DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 97-130-2]

AgrEvo USA Co.; Availability of Determination of Nonregulated Status for Sugar Beet Genetically Engineered for Glufosinate Herbicide Tolerance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that AgrEvo USA Company's sugar beet designated as Transformation Event T120-7, which has been genetically engineered for tolerance to the herbicide glufosinate, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by AgrEvo USA Company in its petition for a determination of nonregulated status and an analysis of other scientific data. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: April 28, 1998.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, and the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690-2817 to facilitate entry into the reading room.

FOR FURTHER INFORMATION CONTACT: Dr. Ved Malik, Biotechnology and Biological Analysis, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-6774. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-4885; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On December 2, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97-336-01p) from AgrEvo USA Company (AgrEvo) of Wilmington, DE, seeking a determination that sugar beet

(*Beta vulgaris* L.) designated as Transformation Event T120–7 (event T120–7), which has been genetically engineered for tolerance to the herbicide glufosinate, does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On February 6, 1998, APHIS published a notice in the **Federal Register** (63 FR 6148–6149, Docket No. 97–130–1) announcing that the AgrEvo petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject sugar beet and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether this sugar beet posed a plant pest risk. The comments were to have been received by APHIS on or before April 7, 1998. APHIS received no comments on the subject petition during the designated 60-day comment period.

Analysis

Event T120–7 sugar beet has been genetically engineered to contain a synthetic version of the *pat* gene derived from *Streptomyces viridochromogenes*. The *pat* gene encodes the enzyme phosphinothricin-N-acetyltransferase (PAT), which confers tolerance to the herbicide glufosinate. Expression of the *pat* gene is controlled by 35S promoter and terminator sequences derived from the plant pathogen cauliflower mosaic virus. Event T120–7 sugar beet also contains the *aph(3')II* or *nptII* marker gene used in plant transformation.

Expression of the *nptII* gene is controlled by gene sequences derived from *Agrobacterium tumefaciens*, and analysis indicates that the NPTII protein is expressed in certain parts of the subject sugar beet plants. The *A. tumefaciens* method was used to transfer the added genes into the parental sugar beet line.

The subject sugar beet has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of this sugar beet conducted under APHIS permits since 1994 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of event T120–7 sugar beet.

Determination

Based on its analysis of the data submitted by AgrEvo, and a review of other scientific data and field tests of the subject sugar beet, APHIS has determined that event T120–7: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than sugar beet developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture. Therefore, APHIS has concluded that the subject sugar beet and any progeny derived from crosses with other sugar beet varieties will be as safe to grow as sugar beet in traditional breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that AgrEvo's event T120–7 sugar beet is no longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the subject sugar beet or its progeny. However, importation of event T120–7 sugar beet or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that AgrEvo's event T120–7 sugar beet and lines developed from it are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT**.

Done in Washington, DC, this 30th day of April, 1998.

Craig A. Reed,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 98–12125 Filed 5–6–98; 8:45 am]

BILLING CODE 3410–34–P

AgrEvo USA Company Petition 97-336-01p for Determination of Nonregulated Status for Transgenic Glufosinate Tolerant Sugar Beet Transformation Event T120-7

Environmental Assessment and Finding of No Significant Impact

April 1998

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 97-336-01p) received from AgrEvo USA Company seeking a determination of non-regulated status for their genetically engineered glufosinate-ammonium (glufosinate) tolerant sugar beet designated as Transformation event T120-7 under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers resistance to the phosphinothricin herbicide, glufosinate. Based on the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact (FONSI) on the environment from the unconfined cultivation and agricultural use of event T120-7 and its progeny.

Arnold Foudin for

Rebecca A. Bech
Assistant Director
Scientific Services
Operational Support
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: APR 28 1998

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 97-336-01p) from AgrEvo USA Company (AgrEvo) seeking a determination of non-regulated status for their transgenic glufosinate tolerant sugar beet designated as Transformation Event T120-7 (event T120-7). AgrEvo seeks a determination that event T120-7 and its progeny do not present a plant pest risk and, therefore, are no longer regulated articles under regulations at 7 CFR Part 340. Event T120-7 is sugar beet, *Beta vulgaris ssp. vulgaris*, containing a stably integrated gene which encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient in the herbicide glufosinate-ammonium, to an inactive form, thereby conferring tolerance to the herbicide. The *pat* gene in event T120-7 is a synthetic version of the native gene isolated from *Streptomyces viridochromogenes*. The gene was introduced into sugar beet calli using disarmed *Agrobacterium tumefaciens*. Southern blot and polymerase chain reaction (PCR) analyses confirm that the incorporation has been limited to DNA sequences contained within the T-DNA borders and that event T120-7 contains a single, stably integrated copy of the *pat* gene.

No differences in event T120-7 sugar beet compared to nontransformed counterpart beets as well as standard commercial sugar beet varieties growing in nearby fields were found in the agronomic characteristics, plant emergence and seedling vigor. Event T120-7 has also been field tested extensively in Canada, Western and Eastern Europe, and in the former Soviet Union. Field trial reports from these tests demonstrate that the transformed line did not exhibit weedy characteristics, and does not cause any harm to nontarget organisms or the general environment.

An environmental assessment (EA) was prepared prior to granting field test permits involving the event T120-7. The EA for the previous introductions of event T120-7 addressed plant pest risk issues relative to the conduct of field trials under physical and reproductive confinement. This EA specifically addresses the potential impacts of event T120-7 to the human environment through unrestricted use in agriculture. The U.S. Environmental Protection Agency (EPA) has the authority over the potential uses of the herbicide glufosinate ammonium (Basta®, Ignite®, Rely®, Liberty®, Harvest®, and Finale®) in conjunction with event T120-7 through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

APHIS has considered the information provided by AgrEvo in its petition as well as other scientific data, information, and comments received from the public relating to potential plant pest risk and related environmental impacts of event T120-7. An evaluation of the potential for significant impact to the human environment through the unconfined, agricultural use of event T120-7 resulted in a Finding of No Significant Impact (FONSI) by APHIS. This conclusion is based upon (1) the nature of the genetic

modification; (2) the fact that sugar beet has weedy relatives with which it may interbreed in the United States and its territories but other herbicides exist to control such weeds if they become glufosinate-ammonium tolerant and (3) the fact that this modification will not increase the weediness potential of the sugar beets or negatively affect any nontarget or beneficial organisms. In conjunction with the FONSI, APHIS has made the determination that event T120-7 and its progeny do not pose a plant pest risk and are, therefore, determined to be no longer regulated articles according to 7 CFR 340. The determination document is in Appendix A.

II. INTRODUCTION

This EA examines potential environmental impacts from the unrestricted introduction of event T120-7. Event T120-7 and its progeny has been field tested under permits from APHIS in primary sugar beet growing regions of the USA since 1994. In total, 68 trials have been conducted under USDA authorization 94-054-06r, 94-347-01r; 96-052-02r; and 97-029-01r. AgrEvo based its petition, in part, on the data gathered from these trials. Field trial reports from these tests demonstrate no deleterious effects on plants, nontarget organisms, or the environment as a result of these field releases. All field trials were performed under physical and reproductive confinement. Further discussions of the biology of sugar beet, as well as of the genetic components of event T120-7, are found in the determination document (Appendix A). Because this information is included in Appendix A, it will not be described in detail in the body of this document.

