AEIC Spring Meeting 2017

April 19-20

Santa Clara, CA

Hosted by: Thermo Fisher

P.L. Hunst, AEIC Secretary

AEIC Business Meeting

ACTION ITEMS

Topic	Action
Composition Working Group	Continue the good work!
Gene Editing	AEIC to prepare technical comments for FDA Request for Information: DUE June 19
	AEIC to prepare a white paper on limits of detection of various types of products
	AEIC to consider an informative paper discussing rebooting of the discussion on modern breeding and multinational agreements on thresholds and comparison of gene editing to mutations that occur anyway
AEIC website	Examine website for areas of improvement of information, i.e., updating of text (Matt Cheever, Denise Thiede)
	Relevant pictures needed
	Update to standard slide sets available on website
	AEIC to consider putting together a slide set explaining sampling and testing for a public/non-specialist audience
ISO activities	Isothermal DNA methods standard (volunteers: Dan Kephart, Tao Geng, Alex Eads, Anna Rice, Ray Shillito)
	Biobanking – inform Ray S. if interested in this topic: deadline for next steps is May 4.
	Contact Ray S. if interested in participating in standards setting

1) **Secretary's Minutes of AEIC Fall Meeting 2016**: A motion was made, seconded and voted positive to approve the minutes.

2) **Treasurer Report (D. Layton):** A motion was made, seconded and voted positive to accept the final 2016 report and the projected 2017 budget.

	Projected	Actual
2016		
Beginning Balance	$32757	$32757
Dues	9500	10550
Interest	250	
TOTAL revenue	9750	10550
Expenditures		
Scientific paper	2000	
DE franchise tax	25	25
ANSI/ISO	2900	2900
Board Mtg	700	702
Spr Meeting	4500	12085
Website	2000	2251
Credit card processing	596	595
Fall mtg	4500	2463
Graphic design		
Reprints (brochure)		
Subscriptions	100	
Miscellaneous	100	70
TOTAL Expenditures	17421	21091
Ending Balance TOTAL	25087	22215.85
2017		
Beginning Balance	22215	22215
Dues	9500	3700 (to date)
TOTAL Revenue	9500	3700 (to date)
Expenditures		
Scientific paper	2000	
DE franchise tax rpt	25	25
ANSI/ISO	2900	2900
Board Meeting	800	451
Spring Meeting	2000	(1792)
Website	3000	1802
Credit card processing	600	75
Fall Meeting	4500	
Subscriptions	100	
Misc	100	
TOTAL Expenditures	16025	3460
Ending Balance	15691	22455

A discussion was initiated by the Board concerning a projected shortfall of funds in the coming year due to the cost of meetings. The membership suggested that a meeting registration of $50/person should be charged to help defray meeting costs. Since the AEIC website is able to process online registration and payment for the group dinner, this would just be an add-on to the form. The host company, which usually contributes $1500-4500 to the meeting expenses, would still have their dues for the following year waived. However, the host company attendees would all be subject to the $50/person registration fee.

The other item discussed was the "tweaking" of AEIC dues. The AEIC Board suggested the following for dues:

	Previous	Potential
Large (>1000)	$500	$1250
Medium (≥50 to <1000)		$750
Small (<50)	$250	$250
Associate	$50	$50
Individual	$100	$100

After membership discussion, a motion was made to charge $50/person meeting registration fee and raise the dues for large companies (≥1000 employees) to $1000/year from the current $500/year dues and add the "Medium" companies category. All other dues would remain the same. The motion was seconded and voted positive by the membership. The dues fee structure will now be:

Companies	Previous dues amount	New dues amount
Large (≥1000 employees)	$500	$1000
Medium (≥50 to <1000 employees)		$500
Small (<50 employees)	$250	$250
Associate members	$50	$50
Individual members	$100	$100

3) **AEIC Website Update (D. Layton):**

There is a new webmaster who is helping the organization. The hosting site has been changed to "Go Daddy" for additional security features. The Board is asking the membership for new pictures that can be included on the website that represent what we do. The Board is also asking for volunteers to review the text on the website and suggest updates/changes. Matt Cheever (Bayer) and Denise Thiede (Eurofins BDI) have volunteered to assist in this.

