Labeling Regulations and Methods of Analysis

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Introduction

Adequate analytical methods for nutrients in foods, food ingredients, and food products are the basic first step in determining the nutritional adequacy of a food supply. Whether the nutrition data is ultimately used to educate consumers with information on the food label, or to build databases to study correlation between nutrient(s) and deficiency diseases, the assay used to provide the data must adequately determine the analyte of interest. AOAC International (formerly the Association of Official Agricultural Chemists and then the Association of Official Analytical Chemists) has been systematically validating methods for nutrition analysis for over 100 years. These validated methods provide competent laboratories with a means of supplying dependable data for nutrition labels and/or databases regarding the nutrition content of foods and food products.

Today I would like to cover three areas. I would like to cover the process and criteria for validation and acceptance of a method as an AOAC Official Method. Second, I would like to review a recent assessment of the adequacy of the Official Methods that are available for Nutrition Labeling and generating data for Nutrition Databases. And third, I would like to present an idea for a methods validation scheme that might potentially be used to improve the method validation process for foods, providing better comparative data and more rugged methods for laboratories to use.

Procedures of AOAC International

Although it started as a group of agricultural regulatory chemists, AOAC International has a membership that now includes scientists from all walks of life, from over the entire globe, interested in improving analytical methodology and results. In the late 1800's, AOAC started publishing "Official Methods" as United States Department of Agriculture bulletins. By 1920, the AOAC's volume of validated methods had grown to the point where it warranted its own volume, and the *Official Methods of Analysis* was established. It has been revised and updated every five years since.

AOAC has adopted the collaborative study as a means of validating methods and evaluating their performance. A complete peer review system, including study of the method in multiple peer laboratories and multilevel peer review of the study results assures the validity of a proposed method for its intended purpose. The key to rugged effective validated methods in AOAC lies with the Associate Referee. The associate referee, appointed on the basis of his or her expertise in an analytical area, develops an analytical method to meet a need, or through knowledge of the literature selects an applicable method for study. After a requisite number of laboratories have been found to carry out a collaborative, the associate referee distributes methodology and samples, collects the data, develops a study report and submits a recommendation for method adoption to the Association. Assisting the Associate Referee is the General Referee who brings a broad knowledge base to bear on the study and its results.

When the General Referee and the Associate Referee agree that a method performs sufficiently well to be considered as an Official Method, the method is submitted to an AOAC statistician and a safety advisor for review. Upon completion of these reviews, the method is sent to an appropriate Methods Committee for review and recommendation regarding Official Status. Methods committees are constituted of members chosen for their broad expertise in a given analytical area such as Food Nutrition, Food Toxins, or Drug Residues. Recommendation to the Official Methods Board to adopt a method as Official First Action

requires agreement of two thirds of the members of the Methods Committee. If members of the Methods Committee raise significant issues with the method, the method cannot be recommended for Official Status until the issues have been addressed by the Associate Referee. Upon recommendation from the Methods Committee, the method is considered for Official Status by the Official Methods Board. The Board reviews the actions taken on the method, the review process, and assures consistency between methods and between methods committee reviews. If the method is given First Action Official Status, it is published in the Official Methods of Analysis. After first action status for two years, methods which have no unresolved negative comments or issues can be considered for Final Action Status, a status achieved through balloting by the entire AOAC International membership. There is no difference in the Official Status of Methods, whether first action or final action. Final action only indicates that a method has withstood some test of time with no substantive issues raised regarding its performance. As you can see, any method achieving Official Status through the AOAC process has had both substantial performance testing in multiple laboratories and peer review by scientists who are experts in the analytical area. In addition, it has had intense scrutiny by scientists in related endeavors.

Criteria for acceptance of a method for Official status are well established. The method must be submitted to participating laboratories written exactly as it is intended to be run. Participating laboratories are expected to run the method exactly as written. For a given collaborative study, participation by no fewer than 8 laboratories analyzing a minimum of five sample materials is required for quantitative methods. For qualitative methods, no fewer than fifteen laboratories analyzing a minimum of 2 analyte levels per matrix, 5 materials per level, and 5 negative controls are required. Obviously in both cases participation by more laboratories and the inclusion of more samples is encouraged. In extenuating circumstances, i.e. a particular method being considered has significant regulatory or commercial importance, but can only be carried out in five laboratories anywhere because only they have key instrumentation etc., special consideration is given. Obviously, such circumstances are rare.

After the collaborative study is complete, statistical outliers (laboratories and/or data points) are removed. Rejection of more than 2/9 ths of the data is considered excessive without an explanation, i.e. failure to follow the method by a laboratory. Since method performance will vary depending on analyte, matrix, and/or quantity, there are no hard and fast criteria for method acceptance or rejection. The combined judgment applied by scientific peer review throughout the entire method validation process serves to provide stringent criteria to be met for acceptance as Official Methodology. Experts from government, academia, industry, and associations, cognizant of the ultimate use of the methods being validated and of guidelines for adequate method performance, work in concert to produce top quality methods for the analytical community to use.