Prior to issuing a permit for a field release, APHIS analyzes the potential impacts associated with the proposed introduction and prepares an environmental assessment that documents the environment analysis in accordance with regulations and guidelines implementing the National Environmental Policy Act (NEPA) of 1969, as amended (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372). APHIS also evaluates cumulative impacts to the human environment from its determination of nonregulated status.

A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. The transgenic sugar beet plants described in the AgrEvo petition have been considered regulated articles because they contain certain noncoding regulatory sequences (DNA) derived from known plant pathogens listed in 7 CFR Part 340.

III. PURPOSE AND NEED

The purpose of this EA is to ascertain whether the approval of a petition submitted to USDA/APHIS for the determination of nonregulated status of event T120-7 (that

would allow their unconfined introduction into the environment) will present any plant pest risk or have any significant impact on the environment.

A petition was submitted to APHIS pursuant to regulations codified in 7 CFR Part 340 entitled "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." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted data and to seek a determination that a particular regulated article does not present a plant pest risk and should no longer be regulated.

If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be approved for the unregulated introduction (importation, interstate movement, and release into the environment) of the articles or their progeny in question without any prior permit or notification from APHIS.

Effects associated with the potential uses of the herbicide glufosinate in conjunction with event T120-7 are outside the scope of the regulatory authority of APHIS. APHIS determination does not constitute authorization to use glufosinate on event T120-7. The EPA has authority over the use in the environment of pesticidal substances, including herbicides, under FIFRA; specifically, EPA has jurisdiction over registration of glufosinate for use on transgenic sugar beets as well as other transgenic and nontransgenic crops. EPA considers both human health and safety as well as nontarget effects of the herbicide and its breakdown products in making a decision on registration of a herbicide.

IV. ALTERNATIVES

In the course of preparing the environmental assessment for the AgrEvo petition, APHIS considered the following three alternatives: (1) deny the petition, so that event T120-7 would continue to be regulated under 7 CFR Part 340; (2) approve the petition, with geographical limitations; and/or (3) approve the petition so that event T120-7 would no longer be regulated when grown in the United States and its territories. Based on the biology of sugar beet, the nature of the genetic change, data and information presented by AgrEvo, scientific literature, and information and comment provided by the public, APHIS could find no basis for denying the petition (Alternative 1) or for imposing geographical limitations on the use of event T120-7(Alternative 2).

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of event T120-7 in the absence of confinement.

Potential Impacts Based On Increased Weediness Of Event T120-7 Relative To Traditionally Bred Sugar beets

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje *et al.* (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

The parent plant in this petition, *Beta vulgaris L. ssp. vulgaris* L., does not show any especially weedy characteristics. It is unlikely that as a result of cross-pollination descendants of crosses between transgenic and wild sugar beets would become part of breeding material or become established in any sugar beet ecological community (Lewellen 1998). In a review of the State Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act, no reference was found regarding "wild" sugar beets or wild sugar beet relatives as either restricted or noxious weeds (Holm et al. 1979). This demonstrates that sugar beet does not have the necessary attributes which could allow it to become a serious weed problem in sugar beet growing areas.

Sugar beet is a biennial crop. The second season's crop produces seed. However, certain conditions such as low temperatures after planting and longer day length can cause the sugar beet to "bolt" or produce a seed stalk during the first growing season (Bell, 1946; Jaggard, Wickens, Webb and Scott, 1983; Durrant and Jaggard, 1988). These situations exist in Europe, especially England when growers seed too early in the spring. They also can occur in California where sugar beets are often seeded during the fall and winter months or when spring planted crops are overwintered. Bolters are a problem in the current planted crop because, although inflorescences (bolting stalk) may be cut off, the bolted plants contain more lignin in their roots and sugar yield could be reduced by 50% (Smith, 1987; Scott and Wilcockson, 1976; and Jaggard et al. 1983). While bolting can cause a problem in any given year it does not lead to any increase in weediness potential of sugar beet. Unwanted seed germination in sugar beet is controlled by various methods such as cutting at the base of the stalk, or treatment with non-selective herbicides.

Potential Impacts From Out crossing Of Event T120-7 to Wild Relatives

Wild species of sugar beets have been reported in the region surrounding the Mediterranean Sea and in the Caucasus Mountains of Russia and into Turkey and Iran. Wild species may also be found as far west as the Canary Islands (Doney, 1996; Cooke and Scott, 1993). Some relatively small wild populations of *B. macrocarpa*, *Beta vulgaris L.*ssp. *maritima L.* , and *B. vulgaris L.* ssp. *vulgaris L.* have become established in California due to the mild climate. The populations of *Beta vulgaris L.* ssp. *vulgaris L.*, *B. vulgaris L. ssp. maritima L.* and *B. macrocarpa* probably developed from seed contaminants or from seed intentionally imported into California. No wild populations of *Beta* have been reported in the U.S. outside of California.

In 1928 Carsner, as referenced by McFarlane (1975), reported wild beet populations in Imperial, Santa Clara, Ventura, San Bernardino, Los Angeles, and Orange Counties of California. Carsner speculated that these beets were either *B. vulgaris L. ssp. maritima L.* or hybrids between *B. vulgaris L. ssp. maritima* L. and *B. vulgaris L.* ssp. *vulgaris L.* McFarlane, (1975) identified the wild beets in Imperial County as *B. macrocarpa* rather than *B. vulgaris ssp. maritima.* These populations have been established for many years and are not spreading. *Beta macrocarpa* is a species that occurs naturally in the Canary Islands and along the Mediterranean coastline. Seeds of *B. macrocarpa* may have been imported as contaminants in seed or in feed grain. McFarlane, (1975) reported the existence of numerous naturally occurring hybrids between plants of *B. macrocarpa* and *B. vulgaris L.* ssp. *vulgaris L.* in the Imperial Valley. .

Dahlberg and Brewbaker, (1948) referred to the population of *B. vulgaris L. ssp. maritima L.* in Santa Clara County as the "Milpitas wild beet". Seed brought in by the Franciscan Fathers when they established the Santa Clara and other missions in the late 1700's may be the source of these wild relatives. Sugar beets are no longer commercially grown in this area where these beets were found. Johnson and Burtch, (1958) describe sugar beets which evolved into annual plants and became a weed problem in California. Recent surveys localize such populations in the Gilroy/Hollister area where sugar beet is grown.

Abe (1988) reported that *B. vulgaris L.* ssp. *vulgaris L.* and *B. macrocarpa* do not readily produce viable hybrids. Crosses with species outside the *Beta* type are made, with difficulty, using special plant breeding techniques. Due to the biennial nature of sugar beets the risk of gene transfer from transgenic sugar beets to weedy relatives is remote. Since bolting beets are uncommon except in fields or plots grown specifically for seed production, there is little opportunity for uncontrolled pollen flow due to adequate isolation distances. Even if gene transfer were to occur from event T120-7 to wild beets, other herbicides can be used to control such glufosinate-tolerant wild beets (Lewellen 1998 personal communication).

Potential Impact On Nontarget Organisms Including Beneficial Organisms Such As Bees And Earthworms

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of event T120-7. Glufosinate tolerant soybean that produce the same enzyme (PAT) are already commercialized and have proven safe and similar to their non-transgenic parents. The enzyme that confers glufosinate resistance in sugar beet is normally not present in sugar beets and is not known to have any toxic property. Field observations of event T120-7 revealed no negative effects on nontarget organisms. The lack of known toxicity for this enzyme suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrates makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms. In addition, there is no reason to believe that the presence of event T120-7 would harm any threatened or endangered species in the United States.

