AEIC Fall 2003 Meeting Minutes

P.L. Hunst, AEIC Secretary

The AEIC 2003 Fall Meeting was held at the Kellogg Hotel and Conference Center in East Lansing, Michigan and was hosted by Neogen Corp. (Frank Klein). Jim Herbert, President of Neogen welcomed the attendees (14 member companies, USDA GIPSA, USDA AMS, Eurofins EU) to East Lansing and the beautiful fall weather.

AEIC Business Meeting

The Secretary's minutes from the 2003 Spring Meeting were accepted.

Dean Layton gave the treasurer's report. The balance prior to expenses was $37763. Dues of $6800 were added and expenses of $9986 were substracted leaving a balance of $34712. There are $1650 of outstanding dues. The projected expenditures are $17673 which would leave a balance of $18689. A motion was made, seconded and approved to accept the report.

Membership:

	Paid	Unpaid	
Large Corp. Members	13	2	(BASF, Medallion)
Small Corp. Members	11	2	(Biogenetic Services, UNE)
Associate Members	3	1	
Affiliate Members	11		
Individual Members	2	1	(J. Sharp)

A request was made to extend the mailing list to past meeting speakers and other interested parties. If anyone has suggestions for other persons who should be included on the mailing list, they are to send them to the AEIC Secretary (P. Hunst).

AOCS Update: AOCS Technical Services publishes official methods and recommended practices. It also oversees a lab proficiency program, approved chemist program, soybean quality trait intiative, NOPA lab program and performance reference samples.

DNA Paper (M. Lipp): The paper is still being worked on. Monthly phone calls have been scheduled and currently working with CropLife America to publish. Expect to be completed by January, 2004.

USDA GIPSA Update (R. Jenkins): GIPSA is currently cooperating with NIST and Bayer CropScience on a DNA quantification project. The objective is to use Qiagen kits and extract DNA from ground soybean. After clean-up steps, the DNA will be split three ways and quantified using inductively coupled plasma-optical emission spectoscopy, gravimetric analysis, absorbance at 260/280, luciferase, fluorescent intercalating dyes and shearing studies will also be conducted.

A white paper has been written on reference materials by D. Kendall based on the workshop held in June, 2003 and has been sent to USDA upper management for a decision. The decision will determine whether USDA collaborates with NIST. The recommendations from the workshop were that the reference materials should be ground materials and should serve both DNA and protein purposes.

For the proficiency program, samples (corn and soybean) were sent out again in September, 2003. Results are being posted on the GIPSA website (currently, results through February, 2003 are posted—May, 2003 results are still being reviewed).

ISO/TAG (R. Shillito): For the sampling document, the proposal will be discussed and the CEN sampling document.

Spring 2004 AEIC Meeting: R. Shillito will follow up with Eurofins to see if they still want to host the meeting in Memphis, TN. Gina Clapper also volunteered AOCS to host either the spring or fall meetings in Champaign, IL.

Proposed topics:

- Understanding grain development (maize/cereals): morphology/biology
- IRMM and standards (M. Lipp)
- EU validation program for protein/DNA methods (JRC ISPRA—M. Lipp)
- Reference genes (F. Spiegelhalter)
- Mass spectroscopy for characterization of GM crops (proteomics, metabolomics)
  --suggestion of Mark Engle (Dept. of Ag., FL)
- Antibody production by different systems (phage display, etc—J. Stave will follow up)
- PNA (M. Lipp will follow up with U. Lungren)

The proposed timing of the meeting is April.

Presentations:

The first presentation was given by **Stacy Charlton (Syngenta; President of AEIC)** on the topic of sampling issues and detection of biotech products. Sampling is cost effective since to have 100% certainty of GM vs. non-GM would require the testing of every particle (seed). This is impractical since testing is destructive. Therefore, correctly designed sampling procedures provide high levels of confidence that the sample is representative of the whole lot. Probability theory assumes a random distribution, however, biotech products are distributed hetergeneously (non-randomly). Due to random chance the proportion of biotech positive particles in the sample may differ from the bulk lot. The practical solution to overcome this is to take a representative sample of the lot. For threshold testing of bulk grain, knowing the number of particles in the sample allows a statistical estimation of the probability that the lot (or truckload, boat, etc) is above or below the threshold. As the desire to detect lower thresholds increases, this creates pressure to work with increasing sample sizes. However, the limitation of the analytical procedure to handle larger sample sizes results in countervailing pressures. Collecting and analyzing multiple samples of the same lot accommodates these conflicts but it is more expensive. In quantitative testing, it is not simply the presence or absence that is being determined. It is desirable to know the variation around the mean and obtaining larger sample sizes will approximate the mean more closely. Improper sampling may lead to false negatives or false positives, imprecise and variable results. Bulk sampling must be representative and the sample size is a balance between sensitivity, cost, buyer's/seller's risk. Subsampling is dependent on the number of particles and the size of the particles to obtain the most accurate results.

