

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

**January 21, 2009**

**Marriott, Tucson, AZ**

Present at the meeting were:

<b>NAME</b>	<b>E-MAIL ADDRESS</b>	<b>ORGANIZATION</b>
Aaron Price	<a href="mailto:aaron.price@inspection.gc.ca">aaron.price@inspection.gc.ca</a>	CFIA
Andy Crawford	<a href="mailto:nutroandy@aol.com">nutroandy@aol.com</a>	
Charles Staff	<a href="mailto:Chstaf01@louisville.edu">Chstaf01@louisville.edu</a>	Distillers Grain Council
Chris Hudalla	<a href="mailto:Chris_Hudalla@waters.com">Chris_Hudalla@waters.com</a>	Waters
Dale Hill	<a href="mailto:Dale_Hill@ADMWorld.com">Dale_Hill@ADMWorld.com</a>	ADM
David Heller	<a href="mailto:davidheller@fda.hhs.gov">davidheller@fda.hhs.gov</a>	FDA
Frank Jaramillo	<a href="mailto:Frank.Jaramillo@uky.edu">Frank.Jaramillo@uky.edu</a>	KY Dept of Ag
Gail DeGreeff	<a href="mailto:gaildegreeff@princeagri.com">gaildegreeff@princeagri.com</a>	Prince
Jay Gandhi	<a href="mailto:jgandhi@metrohmusa.com">jgandhi@metrohmusa.com</a>	Metrohm
John D. McCurdy	<a href="mailto:john.mccurdy@fda.hhs.gov">john.mccurdy@fda.hhs.gov</a>	FDA / CVM
Kevin Armbrust	<a href="mailto:armbrust@mscl.msstate.edu">armbrust@mscl.msstate.edu</a>	MS Dept of Ag
Lars Reimann	<a href="mailto:larsreimann@EurofinsUS.com">larsreimann@EurofinsUS.com</a>	Eurofins Scientific, Inc.
Mark Coleman	<a href="mailto:mcoleman@lilly.com">mcoleman@lilly.com</a>	Elanco
Marlene Petersen	<a href="mailto:mpetersen@msccompany.com">mpetersen@msccompany.com</a>	Milk Specialties
Mary Koestner	<a href="mailto:Mary.koestner@mda.mo.gov">Mary.koestner@mda.mo.gov</a>	MO Dept of Ag
Melton Bryant	<a href="mailto:mbryant@uky.edu">mbryant@uky.edu</a>	KY Dept of Ag.
Nancy Thiex	<a href="mailto:Nancy.thiex@sdstate.edu">Nancy.thiex@sdstate.edu</a>	SD Dept of Ag
Paul Mostyn	<a href="mailto:Paulm@Westway.com">Paulm@Westway.com</a>	Westway
Ray Niles	<a href="mailto:Raymond.niles@fda.hhs.gov">Raymond.niles@fda.hhs.gov</a>	FDA
Reggie Roybal	<a href="mailto:Reggie.Roybal@ag.state.co.us">Reggie.Roybal@ag.state.co.us</a>	CO Dept of Ag.
Rob Jeppsen	<a href="mailto:rjeppesen@albionminerals.com">rjeppesen@albionminerals.com</a>	Albion Advanced Nutrition
Rod Noel	<a href="mailto:noelr@Qpurdue.edu">noelr@Qpurdue.edu</a>	IN Dept of Ag
Sandy Flick	<a href="mailto:Sandy.Flick@alpharma.com">Sandy.Flick@alpharma.com</a>	Alpharma, Inc.
Tim Herrman	<a href="mailto:tjh@otsc.tamu.edu">tjh@otsc.tamu.edu</a>	TX Dept of Ag
Ursula Gage	<a href="mailto:Ursula.gage@kda.ks.gov">Ursula.gage@kda.ks.gov</a>	KS Dept of Ag
Vicki Siegel	<a href="mailto:vsiegel@purdue.edu">vsiegel@purdue.edu</a>	IN Dept of Ag

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

Approval of Agenda – The attached agenda was approved with the following additions: DDG, Aflatoxin methods, CLA methods, enzyme methods, melamine methods;

Approval of Minutes from the September Meeting –The Minutes from the meeting held September 2009 in Dallas, TX (attached) was approved

Progress report from the Fertilizer Community in its dealings with AOAC – Lars Reimann summarized a PowerPoint presentation provided by Bill Hall. The group had collected approx. \$200,000 to cover the

validation/updating of approx. 8 methods. AOAC's fee schedule for Organizational Affiliates is \$20,000 for a method validation, \$10,000 for a method change and \$2,000 for a small (editorial) change.

AOAC Update – Nancy Thiex reported that AOAC called and told her that AOAC would no longer process methods for free. Doug Hite had promised to assist in fundraising when needed. Nancy believed that the next method ready for AOAC approval would be a method for quantifying starch.