4) **ISO/TC 34/SC 16 Update (R. Shillito):**

The U.S. TAG meeting was held on April 18. Each country has 1 vote within ISO and the TAG represents the U.S. (who is also the chair country). The goals of the TAG include:

- Gather and represent the US postion on items being considered by ISO/TC 34/SC 16
- De-emphasize concentration in standards on GMOs and concentrate more on analytical technology;
- Prevention of regional issues being incorporated.

The TAG requires timely responses to inquiries within an accepted timeline and all points of view are accepted in forming the TAG position (consensus support of the position). All activities are open and may be inspected.

The key items from the TAG meeting were as follows:

- ISO/DIS 13484: plant pests which is being led by USDA
- ISO/WD 20387:2016: biobanking standards scope which is overlapping into TC 34/SC 16.
- TC276: developing standards for DNA quality and quality of synthetic DNA (oligos)
- Determination of performance charactertistics of qualitative measurement methods
- Meat speciation

- Semi-quantitative statistics evaluation of GMO seed

The 7th Plenary Meeting of SC 16 will be in Washington, DC on Sept 6-8. It is open to register for the meeting. AEIC members are encouraged to attend to get a view as to how ISO works.

4) **Other Meetings of Interest**

The ISTA Meeting will be held in Colorado in June and will have a symposium on seed purity testing. The AACCI will be held in San Diego in October and will have a symposium on GMO food ("Protecting your breakfast").

5) **AEIC Fall Meeting 2017**

The Fall Meeting dates will be Oct 4-5 and the meeting will be held in Indianapolis (hosted by Dow AgroSciences). The main focus of the meeting will be seed testing (what we do for purity testing, etc) and an exploration of where seed testing is going (DNA, protein, herbicide, etc). Other suggested topics included:

- Re-visit the breakout topics from the Spring Meeting

Seed health testing

- Testing for biologicals (what methods, how to support, etc)
- Oil testing (detection of GM)

6) **Composition Working Group Udpate (N. Gillikin):**

The Composition Working Group (CWG) met the day prior to the Spring Meeting. Nancy announced that the ELLA method (for lectin analysis) was as an AOCS Official Method in January, 2017. The CWG is working on a cyclopropenoic fatty acid method which was accepted by AOCS as a Recommended Practice. The CWG is planning to do more work and re-submit the method for Official Method consideration. Fred C. (EPL) is heading up work on a multiplex method for fat soluble vitamins. For endogenous allergens, the CWG discussed the possibility of a ring trial for Gly m 7 soybean allergen and then publishing a comparison of the methods. Luke M. (Covance) is working on a MS method for trypsin inhibitor. A review of methods for proximate methods has begun, starting with methods used to generate data for the ILSI Crop Composition database. Anders T. (Eurofins) is working on the making the case for use of NIR proximates. The CWG co-chairs will rotate every two years. Carl M. (DuPont Pioneer) and Luke M. (Covance) will be the upcoming co-chairs and Nancy G. will rotate off

7) **Topic Breakout Session**

The meeting attendees were broken up into two groups. Group 1 discussed the topic of "Should we chase zero?" (moderated by John L. [Covance]) and Group 2 discussed the topic of "Gene editing: how large a change is relevant and what are the consequences?" (moderated by Clara A. [DuPont Pioneer] and Ryan J. [BASF]).

Conclusions of Group 1: Thresholds for GM are low (0.9% in EU) and cause trade issues unless there are bilateral agreements between countries. Questions from a consumer perspective:

- When does a GMO become just an ingredient?
- Can we get agencies to talk about this?
- Will GMO mentality be lost eventually from public discourse?

Conclusions of Group 2: What is the reason for testing—regulatory, public perception? There is difficulty in testing for changes, particularly in bulk grain. Some SNPs cannot be detected due to where they are located in the genome so what if a detection method cannot be developed? There was a suggestion that AEIC should author a paper on genome editing as a natural biological approach: should there be testing? What would be the problems in trying to develop methods? How to convince people who do not want to be convinced? Tao G. (Monsanto) and Frank S. (Eurofins) agreed to head a group to work on the technical white paper. Ray S. (Bayer) will head a group to put together a response from AEIC to FDA's Request for Information (due June 19). Alan McHughen (UC-Riverside) will ask around to see if an academic would be interested in putting together a paper on gene editing is a natural process.

