DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE
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Subject: Glufosinate-Tolerant Sugar Beet, Transformation Event T120-7

Keywords: sugar beet (*Beta vulgaris*), herbicide-tolerant, glufosinate-tolerant, *Streptomyces viridochromogenes pat* gene, phosphinothricin-N-acetyltransferase, aminoglycoside 3'-phosphotransferase II (APH(3') II, also known as NPT II), kanamycin resistance gene (*kan*^{r}).

Background
AgrEvo USA Company initiated a consultation with FDA regarding this product in September of 1996. On June 19, 1998, AgrEvo provided summary information to support its safety and nutritional assessment of a new glufosinate-tolerant sugar beet line containing transformation event T120-7.

Intended Effect and Food/Feed Use
The intended effect of the genetic modification is to render sugar beet (*Beta vulgaris*) plants tolerant to the non-selective herbicide glufosinate-ammonium (glufosinate). Glufosinate's herbicidal activity is due to its ability to inhibit the enzyme glutamine synthetase. Glutamine synthetase is the only enzyme in plants that can detoxify ammonia released by photorespiration, nitrate reduction, and amino acid degradation. AgrEvo used the *pat* gene which encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) to confer herbicide tolerance. PAT converts glufosinate to a nonherbicidal derivative.

Sugar beets are cultivated for the fleshy, bulbous root, which normally contains 10-15% sucrose. The processing of sugar beets yields products for use in both human and animal foods. Sugar, which is used in a variety of foods, is a primary product obtained from sugar beets. By-products from sugar processing are sugar beet molasses, dried sugar beet pulp, and sugar beet fiber. Sugar beet molasses is used for production of yeast, chemicals, pharmaceuticals, and in animal feeds. Dried sugar beet pulp is used in the diets of cattle, both dairy and beef, sheep. Sugar beet fiber is used in human food as a source of fiber. A small quantity of sugar beet tops is also used as an animal feedstuff.

Molecular Alterations and Characterization
AgrEvo reports that to produce the glufosinate-tolerant sugar beet, the company, PLANTA Angewandte Pflanzengenetik und Biotechnologie GmbH, Einbeck, Germany, transformed sugar beet line R01. PLANTA used *Agrobacterium tumefaciens* mediated transformation to incorporate foreign DNA into the sugar beet genome. The vector system used for the transformation consisted of an *A. tumefaciens* strain and two plasmids, a nononcogenic Ti-plasmid and a binary plasmid. The binary plasmid contained a modified *pat* gene, and the

kanamycin resistance gene *(kan', also known as aph(3') II, aphA-2, npt II and neo')* between T-DNA border sequences. The *pat* gene is a synthetic version of the gene isolated from *S. viridochromogenes*, strain Tü 494. While the nucleotide sequence of *pat* was synthesized using codons preferred by plants, the amino acid sequence of the enzyme was unchanged. The *pat* gene is under the control of the 35S promoter and terminator sequences derived from Cauliflower Mosaic Virus (CaMV). The *kan'* gene is under the control of the nopaline synthase (nos) promoter.

To determine the nature, number and molecular stability of insertions which occurred in transformation event T120-7, AgrEvo performed Southern blot and PCR analyses. According to AgrEvo, the results indicate that one copy of the DNA between the T-DNA borders was inserted and hence, one copy each of *pat* and *kan'* genes was integrated into the sugar beet genome.

To demonstrate that the integrated DNA was stable over several generations, AgrEvo compared the original transformant (T120-7) with four progenies. AgrEvo produced the progenies either by self-pollination or crosses with nontransgenic sugar beet lines. From Southern blot analyses and segregation data, AgrEvo concludes that the inserted sequences and traits were stably maintained through multiple generations.

Expressed Proteins

AgrEvo determined the levels of PAT and APH(3')II in the transgenic sugar beet line T120-7 by enzyme linked immunosorbent assays (ELISA). In the transgenic sugar beet plants, the average levels of PAT are 2.01 µg PAT/g of crude protein in roots and 6.44 µg PAT/g of crude protein in tops. In pulp, molasses and refined sugar, PAT was not detected by ELISA. The average levels of APH(3')II are 0.29 µg APH(3')II/g of crude protein in roots, 0.29 µg APH(3')II/g of crude protein in tops, and 0.18 µg APH(3')II/g of crude protein in pulp. In molasses and refined sugar, APH(3')II was not detected by ELISA.

Allergenic or Toxic Potential of Introduced Proteins

AgrEvo states that the safety of PAT was addressed previously in several completed consultations between AgrEvo and FDA (BNF0023, 0029, 0046 and 0055). AgrEvo concluded in these consultations that the presence of PAT in small amounts in transgenic crop plants does not raise any safety concerns. AgrEvo reports that very small amounts of PAT were detected by ELISA in its transgenic sugar beet line T120-7. PAT is heat and pH labile, and it is extremely labile in the digestive fluids of cattle and simulated human digestive fluids. AgrEvo concludes that PAT present in sugar beet derived from event T120-7 will not pose any increased risk of allergenicity or toxicity.

APH(3')II is regulated as a food additive under 21 CFR 173.170 and 21 CFR 573.130 for use as a processing aid in the development of new varieties of tomato, oilseed rape and cotton.

