Association's standing or special boards, committees, subcommittees, task forces, or task groups.

ARTICLE VIII
Official Methods of Analysis

The Board of Directors (BoD) is empowered to develop written policies and procedures for the study, adoption, and change in status of the Official Methods of Analysis of AOAC INTERNATIONAL. Implementation of the policies and procedures shall be delegated to an Official Methods Board (OMB).

Section 1. Composition of the Official Methods Board

The Official Methods Board shall consist of a chair and a vice chair, and members who are recommended by the chair. The chair, vice chair and members are appointed by the President of AOAC INTERNATIONAL. The OMB shall be composed of members representing a balance of government, industry, and academia as appropriate to the scope of the group and shall not be dominated by any single interest.

Section 2. Purpose of the Official Methods Board

The OMB shall serve the Association in a scientific and advisory capacity on methods and the process of their adoption. The OMB shall be responsible for implementation of procedures adopted by the BoD, according to the principles in section 3 below.

Section 3. Principles of the Official Methods Program

A. Adequate records of technical data, discussions, and decisions on the study, adoption, and change of status of Official Methods of Analysis shall be maintained for a reasonable time.

B. Timely notice of proposed method studies, adoption, or change in status shall be published in an Association publication that is circulated to the members.

C. Opportunity shall be provided for materially interested parties to submit input during method study and adoption procedures and to submit comments on the adoption, use of, or change in status of specific methods.

D. Methods submitted to the OMB for inclusion in the OMA shall be thoroughly studied, scientifically reviewed, and available in published form prior to adoption as Final Action by the OMB.

E. The OMB shall adopt methods as Final Action.

ARTICLE IX
Meetings

Section 1. Annual Meeting

The annual business meeting of the Association shall be held at the time and place decided by the Board of Directors. A special meeting of the entire Association may be called by the Board of Directors; announcement thereof shall be made at least thirty (30) days prior to the time of said meeting.

Section 2. Quorum

One hundred Individual Members who are present in person or by proxy and entitled to vote shall constitute a quorum at any meeting of the Association which is duly called pursuant to the provisions of these Bylaws.

ARTICLE X
Voting

Section 1. Voting by Ballot

By direction of the Board of Directors, unless otherwise required by these Bylaws or conducted under alternative procedures established under these Bylaws, voting on any matter, including the election of officers and directors, the election of Honorary Members, amendment of the Bylaws, and the approval of dues, may be conducted by ballot of the voting membership by any of the following means (whichever is deemed appropriate at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission, and the question(s) thus presented shall be determined according to the votes received, provided in each case votes of at least five (5) percent of the voting membership shall be received. Any and all action taken in pursuance of a vote by any of the means indicated above (whichever the Board deemed appropriate at the time)

in each case shall be binding upon the Association in the same manner as would be action taken at a duly called meeting and shall become effective, unless otherwise provided for in these Bylaws or otherwise stated in the ballot, on the day following certification of the vote.

Section 2. Voting by Proxy

At any duly called meeting of Individual Members, a member-of-record, as determined thirty (30) days prior to any meeting and who is entitled to vote, may vote by proxy executed in writing by the Individual Member or his or her duly authorized attorney-in-fact. No proxy shall be valid for more than eleven (11) months after the date of its execution unless otherwise provided in the proxy.

ARTICLE XI
Earnings and Assets

Section 1. Non-Profit Status

A. Regardless of any provision of the Bylaws which may be construed otherwise:

[1] No part of the net earnings of the Association shall under any circumstances inure to the benefit of any member or individual.

[2] The Association shall not be operated for a private profit.

B. On lawful dissolution of the Association and after settlement of all just obligations of the Association, the Board of Directors shall distribute all remaining assets of the Association to one (1) or more organizations selected by the Board of Directors which have been held exempt from Federal Income Tax as organizations described in section 501(c)(3) of the Internal Revenue Code of 1954.

Section 2. Political Activities

A. No substantial part of the Association's activities shall consist of carrying on propaganda or otherwise attempting to influence local, state, or national legislation. All activities of the Association shall be determined by the Board of Directors.

B. The Association shall not participate or intervene in any manner in any campaign on behalf of any candidate for a political office.

ARTICLE XII
Sections

Section 1. Sections

The Board of Directors shall set geographic limits and grant authority to groups of Individual Members of the Association residing or working in the same geographical areas for the establishment of Sections.

Section 2. Purpose of Sections

The purpose of Sections shall be to promote and further the purpose of the Association.

Section 3. Membership in Sections

Individuals interested in the purpose of the Section shall be eligible for Section membership. Only Individual Members of the Association shall be eligible for election to the Executive Committee of the Section.

Section 4. Bylaws of Sections

Subject to approval of the Board of Directors, each Section shall adopt, for its own governance, bylaws not inconsistent with these Bylaws.

Section 5. Dissolution of Sections

When any Section shall cease to function as a Section for a period of more than one year, or if its membership shall be less than ten (10) Individual Members of the Association for a period of one (1) year, the Board of Directors may terminate the existence of such Section.

Section 6. Actions of Sections

No act of a Section or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XIII
Technical Divisions

Section 1. Purpose

Technical Divisions shall represent communities of interest within the Association which have the purpose of furthering the purpose of the Association through the development of the analytical sciences either in a commodity-based or scientific discipline-based field. Their activities shall not duplicate the organizational structure nor conflict with the policies or procedures for the adoption of official methods of analysis by the Association.

Section 2. Creation, Combination, Discontinuance, or Change

Technical Divisions may be created, existing Technical Divisions may be combined or discontinued, or the name of a Technical Division may be changed under policies and procedures adopted by the Board of Directors. Each Technical Division shall adopt bylaws not inconsistent with these Bylaws. The jurisdiction of each Technical Division shall be described in its bylaws. No act of any Technical Division or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XIV
Indemnification

The Association shall have the power to pay, by indemnity, reimbursement, or otherwise, to or for the use of any person designated by resolution of the Board of Directors who was or is a party or is threatened to be made a party to any threatened, pending, or completed action, suit, or proceeding, whether civil, criminal, administrative, or investigative (other than an action by or on behalf of the Association), by reason of the fact he or she is or was a director, officer, committee member, employee or agent of the Association, or was serving as such for another at the request of the Association, against expenses (including legal, accounting, witness and other), judgments, fines, and amounts paid in settlement so long as such person was not found by a court of competent jurisdiction to have been willfully negligent of the interests of the Association or such person had reasonable cause to believe that his or her conduct was lawful.

ARTICLE XV
Parliamentary Authority

The rules contained in the current edition of *Robert's Rules of Order Newly Revised* shall govern the Association in all cases in which they are applicable and in which they are not inconsistent with these Bylaws or any special rules of order the Association may adopt.

ARTICLE XVI
Amendments to the Bylaws

These Bylaws may be amended, repealed, or altered, in whole or in part, by a three-fourths vote: (a) of the Individual Members at any annual business or duly called special meeting of the Association, provided notice of any amendment proposed for consideration shall be sent by any of the following means (whichever may be deemed appropriate at the time): mail, telephone call, telegram, cablegram, electronic mail or other means of electronic or telephonic transmission to the last recorded address or number of each Individual Member at least thirty (30) days prior to the date of the meeting; or (b) by approval of the Individual Members through ballot sent by any means indicated above in accordance with the provisions of Article X, Voting.

All proposed amendments of these Bylaws shall be presented in writing to the Board of Directors. The Board shall present the proposals to the Association membership, with recommendations. All amendments to the Bylaws, unless otherwise stated, will become effective at the adjournment of the meeting where action is taken or on the day following the certification of a vote by mail ballot.

AOAC INTERNATIONAL
POLICY ON THE USE OF THE
ASSOCIATION NAME, INITIALS,
IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

Introduction

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.

The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.

The Scientific Association Dedicated to Analytical Excellence®

Policy

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

> "The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.
>
> The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for the purposes for which permission has been specifically granted.
>
> The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization."

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a Member of the Association.

Instructions

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.

2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.

3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.

5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.

6. Do not print one part of the logo or insignia in one color and other parts in another color.

7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, its elected and appointed officers, staff, sections, or committees; except by special permission.

   Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

   Copies of all correspondence using AOAC letterhead or conducting AOAC official business, whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

   Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.

2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.

3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

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Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

AOAC INTERNATIONAL
ANTITRUST POLICY
STATEMENT AND GUIDELINES

Introduction

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on_going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

Responsibility for Antitrust Compliance

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance __ which includes avoidance of even an appearance of improper activity __ belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

Antitrust Guidelines

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits.

Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment.
Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard.

2. Don't discuss with competitors your own or the competitors' prices, or anything that might affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.

3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.

4. Don't disclose to others at meetings or otherwise any competitively sensitive information.

5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.

6. Don't stay at a meeting where any such price or anti_competitive talk occurs.

7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.

8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.

9. Do send copies of meeting minutes and all AOAC_related correspondence to the staff member involved in the activity.

10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

Conclusion

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

* * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: March 11, 1991
Revised October 1996

AOAC INTERNATIONAL

POLICY AND PROCEDURES ON

VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

Illustrations of Conflicts of Interest

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.

2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.

3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

AOAC INTERNATIONAL

Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)

Meeting at the Gaithersburg Marriott Washingtonian Center

9751 Washingtonian Boulevard, Gaithersburg MD 20878, USA

STAKEHOLDER PANEL - DRAFT MEETING AGENDA

Wednesday, March 15, 2017

Meeting Start Time: 8:30AM (Eastern US)

SPIFAN Chair: Darryl Sullivan

(Covance Laboratories)

Location: C/D/E

(Registration Opens at 7:30AM)

I. INTRODUCTION (Goodwin/Sullivan – 8:30AM-8:45AM)

Jonathan Goodwin (AOAC)/Darryl Sullivan (Covance) will call the Stakeholder Panel meeting to order and introduce/welcome all participants. In addition, the AOAC policies for Antitrust, Volunteer Conflict of Interest, and Use of Association Name and Insignia will be reviewed.

II. AOAC SPIFAN OVERVIEW (Sullivan – 8:45AM-9:15AM)

Darryl Sullivan (Covance) will provide an overview of the activities, accomplishments and achievements of SPIFAN I & II including methods adopted First/Final Action *Official Methods*^{SM} since the last stakeholder meeting.

III. UPDATE ON INTERNATIONAL ACTIVITIES

a. **Codex Review and Adoption of AOAC SPIFAN Methods (Sullivan/Rankin – 9:15AM-9:45AM)**

Darryl Sullivan (Covance) and Robert Rankin (INCA) will provide updates and lead discussions on the progress of AOAC SPIFAN methods through the Codex system.

b. **AOAC/ISO/IDF Cooperative Update (Konings/de Vreeze/Evers – 9:45AM-10:15AM)**

Erik Konings (Nestlé/ISO) & Marcel de Vreeze (ISO) and Jaap Evers (IDF) will provide an update on ISO/IDF activities.

c. **Whey Protein: Casein Ratio (WPC) (Feng – 10:30AM-11:00AM)**

Ping Feng (Wyeth) will provide information/update on lab performance of the Whey Protein: Casein Ratio (WPC) method.

IV. NUTRIENT WORKING GROUP UPDATES

a. **Carotenoids* (11:00AM-11:30AM)**

Greg Hostetler (Perrigo) will present proposed revisions to Standard Method Performance Requirements (SMPR®) for Determination of Selected Carotenoids in Infant and Adult/Pediatric Nutritional Formula (AOAC SMPR 2014.014).

b. **Folate* (11:30AM-12:00PM)**

Erik Konings (Nestlé) will present proposed revisions to Standard Method Performance Requirements (SMPR®) for Folate in Infant Formula and Adult/Pediatric Nutritional Formula (AOAC SMPR 2011.006).

V. NEW WORKING GROUP LAUNCH

a. **2- and 3-MCPD and glycidyl esters (GE)* (12:00PM-12:30PM)**

Jan Kuhlmann (SGS Germany GmbH) will present a launch presentation including technical information, regulatory requirements, and a proposed fitness for purpose for 2 and 3-monochloro-1,2-propanediol (2- and 3-MCPD) and glycidyl esters (GE) in infant formula.

VI. UPDATE ON EXPERT REVIEW PANEL (ERP) STATUS (12:30PM-12:45PM)

An update will be provided on the March 16, 2017 AOAC SPIFAN ERP.

VII. TIMELINES/DEADLINES/WRAP-UP (12:45PM-1:00PM)

Darryl Sullivan (Covance)/Alicia Meiklejohn (AOAC) will provide a timeline of AOAC SPIFAN activities including upcoming deadlines, review of any action items, and additional questions. Robert Rankin (INCA) will discuss plans for future endeavors.

MEETING ITINERARY:

REGISTRATION (7:30AM)

MEETING START TIME (8:30AM)

MORNING BREAK (10:15AM)

LUNCH ON YOUR OWN

AFTERNOON BREAK (2:30PM)

MCPD/GE WORKING GROUP MEETING (3:00PM)

AOAC INTERNATIONAL

Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN)

Meeting at the Gaithersburg Marriott Washingtonian Center

9751 Washingtonian Boulevard, Gaithersburg MD 20878, USA

2- and 3-MCPD & GE WORKING GROUP
DRAFT MEETING AGENDA

Wednesday, March 15, 2017

Meeting Start Time: 3:00PM (Eastern US)

Working Group Chair: Jan Kuhlmann

(SGS Germany GmbH)

I. WORKING GROUP DISCUSSION (Working Group Chair – 3:00PM-5:00PM)

a. Standard Method Performance Requirement (SMPR®) Orientation (Coates)

b. Review of Endorsed Fitness-for-Purpose (Kuhlmann)

c. Standard Method Performance Requirement (SMPR®) Development (Kuhlmann/Coates)

II. IDENTIFICATION OF ADDITIONAL GLOBAL EXPERTS (Meiklejohn)

Alicia Meiklejohn (AOAC) will discuss identifying additional experts for this project.

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Darryl Sullivan, Covance Labs

SPIFAN & Working Group Chair

Darryl Sullivan is the Director of Industry and Regulatory Affairs for the Food Solutions Division at Covance Laboratories. Mr. Sullivan acts as the primary liaison with food, nutritional and dietary supplement companies as well as providing expertise on designing comprehensive testing programs to meet scientific and regulatory requirements. In this role, he is often called upon as an expert witness for litigation and dispute resolution. He has managed numerous different departments at Covance including lab operations, research and development, client services, sample management, sample preparation and study direction, as well as a satellite laboratory in Michigan.

Mr. Sullivan received his BS from the University of Wisconsin-Madison and has more than 35 years of experience in laboratory testing of food and dietary supplements. He is considered to be an expert in the field of validation of analytical methods, having served for three years as Chair of the AOAC INTERNATIONAL Official Methods Board. Mr. Sullivan was a member of the Task Force that redesigned the AOAC Standards Development Process. He is currently the Past President and Secretary of the AOAC INTERNATIONAL Board of Directors, and the Chair of the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals. He is also the Chair of the AOAC Stakeholder Panel on Dietary Supplements. He is a former member of the Board of Directors of the AOAC Research Institute. He is the Chair of the Analytical Laboratories Committee of the American Herbal Product Association, a member of the USP Expert Committee for Dietary Supplements, and is a member of the Joint Committee on Dietary Supplements of NSF. He is a member of the CRN Regulatory Affairs Committee and the NPA ComPLI Committee. Mr. Sullivan has developed and validated hundreds of analytical methods in the areas of nutrient and residue testing, and is the author of more than 75 publications and 100's of scientific presentations. In addition, he is the Past Chair of the AOAC Presidential Task Force on Dietary Supplements and co-editor of the book Methods of Analysis for Nutrition Labeling. He is also the co-editor of the book Improving Import Food Safety.

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STAKEHOLDER PANEL ON INFANT FORMULA & ADULT NUTRITIONALS (SPIFAN)

SPIFAN OVERVIEW

Darryl Sullivan

Covance Laboratories

Rockville, MD

March 15, 2017

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Outline

- Background and Overview
- About AOAC SPIFAN
- AOAC SPIFAN Accomplishments
- Activities Since September 2016
- AOAC SPIFAN Activities at AOAC Mid-Year Meeting
- AOAC Standards Development Process

Background and Overview

- AOAC infant formula methods were validated in 1980s
- New formulas exposed some gaps in validated methods
- Infant formula is highly regulated around the world

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Background and Overview (con't)

Agreement with INCA signed in 2010

- Create AOAC voluntary consensus standards for methodology for 15 sets of nutrients
- Evaluate and recommend "best" methods
- AOAC established the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) to develop the voluntary consensus standards

Second agreement with INCA signed in June 2013

- Create standards for methodology for 9 sets of nutrients
- 7 stakeholder panel, WGs, and ERP meetings between 9/2013 and 9/2016
- No cost extension signed in December 2016 through March 2017

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SPIFAN Working Groups

WG on Vitamin A	Jon DeVries	WG on Whey protein: Casein	Lei Bao & Shane Rutherfurd
WG on Vitamin D	Don Gilliland	WG on Fatty acids	Mark Hill
WG on Vitamin B12	Esther Campos-Gimenez	WG on Minerals & Trace Elements	Eric Poitevin
WG on Folic acid	Erik Konings	WG on Biotin	George Joseph/Jean-Luc Deborde
WG on Inositol	Karen Schimpf & Harvey Indyk	WG on Vitamin K	Sneh Bhandari
WG on Vitamin E	Jon DeVries	WG on FOS/GOS	Sean Austin
WG on Nucleotides	Brendon Gill	WG on Amino acids	Wesley Jacobs & Ping Feng
WG on Ultra Trace Minerals	Joe Thompson	WG on Carotenoids	Greg Hostestler
WG on Vitamin C	Jayasharee Arcot & Lalitha Gowda	WG on Fluoride and Chloride	Christopher Blake
WG on Choline	Sneh Bhandari & Rajesh Girdhar/ Nick Cellars	WG on Vitamins B_1, B_2, B_3, and B_6	Louis Salvati
WG on Pantothenic acid	Shang-Jing Pan	WG on SPIFAN Reference Materials	Dan Schmitz/Wayne Wargo
WG on Iodine	Darryl Sullivan	WG on SPIFAN Pesticide Contaminants	Joe Boison
WG on Carnitine	John Austad & Guenther Raffler	WG on SPIFAN MCPD & GE **(NEW)**	Jan Kuhlmann [5]

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AOAC ERPs for SPIFAN Methods

AOAC ERP for SPIFAN Nutrient Methods

- Chair: Darryl Sullivan

AOAC ERP for SPIFAN Whey Protein – Casein Ratio Methods

- Chair: Sarwar Gilani and Nana Farkye

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Completed Voluntary Consensus Standards Developed

SPIFAN I (SMPRs) 2011 – 2013

1. Vitamin A
2. Vitamin B12
3. Vitamin D
4. Folate
5. Inositol
6. Vitamin E
7. Whey Protein : Casein
8. Fatty Acids (ISO)
9. Carnitine
10. Vitamin C (India 2012)
11. Choline (India 2012)
12. Pantothenate
13. Iodine
14. Ultra Trace Minerals (Mo, Se, Cr)
15. Nucleotides

SPIFAN II (SMPRs) 2013 – 2016

16. Vitamin K
17. FOS
18. GOS
19. Biotin
20. Minerals
21. Amino Acids
22. Carotenoids
23. Fluoride
24. Chloride
25. Vitamin B1 (thiamine)
26. Vitamin B2 (riboflavin)
27. Vitamin B3 (niacin)
28. Vitamin B6 (pyridoxine)

AOAC Working Group Initiative (SMPRs) 2015

29. Compound 1080

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AOAC SPIFAN Accomplishments

29	Voluntary Consensus Standards – Standard Method Performance Requirements (SMPRs®)
51	First Action *Official Methods of Analysis*℠ adopted using SMPRs
11	First Action *Official Methods of Analysis*℠ recommended for Final Action status
11	Number of Final Action *Official Methods of Analysis*℠ Approved
9	Number of Final Action *Official Methods of Analysis*℠ submitted to Codex*

Agreement with ISO to participate in each other's standards development activities – *for milk and milk products*

*AOAC, ISO, and IDF jointly submitted methods to Codex

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Activities Since September 2016

Working Groups re-engaged to revise SMPRs for carotenoids and folate

- Carotenoids and folate working groups met and revised the SMPRs
- Fluoride was included in the Call for Methods

Codex and International Activities

- Vitamin C method was submitted to CCNFSDU in December
- CCNFSDU Meeting in December 2016 – update to be provided during this meeting
- ISO and IDF Activities Updates will be provided during this meeting

AOAC and INCA signed a no-cost extension for SPIFAN II, SPIFAN III negotiations continue

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Activities Since September 2016 (con't)

ERP for SPIFAN Nutrient Methods

- ERP down-selected between two First Action FOS methods for MLT
- Review team reviewed submitted methods for Amino acids and Carotenoids
- OMB moved 2015.06 (MTE) and 2014.02 (vitamin B12) to Final Action status

AOAC Nutrients in Infant Formula and Adult Nutritionals Proficiency Testing

- New program will include vitamins A, E, B12, fatty acids, iodine, nucleotides, Pantothenic Acid, chromium, selenium and molybdenum (UTM) and myo-inositol
- Projected March 2017 start date has been postponed until June 2017
- Enrollment is open from the AOAC website

New Work and Future Endeavors

- New working group initiative, sponsored through INCA for MCPD and GE in infant formula
- AOAC and INCA in negotiation for SPIFAN III

AOAC Mid-Year Meeting Activities

- Working Group Chair proposals for revisions to the SMPRs for carotenoids and folate
- Updates on international activities
  - Experience of methods going through Codex process
  - ISO and IDF Activities Update
  - Update on Whey Protein-Casein Ration Method in China
- Update on AOAC Proficiency Testing Program for Infant Formula
- Launch of new SPIFAN Working Group on MCPD & GE
  - Working Group to meet following SPIFAN meeting
- Ideas for SPIFAN future endeavors
- ERPs meet on Thursday, March 16, 2017 to review methods for First Action, review of First Action OMAs for Final Action recommendation

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This SPIFAN Meeting

Stakeholders will be asked to demonstrate consensus on the following:

- Draft revision of the SMPR for Carotenoids
- Draft revision of the SMPR for Folate
- Draft Fitness for Purpose Statement for MCPD & GE

Working Group on MCPD and GE

- Will have initial meeting following SPIFAN meeting
- Subsequent meetings will be via teleconference

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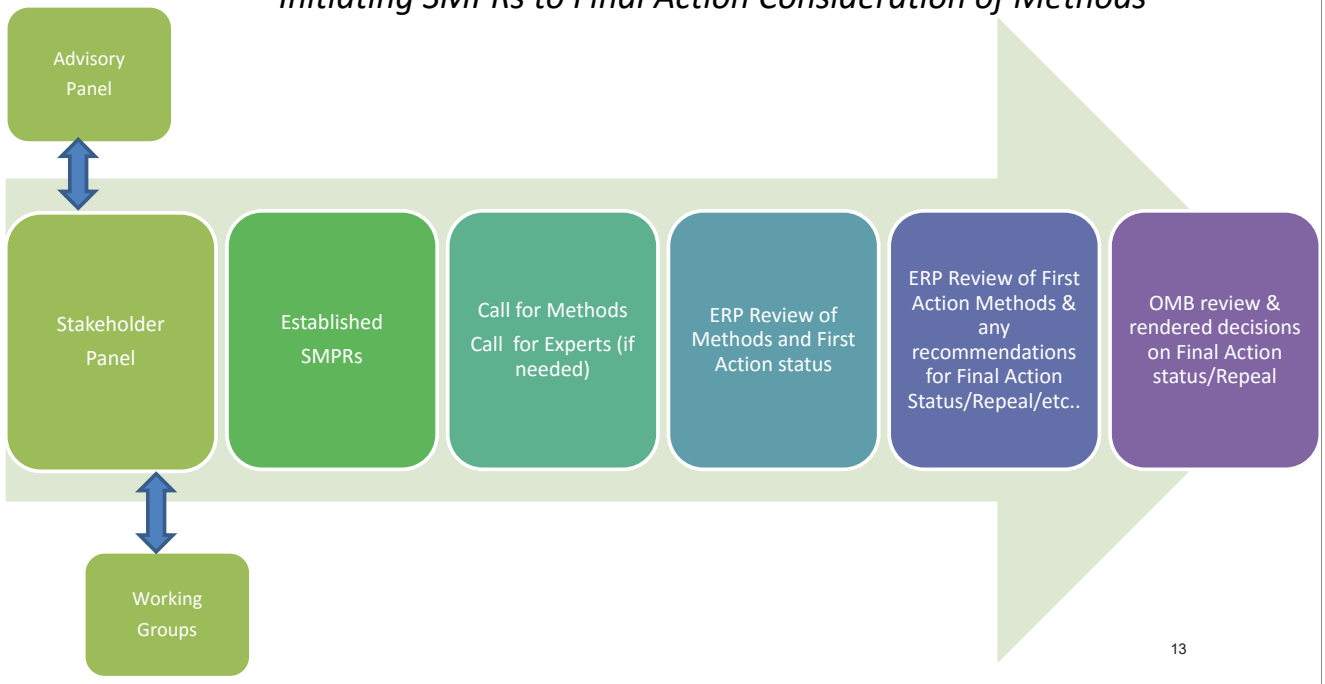
AOAC Standards Development

Initiating SMPRs to Final Action Consideration of Methods



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Stakeholder Panel Working Groups

- Present background and history on nutrient methods for stakeholder panel
- Develop draft SMPR
- Will present motions to the stakeholder panel on components of the standard method performance requirements
- Can participate in SPIFAN related in-person meetings

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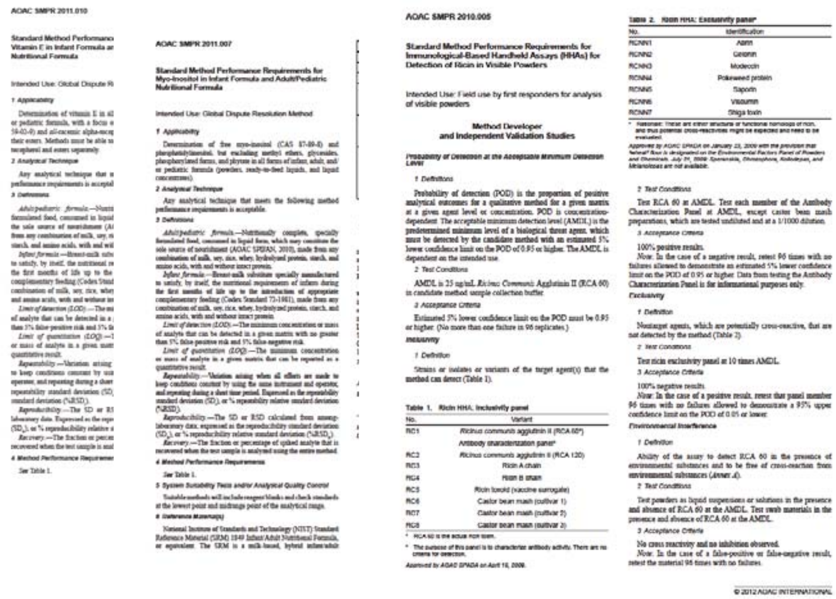
Standard Method Performance Requirements

- ***Commonly referred to as - SMPRs***


AOAC SMPR 2011.007

Standard Method Performance Requirements for Myo-Inositol in Infant Formula and Adult/Pediatric Nutritional Formula

AOAC SMPR 2010.005

Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of Ricin in Visible Powders

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SMPRs

- Documents a community's analytical method needs
- Very detailed description of the analytical requirements
- Includes method acceptance requirements
- Used to qualify methods for AOAC approval in the *Official Methods*[SM] program
- Published as a standard

Performance requirements parameters for quantitative methods

- Analytical Range
- Limit of Detection
- Limit of Quantitation
- Repeatability
- Recovery
- Reproducibility

4. Method Performance Requirements

Analytical range	0.01–5.0[a]	
Limit of detection (LOD)	≤0.004[a]	
Limit of quantitation (LOQ)	≤0.01[a]	
Repeatability (RSD_r)	0.01[a]	≤15%
	0.2[a]	≤7%
	0.5[a]	
	5.0[a]	
Recovery	0.01[a]	90–110%
	0.2[a]	
	0.5[a]	
	5.0[a]	
Reproducibility (RSD_R)	0.3	≤11%
	0.6	
	1.0	
	2.5	
	5.0	
Concentrations apply to (*1*) "ready-to-feed" liquids "as is"; (*2*) reconstituted powders (25 g into 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight.		
[a] µg/100 g expressed as cyanocobalamin in reconstituted final product.		

