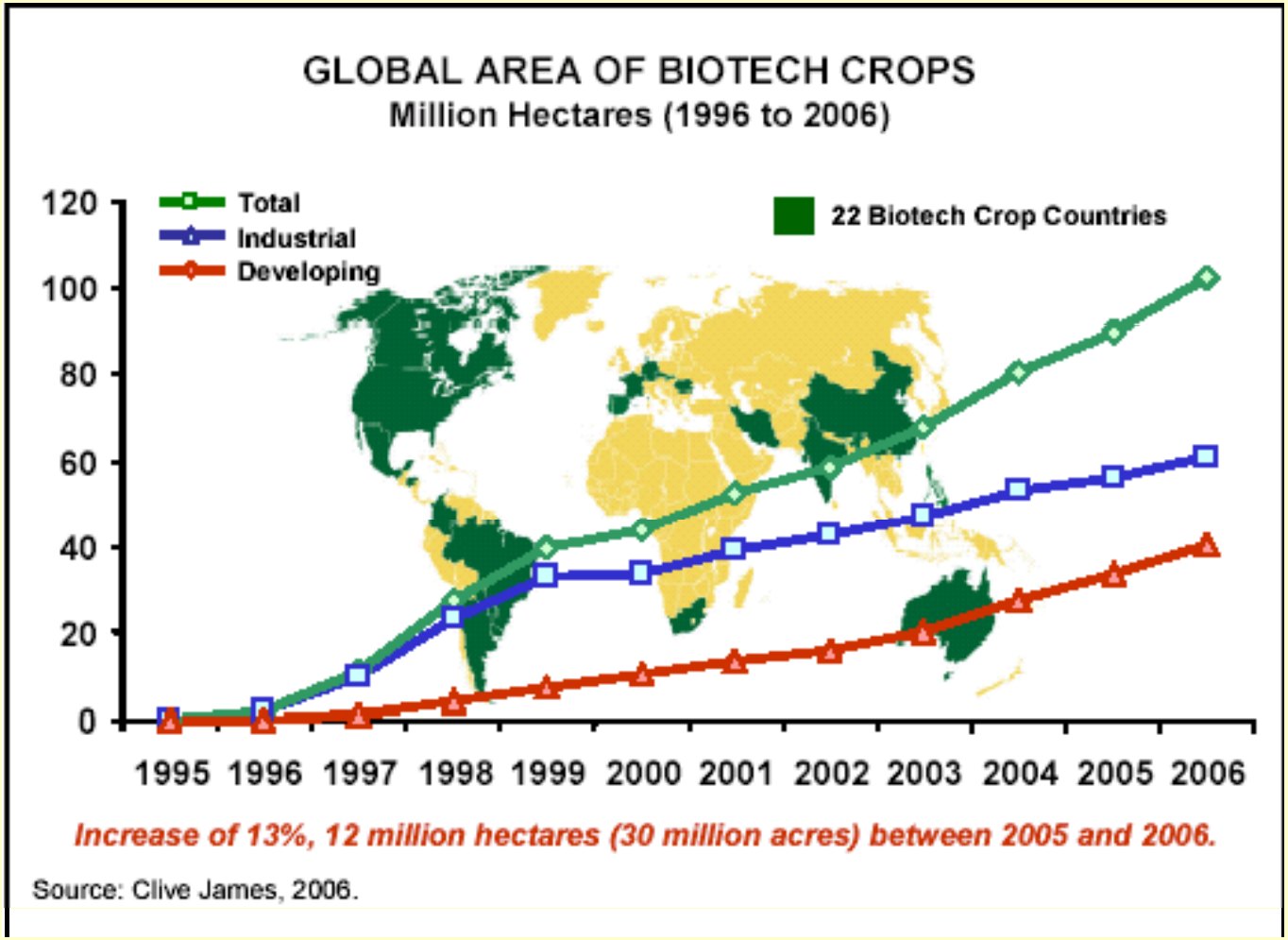
Tips for Biotech Trial Conduct

James A. Mickelson, Ph.D.
Research Scientist
Pioneer Hi-Bred International, Inc.
Johnston, Iowa

PIONEER
A DuPont Company

DUPONT
The miracles of science

World-Wide GMO Acreage



PIONEER
A DuPont Company

DUPONT
The miracles of science

Introduction

- This increase in GMO acreage is the result of the demonstration of the benefits of GMO crops and increasing acceptability of GMO crops worldwide.
- Because of this, GMO discovery efforts and commercial development of new GMO traits continues to increase.
- GMO trait approval process is very complex. Regulations are unique in each country and often change. Data requirements are large.
- Increasing opportunities for GLP Contract Research Organizations to conduct GMO field studies

