

**MINUTES FROM THE MEETINGS OF
AAFCO'S LABORATORY METHODS AND SERVICES COMMITTEE**

August 3, 2008 8:00-5:30

at

Hilton Hotel, Nashville, TN

Present were:

NAME	E-MAIL ADDRESS	ORGANIZATION
Aaron Price	pricea@inspection.gc.ca	CFIA
Allen Williams	Allen.williams@state.tn.us	TN Dept of Ag
Andy Crawford	andy@crawford.org	Nutro Products
Bill Hall	Bill.Hall@mosaicco.com	Mosaic
Bob Jeppsen	rjeppsen@albion-an.com	Albion Advanced Nutrition
Brenda Snodgrass	Brenda.Snodgrass@oda.state.ok.us	OK Dept of Ag
Brian Layton	blayton@ankom.com	Ankom
Charles Staff	Chstaf01@louisville.edu	Distillers Grain Tech. Council
Dale Hill	Dale_Hill@admworld.com	ADM
Gina Clapper	ginac@aocs.org	AOCS
John D. McCurdy	john.mccurdy@fda.hhs.gov	FDA/CVM
Jose Rodriguez	Jmr385@msstate.edu	MS Dept of Ag.
Kenneth McManus	McManuK@mda.state.md.us	MD Dept of Ag.
Kyung-Min Lee	kml@otsc.tamu.edu	Office of Texas State Chemist
Lars Reimann	larsReimann@EurofinsUS.com	Eurofins Scientific, Inc.
Leon Clark	leonclark@eurofinsus.com	Eurofins
Lu Wetzler	luann.wetzler@nebraska.gov	NE Dept of Ag
Mark Coleman	mcoleman@lilly.com	Elanco
Mary Beth Hall	marybeth.hall@ars.usda.gov	USDA-ARS
Mary Koestner-Dorge	Mary.Koestner@mda.mo.gov	MO Dept of Ag
Melton Bryant	mbryant@uky.edu	University of KY
Nancy Thiex	Nancy.Thiex@sdstate.edu	SD Dept of Ag
Parvan Dasari	Roger.dasari@uky.edu	KY Dept of Ag
Richard TenEyck	rteneck@oda.state.or.us	OR Dept of Ag.
Roger Hoestenbach	rdh@otsc.tamu.edu	Office of Texas State Chemist
Saber Ahmed	Ahmed@Agri.ohio.gov	Ohio Dept of Ag
Sandy Flick	Sandy.Flick@alpharma.com	Alpharma
Sharon Webb	sharon.webb@uky.edu	University of KY
Tom Jenkins	tjnks@clemson.edu	Clemson University
Vernon Pabst	Vernon@Spectrumanalytic.com	Spectrum Analytic
Vicki Siegel	vsiegel@purdue.edu	Office of IN State Chemist
Will Donaldson	wd@mp-ic.com	Metrohn-Peak
William Friz	wfriz@agri.ohio.gov	OH Dept of Ag

Nancy Thiex opened the meeting. After introductions the following issues were covered:

Approval of Agenda – The agenda was approved following the addition of amino acid as an item. (attached).

Approval of Minutes from the January Meeting –The Minutes from the meeting held on January 30, 2008 in San Antonio, TX was approved. (attached)

Annual Report – Thiex mentioned that an Annual Report had been submitted to the AAFCO Board. (attached).

Method Updates –

Lasalocid, Oxytetracycline and Decoquinatate - Thiex reported that they had all received AOAC “First Action” status and that study manuscripts would soon be published.

Neomycin, multi-analyte mycotoxin (corn) and Carbadox - Thiex reported that they had all had been submitted to AOAC for publication.

Tylosin – No update. A discussion followed as to the scope of the method validation effort – should it include soluble and injectable materials. At issue was the expected low bias between an HPLC and a microbiological activity method due to the complex nature of the drug (multiple components). It was agreed to include type A material, but disregard injectables and solubles and to include a statement in the preamble to the finished method addressing the bias on high potency materials between the HPLC method and the official microbiological method.