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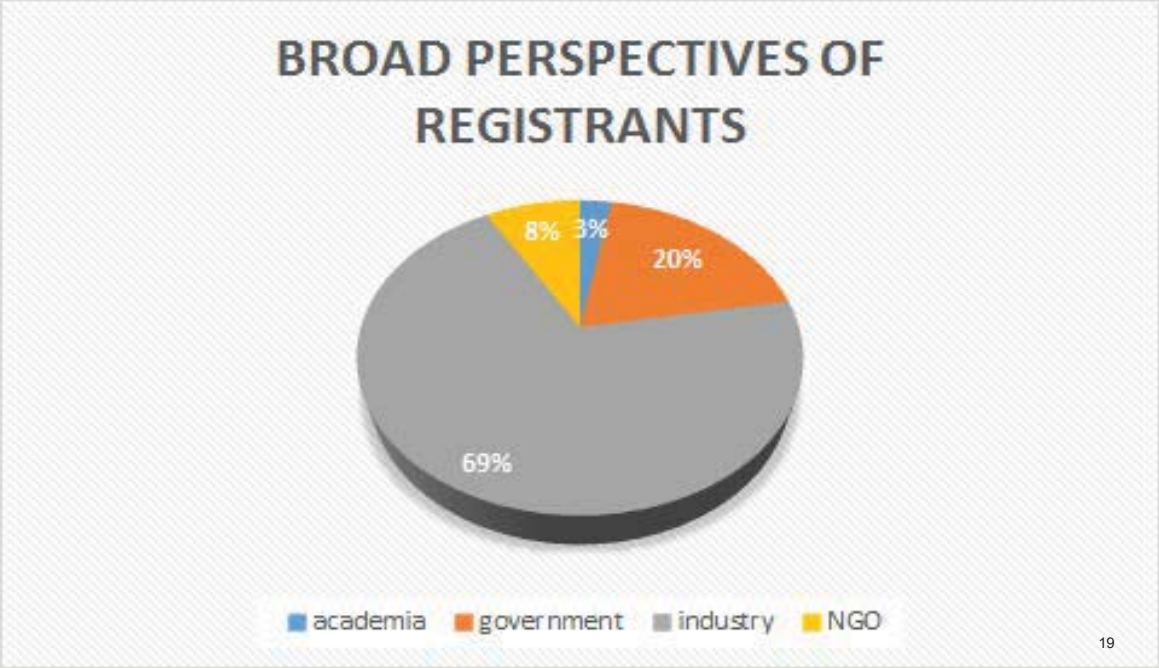
SPIFAN Organizational Registrants

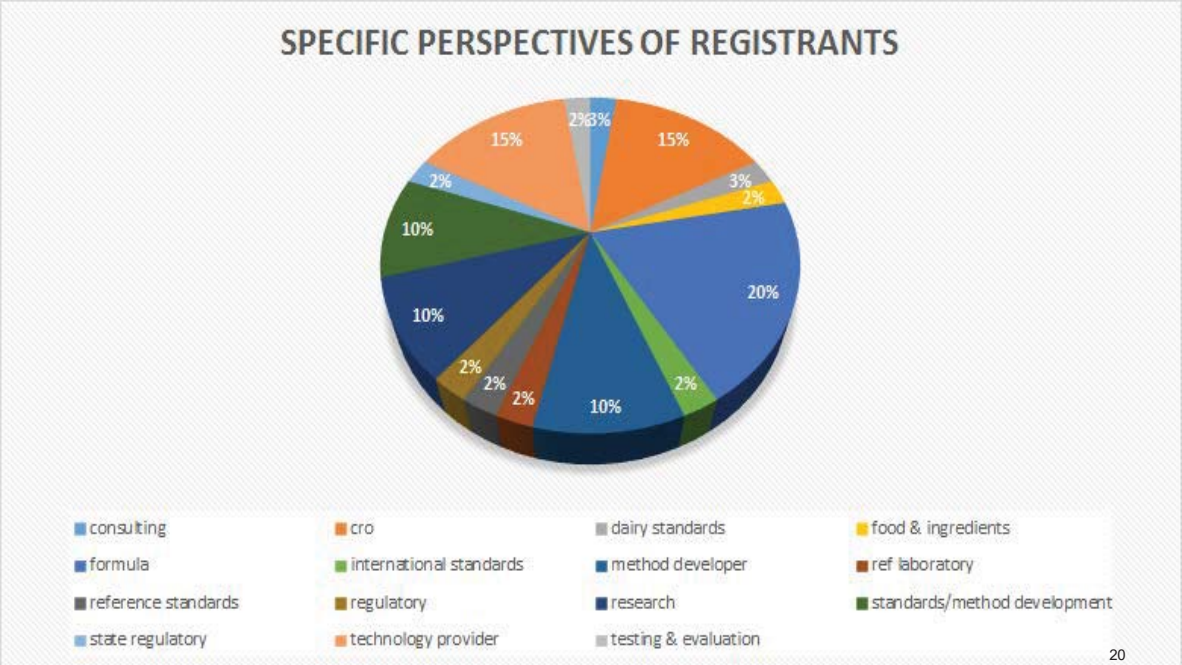
Abbott Nutrition
Agilent Technologies Brasil Ltda.
Agilent Technologies, Inc.
Archer Daniels Midland Company
AsureQuality, New Zealand
Ausnutria Hyproca
BioAnalyt GmbH
Covance Laboratories
Danone
DuPont Nutrition & Health
Eurofins
First Source Laboratory Solutions LLP
Florida Department of Agriculture And Consumer Services
Fonterra Co-operative Group Ltd.
Food Consulting Services
FrieslandCampina
GAAS Analytical
IDF
Infant Nutrition Council of America (INCA)
ISO
LATU - Chromatography and Mass Spectrometry
Department-Method Development Depart
Mérieux NutriSciences
National Institute of Industrial Technology - Food Science Centre
National Institute of Nutrition and Seafood Research
Neogen Corporation
Nestle Research Center
Office of Dietary Supplements, NIH
Perrigo / PBM Nutritionals
R-Biopharm Rhone Ltd
Rheonix
RIKILT
SGS Germany GmbH
Shimadzu Scientific Instruments, Inc.
US FDA
US NIST
VUV Analytics, Inc
Waters Corporation

As of March 1, 2017

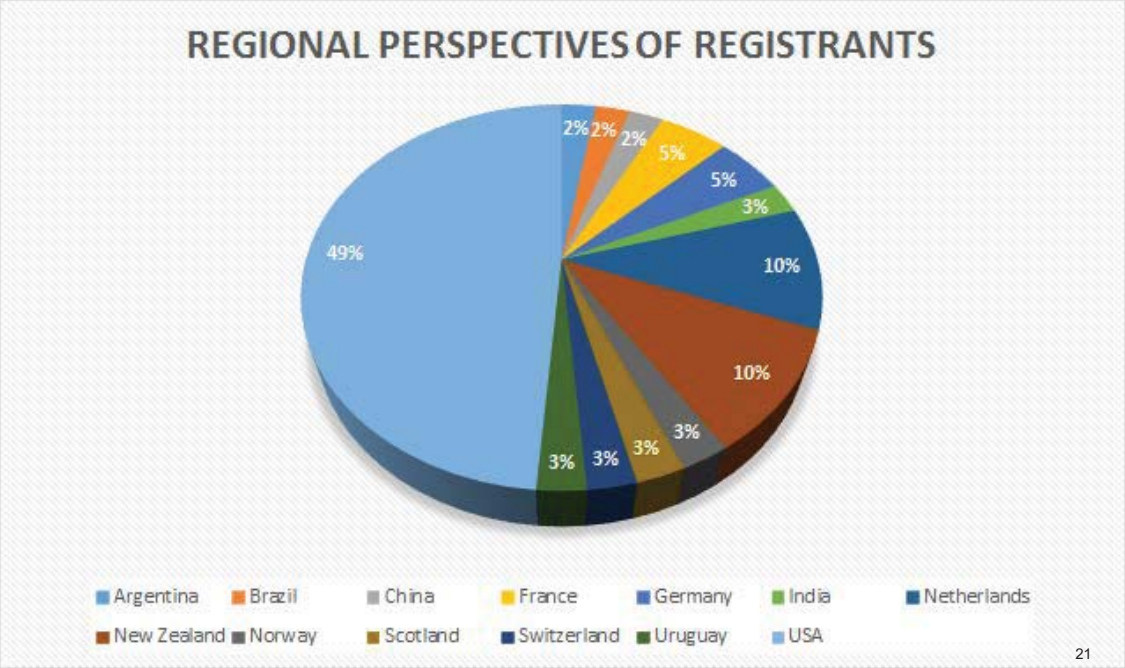
SPIFAN Registrants by Broad Perspectives



SPIFAN Registrants by Specific Perspectives



SPIFAN Registrants by Regions



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SPIFAN Representative Voting Members

RIKILT	Perrigo-PBM Nutritionals
US FDA	Danone
LATU	Agilent
FL Dept. Ag.	SCIEX
US NIST	Waters/Shimadzu
INTI	Phenomenex/VUV Analytics
ISO	R-Biopharm Rhone
IDF	First Source Laboratory Solutions
Abbott Nutrition	AsureQuality
Mead Johnson	Merieux NutriSciences
Nestle	Eurofins
FrieslandCampina	SGS Germany
Fonterra	

alternates

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Documentation and Publications

Documentation

- AOAC carefully documents the actions and decisions of the Expert Review Panel
- AOAC will prepare summaries of the meetings
  - Communicate summaries to the Expert Review Panel members and method authors
  - Publish summaries in the *Referee* section of AOAC's *Inside Laboratory Management*

Publication

- AOAC publishes adopted methods in:
  - *Official Methods of Analysis of AOAC INTERNATIONAL*
  - *Journal of AOAC INTERNATIONAL*
- AOAC publishes the status of methods in the Referee section of AOAC's *Inside Laboratory Management*

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Related Roles and Responsibilities

- Official Methods Board
  - Vet and approve stakeholder panel chair & representative voting members for each meeting
  - Vet and approve ERP membership
  - Review ERP recommendations and render decisions regarding Final Action, Repeal or continuance
  - Assign OMB representative to serve as a resource to stakeholder panels and ERPs
- AOAC Stakeholder Panels
  - Meet in person to develop standards
  - Assign working groups to draft standards method performance requirements
  - Vetted representative voting members demonstrate consensus on behalf of stakeholders
- AOAC Working Groups
  - Draft standards, reconcile comments, present draft standards to stakeholder panel for approval
- AOAC Expert Review Panels
  - Review methods and meet in person to render decisions on methods for First Action Official Methods status.
  - Track First Action Official Methods and modify, if necessary
  - Recommend First Action methods ≤ 2 years to OMB for Final Action, continuance, or Repeal
- AOAC Staff
  - Business direction
  - Manage/coordinate stakeholder panel, working group and expert review panel activities and meetings
  - Issue any calls for experts and methods
  - Provide trainings and orientations
  - Maintain website and communication
  - Document and publish actions and decisions
  - Publish approved standards and methods
  - Coordinate comments on standards and methods

Contact Information

SPIFAN Chair:

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Covance Laboratory
Email: darryl.sullivan@covance.com
Telephone: (608) 232-2711

Jonathan Goodwin – Interim Executive Director, jgoodwin@aoac.org, (301) 924-7077 x104

Alicia Meiklejohn – Director, Scientific Business Development, ameiklejohn@aoac.org, (301) 924-7077 x101

Scott Coates – Chief Science Officer, scoates@aoac.org, (301) 924-7077 x137

Deborah McKenzie – Sr. Director, Standards Development & Research Institute, dmckenzie@aoac.org, (301) 924-7077 x157

Delia Boyd – Program Manager, Standards Development, dboyd@aoac.org, (301) 924-7077 x126

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Questions?

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Robert Rankin, INCA

Robert Rankin is Executive Director of the Infant Nutrition Council of America (INCA), an association of manufacturers and marketers of formulated nutrition products, including infant formulas and adult nutritionals. Robert has worked with INCA since 2005, and has addressed a number of regulatory, legislative, technical and other issues on behalf of the infant formula industry. Robert works closely with officials at key US Government agencies including the US Food and Drug Administration and US Department of Agriculture. He has also worked with the Centers for Disease Control and Prevention and other US/international government agencies on industry issues. He has extensive experience in the development and communication of industry positions and has testified before U.S. state legislatures, the World Health Organization and local authorities.

Since 2010, Robert has managed Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Project on behalf of the infant formula industry. Through SPIFAN, voluntary consensus standards and internationally recognized methods of analysis for over 40 nutrients in infant formula have been developed with the ultimate goal of having the methods adopted by Codex Alimentarius as Type II dispute resolution methods.

Robert Rankin is a Vice President at Kellen, a global professional services firm specializing in trade associations, professional societies and communications. In addition to INCA, Robert also serves as President of the Calorie Control Council and Executive Director of the International Food Additives Council. Prior to Kellen, Robert spent two years at the Grocery Manufacturers Association where he worked in the Federal Affairs and Scientific & Regulatory Departments. Robert has a BA in Public Policy Studies from Duke University and lives in Maryland with his wife and two children.

Codex Review and Adoption of AOAC SPIFAN Methods

Robert Rankin
Executive Director
Infant Nutrition Council of America

Background

- SPIFAN began as a result of trade disputes over nutrient levels in products and because Codex infant formula methods were outdated and/or not properly validated
- Original SPIFAN Goal = develop new methods to replace existing Codex Type II methods for dispute resolution purposes
- Manufacturers produced SPIFAN sample materials which were used for SLV and MLT studies
- AOAC partnered with ISO and IDF to collaborate on SPIFAN methods
- Joint AOAC/ISO/IDF SPIFAN methods submitted to Codex as Type II

CODEX Progress to Date

- INCA formed SPIFAN Codex Strategy Group to discuss plans/actions
- INCA worked with US Delegation to the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) to explain SPIFAN and gain support for introducing SPIFAN methods
- SPIFAN Codex Strategy Group developed supporting materials and conducted outreach to key Member Country delegations
- SPIFAN stakeholders (INCA, AOAC, ISO, IDF, industry reps) attend Codex meetings to lobby delegations

CODEX Progress to Date (Nov 2015)

- CCNFSDU considered 8 SPIFAN methods
  - Vitamins A/E
  - Vitamin B12
  - Pantothenic Acid
  - Myo-inositol
  - Fatty Acids
  - Nucleotides
  - Iodine
  - Chromium/Molybdenum/Selenium
- All eight were referred to the Codex Committee on Methods of Analysis and Sampling (CCMAS) for technical review and typing in February 2016

CODEX Progress to Date (Feb 2016)

- CCMAS endorsed methods for Vitamin A, Pantothenic Acid, Nucleotides and Iodine as Type II
- CCMAS endorsed method for Cr/Mo/Se as Type III
  - Also noted no methods meet Codex minimum level criteria so suggested criteria approach for these analytes
- CCMAS endorsed methods for Vitamin B12 and Fatty Acids as Type II but requested CCNFSDU clarification regarding existing methods
- CCMAS endorsed methods for Vitamin E and Myo-inositol provided CCNFSDU confirms forms are in accordance with Codex Infant Formula Standard

CODEX Progress to Date (Jul 2016)

- Codex Alimentarius Commission (CAC) originally proposed to adopt methods for Vitamin A, Pantothenic Acid, Nucleotides as Type II
  - INCA petitioned to also have method for Iodine adopted as Type II and Cr/Mo/Se adopted as Type III
- US Codex office supported our position and CAC adopted:
  - Vitamin A, Pantothenic Acid, Nucleotides, and Iodine as Type II
  - Cr/Mo/Se as Type III

CODEX Progress to Date (Dec 2016)

- SPIFAN Codex Strategy group:
  - Developed strong responses to CCNFSDU regarding Vitamin B12 and Fatty Acids
  - Developed robust rationale in support of Vitamin E and Myo-Inositol as Type II
  - Abbott conducted additional testing re Cr/Mo/Se method
- CCNFSDU agreed:
  - Make B12 and Fatty Acid methods Type II
  - Make Vitamin E and Myo-inositol methods Type II
  - Advance Cr/Mo/Se method as Type II
- CCNFSDU also introduced Vitamin C method

Next Steps (May 2017)

- CCMAS consider recommendation from CCNFSDU, including new LOQ data for Cr/Mo/Se method and SPIFAN argument against criteria method, and possibly recommend as Type II
- CCMAS review Vitamin C method and possibly recommend as Type II

Next Steps (Jul 2017)

- CAC adopt the following methods as Type II:
  - Vitamin B12
  - Fatty Acid Profile
  - Vitamin E
  - Myo-inositol
- CAC may adopt the following as Type II:
  - Chromium/Molybdenum/Selenium
  - Vitamin C

Additional Next Steps (Dec 2017)

- Possible introduction of additional methods at CCNFSDU:
  - Biotin
  - Chloride
  - Minerals and Trace Elements
  - Vitamin D

Upcoming CODEX Meetings

- CCMAS 38 – May 8-12, 2017 (Budapest)
- CAC 40 – July 17-22, 2017 (Geneva)
- CCNFDSU 39 – December 4-8, 2017 (Berlin)

Questions?

Thank you!

Robert Rankin
Executive Director
Infant Nutrition Council of America
rrankin@kellencompany.com

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Erik Konings, Nestlé

Working Group Chair

Erik Konings studied higher professional laboratory education with majors in analytical and clinical chemistry. After graduating in 1984, he started his professional career at the then called Food Inspection Service in Maastricht, the Netherlands. In 2001 he completed his PhD study “Dietary folates in human nutrition” at Maastricht University. During this study, he obtained an MSc-degree in epidemiology. He is (co)author of more than 30 scientific publications. In September 2008, he started at the European Food Safety Authority (EFSA) in Parma, Italy, for a secondment as Scientific Officer at the Data Collection and Exposure Unit, and from there accepted, in June 2009, a position at the Nestlé Research Centre in Lausanne, Switzerland, currently in a role as Food Safety & Quality expert. He is active in several Standard Developing Organisations as AOAC INTERNATIONAL (Past-President), ISO, CEN, and IDF, and participates in the Codex Committee on Methods of Analysis and Sampling (CCMAS).

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Jaap Evers, Fonterra

IDF Representative

Jaap has over 30 years of combined experience in analytical R&D and methodology development, quality assurance, global harmonisation of analytical standards and regulatory advocacy. He started his career in 1984 as a research chemist in an industrial laboratory in the Netherlands and joined the New Zealand dairy sector in 1988 where he had several senior technical, R&D and managerial roles. He holds two 0.5 FTE roles, i.e. *Senior Manager Regulatory Affairs* in Fonterra's corporate regulatory team, and *Leader – Global Standards* for the International Dairy Federation. Both roles focus strongly on international harmonization of standards affecting the global dairy sector.

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STAKEHOLDER PANEL ON INFANT FORMULA & ADULT NUTRITIONALS (SPIFAN)

AOAC-ISO-IDF Collaboration

Erik Konings, Marcel de Vreeze, Jaap Evers

Gaithersburg, MD

March 15, 2017

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Status methods within ISO/IDF

- ISO TC 34 WG 14 (Vitamins, carotenoids, and other nutrients)
- ISO TC 34 SC 5 (Milk & Milk products), IDF

ISO TC 34/WG 14

Agreed Work Items SPIFAN	Nutrient	Stage ISO draft	Expected pub. DIS	Expected pub. final version
To be completed	Vitamin C	ISO/DIS 20635		June 2017 (before CAC)
1	Vitamin D	ISO/CD 20636	April 2017	December 2017
2	Biotin	ISO/NWIP	October 2017	June 2018
3	Vitamin K	ISO /CD 21446	November 2017, If MLT data available	July 2018
	Vitamins B	ISO/NP 21470	November 2017, If MLT data available	July 2018
	Choline/carnitine	ISO/NP 21468	November 2017, If MLT data available	July 2018
Not started	Folates			
Not started	Carotenoids			

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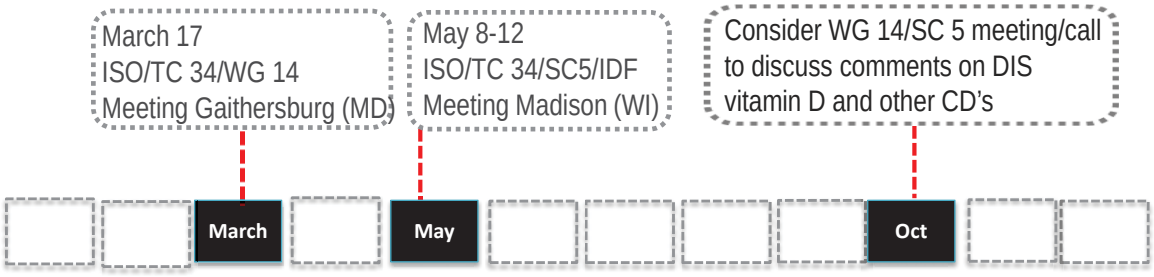
ISO TC 34/SC 5/IDF

Agreed Work Items SPIFAN	Nutrient	Stage ISO draft	Expected pub. DIS	Expected pub. final version
1	Chloride	ISO/NP 21422	April 2017	December 2017
2	Minerals and trace elements by ICP-AES	ISO/NP 15151	April 2017	December 2017
3	Minerals and trace elements by ICP-MS	ISO/NP 21424	April 2017	December 2017
4	FOS	ISO/NWIP		

2017 timeline ISO/IDF meetings



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Summary Status

- Decision needed on next work items for ISO TC 34/WG14.
- In principle all relevant methods can be available as ISO/DIS by next submission to CCNFSDU.
- ISO Feedback on methods available in October 2017, last alignment with AOAC methods before finalization for CCNFSDU submission.

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Ping Feng, Wyeth Nutrition

Working Group Chair (Amino Acids)

Ms. Ping Feng joined Wyeth Nutrition as a Biochemist in 1991. Since then, she has contributed to several infant formula protein innovations including first age products with hydrolyzed protein, soy protein, and whey protein with enriched alpha-lactalbumin. Each of these innovations was supported by the development and validation of methods for amino acid analysis, individual intact protein quantification and molecular weight profile determination. In preparation for product launches, she transferred analytical assays to Quality labs of the lead manufacturing site. In 2006, Ms. Feng was appointed as manager of the protein analytical lab and in this role, provided managerial and technical leadership. Her bilingual skills in English and Mandarin have also been instrumental in the technical support she provides to Quality and Regulatory teams in China.

Ms. Feng's laboratory expertise includes proficiency with HPLC, UPLC, and all aspects of protein and amino acid analysis in infant formula matrix and biological samples. Her methods for alpha-lactalbumin using gel permeation chromatography and amino acids using UPLC have been presented at AOAC. Her 9- country human milk amino acid composition results have been presented at Experimental Biology.

In August 2013, Ms. Feng was awarded **2013 Single Laboratory Validation Study of the Year** by AOAC for the study entitled*: AOAC Official Method 2012.08 Whey Protein Content in Milk-Based Infant Formula Finished Products Using Amino Acids Calculation Method.* The related method was published at *Journal of AOAC International* (2013) 98, 4, 795-797.

After leaving Wyeth Nutrition in June 2013 due to the lab closing, Ms. Feng currently works as a consultant for analytical method validation following ICH guidelines at Alcon/Novartis in Fort Worth, TX.

Prior to joining Wyeth, Ms. Feng worked as a project liaison in Ministry of Petroleum Industry of China and a chemical engineer in the environmental industry. Ms. Feng graduated from Beijing Institute of Technology with a BSc in 1982 in Chemical Engineering and a MS from West Chester University in 1990 in Analytical Chemistry. She also completed course work for Drug Development in QA/RA and obtained a Certificate from Temple University.

WHEY PROTEIN DETERMINATION METHOD AND GB IN CHINA

Ping Feng, Wyeth Nutrition

March 14, 2017

Wyeth | Nutrition

GB's Definition and Coverage

- GB is the abbreviation of national standard in Chinese.
- GB may represent a series of regulations for products;

  Example:

  GB 10765-2010 states that "as to milk-based first age infant formulas, the content of whey protein should be ≥60%".
- GB can also represent a series of analytical methods.

  Example:

  Draft GB 5413 contains two analytical methods for the determination of whey protein content in infant formulas: Method I based on LC/MS; Method II based on amino acids.

GB Analytical Methods (1)

- Most current GB analytical methods for infant formulas are:
  - Either modified from or replacing existing methods
  - Reviewed and updated every 5 years
- The methods are assigned (after evaluating applications) with government budget, developed and have to be submitted by Chinese government labs through a special government channel for guaranteed objectiveness and fairness.
- Submitted methods will be evaluated by a group of experts (same as ERP), and finally approved by an administrative authority body.

GB Analytical Methods (2)

- Submitted methods should be scientifically sound with literature sources; not conflict, cross and overlap with other current regulations and GBs.
- Submitted methods should be compared to national and international related standard methods; similarity and differences should be detailed. If differences exist, a scientific explanation is needed.
- If the method is innovative, 3 qualified labs (other than submitter) are needed for the validation;
- If the method is the same level as the international standard, 1 lab is needed for the validation;
- If the method is a modification from an international standard, at least 1 more lab (other than the submitter) is needed for the validation.

