BENSON HILL

BIOSYSTEMS

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Mr. Michael Firko
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road, Unit 98
Riverdale, MD 20737

Mr. Firko:

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Re: Confirmation that BHB Hi-Yield Maize is not a regulated article

Benson Hill Biosystems, Inc. (Benson Hill) is developing technology that will enable food crops to be more efficient and productive as food and feed. One of the products that Benson Hill is focused on is genetically modified maize (*Zea mays* L.) ("BHB Hi-Yield Maize"). BHB Hi-Yield Maize is designed to have higher photosynthetic efficiency and/or capacity than conventional maize varieties, thereby offering a higher yield potential with the same or lesser agricultural inputs.

Because maize is not a plant pest or an invasive species, the genetic elements used to generate BHB Hi-Yield Maize are all sourced from fully classified organisms, and the transformation process does not introduce any plant pest DNA components, there is no scientifically valid basis for concluding that BHB Hi-Yield Maize is, or will become, a plant pest within the meaning of the Plant Protection Act (PPA).[1] Benson Hill therefore asserts that under current regulations, BHB Hi-Yield Maize is not a regulated article within the meaning of 7 CFR §340.1 because it does not satisfy any of the regulatory criteria that would subject it to the oversight of the USDA's Animal Plant Health and Inspection Service (APHIS).

Before proceeding further with product development, Benson Hill requests that APHIS confirm that BHB Hi-Yield Maize, modified without any plant pest elements (as described more fully in Table 1 below), should not be considered a regulated article within the meaning of the

[1] Plant Protection Act; 7 U.S.C. §7701, et seq. (2000)

current regulations. If the agency does not concur with Benson Hill's interpretation of the current regulations, Benson Hill requests that the Agency provides us with its scientific rationale for concluding that BHB Hi-Yield Maize is or will become a plant pest.

I. Transformation Background

To further assist APHIS in understanding the origin of BHB Hi-Yield Maize, a summary of information on the recipient plant, as well as the genetic and technical elements used to modify the recipient plant to make BHB Hi-Yield Maize, is provided below.

A. BHB Hi-Yield Maize (*Zea mays* L.)

Transformation of maize, using purified DNA that is transferred by biolistic (gene gun) methods, results in stably integrated DNA. Purified DNA containing the genetic elements to be integrated into the maize genome is co-bombarded along with purified DNA encoding a specially designed homing endonuclease based on the I-CreI meganuclease produced by *Chlamydomonas reinhardtii*. The derived meganuclease that will be used for these experiments has been evolved in the laboratory to show an altered sequence specificity, resulting in a meganuclease that recognizes the intended sequence in the maize genome and creates a double-stranded break at the intended cut site. Transient expression of I-CreI meganuclease forms a double stranded break in maize genomic DNA [ ]. Using the genetic elements described in Table 1 as a DNA repair template, the genetic elements in Table 1 are used to guide stable integration of the desired sequences into the maize genome at the target loci by homologous recombination. The genetically enhanced materials express the BHB Hi-Yield trait, which is designed to increase photosynthetic efficiency and/or capacity resulting in a higher yielding maize crop. Table 1 below describes each genetic element and identifies its respective sources and functions:

Table 1. Genetic Elements in BHB Hi-Yield Construct for Biolistic Transformation of Maize.

GENETIC ELEMENT	SOURCE	FUNCTION
Plasmid Number 1		
Zm genomic DNA	*Zea mays*	[ ]
[ ]	[ ]	[ ]
Zm genomic DNA	*Zea mays*	[ ]
Multi-Cloning	Synthetic Sequence	Contains sequences to facilitate cloning