Consideration Of Potential Environmental Impacts Associated With The Cultivation Of Event T120-7 Outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with the potential approval of event T120-7. Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of this sugar beet line or its progeny.

Any international traffic in sugar beet would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (105 countries as of October, 1996). The treaty, now administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into force on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS has played a role in working toward harmonization of biosafety and biotechnology

guidelines and regulations included within the RPPO for our region, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection.

APHIS participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. In addition, APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner. In the course of these wide-ranging studies and interactions, APHIS has not identified any impacts on the environment that might be relevant to the unconfined cultivation of event T120-7 sugar beet in the United States and its territories, or abroad.

Potential impacts on biodiversity.

Genetically engineered event T120-7 sugar beet is no more likely to become weed than lines developed by traditional breeding techniques. It is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which it may interbreed. It will not harm threatened and endangered species and non-target organisms. Based on this analysis, APHIS concludes that there is no potential impact of event T120-7 on biodiversity.

Potential impacts on agricultural and cultivation practices.

Based on APHIS analysis, there is unlikely to be any significant adverse impact on agricultural practices associated with the use of this line. The introduction of event T120-7 in agriculture offers the opprtunity of no till cultivation of sugarbeet leading to decreased soil erosion and thereby soil sustainability.

Potential damage to raw or processed agricultural commodities.

An analysis of the components and processing characteristics of this line reveals no differences in any component that could have an indirect plant pest effect on any raw or processed plant commodity.

VI. CONCLUSIONS

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of a proposed action, i.e, reaching the determination that event T120-7 have no potential to present a plant pest risk and should no longer be considered a regulated article under the regulations at 7 CFR Part 340. After careful analysis of the available information, APHIS concludes that its

proposed action should not have a significant impact on the environment and that the proper alternative is to approve the petition so that event T120-7 would have a nonregulated status when grown in the United States and its territories. APHIS has identified no factors that would suggest any impact to the environment of the United States and its territories. While isolated environments, such as are found in Hawaii, Puerto Rico, or in territories or possessions of the United States, have fragile ecologies that have frequently been damaged through human intervention, APHIS has determined that in these environments event T120-7 will have impacts no different from traditional sugar beet varieties that are not subject to petition requirements under 7 CFR Part 340 before they enter agriculture. Sugar beet at present is not grown in Hawaii or Puerto Rico. This conclusion is based on factors discussed herein or in the determination included as appendix A, as well as the following factors:

1. Neither the glufosinate resistance gene nor its product or the regulatory sequences confer on event T120-7 or its progeny any plant pest characteristic. A *pat* gene that confers tolerance to the herbicide glufosinate has been inserted into a sugar beet chromosome in sugar beet lines. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination. Sexually compatible wild species of sugar beets in the United States and its territories are rare. Even if out crossing produced glufosinate-tolerant weeds, other herbicides are on the market to control them..

2. The gene that confers tolerance to the herbicide, glufosinate, will not provide event T120-7 or its progeny with any measurable selective advantage over nontransformed sugar beet plants in their ability to disseminate or to become established in the environment. There is no reason to believe that event T120-7 exhibit any increased weediness relative to that of traditional varieties or the unmodified parental lines.

3. The use of event T120-7 or its progeny in agriculture will not lead to an increase in weediness in any plant with which it can successfully interbreed.

4. There is no reason to believe that the use of event T120-7 or its progeny in agriculture will have a significant impact on any beneficial organisms in the environment or on any threatened or endangered species.

5. The use of event T120-7 or its progeny in agriculture will not cause damage to raw or processed agricultural commodities.

VII. REFERENCES.

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VIII. PREPARERS AND REVIEWERS

Biotechnology and Biological Analysis

Rebecca Bech, Assistant Director
Subhash Gupta, Ph.D., Biotechnologist
David S. Heron, Ph.D., Biotechnologist
Susan Koehler, Ph.D., Biotechnologist
James Lackey, Ph.D., Biological Safety Officer
Vedpal Singh Malik, Ph.D., Biotechnologist (Principal Reviewer)
Sivramiah Shantharam, Ph.D., Team Leader
James L. White, Ph.D., Team Leader, Plants Branch
Shirley Ingebritsen (Reviewer)

IX. AGENCY CONTACT

Ms. Kay Peterson, Regulatory Analyst
Biotechnology and Scientific Services
USDA, APHIS, BSS
4700 River Road, Unit 147
Riverdale, MD 20737-1237
mkpeterson@aphis.usda.gov
Phone: (301) 734-7612
Fax: (301) 734-8669

APPENDIX A

DETERMINATION OF NONREGULATED STATUS FOR TRANSGENIC GLUFOSINATE-AMMONIUM TOLERANT SUGAR BEET TRANSFORMATION EVENT T120-7

Petitioner: AgrEvo Company USA, Wilmington, Delaware
Petition Number: 97-336-01p

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Biotechnology and Biological Analysis
Riverdale, Maryland

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) on reviewing the AgrEvo U.S.A. Company's (AgrEvo) petition 97-336-01p, has concluded that the glufosinate-tolerant sugar beet designated as Transformation Event T120-7 (event T120-7) and its progeny do not present a plant pest risk, and are, therefore, determined to be no longer regulated articles under regulations at 7 CFR part 340. The applicant is no longer required to obtain a permit or notify APHIS for the unrestricted introduction of event T120-7 and its progeny lines into the environment within the continental United States and its territories. Exportation of such lines still will remain regulated according to Foreign Quarantine Notice regulations at 7 CFR 319. Variety registration and/or seed certification of glufosinate-tolerant lines of sugar beet may involve future actions by the U. S. Plant Variety Protection Office and State Seed Certification officials.

The AgrEvo petition was submitted to APHIS on December 2, 1997. On February 6, 1998, APHIS announced the receipt of the AgrEvo petition in the *Federal Register* (63 FR 6148-6149, Docket Number 97-130-1) seeking comments from the interested public. The public comment period ended on April 7, 1998. The AgrEvo petition sought regulatory relief for event T120-7 and its progeny lines from the regulations at 7 CFR 340. In the *Federal Register* notice, APHIS indicated its role in the process of reviewing the AgrEvo petition and the roles of other Federal agencies, such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) in regulating glufosinate-tolerant sugar beet lines, food products derived from them, and the potential herbicide use of glufosinate on these lines. APHIS received no comments on the subject petition during the designated 60-day comment period.

Event T120-7 sugar beet is glufosinate-tolerant due to the chromosomal integration of a synthetic phosphinothricin acetyltransferase (*pat)* gene originally isolated from *Streptomyces viridochromogenes*, a soil microbe. The parent line is the tissue culture line R01, which was transformed with the vector pOCA18/Ac using *Agrobacterium tumefaciens* mediated transformation. The subject sugar beet line also contains the selectable marker gene aminoglycoside (3') phosphotransferase type II, *nptII*, from transposon Tn5 of *Escherichia coli*. The introduced genes also have accompanying DNA regulatory sequences that modulate their expression. Event T120-7 sugar beet is considered a regulated article because it contains regulatory sequences from the plant pests *A. tumefaciens* and cauliflower mosaic virus (CaMV).