GB Analytical Methods (3)

- However, when updating the method or for a new analyte, GB will encourage international literature search; and internationally published or accepted methods are respected, such as by AOAC.
- An AOAC adopted method will be the first consideration.
- Published in Journal of AOAC is the next.
- If an international standard is adopted, a full Chinese translation is needed.
- CFSA (China national center for Food Safety risk Assessment) is in charge of method evaluation.

GB Method Validation Guideline

- Specification
- LOD: 3 x SD (blank) or 3 x S/N.
- Linearity:
  Cover at least 5 concentration points, including LOQ;
  Analytical range cover the analytical concentration;
  $R^2 \geq 0.99$.
- Accuracy:
  Either: Trueness (known concentration reference standards);
  Or 3 levels of spike-recoveries, including LOQ, in sample concentration and highest concentration with at least 3 replicates for each level.
- Precision:
  Repeatability, 6 replicates for each concentration level;
  Reproducibility, 3 labs other than method submitting lab.
- Robust (optional)
- Uncertainty (optional)

Whey protein analysis method using SDS-CGE

- Wyeth/Nestle has developed a whey protein analysis method using SDS-CGE responding to the need of the Chinese regulation of GB 10765-2010 for “milk based first age infant formulas, the content of whey protein should be ≥60%”.
- The method is innovative (no similar method for infant formulas published or used before); a single lab validation by Wyeth/Nestle and an MLT with 4 qualified Chinese government labs have been finished, and the results are promising.
- A linearity test by Wyeth/Nestle, 3 levels spike-recovery tests with 4 labs, and precision test with repeatability, intermediate precision and reproducibility by 4 labs were done following combined GB guidelines and AOAC requirements.

  note: not all validation parameters are tested since the method is not a quantification method based on a calibration curve, but on area normalization.
- Although an officer has commented that fit for purpose methods proposed by reputable manufacturers should be considered, the method still needs to be submitted via a Chinese government lab.

Summary of Method Validation Results for Whey Protein Analysis Using SDS-CGE(1)

Parameter	Acceptance criteria (SPIFAN/SMPR)	Results
Accuracy	The % recovery must lie within the range 95% - 105% of theoretical	% Recovery Range 97.6% - 102.2% in 3 levels and in 4 labs
Precision – Repeatability	%RSD ≤ 3.0% for whey protein g/100 g protein	0.2% – 2.1% RSD in 4 labs for 4 different infant formula samples
Precision- Intermediate Precision	%RSD ≤ 3.0% for whey protein g/100 g protein	0.3% - 2.2% RSD in 4 labs for 4 different infant formula samples
Precision – Reproducibility	%RSD ≤ 6.0% for whey protein g/100 g protein	0.9% - 3.8% RSD in 4 different labs for 21 different infant formula samples

Summary of Method Validation Results for Whey Protein Analysis Using SDS-CGE (2)

Parameter	Acceptance criteria (SMPR)	Results
Specificity – Matrix Interference	Electropherograms from injections of purified water and processed formulation matrix without protein ingredients will be evaluated for presence of peaks at the migration times corresponding to analyte proteins related peaks	No interfering peaks were observed for purified water and processed formulation matrix
Quantitation Limit	≤10 whey protein g/100 g protein	20%
Linearity	The R^2 must be ≥0.99. The residuals on the residual plot should be randomly distributed around zero.	Linear of R^2 0.993-0.999 for the area ratio of whey protein to casein Logarithm of R^2 0.993-0.996 for whey protein as % of total protein.
Range		Range – 20% to 80% of whey protein in total protein in infant formulas in tested linear range

Thank you!

The raw data of the SLV and the MLT of 4 Chinese labs are available upon request.

The SLV is published on J. AOAC, the electronic version can be found with link:
http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac/pre-prints/content-16-0344
The manuscript is being printed in the Mar/Apr 2017 Journal issue, 16-0344.R1 - Quantification of whey protein content in infant formulas by SDS-CGE (Sodium Dodecyl Sulfate – Capillary Gel Electrophoresis): First Action 2016.15 Single Lab Validation.

- Ping Feng, Wyeth Nutrition, 30F, CITIC Square, 1168 Nan Jing Rd. (W), Shanghai 200041, China
  Email: ping.feng@wyethnutrition.com; phone: 18521400044
- Christophe Fuerer, Nestec SA, Lausanne, Switzerland; Email: Christophe.Fuerer@rdls.nestle.com
- Adrienne McMahon, Wyeth Nutrition, Askeaton, Ireland; Email: Adrienne.Mcmahon@wyethnutrition.com

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Gregory Hostetler, Perrigo

Working Group Chair (Carotenoids)

Dr. Gregory Hostetler earned his B.S. in Horticulture from the University of Washington, M.S. in Pomology from Cornell University, and his Ph.D. in Food Science from the Ohio State University. His academic research focused on the effects of food processing on the absorption of phytochemicals and the role of phytochemicals in attenuating chronic inflammation. Since joining the Analytical Research and Development team at Perrigo Nutritionals, his work has included analysis and method development for vitamins, carotenoids, lipids, and allergens. Greg has been active in AOAC since 2011 and also serves in the SPIFAN Matrices Working Group and the SPSFAM Allergens Working Group.

AOAC SMPR 2017.XXX; Version 2; February 9, 2017

Method Name: **Determination of α-Carotene in Infant and Adult/ Pediatric Nutritional Formula**

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. **Applicability:**

Determination of total[1] α-carotene (CAS 7488-99-5), in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2. **Analytical Technique:**

Any analytical technique that meets the following method performance requirements is acceptable.

3. **Definitions:**

Accuracy (Corresponds to the VIM definition for "trueness").

The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

α-Carotene

IUPAC name: 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-2-en-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene, CAS number: 7488-99-5). Figure 1.

Infant formula

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 (Codex Standard 72 – 1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

[1] Include *cis* and *trans* isomers if they are separated

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period.

Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

4. **Method Performance Requirements:**
See Table 1.

Table 1. Method Performance requirements[a]

Analytical range	1–50[b]
Limit of Quantitation (LOQ)	≤ 1[b]
Recovery	90-110%
Repeatability (RSD_r)	8%
Reproducibility (RSD_R)	15%
[a]Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.	
[b] µg /100 g reconstituted final product	

5. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range. Methods must be capable of resolving α-carotene from lycopene and β-carotene.

6. **Reference Material(s)**: Neither NIST nor JRC produce a certified reference material for α-carotene in infant formula.

7. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.

8. **Maximum Time-To-Result**: No maximum time.

Figures:

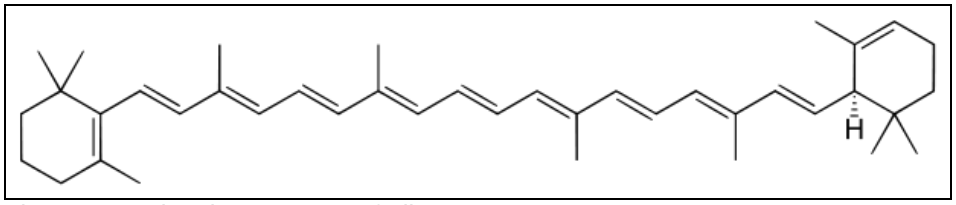

Figure 1: Molecular structure of all-*trans* α-carotene

AOAC SMPR 2017.XXX; Version 3; February 9, 2017

Method Name: Determination of β-Carotene in Infant and Adult/ Pediatric Nutritional Formula

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. Applicability:

Determinations of all-*trans* β-carotene (CAS 7235-40-7) and *cis* isomers of β-carotene in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2. Analytical Technique:

Any analytical technique that meets the following method performance requirements is acceptable.

3. Definitions:

Accuracy (Corresponds to the VIM definition for "trueness").

The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

β-Carotene

All-*trans* beta-carotene (IUPAC name: 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene, CAS number: 7235-40-7) and its *cis* isomers. Figure 1.

Infant formula

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 (Codex Standard 72 – 1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period.

Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

4. Method Performance Requirements:
See Table 1.

Table 1. Method Performance requirements[a]

Analytical range	1–1300[b]
Limit of Quantitation (LOQ)	≤ 1[b]
Recovery	90-110%
Repeatability (RSD_r)	
1-100[b]	8%
>100-1300[b]	5%
Reproducibility (RSD_R)	
1-100[b]	15%
>100-1300[b]	10%
[a]Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.	
[b] µg /100 g reconstituted final product; range and LOQ are based on total of *cis*+*trans* isomers.	

5. System suitability tests and/or analytical quality control:
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range. Methods must be capable of resolving β-carotene from α-carotene and lycopene.

6. **Reference Material(s)**:
SRM 1869. Please contact Dr. Melissa Phillips, Research Chemist, NIST for materials at melissa.phillips@nist.gov or (301) 975-4134.

7. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.

8. **Maximum Time-To-Result**: No maximum time.

Figures:

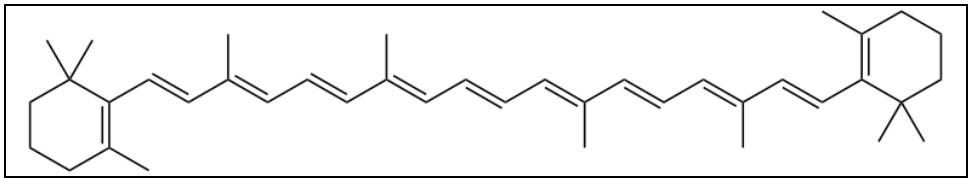

Figure 1: Molecular structure of all-*trans* β-Carotene

AOAC SMPR 2017.XXX; Version 3; February 9, 2017

Method Name: **Determination of Lutein in Infant and Adult/ Pediatric Nutritional Formula**

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. **Applicability:**

Determinations of all-*trans* lutein (CAS 127-40-2) and *cis* isomers of lutein in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2. **Analytical Technique:**

Any analytical technique that meets the following method performance requirements is acceptable.

3. **Definitions:**

Accuracy (Corresponds to the VIM definition for "trueness").

The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula

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 (Codex Standard 72 – 1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

Lutein

All-*trans* lutein (IUPAC name: (1R)-4-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18-[(1R,4R)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethylcyclohex-3-en-1-ol, (CAS number: 127-40-2) and its *cis* isomers. Figure 1.

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period.

Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

4. **Method Performance Requirements:**
See Table 1.

Table 1. Method Performance requirements[a]

Analytical range	1–800[b]
Limit of Quantitation (LOQ)	≤ 1[b]
Recovery	90-110%
Repeatability (RSD_r)	
1-100[b]	8%
>100-800[b]	5%
Reproducibility (RSD_R)	
1-100[b]	15%
>100-800[b]	10%
[a]Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.	
[b] µg /100 g reconstituted final product; range and LOQ are based on total of *cis*+*trans* isomers.	

5. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range. Methods must be capable of resolving lutein from zeaxanthin.

6. **Reference Material(s)**:
SRM 1869. Please contact Dr. Melissa Phillips, Research Chemist, NIST for materials at melissa.phillips@nist.gov or (301) 975-4134.

7. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.

8. **Maximum Time-To-Result**: No maximum time.
s

Figures:

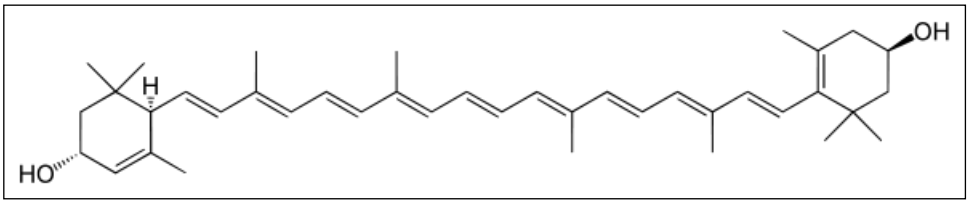

Figure 1: Molecular structure of all-*trans* lutein.

AOAC SMPR 2017.XXX; Version 3; February 9, 2017

Method Name: **Determination of Lycopene in Infant and Adult/ Pediatric Nutritional Formula**

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. **Applicability:**

Determination of total[1] Lycopene (CAS 502-65-8) in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2. **Analytical Technique:**

Any analytical technique that meets the following method performance requirements is acceptable.

3. **Definitions:**

Accuracy (Corresponds to the VIM definition for "trueness").

The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula

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 (Codex Standard 72 – 1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

Lycopene

IUPAC name: (6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene, CAS number: 502-65-8. Figure 1.

[1] Include *cis* and *trans* isomers if they are separated

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period.

Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

4. **Method Performance Requirements:**
See Table 1.

Table 1. Method Performance requirements[a]

Analytical range	1–50[b]
Limit of Quantitation (LOQ)	≤ 1[b]
Recovery	90-110%
Repeatability (RSD_r)	8%
Reproducibility (RSD_R)	15%
[a]Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.	
[b] µg /100 g reconstituted final product	

5. **System suitability tests and/or analytical quality control:**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range. Methods must be capable of resolving lycopene from α-carotene and β-carotene.

6. **Reference Material(s)**:
SRM 1869. Please contact Dr. Melissa Phillips, Research Chemist, NIST for materials at melissa.phillips@nist.gov or (301) 975-4134.

7. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.

8. **Maximum Time-To-Result**: No maximum time.

Figures:

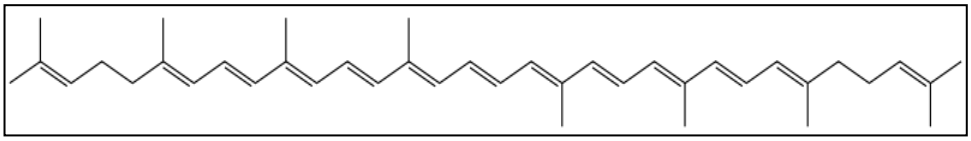

Figure 1: Molecular structure of lycopene.

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Approval of SMPR Revision for:

Carotenoids:

Lutein, β-Carotene, α-Carotene, & Lycopene

Greg Hostetler, Perrigo Nutritionals (Vermont, USA)

Gaithersburg, MD

March 15, 2017

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Agenda

- **Review**
  - Separate SMPRs for individual carotenoids
  - Comments / Responses
- **Motion to Adopt SMPRs**

Analyte Definitions

- **Descriptions of the analytes:** lutein (cis + trans), β-carotene (cis + trans), α-carotene, lycopene
- **Rationale:** Carotenoids are currently added to infant formula and adult nutritionals.
- **Implications:** Quantify cis/trans isomers for nutritional implications, quantify total to meet regulatory requirements

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Specific Performance Claims

- **Analytical ranges:**

		Updated
– Lutein	~~1-1300~~	1-800 µg/100 g
– β-Carotene	1-1300	1-1300 µg/100 g
– α-Carotene	~~1-1300~~	1-50 µg/100 g
– Lycopene	~~1-1300~~	1-50 µg/100 g

- **LOD:** not specified
- **LOQ:** ≤1 µg/100 g reconstituted final product

Specific Performance Claims

- **Repeatability**:
  1-100 µg/100 g ≤8% RSD
  >100 µg/100 g ≤5% RSD

- **Recovery**: 90-110% from spiked samples

- **Reproducibility**:
  1-100 µg/100 g ≤15% RSD
  >100 µg/100 g ≤10% RSD

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Stakeholder Comments

Draft SMPRs were posted on AOAC web-site for Stakeholder comment(s).

No comments were received for the Draft SMPRs.

Motion for Approval

I ask for a motion to approve the SMPRs for lutein, β-carotene, α-carotene, and lycopene.

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Questions??

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Erik Konings, Nestlé

Working Group Chair

Erik Konings studied higher professional laboratory education with majors in analytical and clinical chemistry. After graduating in 1984, he started his professional career at the then called Food Inspection Service in Maastricht, the Netherlands. In 2001 he completed his PhD study “Dietary folates in human nutrition” at Maastricht University. During this study, he obtained an MSc-degree in epidemiology. He is (co)author of more than 30 scientific publications. In September 2008, he started at the European Food Safety Authority (EFSA) in Parma, Italy, for a secondment as Scientific Officer at the Data Collection and Exposure Unit, and from there accepted, in June 2009, a position at the Nestlé Research Centre in Lausanne, Switzerland, currently in a role as Food Safety & Quality expert. He is active in several Standard Developing Organisations as AOAC INTERNATIONAL (Past-President), ISO, CEN, and IDF, and participates in the Codex Committee on Methods of Analysis and Sampling (CCMAS).

AOAC SMPR® 2011.006

***Standard Method Performance Requirements* for Folate in Infant Formula and Adult/Pediatric Nutritional Formula**

Intended Use: Global Dispute Resolution Method

1 Applicability

Determination of total folate [supplemental folic acid (CAS 59-30-3) and/or 5-methyl-tetrahydrofolate (CAS 68792-52-9), endogenous 5-methyl-tetrahydrofolate polyglutamates, 5- formyl-tetrahydrofolate, and 5-formyl tetrahydrofolate polyglutamates] in all forms (powders, ready-to-feed liquids, and liquid concentrates) of infant, adult, and pediatric nutritional formula.

2 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

3 Definitions

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment (AOAC SPIFAN, 2010), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula.—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 (Codex Standard 72-1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of detection (LOD).—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

Limit of quantitation (LOQ).—The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation ($\%RSD_r$).

Reproducibility.—The SD or RSD calculated from among-laboratory data; expressed as the reproducibility standard deviation (SD_R), or % reproducibility relative standard deviation ($\%RSD_R$).

Recovery.—The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples and check standards at the lowest point and midrange point of the analytical range.

6 Reference Material(s)

National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1849a Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849a consists of 10 packets, each containing approximately 10 g of material.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*^{SM}.

8 Maximum Time-to-Signal

No maximum time.

Approved by the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN). Final Version Date: April 5, 2011. Effective Date: June 29, 2011.

Table 1. Method performance requirements[a]		
Analytical range	0.50–300[b]	
Limit of detection (LOD)	≤0.10[b]	
Limit of quantitation (LOQ)	≤0.50[b]	
Repeatability (RSD_r)	0.50–21.5[b]	≤11%
	>21.5[b]	≤7%
Recovery	90–110%	
Reproducibility (RSD_R)	0.5–21.5[b]	≤32%
	>21.5[b]	≤16%
[a] Concentrations apply to (*1*) "ready-to-feed" liquids "as is"; (*2*) reconstituted powders (25 g into 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight.		
[b] µg/100 g expressed as folic acid in reconstituted final product.		

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Approval of SMPR Revision for:

Folate

Erik Konings

Gaithersburg, MD

March 15, 2017

The Scientific Association Dedicated to Analytical Excellence®

Agenda

- **Review**
  - SMPR
  - Comments / Responses WG/Stakeholders
- **Motion to Adopt SMPR**

Conclusions of SLV

- 5-CHO-THF quantified in soy-based formulas, after conjugase treatment up to ≈ 10% of total folates
- 5-MTHF quantified in milk-based formulas, after conjugase treatment up to ≈ 14% of total folates
- THF, 10-CHO-FA, 10-CH3-FA < LOQ
- 5,10-CH=CH-THF in one milk-based sample < 6.5% total folates, not confirmed in SLV 2

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Adaptation Applicability SMPR 2011.006

1 Applicability

Determination of total folate [supplemental folic acid (CAS 59-30-3) and/or 5-methyl-tetrahydrofolate (CAS 68792-52-9), ~~and~~ endogenous 5-methyl-tetrahydrofolate polyglutamates, 5- formyl-tetrahydrofolate, and 5-formyl tetrahydrofolate polyglutamates] in all forms (powders, ready-to-feed liquids, and liquid concentrates) of infant, adult, and pediatric nutritional formula.

Additional adaptations SMPR 2011.006

OLD

- *Limit of detection (LOD)*.—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

NEW

- *Limit of Detection (LOD)*
  The smallest amount or concentration of an analyte that can be estimated with acceptable reliability. Determined as: LOD = blank mean + 3 standard deviations of ten independent analyses of blank or blank spiked at low level (to be agreed upon by Study Directors) (if there is no detectable blank signal). See reference to Appendix L in section 7. Validation Guidance.

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Additional adaptations SMPR 2011.006

- ***6 Reference Material(s)***

National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1849a Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849a consists of 10 packets, each containing approximately 10 g of material. ~~Certified value of folic acid in NIST 1849 is 2.11 (±0.13) mg/kg. Note: The reference value for NIST 1849 is defined in terms of folic acid. The performance parameters in this SMPR are intended for folate and 5-methyl-tetrahydrofolate polyglutamate. Some discrepancy may be expected.~~

Stakeholder Comments

Draft SMPRs were posted on AOAC web-site for Stakeholder comment(s).

0 comment(s) were received for the Draft SMPR.

The Scientific Association Dedicated to Analytical Excellence®

Motion for Approval

I ask for a motion to approve the SMPR for Folate.

Questions??

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Katerina Mastovska, Covance Labs
AOAC Working Group Chair

Dr. Katerina (Kate) Mastovska is an Associate Scientific Director at Covance Food Solutions, where she leads the Chemistry Solutions Global Research, Development and Innovation group. Prior joining Covance Laboratories in 2009, she worked at the U.S. Dept. of Agriculture (USDA) and served as an expert in the United Nation's Food and Agricultural Organization (FAO) panel of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Dr. Mastovska is a Fellow of the AOAC Int. Among other activities at the AOAC Int., she is an Official Methods Board member and a former co-chairs the AOAC Chemical Contaminants and Residues Community. Dr. Mastovska has authored/co-authored more than 60 scientific publications (journal articles, book chapters, and monographs). She received her Ph.D. in Food Chemistry and Analysis from the Institute of Chemical Technology in Prague, Czech Republic.

AOAC MIDYEAR MEETING

AOAC INTERNATIONAL 7th Annual Midyear Meeting, March 13-17, 2017

AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN)

Current state of MCPD and glycidol analysis in infant formulae and related foods

J. Kuhlmann

Contents

Structure of the presentation

I a 1. Background and History

History

Free 3-MCPD *(beside 2-MCPD, 1,3-DCP & 2,3-DCP) has been discovered as food contaminant in the 1970s.*

*The occurrence of **2- & 3-MCPD esters and glycidyl esters** in oils, fats and oil/fat containing foods is a newer topic discovered between 2004 and 2011:*

Svejkovska B. et al.: Esters of 3-Chloropropane-1,2-diol in Foodstuffs; *Czech J. Food Sci.* 22 (5), **2004**, 190-196

Divinova V. et al.: Determination of Free and Bound 3-Chloropropane-1,2-diol by Gas Chromatography with Mass Spectrometric Detection using Deuterated 3-Chloropropane-1,2-diol as Internal Standard; *Czech J. Food Sci.* 22 (5), **2004**, 182-189

Zelinkova Z., et al.: Fatty esters of 3-chloropropane-1,2-diol in edible oils. *Food Addit. Contam.*, **2006**, 23, 1290-1298

Ilsi Europe Report Series: Summary Report of a Workshop held in February **2009**, Brussels, Belgium: "Dr. Seefelder reported on studies to investigate the formation of 2-MCPD during the deodorisation step of oils."

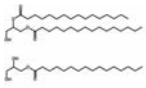
Weisshaar R., Perz R.:Fatty acid esters of glycidol in refined fats and oils; *Eur. J. Lipid. Sci. Technol*. 112, **2010**, 158-165

Kuhlmann J.: Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils; *Eur. J. Lipid. Sci. Technol*. 113, **2011**, 335-344

I a 1. Background and History

Background: what is 3-MCPD- & glycidyl esters in edible oils & fats?

Naturally occurring minor components e.g.:



Mono- & Diacylglycerides

Mycotoxins

Phospholipids

Free fatty acids

Colour compounds

Phytosterols

Trace elements

Volatiles

Major components:

Triacylglycerides

Contaminating minor components e.g.:

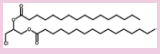
Pesticide residues

PAHs, Dioxins

Plasticisers

Mineral oils (MOSH/MOAH)

3-MCPD ester, 2-MCPD ester, glycidyl ester

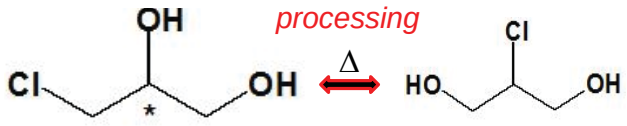


Heat-induced processing contaminants / Food-bourne contaminants

I a 2. Chemical Structures

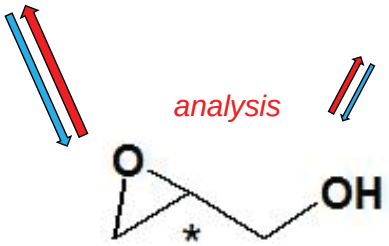
Chemical structures of the analytes

Free analytes



3-MCPD
3-*mono*-chloropropane-1,2-diol
3-Chloropropane-1,2-diol

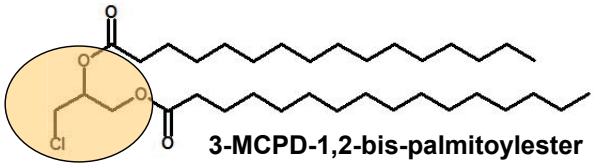
2-MCPD
2-*mono*-chloropropane-1,3-diol
2-Chloropropane-1,3-diol



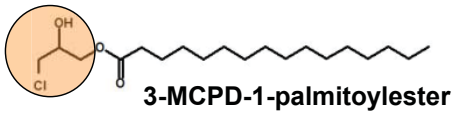
Glycidol
(2,3-Epoxi-1-propanol)

(Fatty acid) Bound analytes

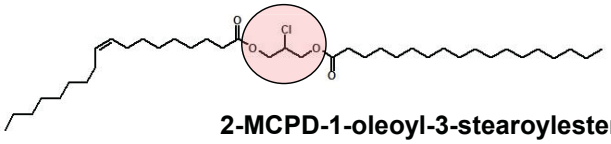
just examples, all fatty acids of an oil/fat might be present



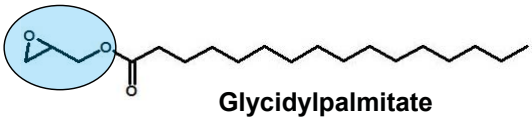
3-MCPD-1,2-bis-palmitoylester



3-MCPD-1-palmitoylester



2-MCPD-1-oleoyl-3-stearoylester

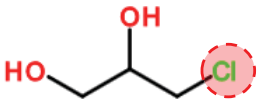


Glycidylpalmitate

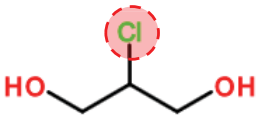
I a 3. Why it is an issue

Potential hazards of free 2-MCPD, 3-MCPD & glycidol

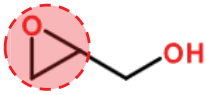
Toxicity of free 3-MCPD or glycidol is related to a **chloride** or an **epoxy group** at the molecular backbone.



3-MCPD



2-MCPD



Glycidol

glycidol: *probably carcinogenic to humans 2A* [1)] **(genotoxic)**

3-MCPD: *possibly carcinogenic to humans 2B* [2)]

2-MCPD: *No official classification available*

In regard to risk assessment (and product quality) **glycidol is the more problematic compound**!