Site		
Modified pMDC99 vector backbone	Synthetic Sequence	Vector backbone used for cloning (Curtis and Grossniklaus, 2003)
aph3 Gene	*Escherichia coli* K-12	Provides kanamycin resistance for plasmid maintenance in *E. coli*
pVS1 replicon	*Pseudomonas fluorescens* plasmid pVS1	Region for plasmid replication in *Agrobacterium*
BR322 origin of replication	*E. coli*	Origin of replication for plasmid maintenance in bacterial cells
Plasmid Number 2		
[ ]	*Zea mays*	Drives transient expression of the meganuclease gene
Meganuclease gene	*Chlamydomonas reinhardtii*	Produces a double-stranded break at a pre-determined site in the maize genome to guide site-specific integration of the desired sequence
[ ]	*Zea mays*	Terminates transcription of the meganuclease gene
Multi-Cloning Site	Synthetic Sequence	Contains sequences to facilitate cloning
Modified pMDC99 vector backbone	Synthetic Sequence	Vector backbone used for cloning (Curtis and Grossniklaus, 2003)
aph3 Gene	*Escherichia coli* K-12	Provides kanamycin resistance for plasmid maintenance in *E. coli*
pVS1 replicon	*Pseudomonas fluorescens* plasmid pVS1	Region for plasmid replication in *Agrobacterium*
BR322 origin of replication	*E. coli*	Origin of replication for plasmid maintenance in bacterial cells

[ ] A description of the pMDC99 vector and of the modifications made to this vector backbone at Benson Hill Biosystems is below.

The pMDC99 plant transformation vector was derived from the pCambia series of vectors, as described by Curtis and Grossniklaus (Plant Physiol 133: 462-469). [ ]

The kanamycin resistance gene in the pMDC99 vector backbone that is used for maintenance of the plasmid in *E. coli* is found in many different bacterial species, including *E. coli* itself (Genbank entry CAA67773.1), as determined from a BLAST search using the amino acid sequence encoded by the kanamycin resistance gene in this plasmid.

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B. Recipient Maize (*Zea mays* L.)

Maize is not a federal noxious weed.[2] It is a non-native, domesticated variant of a Mesoamerican grass species and is listed as an agricultural seed (7 CFR §361). It is extensively grown in the United States for animal feed, fuel, food, and industrial uses.[3]

II. APHIS' Interpretation of Its 7 CFR §340 Regulation Dictates a Finding that BHB Hi-Yield Maize is Not a Regulated Article

A. APHIS Has Been Clear That Not All Transgenic Plants Are Subject to Regulatory Oversight

APHIS defines a "regulated article" as (Part 340.1):

> Any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in §340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator, determines is a plant pest or has reason to believe is a plant pest. Excluded are recipient microorganisms which are not plant pests and which have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions.[4]

Consistent with the PPA's definition of a plant pest, APHIS further defines a "plant pest" as:

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[2] http ://plants.usda.gov/ java/invasiveOne?startChar=Z

[3] http://www.ers.usda.gov/topics/crops/corn/background.aspx

[4] Well-characterized and contains only non-coding regulatory regions (e.g., operators, promoters, origins of replication, terminators, and ribosome binding regions). The genetic material added to a microorganism in which the following can be documented about such genetic material: (a) The exact nucleotide base sequence of the regulatory region and any inserted flanking nucleotides; (b) The regulatory region and any inserted flanking nucleotides do not code for protein or peptide; and (c) The regulatory region solely controls the activity of other sequences that code for protein or peptide molecules or act as recognition sites for the initiation of nucleic acid or protein synthesis. (7 CFR §340.1).

> *Plant pest*. Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants[5].

APHIS further claims that its regulations are consistent with the Coordinated Framework, because they apply "only [to] genetically engineered organisms or products which are plant pests or for which there is a reason to believe are plant pests, and not to ...an organism or product merely because of the process by which it was produced."[6] APHIS has further stated that its concern arises only "when an organism or product is altered or produced by genetic engineering and one or more of its constituents (donor, vector/vector agent or recipient) comes from a family or genus of organisms known to contain plant pests.... This is because... there is a risk that certain undesirable traits may be transferred to the new organism and may survive when the organism is released into the environment."[7]

APHIS reiterated this policy on several occasions, first when it introduced its notification and permit process for the confined release of transgenic organisms[8], and again during the proposed revision to its regulations[9]. It has been clear that not all transgenic plants are to be regulated, and those that are belong to the limited group of "plant pests" as defined in the regulations.

B. Hi-Yield Maize Does Not Fall Within the Regulatory Definition of a "Regulated Article."

Under APHIS regulations, a transgenic organism is considered a "regulated article" "if the donor organism, recipient organism, or vector agent(s) belongs to a genera or taxa designated in 7 CFR §340.2, *and* the organism meets the definition of a plant pest (emphasis added)." The language of the regulation requires that both criteria must be met to satisfy the definition of a regulated article.