APHIS regulations at 7 CFR 340, which were promulgated pursuant to the authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, inter-state movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subjected to the regulatory oversight of 7 CFR 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate the submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated under 7 CFR part 340. If the Agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question.

Event T120-7 and its progeny have been considered "regulated articles" under 7 CFR 340 because they contain components or DNA sequences from organisms considered to be plant pathogens, and are on the list of regulated articles (organisms). In this instance DNA sequences from well-known plant pathogens like *A. tumefaciens* and CaMV have been used to create event T120-7, rendering them to be regulated articles. Field tests of event T120-7 have been conducted with APHIS approval since 1994. AgrEvo submitted its petition after the completion of field tests of event T120-7 under APHIS permits and notifications. Event T120-7 has also been field tested extensively in Canada, Western and Eastern Europe, and in the former Soviet Union. All field trials were performed under conditions of physical and reproductive confinement.

APHIS has determined that event T120-7 does not present a plant pest risk and will no longer be considered a regulated article, under APHIS regulations at 7 CFR Part 340. Based on an analysis of data provided to APHIS by AgrEvo, as well as other scientific data, the agency concluded that event T120-7: (1) exhibits no plant pathogenic properties; (2) are no more likely to become a weed than its non-engineered parental varieties; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities, and (5) are unlikely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture, or threatened and endangered species.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et.seq.)* and pursuant implementing regulations (40 CFR 1500-1508, 7 CFR Part 1b; 7 CFR Part 372).

The body of this document consists of two parts: (1) background information which provides the regulatory framework under which APHIS has regulated the field testing, interstate movement, and importation of event T120-7, as well as a summary of comments provided to APHIS on its proposed action and (2) analysis of the key factors relevant to APHIS decision that event T120-7 does not present a plant pest risk.

II. BACKGROUND

A. USDA Regulatory Authority

APHIS regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C.) 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is deemed a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in section 340.2 of the regulations and is also a plant pest, if it is unclassified, or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk.

Prior to the introduction of a regulated article, the applicant is required under § 340.1 of the regulations to either (1) notify APHIS in accordance with section 340.3 or (2) obtain a permit in

accordance with section 340.4. Introduction under notification (Section 340.3) requires that the introduction meet specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under Section 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

The FPPA gives USDA authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. The PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants which may harbor injurious pests or diseases and requires that they be grown under certain conditions after importation. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties and to demonstrate that although they were developed by using components from plant pests, they do not possess plant pest characteristics.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations entitled "Petition Process for Determination of nonregulated Status" provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

APHIS believes it is prudent to provide assurance prior to commercialization that organisms, such as event T120-7, that are developed in part from plant pest sequences, do not present any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that event T120-7 and its progeny are no longer regulated articles is based in part on evidence provided by AgrEvo concerning the biological properties of event T120-7 and its similarity to other varieties of sugar beet grown using standard agricultural practices for commercial sale or private use.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant pest or that plants or genes are pathogenic. The regulations, instead, are based on the premise that when plants are developed using biological vectors from pathogenic sources, transforming material from pathogenic sources, or pathogens as vector agents, that they should be evaluated to assure that there is not a plant pest risk. For each field test, APHIS performs a review that allows a verification of the biology and procedures used, assesses the degree of uncertainty and familiarity, and allows the identification of any predictable hazards. The overall aim of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 is to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight and to enable any issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms for the safe utilization of the technology in agriculture. A

certification that a genetically engineered organism does not present a plant pest risk means that there is reasonable certainty that the organisms cannot directly or indirectly cause disease, injury, or damage either when grown in the field, or when stored, sold, or processed. This approach is considerably broader than a narrow definition of plant pest risk arising from microbial or animal pathogens, including insect pests. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, clearly come under what is meant by direct or indirect plant pest risk. In APHIS regulations at 7 CFR Part 340, a "plant pest" is defined as: "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."

Lack of plant pest risk may be concluded when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. Evidence presented by AgrEvo bears on all of these topics. In addition, because the AgrEvo petition seeks a determination regarding event T120-7, it should be established that there is a reasonable certainty that any new sugar beet varieties bred with event T120-7 will exhibit plant pest properties not substantially different from any observed for sugar beets in traditional breeding programs or as seen in the development of event T120-7.

B. Other Federal Agencies Regulatory Authority

The EPA regulates the use of pesticide chemicals, including herbicides, in the environment. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended (7 U.S.C. 136 *et seq.*), EPA has the authority to regulate the testing, sale, distribution, use, storage, and disposal of pesticides. Before a pesticide may be sold, distributed, or used in the United States, it must be registered under FIFRA Section 3. For a pesticide that is already registered, the use of the pesticide on a new crop plant (i.e., use on a crop for which the pesticide is not already registered) requires EPA approval of an amendment to the registration. In determining whether to approve the new use of the pesticide, EPA considers the possibility of adverse effects to human health and the environment. Under the Federal Food, Drug and Cosmetic Act as amended (FFDCA) (21 U.S.C. 301 *et.seq.*), EPA also has responsibility for establishing tolerances for pesticide residues on food or feed. However, any new use of the herbicide on sugar beet would require the approval by EPA of an amendment to the registration under FIFRA and a tolerance establishment under FFDCA.

The FFDCA provides FDA with authority to ensure the safety and wholesomeness of all food(s), other than meat and poultry. The FDA policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from event T120-7 is under the jurisdiction of the FDA.

III. PUBLIC COMMENTS

APHIS received no comments on the AgrEvo petition during the designated 60-day comment period that ended on April 7, 1998.

IV. BIOLOGY AND CULTIVATION OF SUGAR BEET

Brief discussions of the biology of sugar beet and sugar beet cultivation practices follow in the next section to help in the subsequent analysis.

A. History and Uses of Sugar Beet

The beet (*Beta vulgaris*) root was a common element of the Egyptian diet during the building of the pyramids. Its use as a source of sugar, however, was not discovered until the middle of the 18th Century in Europe (Smith, 1980). Today, 26% of the world sugar consumed and 47% of the U.S. sugar consumed is produced from sugar beets (USDA Agricultural Statistics, 1997). The by-products from the processing of sugar beets include sugar beet pulp and molasses. Sugar beet pulp is dried and pelleted for use as a livestock feed. Molasses is also used as livestock feed to make feedstuffs such as hay more palatable. Sugar beet tops are used in a limited amount as livestock feed (cattle, sheep) when they are left in fields for grazing.

In the United States five (5) major sugar beet growing regions listed in order of sugar beet acreage are: 1) the Red River Valley of Minnesota and North Dakota, 2) Southern Idaho, 3) the "Intermountain" region, consisting of parts of Montana, Wyoming, Nebraska and Colorado, 4) the Imperial and Central Valleys of California, and 5) the "thumb" of Michigan. In the semiarid areas of the western region, including Montana, Wyoming, Nebraska, Colorado, Texas, Idaho, Oregon, Washington, and California, inadequate spring and summer rains necessitate irrigation. In California irrigated sugar beets are grown under a wide range of soil and climatic conditions and may be grown as a summer or winter crop. In 1996 in the USA sugar beets were grown (harvested) on 1,322,900 acres, with more than 99% grown primarily in twelve states. Minnesota and North Dakota grew 663,300 acres (50%). The Imperial Valley of California and southern Idaho together grew 266,000 acres (20%), Michigan grew 130,000 acres (10%), and the Intermountain states of Colorado, Nebraska, Montana, Wyoming grew 16.4%. Thus, in 1996, these five regions accounted for 96.4% of the United States sugar beet production (USDA Agricultural Statistics, 1997).