The next presentation was given by **Bert Popping (Eurofins Scientific Group)** and dealt with the detection and quantification of allergens in cereal products using molecular/immunological techniques. An allergy is a misguided reaction of the immune system which causes mass production of IgE antibodies and the activation of histamine. In food allergies, the body mistakes food for a foreign object. The symptoms may range from a minor itching/rash to severe reactions resulting in death. Food allergies are a liability to companies and a real health issue for sensitive people. Food recalls for food allergies have resulted from non-declared ingredients due to intentional substitution (using cheaper products), accidental cross-contamination and wrong assumptions (i.e., allergen is no longer present after heat treatment—most allergens are heat stable). In the EU, the amended food directive 2000/13/EC directs that all allergens must be labeled. This includes cereals containing gluten and certain nuts (walnuts, hazelnuts, almond, etc.). Testing for food allergens has employed ELISA, rocket immunelectrophoresis, PCR ELISA, PCR, real-time PCR. Of these, ELISAs are the most prevalent since the cost is reasonable, easy

to conduct, high throughput, require no expert skills and can be quantitative. On the other hand, there is also the potential for cross-reactions and matrix effects. For PCR-ELISA, there are some commercial kits available. It is important to note that in these procedures, the PCR and post-PCR reactions cannot be performed in the same room, otherwise, false positives will occur. Currently, method harmonization is ongoing between LMBG Immunology Group, GDCh MolBio Immunochemistry Group, DIN Allergen (CEN), CEN Working Group 12 and through AOAC-Presidential Task Force on Food Allergens.

Deana Namuth (U. of Nebraska) gave an introduction to the Library of Crop Technology—an educational website. The objective of the website was to make available learning objects which are portable and to make these available to information disseminators such as teachers, extension specialists, etc. The focus is trying to create something more sustainable than other websites, i.e., an electronic library. The learning objects may be checked out and used. This is particularly conducive for long distance students. There are learning modules on a variety of topics and for a variety of audiences. The address of the website is: www.croptechnology.unl.edu/.

Kirk Remund (Monsanto) talked about statistical methodologies for adventitious presence testing using quantitative methods. The work that Kirk described was a collaboration with Laffont (Pioneer), Wright (Pioneer) and Simpson (Monsanto) and dealt with the implementation into Seedcalc of quantitative methods. Again, the premise is that we cannot guarantee 100% conventional seed unless we test 100% of the seed. The decision of conventional vs. GM seed using an analytical method and sampling is supported by the probability calculations giving the probability of making a wrong decision. The implementation of the quantitative version will be via the ISTA website (www.seedtest.org/STA/sta_toolbox.cfm) which allows access to Seedcalc and Qualstat. USDA GIPSA also has Seed Planner available at www.usda.gov/gipsa/biotech/samplingplan1.xls. The beta version of Seedcalc will soon be availabe and for those who wish to be beta-testers, they should contact Kirk Remund at Monsanto.

Michael Sussman (USDA AMS) gave an overview of the USDA Ag Marketing Service Science & Technology, Field Laboratory Services, National Science Laboratory. AMS has oversight of seed regulation, organic program, facilitates strategic marketing of agricultural products and provides testing services and grants intellectual property rights for certain plant varieties. AMS programs include tobacco, cotton, dairy (grading program), fruit & vegetables, livestock and seed, poultry (grading program), science & technology, transporation & marketing. There is currently a proposed memorandum of understanding between GIPSA and AMS. The purpose of the MOU is to increase efficiencies and reduce duplication of effort; make progress toward standardization of testing methods and within USDA marketing and regulatory programs (MRP), establish clear contact points for biotech needs. The defined roles are:

AMS: fruits, vegetables, cotton, tobacco, poultry, dairy products and seeds
GIPSA: grains and oilseeds, intellectual property coordination

AMS' roles include the establishment of methods and policies for sampling seeds and other commodities; proficiency tests, check samples and accreditation; rapid test evaluation programs (will pick up as needed from GIPSA); asssist industry to meet export requirements and product labeling claims for biotech products; and method development (as needed).

Andre Silvanovich (Monsanto) gave a presentation on single amino acid differences and immunoassays for their detection. The example was NK603, Monsanto's Roundup Ready corn event. In NK603, two gene cassettes were inserted which contained the gene for CP4 EPSPS, however, one cassette contained the 35S promoter and one cassette contained the rice actin promoter. Following transformation, it was found that the protein produced from the rice actin promoter cassette contained a single amino acid substitution (proline was substituted for leucine). The substitution was found to not alter the structure of the enzyme since the substitution occurred in a variable loop region which is not relevant for enzyme activity. The desire was to determine how much of the protein with the proline substitution was present in the NK603 corn. Two

peptides were manufactured—one containing the proline and one containing the leucine. Antibodies were generated by injection into rabbits. The leucine peptide gave no immune response, however, the proline peptide was highly immunogenic. On gels, it was found that the proline version of the CP4 EPSPS had a slightly changed electrophoretic mobility. Mass spectrometry showed no difference in molecular weight, however. By using the peptide antibodies, it was found that ~25% of the CP4 EPSPS found in NK603 corn was the proline version.

Ray Shillito (Bayer CropScience) gave an overview of the crop composition database compiled by a task force of the ILSI Food Biotechnology Committee. The members of the task force included Syngenta, Renessen, Pioneer/DuPont, Monsanto, Dow AgroSciences and Bayer CropScience. The objective of the database were to understand the variability in the composition of crops, use a reference for comparison of composition data and use a baseline of data. Each data point in the database is associated with a referenced method. The data is in consistent units and was derived from controlled field trial experiments carried out at multiple sites with replicated plots. The content of the database is currently only non-GM crops and only corn and soybean data for the moment. Currently, data points exist for 102 analytes and there are 1446 data sets comprising 53221 data points. In the near future, data will be added for cotton, canola, rice, etc. The database may found at: www.cropcomposition.org.