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

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Authored by:

The GLP/GE Crop Working Group,
A Work Group of the National Alliance of Independent Crop Consultants

BASF: M. Bethea-Ngom and D. Huntsinger
Bayer CropScience: K. Hill and S. Mackie
Dow AgroSciences: T. Froggatt^
DuPont Pioneer: A. Leslie and J. Schmidt
Monsanto: R. Barlow and K. Karunanandaa
Syngenta: J. Jarrell and D. Negrotto

^ Formerly with Dow AgroSciences

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I. SUMMARY

This document provides a harmonized recommendation for the interpretation of the EPA GLP definitions for the test, control, and reference substances relative to studies conducted on genetically-engineered crops. The selected studies presented in this document may be submitted to the EPA, USDA, and/or to governmental agencies worldwide. If these studies are conducted in the United States, they are conducted in accordance with the EPA GLPs. The selected studies presented and the discussion regarding the EPA GLP definitions are focused on when the test substance is defined as "The Event" (*i.e.* the product being registered).

II. INTRODUCTION

Genetically-engineered crops on the market today or currently under development are typically intended to improve a crop's pest management characteristics (*e.g.*, insect or disease resistance, herbicide tolerance), agronomic traits (*e.g.*, improved nutrient utilization, drought and other stress tolerances, increased yield), or improve or change a crop's quality traits (*e.g.*, enhanced nutritional value, introduction of novel oil production, improved harvest or processing qualities). The agricultural biotechnology sector is one of the most heavily regulated industries in the world. Genetically-engineered (GE) crops must undergo an exhaustive series of regulatory studies to evaluate environmental and human safety before being released onto the market. The 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; hereafter, EPA GLPs), and those conducted in OECD member countries typically follow OECD Principles of Good Laboratory Practice. Although there are minor differences in the sets of GLP standards, all provide a framework within which studies are planned, performed, monitored, recorded, reported and archived. This document will focus on studies on GE crops conducted in accordance with the EPA GLPs.

The current version of the EPA GLPs was published in the Federal Register in 1989, and is in place to ensure the quality and integrity of studies and data submitted to support applications for research or marketing permits for pesticide products. The test substance, as defined by the EPA GLPs, is a substance or mixture administered or added to a test system in a study, which is the subject of an application for a research or marketing permit supported by the study. The test substance may be an active ingredient, or it may be an impurity, degradation product, metabolite, or radioactive isotope of the active ingredient. The EPA GLP requirements for the test substance are intended to confirm the identity, and demonstrate the integrity of the substance before and during its use in a study. These requirements include appropriate characterization and demonstration of stability; methods to prevent contamination, damage, or degradation during handling and use; demonstration of solubility and adequate mixing with any carriers used; and retention of samples.

The process of genetically engineering a food/feed crop includes several steps, beginning with the plant cell transformation where a DNA sequence is inserted into the genome of the

recipient crop to create an Event. Event refers to the unique DNA recombination that took place in one plant cell, which was then used to generate an entire transgenic plant. The transgenic Event is identified by an abbreviation (e.g. Bt11, MON863). Events can be introduced to other cultivars by breeding. This is why certain Events (*e.g.* MON810) are available in many different cultivars.

An Event is identified by the DNA sequence of the inserted novel gene or genes, along with its flanking sequence, in the plant genome of the GE crop. Subsequently, the crop produces a downstream product based on the inserted DNA. Generally, many Events are produced and evaluated over time, culminating with a single Event that becomes the commercial product. This multi-stage process often takes more than ten years, and results in a "test substance" that is intrinsically a component of a plant, plant tissues, or plant cells.

The first GE crops were introduced to the market in 1994, well after the EPA GLPs were adopted. Originally developed for studies conducted in support of chemical pesticide registrations, the definitions included in the EPA GLPs have required interpretation to be applicable to GE crops. For example, the experimental start date is defined as the first date the test substance is applied to the test system. However, it is often not possible, practical, or informative to conduct studies on the inserted DNA when it is first introduced into a single plant cell's DNA. A consistent interpretation of the test substance definition is needed for practical application in studies conducted in accordance with the EPA GLPs.

III. OBJECTIVE

The objective of this document is to provide a harmonized recommendation for the interpretation of the EPA GLP definitions for the test, control, and reference substances relative to studies conducted under EPA GLPs for GE crops. Additionally, test substance characterization, when the test substance is defined as the Event, is described.