B. Taxonomy and Habit of Sugar Beets

Sugar beets are a member of the family *Chenopodiaceae* (Goosefoot Family) which also includes the leaf beet (Swiss chard), and the red table beet (fodder beet), from which the sugar beet was derived (Hymowitz and Singh 1987; Cooke and Scott, 1993). Members of this family are dicotyledonous and usually herbaceous in nature. They are also halophytes and many of the family's weed species are found along sea coasts and near brackish marshes. Several members of this family may also be found invading crops. These include Lambsquarters (*Chenopodium album*), Mexican fireweed (*Kochia scoparia*) and Russian thistle (*Salsola kali*) (Fernald, 1950). Agriculturally important plants of the genus *Beta* belong to the species *vulgaris*. The genus *Beta*,

including the wild relatives, is divided into four sections as shown in Table 1. (Smith, 1987, Panella, 1996):

TABLE 1: Species of the Genus *Beta*

SECTION	SPECIES	CHROMOSOME NUMBERS
Beta	*B. vulgaris* L. subsp. *vulgaris* L. subsp. *maritima* L. subsp. *adanensis* (Pamuk.) *B. macrocarpa* (Guss.) *B. patula* (Ait.)	 2n = 2x = 18 2n = 2x = 18 2n = 2x = 18 2n = 2x = 18 2n = 2x = 18
Corollinae	*B. lomatogona* (F.et Mey) *B. trigyna* (W. et Kit) *B. corolliflora* *B. macrochiza* (Stev.) *B. intermedia* (Bunge)	2n = 2x = 18, 4x = 36 2n = 4x = 36, 5x =45, 6x = 54 2n = 2x = 18 2n = 4x = 36 2n = 2x = 18
Procumbentes	*B. patellaris* (Moq.) *B. procumbens* (Chr. Sin.) *B. webbiana* (Moq.)	2n = 4x = 36 2n = 2x = 18 2n = 2x = 18
Nanae	*B. nana* (Boiss. & Hldr.)	2n = 2x = 18

Beta is considered an Old World genus basically confined to the Mediterranean Basin and Middle East. The genus has been organized into four sections: *Beta* (formerly *Vulgares*), *Corollinae*, *Procumbentes* (formerly *Patellares*), and *Nanae*. The taxonomy of the *Beta* section has recently been revised based upon morphometric analysis of variation, allozyme differentiation, and evaluation of available herbarium specimens. The section consists of three species: *B. vulgaris L.*, a large and variable species containing both cultivated and wild materials *B. macrocarpa;* and *B. patula. Beta vulgaris* L. is subdivided into three subspecies: subsp. *vulgaris L.*, containing all cultivated materials; subsp. *maritima L.*, a large and variable group of plant types; and subsp. *adanensis*. So called Weed beets are classified as *B. vulgaris L.* subsp. *maritima L.* (Letschert, et. al., 1994).

The sugar beet, as originally developed, was a diploid with 18 chromosomes (2x). Commercial exploitation of polyploidy in sugar beets began in Europe in the 1940s with the development of anisoploid varieties. Such varieties were actually mixtures including diploid, triploid and tetraploid individuals, and were produced by interpollination of diploid and tetraploid seed-parents. The use of cytoplasmic sterility in conjunction with polyploidy allowed the production of triploid varieties. Currently there are diploid, triploid, and anisoploid varieties available. Sugarbeet plants with higher ploidy levels notably auto-hexaploids and auto-octoploids have been produced experimentally but are not used commercially.

Hybridization between *B. vulgaris L.* subsp. *vulgaris L and B. vulgaris L.* subsp. *adanensis or B. patula* have not been reported. *However* McFarlane, (1975) reported that hybrids between *B. vulgaris L.* subsp. *vulgaris L.* and *B. macrocarpa* are rare due to different flowering times of the species. Abe and Ui (1986) reported pollen sterility and seed abortion in the F1 generation of crosses between *B. macrocarpa* and *B. vulgaris L.* subsp. *vulgaris L.* and *B. vulgaris L.* subsp. *maritima L.* In later generations segregation for chlorosis, hybrid weakness and sterility was observed, whereas distorted segregation occurred in the backcrossed progenies (Abe & Tsuda., 1988). Lange & De Bock, (1989) produced triploid and tetraploid hybrids between tetraploid *B. macrocarpa* and diploid or tetraploid cytotypes of *B vulgaris*. The triploids were nearly fully sterile, attributable to meiotic irregularities resulting from the triploidy. The tetraploid hybrids exhibited a somewhat better fertility. An F2 generation showed partial hybrid dwarfness, partial fertility, as well as segregation for earliness and coloration of the hypocotyl. The fitness of these hybrids in competition with event T120-7 in an agricultural environment is not known.

Successful hybridizations between *B. vulgaris* and species in the section *Corollinae* but not of *Nanae* have been carried out by several investigators. It is generally not necessary to use bridge species, although *B. vulgaris L.* subsp. *maritima L* has been successfully used to introduce traits from *B. trigyna* into sugar beet. Most of the hybrids with species of section *Corollinae* showed apomictic reproduction. The three species of section *Procumbentes* can be hybridized with *B. vulgaris* only with great difficulty. The hybrids become necrotic and die at the seedling stage. They either do not develop secondary roots, or they have poor vascular connection between the roots and the shoots. Such obstacles could be overcome by grafting the hybrids onto cultivated beet plants or by using fodder beets, mangels,*B. vulgaris L.* subsp. *maritima L* as bridge species. The surviving hybrids are difficult to backcross with *B. vulgaris L.* subsp. *vulgaris L.* Pollen sterility in F1 and back crosses results from abnormal meiosis. Chromosome lagging, multiple spindles, bridges and ejected chromosomes have been frequently observed causing lack of fertility or embryo abortion. The chromosomes of the species of section *Procumbentes* do not pair with those of section *Beta* (Van Geyt et. al., 1990) and hybrids of them may be unstable and sterile.

The agriculturally important sugar beet, *B. vulgaris L.* subsp. *vulgaris L*, is a herbaceous dicot which usually completes its life cycle in two years (Smith, 1987). Wild populations of *B. vulgaris* consist of annual, biennial, and perennial species, but only the biennial type has been developed for sugar beet production (Hecker and Helmerick, 1985). Sugar beets produce a fleshy, bulbous taproot during the first growing season which is the sugar producing crop. During the second growing season, following an overwintering period of cold temperature, the sugar beet plants flower and produce seed (Smith, 1987).

