USDA/APHIS Environmental Assessment

Monsanto Company and KWS SAAT AG Petition 03-323-01p for Determination of Non-regulated Status for Roundup Ready® Sugar Beet Event H7-1

October 2004

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 03-323-01p) received from Monsanto Company and KWS SAAT AG seeking a determination of non-regulated status for their genetically engineered sugar beet designated as event H7-1 (OECD unique identifier KM-ØØØH71-4) under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers tolerance to the herbicide glyphosate.

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

Date:

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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 03-323-01p) from Monsanto Company (St. Louis, MO) and KWS SAAT AG (Einbeck, Germany) (hereafter KWS) seeking a determination of non-regulated status for their transgenic Roundup Ready® sugar beet designated as event H7-1 (OECD unique identifier KM-ØØØH71-4). Monsanto Company and KWS seek a determination that event H7-1 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 H7-1 was engineered to be glyphosate tolerant by inserting a gene for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the sugar beet genome. The gene is from the common soil bacterium *Agrobacterium* sp. strain CP4 and was introduced into these sugar beets via an *Agrobacterium*- mediated transformation protocol.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of event H7-1. It does not address the separate issue of the potential use of the herbicide glyphosate in conjunction with these plants. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Food and Drug Administration (FDA) has authority over food and feed issues of all genetically improved plants used as food or feed.

Field trials of H7-1 sugar beet have been conducted under APHIS notification procedures (7 CFR 340.3). In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR 372), this EA has been prepared prior to issuing a determination of nonregulated status for H7-1 sugar beet in order to specifically address the potential for impact to the human environment through unconfined cultivation and use of the regulated articles in agriculture.

A previous petition for determination of non-regulated status was granted for glyphosate tolerant sugar beets in 1998 (Petition 98-173-01p from Novartis Seeds and Monsanto Company). That sugar beet event, GTSB77, contained, in addition to the *Agrobacterium cp4 epsps* gene, a ß-glucuronidase gene from *E. coli* and a partial glyphosate oxidoreductase gene from *Ochrobactrum anthropi*, another common soil bacterium. Event GTSB77 sugar beet has not, however, been grown commercially in the U.S.

II. INTRODUCTION

A. Development of event H7-1 Sugar Beet

Monsanto and KWS have submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS (APHIS number 03-323-01p) for genetically engineered sugar beets that are tolerant to the broad spectrum herbicide glyphosate. Glyphosate tolerant sugar beets would offer farmers a new option for weed control.

The management of weeds in sugar beet fields can be an expensive, labor intensive, and sometimes complicated operation. Often farmers use pre-emergent herbicides that will stop weed seeds from germinating. However, this assumes that weeds will always be a problem in all parts of the field. With H7-1, farmers will have the option of applying herbicide after weeds have germinated and only in the areas of the field where there are weeds. Glyphosate is one of the most environmentally friendly herbicides.

These sugar beets were genetically engineered to be glyphosate tolerant by inserting a gene (from *Agrobacterium* sp. strain CP4) that codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the sugar beet genome. This gene, along with its regulatory sequences, was introduced into these sugar beets via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure that has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

APHIS authorized the first field testing of these sugar beets starting in 1998 and they have been field tested in the United States under the APHIS authorization numbers noted in Appendix B. Event H7-1 sugar beets have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. APHIS Regulatory Authority.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. 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. These sugar beets have been considered regulated articles because they contain non-coding DNA regulatory sequences derived from plant pathogens and the vector agent used to deliver the transforming DNA is a plant pathogen.

Section 340.6 of the regulations, entitled "Petition 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 therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. U.S. Environmental Protections Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority

This genetically engineered sugar beet plant and the use of glyphosate are also subject to regulation by other U.S. government agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA.

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Monsanto/KWS submitted a food and feed safety and nutritional assessment summary for event H7-1 in April 2003. A final FDA decision is pending.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of H7-1 sugar beets as regulated articles under APHIS regulations. The developer of these sugar beets, Monsanto and KWS, submitted a petition to USDA, APHIS requesting that APHIS make a determination that these sugar beets shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not come to a determination that these sugar beets are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of H7-1 lines of glyphosate tolerant sugar beets. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of glyphosate tolerant sugar beets.

B. Determination that H7-1 Sugar Beets are No Longer Regulated Articles, in Whole

Under this alternative, these glyphosate tolerant sugar beets would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of glyphosate tolerant sugar beets. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq*.; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

C. Determination that H7-1 Sugar Beets are No Longer Regulated Articles, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

1. <u>Approval of some but not all lines requested in the petition</u>. In some petitions, applicants request deregulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others. This request is only for the single event H7-1 and its progeny.

2. <u>Approval of the petition with geographic restrictions</u>. APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved line could only be grown without APHIS authorization in certain geographic area.