INVITED TALKS

Regulation based on product vs process (A. McHughen, UC Riverside):
The process of making a GM plant is not an issue of safety but countries still regulate on process rather than end product. In the US, FDA is responsible for food/feed safety, USDA for whether there are plant pest features, and EPA for whether there are pesticidal properties. The Canadian agencies (CFIA, HC) are assessing plants with novel traits. In Argentina, new breeding techniques (NBT) such as gene editing, do not trigger a regulatory review. The National Academy of Sciences in the US is pushing for a move to regulating based on product and familiarity. The USDA proposed revision to regulations embraces "NoForn", i.e., "nor foreign DNA", which would impact about 30 crops such as CRISPR corn and mushrooms. FSANZ (Food Standards Australia New Zealand) has recommended that simple deletions be exempted from regulation. In the EU, academics have recommended "NoForn" but the anti-GMO NGOs refer to them as "stealth GMOs". The bottom line is confusion and disarray around the technologies. The impact of incompatibility among agencies/regulations is that technology developers may move to easier regulatory spheres. International trade disruptions may occur if one country exempts a certain NBT but other importing countries still want to regulate the NBT.

What are GMOs? There is no standard definition. The US has not standard definition but other countries usually define it based on the process used to create the product. NBTs focus the regulatory trigger debate back to end product vs the process used to create it. NBTs allow changes to one nucleotide with no foreign DNA insertions making them virtually undetectable. These are indistinguishable products from those created by mutation breeding. NBTs have a similar risk profile to conventional breeding.

In summary, the real hazards are presented by end products and not the processes used to make them. Regulatory oversight should be commensurate with the degree of risk posed. Many jurisdictions regulate based on processes rather than products. Countries' GMO/NBT policy maintains the inefficient regulatory structure and exposes consumers and the environment to greater risks than necessary since the real food hazards (mycotoxins, E. coli, salmonella, listeria, Clostridium,etc) are not sufficiently addressed. NBTs will probably not change the current regulatory policies for technologies.

The Regulatory environment for new technologies in livestock (A. Van Eenennaam, UC-Davis):
The USDA definition of GMO is the manipulation of organisms genes by introducing, eliminating or rearranging specific genes using methods of modern molecular biology. Animal feed contains 90% genetically engineered (GE) crop commodities. There is a lot of misleading marketing of food products

occurring. Products appear with voluntary labels such as "gluten-free, non-GMO" water. Currently, there are no GE animals on the market (except the glo-fish sold in pet stores).

AquaBounty GE salmon was first generated (founder female) in 1989 and there was no reaction from the public. In the 1990s, AquaBounty decided to license the salmon and sell. FDA is now regulating it as an animal drug, however, the fish is not the drug—the gene is the drug. The company started the regulatory process with FDA in 2001. In May 2016, Canada approved the salmon for sale. In Dec 2016, US FDA billed AquaBounty $113,000 animal drug user fee for their approved animal drug (salmon) product despite FDA's continued ban on the import/sale of the salmon fillets.

New breeding technologies (NBT) allow developers to make precise insertion or modification using donor template DNA similar to nature's repairing of double-strand cuts through non-homologous end-joining which results in mutations. In plants, mutations are caused by the use of chemicals and/or radiation to create double-stranded random breaks. These plants are used in organic production systems.

In animals, NBTs have been used against the PRRS virus in pigs which causes $600 million in losses in the US each year. The gene was knocked out that produces the protein in the pig that allows the viral infection. The company is now planning to take this through the FDA drug process. CRISPR has been used in cows to fight tuberculosis. The company Recombinetics has used TALENS to solve the problem of de-horning (polling) dairy cows. Horns are a detriment in the dairy business as the cows can hurt each other and their human handlers. Recombinetics has used NBT to knock out the genes for horns. Much more humane than burning the horn cells in calves. In 2017, US FDA draft guidance considers all gene edited animals whose genomes have been altered intentionally to be drugs. This guidance is not safety or product-based. Polled animals (hornless) are not a safety hazard for food consumption as US consumers eat polled beef cattle all the time.