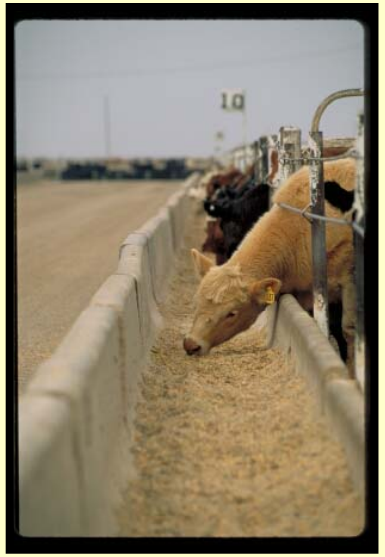
Introduction

- Contract Research Organizations (Management, PI's, and QA) must take a different approach and have different expectations when conducting GMO studies compared to pesticide residue studies.
- Similarities between Residue studies and GMO studies:
  - Field studies conducted according to GLP or GLP-like standards
- Differences between Residue studies and GMO studies:
  - Study objectives
  - Study design
  - Test substances
  - Tissue samples
  - Methods of sample and data collection
  - Regulatory compliance and isolation/containment

Introduction

Common GMO Study Types:

- Nutrient Composition
- Protein Expression
- Agronomic Characteristics
  - CEA studies (Composition, Expression, Agronomics)
- Test, Control, Reference Production
- Non-Target Organisms
- Other



PIONEER
A DuPont Company

DUPONT
The miracles of science

Considerations When Conducting GMO Studies

- Test and control substances
- Study Design
- Hand Pollinations
- Herbicide applications
- Protein expression tissue sampling
- Nutrient composition tissue sampling
- Agronomic characteristics data
- Data collection and documentation
- USDA and CFIA compliance requirements

PIONEER
A DuPont Company

DUPONT
The miracles of science

Considerations When Conducting GMO Studies

Test and Control Substances in GMO Studies:

- Test Substance = transgenic seed
- Control Substance = non-transgenic seed
- Herbicides that may be applied as treatments in the study are not Test Substances
- Documentation and handling of seed receipt is important

Considerations When Conducting GMO Studies

Seed used in GMO studies:

- Often available in very limited quantities
- May arrive late (i.e. just back from a winter nursery production) and result in late planting – implications for pest management, sampling and harvest timing
- May not be of optimal relative maturity for all trial sites
- May not be advanced or commercial hybrid like material
- Study may require use of an inbred
- May be segregating for the trait
- USDA or CFIA compliance requirements – additional complexity for shipping, receiving, handling

Considerations When Conducting GMO Studies

Study Design

- Small plots, replicated
- Cone planter is ideal for planting - easy to switch seed, complete clean out between plots
- Border rows
  - Internal border rows – commercial seed, buffer between plots
  - External border rows – physical barrier for pollen flow

Considerations When Conducting GMO Studies

Conducting Hand Pollinations in GMO studies:

- Goal is to obtain successful hand pollinations while preventing contamination. Hand pollinated plants are used for composition and expression grain samples.
- Requires training if not experienced at pollinating
- Time consuming – requires additional help
- Occurs at peak workload time – often sampling during pollination
- Cannot put off to the next day
- Weather conditions have a large effect on timing and success
- Lines may be poor pollinators or have poor nick
- Quality is important, experience is valuable

Considerations When Conducting GMO Studies

Herbicide Applications in GMO studies:

- Herbicide treatments may be applied in GMO studies.
- Herbicides used may not be characterized (no COA), and do not need typical GLP shipping documentation (e.g. COC) or storage records (since it is not test substance).
- Although herbicide is not the test substance, still require calibration and documentation to ensure accurate applications.
- Need to be careful of drift – herbicide resistant plots located adjacent to non-resistant plots or border rows.

Considerations When Conducting GMO Studies

Protein Expression Sampling in GMO studies:

- Goal is to characterize protein expression in the plant throughout the growing season. Data used in food/feed safety and environmental risk assessments.
- A wide range of tissues may be sampled at various growth stages.
- Example – Corn
  - Tissues: Leaf, Root, Stalk, Pollen, Whole Plant/Forage, Grain
  - Stages: V6, V9, R1, R4, R6

PIONEER®
A DuPont Company

Considerations When Conducting GMO Studies

Considerations for Protein Expression Sampling Methods:

- Protocol (or SOP) should provide very detailed and specific instructions for sample collection, storage, and shipping.
- Collection of protein samples may take a lot of time. Dependent on number of samples, type of samples, method of collection, documentation required, etc.
- Proteins can degrade very quickly. Factors include: inherent stability of target protein, temperature, time.

Considerations When Conducting GMO Studies

Considerations for Protein Expression Sampling Methods:

- In most cases, it is critical to freeze tissue samples very soon after sampling. Usually sample placed on dry ice in the field immediately after collection of each sample.
- Protocol may require samples to be frozen within specified number of minutes after collection. Documentation of this may be requested.
- After samples are collected and have been frozen, it is also critical to maintain samples frozen. Freeze – thaw – freeze conditions can rapidly degrade proteins. Thawed samples have no value.
- Need to be very careful to maintain samples frozen when moving, sorting, or preparing samples for shipment. Some samples (e.g. leaf) can thaw within a couple minutes when exposed to ambient temperatures.