IV. APPLICATION OF EPA GLPS FOR GE CROPS

Many studies are conducted to characterize the outcome of the transformation on a molecular level. These include determination of the Event identity, the number of copies of the DNA inserted into the plant genome, and whether plasmid backbone DNA is inserted into the

plant genome. For these characterization-type studies, a test system is not applicable because the DNA is being analyzed independently of the crop. A summary of the test/control/reference substance descriptions for these molecular characterization studies is shown in Table 1.

Other studies are conducted on the GE crop as the test system to determine if there is any impact from the downstream product of the Event, including how much is being expressed, and whether the GE test system is equivalent to its non-GE, near-isogenic counterparts. A summary of the test/control/reference substance descriptions for these types of studies is shown in Table 2.

A conventional counterpart (*i.e.,* an untransformed line that does not contain the Event) may be used as a control substance. Non-GE commercial varieties may be used as reference substances in agronomic and composition studies, and may also be used in other studies as additional controls. For studies requiring laboratory analyses, additional reference substances, such as analytical standards, may be used as required by the specific analytical methods.

The following recommended definitions apply to the types of studies referenced in Tables 1 and 2. Only those sections of the EPA GLP regulations that were considered to require interpretation were addressed by the Working Group.

- 40 CFR § 160.3: *Test substance* means a substance or mixture administered or added to a test system in a study, which substance or mixture:

  (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, or radioactive isotope of a substance described by paragraph (1) of this definition, or some other substance related to a substance described by that paragraph, which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described by that paragraph.

  40 CFR § 160.3: *Test system* means 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. "Test system" also includes appropriate groups or components of the system not treated with the test, control, or reference substance.

  <u>Working Group Recommendation</u>: Upon creating a GE crop, the test substance and test system are inherently connected (DNA inserted into the genome of the crop). Therefore, in

certain studies the Event is the test substance and the test system is the crop. When discussing the Event, it is associated with the crop transformed with the DNA (e.g. Event ABC maize, Event CDE soybean, etc.). For the molecular characterization studies shown in Table 1, 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. For the studies shown in Table 2, a test system is defined, since the effect of the test substance on the test system is being evaluated (i.e., phenotypic characterization).

- 40 CFR § 160.3: *Control substance* means any chemical substance or mixture, or any other material other than a test substance, feed, or water, that is administered to the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance for known chemical or biological measurements.

  Working Group Recommendation: As shown in Tables 1 and 2, the control substance may be a conventional counterpart depending on the type of study being conducted, i.e. the test system which does not contain the Event. Although the control substance is not something applied to the test system, as stated in the GLP definition, the conventional counterpart is often used to evaluate whether or not 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.

- 40 CFR § 160.3: *Experimental start date* means the first date the test substance is applied to the test system.

  Working Group Recommendation: If there is an in vivo *phase of the study, the experimental start date is the date the crop is planted; otherwise, it is the first date raw data is collected.*

- 40 CFR § 160.3: *Reference substance* means any chemical substance or mixture, or analytical standard, or material other than a test substance, feed, or water, that is administered to or used in analyzing the test system in the course of a study for the purposes of establishing a basis for comparison with the test substance for known chemical or biological measurements.

  Working Group Recommendation: As shown in Tables 1 and 2, a reference substance may be a reference variety of differing genetic background than the test and control substances of the same crop species. Although the reference substance is not something administered to the test system or used in analyzing the test system in the course of a study, as stated in the GLP definition, the reference variety is often used for a basis of comparison to show the GE Crop is comparable to the conventional crop which has a history of safe use.

  An analytical standard may also be a reference substance.

- 40 CFR § 160.3: *Specimens* means any material derived from a test system for examination or analysis.

  Working Group Recommendation: Samples collected from the crop for examination or analyses are specimens. The terms "sample" or "specimen" may be used interchangeably.

- 40 CFR § 160.105(a): The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each batch and shall be documented before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented by the sponsor or the testing facility, and the location of such documentation shall be specified.

  Working Group Recommendation: When the test substance is defined as the Event, the sequence of the insert and flanking regions is determined for characterization. Although the regulation states this information must be determined before its use in a study, this may not be possible for GE crops. However, materials used in the studies shown in Tables 1 and 2 are tested for the presence of the Event; this testing may be performed before the materials are used in a study, or during the course of a study. Test substance characterization will be completed before submission to the regulatory agency.

  When the control and/or reference substances are defined as the test system which does not contain the Event (i.e., there is no substance added to the test system), there is no characterization required.