In summary, breeding programs increasingly utilize a combination of advanced reproductive technologies and genomic tools to accelerate the rate of genetic gain in animals. This involves the use of *in vitro* processes and has produced a number of useful traits. Regulatory processes must be in proportion to risk and consistent with science. GE regulatory burdens are impossibly high. Regulatory language is ambiguous to gene editing and there is an urgent need to determine appropriate regulatory framework for use of gene editing in agricultural breeding programs.

The International Food Technologists Society is releasing a move entitled "Food Evolution" on June 24 which is designed for the general public. The movie does not tell people what to think but how to think about food. The movie is narrated by Neil Degrasse Tyson and Bill Nye also appears in it.

From freezers to farms: Advancing biological assets to farm-ready IPM solutions (A. Vasavada, Marrone BioSciences):

Marrone BioInnovations (MBI) was incorporated in 2006 and currently has 6 commercially available products. There are 100 employees with 34 of these in R&D. The company has a library of 18,000+ microorganisms and operates a fermentation facility in Bangor, MI. Commercial sales occur in North America, Latin America, Mideast and Asia. Pipeline products include bioherbicides, downy mildew fungicide, biofumigant and anti-transpirant. The company has 34 US-issued patents and 153 issued foreign patents. MBI partners with several large companies such as Syngenta, FMC, Scotts and Koch Industries.

MBI products are based on microorganisms in sprayable formulations. The market is driven by consumers feeling that organics are needed and integrated pest management (IPM) is useful to meet the challenges for sustainable agriculture.

Biopesticides are microbials (fungi, protozoa, viruses) and biochemicals (plant extracts, pheromones, soaps, fatty acids). Biostimulants help to regulate the genes in plants and biofertilizers provide nutrients. Biopesticide growth has increased because of crop higher yields/quality, no residues and good for export markets, used to manage and delay resistance, worker safety and production flexibility, lower development costs and can be used in organic production. The process for biopesticide is to a) isolate microorganisms, b) ferment organisms and bioassay broths, c) perform biological testing against weeds, plant pathogens, nematodes, viruses, etc. and d) perform natural product and analytical chemistry to find new chemicals.

MBI has a microbe-centered R&D SWAT teams for support of products. These teams understand genetics, characterize active chemistry, improve manufacturing processes and develop new formulations. The goal is to develop farmer-friendly formulations and then to develop and scale the manufacturing process. Process analytics is used to make targeted improvements.

The product Grandevo is *Chromobacterium subtsugae* which was isolated from US forest soil by the USDA. It stops feed and reproduction of many chewing and sucking insects, mites, flies. It has been registered at EPA and has approval in Mexico. It has been submitted to the EU and Canada. It has a novel mode of action (MOA), causing gut disruption, agitation/repellency, reduction in egg laying and hatching.

Venerate is a product containing *Burkholderia rinojensis*. It has a broad spectrum of action against chewing and sucking insects, mites, flies and nematodes. There are multiple MOAs with a slow kill action (+7 days). Effects include exoskeleton degradation, mouting interference and stunting. It controls through contact and ingestion and is active against adults and larvae.

Majestene is based on the same organism present in Venerate. It kills nematodes in potato fields and is effective as soil and seed treatment at planting. Other products from MBI include Regalia (biofungicide which is a mixture of 10 volatile compounds), MBI110 (*Bacillus amyloliquefaciens*), MBI010 (controls Palmer amaranth).

Are we ready for genetically modified wine and vegetables? (A. Porterfield, Genetic Literacy Project):
Andrew Porterfield is a journalist from the Genetic Literacy Project who feels wine stories can help get message across about genetic engineering. Opposition to GE grapes is more based on the traditions of the viticulture industry rather than safety concerns. There are thousands of wine varieties available for culture, however, only a handful have been used over 8000 years. New traits cannot be conventionally-bred since this results in a new variety. The industry is hyper-focused on quality and tradition so new varieties are not that welcome. But this also leads to increasing risk of disease such as downy mildew, Pierce's disease, etc.

