Sponsor Focus: Crucial Points for Field & Compliance Data
Overview

- Regulations for biotech trials
- Types of biotech studies
- Test Substance & Test System: What these are for biotech trials
- Things to consider when conducting biotech trials
Regulations

- Pesticides are regulated under EPA
  - Includes plant incorporated protectants
  - Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)
  - Trials conducted under 40 CFR Part 160
- Plant-produced traits regulated under USDA
  - Animal & Plant Health Inspection Service (APHIS): 7 CFR 340
  - APHIS' Biotechnology Regulatory Services (BRS) regulates the introduction (importation, interstate movement, and release into the environment) of genetically engineered organisms that may pose a risk to plant health
- Plant-produced trait destined for food or feed regulated under FDA
  - Voluntary consultation
  - All of the food/feed products commercialized to date have gone through the consultation process
  - FDA opinion after all questions answered posted on their website
Types of Biotech Trials

- Protein Expression
  - Developmental
  - Comparative
- Compositional Analysis
- Agronomic Assessment
Types of Biotech Trials – Developmental Protein Expression

- Usually two or three entries
- Multiple locations
- Trait-specific treatment may be required
- Tissue samples are collected from multiple plants at multiple developmental time points over the life of the crop
- Tissue samples are powdered, extracted, and the amount of transgenic protein is determined
- The data is used to understand when the protein is expressed, where it is expressed, and at what levels
- Information is used in risk assessments and for non-target organism studies (how high should the dose be?)
Types of Biotech Trials – Comparative Protein Expression

- Multiple entries
  - Combined (A x B x C)
  - Single (Event A, Event B, Event C)
- Replicate plots, randomized complete block design
- Tissue samples are collected from multiple plants at multiple developmental time points
- Tissue samples are powdered, extracted, and the amount of transgenic protein is determined
- The data is used to determine if there are statistical differences between the combined and single events
Types of Biotech Trials – Compositional Analysis

- Multiple entries
- Replicate plots, randomized complete block design
- Trait-specific treatment may be required
- Grain and forage composite samples collected
- Samples analyzed for nutrients and anti-nutrients
- Data is statistically analyzed to determine if there are differences between the transgenic entry and the control entry
- Data is compared to data in ILSI database; is data within range?
- Information is used to ensure nutrients in the transgenic crop are equivalent to that in the conventional crop and that the overall quality of the crop is not affected
Types of Biotech Trials – Agronomic Assessment

● Multiple observations taken throughout the season
  - Phenotypic and agronomic traits, such as:
    • Germination/emergence
    • Days to flowering
    • Plant height
    • Lodging
    • Days to maturity
    • Yield
  - Ecological stress observations, e.g. insect damage, disease incidence, and abiotic stressors
  - Final stand count
● Data is used to determine if the transgenic entry is equivalent to the control entry
Test Substance: EPA Definition

- 40 CFR Part 160.3 defines a test substance as:
  - A substance or mixture administered or added to a test system in a study which:
    - (1) Is the subject of an application for a research or marketing permit supported by the study, or is the contemplated subject of such an application; or
    - (2) Is an ingredient, impurity, degradation product, metabolite, radioactive isotope of, or some other substance related to, a substance described in (1), which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described in (1)
Test Substance: What is it?

- For conventional trials (i.e. crop protection trials), the test substance is the chemical used to spray the test system or the chemical applied as a seed treatment, which will ultimately be tested for in laboratory studies.

- For biotech trials, the test substance is the transgenic seed to be planted.

- For the master schedule, the test substance should be shown as the transgenic seed designation, i.e. the Event name.
  - For example: Event A or A x B x C.
Test System: EPA Definition

- 40 CFR Part 160.3 defines a test system as:
  - Any animal, plant, microorganism, chemical or physical matrix, including but not limited to soil or water, or subparts thereof, to which the test, control, or reference substance is administered or added for study.
  - It also includes appropriate groups or components of the system not treated with the test, control, or reference substance.
Test System: What is it?

