AEIC Spring Meeting '99
Princeton, New Jersey

P.L. Hunst, Secretary

The 1999 AEIC Spring Meeting was hosted by FMC (Audrey Chen) at the Nassau Inn in Princeton, NJ on April 8-9. Eighteen companies were present with about 25 persons attending.

On Thursday, a series of presentations, organized by the Biotech Subcommittee of the AEIC, were given. These were a prelude to the presentation by the subcommittee at the end of the day concerning the Biotech Initiative of AEIC. The presentations were meant to give everyone a background in the issues facing people entering the biotech arena and to give a knowledge base to all members of AEIC.

The first presentation was given by Kim Magin (Monsanto) which was an overview of how immunoassays are used in the commercialization of biotech products. For product development, qualitative and semi-quantitative assays are used for screening, transformant identification, line selection, plant breeding, quality control. For safety assessment of the product, quantitative assays are used to determine expression data of field material for regulatory approvals. For the approved product, qualitative and semi-quantitative assays are used in screening, quality control, product stewardship, intellectual property, enforcement, protein based labeling. Immunoassays are used for regulatory purposes for generating product characterization data, assess food, feed and environmental characteristics, calculate concentration for toxicology studies, obtain tolerance exemption for insecticidal proteins, etc. Immunoassays are developed and optimized for each target protein. For protein extraction, a buffer for each given tissue type must be used for each crop. The impact of each buffer must be determined for extraction efficiency and any effects on the assay itself. The buffer to tissue ratio is also very important determination for the assay. The aspects of validation for an assay are: precision--interplate, intraplate and interassay; accuracy--extraction efficiency, matrix effects, spike/recovery, parallelism, stability of protein in extracts; sensitivity--LOD/LOQ; specificity--other similar proteins in matrix that may react with antibodies.

The second presentation dealt with regulatory oversight of biotechnology in the US and how immunoassay data is used. The presentation was presented by Penny Hunst/Sue MacIntosh (AgrEvo). In the US, the USDA, USEPA and USFDA have oversight over biotech products. The USDA regulates biotech products under the Plant Quarantine Act and Plant Pest Act. Under these, regulated biotech products can only be imported, moved interstate and released environmentally through permits and/or notifications which are submitted by the companies to the agency. The USDA also grants nonregulated status to biotech products upon submission and approval of a petition. The petition must contain information on the molecular characterization of the inserted DNA, the stability of the inserted DNA, agronomic performance, Mendelian inheritance of the inserted traits, expression of the inserted genes (expressed proteins). ELISA data is generally submitted

to elucidate the expression products of the inserted genes. When the petition is approved, USDA no longer regulates further agricultural production of the biotech plant product.

The FDA has oversight of biotech plant products through the FFDCA (Federal Food, Drug, Cosmetic Act). In 1992, the FDA issued their statement of policy on transgenic plants. Under this, they stated that FFDCA does not give the agency authority to approve whole foods. Producers are liable for the safety of transgenic food products. This has resulted in a voluntary premarket consultation process with the agency. The document that is submitted to the FDA is a Food and Feed Safety Assessment and contains the same information as submitted to the USDA. In addition, the assessment contains composition data--proximate analyses and amino acid analyses. Proximates are carbohydrates, fiber, crude protein, ash, fatty acids, etc. The FDA is most interested to determine if the transgenic plants are "subtantially equivalent" to nontransgenic counterparts and this assessment is based on the composition data. If the transgenic plant is found to be not substantially equivalent, then the transgenic plant would be considered a food additive and subject to the review process for food additives. Since the FDA cannot regulate whole foods, the outcome of the premarket consultation procedure is a "favorable consultation" from the agency.