There have been attempts to modify wine. Rong Di, Rutgers, used CRISPR to produce downy mildew resistant grapes. Other researchers have made a synthetic wine yeast pathway and others have developed hangover-free wine. Organic wine producers are having issues due to copper sulfate buildup

in the soils. There has also been detection of conventional pesticides in vineyards but is this really drift from non-organic vineyards or cheating by organic producers? Many viticulturists would like a mix of organic soil treatment and GMO use to help solve issues.

How do we get the GM message across? Opponents of GM are very political as they build alliances and gather expertise. It is not just about the data for them. Opposition to GM can be cultural, attitudinal and political. In a study by Clotaire Repaille, who was hired to find messaging for biotechnology, the recommendation was to call it more natural and not use GM. In semi-focus groups, the attitude toward food differed greatly between US and EU. The EU thinks food is life and is their identity whereas in the US, food is viewed as 'just a fuel'. People come into arguments from their own space and to communicate we must find out what is happening in their space.

People love scientists and scientists should use their personalities to connect to people. Talk about how problems are approached and solved; tell stories about how an experiment started and the questions that were raised/answered; relate directly to things they use, i.e., how genetics have changed corn and strawberries; and blur the boundary between 'natural' and 'artificial'. Everyone needs to use public relations tactics such as homework, research, interviews and polls. Try to discern what messages will stick and the best way to reach people. And don't stop. According to the 700 impression rule, 3500 is the number of messages that the average person receives/day. In order to get a message across to a person, that person must hear it 700 times to get it.

People do not know about GM. In the 2016 Pew study, 71% of respondents said they knew little to nothing about GM, 48% think GM has no effect on health and 39% think GM has worst health effects. Most views of people are soft and are dependent on how the question is phrased. Aim to influence the 71% who know little to nothing about GM.

Incorporating environmental data into agricultural management (A. Melnitchouck, Bayer):
Digital farming incorporates analytics, digital marketing, etc. The Bayer scope of digital farming includes the technologies related with data of geographical coordinates. Agriculture is always region and site-specific. The complexity of the data is huge. For instance, the yield variability within a field in Kentucky was 507% and in a field in Canada it was 1164%. Yield is the biggest unpredictable dataset.

Industry always over-estimates the impact of new technology in the short term, and then under-estimates its impact over the long term (Bill Gates). Gartner's hype cycle for emerging technologies is: Technology trigger→peak inflated expectations→trough of disillusionment→slope of enlightenment→peak of acceptance. What does the ag industry expect? Farmers want 'easy' button but it is not so easy. Sense (remote sensing) →act (acting tools) → decide (evaluate).

At Bayer, it is a collaborative approach as something cannot be built that takes care of everything. We just have to make sure the pieces of the puzzle fit. Bayer is starting to offer first digital solutions in various geographies. Digital farming group is located in Germany with sites in the US, Brazil, Asia, etc. Scouting is done with mobile apps to help the farmer manage fields. Remote sensing is relied on due to its scalability and efficiency. Satellite imagery detects variability of biomass which can be combined with other data to determine site risks for disease. Analyzing field trials takes 0.5 seconds to determine low and high potential yield zones. Digital farming provides better prevention of crop damage since 25% of all weather-related harvest damage can be prevented with prognostic weather modeling and precision ag techniques.

For scouting, Bayer is investing in imaging technologies to know where the weeds are growing to help the grower make the right decision on control. Scouting helps to identify and quantify damage on leaves. The zone spray program identifies field sections where weeds are growing and then suggest treatments. This is coming to market in Canada, France and Germany. The future is here with digital farming by offering outcomes instead of just crop protection products.

Molecular breeding in vegetables (J. Djordevic, Bayer): Bayer vegetables is part of the Bayer Crop Science business. Nunhems vegetable seeds has been part of Bayer since 2002 and has 2100 employees in 45 countries. Nunhems works on 24 crops and produce product in 23 countries. In 2016, Nunhems generated 428 billion euros.