No **MRL** or **TDI** applies; risk estimation is based on **MOE**!
(Maximum Residue Limit) (Tolerable Daily Intake) (Margin of Exposure)

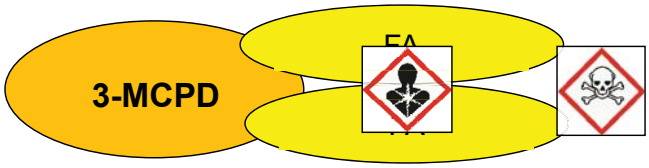
Consumers intake should be "as-low-as-reasonably-achievable" **ALARA**

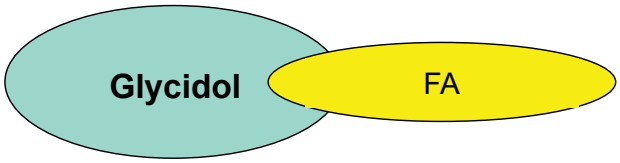
[1)] Opinion N° 007/2009, BfR, March, 10th, 2009, [2)] Opinion N° 006/2013, BfR, April, 3rd, 2012

I a 3. Why it is an issue

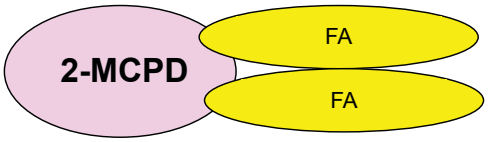
Potential hazards of ester-bound MCPD & glycidol

Do fatty acid esters of MCPD and glycidol are estimated to show toxic properties similar to the free anylates ?
What happens after intake?





After digestion

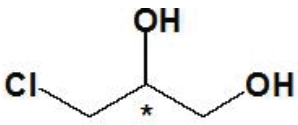


During digestion the toxicologically relevant core components are released by ester hydrolysis!

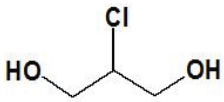
I c. Analytical challenges specific to: Analyte

What is the challenge?

Free analytes



3-MCPD

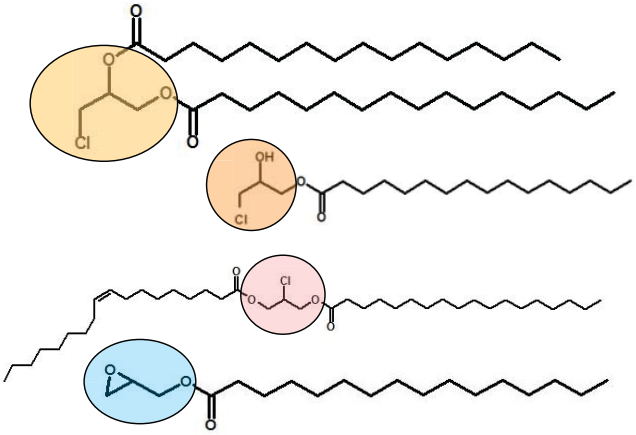


2-MCPD

Clear target structures!

(Fatty acid) Bound analytes

just examples, all fatty acids of an oil/fat might be present

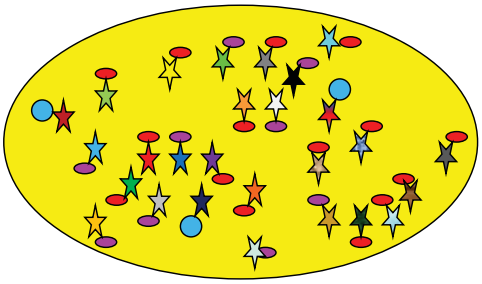


How do you receive sensitive and quantitative data about the core structures of an unknown and complex mixture of derivatives?

I b 1. Description of analytical methods

Direct analysis of the bound analytes: determination of the single original esters

glycidol
3-MCPD
2-MCPD
Fatty acid(s)

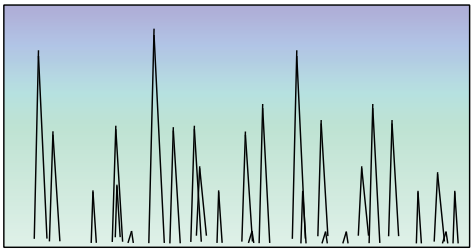


Hypothetic oil
Contains only 3 relevant fatty acids

This yields up to 27 analytes

3 Glycidyl ester
9-MCPD mono-ester
15 MCPD di-ester

Matrix removal in the majority of applications



Chromatogram displays up to 27 analytes!

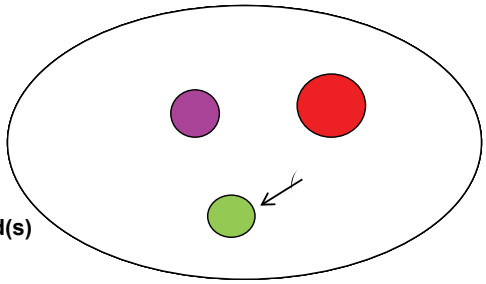
LC-MS / **LC-MS²** / LC-MS-TOF

Direct analysis – indirect quantification:
From every detected ester the amount of core analyte is calculated via molecular weights. Then the single 2-MCPD-, 3-MCPD- and glycidol contents are added up.

I b 1. Description of analytical methods

Indirect analysis of free and bound analytes: determination of the released core components

glycidol
3-MCPD
2-MCPD
fatty acid(s)



Hypothetic oil
Contains only 3 relevant fatty acids

ester cleavage (***alkaline***, ***acidic***, ***enzymatic***)

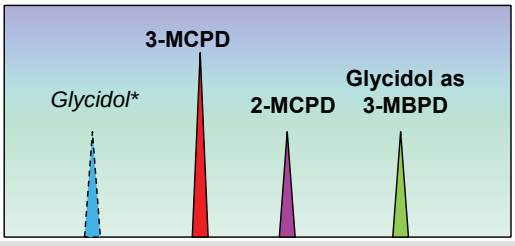
matrix clean-up *(e.g. l/l-extraction)*

glycidol conversion *(into MXPD)*

Derivatisation*
(e.g. HFBA/acetone/PBA)

GC-MS

Indirect analysis – direct quantification:
The target analytes can be quantified directly via internal standards



I b 1. Description of analytical methods

Some of the analytical approaches available for the bound analytes in oils and fats.

Indirect determination
(ester cleavage releases the 3 core analytes, GC-MS)

alkaline

- Early **DGF C-III 18 (09)** Σ 3-MCPD + glycidol **DGF C-VI 17 (10);** fast
- Late **DGF C-III 18 (09) A,B** A: Σ 3-M + g, B: 3-MCPD Withdrawn by DGF
- **BfR method 09** 3-MCPD fast
- **DGF C-VI 18 (10) A, B** A: Σ 3-M + g, B: 3-MCPD **AOCS Cd 29c-13;** fast
- Küsters et al. 2010 3-MCPD, Glycidol fast
- SGS **"3-in-1"** Kuhlmann 2011 3-MCPD, 2-MCPD, Glycidol **AOCS Cd 29b-13;** slow

acidic

- Divinova et al. 2004 Zelinkova et al. 2006 3-MCPD; slow
- **BfR method 08** 3-MCPD slow
- **"Unilever"** Ermacora et al. 2013 3-MCPD, 2-MCPD, Glycidol **AOCS Cd 29a-13;** slow
- Myasaki et al. 2012 3-MCPD, 2-MCPD, Glycidol fast
- Koyama et al. 2015 3-MCPD, Glycidol fast

enzymic

Validated methods

Direct determination
(determination of a selected number of contaminant esters)

Dilute & shoot

- Blumhorst et al. 2011 **GE** LC-MS²
- Haines et al. 2011 **3-MCPD-E, GE** LC-MS²

SPE or SPE²

- Masukawa et al. 2010/11 **GE** SPE²; LC-MS: **AOCS Cd 28-10**
- Granvogl et al. 2011 **GE** SPE; LC-MS²
- Dubois et al. 2011 **3-MCPD-E, 2-MCPD-E, GE** SPE²; LC-MS²
- Steenbergen et al. 2013 **GE** l/l; LC; GC/MS
- MacMahon et al. 2013 **3-MCPD-E, 2-MCPD-E, GE** 2 x SPE²; 2 x LC-MS²

Validated Methods covering MCPD & glycidol

EU commission recommends to use the AOCS Official Methods **Cd 29a,b,c-13** not only for analysis of bound 2- & 3-MCPD and glycidol in oils and fats but also in oil- & fat containing foods.
LOQ = 0.1 mg/kg in the oil/fat fraction
LOQ ≤ 0.01 mg/kg in foods with ≤ 10 % of fat.

I b 1. Description of analytical methods

General approaches for the analysis of *complex composed foods*

Two principal ways might be used for routine analysis of complex matrices:

Fat extraction
prior to analysis with an AOCS method.
Extraction suitable?
Impact on ruggedness/trueness?
Free MCPD included?

No fat extraction:
takin[illegible] sam[illegible] to an A[illegible]d.
Impact on ruggedness/trueness?
Free MCPD is included.

This approach applies for easy to extract foods but not for infant formula

Some points have to be checked!

I b 1. Description of analytical methods

Methods applied to infant formulae

BfR

Addition of internal standards & extraction:

•Accelerated Solvent Extraction (ASE)
petrolether / acetone / *iso*-hexane (2+2+1, v/v/v)
103 bar – 125° C*
changed to:
acetone : *iso*-hexane 4:1 (v,v)
1500 psi – 100° C

Analysis of bound analytes:

•Early DGF* or Divinova
changed to:
• „Unilever method“
AOCS Cd29a-13
changed to:
•„SGS 3-in-1“ method
AOCS Cd29b-13

** BfR-method 22: validated for bd. 2- & 3-MCPD in infant formula etc. 2010-2012*

EC-JRC

Extraction:

•Pressurised Liquid Extraction (PSE)
Ambient pressure, 40° C
tert-butylmethyl ether for all foods
changed to:
petrolether / acetone / *iso*-hexane (2+1+2, v/v/v) for **Infant formula**
changed to additional application of:
•Solid-Phase-Extraction (SPE)*
n-hexane : ethylacetate (85+15, v/v)

Addition of internal standards & analysis of bound analytes:

• „Unilever method“
AOCS Cd29a-13

** Method validation trial 2017*

Addition of internal standards & extraction:

•Liquid/Solid Extraction
n-hexane / acetone / (1+1, v/v)
30 s shaking, 5 min ultra-sonic bath

Analysis of free analytes:

• GC-MS analysis of PBA-derivatives, comparable to AOCS methods Cd 29b,c-13

I b 1. Description of analytical methods

Methods applied to infant formulae

FDA

Extraction:

•Liquid/Liquid Extraction
ethyl acetate / water (1+1, v/v)
35° C + 500 RPM 1,5 h , 2 times repeated for 30 min
1st extraction: 2 x 20 min centrifugation @ 14.500 rpm
2nd/3rd extraction: 1 x 20 min centrifugation @ 14.500 rpm

Addition of internal standards & analysis of bound analytes:

•Direct analysis:
MacMahon et al. 2013
2 x SPE²; 2 x LC-MS²

Applied for analysis of ester-bound 3-MCPD & ester-bound glycidol in infant formula.
Method directly applicable to liquid samples

SGS „5-in-2“

Addition of internal standards & extraction:

•Heat-Ultrasonic-Pressure-supported-Solvent Extraction (HUPsSE)
methanol 15 min ultrasonic bath @ 65° C
(mini-ultra turrax in case of agglutination)
methanol/*tert*-butylmethyl ether (1+1, v,v) 15 min ultrasonic bath @ 65° C
tert-butylmethyl ether 15 min ultrasonic bath @ 65° C

• Liquid/Liquid separation of bound & free analytes

Analysis of bound analytes:

• „SGS 3-in-1“ method *modified*
AOCS Cd29b-13 *modified*

Analysis of free analytes:

• GC-MS analysis similar to AOCS method Cd 29b-13

** Method applied on behalf of the Federal German Ministry of Food and Agriculture for investigating the occurrence of free and ester bound 2- and 3-MCPD and ester-bound glycidol in various foods, including 220 infant formulae from the German market in 2016. Results were in parallel reported to EFSA. Also the Svedish Authorities requested the „5-in-2“ methodology for analysis of infant nutrition. Same request from the Danish Authorities is currently in progress.*

2. Limitations / problems of methods

A general view on limitations / problems

Indirect analysis:

- The analytes easily can be converted into each other. Indirect methods must include techniques to suppress and/or control these interconversions.
- No information on original ester structures
- The **„Unilever-method“** AOCS Cd 29a-13 might give glycidol-overestimations when applied to aged or extracted oils and fats or to foods.

Direct analysis:

- So far not sufficient reference compounds/internal standards for poly-unsaturated, medium and short length fatty acid MCPD or glycidol derivatives.
- The high number of isomeric analytes results in chromatographic challenges.
- Larger costs for reference and standard compounds.

Extraction:

- Some implemented extraction techniques have not been tested for the fate of eventually occurring **free MCPD**.
- For infant formulae a strong extraction efficiency is required (next page).

I c. Analytical challenges specific to: 2 Matrix

What is the challenge with analyte extraction from infant formula?

1.: The extraction of analytes is much harder to achieve – in comparison to other foods.

Consequence: Sample spiking with the analytes does not serve for determination of method performance criteria like recoveries, precision, trueness…!

2.: Infant formulae can show very different extractability: What suits for the one product may not serve for another one!

Consequence: Method validation should be carried out by comparing new extraction techniques with well established approaches like the extraction according to Röse-Gottlieb. Validation should also include a representative set of different samples!

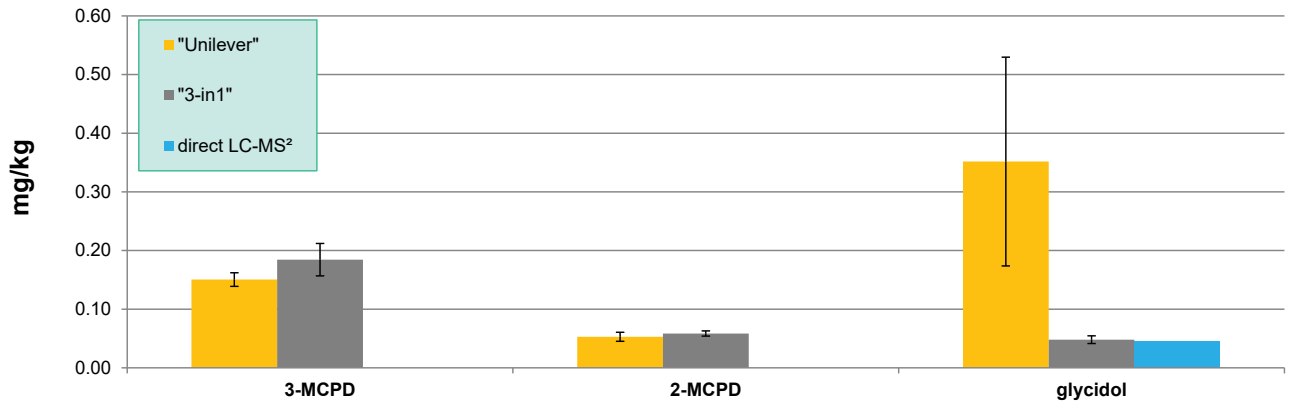
I c. Analytical challenges

Practical experiences, example

2014-2015 results from an inofficial interlaboratory comparison focusing on fats extracted from infant formula: „Unilever“ vs. „3-in-1“ & direct LC-MS² method.

Infant formula sample B

2 Laboratories (BfR/SGS): various extractions (BfR-ASEII, Röse-Gottlieb, HUPsSE)



The “Unilever-method” gave inconsistent glycidol values in fat extracted from aged infant formula.

Low extraction yields (< 20 %) were observed using PSE-US (tBME) for infant formula (data not shown).

II. Regulatory Information

a. Regulatory organizations

Some organisations being active in the fiele with direct or indirect impact on regulations for MCPD/glycidol:

European Commission (EC) formerly: **Commission of the European Communities**

European Food Safety Authority (EFSA) formerly: **Scientific Committee for Food (SCF)**

Part of EFSA: **The Scientific Panel on Contaminants in the Food Chain (CONTAM)**

German Federal Institute for Risk Assessment (BfR)

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

U.S. Food & Drug Administration (FDA)

Health Canada

II. Regulatory Information

b. Recommendations *in the EU*

COMMISSION RECOMMENDATION of 10 September 2014 on the monitoring of the presence of 2 and 3-monochloropropane-1,2-diol (2 and 3-MCPD), 2- and 3-MCPD fatty acid esters and glycidyl fatty acid esters in food (Text with EEA relevance) (2014/661/EU)

1. *Member States should, with the active involvement of feed and food business operators, perform monitoring for the presence of 2 and 3-MCPD, 2 and 3-MCPD fatty acid esters and glycidyl fatty acid esters in food, and particularly in:*

   (a) …,
   (b) foods for particular nutritional uses as defined in Directive 2009/39/EC of the European Parliament and of the Council (1) and intended for infants and young children, **including infant- and follow on formulae** as defined in Commission Directive 2006/141/EC (2) and dietary foods for special medical purposes as defined in Commission Directive 1999/21/EC (3) intended for use by infants,
   (c) - (f)…..

II. Regulatory Information

b. Regulations (MCPD)

SCF opinion 1994: TDI Free 3-MCPD = **2 µg/kg x bw x d**; http://ec.europa.eu/food/fs/sc/scf/out91_en.pdf

EU-Regulations

free 3-MCPD ≤ 20 µg/kg in soy sauce (or HVP); **EU 466/2001**

free 3-MCPD ≤ 100 µg/kg in glycerol used as food additive; **EU 232/2012**

BfR 2007: TDI Bound 3-MCPD = **2 µg/kg x bw x d**; BfR opinion 047-2007

May 2016: EFSA opinion on 2- & 3-MCPD and glycidol; j.efsa.2016.4426:

- From toxicological perspective the free and bound analytes are considered to be equivalent on molar base.
- "*The CONTAM Panel established for 3-MCPD a Tolerable Daily Intake (TDI) of 0.8 µg/kg bw per day and concluded that this TDI constitutes a group TDI for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents). …* "

Nov. 2016: JECFA (JECFA/83/SC) defined a TDI for 3-MCPD to be **4 µg 3-MCPD/kg x bw x d**
MOE for glycidol remains unchanged.

II. Regulatory Information

b. Safe levels - special focus on infant formula

Glycidol:

BfR 2009: In order not to fall below a MoE of 10.000 the content of glycidol in infant formula should not exceed 67 µg/kg in the fat phase. BfR opinion 007-2009

≈18 µg/kg product with fat content of 25 %

3-MCPD

The BfR assumes for infants a maximum daily uptake of 6 g fat/kg x bw.

	Infant formula: max 3-MCPD content in order to be below the TDI	
TDI 3-MCPD	fat phase	dry product (25 % fat)
µg/kg bw d	µg/kg	µg/kg
4	667	167
2	333	83
0.8	133	33

II. Regulatory Information

Do we expect MRLs?

Draft for MRLs in the EU based on a TDI (3-MCPD) of 0.8 µg/kg x bw x d	
Sum of Free 3-monochloropropane-diol (3-MCPD) and 3-MCPD fatty acid esters, expressed as 3-MCPD	**Maximum level (µg/kg)**
Infant formula and follow-on formula (powder)	125 until 30th June 2019 50 as from 1st July 2019
Infant formula and follow-on formula (liquid)	15 until 30th June 2019 6 as from 1st July 2019
Glycidyl fatty acid esters expressed as glycidol	**Maximum level (µg/kg)**
Infant formula and follow-on formula (powder)	75 until 30th June 2019 30 as from 1st July 2019
Infant formula and follow-on formula (liquid)	10 until 30th June 2019 4 as from 1st July 2019

Due to the nonconformity of TDIs the EC advised EFSA to review the calculation of TDI for 3-MCPD. It is expected that the implementation of maximum levels for glycidol remains unchanged.

Proposed Fitness for Purpose

The method should be applicable to the quantitative determination of free 2- and 3-MCPD, 2- and 3- MCPD esters (expressed as 2- and 3-MCPD, respectively) and glycidyl esters (expressed as glycidol) in infant formula, follow-on formula, and – if applicable – also adult nutritionals

- The extraction should be sufficient to isolate the analytes in a satisfying manner from all merchantable products.
- The applied analytical approach should be classified as “state of the art” and should have received international acceptance.
- The method should be sensitive in a way that recent and future maximum residue limits of the analytes are covered.
- Needless to say that the method should suit for routine analysis. This means that beside the required scientific characteristics also turn-around-time, costs, practicability, sustainability etc. should be considered as important criteria.

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Thank you for your kind attention!

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Robert Rankin, INCA

Robert Rankin is Executive Director of the Infant Nutrition Council of America (INCA), an association of manufacturers and marketers of formulated nutrition products, including infant formulas and adult nutritionals. Robert has worked with INCA since 2005, and has addressed a number of regulatory, legislative, technical and other issues on behalf of the infant formula industry. Robert works closely with officials at key US Government agencies including the US Food and Drug Administration and US Department of Agriculture. He has also worked with the Centers for Disease Control and Prevention and other US/international government agencies on industry issues. He has extensive experience in the development and communication of industry positions and has testified before U.S. state legislatures, the World Health Organization and local authorities.

Since 2010, Robert has managed Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Project on behalf of the infant formula industry. Through SPIFAN, voluntary consensus standards and internationally recognized methods of analysis for over 40 nutrients in infant formula have been developed with the ultimate goal of having the methods adopted by Codex Alimentarius as Type II dispute resolution methods.

Robert Rankin is a Vice President at Kellen, a global professional services firm specializing in trade associations, professional societies and communications. In addition to INCA, Robert also serves as President of the Calorie Control Council and Executive Director of the International Food Additives Council. Prior to Kellen, Robert spent two years at the Grocery Manufacturers Association where he worked in the Federal Affairs and Scientific & Regulatory Departments. Robert has a BA in Public Policy Studies from Duke University and lives in Maryland with his wife and two children.

AOAC SPIFAN Future Endeavors

Robert Rankin
Executive Director
Infant Nutrition Council of America

SPIFAN Progress to Date

- SMPRs for all priority nutrients/groups of nutrients
- First Action Methods for 22 nutrients/groups of nutrients
  - Still need ***Amino Acids***, some ***Carotenoids***, Fluoride, GOS and Vitamin B3
- 12 AOAC Final Action Methods
  - Possibly 4 more tomorrow (Biotin, Chloride, MTE, Vit D)
- 4 SPIFAN methods adopted by CODEX as Type II
  - Vit A, Panto, Nucs, Iodine
  - 4-6 more in July 2017 (Vit B12, Fatty Acids, Vit E, Myo-inositol, Cr/Mo/Se, Vit C)

SPIFAN Progress (as of 3/15/17)

	SMPR Approved	Oficial First Action	Official Final Action	ISO/IDF Standard	Codex Adoption
Vitamin A					
Vitamin E					
Vitamin D					
Vitamin B12					
Folate					
Inositol					
Nucleotides					
Cr/Mo/Se					
Vitamin C					
Choline					
Pantothenic Acid					
Carnitine					
Iodine					
Fatty Acids					
Biotin					
Vitamin K					
FOS					
GOS					
Minerals and Trace Elements					
Amino Acids					
Carotenoids					
Chloride					
Fluoride					
Vitamin B1					
Vitamin B2					
Vitamin B3					
Vitamin B6					

	Work that has been completed
	Work to be possibly completed by March 2017
	Work to be possibly completed by September 2017
	Work still to be completed after September 2017

Still to Come

- AOAC First Action Methods
  - Amino Acids, Some Carotenoids, Fluoride, GOS, Vitamin B3
- AOAC Final Action Methods and ISO/IDF Standards
  - Biotin, Chloride, Vit D, Choline, Carnitine, Vit K, B Vits, Folate, Amino Acids, Carotenoids, Fluoride, FOS, GOS
- CODEX adoption of AOAC/ISO/IDF methods for all remaining nutrients/groups of nutrients
- SPIFAN III

SPIFAN III

- Complete original SPIFAN goals (First Action Methods, Final Action Methods, ISO/IDF Standards, CODEX adoption)
- Forum to evaluate new methods/technologies/ opportunities
- Facilitate global awareness, acceptance and support of SPIFAN process & use of SPIFAN methods
- Expand vested stakeholders
- Ensure *OMA* Chapter 50 reflects SPIFAN progress

Questions?

Thank you!

Robert Rankin
Executive Director
Infant Nutrition Council of America
rrankin@kellencompany.com

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

Scott Coates, Ph.D.

AOAC INTERNATIONAL (CSO)

Scott Coates is a 21-year AOAC veteran now serving as the AOAC Chief Scientific Officer. Dr. Coates joined AOAC in 1992 as a manager for the AOAC Research Institute, and ran the *Performance Method Tested* program until 2009 when he was promoted to become AOAC's Chief Scientific Officer (CSO). As CSO, Coates is involved in every major AOAC project, ranging from biological threat agent detection, food/environmental microbiology, and nutritional chemistry of infant formula. Coates was the lead author of the *Guideline for Standard Methods Performance Requirements* in the 19th edition of the *Official Methods of Analysis.* Coates is a University of Maryland alumni has a B.S. in microbiology and a M.S. in Biotechnology Management.

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STAKEHOLDER PANEL ON INFANT FORMULA & ADULT NUTRITIONALS (SPIFAN)

Standard Method Performance Requirements (SMPR®) Orientation

Scott Coates (CSO)

AOAC INTERNATIONAL

Gaithersburg, MD

March 15, 2017

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- Introduction
- Background
- Format
- Process
- Guideline for Development of SMPRs
- Performance parameters

Standard Methods Performance Requirements

- Commonly referred to as:
  - SMPRs
  - "Smipper"s

STANDARD METHOD PERFORMANCE REQUIREMENTS AOAC INTERNATIONAL (2011)

AOAC SMPR 2011.006

Standard Method Performance Requirements for Folate in Infant Formula and Adult/Pediatric Nutritional Formula

Approved by: Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN)

Final Version Date: April 5, 2011

Effective Date: April 5, 2011

Intended Use:

1. Applicability

Determination of total folate [supplemental folic acid (CAS 59-30-3) or 5-methyl-tetrahydrofolate (CAS 68792-52-9), and endogenous 5-methyl-tetrahydrofolate polyglutamate] in all forms (powders, ready-to-feed liquids, and liquid concentrates) of infant, adult, and pediatric nutritional formula.

2. Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

3. Definitions

Adult/Pediatric Formula

Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment (AOAC SPIFAN, 2010), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant Formula

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 (Codex Standard 72-1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r), or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R), or % reproducibility relative standard deviation (%RSD_R).