The EPA has oversight on pesticidal plants only under FIFRA (distribution and sale of a pesticide) and FFDCA (setting tolerance limits on pesticide residues on or in food plants). All data submitted to the EPA should be done under GLP. Although GLPs do not dictate specific validation parameters for analytical methods, industry standards for validation for immunoassays and other analytical methods include stability of target molecule in matrix, LOD/LOQ determination, precision, accuracy, ruggedness, etc. Immunoassay data is used in EPA submissions for elucidating the expression level of the pesticidal protein and assists the agency in determining tolerances or exemptions thereof. For insecticidal plants, an insect resistance management (IRM) plan is also part of the submission to the agency. There are 7 elements of IRM that have to be addressed: pest biology; strategy deployment (refuges); monitoring plans (for field failure response, sales response); product fit with current integrated pest management practices; communication/education of users; and future directions of research (dual gene products).

In general, the data requirements for submissions for other countries are very similar to the US data requirements. One difference is that composition data must be generated in each country and must be conducted over a 2 year period. In addition to composition data, other countries also require feeding study (chickens--45 day test). Other countries also require more molecular data if the insertion of the gene is complex, i.e., more than one copy of the gene. This case usually occurs when the DNA is inserted via a direct method such as the DNA gun.

Ron Lirette (Monsanto) gave an overview of the DNA detection method known as PCR (polymerase chain reaction). PCR is a target amplification method: uses enzyme to replicate the target; sensitivity is unlimited; method is subject to interference by contaminants. Why is amplification of DNA necessary? Inserted DNA sequences are present at one copy/cell while proteins are present at much higher numbers/cell. Radioactive analytical methods are available which have this detection capability, however, they are cumbersome, time consuming and expensive. Non-radioactive

detection systems are not adequate to reliably quantitate DNA at one copy/cell. Amplification offers sensitivity and time advantages, as well as multiple analyte detection. The PCR method uses a thermostable DNA polymerase and two oligonucleotides (that are specific for the target DNA sequence) to replicate the target sequence in an exponential fashion through thermal cycling. Sensitivity in the method is controlled by the number of thermal cycling; the template and primer concentrations; PCR product detection method. PCR is difficult to validate because it is not a closed system. Primers are the only portion which is validated. PCR is a poorly quantitative method--it is qualitative at best. Companies are working on quantitative PCR in order to use for labeling requirements in the EU.

There are other DNA detection methods that are beginning to be used other than PCR. Ligase chain reaction (LCR) is a method that allows for enzymatic amplification of a short target sequence using two oligonucleotides primers and a thermostable DNA ligase. The two primers are designed such that the 3' end of one primer is directly next to the 5' end of the other. This is done for both strands of DNA so four primers are needed. The template DNA is heat dentured and primers are allowed to anneal to the template DNA. The thermostable ligase, present in the reaction mix, joins the primers together, yielding a product that is twice the size. The ligase products serve as templates for the next reactions. LCR is sensitive to mis-matched bases in the primer sequence therefore, the method is useful for mutation detection. It is extremely specific in that it can detect a single base difference between templates.

Another method is called strand displacement amplification (SDA). This is an isothermal process that uses a restriction endonuclease and an exonuclease-deficient DNA polymerase to amplify a target DNA sequence to levels which are equivalent to PCR product generation. The detection of products is accomplished through agarose electrophoresis, fluorescence polarization and Southern blots. The specificity of the method is dictated by the primer sequences and is influenced by reaction conditions.

A comparison of the three methods is given below:

PCR	LCR	SDA
widely used	limited use	limited use
broad literature base	limited literature base	limited literature base
requires thermocylcer	requires thermocylcer	no thermocycler required
simple reaction components	simple reaction components	complex reaction components
tightly patented	tightly patented	tightly patented
time consuming	time consuming	rapid

Of all the procedures, PCR is the most widely used, tested and accepted. Future amplification strategies will be faster, cheaper, have higher throughput, and have improved quantitative capabilities.