The 24 crops have 1200 varieties. Tomato is the #1 crop. There are a plethora of variants in size and color. Nunhems has developed a melon variety which can be shipped ripe in the EU. The golden cantaloupe will be coming soon to the US market. The 'crispy pear concept' melon has a white flesh like a pear but is a melon.

Breeding is art and science. New technologies (marker-assisted breeding) help to make the breeding faster. Markers are a necessary tool which shorten the odds of conventional breeding of a desired trait. Using conventional breeding with a plant that has 20 genes, a breeder would need 400,000 acres to see all combinations. Marker-assisted (MAS) breeding makes this process much easier and requires much less land. For example, MAS was used to develop a marker to enable fast introgression of cucumber green mottling virus resistance gene from southern EU cucumber varieties to northern EU varieties. Another example in tomato was the transference of genes for resistance to 10 different diseases into one variety. Using MAS, this took 4 years which is a very short timeline for a variety. Products that are being worked on now will reach the market in 10 years.

Scalable microbiome diagnostics for crop production (P. Parameswaran, Trace Genomics): Trace Genomics is focusing on one problem in food/agriculture—how to feed 10 billion people by 2050. Harmful and beneficial microbes + nutrient potential = soil. There are 100 million species of bacteria and 10,000 species of fungi in soil. Trace Genomics is making it easier for growers to get a snapshot of soil microbes. The company has developed a kit which allows growers to send in a soil sample to a lab for analysis. The company has an agronomically predictive database which combines machine learning + predictive analytics. This results in broad/deep microbial profiling. The online report to a grower presents disease risk and soil health. Growers will soon have a "recommendation" piece to the report for recommendation of seed coatings sprays, etc. The machine engine digests data in any form and allows to determine the drivers based on the microbiome but also informs the interaction with weather, genetics and environment. More information is available at info@tracegenomics.com.

AgriSeq GBS for breeding, parentage, traceability applications (C. Adams, Thermo Fisher): Thermo Fisher has 55,000 employees and produces $18 billion in revenue. The areas of the company are analytical instruments, life sciences solutions, lab products and services and specialty diagnostics. Life sciencies solutions deals with GMO testing, plant and animal genotyping, QTL mapping, plant and animal gene expression and synthetic biology. Thermo Fisher life sciences solution wants to be a scientific partner and not just the tech provider.

GBS is genotyping-by-sequencing. There are two approaches: a) restriction enzyme mediated and b) amplicon re-sequencing. Thermo Fisher uses the amplicon re-sequencing approach. Both reduce the complexity of the genome and promote SNP discovery. The main differences are the number of SNPs interrogated/sample, inconsistent data output, sample throughput possibilities (ease of automation) and cost per sample.

AgriSeq by Thermo Fisher has pricing to meet market needs. The process is selection of targets → construction of library → preparation of template → sequencing → data analysis. The sequencing is based on nexgen sequencing (NGS). AgriSeq is powereful, flexible and automation friendly. As little as 1 ng DNA can be used and crude samples can also be used. Sequencing is done by the Ion S5 XL system which is rapid with high computing power and quick data output. Innovations are being developed to drive efficiencies to lower running costs. These include robust performance with lysates and miniaturization of reaction volumes.

Thermo Fisher offers a pilot program in which the customer provides gDNA or crude lysates. There is a 3 month turnaround. Currently, there are animal and plant pilots going on (cucumber, soy, canola, tomato, rice, corn). All of these are marker-assisted breeding.

Participants in the Meeting:

Agdia
Bayer
Marrone BioInnovations
Eurofins Nutrition Analysis Center
Romer
EnviroLogix
GeneticID
DuPont Pioneer
Neogen
Covance
Eurofins Biodiagnostics
Eurofins GeneScan
Dow AgroSciences
SynTech Research
North Carolina State University
SGS
OMIC USA
BASF
Monsanto
Merieux NutriSciences
Thermo Fisher
LGC Genomics
UC-Davis
UC-Riverside
Trace Genomics
Genetic Literacy Project
Illumina
Syngenta

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Special thanks to Fred Claussen (EPL Bio Analytical Services) for taking notes.