Nutrition Labeling-Methods Needs

I would now like to switch gears and discuss some recent activities undertaken by AOAC related to nutrition analysis. With the recent passage in the USA of the Nutrition Education and Labeling Act, concern arose amongst food consumers, producers, regulators, and laboratories providing nutrition analytical services, regarding the availability and adequacy of validated analytical methods to meet the requirements of the labeling act.

First a bit of history on the act itself. The act was passed by congress in November of 1990. It required the US Food and Drug Administration to promulgate proposed regulations for nutrition labeling of nearly all foods sold in the US. The US Department of Agriculture, although not legally required to do so, initiated activities to adopt labeling regulations essentially equivalent to those of the USFDA. The proposed regulations of November 1991, were open for comments with final regulations due in November

of 1992. The final regulations were actually issued in January 1993, with an effective date of May 8, 1994 (July 8, 1994 for products under USDA jurisdiction). A few definitions might be in order here. NLEA stands for Nutrition Labeling Education Act, but as the long comment period went on, nutritionists were obviously excited and it became known as the Nutrition Lobbyist Enjoyment Act. The act will have a significant impact on Industry, Consumers, and Government Agencies. It is estimated that it will cost industry upwards of $1.5 Billion for the relabeling required, an estimated $1500 per product for small firms and $900 per product for large firms. Analytical cost will probably range from $750 for the 40% of US foods that need label changes to $1800 for the 60% of foods that had not been previously labeled. Research and development costs for products that will be modified somewhat for marketing advantages under the provisions of the act are hard to estimate, but run anywhere from $20,000 to $400,000 per product. Typically two to five months will be needed to redesign and print new packages. For consumers the cost of relabeling will be passed along in higher product prices. No money has been allocated for "Education", so it is expected that significant consumer confusion will exist after the label changes occur. Governmental agencies will incur extra costs for interpretation, analysis, and enforcement of the act.

As I said before, the effective date for NLEA is May 8, 1994 (this may even be moved to August 8, 1994 if President Clinton signs the bill currently passed by Congress), however other aspects of labeling have different effective dates, i.e. juice labeling in May, 1993, health claims in May 1993, and metric weight declarations in February, 1994. With the interpretation and explanation necessary for such a broad act, NLEA has occasionally been referred to as the National Lawyers Employment Act, or the that NFPA (National Food Processors Association) Loves (the) Extra Attention. The NLEA requires the mandatory nutrition labeling of most products and allows specified uses of nutrient descriptors and health claims related to nutrition.

I would next like to spend a bit of time discussing the format of the label, and some of the nutrient content claims. The label format(s) are rigidly specified for NLEA. Mandatory labeling is required for Calories, calories from fat, total fat, saturated fat, cholesterol, sodium, total carbohydrate, dietary fiber, sugars, protein, vitamin A, vitamin C, calcium, and iron. Voluntary labeling is allowed for calories from saturated fat, polyunsaturated fat, monounsaturated fat, stearic acid (USDA products), potassium, soluble fiber, insoluble fiber, sugar alcohols, other carbohydrates, thiamin (B1), riboflavin (B2), niacin, vitamin D, vitamin E, folate, vitamin B12, phosphorous, iodine, magnesium, zinc, copper, biotin, and pantothenic acid.

Labels will list the quantity of a given nutrient, along with a % of daily value guideline for the consumer to use for comparison. The % of daily value is determined against either a Reference Daily Intake (RDI) value (typically for micronutrients) or against a Daily Reference Value (typically for macronutrients). For example, the daily reference value (based on 2000 calories/day) for fat is 65 g, for saturated fat is 20 g, for cholesterol is 300 mg, and for dietary fiber is 25 g. To encourage consistency in reporting of daily values, reference amounts relating to serving sizes have been published for common food items. Reference amounts are typically in common household units.

Nutrient claims can be made regarding the food product. However, if fat, saturated fat, cholesterol, or sodium exceed certain levels, this must be disclosed on the package along with the nutrient claim. Adequate analytical methods are obviously needed to assure compliance both with the spirit of the nutrient claim, as well as monitoring the disclosure level compliance.

Seven health claims are currently allowed on the food package label. Fiber containing grain products, fruits, and vegetables, and a reduction in the risk of cancer. Fruits, vegetables, and grain products that contain fiber (particularly soluble fiber), and the risk of coronary heart disease. Fruits and vegetables and cancer. Calcium and osteoporosis. Dietary saturated fat and cholesterol and the risk of coronary heart

disease. Dietary fat and cancer. Sodium and hypertension. If certain levels of fat, saturated fat, cholesterol, or sodium are exceeded in the product, then health claims cannot be made.