The next presentation was given by Markus Lipp (JRC-Italy). The JRC (Joint Research Commission) works for the EU Commission. The Commission is organized into general directorates--the JRC is one of these directorates. The JRC is the scientific branch and

has been established so as not to be influenced by national preferences, etc. The Commission looks to the JRC for scientific input and to ask scientific questions. There are several JRC locations: Germany, Belgium, Italy and the Netherlands. Markus works in the Food Products Unit which has the mission to: 1)develop, harmonize, validate analytical methods; to monitor chemical, physical and biological parameters; and to disseminate results in the field of food and other consumables; 2) to serve the safety of the consumer, to detect frauds and to proof compliance with labeling in order to support EU policies. This is achieved by collaboration with EC General Directorates and by a proactive interaction with European institutions, organizations and industries through networking with member states' labs. This work is underpinned by appropriate R&D activities.

The projects of the Food Products Unit include:

1) safety control: feeding stuff (mycotoxins, parameters important within frame of BSE [mad-cow disease])
food (natural toxicants, pesticides)
food contact material (reference collection)
childcare products (phthalates)

2) quality control: BEVABS (wine, alcohol, spirit drinks)
authenticity proof (fruit juices, honey)
compliance with labeling (chocolate, dairy products, organic food)

3) GMOs in food: labeling

The legislative background for GMOs in food is as follows:

1997: novel foods regulation (258/97)—every product not on the market prior to this date are subject to this regulation.

1998: Roundup-Ready Soybean and Novartis Bt176 corn are regulated under 1139/98—due to the fact that these products were on the market prior to the novel foods regulation.
Regulation 90/219 enacted for GMO microorganisms.
Regulation 90/220 covers the deliberate release of GMO products into the environment.

The information and text for these regulations may be found on the internet at europa.eu.int.

Markus then went over the procedures for obtaining clearance for GMO products in the EU. For equivalent products, a complete assessment is not required. Basically, a notification is submitted to the Commission. The Commission sends the notification out to the member states who have 60 days to respond. If there is not response from the member states, the notification is approved. However, it should be noted that if a product is cleared under the novel foods regulation, this does not allow the deliberate release into the environment (90/220). The release is a separate procedure. For those products which are not equivalent, a full application under the novel foods regulation is required. The applicant (company) submits a full dossier via a national authority (member state). The

dossier is reviewed by the Food Assessment Body. After the review, the dossier is sent to the Commission and then out to the member states. Any of the member states may request additional assessments for the product(s). If member states agree with the review and any additional assessments that were requested, a decision is granted. If the member states do not agree after 3 months (in theory), the initial assessment is implemented. The process is currently taking as much as 2 years.

For labeling of GMO foods, there is no clear official procedure in the EU. A draft procedure has been written by the Swiss and a decision on this is expected in June, 1999. In this procedure, a threshold of 1% for GMOs in ingredients and additives will be allowed. For negative labeling, the GMO content must be below 1%, there must be complete documented evidence of the level and the GMO must exist.

For the EU, a draft procedure is expected in the summer, 1999. This draft will amend regulation 1139/98. The threshold will be proposed to be 2% GMO based on ingredients for soybean and maize. There will no negative labeling in this draft. Either protein or DNA analyses may be used. These may include PCR, direct hybridization, DNA chips, etc. and ELISA, Western blots, capillary electrophoresis, HPLC, MS, etc.

Further down the "pipeline", the EU will look at negative labeling, thresholds for other GMOs in a case by case decision, food additives and colorants, and a negative list. The negative list would be for products which are highly refined oils or starch hydrolysates. However, the EU must define what is meant by "highly refined".

One problem with the draft documents is how to determine percentage of GMO in weight percentage? The answer to the question is method specific. For proteins, it is the amount of extracted antigen. For DNA, it is the amount of extracted GMO DNA, amount of GMO-DNA with respect to plant specific DNA (double competitive PCR). The problem is how to relate the answer to the question.