Considerations When Conducting GMO Studies

Nutrient Composition Sampling in GMO studies:

- Goal is to demonstrate substantial equivalence in the nutritional composition of the GMO crop compared to non-GMO isoline crop.
- Typical tissues collected for nutrient composition analysis are Grain and Forage.
- Compositional analytes may include various proximates, fiber, amino acids, fatty acids, minerals, vitamins, saccharides, secondary metabolites, and anti-nutrients.
- Protocol should provide specific instructions for sample collection, storage, and shipping.
- Samples are frozen after collection, but are not as sensitive as protein samples. Compositional analytes typically do not degrade as easily as proteins.

Considerations When Conducting GMO Studies

Agronomic Characteristics for GMO studies:

- Goal is to demonstrate substantial equivalence in agronomic characteristics of the GMO crop compared to non-GMO isoline crop.
- Examples:
  - Plant population
  - Time to silking or pollen shed
  - Pollen viability
  - Plant height
  - Lodging
  - Disease incidence
  - Insect damage

PIONEER
A DuPont Company

DUPONT
The miracles of science™

Considerations When Conducting GMO Studies

Data collection and documentation for GMO studies:

- Sponsors may use paper or electronic notebooks
- Data collection and documentation may be extensive
- Study complexity and uniqueness - notebook should be tailored to each study
- Notebook may contain errors, require additions, etc. Contact Study Director if Notebook does not match the protocol.

Considerations When Conducting GMO Studies

USDA and CFIA compliance:

- Permits required to ship seed and various tissues. Sponsor may also require additional (internal) forms for shipping.
- Permit required to conduct field study (release permit).
- Isolation requirements can be one of the biggest challenges in conducting GMO studies (e.g. corn - 660 ft to any other corn). Can be difficult to find isolation. Requires planning and checking with neighbors.
- Post harvest monitoring requirements – typically extend into the following year
- Compliance documentation required
- USDA and CFIA inspections/audits for compliance
- Compliance aspect of conducting GMO studies takes time and resources

Considerations When Conducting GMO Studies

- Test and control substances
- Study Design
- Hand Pollinations
- Herbicide applications
- Protein expression tissue sampling
- Nutrient composition tissue sampling
- Agronomic characteristics data
- Data collection and documentation
- USDA and CFIA compliance requirements

Tips for Conducting GMO Studies ?

PIONEER
A DuPont Company

DUPONT
The miracles of science™

Tips for Conducting GMO Studies

Advice for CRO Management, Principal Investigators, and QA:

- Study changes may happen.
  - Late requests for additional entries
  - Late requests requests for additional data collection
  - Numerous amendments
  - Cancellations
- Reasons for changes are numerous
  - Changing regulatory requirements
  - Changing business strategy/commercialization plans
  - Seed considerations
- Sponsors dislike last minute changes just as much as CRO's.
- Be ready and willing to handle changes.

PIONEER
A DuPont Company

DUPONT
The miracles of science™

Tips for Conducting GMO Studies

Advice for CRO Management, Principal Investigators:

- Plan for high workload
- Be ready to handle study changes
- Read protocols carefully ahead of time
- Conduct a walk through of study activities ahead of time
- Don’t take on too much work. Workload should depend on experience, number of staff, and resources available
- Communicate with the Study Director – don’t hesitate to ask questions

Tips for Conducting GMO Studies

Advice for Principal Investigators - Sampling GMO studies:

- Read and understand protocol prior to the day of sampling. Allows time to ask SD questions if needed and plan for the sampling.
- If sampling methods are new to you, a practice sampling may be helpful. Helps determine time required, number of people, equipment and supplies needed, etc.
- Check for correct supplies, especially labels prior to day of sampling. Allows for time to have correct supplies/labels re-sent if needed.

Tips for Conducting GMO Studies

Advice for field site QA for auditing GMO studies:

- Learn about GMO studies. Understand what the critical aspects are.
- Tailor audits to the study type. Develop specific forms and practices for auditing GMO studies.
- Critical phases for auditing - planting, sampling, pollination, are often the most important.

Tips for Conducting GMO Studies

What can the Study Director/Sponsor do?

- Provide a well written protocol
- Provide a good field notebook for documentation
- Ensure seed and supplies are provided in a timely manner
- Communicate well and be available to answer questions

PIONEER
A DuPont Company

DUPONT
The miracles of science™

Final Thoughts on Conducting GMO Studies

- A large investment is made in each study
- A lot of responsibility is placed with CRO
- Good planning and excellent communication by Sponsor and CRO are critical to success!

PIONEER
A DuPont Company

DUPONT
The miracles of science™

Questions and Comments?

PIONEER
A DuPont Company

DUPONT
The miracles of science