The proposed nutrition regulations do allow the use of databases for generating label information, however, the extent of such usage is still open to question, due to questions regarding the quality and reliability of data in such databases.

For added nutrients (referred to as Class I nutrients), the nutrient must be present at 100% or greater than declared. For naturally occurring nutrients, (Class II), the nutrient must be present at a level at least 80% or greater than declared, but less than or equal to 120% of declared. Examples of nutrients that must be greater than 80% of declared are dietary fiber and potassium. Examples of nutrients that must be less than 120% of declared are fat, saturated fat, and sugar. Analytical variability is taken into account for enforcement, so well characterized validated methods are necessary for compliance monitoring.

AOAC Response

To deal with concerns regarding availability of adequate methods to meet the needs of NLEA, a special task force of the AOAC with members drawn from regulatory agencies, the food industry, academia, and analytical suppliers was formed. I will give a brief history of the task force, discuss the methods reviewed by the task force, and report the results of the task force efforts. The objectives of the task force were: 1). Determine which Official Methods are adequate to meet current nutrition labeling analysis requirements. 2). Determine which Official Methods need revisions or modifications to meet current nutrition labeling analysis requirements. 3). Determine which nutrient/matrix combinations require the development and validation of Official Methods. 4). Propose means by which AOAC International can supply needed methods and/or modifications. 5). Identify means by which reference materials might be incorporated into AOAC Official methods and into the validation process for AOAC Official methods, further assuring the quality and performance of those methods.

The task force began informally at the AOAC Annual International Meeting in 1991, and was formally appointed by the board of directors in December of that year. Efforts were initiated immediately to obtain feedback regarding the status of Official Methods used for nutrition labeling. A survey was conducted of laboratories carrying out nutrition analysis and using AOAC methods. An information gathering session was also held in March of 1992. A number of task force meetings were held in the succeeding months to carry out the assigned objectives and fulfill the task force's mission.

Under the proposed nutrition labeling regulations, up to 54 nutrition related items were either required or could be placed on the label. Everything from A (ash) to Z (zinc). To organize the task of evaluating methods for these analytes, the task force divided foods into 20 different matrix groups that were felt at the time to cover the scope of foods and food products. This resulted in 1080 analyte matrix combinations to be assessed regarding availability of adequate methods. NLEA immediately took on a new definition-Need Lotsa Extra Analysts. Individual committee members took upon themselves assignments to review AOAC methods on an analyte/matrix basis. After this preliminary review was done, the entire task force, along with aid solicited from others, reviewed the assessments of the individual members. The analyte/matrix grid of adequate methods began to fill in. As the task force progressed, the information being generated was regularly reported in the Referee to keep the AOAC membership informed of progress and to allow feedback. For example the assessment of adequate methods under the proposed regulations was published in the July 1992 issue of the Referee.

Initial review of adequate methods under the proposed regulations, indicated that 947 of the 1080 possible matrix/analyte combinations had adequate methods. This meant that 88% of the methods needs were addressed. In some cases, the Official Methods were deemed adequate for the need, but newer technologies can be brought to bear on the analyte/matrix combination to provide better methods at this point in time. An example might be vitamin A. The Carr-Price method provides adequate results for labeling purposes, however most laboratories today would rather use high pressure liquid chromatography and avoid handling the corrosive antimony trichloride. Therefore, although the task force accepted the adequacy of the Carr-Price method, it is recommending that validation of HPLC methods be undertaken.

As the list of adequate methods was being generated, a complementary list (or shall we say "uncomplimentary" list) of methods in need of validation or revision was also developed. This was published in October 1992 to alert members of methods needs.

Special Nutrition Labeling Issues

As the task force evaluated methods for nutrition analysis, a number of issues were raised. In particular, issues regarding methods for fat, dietary fiber, moisture, carbohydrates, standards and reference materials for Official Methods, and the need for a clear-cut means of determining if a method is applicable to all foods. Subcommittees of the task force were formed to address each of these issues.

Fat has traditionally been analyzed by a variety of methods depending upon matrix, analyst carrying out the analysis, and intended use of the resulting data. Typically, the result was dependent upon determination of some solvent soluble (solvents varied depending on the method) fraction of the food being analyzed. The task force realized that a single concise definition for fat was needed. AOAC International does not set definitions for nutrients, but provides validated analytical methods to quantitate defined nutrients. Therefore, the subcommittee recommended, and the task force concurred, that the regulatory agencies, the USDA and FDA, adopt a single concise definition for fat. The agencies responded by adopting a definition of fat as the sum of the fatty acids (regardless of source) in the food expressed as triglycerides. This concise definition provides a "gold standard" if you will for evaluating fat analysis methods in the future.