## METHOD TOPICS

### Method Progress Reports – FDA Submissions:

- Lasalocid – The data package was submitted to FDA as well as a letter from Alpharma requesting the method being considered an alternative method. Submission date around Dec 6, 2008. FDA responded that the request was classified as CBE30, that the method could be used but that FDA reserved the right to withdraw its approval pending a more thorough review of the package. Sandy Flick explained that this meant we could expect more detailed feedback within the next 120 days. Dennis McCurdy promised to stay in touch with the reviewer and share any feedback with Nancy Thiex. It was agreed to delay the submission of additional data packages pending the completion of FDA's review to ensure that the current template for putting packages together was appropriate.
- Oxytetracycline – Package ready for submission to FDA
- Decoquinatone – Package ready for submission to FDA
- Sulfamethazine – When it is clear what information is needed to be included in a data package Victoria Siegel will extract such information from the data files archived by Bob Smallidge.

### Method Progress Reports – Other:

- Neomycin, carbadox / pyrantel tartrate, tylosin, amprolium, MGA, virginiamycin (residue in DDGs) – Thiex reported that the volunteers associated with these projects had experienced difficulties finding the time and resources to further these projects since the September meeting

### Presentations:

- Eric Hoffman summarized the changes to the LABDIR and FOODSHIELD websites. Industry, government and academia each has separate but interconnectable databases. The databases currently cover US and Canada but other countries will likely be included in the future. People interested in getting access and to be listed should contact (preferably via e-mail) either Mr. Hoffman ([ehoffman@ejhassociates.com](mailto:ehoffman@ejhassociates.com)) or Carrie Rigdon ([crigdon@foodshield.org](mailto:crigdon@foodshield.org)) or call the helpdesk (651.319.9178) for assistance. It was agreed to use these databases as the conveyer of information related to the AAFCO Lab Methods and Services Committee, the AAFCO Check Sample Program and activities associated with the AOAC Agriculture Community and reduce the AGLABS web site to simply show a link to the other sites.

#### Action Items:

- Minutes from meeting and associated documents to be placed on FoodShield
- State Labs to review and update information specific to their facilities.
- David Heller provided an update regarding the determination of antimicrobial residues in DDG (wet and dry) as well as distillers' grains solubles / stillage. He credited Dr. Hema de Alvis for having done most of the work. The method is based on HPLC-Ion trap (good

approach for detection, weak for quantitation). It covers 8 compounds with LOQ's of around 0.1-2.5PPM and a recovery between 60-100% (performance data based on spikes rather than incurred samples). Heller highlighted important issues such as keeping the samples frozen prior to time of analysis, addition of EDTA to the extraction medium, and attention to the pH of the extraction medium. He expected that this method would be FDA's method of choice for a survey of DDG products in the market place. Heller was also VERY interested in receiving samples containing incurred residues including working with facilities willing to take part in studies addressing the fate of antimicrobials in the processes associated with the manufacture of DDG.

Individuals interested in receiving a copy of Heller's presentation should send him an e-mail. Heller also reported that he likely next would be involved with the development of a multianalyte screen covering mycotoxins using LC-MS/MS.

- Chris Hudalla presented an update on his work with the identification of simple sugars in pet foods and equine feeds using a 50% acetonitrile in water as the extraction solution and HPLC-ELS for the quantitation. His current approach had defacto LOQ's around 0.3% and did not seem to cover maltose well.
- Jay Gandhi presented an update on his work with the identification of simple sugars in pet foods and equine feeds using ion exchange chromatography coupled with electrochemical detection. Gandhi presented data indicating different recoveries based on extraction solvents (water/ethanol; water; water /acetonitrile). Discussion followed about the possibility of conversions of low DP sugars with time in the different extraction scenarios.

## REPORTS/TOPICS:

Carbohydrate definitions and methodology – Reimann reported that AAFCO had accepted the definition for Dietary Starch and Fructans. The two presentations Dr. Gandhi and Dr. Hudalla highlighted the difficulties associated with the analysis of simple sugars in a feed matrix. It was clear that we are still far from having good methods for the extraction and quantification of those compounds. Reimann reported that R-Biopharm also had received the same set of samples for analysis but that they had not returned any results.

Thiex reported that Dr. Mary Beth Hall was proceeding with the validation of a starch method and that method needs statements were being prepared for both starch and fructans.

Action Item:

- Thiex to review the paperwork received with the samples and provide Gandhi and Hudalla with a summary characterizing the samples (e.g., samples A through E are equine feeds while samples F through K are pet foods)
- As part of the development of the needs statement Aaron Price will contact Mary Beth Hall to discuss fructans in cold weather grasses

**Ankom Method for Fiber in DDGs** – Thiex reported that she had responded to Brian Layton's request for help addressing the statistical evaluation of comparison data with help from Paul Wehling, w/ General Mills. Feedback addressing appropriate *delta* values and grinding information was sent to Layton as well but to date no response or follow-up has been received.