Recovery

The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

4. Method Performance Requirements

Analytical range	0.50–300[a]	
Limit of detection (LOD)	≤0.10[a]	
Limit of quantitation (LOQ)	≤0.50[a]	
Repeatability (RSD_r)	0.50[a]	≤11%
	21.5[a]	≤7%
	43.0[a]	
	64.0[a]	
	85.0[a]	
Recovery	0.5	90–110%
	21.5[a]	
	43.0[a]	
	64.0[a]	
	85.0[a]	
Reproducibility (RSD_R)	0.5[a]	≤22%
	21.5[a]	≤16%
	43.0[a]	
	64.0[a]	
	85.0[a]	

Concentrations apply to (1) "ready-to-feed" liquids "as is" (2) reconstituted powders (25 g into 200 g water), and (3) liquid concentrates diluted 1:1 by weight.

[a] µg/100 g expressed as folic acid in reconstituted final product.

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the applicability range.

6. Reference Material(s)

NIST Standard Reference Material ® (SRM) 1849 Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849 consists of 10 packets, each containing approximately 10 g of material. Certified value of folic acid in NIST 1849 is 2.11 (±0.13) mg/kg.

Note: The reference value for NIST 1849 is defined in terms of folic acid. The performance parameters in this SMPR are intended for folate and 5-methyl-tetrahydrofolate polyglutamate. Some discrepancy may be expected.

7. Validation Guidance

Recommended level of validation (*Official Methods of Analysis*℠)

8. Maximum Time-to-Signal

No maximum time.

v8: March 13, 2011

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SMPRs

- documents a community's analytical method needs.
- very detailed description of the analytical requirements.
- includes method acceptance requirements.
- published as a standard.

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Uses of SMPRs

- Basis for method acceptance and approval.
- Guidance to method developers for the development of new methods.
- Advance the state-of-the-art in a particular direction.
- Address specific analytical needs.
- Allow AOAC to reach a broader community of method developers and users.

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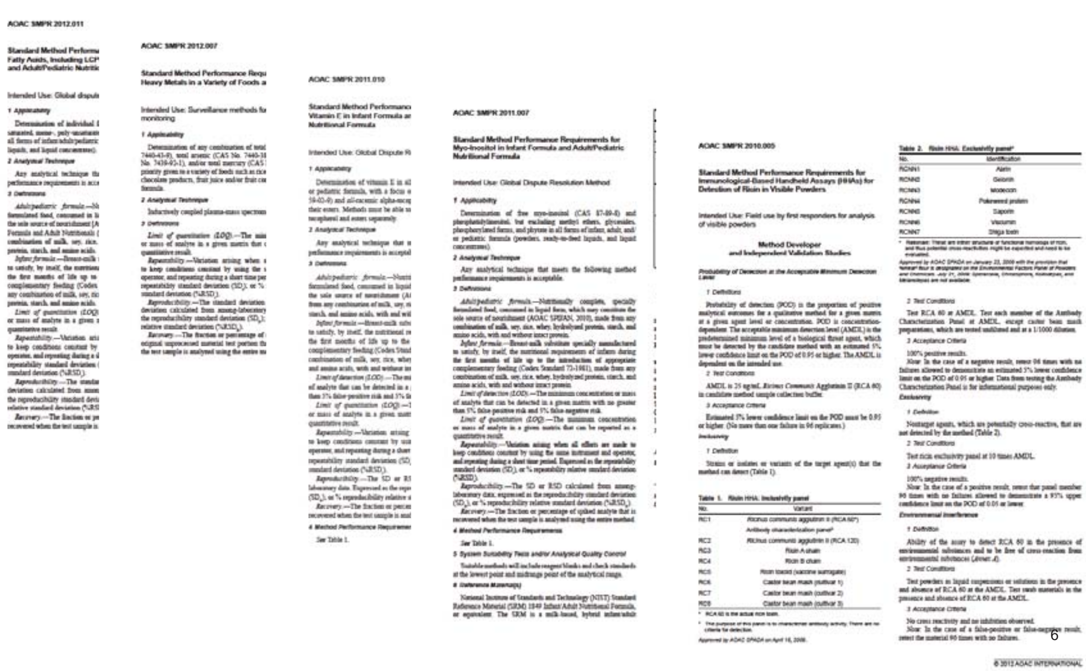
AOAC has adopted 65+ SMPRs



SMPR Format

- Intended use
- Applicability
- Analytical technique
- Definitions

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SMPR Format

- System suitability
- Reference materials
- Validation guidance
- Maximum time-to-determination
- Method performance requirements table

AOAC SMPR 2012.002

Standard Method Performance Requirements for Whey Protein:Casein Ratio in Infant Formula

Intended Use: Global dispute resolution method

1 Applicability

Determination of total whey proteins, including hydrolyzed forms, as a percent of protein content (protein content as defined by appropriate regulatory agencies). 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).

2 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

3 Definitions

Infant formula.—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 (Codex Standard 72–1981), made from any combination of milk, whey, hydrolyzed milk protein, starch, and amino acids, with and without intact protein.

Whey protein.—For the purpose of this SMPR, whey protein is defined as the proteinaceous components obtained from milk after removal of casein components by various processing technologies.

Limit of detection (LOD).—The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

Limit of quantitation (LOQ).—The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Repeatability.—Variation arising when all efforts are made ... conditions constant by using the same instru...

Table 1. Method performance requirements		
Analytical range	20–100*	
Limit of quantitation (LOQ)	≤10*	
Repeatability (RSD_r)	20–100*	≤3%
Recovery	95 to 105% of theoretical	
Reproducibility (RSD_R)	20–100*	≤6%

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

operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

Recovery.—The fraction or percentage of analyte that is recovered versus a known amount in a test sample when analyzed using the entire method.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include check standards at the lowest point and midrange point of the analytical range.

6 Reference Material(s)

To be determined.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*℠.

8 Maximum Time-to-Re...

No maxi...

SMPRs are published in the OMA.

SMPR ID numbers use the year and 3 numerals.

OMA ID numbers use the year and 2 numerals.

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SMPRs can be developed for all types of methods:

Quantitative methods

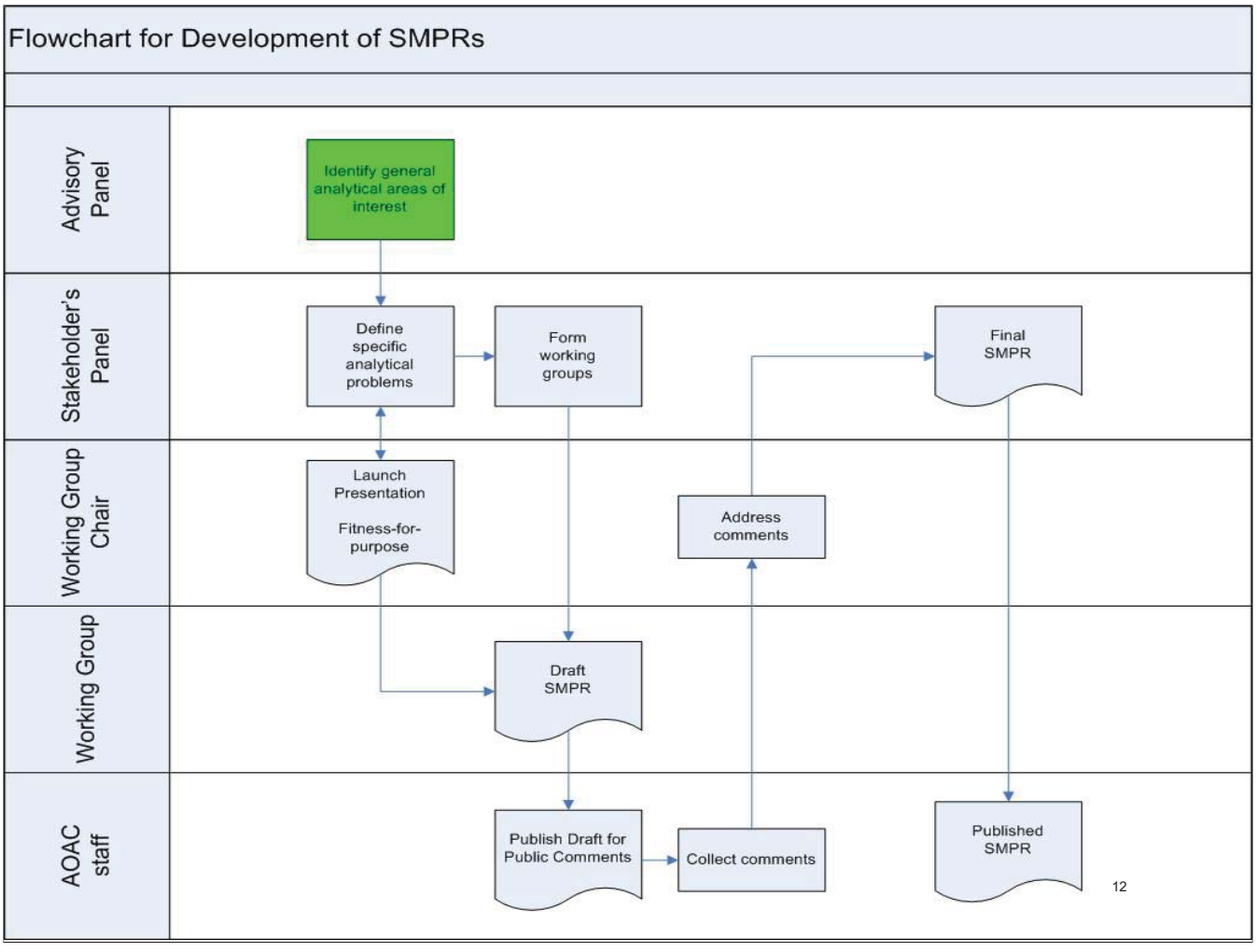
- Trace components: arsenic in food.
- Main components: nutrients in infant formula.

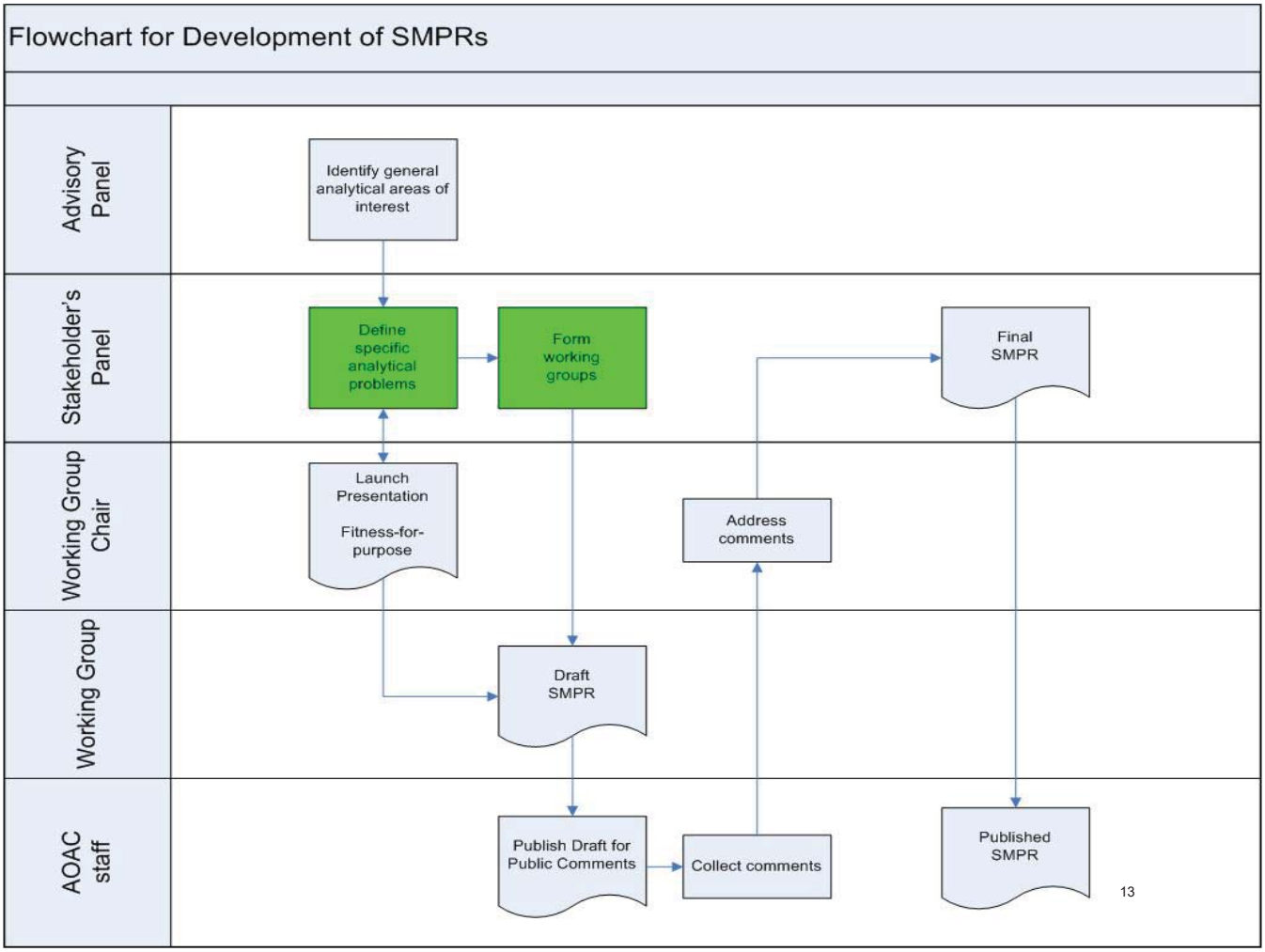
Qualitative methods

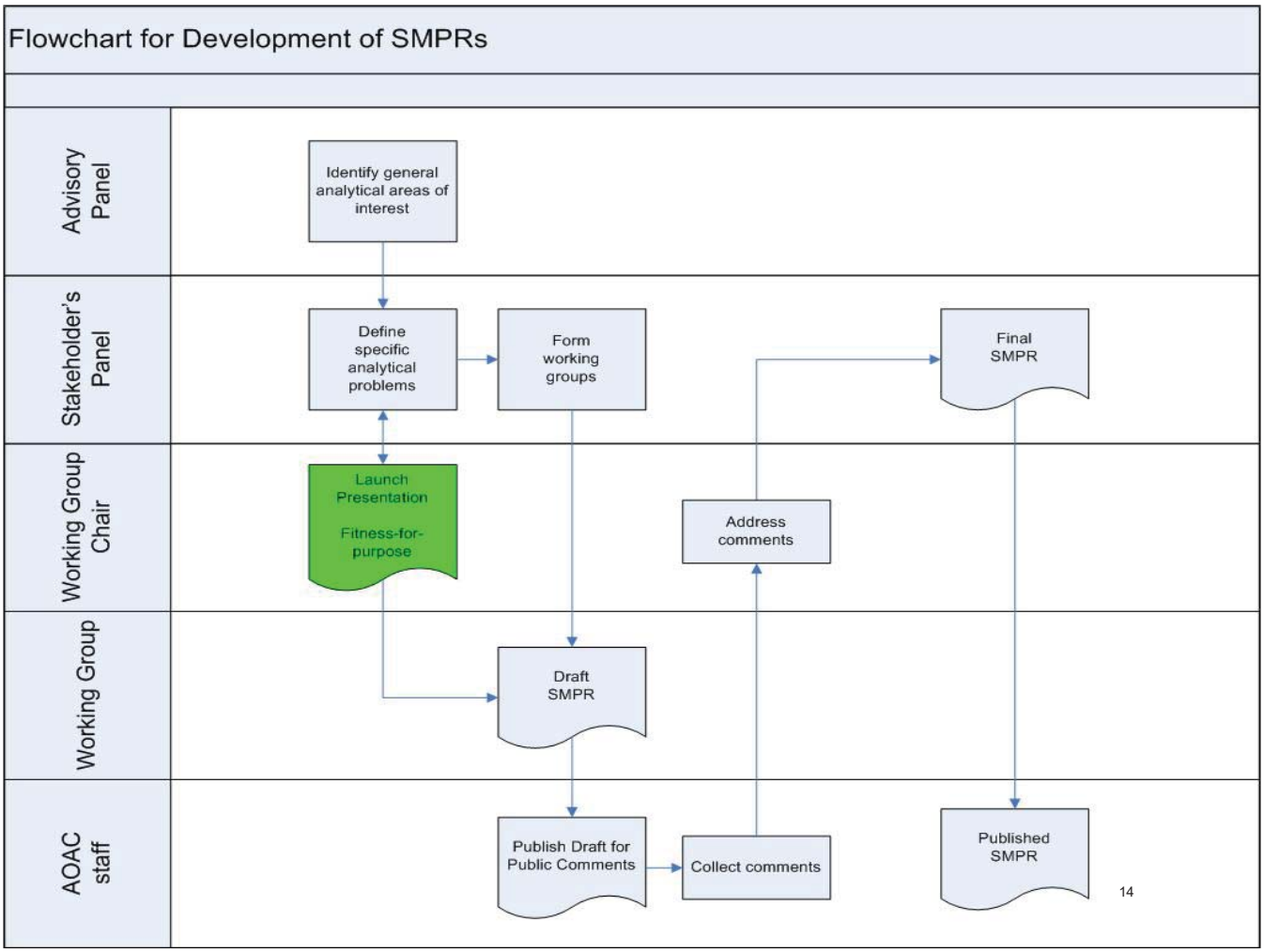
- Trace components: *Listeria* in cheese.
- Main components: chondroitin sulfate.

Identification methods: PDE5-Inhibitors in supplements.

