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March 17, 2015

Dr. Michael J. Firko
Deputy Administrator, Biotechnology Regulatory Services
4700 River Road, Unit 98
Riverdale, MD 20737

RE: Confirmation of regulatory status

Dear Dr. Firko:

I am writing to seek confirmation from Biotechnology Regulatory Services that a new maize crop line developed by Agrivida, Inc., does not meet the definition of a regulated article for purposes of 7 CFR Part 340. Agrivida, Inc. is a biotechnology company focusing on the development of new crop varieties targeted for the animal nutrition and agricultural processing industries.

Agrivida has introduced into maize a meganuclease enzyme that specifically recognizes and creates a deletion in exon [redacted] of the endogenous maize [redacted] gene. Since the gene responsible for expressing the meganuclease was introduced into the maize genome at a position that was not genetically linked to the target [redacted] gene, it was then possible to segregate the introduced DNA from the variant [redacted] allele following crosses with wild-type maize plants. In this manner, all introduced DNA was eliminated from those progeny plants containing the variant [redacted] allele.

The process used to generate maize plants with the variant [redacted] allele involved the introduction of a meganuclease transgene via [redacted] mediated transformation, utilizing the [redacted] vector [redacted] and the [redacted] strain [redacted]. The meganuclease expression cassette and the plant selectable marker included:

Meganuclease expression cassette

- [redacted] promoter
- A [redacted] sequence derived from [redacted]
- A meganuclease from [redacted]
- [redacted] terminator from [redacted]

Selectable marker expression cassette

- [redacted] promoter
- The [redacted] gene encoding [redacted] from [redacted]
- [redacted] terminator from [redacted]

Following crosses to wild-type maize plants to segregate away the meganuclease [redacted] sequences, the absence of all introduced DNA sequences in the variant [redacted] segregating lines was confirmed by both PCR and Southern blot analyses. We submit that the resulting null-segregant (NS) lines, which contain no introduced DNA sequences, are not, and would present no risk of creating, a plant pest, as defined under the Plant Protection Act.

In wild-type maize plants, [redacted] phosphorylates starch, priming starch mobilization through the actions of several enzymes in a process that mediates the diurnal turnover of transient starch in maize. While heterozygous plants that contain one wild-type allele and one variant [redacted] allele behave as wild-type plants, with no significant phenotypic variation, homozygous maize plants expressing the variant [redacted] allele accumulate substantially higher levels of starch in their leaves and stalks than comparable wild-type plants. The increased starch phenotype observed in the variant [redacted] maize plants results from the presence of the homozygous mutant [redacted] allele, which causes gradual accumulation of starch in leaf and stalk tissue over the life span of these plants.

Because maize plants with the variant [redacted] allele, derived from our targeted gene deletion strategy, contain no foreign DNA sequences and are modified only by specific removal of base pairs in exon [redacted] of the endogenous [redacted] gene, we submit that these modified materials do not comprise APHIS-regulated articles, for purposes of 7 CFR Part 340.[1]

Please confirm that this understanding is correct.

Sincerely,

R. Michael Raab
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[1] We note that APHIS has reached similar conclusions with respect to other analogous products. *See, e.g.,* Letter from Michael C. Gregoire (BRS) to Gary W. Rudgers (Dow AgroScience), March 8, 2012 (APHIS agrees that maize plants that have been engineered to contain a gene deletion by using zinc finger nuclease technology are not regulated articles for purposes of 7 CFR Part 340). *See also* Letter from Michael C. Gregoire (BRS) to Sally Mackenzie (University of Nebraska), June 6, 2012 (APHIS confirms that null segregant lines derived from plants that were genetically engineered utilizing *Agrobacterium*-mediated transformation, but that contain no transgenic material or plant pest sequences, are not regulated articles for purposes of 7 CFR Part 340).