Introductions, Antitrust Statement and CWG Mission Statement

Review of Notes from 2016 Meeting

ELLA Lectin Method Status Update: Elisa Leyva-Guerrero (Monsanto)

- New ELLA lectin method was unanimously approved as an official AOCS method (Ac 6-16) in January and will appear in the 7th edition of AOCS methods this spring.
- Collaborative study statistics will appear in the official method.
- The ELLA method (JAOCS) is currently being cited in the ILSI Crop Composition Database.

Nice work folks!

Cyclopropenoid Fatty Acids/Nutritional Fatty Acids Method: Barb Mitchell (Covance)

- The method was accepted as a Recommended Practice and will be published as such in the 7th edition of AOCS methods.
- The collaborative study design should have included duplicate sample analyses by lab participants. Barb also noted that inter-lab variability was high, possibly due to expression of results on a % of total lipid basis. A variety of crude fat extraction procedures were used in the collaborative, leading to variable total fat results.
- Barb suggested direct saponification of seed test portions instead of using a preliminary crude fat extraction. She was uncertain if that approach would work.
- Other suggestions included specifying a specific crude fat extraction procedure in the CPFA method, or specifying several equivalent crude fat methods from which to choose.

We've made good progress on this.

Actions:

Lance Workman, Barb, Keith Persons, and Mike Dowd discussed plans for a new collaborative to achieve official method status (AOCS annual meeting):

1) **Each sample should be run in duplicate as well as blind duplicates (these were included in the previous study)**
2) **Additional samples to extend the range of CPFA analyzed – Mike will look for seed from a different species, and Barb will mix crude cottonseed oil with another vegetable oil to create lower levels.**

Barb to test direct saponification of seed, fine tune a helium carrier gas conditions (some collaborating labs could not use hydrogen), and investigate 1-2 different columns to improve the separation.

All: we need collaborators, please let Barb, Luke or Martha Jennens know if you are willing to participate. There will also be another collaborative study for total gossypol by HPLC, if anyone would like to participate.

CWG needs some resolution on best crude fat methods to use. This could then be applied to CPFA for consistency in extraction if direct saponification doesn't work. One other possibility is to run the collaborative on oil only (Covance would extract from seed, and send the oil to the collaborators).

Allergens: Tao Geng (Monsanto)

- Tao shared results of a Sponsor survey conducted to assess the current status of allergen assays. Most sponsors are measuring the same allergens except for gly m 1 and gly m 7. EFSA has indicated it expects those to me measured as well.
- Gly m 7 determination is needed by all Sponsors. A collaborative ring trial and publication of results comparing different methods was proposed.
- Everyone is either using LC-MS/MS or moving towards using LC-MS/MS.
- Some sponsors are measuring Kunitz Trypsin Inhibitor directly by LC-MS/MS while others rely on the trypsin inhibitor assay.
- EFSA is requesting an allergen database. ILSI doesn't want to include allergens in the Crop Composition Database because it would legitimize the concept of allergenicity.

Actions: Tao will explore organizing a collaborative ring trial for gly m 7

Trypsin Inhibitor: Luke Muschinske (Covance)

- Current methodology is based on a colorimetric activity assay. Methods cited in the ILSI database included ISO 14902, AACC 22-40 and AOCS Ba 12-75.
- Current methods measure consumption of trypsin added to a seed extract. Differing incubation times and activity of added trypsin lead to high variability. Standardization of trypsin activity would be helpful.
- Direct measurement of trypsin inhibiting proteins by LC-MS/MS:
- In soy, 2 Kunitz inhibitors and 7 Bowman-Birk inhibitors are known. Possibly enough homology to utilize the same peptides, or even a single peptide in an LC-MS/MS assay to cover all proteins in a given class.
- It was suggested that only representative proteins be measured. However, this was considered too risky for data submissions by some. Another opinion was that specific trypsin inhibitor measurements should be discouraged altogether.
- What about trypsin inhibitors in other crops? Have specific proteins been identified. TIU values in maize, for example, are very low. Raises the question if trypsin inhibiting proteins are present at all.