Sugar beets are planted as a plant population of approximately 75,000 plants per hectare (Cooke and Scott, 1993). Initial growth of the sugar beet seedling shows a greater early development of leaf tissue. Six weeks following emergence, the plant has 8-10 leaves and still has a very small root. From this stage on, both leaf and root tissue develop simultaneously with the root eventually becoming a greater proportion of the plant's dry weight. The sugar beet root develops in a series of concentric rings of vascular and parenchyma tissue. Root size develops by increased cell multiplication and cell growth. A greater concentration of sucrose can be found in the smaller cells within the vascular region than in the larger cells of the parenchyma region (Milford, 1973). With the proportion of vascular to parenchyma cells fixed, this may explain the reason that

breeders have only been able to develop sugar beet cultivars with the highest fresh weight concentration of sugar being around 18.0% (Cooke and Scott, 1993). Several hypotheses for this phenomenon have been stated, but no one knows for certain the reason why the parenchyma layer cells contain less sugar (Milford, 1973). One theory is that both vascular and parenchyma cells produce the same absolute amount of storage sugar, with the concentration thus being greater in the smaller (vascular) cells. Whatever the reason, data indicates that there is an inverse relationship between the weight of sugar beets produced per unit area and the percentage of sugar produced. Recurrent and reciprocal recurrent selection techniques have not changed this inverse relationship between sucrose yield and root weight yield (Hecker, 1978).

Soon after new growth begins during the second growing season, the vernalized root bolts. Bolting occurs as the reproductive stage is initiated, with the plant producing an elongated stem, or tall angular seed stalk. A large petiolate leaf develops at the base of the stem with small leaves, less petiolate leaves and finally sessile leaves develop further up the stem. At the leaf axils secondary shoots develop forming a series of indeterminate racemes. These flowers are sessile and occur singly if monogerm, or in clusters, if multigerm (Smith, 1987). The terms multigerm and monogerm refer to ripened fruit and not seed.

Flowers are perfect including a tricarpellate pistil surrounded by the five stamens and a perianth of five sepals. The sugar beet flower contains no petals. Below and surrounding each flower is a slender green bract. Mature flowers begin anthesis about 5-6 weeks following the initiation of reproductive growth. Anthesis continues for a period of several weeks. Each fruit contains either a single seed or twin embryos. Pollination is carried out mainly by wind and, to a small degree, by insects. The primary method of pollination is cross pollination because of the lack of synchrony between pollen release and receptiveness of the stigma (Cooke and Scott, 1993). Approximately six weeks following full flower bloom, the seed crop is ready for harvest. Changes in color of the seedstalk and foliage and shattering of the earliest maturing seeds are good indications of harvest time. The harvesting of seed of monogerm cultivars and those resistant to bolting is harder than the harvest of multigerm cultivars. In these cultivars the seed may be ready for harvest while the leaves and stalk are still green. In this case, seed development is the primary indicator of harvest readiness; i.e., when the seed is in the moderate to hard dough stage, then the crop is ready to harvest. (Smith, 1980).

C. Breeding of Sugar Beets

The genetic base of sugar beet germplasm is limited because it was derived from the variation expressed in the white fodder beets of Europe. Spontaneous hybridization with cultivated leaf-beet types and wild *B. vulgaris L.* subsp. *maritima L.* may have contributed additional variation. Due to the difficulty to hybridize *Beta vulgaris* subsp. *vulgaris L.* outside the *Beta* section, the genetic base of sugar beets remains narrow (Panella, 1996). Today, private seed companies dominate sugar beet breeding, concentrating on varieties which produce high sucrose yield, disease and pest resistance, and herbicide tolerance. Cytoplasmic male sterility (CMS) is used to develop male-sterile or female parental lines which allow the breeding of hybrid cultivars. CMS lines are the male-sterile equivalents of O-type (or maintainer) lines. Commonly, a monogerm O-type of one line will be hybridized with the monogerm male-sterile equivalent of another line to

produce a monogerm male-sterile F1. This F1 then is used as the seed parent in crosses with diploid or tetraploid pollinator lines.

In the U.S. all sugar beet cultivars are monogerm hybrids. The use of monogerm sugar beet seed has greatly reduced the major effort needed to thin clusters of sugar beet seedlings. Monogerm seed was developed primarily through the work of displaced Russian scientist, V. F. Savitsky. In 1950-52 he developed two monogerm lines, SLC 101 and SLC 107, and found that the monogerm trait is controlled by a single recessive gene labeled *mm* (Coons et al. 1955). In North America, most commercial hybrids are either diploid, triploid or anisoploid in nature. Three types of hybrids have been developed. These include single, double and three-way crosses. The three-way crosses have become the dominant hybrid cultivars in the U.S. These hybrids combine desired yield, beet quality and disease resistance attributes (Smith, 1987; Hecker and Helmerick, 1985). Comparisons between equivalent diploid and triploid hybrids show similarity in yield of roots but, the triploids show less severe bolting (McFarlane, Skoyen and Lewellen, 1972). In a series of yield trials with diploid, triploid and tetraploid cultivars, the triploids produced the greatest level of sucrose. Sugar beets have a high level of self-incompatibility but there is a self-fertility gene which, when introduced, can create plants which are self-fertile (Smith, 1987).

Sugar beet seed production must ensure the isolation of the flowering sugar beet plants from foreign pollen. The Oregon Seed Certification isolation distance between sugar beets with different pollen sources is 3200 feet. The certification distance between sugar beets and pollinators of other *Beta* species (i.e., fodder beet, red beet, Swiss chard) is 8000 feet. Most U.S. commercial sugar beet seed production is carried out in northwestern Oregon in the Willamette Valley. The climate in the Willamette Valley is close to ideal for producing sugar beet seed. Strip plantings are made of female plants (CMS) and male pollinator plants. Wider strips are planted of the CMS parent than the pollinator parent. The roots produced by these plants are allowed to overwinter. During the second season of growth, the reproductive stage takes over and seed is produced on the bolting stalks of these plants. The climate in Oregon is cold enough for vernalization to take place during the winter but normally not cold enough to kill sugar beet roots. (Dexter 1996, personal communication).

V. PLANT PEST RISK ASSESSMENT AND THE DETERMINATION

The primary transformation event T120-7 is derived from the transformation of tissue culture line R01. Through traditional breeding with these fertile transformation events, individuals homozygous at the *pat* locus have been produced. These have been crossed with both commercially available public inbred lines and proprietary inbred lines. Traditional backcrossing and breeding will be used to continue to transfer the glufosinate-ammonium resistance locus in event T120-7 to a wide range of sugar beet varieties. Transformation event T120-7 has been field tested by AgrEvo USA Company since 1994 in the primary sugar beet growing regions of the United States. These tests have been conducted at approximately 68 sites under field release authorizations granted by APHIS (USDA authorizations: 94-347-01r; 96-052-02r; and, 97-029-01r). Transformation event T120-7 has also been field tested extensively in Europe, including Germany, Great Britain, France, and the Former Soviet Union. T120-7 has also been field tested in Canada. The great majority of the trials have been efficacy trials in which the plants have been sprayed with different rates of glufosinate-ammonium. When sprayed with the herbicide, plants, in trials worldwide, exhibited a tolerance to glufosinate-ammonium tolerance, indicating that the

gene is stably integrated and expressed. Based on information on the biology of sugar beet, data presented by AgrEvo and scientific data on other topics relevant to a discussion of plant pest risk, APHIS concluded the following regarding the properties of event T120-7.

A. The introduced genes, their regulatory sequences and their products in Event T120-7 do not present a plant pest risk

Construction of the vector pOCA18/Ac used to obtain the transgenic sugar beet event T120-7 is described in detail in Olszewski et al. (1988). The backbone of this plasmid is the broad host range vector pRK290. The synthetic version of *pat* gene originally characterized from *Streptomyces viridochromogenes* fused to promoter and terminator of CaMV was inserted into the genome of event T120-7.