SMPR Process




Flowchart for Development of SMPRs
Advisory Panel
Stakeholder's Panel
Working Group Chair
Working Group
AOAC staff
Identify general analytical areas of interest
Define specific analytical problems
Form working groups
Final SMPR
Launch Presentation
Fitness-for-purpose
Address comments
Draft SMPR
Publish Draft for Public Comments
Collect comments
Published SMPR
13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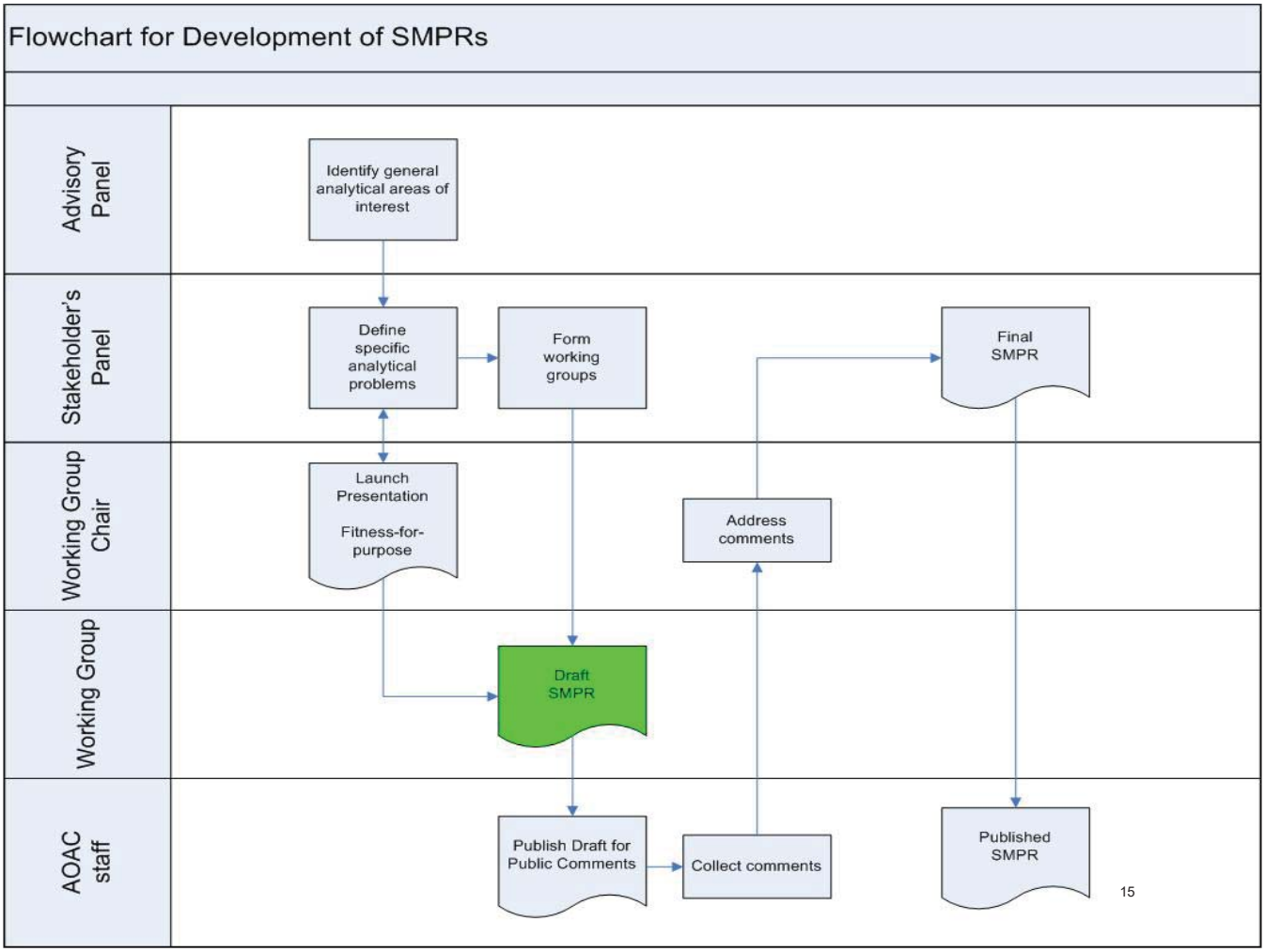
Published SMPR
14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Stakeholder's Panel
Working Group Chair
Working Group
AOAC staff
Identify general analytical areas of interest
Define specific analytical problems
Form working groups
Final SMPR
Launch Presentation
Fitness-for-purpose
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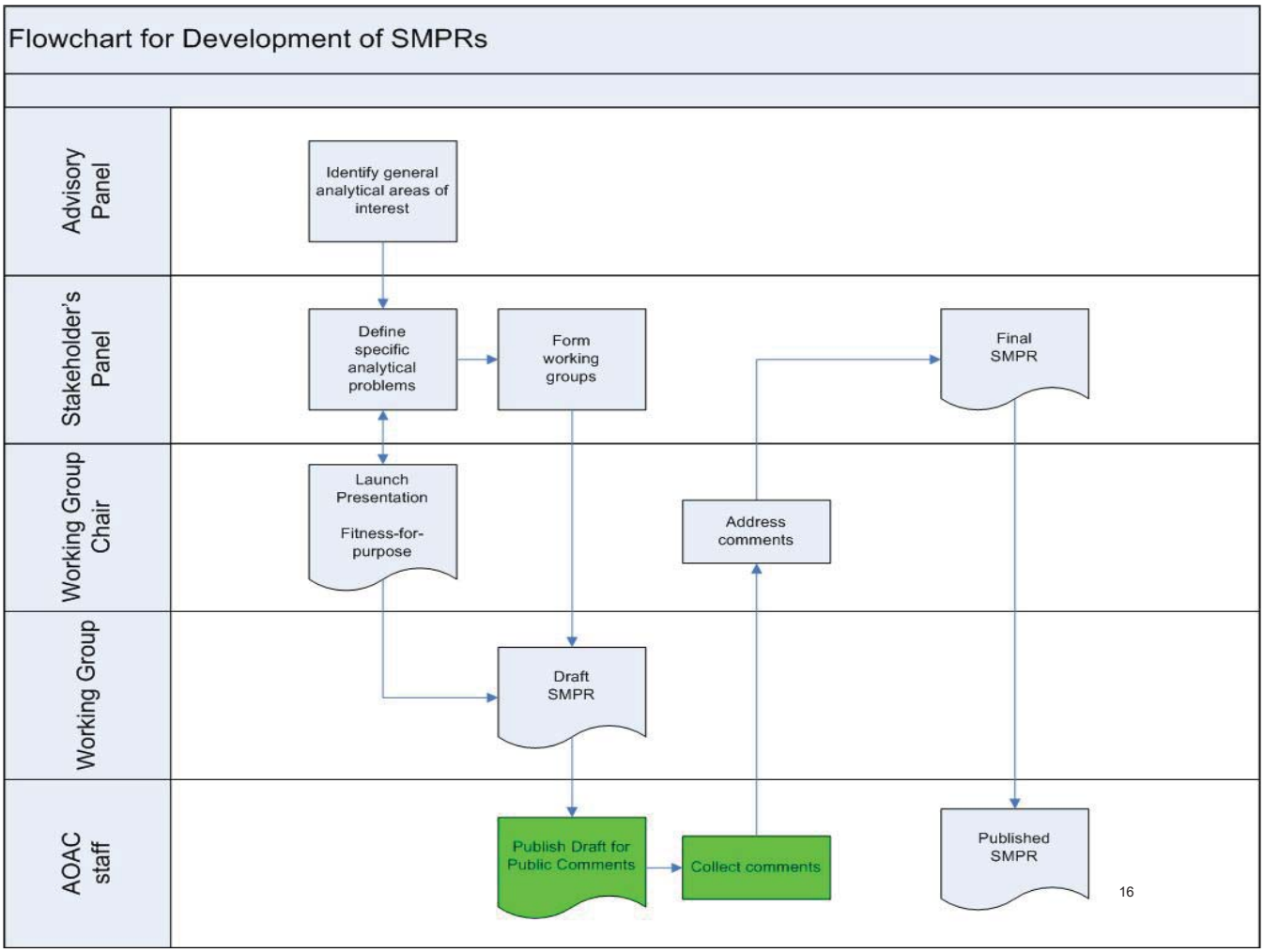
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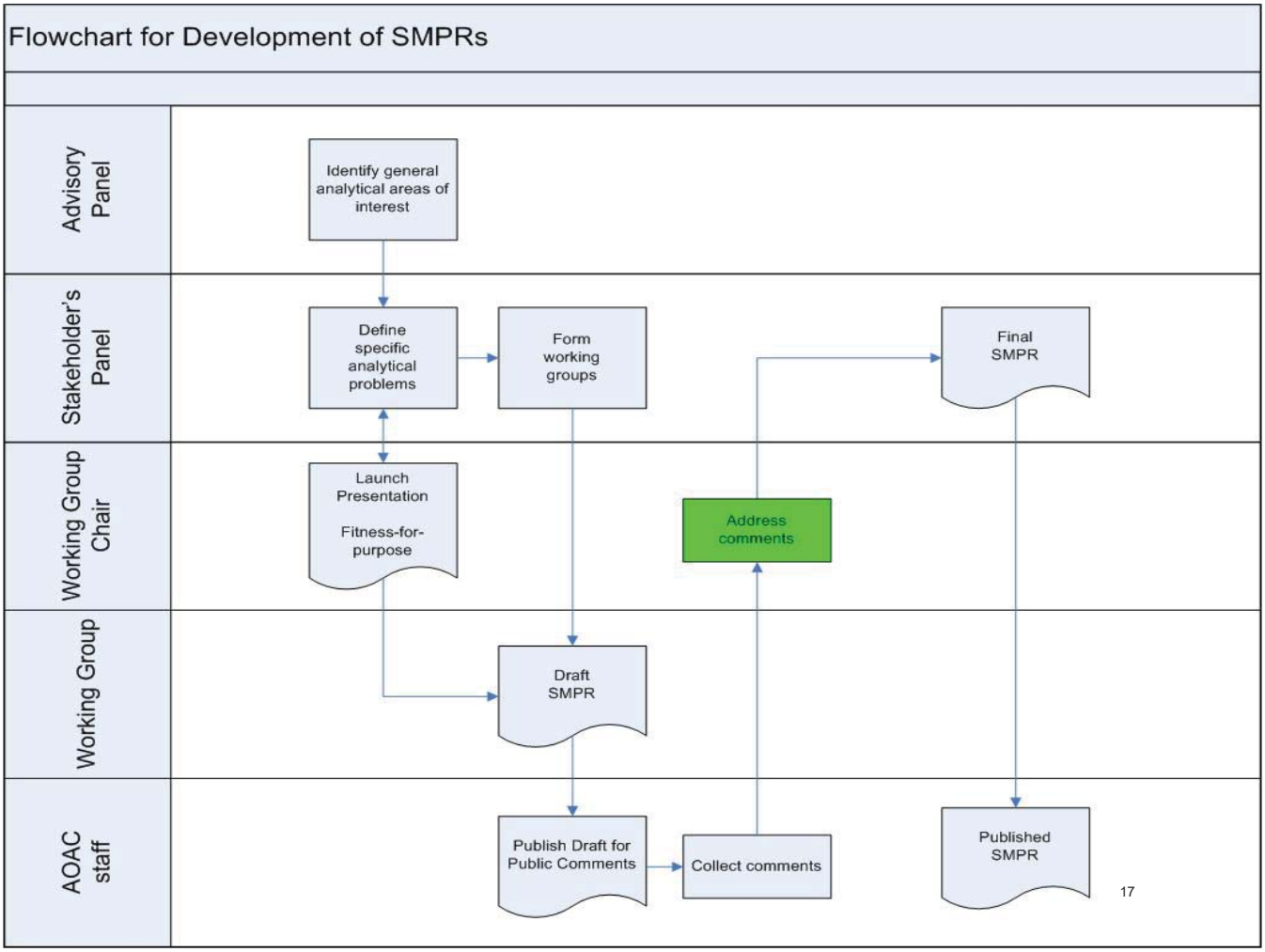
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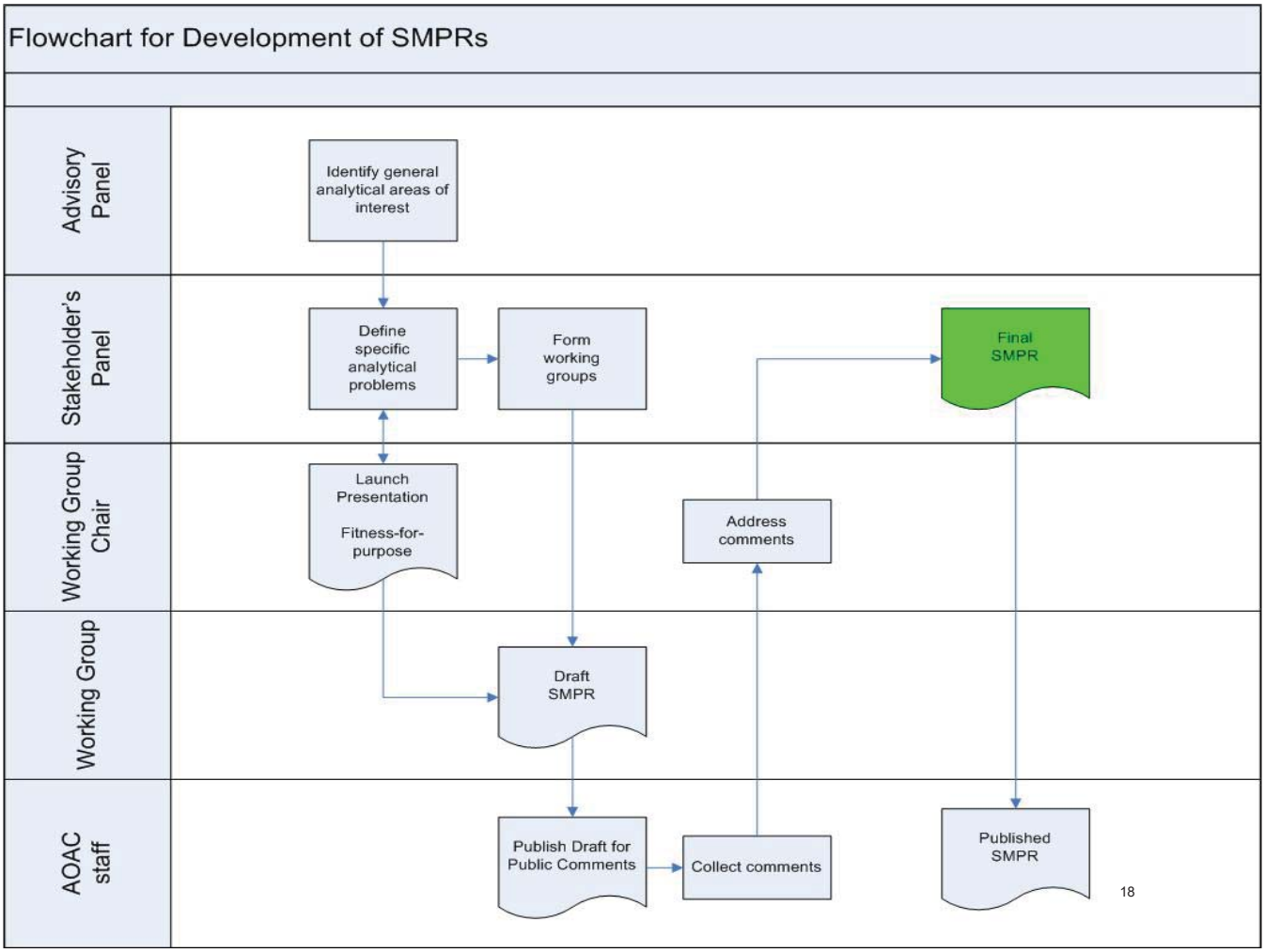
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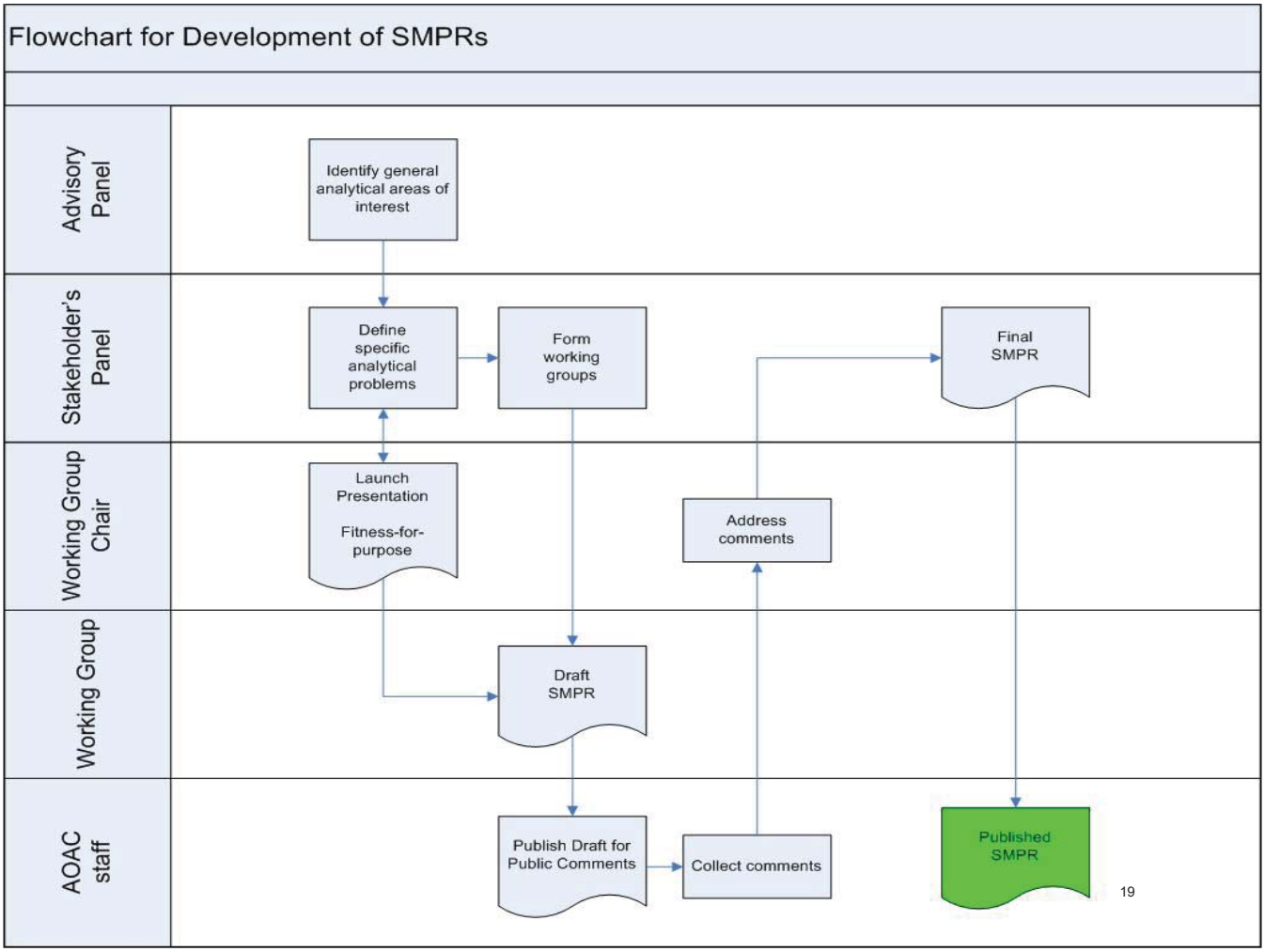


Fitness-for-Purpose

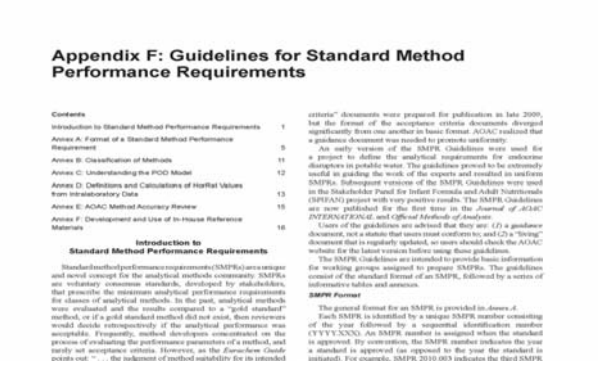
- Very early in process
- General statement of method performance
- No or few acceptance criteria
- 1 or 2 paragraphs
- No formal format
- Not a standard

SMPR

- A deliverable
- Very detailed specification of method performance requirements
- Acceptance criteria
- 2 to 3 pages
- Standard format
- Formal AOAC standard
- Published in the OMA

Appendix F: Guideline to SMPRs

- **Complete** guidance describing SMPRs and general validation requirements.
- 19th ed. of OMA
- On-line at: http://www.eoma.aoac.org/app_f.pdf



Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

Standard method performance requirements (SMPRs) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods. In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable. Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria. However, as the *Eurachem Guide* points out "... the judgment of method suitability for its intended criteria" documents were prepared for publication in late 2009, but the format of the acceptance criteria documents diverged significantly from one another in basic format. AOAC realized that a guidance document was needed to promote uniformity.

An early version of the SMPR Guidelines were used for a project to define the analytical requirements for endocrine disruptors in potable water. The guidelines proved to be extremely useful in guiding the work of the experts and resulted in uniform SMPRs. Subsequent versions of the SMPR Guidelines were used in the Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN) project with very positive results. The SMPR Guidelines are now published for the first time in the *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis*.

Users of the guidelines are advised that they are: (*1*) a guidance document, not a statute that users must conform to; and (*2*) a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using these guidelines.

The SMPR Guidelines are intended to provide basic information for working groups assigned to prepare SMPRs. The guidelines consist of the standard format of an SMPR, followed by a series of informative tables and annexes.

SMPR Format

The general format for an SMPR is provided in *Annex A*.

Each SMPR is identified by a unique SMPR number consisting of the year followed by a sequential identification number (YYYY.XXX). An SMPR number is assigned when the standard is approved. By convention, the SMPR number indicates the year a standard is approved (as opposed to the year the standard is initiated). For example, SMPR 2010.003 indicates the third SMPR

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Performance parameters

Quantitative methods

- Analytical range
- Limit of detection
- Limit of Quantitation
- Repeatability
- Recovery
- Reproducibility

4. Method Performance Requirements

Analytical range	0.01–5.0[a]	
Limit of detection (LOD)	≤0.004[a]	
Limit of quantitation (LOQ)	≤0.01[a]	
Repeatability (RSD_r)	0.01[a]	≤15%
	0.2[a]	≤7%
	0.5[a]	
	5.0[a]	
Recovery	0.01[a]	90–110%
	0.2[a]	
	0.5[a]	
	5.0[a]	
Reproducibility (RSD_R)	0.3	≤11%
	0.6	
	1.0	
	2.5	
	5.0	
Concentrations apply to (*1*) "ready-to-feed" liquids "as is"; (*2*) reconstituted powders (25 g into 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight.		
[a] µg/100 g expressed as cyanocobalamin in reconstituted final product.		

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Qualitative methods

- Probability of Detection (POD)
- Acceptable Minimum Detection Level (AMDL)
- Inclusivity
- Exclusivity

Summary

- SMPRs provide a logical way to define what we need in a method.
- SMPRs provide a way to standardize inclusivity/exclusivity panels.
- The process allows a community to agree on and set the minimum performance requirements for a class of methods.

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Summary

- SMPRs provide an objective standard to judge candidate methods.
- SMPRs are unique in the analytical community.
- AOAC and its volunteers have produced 65+ SMPRs in 5 years, even for the toughest analytes.

Don’t worry -

- It’s a great process.
- We’ll be there at your side every step of the way.

The Scientific Association Dedicated to Analytical Excellence®

Questions ?

Appendix F: Guidelines for *Standard Method Performance Requirements*

Contents

Introduction to *Standard Method Performance Requirements*

Standard Method Performance Requirements (SMPRs®) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods. In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable. Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria. However, as the *Eurachem Guide* points out: " . . . the judgment of method suitability for its intended use is equally important . . ." (1) to the evaluation process.

International Voluntary Consensus Standards

An SMPR is a form of an international, voluntary consensus standard. A standard is an agreed, repeatable way of doing something that is published as document that contains a technical specification or other precise criteria designed to be used consistently as a rule, guideline, or definition. SMPRs are a *consensus* standards developed by stakeholders in a very controlled process that ensures that users, research organizations, government departments, and consumers work together to create a standard that meets the demands of the analytical community and technology. SMPRs are also *voluntary* standards. AOAC cannot, and does not, impose the use of SMPRs. Users are free to use SMPRs as they see fit. AOAC is very careful to include participants from as many regions of the world as possible so that SMPRs are accepted as *international* standards.

Guidance for Standard Method Performance Requirements

Commonly known as the "SMPR Guidelines." The first version of the SMPR Guidelines were drafted in 2010 in response to the increasing use and popularity of SMPRs as a vehicle to describe the analytical requirements of a method. Several early "acceptance criteria" documents were prepared for publication in late 2009, but the format of the acceptance criteria documents diverged significantly from one another in basic format. AOAC realized that a guidance document was needed to promote uniformity.

An early version of the SMPR Guidelines were used for a project to define the analytical requirements for endocrine disruptors in potable water. The guidelines proved to be extremely useful in guiding the work of the experts and resulted in uniform SMPRs. Subsequent versions of the SMPR Guidelines were used in the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) project with very positive results. The SMPR Guidelines are published in the *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis*.

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SMPR Format

The general format for an SMPR is provided in *Annex A*.

Each SMPR is identified by a unique SMPR number consisting of the year followed by a sequential identification number (YYYY.XXX). An SMPR number is assigned when the standard is approved. By convention, the SMPR number indicates the year a standard is approved (as opposed to the year the standard is initiated). For example, SMPR 2010.003 indicates the third SMPR adopted in 2010.

The SMPR number is followed by a method name that must include the analyte(s), matrix(es), and analytical technique (unless the SMPR is truly intended to be independent of the analytical technology). The method name may also refer to a "common" name (e.g., "Kjeldahl" method).

The SMPR number and method name are followed by the name of the stakeholder panel or expert review panel that approved the SMPR, and the approval and effective dates.

Information about method requirements is itemized into nine categories: (*1*) intended use; (*2*) applicability; (*3*) analytical technique; (*4*) definitions; (*5*) method performance requirements; (*6*) system suitability; (*7*) reference materials; (*8*) validation guidance; and (*9*) maximum time-to-determination.

An SMPR for qualitative and/or identification methods may include up to three additional annexes: (*1*) inclusivity/selectivity panel; (*2*) exclusivity/cross-reactivity panel; and (*3*) environmental material panels. These annexes not required.

Informative tables.—The SMPR Guidelines contain seven informative tables that represent the distilled knowledge of many years of method evaluation, and are intended as guidance for SMPR working groups. The informative tables are not necessarily AOAC

policy. SMPR working groups are expected to apply their expertise in the development of SMPRs.

Table A1: Performance Requirements. Provides recommended performance parameters to be included into an SMPR. Table A1 is organized by five method classifications: (*1*) main component quantitative methods; (*2*) trace or contaminant quantitative methods; (*3*) main component qualitative methods; (*4*) trace or contaminant quantitative methods; and (*5*) identification methods. The table is designed to accommodate both microbiological and chemical methods. Alternate microbiological/chemical terms are provided for equivalent concepts.

Table A2: Recommended Definitions. Provides definitions for standard terms in the SMPR Guidelines. AOAC relies on *The International Vocabulary of Metrology Basic and General Concepts and Associated Terms* (VIM) and the International Organization for Standadization (ISO) for definition of terms not included in Table A2.

Table A3: Recommendations for Evaluation. Provides general guidance for evaluation of performance parameters. More detailed evaluation guidance can be found in *Appendix D, Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2); *Appendix I, Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (3); *Appendix K, AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (4); Codex Alimentarius Codex Procedure Manual (5); and ISO Standard 5725-1-1994 (6).

Table A4: Expected Precision (Repeatability) as a Function of Analyte Concentration. The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation (RSD) of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides target RSDs for a range of analyte concentrations.

Table A5: Expected Recovery as a Function of Analyte Concentration. Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 1 ppb to 100%.

Table A6: Predicted Relative Standard Deviation of Reproducibility ($PRSD_R$). This table provides the calculated $PRSD_R$ using the Horwitz formula:

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction.

Table A7: POD and Number of Test Portions. This table provides the calculated probability of detection (POD) for given sample sizes and events (detections). A method developer can use this table to determine the number of analyses required to obtain a specific POD.

Informative annexes.—The SMPR Guidelines contain informative annexes on the topics of classification of methods, POD model, HorRat values, reference materials, and method accuracy and review. As with the informative tables, these annexes are intended to provide guidance and information to the working groups.

Initiation of an SMPR

See Figure 1 for a schematic flowchart diagram of the SMPR development process.

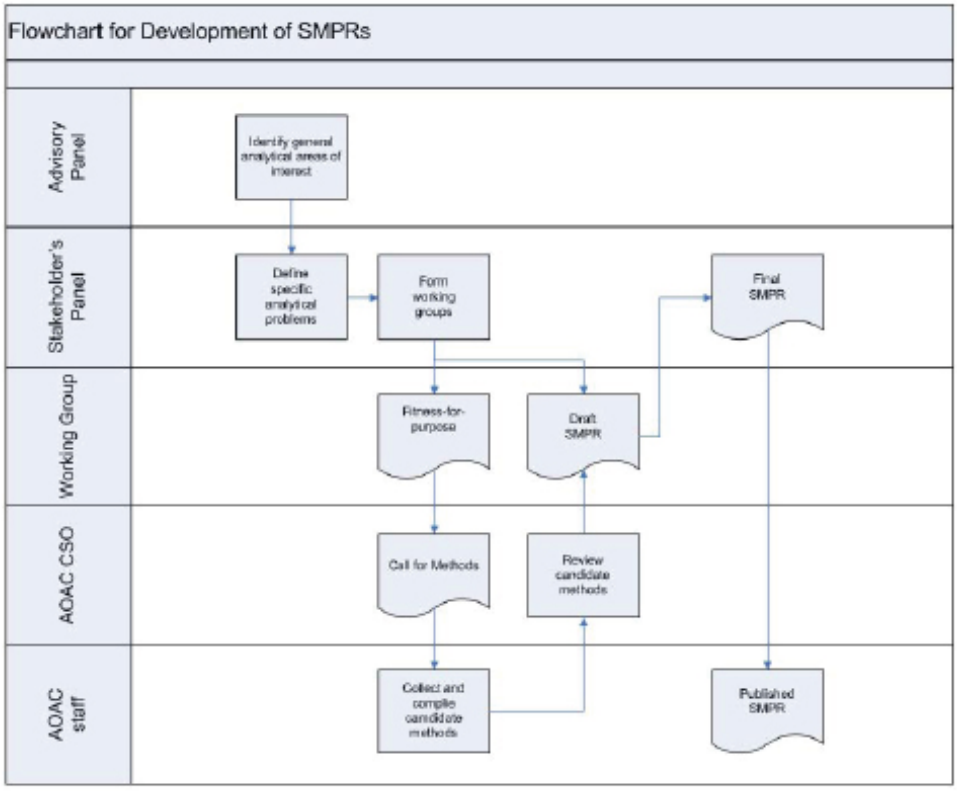

Figure 1. Schematic flowchart diagram of the SMPR development process.

Advisory panels.—Most commonly, an SMPR is created in response to an analytical need identified by an advisory panel. Advisory panels normally consist of sponsors and key stakeholders who have organized to address analytical problems. Usually, the advisory panel identifies general analytical problems, such as the need to update analytical methods for determination of nutrients in infant formula. An advisory panel, with the input of appropriate subject matter experts, also prioritizes the specific analytical problems within the general topic. This panel is critical in planning for the stakeholder panel meeting.

Stakeholder panels.—After an advisory panel has identified a general analytical problem, AOAC announces the standards development activity, identifies stakeholders, and organizes a stakeholder panel. Membership on a stakeholder panel is open to anyone materially affected by the proposed standard. AOAC recruits scientists to participate on stakeholder panels on the basis of their expertise with the analytical problem identified by the advisory panel. Experts are recruited from academia, government, nongovernmental organizations (such as ISO), industry, contract research organizations, method developers, and instrument/equipment manufacturers. AOAC employs a representative voting panel model to ensure balance with regards to stakeholder perspective, and to ensure that no particular stakeholder perspective dominates the proceedings of the stakeholder panel. All stakeholder candidates are reviewed by the AOAC Chief Scientific Officer (CSO) for relevant qualifications, and again by the Official Methods Board to ensure that the stakeholder panel is balanced and all stakeholders are fairly represented.

Stakeholder panels are extremely important as they serve several functions: (*1*) identify specific analytical topics within the general analytical problem described by the advisory panel; (*2*) form working groups to address the specific analytical topics; (*3*) identify additional subject matter experts needed for the working groups; (*4*) provide oversight of the SMPR development; and (*5*) formally adopt SMPRs originally drafted by working groups.

Working groups.—Working groups are formed by the stakeholder panel when a specific analytical topic has been identified. The primary purpose of a working group is to draft an SMPR. Working groups may also be formed to make general recommendations, such as developing a common definition to be used by multiple working groups. For example, SPIFAN formed a working group to create a definition for "infant formula" that could be shared and used by all of the SPIFAN working groups.

The process of drafting an SMPR usually requires several months, and several meetings and conference calls. An SMPR drafted by a working group is presented to a stakeholder panel. A stakeholder panel may revise, amend, or adopt a proposed SMPR on behalf of AOAC.

Fitness-for-Purpose Statement and Call for Methods

One of the first steps in organizing a project is creating a fitness-for-purpose statement. In AOAC, the fitness-for-purpose statement is a very general description of the methods needed. It is the responsibility of a working group chair to draft a fitness-for-purpose statement. A working group chair is also asked to prepare a presentation with background information about the analyte, matrix, and the nature of the analytical problem. A working group chair presents the background information and proposes a draft fitness-for-purpose statement to the presiding stakeholder panel. The stakeholder panel is asked to endorse the fitness-for-purpose statement.

The AOAC CSO prepares a call for methods based on the stakeholder panel-approved fitness-for-purpose statement. The call for methods is posted on the AOAC website and/or e-mailed to the AOAC membership and other known interested parties. AOAC staff collects and compiles candidate methods submitted in response to the call for methods. The CSO reviews and categorizes the methods.

Creating an SMPR

Starting the process of developing an SMPR can be a daunting challenge. In fact, drafting an SMPR should be a daunting challenge because the advisory panel has specifically identified an analytical problem that has yet to be resolved. Completing an SMPR can be a very rewarding experience because working group members will have worked with their colleagues through a tangle of problems and reached a consensus where before there were only questions.

It is advisable to have some representative candidate methods available for reference when a working group starts to develop an SMPR. These methods may have been submitted in response to the call for methods, or may be known to a working group member. In any case, whatever the origin of the method, candidate methods may assist working group members to determine reasonable performance requirements to be specified in the SMPR. The performance capabilities of exisiting analytical methodologies is a common question facing a working group.

Normally, a working chair and/or the AOAC CSO prepares a draft SMPR. A draft SMPR greatly facilitates the process and provides the working group with a structure from which to work.

Working group members are advised to first consider the "intended use" and "maximum time-to-determination" sections as this will greatly affect expectations for candidate methods. For example, methods intended to be used for surveillance probably need to be quick but do not require a great deal of precision, and false-positive results might be more tolerable. Whereas methods intended to be used for dispute resolution will require better accuracy, precision, and reproducibility, but time to determination is not as important.

Once a working group has agreed on the intended use of candidate methods, then it can begin to define the applicability of candidate methods. The applicability section of the SMPR is one of the most important, and sometimes most difficult, sections of the SMPR. The analyte(s) and matrixes must be explicitly identified. For chemical analytes, International Union of Pure and Applied Chemistry (IUPAC) nomenclature and/or Chemical Abstracts Service (CAS) registry numbers should be specified. Matrixes should be clearly identified including the form of the matrix such as raw, cooked, tablets, powders, etc. The nature of the matrix may affect the specific analyte. It may be advantageous to fully identify and describe the matrix before determining the specific analyte(s). It is not uncommon for working groups to revise the initial definition of the analyte(s) after the matrix(es) has been better defined.