Actions:

Luke to send method references cited for possibly enhancing current methodology to group.

Luke to follow up with John Lawry about investigating how homology translates to measure peptides by LC-MS/MS.

Method Review - Proximates: Nancy Gillikin (Bayer)

- Nancy provided follow-up to the conference all held prior to the meeting.
- Methods for ash and calories (calculation) appear to be ok for now. Differing combustion times and temperatures for ash are of potential concern.
- A reference citation in the ILSI database for carbohydrates has been corrected.
- Combustion/Dumas (inorganic plus organic N) vs. Kjeldahl (organic N) techniques were discussed. In general, as one would expect, Kjeldahl techniques yield slightly lower total nitrogen values for seed than combustion methods. Differences are larger for forage.
- Concern was expressed regarding EFSA acceptance of combustion data.
- Appears that Kjeldahl will remain as the accepted technique for nitrogen/protein measurements for composition studies.
- For crude fat, Anders Thomsen (Eurofins) provided some guidance on solvent extraction (pet. Ether/soxhlet or butt tube) vs. acid hydrolysis/ethyl ether extraction. He indicated that hydrolysis methods should be discouraged for seed. He was less certain about the appropriate method for forage.

Actions:

Proximate group to focus on the topic of crude fat extractions to come to a consensus on best 2 or 3 approaches (per matrix) that give equivalent results. Maybe schedule 2 to 3 meetings between now and the next AEIC meeting??? Discussion on other proximates can follow.

Fat-Soluble Vitamins Multiplex: Fred Claussen (EPL Bio Analytical Services)

- Previous meeting: Isotopically labeled internal standards were the preferred solution to MS suppression encountered in recovery experiments. However, internal standards for the minor tocopherols (beta, delta, gamma) are not commercially available without custom synthesis ($$$). It was suggested that the minor tocopherols be eliminated from the method scope.
- Results of a Sponsor survey regarding the need for minor tocopherols in data submissions was presented. All Sponsors indicated that only alpha-tocopherol was needed in most cases for canola, cotton, maize and soy. The exception being for events where fat metabolism is targeted. In these cases, current individual methods for determining vitamin K_1, beta-carotene and all four tocopherols should be used.
- Scope of the new multiplex method has been revised to exclude the minor tocopherols. Exclusion of the minor tocopherols from the multiplex method will allow use of isotopically labeled internal standards. Fred provided commercial sources and approximate costs for isotopically labeled alpha-tocopherol, beta-carotene and vitamin K_1.

- Data on linear range of each analyte using internal standards was presented. Preliminary bridging data using internal standards was also presented. Alpha-tocopherol showed significant improvement over results without internal standard. However, vitamin K_1 results were lower than previous bridging experiments without internal standard.

Actions:

Work will continue with Fred and Co. at EPL. Modified scope should provide a higher probability of success.

Inositol - Phytate Discussion: Nancy Gillikin (Bayer)

- As per previous discussions, wide range of concentrations appearing in the ILSI data base due to use of methods that measure free inositol and total inositol (free + phytate + other minor forms). There are a few total inositol values in the ILSI database that were reported as "<LOQ", and these should be addressed.
- Total and free inositol methods will be identified specifically in the ILSI database.

NIR for Proximates: Nancy Gillikin (Bayer)

- Bayer EU has submitted NIR data for proximates to EFSA, and the data was accepted. CWG could make a case for use of NIR for composition studies. A publication with comparative data to conventional methods would be needed.
- General calibration concepts were discussed, including use of USDA and other calibration curves, and ongoing calibration to capture geographic and varietal differences.

Actions:

Anders Thomsen volunteered to lead an effort to investigate steps needed to encourage regulatory acceptance of NIR proximates data (and possibly other anlaytes). Conversation should include strategy to make calibration practices practical as well as consistent and scientifically sound.

Co-Chair Change: Carl Maxwell (DuPont Pioneer)

- Luke Muschinske will replace Nancy Gillikin as co-chair of the CWG. Carl will remain as co-chair for one more year.

As co-chairs continue to rotate in and out, it was generally agreed that at least one co-chair should be from a Sponsor company.