FDA evaluated APH(3')II as a food additive in response to a petition filed by Calgene, Inc. At the time the petition was filed, the use of APH(3')II as a processing aid in the development of new plant varieties was new and a record of safe use in foods for human or animal consumption had not yet been established nor had its use in foods been evaluated. Since then, scientific studies and evaluations regarding the use of APH(3')II in new plant development have been performed. In FDA's review of APH(3')II, FDA concluded that APH(3')II will not compromise the efficacy of antibiotic treatment, the probability of transfer of the *kanr* gene consumed as a component of crops to microorganisms in the gastrointestinal tract or in the environment is remote, and APH(3') II does not have any properties that would distinguish it toxicologically from any other phosphorylating enzymes in the food supply. On September 8, 1998, FDA issued a draft guidance on the use of antibiotic resistance marker genes in transgenic plants together with a report of consultations the agency undertook with several outside experts that formed the basis of the draft guidance. The report concluded that there was consensus among the experts that the *kanr* gene is safe to use as a selectable marker in the development of transgenic crops.

In its safety assessment for its transgenic sugar beet, AgrEvo evaluated the safety of kanamycin resistance gene (*kanr*) for use as a selectable marker in sugar beets. AgrEvo noted that FDA, in its approval of a food additive petition submitted by Calgene, Inc., concluded that no limits other than good manufacturing practice are needed to ensure the safety of the petitioned use of APH(3')II in transgenic tomato, oilseed rape, and cotton crops. AgrEvo states that the scientific reasoning and basis that FDA relied upon for approval of the use of APH(3')II as a processing aid in the development of new varieties of tomato, oilseed rape and cotton would also apply for use of APH(3')II found in transgenic sugar beet crops. AgrEvo calculated a dietary exposure estimate for APH(3')II from sugar beet tops. The estimated values for daily exposure to APH(3')II in cattle show that with the assumption that all of the protein is active, cattle would be exposed to extremely small amounts of the enzyme, less than 0.001 mg/kg body weight/day. If only amounts of active APH(3')II are considered, potential intake is reduced by almost an order of magnitude to less than 0.0003 mg active protein /kg body weight/day. AgrEvo claims that APH(3')II is generally recognized as safe (GRAS) for use in the development of new varieties of transgenic plants, and that AgrEvo's use of APH(3')II in its transgenic sugar beet is comparable to the uses previously considered safe.

Compositional Analysis

The intent of the genetic modifications made by AgrEvo was to produce a herbicide tolerant sugar beet. AgrEvo did not anticipate any other effect from the introduction of the transgenes into the sugar beet. To confirm this expectation, AgrEvo performed proximate and mineral analyses on the transgenic sugar beet T120-7 and nontransgenic sugar beet controls. Samples were grown at a number of different sites. AgrEvo performed analyses on raw agricultural commodities which include the sugar beet root and tops.

AgrEvo analyzed sugar beet roots and tops for the following constituents: crude fat, crude protein, crude fiber, ash, carbohydrates, calorie, calcium, magnesium, phosphorus, potassium, and sodium. In the beet roots AgrEvo observed significant differences in caloric, crude fiber and calcium contents between transgenic sugar beet and combined nontransgenic samples. However, when AgrEvo performed two-way analysis of variance (ANOVA) to test for the effect of location and type, a statistically significant difference between transgenic and nontransgenic samples was observed only for fiber content. For sugar beet tops, AgrEvo observed some statistically significant differences between transgenic and nontransgenic samples at individual locations.. However, AgrEvo observed no statistically significant differences between transgenic and nontransgenic samples when a two-way ANOVA was used to test for the effect of type and region. AgrEvo noted that growing location had a significant association with most nutrient concentrations.

AgrEvo also performed analyses on processed fractions which include refined sugar, molasses, and dried pulp. The parameters that AgrEvo analyzed were the proximate variables, fatty acids, amino acids, minerals, and sugar profiles. AgrEvo excluded from its statistical analyses fatty acids for which the reported values were below 0.01%. ANOVA analyses demonstrated statistically significant differences between transgenic and nontransgenic dried pulp samples for ash and some amino acids. For amino acids, the content was higher in the transgenic sugar beet than the nontransgenic samples. AgrEvo argues that since dried pulp can constitute only up to 25% of the total diet for cattle feed use, the small difference in nutrient content is not expected to have a significant impact on cattle nutrition.

For molasses, AgrEvo observed statistically significant differences between transgenic and nontransgenic samples for fat, protein, calcium, C10:0 and some amino-acids. AgrEvo notes that molasses is not used in poultry or swine feed and constitutes at most 10% of the total diet for cattle. Also, the main use of desugared molasses is primarily to make livestock feedstuffs such as hay more palatable. AgrEvo concludes that the differences between transgenic and nontransgenic samples would not be expected to have a significant impact on the nutritional status of animals. For refined sugar, AgrEvo observed no statistically significant differences between transgenic and nontransgenic samples, except for tryptophan. Most importantly, no significant differences exist between the transgenic and nontransgenic samples with respect to their carbohydrate content which constitutes the major nutrient in refined sugar (>98%).

AgrEvo concludes that although there is some variation between transgenic and nontransgenic sugar beet samples and processed root fractions, none of these differences are significant nutritionally. AgrEvo claims that there is no scientific basis to conclude that transgenic sugar beet is different from commercial nontransgenic sugar beet with regard to compositional and nutritional parameters.

Conclusions

AgrEvo has concluded that its transgenic sugar beet line containing transformation event T120-7 is not materially different in terms of food safety and nutritional profile from sugar beet varieties currently on the market. At this time, based on AgrEvo's description of its data and analyses, the Agency considers AgrEvo's consultation on sugar beet lines containing transformation event T120-7 to be complete.

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