- 40 CFR § 160.105(b): When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the testing facility or the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

  Working Group Recommendation: Studies conducted on multiple generations of the crop address stability of the inserted DNA and subsequent downstream product(s). These studies are conducted independently of safety studies and are conducted to address viability of the product. When the test substance is the Event, solubility is not applicable due to the nature of the test substance.

- 40 CFR § 160.105(c): Each storage container for a test, control, or reference substance shall be labeled by name, chemical abstracts service number (CAS) or code number, batch number, expiration date, if any, and, where appropriate, storage conditions necessary to maintain the identity, strength, purity, and composition of the test, control, or reference substance. Storage containers shall be assigned to a particular test substance for the duration of the study.

  Working Group Recommendation: The test/control/reference substances are typically stored in seed packets prior to planting. Studies conducted in support of GE crops are

often conducted on USDA-APHIS regulated material; therefore, these studies also have to be conducted in accordance with USDA-APHIS regulations. USDA-APHIS regulations support burning or deep burial of excess seeds and seed packets in the field to prevent the possibility of accidental release of a regulated Event. The decision to dispose of seed packets prior to study completion is left to the Sponsor, and should take into account USDA-APHIS regulations.

- 40 CFR § 160.105(d): For studies of more than 4 weeks experimental duration, reserve samples from each batch of test, control, and reference substances shall be retained for the period of time provided by 40 CFR § 160.195.

  Working Group Recommendation: An aliquot of each seed lot or appropriate plant material used in the studies will be retained.

V. CONCLUSIONS

For the studies on GE crops described in this document, the test substance is defined as the Event. A conventional counterpart (*i.e.*, an untransformed line which has no inserted DNA) may be included as a control substance. Reference substances may include commercial comparator varieties. Additional reference substances, such as analytical standards, may be used for studies requiring protein or molecular analyses. 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. 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, and at the same time adhere to the EPA GLP regulatory intent of demonstrating and protecting a substance's identity and integrity before and during its use in a study.

VI. REFERENCES

US Environmental Protection Agency. 40 Federal Code of Regulations Part 160 (Issued 17 August 1989). Good Laboratory Practice Standards.

VII. ACKNOWLEDGEMENTS

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Table 1. Molecular Characterization Studies for an Event^

Study Type	**Sequence of the Insert and Flanking Regions**
Purpose	To determine the identity of the Event
Test System	Not Applicable#
Test Substance	The Event
Control Substance	Conventional counterpart
Reference Substance	Not Applicable

Study Type	**Absence or Presence of Backbone DNA**
Purpose	To determine if plasmid backbone DNA was inserted into the genome of the crop during transformation
Test System	Not Applicable#
Test Substance	The Event
Control Substance	Conventional counterpart*
Reference Substance	Molecular weight markers*

Study Type	**Copy Number**
Purpose	To determine how many copies of the T-DNA were inserted in the genome of the crop during transformation
Test System	Not Applicable#
Test Substance	The Event
Control Substance	Conventional counterpart, if applicable
Reference Substance	Molecular weight markers

Study Type	**Multi-generational Stability**
Purpose	To determine if the DNA inserted into the genome of the crop is stably transmitted across multiple generations
Test System	Not Applicable#
Test Substance	The Event
Control Substance	Conventional counterpart
Reference Substance	Molecular weight markers

Study Type	**Mendelian Inheritance**
Purpose	To determine if the DNA inserted into the genome of the crop is inherited in a Mendelian fashion
Test System	Not Applicable#
Test Substance	The Event
Control Substance	None
Reference Substance	Analytical Standards

^ May be separate studies or combined.
# 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.
* Plasmid DNA + Conventional Counterpart may also be used.

Table 2. Studies Conducted to Assess the Impact of the Event in the GE Test System^

Study Type	**Multi-Generational Expression**
Purpose	To determine expression of the downstream product produced by the Event across multiple generations
Test System	Crop
Test Substance	The Event*
Control Substance	Conventional counterpart
Reference Substance	Analytical standards

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

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

Study Type	**Expression Analysis**
Purpose	Determine levels of the downstream product produced by the Event
Test System	Crop#
Test Substance	The Event*
Control Substance	Conventional counterpart
Reference Substance	Analytical standards

^ May be separate studies or combined.
* Test substance characterization = sequence of the insert and flanking regions.
# Tissues analyzed may have been generated under a separate study.