United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

4700 River Road
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Gary W. Rudgers, PhD
Global Regulatory Leader - New Ventures
Dow AgroScience LLC
9330 Zionsville Road
Indianapolis, IN 46268

Re: APHIS review as to whether *Zea mays* plants with the IPK1 gene deleted using zinc nuclease technology is regulated by APHIS.

Dear Dr. Rudgers:

Thank you for your presentation on March 18, 2010 describing the zinc finger nuclease deletion technology used to create *Zea mays* plants with a nonfunctional IPK1 gene. IPK1 gene encodes inositol-1,3,4,5,6-pentakisphosphate 2-kinase, an enzyme that catalyses the final step in phytate biosynthesis in maize seeds (Shukla et al., 2009). When the IPK1 gene is disrupted using the zinc finger nuclease deletion technology, the resulting phenotype is maize with reduced phytate production. These plants have been described in detail in Shukla et al., 2009.

APHIS regulates the environmental release of certain genetically engineered organisms which are, or are believed to be, plant pests. Regulations for genetically engineered organisms that have the potential to be plant pests, under the Plant Protection Act, are codified at 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." Under the provisions of these regulations, a genetically engineered (GE) organism is deemed a regulated article if it has been genetically engineered from a donor organism, recipient organism, or vector or vector agent listed in §340.2, and the listed organism meets the definition of "plant pest" or is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to believe it is a plant pest.

APHIS has evaluated the ZFN-12 maize plants as described in Shukla et al. 2009. The ZFN-12 plants (Figure 1, Shukla et al., 2009) all have similar deletions to the IPK1 gene, and these deletions were created using zinc finger nuclease technology. The ZFN-12 maize plants containing the induced deletion of sections of the IPK1 gene have reduced phytate production. Phytate is an anti-nutritional component of feed grain. Phytate reduction results in more biologically available phosphorus in feed so animal feed products have a higher nutritional quality. Subsequently, feed with more nutritionally available phosphorus utilized by animals results in decreased amounts of phosphorus that ordinarily leaves agricultural lands as environmental pollution.

As described by Dow during the March 18, 2010 presentation, no plant pest was used to create the ZFN-12 maize plants, which contain deletions at the IPK1 gene. There is no

reason to believe that *Zea mays* containing an IPK1 deletion is a plant pest or is likely to pose a plant pest risk. Therefore, the ZFN-12 maize plants with induced deletions due to the use of zinc finger nuclease technology are not considered regulated articles.

If you have any questions about our review, please call Dr. John Turner, Director of the Policy Coordination Program, at (301) 734-8365.

Sincerely,

Michael Gregoire
Deputy Administrator
Biotechnology Regulatory Services

References

Shukla, V.K., Y. Doyon, J. C. Miller, R. C. DeKelver, E. A. Moehle, S. E. Worden, J. C. Mitchell, N. L. Arnold, S. Gopalan, X. Meng, V. M. Choi, J. M. Rock, Y.-Y. Wu, G. E. Katibah, G. Zhifang, D. McCaskill, M. A. Simpson, B. Blakeslee, S. A. Greenwalt, H. J. Butler, S. J. Hinkley, L. Zhang, E. J. Rebar, P. D. Gregory & F. D. Urnov. 2009. Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. *Nature* **459**, 437-441 (21 May 2009). doi:10.1038/nature07992