### Method Needs Statements and Prioritization:

- Starch – changes to the current version were suggested. It was agreed that an updated version would be included with the Meeting Minutes. Suggestions for comments/corrections and/or additions would be received for the next 14 days. If no feedback is received the version would be considered as “Final”.
- Fructans – updated statement will be sent out for comments and reviewed at the next meeting
- Tylosin – changes to the current version were suggested. It was agreed that an updated version would be included with the Meeting Minutes. Suggestions for comments/corrections and/or additions would be received for the next 14 days. If no feedback is received the version would be considered as “Final”.
- Bacitracin – Aaron Price will try to contact Bill Williams for information about the pertinent homologues to consider and their activity factors. The needs statement will be updated to reflect such information as it becomes available.
- Multi-analyte mycotoxin method – Concerns were expressed that the methods statement as currently written is too demanding. It was suggested that the statement be amended to include language that methods handling MOST of the listed mycotoxins would be considered. It was agreed that an updated version would be included with the Meeting Minutes. Suggestions for comments/corrections and/or additions would be received for the next 14 days. If no feedback is received the version would be considered as “Final”.
- Prohibited Animal Protein – The draft provided by Tim Herrman will be updated to reflect the AOAC guidelines for qualitative test methods and compared with the current method under consideration. The final version as well as the paper describing the method will be reviewed at the next meeting.
- Vitamin D & E – No updates. Thiex offered to try to draft methods statements covering these analytes.
- Pesticide Multi-Analyte Method – Tim Herrman will have a draft for review at the next meeting. A work group may have to be formed to handle the research needed for determining the compounds and associated LOQ's needed.
- Fatty acids in foods – Reviewed draft. It was agreed that the detection limits needed to be reduced to <0.01% for individual fatty acids on an “as is” sample basis. The Committee expressed a wish to evaluate the validation data for the method under consideration especially as it relates to feed matrices and stressed the need for including a variety of animal feeds and ingredients including pet foods and high fat supplements in any collaborative study. The study should also handle difficult to extract matrices such as extruded products, high urea products, fermentation by-products and high-mineral supplements. Finally the question arose if this method would be suitable for determining CLA's as well.

Aaron will contact Dr. Palmquist to convey those concerns.

Quality –No update. Subject carried over to next meeting.

Other issues –

- DDG – Victoria Siegel reported that she had approx 150 lb of well mixed DDG in her freezer that could be used for studies with this matrix including (but not limited to) methods addressing drug residues, sulfur and melamine content.
- Mycotoxin methodologies – a discussion followed as to the experience among the participants with the correlation between test kits and wet chemical methods. Siegel reported that her lab uses test kits to screen samples for aflatoxin, fumonisins, zearalenone, DON and T2 mainly in corn, pet food and DDG. Whenever screening results are within 15 % of regulatory limit such screening results are confirmed by wet chemical methods and in her experience the results correlated reasonably well with fumonisins having the worst correlation (could be off by as much as 50%). The distillers grain matrix did not appear to be a problem as long as a pH adjustment was built into the extraction process. She had not monitored for “false” negative. Kevin Armbrust reported that his lab also screened using test kits and confirmed observations using wet chemical methods but that he did not have any correlation data handy.
- Melamine – The test kits available only focuses on melamine (LOQ as low as 10PPB) which makes them unsuitable for verifying compliance with FDA guidelines recommending levels of <2.5PPM combined for melamine, cyanuric acid, ammeline and ammedine. Both Abraxis and R-Biopharm’s kits appear to work well. Dr. McCurdy reported that biuret (currently an approved feed ingredient for ruminants) could contain as much as 35% cyanuric acid. It was also mentioned that feeds containing cyromazine (Larvadex) could test positive for melamine because melamine is one of the degradation products from cyromazine. Reimann reported that there is talk about putting together a ring-test study to evaluate the different approaches to the determination of melamine and similar compounds in feeds, foods and their ingredients. He would notify the group if this became a reality.
- Enzyme testing – Discussion as to the choice of methodologies for testing samples for specific enzymes since many manufacturers have specific methods for quantifying the activity of their products. It was reported that a recent EU study of the apparent difference between phytase methodologies was published recently in JAOAC.

Respectfully submitted,

**Lars Reimann**

***Eurofins Scientific, Inc.***

Attachments:

- Meeting Agenda
- Lab Minutes 092308
- Metrohm’s presentation
- Method needs statement – Tylosin
- Method needs statement – Dietary Starch
- Method needs statement – Multi-mycotoxin
- Method needs statement – Fructans