In Europe, there are no agencies to approve methods such as the FDA and EPA in the US. The European Commission will not fix methods in legislation. A validation study can be done by anyone with no stamps of approval. There is an urgent need for many different methods, however, there must be a validation procedure to go along with any method. The expectation of any validation of a method is characterization of the performance of the method; quantitative methods are preferred; and qualitative methods should be described very clearly (statistics are a must). For quantitative methods, the results should be reproducibility and repeatability; for qualitative methods, the false negatives/false positives should be elucidated; methods should be measured for robustness; for DNA methods, the primers should be validated; for ELISA, there should be sufficient antibody for one or a few materials.

At this point, the alotted time was expired and Markus had to quit. However, further slides can be viewed in the attachment to these notes. PLEASE NOTE: Markus has requested that his slides do not go any further than within the AEIC member companies. DO NOT distribute them to non-members.

The last talk was given by Dwight Denham (SDI) on industry perspective of the EU food labeling. EU regulation 1139/98 states that "neither DNA nor protein, resulting from genetic modification, is present". Does this mean that both DNA and protein methods must be run on a product? Does protein exist when DNA does not and vice

versa? How do we deal with opposing parties using different methods, i.e., conflicting results between ELISA and PCR? A threshold is being considered, however, do to a lack of determination of this, labeling cannot be implemented or enforced. In the absence of a threshold limit, a secondary market of non-GMO testing has developed to meet identity preserved (IP) GMO-free products throughout the distribution chain. There is also no sampling plan identified. Without a sampling plan, we miss 50% of the total error in making characterization decisions. Distribution groups are making up their own plans, usually with PCR in mind as the method.

GMO detection method reliability for various portions of the food distribution chain are: immunoassay strip tests good for raw agricultural commodities (RACs); ELISA methods are good for RACs and primary ingredients; PCR is good for RACs, primary ingredients and processed foods/ingredients. However, there are no methods for highly processed foods/ingredients.

For methods, endusers must received education on what is the appropriate method for the application. Food companies and others are confused on methods that are currently available, i.e., which should be used on what. PCR labs are claiming that they can test anything, however, PCR is currently only qualitative. Food companies are not receiving reproducible answers from PCR labs. Also, does percent DNA equal percent of protein? How do we correlate the two? Is it percent new protein to total protein? Is it percent new DNA to percent total DNA? Is it weight/weight of GM seed to weight/weight non-GM seed? There are lots of opinions on these questions, however, very little consensus. Another area is the master standard. What should it be---purified protein? In reality, no better standard exists.

The food industry perspective is that because there is no clarification on 1139/98, there is little enforcement by control authorities. In the absence of this clarity, the companies are relying on detectability methods. The purpose of detectability is not to label by confirming GMO-free. Several large food companies are replacing soy protein in products such as infant formula with wheat protein in order to bypass regulations, i.e., no GM wheat so the companies do not have to confirm that products are made without GM protein.

From the consumer perspective, the EU labeling law does not meet their needs. The law does not address ethical concerns. Detectability in foods is not the issue—the source of food ingredients is the issue. Consumers are not against biotechnology—they are against the lack of choice.

There is a need for industry leadership. There needs to be a promotion of a master standard for all methods. There needs to be a development of a reference point for DNA equals protein relationship vs. method performance. There needs to be development of a sampling plan guidance. And there needs to be resolution of testing throughout the distribution chain or at what stage to meet market need in light of consumer and government efforts so that best IP testing market exists for future outbound traits.

Chuck Mihaliak (Dow Agro) presented the AEIC Biotech Subcommittee's proposal as to what AEIC can do. The mission statement for biotech was first presented:

Mission:

To facilitate the proper use of immunoassay technology in the development and approval of GMO plants and derived products, the AEIC Biotech Subcommitte will:

1) develop performance-based method validation guidelines for determination of proteins in GMO plant matrices, food commodities and derived products;
2) serve as a global educational resource to regulatory bodies and endusers (food processors, food companies and other interested industry affiliates);
3) engage in an industry-wide effort of cooperation in obtaining consensus on guidelines for proper use of immunoassay methods;
4) facilitate education regarding development of methods for DNA detection and quantitation.