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[5] 7 CFR §340.1

[6] 51 Fed. Reg. 23352 (proposed rule); 52 Fed. Reg. 22892 (final rule where similar language is used)

[7] 0ffice of Science and Technology Policy's Coordinated Framework for Regulation of Biotechnology, June 26, 1986 (51 Fed. Reg. 23302)

[8] 57 Fed. Reg. 53036 (Feb 1991)

[9] 73 Fed. Reg. 60008, 60010 (Oct 8, 2008)

For BHB Hi-Yield Maize, none of the donor organisms, the recipient organism, or the vectors Benson Hill will utilize to transform maize belong to any taxa identified in §340.2. Further, none of the genetic elements described in Table 1 are sourced from any plant pest. In addition, the recipient organism, maize, is not a plant pest. Therefore, BHB Hi-Yield Maize using the genetic elements identified in Table 1 does not satisfy either of the criteria set forth to qualify as a "regulated article."

Another definition of a "regulated article" includes transgenic organisms that are unclassified or whose classification is unknown. Other types of organisms that could raise concerns are "pathogens, predators or parasites of natural enemies of plant pests or weeds or of commercially available pollinators such as honeybees, bumble bees and alkali bees."[10] However, since the introduced trait enhances photosynthetic efficiency and/or capacity, it does not change the plants' basic biological characteristics and the trait's presence does not produce a plant that would directly feed on, infect, parasitize, or contaminate plants, or adversely affect other organisms that are beneficial to plants.

III. Finding that Hi-Yield Maize is Not a Regulated Article is Consistent With Previous APHIS Determinations.

APHIS has made a number of different determinations that transgenic plants are not "regulated articles." These include, for example:

A genetically engineered petunia that was transformed using genes derived from *Petunia hybrida* and *E. coli* K-12, transferred by biolistics.[11] APHIS determined that the transgenic petunia was not a regulated article because neither the recipient organism nor the donor organism(s) belonged to any of the genera of plant pests listed in Part 340.2. APHIS reconfirmed that transgenic petunia is not a regulated article.[12]

A genetically engineered geranium was modified with wild-type *Agrobacterium rhizogenes* and did not involve the use of recombinant DNA techniques. APHIS concluded that to fall within the definition of a regulated article, the organism must involve a plant pest element AND be modified by recombinant DNA techniques. Therefore, the transgenic geranium is not a regulated article.[13]

[10] 66 Fed. Reg. 51340 (Oct 9, 2001)
[11] BRS letter to New Zealand Crop and Food Limited (dated May 19, 2008)
[12] Email from Mr. Michael Gregoire to M. Boase (April 20, 2010)
[13] BRS letters from Catherine Joyce/John Payne to Dr. John Sanford (Feb 5, 1993/Nov 1994)

A genetically engineered Kentucky bluegrass was modified with genes derived from *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*, using biolistics, to be tolerant to glyphosate. APHIS determined that the transgenic Kentucky bluegrass was not a regulated article because neither the recipient organism nor the donor organism(s) belonged to any of the genera of plant pests listed in Part 340.2.[14]

A genetically engineered plant was modified with targeted gene deletions, caused by DNA repair with or without a DNA template following a double-stranded break by the I-CreI meganuclease. APHIS determined that genetically engineered plants containing targeted gene deletions are not, in most cases, regulated articles because the I-CreI meganuclease used is not from a plant pest and no plant pest sequences are inserted into the plant genome.[15] APHIS stated it would consider genetically engineered plants that use template DNA to repair a double-stranded break on a case-by-case basis.

IV. Summary of Conclusions

In summary, maize is not itself a plant pest, there are no plant pest elements involved in the production of BHB Hi-Yield Maize, and all the native genomes that are sources for the genetic elements that will be used have been fully classified. Therefore, there is no scientifically valid basis to determine that BHB Hi-Yield Maize is or will become a plant pest within the meaning of the PPA.

We look forward to receiving your response, and thank you in advance for your consideration and prompt confirmation of Benson Hill's position that BHB Hi-Yield Maize is not a "regulated article" for the reasons described herein.

Sincerely,

Matthew B. Crisp
President and Chief Executive Officer

[14] USDA letter from Secretary Vilsack to Dr. Richard Shanks (July 1, 2011)
[15] BRS letter from Mr. Michael Gregoire to Thenell & Associates (Dec 16, 2011)