Table 1. Example of method performance table for a single analyte

Analytical range	7.0–382.6 µg/mL	
Limit of quantitation (LOQ)	≤7.0 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%
	≥10 µg/mL	≤6%

Table 2. Example of method performance table for multiple analytes

	Analyte 1		Analyte 2		Analyte 3	
Analytical range	10–20 µg/mL		100–200 µg/mL		200–500 µg/mL	
Limit of quantitation (LOQ)	≤10 µg/mL		≤100 µg/mL		≤200 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%	<10 µg/mL	≤8%	<200 µg/mL	≤10%
	≥10 µg/mL	≤6%	≥10 µg/mL	≤6%	≥200 µg/mL	≤8%

For projects with multiple analytes, for example, vitamins A, D, E, and K in infant formula, it may be useful to organize a separate working group to fully describe the matrix(es) so that a common description of the matrix(es) can be applied to all of the analytes.

For single analyte SMPRs, it is most common to organize the method performance requirements into a table with 2–3 columns as illustrated in Table 1. For multiple analyte SMPRs, it is often convenient to present the requirements in an expanded table with analytes forming additional columns as illustrated in Table 2.

Once the intended use, analytical techniques, and method performance requirements have been determined, then a working group can proceed to consider the quality control parameters, such as the minimum validation requirements, system suitability procedures, and reference materials (if available). It is not uncommon that an appropriate reference material is not available. *Annex F* of the SMPR Guidelines provides comprehensive guidance for the development and use of in-house reference materials.

Most working groups are able to prepare a consensus SMPR in about 3 months.

Open Comment Period

Once a working group has produced a draft standard, AOAC opens a comment period for the standard. The comment period provides an opportunity for other stakeholders to state their perspective on the draft SMPR. All collected comments are reviewed by the AOAC CSO and the working group chair, and the comments are reconciled. If there are significant changes required to the draft standard as a result of the comments, the working group is convened to discuss and any unresolved issues will be presented for discussion at the stakeholder panel meeting.

Submission of Draft SMPRs to the Stakeholder Panel

Stakeholder panels meet several times a year at various locations. The working group chair (or designee) presents a draft SMPR to the stakeholder panel for review and discussion. A working group chair is expected to be able to explain the conclusions of the working group, discuss comments received, and to answer questions from the stakeholder panel. The members of the stakeholder panel may revise, amend, approve, or defer a decision on the proposed SMPR. A super majority of 2/3 or more of those voting is required to adopt an SMPR as an AOAC voluntary consensus standard.

Publication

Adopted SMPRs are prepared for publication by AOAC staff, and are published in the *Journal of AOAC INTERNATIONAL* and in the AOAC *Official Methods of Analysis*℠ compendium. Often, the AOAC CSO and working group chair prepare a companion article to introduce an SMPR and describe the analytical issues considered and resolved by the SMPR. An SMPR is usually published within 6 months of adoption.

Conclusion

SMPRs are a unique and novel concept for the analytical methods community. SMPRs are voluntary, consensus standards developed by stakeholders that prescribe the minimum analytical performance requirements for classes of analytical methods. The SMPR Guidelines provide a structure for working groups to use as they develop an SMPR. The guidelines have been employed in several AOAC projects and have been proven to be very useful. The guidelines are not a statute that users must conform to; they are a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using the guidelines.

References

(1) Eurachem, *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, Validation*, http://www.eurachem.org/guides/pdf/valid.pdf, posted December 1998, accessed March 2012

(2) *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis, Appendix D*, AOAC INTERNATIONAL, Gaithersburg, MD

(3) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (2012) *Official Methods of Analysis,* 19th Ed., *Appendix I, Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*, AOAC INTERNATIONAL, Gaithersburg, MD

(4) *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD

(5) Codex Alimentarius Codex Procedure Manual

(6) International Organization for Standardization, Geneva, Switzlerland

ANNEX A
Format of a
Standard Method Performance Requirement

AOAC SMPR® YYYY.XXX
(YYYY = Year; XXX = sequential identification number)

Method Name: Must include the analyte(s), matrix(es), and analytical technique [unless the *Standard Method Performance Requirement* (SMPR®) is truly intended to be independent of the analytical technology]. The method name may refer to a "common" name (e.g., "Kjeldahl" method).

Approved By: Name of stakeholder panel or expert review panel

Final Version Date: Date

Effective Date: Date

1. Intended Use: Additional information about the method and conditions for use.

2. Applicability: List matrixes if more than one. Provide details on matrix such as specific species for biological analytes, or International Union of Pure and Applied Chemistry (IUPAC) nomenclature and Chemical Abstracts Service (CAS) registry number for chemical analytes. Specify the form of the matrix such as raw, cooked, tablets, powders, etc.

3. Analytical Technique: Provide a detailed description of the analytical technique if the SMPR is to apply to a specific analytical technique; or state that the SMPR applies to any method that meets the method performance requirements.

4. Definitions: List and define terms used in the performance parameter table (*see* Table A2 for list of standard terms).

5. Method Performance Requirements: List the performance parameters and acceptance criteria appropriate for each method/analyte/matrix. *See* Table A1 for appropriate performance requirements.

If more than one analyte/matrix, and if acceptance criteria differ for analyte/matrix combinations then organize a table listing each analyte/matrix combination and its minimum acceptance criteria for each performance criteria.

6. System Suitability Tests and/or Analytical Quality Control: Describe minimum system controls and QC procedures.

7. Reference Material(s): Identify the appropriate reference materials if they exist, or state that reference materials are not available. Refer to *Annex E* (*AOAC Method Accuracy Review*) for instructions on the use of reference materials in evaluations.

8. Validation Guidance: Recommendations for type of evaluation or validation program such as single-laboratory validation (SLV), *Official Methods of Analysis*℠ (OMA), or *Performance Tested Methods*℠ (PTM).

9. Maximum Time-to-Determination: Maximum allowable time to complete an analysis starting from the test portion preparation to final determination or measurement.

Annex I: Inclusivity/Selectivity Panel. Recommended for qualitative and identification method SMPRs.

Annex II: Exclusivity/Cross-Reactivity Panel. Recommended for qualitative and identification method SMPRs.

Annex III: Environmental Materials Panel. Recommended for qualitative and identification method SMPRs.

Table A1. Performance requirements

Classifications of methods[a]				
Quantitative method		Qualitative method		
Main component[b]	Trace or contaminant[c]	Main component[b]	Trace or contaminant[c]	Identification method
Parameter				
Single-laboratory validation				
Applicable range	Applicable range	Inclusivity/selectivity	Inclusivity/selectivity	Inclusivity/selectivity
Bias[d]	Bias[d]	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity
Precision	Precision	Environmental interference	Environmental interference	Environmental interference
Recovery	Recovery	Laboratory variance	Laboratory variance	
Limit of quantitation (LOQ)	LOQ			
		Probability of detection (POD)[e]	POD at AMDL[f]	Probability of identification (POI)
Reproducibility				
RSD_R or target measurement uncertainty	RSD_R or target measurement uncertainty	POD (0)	POD (0)	POI (c)
		POD (c)	POD (c)	
		Laboratory POD[g]	Laboratory POD[g]	Laboratory POI

[a] *See Annex B* for additional information on classification of methods.

[b] ≥100 g/kg.

[c] <100 g/kg.

[d] If a reference material is available.

[e] At a critical level.

[f] AMDL = Acceptable minimum detection level.

[g] LPOD = CPOD.

Table A2. Recommended definitions

Bias	Difference between the expectation of the test results and an accepted reference value. Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias.
Environmental interference	Ability of the assay to detect target organism in the presence of environmental substances and to be free of cross reaction from environmental substances.
Exclusivity	Strains or isolates or variants of the target agent(s) that the method must not detect.
Inclusivity	Strains or isolates or variants of the target agent(s) that the method can detect.
Laboratory probability of detection (POD)	Overall fractional response (mean POD = CPOD) for the method calculated from the pooled POD_j responses of the individual laboratories (j = 1, 2, ..., L).[a] *See Annex C*.
Limit of quantitation (LOQ)	Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.
POD (0)	Probability of the method giving a (+) response when the sample is truly without analyte.
POD (c)	Probability of the method giving a (–) response when the sample is truly without analyte.
POD	Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. Consult *Annex C* for a full explanation.
Probability of identification (POI)	Expected or observed fraction of test portions at a given concentration that gives positive result when tested at a given concentration. Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*.[c]
Precision	Closeness of agreement between independent test results obtained under stipulated conditions. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results.[d]
Recovery	Fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method. There are two types of recovery: (*1*) Total recovery based on recovery of the native plus added analyte, and (*2*) marginal recovery based only on the added analyte (the native analyte is subtracted from both the numerator and denominator).[e]
Repeatability	Precision under repeatability conditions.
Repeatability conditions	Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.
Reproducibility	Precision under reproducibility conditions.
Reproducibility conditions	Conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.
Relative standard deviation (RSD)	$RSD = s_i \times 100/\bar{x}$
Standard deviation (s_i)	$s_i = [\Sigma(x_i - \bar{x})^2/n]^{0.5}$

[a] *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (*Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*), *J. AOAC Int.* **94**, 1359(2011) and *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), *Appendix I*.

[b] *International Vocabulary of Metrology (VIM)—Basic and General Concepts and Associated Terms* (2008) JCGM 200:2008, Joint Committee for Guides in Metrology (JCGM), www.bipm.org.

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int.* **95**, 273–285.

[d] ISO 5725-1-1994.

[e] *Official Methods of Analysis* (current edition) *Appendix D* (*Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*), AOAC INTERNATIONAL, Rockville, MD, USA.

Table A3. Recommendations for evaluation

Bias (if a reference material is available)	A minimum of five replicate analyses of a Certified Reference Material.[a]
Environmental interference	Analyze test portions containing a specified concentration of one environmental materials panel member. Materials may be pooled. Consult with AOAC statistician.
Exclusivity/cross-reactivity	Analyze one test portion containing a specified concentration of one exclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Inclusivity/selectivity	Analyze one test portion containing a specified concentration of one inclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Limit of quantitation (LOQ)	Estimate the LOQ = average (blank) + 10 × s_0 (blank). Measure blank samples with analyte at the estimated LOQ. Calculate the mean average and standard deviation of the results. Guidance[b]: For ML ≥ 100 ppb (0.1 mg/kg): LOD = ML × 1/5. For ML < 100 ppb (0.1 mg/kg): LOD = ML × 2/5.
Measurement uncertainty	Use ISO 21748: *Guidance for the use of repeatability, reproducibility, and trueness estimates in measurement uncertainty estimation to analyze data collected for bias, repeatability, and intermediate precision to estimate measurement uncertainty*.
POD(0)	Use data from collaborative study.
POD (c)	
Repeatability	Prepare and homogenize three unknown samples at different concentrations to represent the full, claimed range of the method. Analyze each unknown sample by the candidate method seven times, beginning each analysis from weighing out the test portion through to final result with no additional replication (unless stated to do so in the method). All of the analyses for one unknown sample should be performed within as short a period of time as is allowed by the method. The second and third unknowns may be analyzed in another short time period. Repeat for each claimed matrix.
Probability of detection (POD)	Determine the desired POD at a critical concentration. Consult with Table A7 to determine the number of test portions required to demonstrate the desired POD.
Probability of identification (POI)	Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*[c].
Recovery	Determined from spiked blanks or samples with at least seven independent analyses per concentration level at a minimum of three concentration levels covering the analytical range. Independent means at least at different times. If no confirmed (natural) blank is available, the average inherent (naturally containing) level of the analyte should be determined on at least seven independent replicates. Marginal % recovery = $(C_f - C_u) \times 100/C_A$ Total % recovery = $100(C_f)/(C_u + C_A)$ where C_f = concentration of fortified samples, C_u = concentration of unfortified samples, and C_A = concentration of analyte added to the test sample.[d] Usually total recovery is used unless the native analyte is present in amounts greater than about 10% of the amount added, in which case use the method of addition.[e]
Reproducibility (collaborative or interlaboratory study)	Quantitative methods: Recruit 10–12 collaborators; must have eight valid data sets; two blind duplicate replicates at five concentrations for each analyte/matrix combination to each collaborator.
	Qualitative methods: Recruit 12–15 collaborators; must have 10 valid data sets; six replicates at five concentrations for each analyte/matrix combination to each collaborator.

[a] *Guidance for Industry for Bioanalytical Method Validation* (May 2001) U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).

[b] Codex Alimentarius Codex Procedure Manual.

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int.* **95**, 273–285.

[d] *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (current edition) *Official Methods of Analysis*, *Appendix D,* AOAC INTERNATIONAL, Rockville, MD, USA.

[e] *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (current edition) *Official Methods of Analysis*, *Appendix K*, AOAC INTERNATIONAL, Rockville, MD, USA.

Table A4. Expected precision (repeatability) as a function of analyte concentration[a]

Analyte, %	Mass fraction (C)	Unit	RSD_r, %
100	1	100%	1.3
10	10^{-1}	10%	1.9
1	10^{-2}	1%	2.7
0.1	10^{-3}	0.1%	3.7
0.01	10^{-4}	100 ppm (mg/kg)	5.3
0.001	10^{-5}	10 ppm (mg/kg)	7.3
0.0001	10^{-6}	1 ppm (mg/kg)	11
0.00001	10^{-7}	100 ppb (µg/kg)	15
0.000001	10^{-8}	10 ppb (µg/kg)	21
0.0000001	10^{-9}	1 ppb (µg/kg)	30

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Rockville, MD, USA.

The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides targets RSDs for a range of analyte concentrations.

Table A5. Expected recovery as a function of analyte concentration[a]

Analyte, %	Mass fraction (C)	Unit	Mean recovery, %
100	1	100%	98–102
10	10^{-1}	10%	
1	10^{-2}	1%	97–103
0.1	10^{-3}	0.1%	95–105
0.01	10^{-4}	100 ppm (mg/kg)	90–107
0.001	10^{-5}	10 ppm (mg/kg)	80–110
0.0001	10^{-6}	1 ppm (mg/kg)	
0.00001	10^{-7}	100 ppb (µg/kg)	
0.000001	10^{-8}	10 ppb (µg/kg)	60–115
0.0000001	10^{-9}	1 ppb (µg/kg)	40–120

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Rockville, MD, USA.

Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 100% to 1 ppb.

Table A6. Predicted relative standard deviation of reproducibility ($PRSD_R$)[a]

Analyte, %	Mass fraction (C)	Unit	RSD_R, %
100	1	100%	2
10	10^{-1}	10%	3
1	10^{-2}	1%	4
0.1	10^{-3}	0.1%	6
0.01	10^{-4}	100 ppm (mg/kg)	8
0.001	10^{-5}	10 ppm (mg/kg)	11
0.0001	10^{-6}	1 ppm (mg/kg)	16
0.00001	10^{-7}	100 ppb (µg/kg)	22
0.000001	10^{-8}	10 ppb (µg/kg)	32
0.0000001	10^{-9}	1 ppb (µg/kg)	45

[a] Table excerpted from *Definitions and Calculations of HorRat Values from Intralaboratory Data*, HorRat for SLV.doc, 2004-01-18, AOAC INTERNATIONAL, Rockville, MD, USA.

Predicted relative standard deviation or reproducibility = $PRSD_R$: Reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction.

This table provides the calculated $PRSD_R$ for a range of concentrations. *See* Annex D for additional information.

Table A7. POD and number of test portions[a,b]

	Sample size required for proportion						
Assume	1. Binary outcome (occur/not occur). 2. Constant probability rho of event occurring. 3. Independent trials (e.g., simple random sample). 4. Fixed number of trials (*N*)						
Inference	95% Confidence interval lies entirely at or above specified minimum rho						
Desired	Sample size *N* needed						
Minimum probability rho, %	Sample size (*N*)	Minimum No. events (*x*)	Maximum No. nonevents (*y*)	1-Sided lower confidence limit on rho[c], %	Expected lower confidence limit on rho, %	Expected upper confidence limit on rho, %	Effective AOQL[d] rho, %
50	3	3	0	52.6	43.8	100.0	71.9
50	10	8	2	54.1	49.0	94.3	71.7
50	20	14	6	51.6	48.1	85.5	66.8
50	40	26	14	52.0	49.5	77.9	63.7
50	80	48	32	50.8	49.0	70.0	59.5
55	4	4	0	59.7	51.0	100.0	75.5
55	10	9	1	65.2	59.6	100.0	79.8
55	20	15	5	56.8	53.1	88.8	71.0
55	40	28	12	57.1	54.6	81.9	68.2
55	80	52	28	55.9	54.1	74.5	64.3
60	5	5	0	64.9	56.5	100.0	78.3
60	10	9	1	65.2	59.6	100.0	79.8
60	20	16	4	62.2	58.4	91.9	75.2
60	40	30	10	62.4	59.8	85.8	72.8
60	80	56	24	61.0	59.2	78.9	69.1
65	6	6	0	68.9	61.0	100.0	80.5
65	10	9	1	65.2	59.6	100.0	79.8
65	20	17	3	67.8	64.0	94.8	79.4
65	40	31	9	65.1	62.5	87.7	75.1
65	80	59	21	65.0	63.2	82.1	72.7
70	7	7	0	72.1	64.6	100.0	82.3
70	10	10	0	78.7	72.2	100.0	86.1
70	20	18	2	73.8	69.9	97.2	83.6
70	40	33	7	70.7	68.0	91.3	79.7
70	80	63	17	70.4	68.6	86.3	77.4
75	9	9	0	76.9	70.1	100.0	85.0
75	10	10	0	78.7	72.2	100.0	86.1
75	20	19	1	80.4	76.4	100.0	88.2
75	40	35	5	76.5	73.9	94.5	84.2
75	80	67	13	75.9	74.2	90.3	82.2
80	11	11	0	80.3	74.1	100.0	87.1
80	20	19	1	80.4	76.4	100.0	88.2
80	40	37	3	82.7	80.1	97.4	88.8
80	80	70	10	80.2	78.5	93.1	85.8
85	20	20	0	88.1	83.9	100.0	91.9
85	40	38	2	86.0	83.5	98.6	91.1
85	80	74	6	86.1	84.6	96.5	90.6
90	40	40	0	93.7	91.2	100.0	95.6
90	60	58	2	90.4	88.6	99.1	93.9
90	80	77	3	91.0	89.5	98.7	94.1
95	60	60	0	95.7	94.0	100.0	97.0
95	80	80	0	96.7	95.4	100.0	97.7
95	90	89	1	95.2	94.0	100.0	97.0
95	96	95	1	95.5	94.3	100.0	97.2
98	130	130	0	98.0	97.1	100.0	98.6
98	240	239	1	98.2	97.7	100.0	98.8
99	280	280	0	99.0	98.6	100.0	99.3
99	480	479	1	99.1	98.8	100.0	99.4

[a] Table excerpted from Technical Report TR308, *Sampling plans to verify the proportion of an event exceeds or falls below a specified value*, LaBudde, R. (June 4, 2010) (not published). The table was produced as part of an informative report for the Working Group for Validation of Identity Methods for Botanical Raw Materials commissioned by the AOAC INTERNATIONAL Presidential Task Force on Dietary Supplements. The project was funded by the Office of Dietary Supplements, National Institutes of Health.

[b] Copyright 2010 by Least Cost Formulations, Ltd. All rights reserved.

[c] Based on modified Wilson score 1-sided confidence interval.

[d] AOQL = Average outgoing quality level.

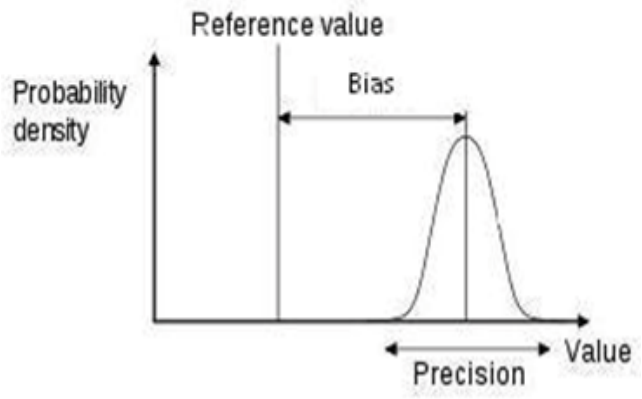

Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

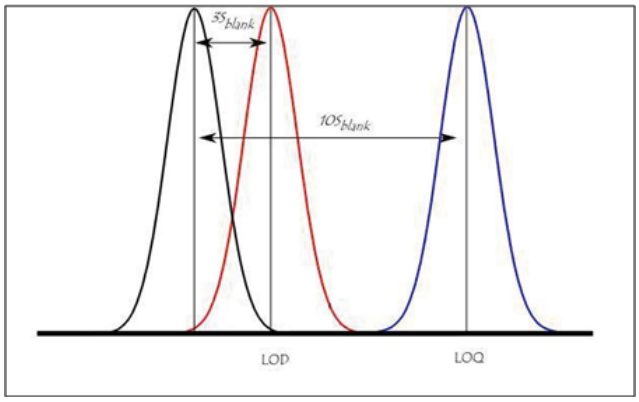

Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

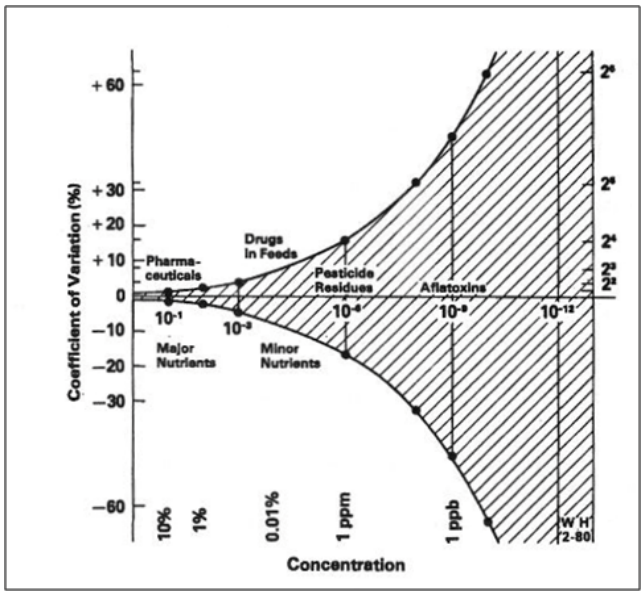

Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int.* 89, 1095(2006)].

ANNEX B
Classification of Methods

The following guidance may be used to determine which performance parameters in Table A1 apply to different classifications of methods. AOAC INTERNATIONAL does not recognize the term "semiquantitative" as a method classification. Methods that have been self-identified as semiquantitative will be classified into one of the following five types:

Type I: Quantitative Methods

Characteristics: Generates a continuous number as a result.

Recommendation: Use performance requirements specified for quantitative method (main or trace component). Use recovery range and maximum precision variation in Tables A4 and A5.

In some cases and for some purposes, methods with less accuracy and precision than recommended in Tables A4 and A5 may be acceptable. Method developers should consult with the appropriate method committee to determine if the recommendations in Tables A4 and A5 do or do not apply to their method.

Type II: Methods that Report Ranges

Characteristics: Generates a "range" indicator such as 0, low, moderate, and high.

Recommendation: Use performance requirements specified for qualitative methods (main component). Specify a range of POD for each range "range" indicator.

Type III: Methods with Cutoff Values

Characteristics: Method may generate a continuous number as an interim result (such as a CT value for a PCR method), which is not reported but converted to a qualitative result (presence/ absence) with the use of a cutoff value.

Recommendation: Use performance requirements specified for qualitative methods.

Type IV: Qualitative Methods

Characteristics: Method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a specified test portion.

Recommendation: Use performance requirements specified for qualitative methods.

Type V: Identification Methods

Characteristics: Method of analysis whose purpose is to determine the identity of an analyte.

Recommendation: Use performance requirements specified for identification methods.

ANNEX C
Understanding the POD Model

Excerpted from AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures, J. AOAC Int. ***94****, 1359(2011) and Official Methods of Analysis of AOAC INTERNATIONAL (current edition), Appendix I.*

The Probability of Detection (POD) model is a way of characterizing the performance of a qualitative (binary) method. A binary qualitative method is one that gives a result as one of two possible outcomes, either positive or negative, presence/absence, or +/–.

The single parameter of interest is the POD, which is defined as the probability at a given concentration of obtaining a positive response by the detection method. POD is assumed to be dependent on concentration, and generally, the probability of a positive response will increase as concentration increases.

For example, at very low concentration, the expectation is that the method will not be sensitive to the analyte, and at very high concentration, a high probability of obtaining a positive response is desired. The goal of method validation is to characterize how method response transitions from low concentration/low response to high concentration/high response.

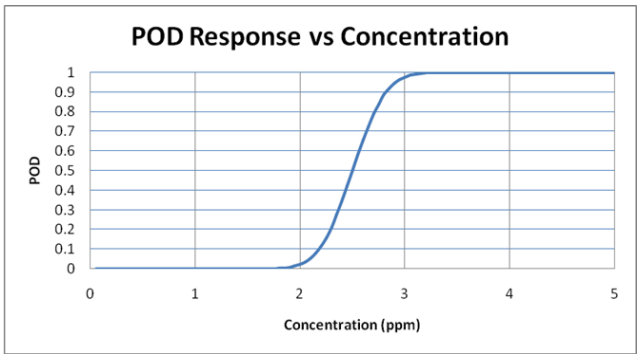

Figure C1. Theoretical POD curve for a qualitative detection method.

POD is always considered to be dependent upon analyte concentration. The POD curve is a graphical representation of method performance, where the probability is plotted as a function of concentration (*see*, for example, Figure C1).

The POD model is designed to allow an objective description of method response without consideration to an a priori expectation of the probabilities at given concentrations. The model is general enough to allow comparisons to any theoretical probability function.

The POD model is also designed to allow for an independent description of method response without consideration to the response of a reference method. The model is general enough to allow for comparisons between reference and candidate method responses, if desired.

Older validation models have used the terms "sensitivity," "specificity," "false positive," and "false negative" to describe method performance. The POD model incorporates all of the performance concepts of these systems into a single parameter, POD.

For example, false positive has been defined by some models as the probability of a positive response, given the sample is truly negative (concentration = 0). The equivalent point on the POD curve for this performance characteristic is the value of the curve at Conc = 0.

Similarly, false negative has sometimes been defined as the probability of a negative response when the sample is truly positive (concentration >0). In the POD curve, this would always be specific to a given sample concentration, but would be represented as the distance from the POD curve to the POD = 1 horizontal top axis at all concentrations except C = 0.

The POD model incorporates all these method characteristics into a single parameter, which is always assumed to vary by concentration. In other models, the terms "false positive," "false negative," "sensitivity," and "specificity" have been defined in a variety of ways, usually not conditional on concentration. For these reasons, these terms are obsolete under this model (*see* Table C1).

The terms "sensitivity," "specificity," "false positive," and "false negative" are obsolete under the POD model (*see* Figure C2).