After discussion by the members, the mission statement was changed to an "initiative" and was revised as follows:

Initiative:
To facilitate the proper use of immunoassay technology in the development, approval and production of GMO plants and derived products, the AEIC Biotech Initiative will:

1) develop performance-based method validation guidelines for detrmination of proteins in GMO plant matrices, food commodities and derived products;
2) serve as a global educational resource to regulatory bodies and endusers (seed companies, growers, food processors, food companies and other interested industry affiliates);
3) engage in an industry-wide effort of cooperation in obtaining consensus on guidelines for proper use of immunoassay methods;
4) facilitate AEIC education regarding development of other methods for detection and quantitation;
5) increase awareness of immunoassay methods as a tool for compliance for GMOs;
6) ensure guidelines are available for validation and use immunoassay methods for GMO determinations;
7) provide a forum for communication and education regarding GMO detection methods;
8) gain industry consensus on proper validation and use of analytical methods for GMO detection.

The action items (and proposed deadlines) for this initiative are:

1) Communicate to the broader AEIC membership to promote buy in and involvement of all AEIC members—Spring, 1999 AEIC Meeting [Completed]
2) Identify issues and associated work products—April, 1999.
3) Identify necessary work groups—April, 1999.
4) Recruit appropriate participants—June, 1999.
5) Communicate AEIC's intention to work on issues and develop guidelines—June, 1999.
6) Generate draft work products for CEN, SFAI Meetings—August, 1999.
7) Finalize draft guidelines—November, 1999.

8) Identify audience for guidelines for development and validation of protein detection methods in ag. biotech products (ACPA, CEN, EPA, JRC, ASTA, IBC, Inst. of Food Technologists, ACS, AOAC, BIO, EuropaBIO, NFPA, etc.)—3rd Qtr., 1999.
9) Identify medium to publish AEIC guidelines—3rd Qtr., 1999.
10) Invite speakers on DNA and other technologies to AEIC Meetings.

The proposed working groups to work on the following action items are:
1) Guidelines for validation of methods for quantitative dry weight immunoassays; quantitative on a protein basis immunoassays; semi-quantitative assays and qualitative assays. Drafts and final documents.
2) Recruit industry participation—generate contacts list, communications materials. Purpose is to invite participation with goal of expanding AEIC membership and to include relevant industry representation in the consortium.
3) SFAI Meeting in September, 1999---moderate a roundtable discussion; prepare and deliver a presentation including the background for the roundtable discussion.
4) Proper application of methods—sampling plans, QA/QC documents during routine use of assays.

On the second day of the AEIC meeting (April 9), Chuck moved that the AEIC endorse the biotech initative. Dean Layton (Envirologix) seconded the motion and the motion was passed by a majority vote of attending members. The scope of the initiative does not include trying to influence legislation. It was felt that member companies could use the information to work in the legislative area themselves. It was also recognized that the sampling issue is not a trivial project. It will require expertise from food companies and from expertise of individual AEIC member companies (statistical support). Sampling should include variation of the assay and all variation of the sampling, method, etc. should be stated in order to legislators (in the EU) to understand the threshold. This allows them to build in the risk factor for the consumer (Markus Lipp). Reference materials are essential in validation and their development is tied to sampling. The guidelines that are developed by AEIC should allow kit manufacturers to produce kits that will yield comparable results to the assays used internally by the ag. biotech companies.

At this point, volunteers were asked for the various working groups. The composition of the working groups are:

1) Validation guidelines (Chuck Milhaliak [Dow Agro]/Kim Magin [Monsanto]—Co-Chairs).
Jim Stave (SDI), Brian Skoczenski (Beacon Analytical), Sarah Hindman (Agdia), Fernando Rubio (Abraxis) and Paul Satoh (Neogen) Dave Grothaus (Poineer).