● For both conventional and biotech trials, the test system is the crop.
Biotech Trials – Things to Consider

- Receipt of Test Substances
- Trial Layout
- Trait-Specific Treatments
- Pollinations
- Sampling
- Sample Shipment
- Post-harvest Monitoring
Biotech Trials – Test Substance Receipt

- Seed is contained in seed packets or other appropriate containers
  - Packets should be labeled per GLPS
    • Identity, storage conditions, expiration date
    • Additional information may also be included
  - Follow retention rule under GLPS
- Things to consider:
  - Chain of custody records
  - Verification that shipment was received at destination intact
  - Process to follow if there is a breach in the container
  - Are the sites where the seed was received and where it will be planted correctly listed on the permit/notification?
Biotech Trials – Test Substance Storage

- Storage location and temperatures documented
- Minimize chance of contamination; separate from non-regulated seed
- Limit access to authorized individuals
Biotech Trials – Trial Layout

- May have multiple entries in a trial depending on type of study
- USDA permit/notification required
  - Are the events to be planted included under permit/notification?
  - Is the total acreage planted under the limit provided in the permit/notification?
- Number of replicated plots per trial and randomization provided
- Number and length of rows provided
- Additional border rows may be required for trait-specific treatment
Biotech Trials – Trial Layout (Cont’d)

- Isolation requirements
  - Types of reproductive isolation, for example:
    - Spatial
    - Early Crop Destruct
    - Detasseling
    - Temporal
    - Border Row Isolation
  - USDA guidance document indicates a 10-foot fallow zone should be added around the trial site
Biotech Trials – Trial Layout (Cont’d)

- Map should include:
  - GPS coordinates of trial site
  - Surrounding crops within isolation distance
  - Distance from trial site to any permanent marker or landmark
- The corners of the trial site should be marked
- Proper cleaning of equipment after field activities
  - Clean on the trial site
  - Acceptable methods:
    - Hand-cleaning
    - Compressed or accelerated air
    - Vacuuming remaining seed
    - High-pressure water
Biotech Trials – Trait-specific Treatments

- Treatments required? On which plots?
- What records are required?
  - Storage location and storage temperatures of chemical to use
  - Identification of adjuvant used, if required
  - Calibration of equipment prior to spraying
  - Rate provided in protocol
    • Documentation indicating correct rate applied
    • Calculations should be provided
  - Environmental conditions at the time of treatment to verify no drift
Biotech Trials – Pollinations

- Hand-pollinations may be required
- Ears have to be bagged prior to tasseling
- Time consuming
- If not done properly, can affect kernel sample
- Ensure measures are taken to prevent cross-contamination between plots and non-regulated fields
Biotech Trials – Sampling

● Ensure understanding of protocol prior to sampling
● Samples collected from plants in center two rows, not including the end two plants
● Protocol will specify
  - Developmental time points
    • e.g., V6, R1, R6
  - Tissues to be collected at each time point
    • e.g., leaf, root, whole plant
● Store samples within a certain amount of time after collection
● Maintain storage conditions to prevent deterioration of protein
● Equipment cleaned after each plot
Biotech Trials – Sample Shipment

- Proper containment/packaging required for regulated plant material
  - seed, root, forage, pollen (i.e. viable material)
- Transfer of Custody form is required
- Inventory list to be included
- Outside of shipping container should indicate USDA permit/notification number
- Shipment on dry ice or by freezer truck
- Prefer for shipments to be made by Wednesday to prevent delays (overnight shipping)
- Detailed shipping instructions provided in protocol
Biotech Trials – Post-harvest Monitoring

● After last samples taken and trial is complete, crop destruct
  - Prevent regulated event from entering food and feed supply
● Monitoring of trial site to ensure volunteers detected and removed
  - Volunteers removed prior to flowering/pollination
● Trial site readily accessible
● Trial site may be planted with crop that allows easy identification of volunteers
  - Corn trial site planted to soybean the following season
Important Things to Remember

- Seed is the test substance
- Make sure applicable permits/notifications are in place before planting or shipping samples
- Clean equipment on the trial site after planting
- Trait-specific treatments should be done in accordance with GLPS
- Pollinations are important
- Make sure samples are collected in accordance with the protocol
  - Roots should be clean!
- Ensure storage conditions are maintained throughout the process to prevent freeze-thaw
- Ensure proper packaging used when shipping
- Volunteers need to be removed before flowering/pollinating
Questions or Comments?