Table C1. Terminology

Traditional terminology	Concept	POD equivalent	Comment
False positive	Probability of the method giving a (+) response when the sample is truly without analyte	POD(0) POD at conc = 0	POD curve value at conc = 0; "*Y*-intercept" of the POD curve
Specificity	Probability of the method giving a (-) response when the sample is truly without analyte	1-POD(0)	Distance along the POD axis from POD = 1 to the POD curve value
False negative (at a given concentration)	Probability of a (–) response at a given concentration	1-POD(c)	Distance from the POD curve to the POD = 1 "top axis" in the vertical direction
Sensitivity (at a given concentration)	Probability of a (+) response at a given concentration	POD(c)	Value of the POD curve at any given concentration
True negative	A sample that contains no analyte	C = 0	Point on concentration axis where c = 0
True positive	A sample that contains analyte at some positive concentration	C > 0	Range of concentration where c > 0

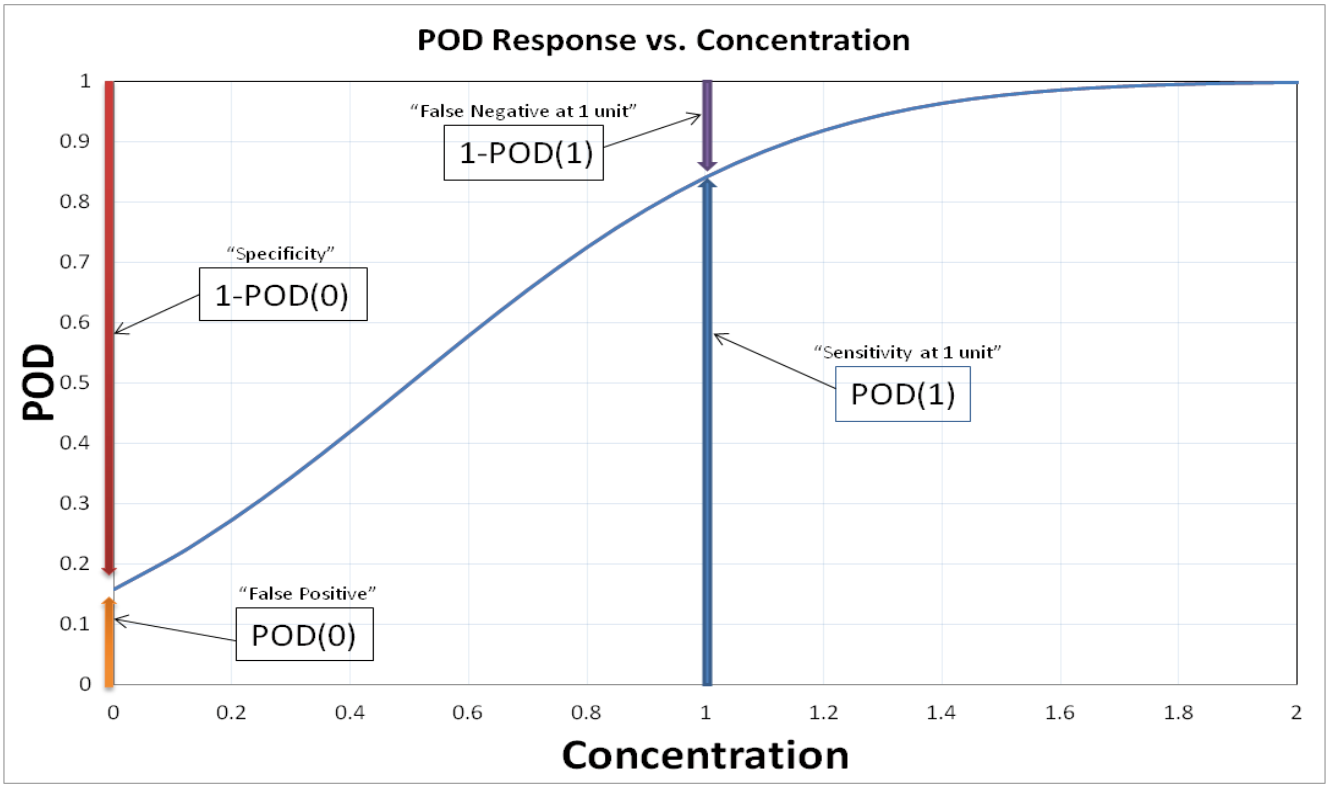

Figure C2. Comparison of POD model terminology to other obsolete terms.

ANNEX D
Definitions and Calculations of HorRat Values from Intralaboratory Data

Excerpted from Definitions and Calculations of HorRat Values from Intralaboratory Data, AOAC INTERNATIONAL, HorRat for SLV.doc, 2004-01-18.

1. Definitions

1.1 Replicate Data

Data developed under common conditions in the same laboratory: simultaneous performance, or, if necessary to obtain sufficient values, same series, same analyst, same day. Such data provides "repeatability statistical parameters."

1.2 Pooled Data

Replicate data developed in the same laboratory under different conditions but considered sufficiently similar that, for the purpose of statistical analysis, they may be considered together. These may include different runs, different instruments, different analysts, and different days.

1.3 Average

0 = Sum of the individual values, x_i, divided by the number of individual values, n.

$$0 = (\Sigma\, x_i)/n$$

1.4 Standard Deviation

$$s_i = [\Sigma(x_i - (\overline{x})^2/n]^{0.5}$$

1.5 Relative Standard Deviation

$$RSD = s_i \times 100/\overline{x}$$

1.5.1 Repeatability Relative Standard Deviation [RSD(r) or RSD_r]

The relative standard deviation calculated from within-laboratory data.

1.5.2 Reproducibility Relative Standard Deviation [RSD(R) or RSD_R]

The relative standard deviation calculated from among-laboratory data.

Table D1. Predicted relative standard deviations

Concentration (C)	Mass fraction (C)	$PRSD_R$, %
100%	1.0	2
1%	0.01	4
0.01%	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

1.6 Mass Fraction

Concentration, C, expressed as a decimal fraction. For calculating and reporting statistical parameters, data may be expressed in any convenient units (e.g., %, ppm, ppb, mg/g, µg/g; µg/kg; µg/L, µg/µL, etc.). For reporting HorRat values, data must be reported as a mass fraction where the units of the numerator and denominator are the same: e.g., for 100% (pure materials), the mass fraction C = 1.00; for 1 µg/g (ppm), C = 0.000001 = (E-6). *See* Table D1 for other examples.

1.7 Predicted Relative Standard Deviation [PRSD(R) or $PRSD_R$]

The reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD(R) = 2C^{-0.15}$$

where C is expressed as a mass fraction. *See* Table D1.

In spreadsheet notation: PRSD(R) = 2 * C ^(–0.15).

1.8 HorRat Value

The ratio of the reproducibility relative standard deviation calculated from the data to the PRSD(R) calculated from the Horwitz formula:

$$HorRat = RSD(R)/PRSD(R)$$

To differentiate the usual HorRat value calculated from reproducibility data from the HorRat value calculated from repeatability data, attach an R for the former and an r for the latter. But note that the denominator always uses the PRSD(R) calculated from reproducibility data because this parameter is more predictable than the parameter calculated from repeatability data:

$$HorRat(R) = RSD_R/PRSD(R)$$

$$HorRat(r) = RSD_r/PRSD(R)$$

Some expected, predicted relative standard deviations are given in Table D1.

2 *Acceptable HorRat Values*

2.1 For Interlaboratory Studies

HorRat(R): The original data developed from interlaboratory (among-laboratory) studies assigned a HorRat value of 1.0 with limits of acceptability of 0.5 to 2.0. The corresponding within-laboratory relative standard deviations were found to be typically 1/2 to 2/3 the among-laboratory relative standard deviations.

Table D2. Predicted relative standard deviations

Concentration (C)	$PRSD_R$, %	$PRSD_r$, %
100%	2	1
1%	4	2
0.01%	8	4
1 ppm	16	8
10 ppb	32	16
1 ppb	45	22

2.1.1 Limitations

HorRat values do not apply to method-defined (empirical) analytes (moisture, ash, fiber, carbohydrates by difference, etc.), physical properties or physical methods (pH, viscosity, drained weight, etc.), and ill-defined analytes (polymers, products of enzyme reactions).

2.2 For Intralaboratory Studies

2.2.1 Repeatability

Within-laboratory acceptable predicted target values for repeatability are given in Table D2 at 1/2 of PRSD(R), which represents the best case.

2.2.2 HorRat(r)

Based on experience and for the purpose of exploring the extrapolation of HorRat values to SLV studies, take as the minimum acceptability 1/2 of the lower limit ($0.5 \times 0.5 \approx 0.3$) and as the maximum acceptability 2/3 of the upper limit ($0.67 \times 2.0 \approx 1.3$).

Calculate HorRat(r) from the SLV data:

$$HorRat(r) = RSD(r)/PRSD(R)$$

Acceptable HorRat(r) values are 0.3–1.3. Values at the extremes must be interpreted with caution. With a series of low values, check for unreported averaging or prior knowledge of the analyte content; with a series of high values, check for method deficiencies such as unrestricted times, temperatures, masses, volumes, and concentrations; unrecognized impurities (detergent residues on glassware, peroxides in ether); incomplete extractions and transfers and uncontrolled parameters in specific instrumental techniques.

2.3 Other Limitations and Extrapolations

The HorRat value is a very rough but useful summary of the precision in analytical chemistry. It overestimates the precision at the extremes, predicting more variability than observed at the high end of the scale (C > ca 0.1; i.e., >10%) and at the low end of the scale (C < E-8; i.e., 10 ng/g; 10 ppb).

ANNEX E
AOAC Method Accuracy Review

Accuracy of Method Based on Reference Material

Reference material (RM) used.—The use of RMs should be seen as integral to the process of method development, validation, and performance evaluation. RMs are not the only component of a quality system, but correct use of RMs is essential to appropriate quality management. RMs with or without assigned quantity values can be used for measurement precision control, whereas only RMs with assigned quantity values can be used for calibration or measurement trueness control. Method development and validation for matrices within the scope of the method is done to characterize attributes such as recovery, selectivity, "trueness" (accuracy, bias), precision (repeatability and reproducibility), uncertainty estimation, ruggedness, LOQ or LOD, and dynamic range. RMs should be chosen that are fit-for-purpose. When certified reference materials (CRMs) are available with matrices that match the method scope, much of the work involved in method development has already been completed, and that work is documented through the certificate. RMs with analyte values in the range of test samples, as well as "blank" matrix RMs, with values below or near detection limits, are needed.

Availability of RM.—Consideration needs to be given to the future availability of the chosen RM. Well-documented methods that cannot be verified in the future due to lack of material may lose credibility or be seen as inferior.

Fit to method scope.—Natural matrix CRMs provide the greatest assurance that the method is capable of producing accurate results for that matrix. When selecting an RM to perform a method validation, analysts should consider the method to material fit. An example of a good fit would be a method for specified organic molecules in infant formula and using an infant formula or powder milk RM. A poor fit would be a method for specified organic molecules in infant formula and using a sediment material.

Stability.—Providing a stable RM can be challenging where analytes are biologically active, easily oxidized, or interactive with other components of the matrix. CRM producers provide assurance of material stability, as well as homogeneity. CRMs are accompanied by a certificate that includes the following key criteria:

(*1*) Assigned values with measurement uncertainty and metrological traceability
(*2*) Homogeneity
(*3*) Stability, with the expiration date for the certificate
(*4*) Storage requirements
(*5*) Information on intended use
(*6*) Identity of matrix

For some RMs, such as botanical RMs, the source and/or authenticity can be a very important piece of information that should be included with the certificate. Even under ideal storage conditions, many analytes have some rate of change. Recertification may be done by the supplier, and a certificate reissued with a different expiration date and with certain analyte data updated or removed.

Definition of CRM.—Refer to the AOAC TDRM document for definitions from ISO Guide 30, Amd. 1 (2008), http://www.aoac.org/divisions/References.pdf.

Information on source of RM is available.—It is the responsibility of the material producer to provide reliable authentication of the RM and make a clear statement in the accompanying documentation. This should be an as detailed listing as possible, including handling of ingredients, identification of plant materials as completely as feasible (species, type, subtype, growing region), etc. This is comparable to other required information on an RM for judging its suitability for a specific application purpose (e.g., containing how much of the targeted analyte, stabilized by adding acid—therefore not suited for certain parameters/procedures, etc.).

Separate RM used for calibration and validation.—A single RM cannot be used for both calibration and validation of results in the same measurement procedure.

Blank RM used where appropriate.—Blank matrix RMs are useful for ensuring performance at or near the detection limits. These are particularly useful for routine quality control in methods measuring, for instance, trace levels of allergens, mycotoxins, or drug residues.

Storage requirements were maintained.—Method developers should maintain good documentation showing that the RM producer's recommended storage conditions were followed.

Cost.—The cost of ongoing method checks should be considered. Daily use of CRMs can be cost prohibitive. Monthly or quarterly analysis of these materials may be an option.

Concentration of analyte fits intended method.—Concentration of the analyte of interest is appropriate for *Standard Method Performance Requirements* (SMPRs®).

Uncertainty available.—Every measurement result has an uncertainty associated with it, and the individual contributions toward the combined uncertainty arise from multiple sources. Achieving the target measurement uncertainty set by the customer for his/her problem of interest is often one of the criteria used in selecting a method for a given application. Estimation of measurement uncertainty can be accomplished by different approaches, but the use of RMs greatly facilitates this part of a method validation.

Demonstration of Method Accuracy when No Reference Material Is Available

If an RM is not available, how is accuracy demonstrated?

There are many analytes for which a CRM with a suitable matrix is not available. This leaves the analyst with few options. For some methods, there may be proficiency testing programs that include a matrix of interest for the analyte. Proficiency testing allows an analyst to compare results with results from other laboratories, which may or may not be using similar methods. Spiking is another technique that may be used. When alternative methods are available, results may be compared between the different methods. These alternatives do not provide the same level of assurance that is gained through the use of a CRM.

Spike recovery.—In the absence of an available CRM, one technique that is sometimes used for assessing performance is the spiking of a matrix RM with a known quantity of the analyte. When this method is used, it cannot be assumed that the analyte is bound in the same way as it would be in a natural matrix. Nevertheless, a certified blank RM would be the preferred choice for constructing a spiked material.

When preparing reference solutions, the pure standards must be completely soluble in the solvent. For insoluble materials in a liquid suspension or for powdered forms of dry materials, validation is required to demonstrate that the analyte is homogeneously

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distributed and that the response of the detection system to the analyte is not affected by the matrix or preparation technique. When a matrix material is selected for spiking, it should be reasonably characterized to determine that it is sufficiently representative of the matrix of interest. Spiked samples must be carried through all steps of the method. Many analytes are bound in a natural matrix and whether the spiked analyte will behave the same as the analyte in a natural matrix is unknown.

Other.—Use of a substitute RM involves the replacement of the CRM with an alternative matrix RM matching the matrix of interest as close as possible based on technical knowledge.

ANNEX F
Development and Use of In-House Reference Materials

The use of reference materials is a vital part of any analytical quality assurance program. However, you may have questions about their creation and use. The purpose of this document is to help answer many of these questions.

- What is a reference material?
- Why use reference materials?
- What certified reference materials (CRMs) are currently available?
- Why use an in-house reference material?
- How do I create an in-house reference material?
- How do I use the data from an in-house reference material?

What Is a Reference Material?

The International Organization for Standardization (ISO) defines a reference material as a "material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials" (1). In plain English, natural-matrix reference materials, such as those you might prepare for use in-house, can be used to validate an analytical method or for quality assurance while you're using your method to analyze your samples. (Natural-matrix materials are not generally used as calibrants because of the increased uncertainty that this would add to an analysis.) The assigned values for the target analytes of an in-house reference material can be used to establish the precision of your analytical method and, if used in conjunction with a CRM, to establish the accuracy of your method.

ISO defines a CRM as a "reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence" (1).

Why Use Reference Materials?

CRMs can be used across the entire scope of an analytical method and can provide traceability of results to the International System of Units (SI). During method development, CRMs can be used to optimize your method. During method validation, they can be used to ensure that your method is capable of producing the "right" answer, and to determine how close your result is to that answer. During routine use, they can be used to determine within-day and between-day repeatability, and so demonstrate that your method is in control and is producing accurate results every time it is used.

Natural-matrix reference materials should mimic the real samples that will be analyzed with a method. They should behave just as your samples would during a procedure, so if you obtain accurate and precise values for your reference material, you should obtain accurate and precise values for your samples as well.

What Certified Reference Materials Are Currently Available?

CRMs are available from a number of sources, including (but not limited to):

- American Association of Cereal Chemists (AACC)
- American Oil Chemists Society (AOCS)
- International Atomic Energy Agency (IAEA)
- Institute for Reference Materials and Measurements (IRMM)
- LGC Promochem
- National Institute of Standards and Technology (NIST)
- National Research Council Canada (NRC Canada)
- UK Food Analysis Proficiency Assessment Program (FAPAS)

A number of websites provide general overviews and catalogs of producers' and distributors' reference materials:

http://www.aocs.org/tech/crm/
http://www.comar.bam.de
http://www.erm-crm.org
http://www.iaea.org/oregrammeslaqcs
http://www.aaccnet.org/checksample
http://www.irmm·ire.be/mrm.html
http://www.lgcpromochem.com
http://www.naweb.iaea.org/nahu/nmrm/
http://www.nist.gov/srm
http://www.fapas.com/index. cfm
http://www.virm.net.

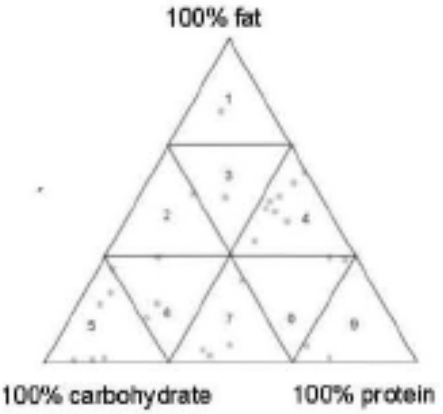
Because new reference materials are produced regularly, it is important to check these websites to determine what is currently available.

Why Use an In-House Reference Material?

There are many benefits to the use of a CRM. CRMs have been prepared to be homogeneous and, if stored under the proper conditions, stable. You are provided with a certified value as well as the statistical data for the concentration of your analyte; this is about as close as you can come to knowing the true value of the concentration of the analyte. The material has been tested by experienced analysts in leading laboratories, so you have the security of knowing that your method is generating values similar to those generated in other competent laboratories. The CRMs from the sources mentioned above are nationally and/or internationally recognized, so when you obtain acceptable results for a CRM using your analytical method, you give credibility to your methodology and traceability to your results.

But there are some drawbacks associated with CRMs. Unfortunately, many analyte/matrix combinations are not currently available. When testing food products for nutrient content, for example, a laboratory can be asked to analyze anything that might be found in a kitchen or grocery store. Reference materials that represent all of the types of foods that need to be tested are not available, and most CRMs are certified for a limited number of analytes. It is important to match the reference material matrix to your sample matrix. (Food examples dominate the discussion

Excerpted from *Development and Use of In-House Reference Materials*, Rev. 2, 2009. Copyright 2005 by the AOAC Technical Division on Reference Materials (TDRM).



below, but the same processes apply to the development of in-house reference materials in other areas of analytical chemistry.)

To demonstrate the applicability of an analytical method to a wide variety of food matrices, AOAC INTERNATIONAL's Task Force on Methods for Nutrition Labeling developed a triangle partitioned into sectors in which foods are placed based on their protein, fat, and carbohydrate content (2, 3). Since ash does not have a great impact on the performance of an analytical method for organic-material foods, and water can be added or removed, it can be assumed that the behavior of an analytical method is determined to large extent by the relative proportions of these proximates. AOAC INTERNATIONAL anticipated that one or two foods in a given sector would be representative of other foods in that sector and therefore would be useful for method assessment. Similarly, one or two reference materials in a given sector (or near each other in adjacent sectors) should be useful for quality assurance for analyses involving the other foods in the sector. The positions of many of the food-matrix CRMs from the sources listed above are shown in the triangle and are provided in the list.

These food-matrix reference materials are spread through all sectors of the triangle, thereby making it likely that you can find an appropriate CRM to match to your samples. Ultimately, however, the routine use of a CRM can be cost prohibitive, and is not really the purpose of CRMs. For example, in order to use NIST's Standard Reference Material (SRM) 2387 Peanut Butter for all mandatory nutrition labeling analyses, you could buy one sales unit (three jars, each containing 170 g material) for $649 (2009 price). If you charge your customer about $1000 for analysis of all mandatory nutrients in a test material, the control material would account for more than 60% of your fees. Therefore, many laboratories have found it more cost-effective to create in-house reference materials for routine quality control and characterize them in conjunction with the analysis of a CRM (4). You can prepare larger quantities of a reference material by preparing it in-house, and you have more flexibility in the types of matrices you can use. There are not many limitations on what can be purchased.

How Do I Create an In-House Reference Material?

There are basically three steps to preparing an in-house reference material: selection (including consideration of homogeneity and stability), preparation, and characterization. Additional guidance through these steps can be provided from the AOAC Technical Division on Reference Materials (TDRM), as well as in ISO Guides 34 (5) and 35 (6).

Sector	RM No.	Matrix
	NIST 1563	Coconut oil
1	NIST 3274	Fatty acids in botanical oils
1	NIST 3276	Carrot extract in oil
1	LGC 7104	Sterilized cream
2	NIST 2384	Baking chocolate
3	NIST 2387	Peanut butter
4	NIST 1546	Meat homogenate
4	LGC 7106	Processed cheese
4	LGC 7000	Beef/pork meat
4	LGC 7150	Processed meat
4	LGC 7151	Processed meat
4	LGC 7152	Processed meat
4	SMRD 2000	Fresh meat
4	LGC 7101	Mackerel paste
4	LGC QC1001	Meat paste 1
4	LGC QC1004	Fish paste 1
5	BCR-382	Wleat flour
5	BCR-381	Rye flour
5	LGC 7103	Sweet digestive biscuit
5	LGC 7107	Madeira cake
5	LGC QC1002	Flour 1
6	NIST 1544	Fatty acids
6	NIST 1548a	Typical diet
6	NIST 1849	Infant/adult nutritional formula
6	LGC 7105	Rice pudding
7	LGC 7001	Pork meat
7	NIST 1566b	Oyster tissue
7	NIST 1570a	Spinach leaves
7	NIST 2385	Spinach
8	NIST 1946	Lake trout
8	LGC 7176	Canned pet food
9	NIST 1974a	Mussel tissue
9	NIST 3244	Protein powder

References

(1) JCGM 200:2008, *International vocabulary of metrology—Basic and general concepts and associated terms (VIM)*, International Bureau of Weights and Measures (www.bipm.org)

(2) Wolf, W.R., & Andrews, K.W. (1995) *Fresenius' J. Anal. Chem*. **352**, 73–76

(3) Wolf, W.R. (1993) *Methods of Analysis for Nutrition Labeling*, D.R. Sullivan & D.E. Carpenter (Eds), AOAC INTERNATIONAL, Gaithersburg, MD

(4) European Reference Materials (2005) *Comparison of a Measurement Result with the Certified Value*, Application Note 1

(5) *ISO Guide 34 General Requirements for the Competence of Reference Material Producers* (2009) 2nd, International Organization for Standardization, Geneva, Switzerland

(6) *Guide 35 Certification of Reference Materials—General and Statistical Principles* (2006) International Organization for Standardization, Geneva, Switzerland

For more information about TDRM, visit http://aoac.org/divisions/tdrm.

Appendix L: AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation

1 General

(**a**) All methods for a given nutrient or nutrient group will be subjected to a common single-laboratory validation (SLV) protocol utilizing the available SPIFAN matrices.

(**b**) SLV protocols may vary somewhat *between* nutrients, depending on the specific demands associated with each.

(**c**) Study directors (SDs) for each nutrient or nutrient group will agree on final details of the required SLV protocol.

(**d**) Suitability criteria indicating method/system performance is acceptable will be generated during SLV.

2 Linearity/Calibration Fit

(**a**) Minimum of six levels (levels to be agreed upon by SDs) that span the desired working range.

(**b**) Relative error of back-calculated concentrations determined within the desired working range. (No specific criterion in standard method performance requirement. Recommend calibration errors to be <5%.)

(**c**) Minimum of three independent experiments. (Independently prepared standards, if feasible.)

3 LOD/LOQ

Ten independent analyses of blank or blank spiked at low level (to be agreed upon by SDs) (if there is no detectable blank signal):

LOD = blank mean + 3 standard deviations

LOQ = blank mean + 10 standard deviations
(concentration of blank to be <10% of the estimated LOQ)

4 Specificity

(**a**) No explicit proposals for evaluating specificity have been suggested.

(**b**) Because useful strategies for doing this vary from analyte to analyte, SDs for each nutrient will agree on acceptable practice.

(**c**) An adequate evaluation of specificity may have already been done for some methods, in which case it would not have to be repeated.

5 Precision

(**a**) All samples selected for precision studies will be analyzed in duplicate on each of 6 days using multiple analysts and instruments as practical for the different days. Fresh reagents and working standards will be used each day. Reports will include information of number of analysts, instruments, etc.

(**b**) Precision data using SRM 1849a should be included for *all* methods. For each nutrient or nutrient group, precision data shall be collected using an appropriate variety of SPIFAN matrices that contain the nutrient or nutrient group (as agreed upon by the SDs). The number of matrices may vary between nutrients.

(**c**) Estimate within-day (repeatability), day-to-day, and overall (intermediate precision) for each sample type. Estimates pooled across sample types may also be useful.

6 Accuracy (Trueness)

(**a**) *Analysis of SRM 1849a*.—Comparison to SRM values may not always be applicable because nutrient definitions are not aligned. SDs will agree on whether this should be part of the accuracy assessment.

(**b**) *Spike recovery.*—(*1*) Recovery will be determined from an appropriate sampling of SPIFAN matrices. Either unfortified (preferably) and/or fully fortified products may be used.

(*2*) Each selected matrix will be spiked at two levels. Recommended spike levels are 50 and 150% of typical target; or 50 and 100% overspikes. SDs will agree on levels used.

(*3*) Spiked and unspiked samples will be analyzed in duplicate on each of 3 days.

(*4*) The overall mean of unspiked samples will be used for computing recoveries.

(*5*) Matrices used for estimating recoveries may or may not coincide with one or more of those selected for precision studies. If there is overlap, then a single 2 × 6 replication of the unspiked matrix covers both requirements for that sample type.

(**c**) *Comparison to reference methods*.—(*1*) This is not required as matter of routine, because the additional effort and lack of appropriate reference methods.

(*2*) SDs may choose to collect reference method comparison data.

The SPIFAN SLV guidelines were approved by the AOAC Expert Review Panel on Infant Formula and Adult Nutritionals in September 2011.

STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS (SPIFAN)

2- AND 3-MCPD & GLYCIDYL ESTERS IN FINISHED INFANT FORMULA PRODUCTS

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