2) Recuiting (Dave Grothaus [Pioneer]—Chair)
Kelly Cullum (SDI), Cynthia Lipton (Zeneca), Sarah Hindman (Agdia)

3) SFAI Meeting Presentation/Roundtable—(Dave Grothaus/Kim Magin—Co-Chairs)
Jim Rittenburg/Dan Kozo (Biocode)

4) Application of Methods—sampling, QA/QC (Joe Dautlick [SDI]—Chair)
Dwight Denham (SDI), Sam Dubelman (Monsanto), Dean Layton (Envirologix), Penny Hunst (AgrEvo).

The AEIC Board will act as an oversight committee for expenses for these working groups. The Board will have to approve all major expenditures and any letters or other communications sent out by each working group.

The AEIC Business Meeting then was started. The Secretary's and Treasurer's reports were approved by the membership. AEIC membership notices will be sent out in April. Rich Lankow gave a follow-up on the EPA Workshop which was held in October, 1998. The 2-day session format was good for the workshop. There was about equal participation on both days by attendees. Responses from attendees indicated that the workshop was viewed as very professional and informative. It was decided that a follow-up letter to Don Marlowe (EPA) should be sent, thanking him for his support in circulating the announcements and other information within the agency. In other business concerning the EPA, it was announced that Pat Nugent would no longer participate in AEIC due to a job change within Dow. SDI will maintain the contact with Barry Lesnick (Office of Solid Waste) which Pat had done previously. Jim Brady will maintain contact with the EPA Office of Water on behalf of the AEIC. The recruiting working group will contact the EPA biotech group (Janet Anderson—Head) and Jeanette von Eamon (EPA-Las Vegas) will be include in on all mailings.

For the SFAI Meeting in Norwich, UK in September, 1999, Dave Grothaus will present an invited talk on GMO detection. Jim Rittenburg is working on assuring that AEIC will have a slot for a talk about the consortium's activities and also an evening roundtable discussion. Kim Magin is also attending to give a Monsanto poster. Either Kim or Dave would present the AEIC talk.

AEIC Dues/Membership: This topic had been briefly discussed at the close of the AEIC Board Meeting in February. The Board decided that the membership should decide if the dues needed to be adjusted to accommodate smaller companies and individuals who wanted to join. The other issue to be discussed was whether the number of affiliate members/company should continue to be limited. After a short discussion, a motion was made by Chris Rankin (DuPont) to change the AEIC by-laws to not limit the number of affiliate members/company. The motion was seconded by Jim Brady (Novartis) and was passed by a majority vote of the attending members. For the dues, a motion was made by Chuck Milhaliak (Dow Agro) that the dues be as follows: $100/yr for individuals; $250/yr for companies of < or equal to 50 employees; $500 for companies of 50+ employees. The motion was seconded by Kim Magin (Monsanto) and was passed by a majority vote of attending members.

AEIC Website (**http://www.Immunochem.org/)** Chuck will have the biotech initiative posted on the website. Slides from the speakers at the current meeting will also be posted on the website under the "Member" area which is password protected. Chuck asked that all the speakers forward their slides to him electronically. Chuck also asked that any relevant "links" to other websites be forwarded to him so that these can be added to the AEIC site.

The 1999 Fall AEIC Meeting will be held September 30-October 1 in Des Moines, Iowa and will be hosted by Pioneer Hi-Bred. The 2000 Spring AEIC Meeting will be hosted by Ricerca and will be held in Painesville, OH. The 2000 Fall AEIC Meeting will be hosted by Beacon Analytical and will be held in Portland, ME.

A thank you was extended to FMC and Audrey Chen for hosting the meeting in Princeton. Chris Ranking will have a plaque made for FMC acknowledging them as a host company.