Virginiamycin – Thiex reported that Dr. Alex MacDonald’s flight had been cancelled after long delays. After a number of failed attempts, the current extraction method under development uses the following sequence: soxhlet extraction with methanol, evaporate to oily residue, re-suspend with methanol-buffer, 2 hexane washes, filter with paper and 0.45um membrane filter, SepPak C-18 SPE clean-up and wash with diluent, elute with 70% methanol, reconstitute and plate. This approach handles most damp and dry DDG samples, yields sharp zones with light yellow edge haze, recoveries of 85-90% at 0.5 ppm, and is reproducible. The procedure is slow and labor intensive and needs “good lab hands”.

Business Issues-

Mycotoxin workshop at Office of Texas State Chemist – Victoria Siegel reported that the meeting had been a great success and that several state labs were in the process of changing methodologies as result of the workshop. A good confirmation method for DON is still lacking.

MW AOAC Workshops – Thiex reported that the Electrode workshop and the ICP Maintenance workshops at the MW AOAC had been a success and referred to the annual report for further details. AAPCO and AAFCO may sometime next year sponsor a method validation course.

AOAC Update – Coleman/Thiex reported that AOAC currently seems more focused on getting contracts involving getting stakeholders together than dealing with methods. OMB has finalized a new validation process including a new “Terms of Reference” for all volunteer positions associated with the validation process and plans on initiating the new process following the Annual meeting in September.

Fertilizer Methods – Bill Hall reported that the fertilizer group had been looking for funding, assembled \$100K+ to date and had presented a proposal to AOAC for using this money to cover the

validation of 10 methods (attached). The proposal received somewhat cool reception from AOAC (Jim Bradford was quoted as saying that "...AOACI is no longer the method validation organization"). AOACI is currently reviewing the proposal with an answer expected VERY soon. (AOAC's response is included in the updated attachment – Reimann)

Sulfur data from AAFCO's Check Sample Program – Andy Crawford presented a comparison of the bias and CV of different sulfur methods over a variety of samples including DDG (attached). Based on the data there seemed to be little bias between the methods and they all showed a relatively high between lab CV (approx 15%). It was discussed how this is an issue with DDG in cattle where DDG's "normal" sulfur content of 0.6% was close to the max level for dairy cattle. Work in the SDSU Lab (Thiex - poster attached) indicates no significant difference among gravimetric, ICP and combustion methods for feed or for DDGs. A discussion followed concerning the challenges associated with the optimal calibration of combustion units.

AOAC OMA Update – Thiex reported that the OMA was being updated by George Latimer and that all suggestion for changes should be sent to him. She also asked for volunteers in this effort in addition to Sharon Webb.

Methods Used in the Analysis of DDGS – Charles Staff reminded Thiex of her promise to survey state labs for their choice of methodology when analyzing DDG and to the extent possible determine the level of compliance with industry recommendations.

ANKOM Methods for DDG – Brian Layton presented a proposal for determining the equivalence of ANKOM methods for crude fat and crude fiber in DDG (attached). A discussion of the impact of the fineness of grind and acceptable bias followed. It was agreed that Layton would discuss setting a narrower acceptability range. SDSU (Nancy Thiex) would execute the comparisons.

Starch Definition – Reimann reported that the starch definition agreed to in the past was rejected at the recent AAFCO Board meeting at our recommendation. Mary Beth Hall had reported that the originally proposed AAFCO definition for starch as

The non-structural storage polysaccharide of plants, an alpha-glucan with the glucose released after gelatinization through the use of purified amylases and amyloglucosidases that are specifically active only on α -(1-4) and α -(1-6) linkages. Its concentration in feed is determined by enzymatically converting the starch component to glucose and then measuring the liberated glucose.

while agreeing with current ISO and FAO definitions for starch did not take into account several important factors including:

1. Current enzymatic methods of starch analysis utilize amyloglucosidase with or without α -amylase, which hydrolyze α -(1-4) and α -(1-6) linkages in α -glucans and maltooligosaccharides.
2. α -glucans in animal feedstuffs may come from non-plant sources. These include animal tissues (glycogen in liver, muscle, and oysters), microbes (yeast glycogen, dextrans in molasses produced by microbes). Methods for measuring the non-plant α -glucans use amyloglucosidase to release glucose which is then measured.
3. Presently, we have no nutritional basis for excluding non-starch sources of α -glucan or maltooligosaccharides; the α -(1-4) and α -(1-6) linkages hydrolyzed by the enzymes used in the starch assays are potentially digestible by enzymes in the small intestine.
4. Restriction of the definition of "starch" to plant sources would force the exclusion of matrices common to animal feedstuffs (meat products, microbial products, molasses) from the analysis for starch. (e.g., AOAC Official Method 958.06 "Starch in Meat" specifically indicates that the method is "Not applicable to liver products.")

