

Application of U.S. EPA GLP Terminology for Selected Studies on Genetically-Engineered Crops

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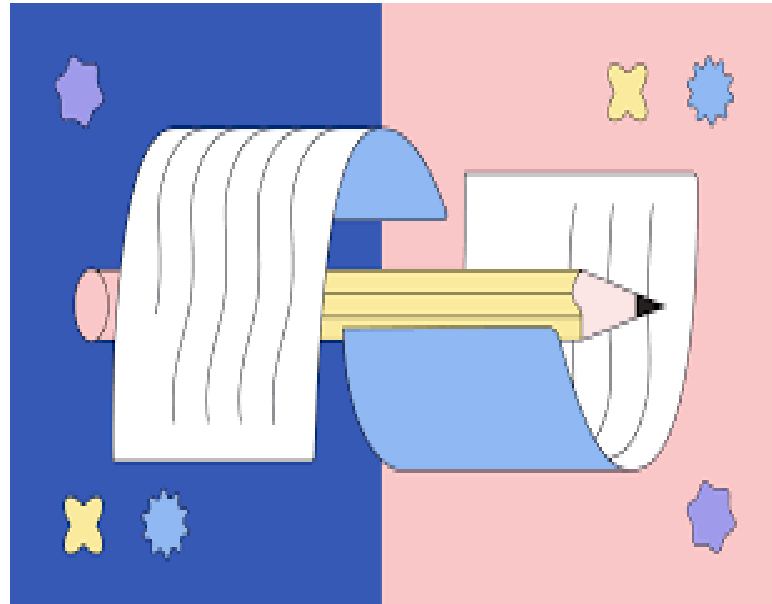
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NAICC Annual Meeting

Industry White Paper Discussions

Agenda

- ▶ Background
- ▶ Objective
- ▶ Terminologies related to stacks
- ▶ Summary
- ▶ Conclusions
- ▶ Acknowledgements



Background

- ▶ Genetically-engineered (GE) crops on the market today, or currently under development, are intended to improve a crop's pest management characteristics, agronomic traits, or improve or change a crop's quality traits
- ▶ An exhaustive series of Product Safety studies are conducted to provide a safety assessment based on
 - ▶ Molecular Characterization
 - ▶ Protein Characterization
 - ▶ Food, Feed, and Environmental Safety
 - ▶ Agronomic and Compositional Equivalence



Background

- ▶ GLPs originally developed for studies conducted in support of chemical pesticide registrations
 - ▶ Definitions included in the EPA GLPs have required interpretation to be applicable to GE crops
- ▶ Studies that are conducted within the United States to support the safety of GE crops and submitted to the U.S. EPA are conducted under U.S. EPA FIFRA Good Laboratory Practices (40 CFR Part 160)
- ▶ First version of this white paper described terminology applied to single Events and was published in 2018
- ▶ Version 2 incorporates breeding stacks

Objective

- ▶ As more companies begin to develop and register GE crops, independently and in collaboration with each other, the need for a common industry approach for interpreting EPA GLP definitions has become more important
 - ▶ E.g. test, control, and reference substances
- ▶ Additionally, the paper describes test substance characterization, when the test substance is defined as the Event in the Breeding Stack



Event A x Event B x Event C

VS.



Event A



Event B



Event C

Test Substance

- ▶ Upon creating a GE crop, the test substance and test system are inherently connected (DNA inserted into the genome of the crop)
- ▶ For Agronomy, Composition and Protein Expression studies, a test system is defined since the effect of the test substance on the test system is being evaluated (i.e., phenotypic characterization)
- ▶ In certain studies, the **Event or Breeding Stack** is the test substance, and the test system is the **crop**
 - ▶ “Molecular Stack” is defined as multiple traits inserted through an initial transformation event; these are registered in the same manner as a single-event product

Control Substance

- ▶ Administered to the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance
- ▶ Determine if there is a matrix effect during analyses or as a basis of comparison to show the GE Crop is substantially equivalent to the conventional counterpart



Reference Substance

- ▶ A reference substance may be a reference variety of differing genetic background (non-GE) than the test and control substances of the same crop species
 - ▶ The reference variety is often used for a basis of comparison to show the GE Crop is comparable to the conventional (non-GE) crop which has a history of safe use
- ▶ An analytical standard may also be a reference substance (positive control)



Summary

- ▶ Studies presented in this document and in the following tables may be submitted to the EPA, USDA, and/or to governmental agencies worldwide
- ▶ A summary of the test, control, and reference substance descriptions for various studies required for registering a Breeding Stack are shown in the following tables.

Molecular Characterization Studies for a Breeding Stack[^]

Study Type	Sequence(s) of the Insert and Flanking Regions*
Purpose	To confirm the integrity of each transgenic insert and the flanking region were preserved during the process of creating the Breeding Stack.
Test System	Not Applicable#
Test Substance	The Breeding Stack
Control Substance	Conventional counterpart ⁺
Reference Substance	Molecular Weight Markers ⁺

Study Type	Integrity of Transgenic Insert**
Purpose	To confirm the integrity of each transgenic insert were preserved during the process of creating the Breeding Stack.
Test System	Not Applicable#
Test Substance	The Breeding Stack
Control Substance	Conventional counterpart ⁺ ; Single Events ⁺
Reference Substance	Molecular Weight Markers ⁺

[^] May be separate studies and possibly conducted by different Sponsors.

* Information is for typical study design; however, protocols may show different information, depending on country-specific submission requirements.

A test system is not applicable because the DNA is being analyzed independently of the crop; however, study protocols may state the test substance or the method is the test system.

+ Control/Reference Substances may not be applicable, depending on methods or study design used.

** Study type required for specific countries. When required for GLP submission purposes, studies will be conducted under GLP regulations.

Studies Conducted to Assess the Impact of the Breeding Stack in the GE Test System[^]

Study Type	Agronomic Assessment
Purpose	Agronomic equivalency between plants containing the Breeding Stack and the conventional counterpart
Test System	Crop
Test Substance	The Breeding Stack*
Control Substance	Conventional counterpart
Reference Substance	Reference Varieties

Study Type	Compositional Analysis
Purpose	Compositional equivalency between plants containing the Breeding Stack and the conventional counterpart
Test System	Crop [#]
Test Substance	The Breeding Stack*
Control Substance	Conventional counterpart
Reference Substance	Reference Varieties (field component) & analytical standards (analytical component)

Study Type	Expression Analysis
Purpose	Determine expression of the downstream product produced by the Breeding Stack
Test System	Crop [#]
Test Substance	The Breeding Stack*
Control Substance	Single Events & Conventional counterpart
Reference Substance	Analytical standards

[^] May be separate studies or combined.

* Test substance characterization = sequence(s) of the insert and flanking regions of the single events contained in the Breeding Stack (see Table 3).

Tissues analyzed may have been generated under a separate study.

Conclusions

- ▶ Test substance is defined as the Event or Breeding Stack
- ▶ Control substance is a conventional counterpart or a single Event
- ▶ Reference substance establishes a basis for comparison with the test substance
 - ▶ May include commercial (non-GE) comparator hybrids or varieties
 - ▶ Analytical standards, may be used for studies requiring protein or molecular analyses
- ▶ Using these proposed definitions would harmonize the interpretation of the EPA GLP definitions for the test, control, and reference substances relative to certain studies conducted for GE crops

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Questions