The 35S promoter and terminator sequences, derived from CaMV, control expression of the *pat* gene. CaMV is a doublestranded DNA caulimovirus with a host range restricted primarily to cruciferous plants. The region of the CaMV genome used corresponds to nucleotides 6909 - 7437 for the promoter and nucleotides 7439 - 7632 for the terminator (Pietrzak et al., 1986). The 35S promoter directs high level constitutive expression in higher plants and is widely used as a promoter for high expression of genes (Harpster et al., 1988). The CaMV sequences, as used in glufosinate-ammonium tolerant sugar beet do not cause the sugar beet to become a plant pest.

The *pat* gene is a synthetic version of the same gene isolated from *Streptomyces viridochromogenes*, strain Tü 494 (Hara et al., 1991). A similar gene (*bar*) isolated from *S. hygroscopicus* (Thompson et al., 1987, Bayer et al., 1972) has been used for creating transgenic plants. Both genes encodes the enzyme phosphinothricin-N- acetyltransferase (PAT), which inactivates glufosinate-ammonium. Since the native *pat* gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesized using codons preferred by plants. The amino acid sequence of the enzyme remains unchanged. The nucleotide sequences of the native and synthetic gene share 70% homology.

The gene for aminoglycoside (3') phosphotransferase type II (APH(3') II) was first isolated from *Escherichia coli* (Beck et al., 1982). It mediates resistance to aminoglycoside antibiotics, e.g. kanamycin. This gene was used to select transformed calli of event T120-7. Its expression was driven by using regulatory sequences isolated from *Agrobacterium tumefaciens* which is a known plant pest. This gene is widespread in nature including organisms found in environment, soil and water. The gene is also widely spread among human and animal pathogens. Even if this gene was to transfer to other organisma no new gene combinations or environmental consequences are expected.

1. AgrEvo has analyzed the physical structure of the integrated genetic material in event T120-7 lines. (See the petition.) This analysis revealed that the vector DNA sequences of DNA was not present in the plant's genome. Southern and PCR analyses indicate that event T120-7 and its progeny contain 1 copy of the T-DNA from vector pOCA18/Ac. Furthermore, DNA from outside the T-DNA borders has not integrated into the genome (Horsch et al.1984, Zambryski 1988). Event T120-7 contains 1 copy of the *pat* and *aph (3') II* genes. The introduced coding regions do not confer a plant pest risk.

2. The introduction of the vector DNA does not present a plant pest risk in in event T120-7.

The vector system used to transfer the *pat* gene into the sugar beet nuclear genome, is based on plant pathogenic bacterium *A. tumefaciens*. It does not contain any sequences from the natural Tumor-Inducing (Ti) plasmid system used for plant infection and gene transfer and therefore are not a plant pest risk.

3. AgrEvo has presented evidence in its petition that the glufosinate resistance gene in the event T120-7 is integrated in its chromosomes and is transmitted through mitosis and meiosis in a Mendelian fashion, i.e., to a fashion consistent with integration of the added material into nuclear-chromosomal DNA. As integrated pieces of plant chromosomes, introduced foreign DNA is subject to the same rules governing chromosomal rearrangements and gene stability as other plant genes.

4. The sugar beet plants have been transformed with the glufosinate resistance gene and a selectable marker gene. The glufosinate resistance gene expressed in event T120-7 lines is a single insert of DNA chimera comprised of a 35S promoter derived from cauliflower mosaic virus, and the nopaline synthase 3' terminator from *A. tumefaciens.* The event T120-7 sugar beet contains the expressible selectable marker gene *aph (3')*II which was codes for aminoglycoside (3') phosphotransferase type II and isolated from *Escherichia coli.* This gene is wide spread relic in nature and is not a novelty to the environment. The promoter, leader, and the terminator sequences are all pieces of DNA sequence that are necessary for the expression of the introduced herbicide resistance and selectable marker genes in sugar beet plants. They themselves do not code for any gene product and are therefore not a plant pest risk.

B. Event T120-7 Sugar Beet Has No Significant Potential To Become A Weed.

Sugar beet does not possess the attributes most commonly found in many weeds (Baker 1965). Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans; but from this core, individual definitions differ (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without being deliberately cultivated). Baker also described many ideal characteristics of weeds. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed these characteristics to develop guidelines to address the weediness potential of transgenic plants. Both authors emphasize the importance of the parent plant and the nature of the specific genetic changes. Sugar beet is a biennial crop that is not persistent in undisturbed environments. The parental lines are not listed as a weed (Holm 1979, 1991), and introduction of the glufosinate resistance trait should not impart any new weedy characteristics. Event T120-7 lines are likely to be grown mostly in areas that are currently under sugar beet cultivation. In typical growing regions for the crop, the parent of event T120-7 has not established as a noxious weed. Even if the farmer is careful with the elimination of bolting plants, some may escape attention. These bolting plants could spread pollen. However, most of their volunteer progeny will be destroyed by winter or controlled by other herbicides. Sugar beet is a poor competitor compared to other plants and farmers can eliminate herbicide tolerant volunteer and weedy beets by crop rotation.

AgrEvo has collected data from greenhouse and field trials to support that the glufosinate tolerant sugar beet has little potential to become a serious or successful weed. Data provided in the petition indicate that the applicant has not observed any significant changes in the number of seeds produced, germination characteristics, final stand, over-wintering capability, or pathogen susceptibility. The event T120-7 has no higher potential to become a weed than its parent (Lee Panella, personal communication to J.R. Stander 1998).

C. Event T120-7 Sugar Beet Will Not Increase The Weediness Potential Of Any Other Plant With Which It Can Breed.

Except for California, sexually compatible wild relatives of the event T120-7 which can form viable hybrids with sugar beet are not found in U.S.A. APHIS considered the locations where sugar beet is grown and the potential for outcrossing to occur with wild or weedy relatives in those areas. Considering the cultural practices and seed production measures, the potential for the progeny of rare outcrosses between sexually compatible wild relatives and event T120-7 to persist in the environment and pose a weedy problem is not more than progeny of crosses between wild relatives and the nontransgenic sugar beets (Boudry et al 1993). It is unlikely that descendants of crosses between transgenic and non-transgenic sugar beets would inadvertantly become part of breeding material or become established in any sugar beet ecological community (USDA/APHIS, 1993).

In the Red River Valley of North Dakota subzero weather during winter months does not allow for the carryover of viable seeds (USDA/APHIS, 1993). Due to the mild climate, wild populations of *B. vulgaris L.* subsp. *maritima L, B. macrocarpa,* and *B. vulgaris L.* subsp. *vulgaris L.* have become established in California. McFarlane, (1975) identified the wild beets in Imperial County of California as *B. macrocarpa* rather than *B. vulgaris L.* subsp. *maritima L. Beta macrocarpa* is a species that occurs naturally in the Canary Islands and along the Mediterranean coastline. McFarlane (1975) reported the existence of naturally occurring hybrids between plants of *B. macrocarpa* and commercial sugar beets in the Imperial Valley. Abe, (1988) and Lewellen (personal communication to J.R. Stander, 1996), however, report that sugar beet and *B. macrocarpa* do not readily produce viable hybrids.