5. Exclusion of maltooligosaccharides (DP>2) would leave these α -linked oligosaccharides commonly found in baked products out of most nutritional analyses. Current efforts are devoted to measurement of “sugars” (DP 1 or 2) and polysaccharides, but not many of the oligosaccharides.

Discussion followed concerning changing the definition to meet nutritional concerns and correlate with the capabilities of available methods. The definition was modified to

“Nutritional” or “Dietary” Starch is an alpha-linked-glucose carbohydrate of or derived from plants, animals and microbes from which glucose is released after gelatinization through the use of purified amylases and amyloglucosidases that are specifically active only on α -(1-4) and α -(1-6) linkages. Its concentration in feed is determined by enzymatically converting the alpha-linked-glucose carbohydrate to glucose and then measuring the liberated glucose.

It was agreed that the new definition would be presented to and discussed at the meeting of the AAFCO Ingredients Definitions Committee. (It was rejected at the meeting – Reimann comment).

Discussion of Replacing the “Crude Fat” Method for the “Fatty Acid Profile” Method for Estimating the Fat Content in Feed and Ingredients – Tom Jenkins reported on Don Palmquist’s findings that estimating the fat content in a sample based on the fatty acid composition was a much more accurate way than though determination of the “crude fat” by solvent extraction. He also summarized Dr. Palmquist’s suggested methodology. Jenkins reported that the AFIA President was the driver behind this project. Gina Clapper reported that AOCS had spent a lot of efforts on developing and validating such methods, including “one pot” type methods. It was agreed that Dr. Palmquist would contact AOCS and compare his method against theirs. Long term it was agreed that AOCS would coordinate validation efforts while AAFCO would contribute with samples of pertinent matrices for use in the validation process.

Contaminants in Feeds – Shannon Jodre, FDA explained FDA’s need for data about the residue content commonly found in feeds and feed ingredients to aid them in prioritizing testing schemes based on risk. He explained that FDA likely would have some money to cover the expense of clerical activities transferring data into a format acceptable to FDA. He asked that labs interested in providing such type of data contact him.

Aflatoxin in Corn using UPLC – Pravan Dasari presented a method (attached) for determining the amount of aflatoxin in corn. He believed the same method would work for other mycotoxins. He also mentioned that an improved affinity column was entering the market place.

Amino Acid Methodology – Clapper reported that AOCS had been contracted by United Soybean Board to develop/identify better (faster) methods for determining the amino acid content (summary attached). AOCS sponsored a symposium on this issue 2 years ago. Currently 4 labs are working on methods based on acid hydrolysates. 3 labs will be submitting date (1 lab by GC/MS; two labs by UPLC-fluorescence). Following the determination of the most appropriate approach for dealing with hydrolysates, focus will be directed on the digestion procedures.

AAFCO’s FDA Master Files – McCurdy summarized the process – AAFCO requests from FDA a Master File # for each drug method. Following the receipt of the Master File # AAFCO submits the method package for inclusion into the file (at least two copies). Data packages should be prepared in accordance with FDA Guide 57 (downloadable from the FDA web site) and submitted with a cover letter on AAFCO letterhead. Important to ensure that AAFCO (Sharon Krebs) retains a copy as well. The

final step is for the Drug Sponsors to request FDA to place a note in their “jacket” referencing the method contained in the AAFCO Master File as an “additional method”.

Currently AAFCO has received Master File numbers for lasalocid (VFM 005-918), oxytetracycline (VFM 005-919), and decoquinatone (VFM 005-920). No method packages have been submitted so far.