The carbohydrates subcommittee determined that methods for total, soluble, and insoluble dietary fiber are adequate. Sugar methods, in particular the HPLC methods with defatting steps, while adequate, should be further studied to assure validity across a broader matrix base. Complex carbohydrates as a nutrition label item had been included in the labeling proposal, but eliminated from the final regulations due to a clear definition of the nutrient, and lack of analytical methods to measure it. The subcommittee (and the task force) recommends a concise definition for complex carbohydrates be adopted and has committed AOAC to validating appropriate methodology at such time as a definition is adopted.

A complete listing of moisture methods, along with their characteristics has been published by the moisture subcommittee. As with complex carbohydrates, a clearer definition of moisture will be helpful in validating more concise methodology for this analyte.

The subcommittee on reference materials published a listing of commercially available reference materials for the nutrients requiring mandatory labeling in August, 1992. The subcommittee further went on to publish "Guidelines for the Preparation of In-house Quality Assurance Materials" in the May, 1993 issue of the Referee. Recognizing that reference materials was an ongoing task with significant follow-up required long after the nutrition labeling task force would be disbanded, the task force supported the formation of the first technical division of AOAC International, namely the Technical Division on Reference Materials. This division will continue the efforts initiated through the task force and will expand

to reference materials beyond food nutrition. This division already has over 125 members and held its first annual meeting in conjunction with the AOAC Annual International Meeting in July.

I'll address the topic of the definition of food in a little bit.

Method Validation Needs

After the final regulations for Nutrition Labeling in the US were issued by the USDA and the USFDA, the task force reassessed methods adequacy and needs. The updated listings were published in the March and April, 1993 issues of the Referee respectively. In particular, methods and/or collaborative studies are needed for beta-carotene, biotin, sugar alcohols, sugars (verification for certain matrices), cholesterol, copper, cyanocobalamine, defatting of samples for dietary fiber, fat (total, saturated, monounsaturated, and stearic acid), folacin, iodine, niacin (microbiological method), pantothenate, protein (eliminate mercury use), pyridoxine, tryptophan (microbiological method), vitamin A, vitamin C (where erythorbate is present), and vitamin E. As I said before, some of these nutrients do have adequate methods, however, the methods are in need of modernization and therefore are recommended for further study.

All this brings to mind yet another definition for NLEA. Per AOAC, we Need Loyal Enthusiastic Analysts to validate appropriate methods.

Systematic Approach to Method Evaluation-Food Triangle

I would like to return now to a question that arose during the task force deliberations. How does one ascertain with reasonable confidence that a method is applicable to all foods without a substantial history of trouble free application to a wide variety of food samples? Clearly, a defined systematic approach might be helpful to assure method ruggedness across all food types while minimizing the analyst's efforts in assessing the method. The task force Subcommittee on Definition of Foods for Analytical Purposes has proposed an approach that is currently being considered by the Foods committees and the Official Methods Board.

The idea of requiring a collaborative study of forty or more samples can be very discouraging, both for the associate referee organizing the study and for potential participants. There are five macronutrient components of any given food; moisture, ash, protein, fat, and carbohydrate. Moisture of nearly all samples can be adjusted if the level affects an assay. Water can be added, or the sample dried. Ash content of a sample usually has little effect on assays, particularly for organic nutrients. Therefore, the remaining three macronutrients, fat, protein, and carbohydrate have the major impact on the effectiveness of an analytical method. If we picture a triangle with fat, protein, and moisture at the apices, all food samples will fit somewhere on that triangle, assuming the sum of fat, protein, and carbohydrate is normalized to 100%, and these components are expressed as a percentage thereof. For example, a sample with 10% fat, 30% carbohydrate, and 10% protein will have normalized values of 20% fat, 60% carbohydrate, and 20% protein.

The triangle can be split equally in nine subtriangles, with any particular nutrient lying between 0-33%, 33-67%, and 67-100% respectively. By choosing eighteen samples (two from each subtriangle), the analyst would be reasonably certain of covering foods characteristic of most foods. To develop further confidence in a method, samples taken from a subtriangle can be purposefully chosen to represent particular characteristics, i.e. for the 67-100% carbohydrate subsection, a high fiber and a high starch sample might be used. For the 67-100% fat section, a dairy or animal fat and a vegetable fat might be chosen. The system could be applied to any nutrient being analyzed by using a Youden pairing technique for

determination of within laboratory variability for the analyte of interest. If difficulty is experienced with getting acceptable results for the method in question for samples from certain subtriangles, this information could be quite helpful for understanding and delineating the cause of the ineffectiveness.

Conclusion

The task force has completed its objectives and reported the results of its deliberations on an ongoing basis in the Referee, the Official organ of AOAC. The final report has been submitted to and accepted by the Official Methods Board and will be published soon in the Journal of AOAC International. The task force disbanded at the July Annual International Meeting of AOAC International.