Johnson and Burtch, (1958) described sugar beets which evolved into annual plants and became a weed problem in California. Recent surveys indicate that such populations are restricted in size and appear to be localized in the Gilroy/Hollister area (Lewellen, personal communication, 1998). This is approximately 20 miles from the 500 acres of commercial sugar beet grown in Santa Clara County (USDA Census, 1992).

The odds of weedy beet outcrossing with commercially grown glufosinate-ammonium tolerant sugar beet are small. Should outcrossing occur, and fertile offspring result which express the trait of herbicide tolerance, these offspring would pose no greater risk of weediness than the present population of *B.vulgaris ssp maritima* in the absence of glufosinate-ammonium application. Such glufosinate-tolerant weeds can be controlled by physical methods or other herbicides like glyphosate.

Sugar beet is a biennial crop. The second season's crop produces seed. Certain conditions such as

low temperatures after planting during the prior harvest season and longer day length can cause the sugar beet to "bolt" or produce a seed stalk during the first growing season (Bell, 1946; Jaggard, Wickens, Webb and Scott, 1983; Durrant and Jaggard, 1988). These situations exist in Europe, especially England when growers seed too early in the spring. They also can occur in California where sugar beets are often seeded during the fall and winter months or when crops planted in the spring are overwintered. Bolters are a problem in the crop planted for sugar because, although inflorescences (bolting stalk) may be cut off, the bolted plants contain more lignin in their roots and sugar yield could be reduced by 50% (Scott and Wilcockson, 1976; and Jaggard et al. 1983). Unwanted seed germination in sugar beet can be controlled by various methods such as hand pulling, or treatment with non-selective herbicides.

In a review of the State Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act, no reference was found regarding "wild" sugar beets or wild sugar beet relatives as either restricted or noxious weeds (Holm 1979). This demonstrates that sugar beet does not have the necessary attributes which could allow it to become a serious weed problem in sugar beet growing areas (Tiedje et al. 1989, Keeler 1989). There are wild species of sugar beets in the region surrounding the Mediterranean Sea and in the Caucasus Mountains of Russia and into Turkey and Iran. Wild species may also be found as far west as the Canary Islands. As discussed previously, no wild relatives exist in the U.S. except in a few locales in California (Doney, 1996; Cooke and Scott, 1993).

Except under herbicide application, the novel trait of glufosinate-tolerance imparted to event T120-7 lines itself or transferred to a wild or weedy relative by outcrossing does not confer any characteristic that is likely to be favored by natural selection, in either managed or unmanaged habitats. Under the circumstances of glufosinate application, event T120-7 its, progeny and out crossed weedy relative could survive but there are other herbicides that are on market to control them.

D. Event T120-7 Sugar Beet Will Not Cause Damage To Raw Or Processed Agricultural Commodities.

Information provided by AgrEvo regarding the components and processing characteristics of event T120-7 lines revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity.

E. Event T120-7 Sugar Beet Is Not Harmful To Beneficial, Threatened or Endangered Organisms.

There is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of event T120-7 lines. The PAT enzyme, expressed in event T120-7 lines of sugar beet , is not known to have any toxic properties. Field observations of event T120-7 lines revealed no negative effects on nontarget organisms, suggesting that PAT enzyme in the tissues of the line is not toxic to beneficial organisms. Knowledge of this enzyme's mode of action, and the lack of known toxicity for this protein suggest no potential for deleterious effects on beneficial organisms, such as bees and earthworms. The high specificity of PAT for its substrate makes it

unlikely that PAT would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

F. Event T120-7 Sugar Beet Will Not Adversely Impact Biodiversity.

As detailed in the sections above, we have concluded that event T120-7 sugar beet is no more likely to become a weed than other sugar beet varieties developed by traditional breeding. It is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which this line may interbreed. In the absence of herbicide treatment, viable offspirings produced from transgenic pollen flow from event T120-7 to weedy relatives would have no fitness enhancement over current populations of wild or weedy beets which occur naturally (Purrington and Bergelson, 1977). It will not harm threatened and endangered species and non-target organisms. Biological diversity is the variety and variability among living organisms and the ecological complexes in which they occur. The glufosinate tolerance trait when present in event T120-7 sugar beet or in any other sexually compatible species would confer no competitive advantage in unmanaged environments, and thus is not expected to have an ecological impact. Based on this analysis, APHIS concludes that the potential impact on biodiversity of event T120-7 sugar beet is equivalent to that of currently commercialized sugar beet varieties.

G. Event T120-7 Sugar Beet Will Not Adversely Affect Current Agricultural Practices

Glufosinate-ammonium, a non-selective herbicide, will provide control of most annual grass and broadleaf weeds in glufosinate-ammonium resistant sugar beet. Glufosinate-ammonium will control larger broadleaf weeds than currently available herbicides, thus allowing more application flexibility when environmental conditions prevent the timely application required by today's herbicides. In addition, glufosinate-ammonium will provide a different herbicide mode of action in the growers' crop rotation, which is important in preventing the build up of herbicide resistant weeds. Glufosinate-ammonium is applied like any other postemergent herbicide used in any other crop. Glufosinate-ammonium tolerant sugar beet will alter current sugar beet cultivation practices in that it will allow for reduced herbicide use than currently is practiced in order to achieve the same crop yield.

VI. CONCLUSION

APHIS has determined that event T120-7 lines developed by AgrEvo will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of event T120-7 lines or their progeny. Importation of event T120-7 lines and nursery stock or seeds capable of propagation is still, however, subject to the restrictions found in the Foreign Quarantine notice regulations at 7 CFR Part 319, just as it applies to any other importation of sugar beet seed. This determination has been made based on data collected from these approved field trials, laboratory analyses and literature references presented herein which demonstrate that:

1. Neither the synthetic (*pat)* gene, its product phosphinothricin-N- acetyltransferase, nor the regulatory sequences confer on event T120-7 or its progeny any plant pest characteristic. Although DNA from pathogenic organisms was used in its development, event T120-7 and plants derived from it are not infected by this organisms. This line can not incite disease in other plants.

2. Event T120-7 is no more likely to become a weed than herbicide-tolerant sugar beets if they were to be developed by traditional breeding techniques. Sugar beet is not a weed, and there is no reason to believe that the introduced gene conferring tolerance to the herbicide glufosinate would enable sugar beet to become a weed pest.

3. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination. Multiple factors ensure that gene introgression from event T120-7 into wild plants in the United States and its territories is extremely unlikely. There are no significant populations of sexually compatible species of beets in the United States and its territories. Existing populations of wild beet relatives in the United States are small and genetically as well as physically isolated. Even in these regions, potential gene introgression from event T120-7 into wild relatives is not likely to increase the weediness potential of any resulting progeny nor adversely effect biodiversity or genetic diversity of related plants any more than would introgression from traditional sugar beet lines.

4. There is no reason to believe that event T120-7 sugar beet will have a significant adverse impact on organisms beneficial to plants or agriculture, or other nontarget organisms, or will harm threatened or endangered species.

5. The use of event T120-7 sugar beet is unlikely to have any significant adverse impact on agricultural practices in the United States. The use of event T120-7 sugar beet or products derived from it will not cause damage to raw or processed agricultural commodities.

APHIS has also concluded that there may be new varieties bred from event T120-7. However, if such varieties are developed they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for glufosinate tolerant sugar beet already field tested, or those observed for sugar beet varieties developed from traditional breeding.

Arnold Foudin for

Rebecca A. Bech
Assistant Director, Scientific Services
Operational Support, Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

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