Sulfamethazine data need to be recompiled and the process for obtaining a Master File number initiated. Lu Ann Wetzler to submit first method package. Siegel to prepare a sulfamethazine method package.

Web Sites – Wetzler reported on the status of web sites covering the group’s activities. It is very difficult to have the AOAC web site updated (VERY low priority within AOACI). Information is available on the AAFCO web site (www.aafco.org) – however access is restricted to regulatory personnel (industry personnel can obtain access through sending a request to Sharon Krebs, AAFCO). The Foodshield web site (www.foodshield.org) has a public area. Information related to Lab Committee is kept updated on the last two web sites.

Quality Sub-Group – Thiex referred to the Annual Report for an update. The Sub-Group has been dissolved. She also suggested that considerations be given to set up a laboratory accreditation program similar to the program offered by American Association of Laboratory Diagnosticians (AAVLD). The purpose of this would be to reduce accreditation costs. Louise Ogden will distribute a questionnaire to evaluate interest among state labs (attached). If there sufficient interest is expressed in investigating such a program, a white paper will be developed.

Sugar Methodology – Will Donaldson presented a method and associated data from the 11 samples provided by AAFCO (attached). Metrohm will submit a short report to the Committee summarizing method as well as an explanation for the apparent analyte loss following sonication in excess of 3 min. Reimann reported that Waters expected to present their suggested approach at the AOAC meeting. Clapper also mentioned the project coordinated by AOCS (summary attached) addressing sugar analyses.

Method Prioritization – Aaron Price summarized the responses he had received so far (attached).

Method Needs Statements – Aaron Price

- Sugars as defined by AAFCO – Motion made and approved that people has to submit changes to Price within two weeks of the distribution of the Meeting Minutes. In absence of comments, the method need statement would be considered as approved.
- Starch as defined by AAFCO – It was agreed that further work on this document be delayed pending receiving an approved definition. Any review should also address necessary LOQ requirements.
- Fructans as defined by AAFCO – Motion made and approved that people has to submit changes to Price within two weeks of the distribution of the Meeting Minutes. In absence of comments, the method need statement would be considered as approved.
- Bacitracin – an issue with quantifying homologues (components) present in this drug (15-20). Price to discuss issue with Bill Williams, Alpha.
- Prohibited animal protein – subject removed from agenda per Tim Herrman’s request.
- Tylosin – Price to update needs statement to include as many factors as possible.

- Vitamin A – Motion made and approved that people has to submit changes to Price within two weeks of the distribution of the Meeting Minutes. In absence of comments, the method need statement would be considered as approved.
- Vitamin D and Vitamin E – Melton Bryant to contact Ken Riter to explore if he is still willing and interested in heading up this effort.
- Pesticide multi-analyte method – Price to follow up with Dr. Tim Herrman and report on issue.
- Amprolium –Price to review the method CFIA currently uses against the Method Needs Statement and identify shortcomings.
- Minerals – Terry Field, CFIA has volunteered to be the project manager for this method.
- Fatty Acids - It was agreed that AOCS would provide a method summary of a method for analyzing the fatty acid content of fats and oils and that no Method Needs Statement was needed. Charles Staff mentioned a method for determining the fatty acids present in DDG ((Michael J. Haas et. Al; J Am Oil Chem Soc **84** (2007) p 963-970)

Respectfully submitted,

Lars Reimann

Eurofins Scientific, Inc.

Attachments:

Agenda August 2008
 AAFCO Lab Minutes 013008
 2008 Lab Committee Annual Report
 Bill Hall's "AOAC Update"
 Andy Crawford's "AAFCOSulphur"
 Nancy Thiex's "Sulfur in Feed and DDG"
 Ankom's "Study Design for ANKOM Equivalency Trial"
 Ankom's Experimental Protocol for Distillers Grains Study
 Pravan Dasari's "Nashville Aflatoxin"
 Gina Clapper's "amino acids aafco"
 Louise Ogden's "Accreditation Survey"
 Will Donaldson's "AAFCO-2008_Sugars in Animal Feed"
 Gina Clapper's "sugars aafco"
 2008 Method Prioritization Survey Results