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of event H7-1 and its progeny in the absence of confinement.

A. Alternative A: No Action

If APHIS takes no action, commercial scale production of event H7-1 and its progeny is effectively precluded. These plants could still be grown in field trials for variety development as they have been for the past several years under APHIS authorizations (notifications). APHIS has evaluated field trial data reports submitted on event H7-1 and its progeny and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics and no effect on the health of other plants. The Agency expects that future field tests would perform similarly.

With respect to commercial production, if APHIS were to take no action, sugar beet growers would still have the same options available to them for weed control in their fields as they currently have. Control measures can be complicated by growth stage of specific weeds, growth stage of the sugar beets and field environmental conditions as some of the herbicides in current use have quite specific applications to grasses and/or broad leaf weeds. USDA/NASS statistics collected from sugar beet growers most recently (2000) document significant use of 12 herbicides, other than glyphosate, used in sugar beet fields in the 11 states surveyed. Planted area in those primary sugar beet-producing states covered 1.56 million acres. Those herbicides are listed in the following table taken from USDA/NASS statistics (http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcs0501.txt.)

Agricultural Chemical	Area Applied	Appli-cations	Rate per Application	Rate per Crop Year	Total Applied
	Percent	Number	Pounds per Acre		1,000 lbs
Herbicides:					
Clethodim	46	2.5	0.04	0.11	77
Clopyralid	74	2.8	0.03	0.09	102
Cycloate	5	1.0	1.84	1.84	139
Desmedipham	94	2.8	0.07	0.18	270
EPTC	6	1.0	2.61	2.64	230
Ethofumesate	37	2.1	0.06	0.14	82
Glyphosate	13	1.1	0.39	0.43	86
Phenmedipham	80	2.6	0.05	0.14	170
Pyrazon	6	1.0	0.82	0.85	76
Quizalofop-ethyl	10	1.6	0.04	0.06	9
Sethoxydim	11	1.7	0.19	0.33	56
Trifluralin	5	1.0	0.65	0.66	55
Triflusulfuron	83	2.7	0.008	0.02	29

States and production acres (in parentheses) included in that survey are CA (98,000), CO (71,500), ID (212,000), MI (189,000), MN (490,000), MT (60,700), NE (78,200), ND (258,000), OR (16,200), WA (28,400) and WY (61,000).

The applicant submitted information in Tables VII-6, VII-7, VII-8, VII-9, and VII-10 of the petition that document the varieties of herbicide label precautions, crop injury warnings, applicator and handler protective equipment required, presence of known tolerant weed biotypes, potential toxicities to fish, aquatic invertebrates and plants and potential water quality effects of the listed herbicides. All of the current herbicides used on sugar beets have more significant issues in one or more categories relating to toxicity or water quality than glyphosate. Growers are averaging well over 2 applications per year of five herbicides on a significant number of acres (clethodim, clopyralid, desmidipham, phenmedipham, and triflusulfuron). Documented chemical herbicide application in that year (2000) totaled over 1.3 million pounds and it is likely that that approximate rate would continue.

In addition to chemical control measures, growers would also likely continue use of tillage and hand weeding for control.

B. Alternative B: Approval of the petition, in whole

If APHIS were to grant the petition for non-regulated status in whole, sugar beet event H7-1 and its progeny would no longer be considered regulated articles. APHIS' assessments of the environmental impacts are discussed in the following sections.

1. Plant pathogenic properties

APHIS considered the potential for the transformation process, the introduced DNA sequences or their expression products to cause or aggravate disease symptoms in sugar beet event H7-1 and its progeny or in other plants. We also considered whether data indicate that unanticipated unintended effects would arise from engineering of these plants. APHIS considered information from the scientific literature as well as data provided by the developer when conducting their field trials.

Recipient organism

The plant material used for development of event H7-1 was KWS proprietary sugar beet line 3S0057. The initial plant, selected for tolerance to glyphosate, was designated H7-1 and the breeding line developed from this event was designated as 6401VH. The breeding history and progeny resulting from line 6401VH can be found in Figure III-2, p. 21 of the petition. Sugar beet is not listed as a Federal Noxious weed or on other weed lists (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html, http://www.nwcb.wa.gov/weed_list/weed_listhome.html, http://www.extendinc.com/weedfreefeed/list-b.htm, http://www.weedawareness.org/weed%20list.html, http://www.ag.ndsu.nodak.edu/weeds/w253/w253-2a.htm#North%20Dakota%20Noxious%20Weeds).

Transformation system

Event H7-1 was developed using a disarmed *Agrobacterium*-mediated transformation system of sterile sugar beet seedling cotyledons. Post-transformation, *Agrobacterium* were eliminated from tissues by a seven week culture on antibiotic-containing medium. Glyphosate was used to select for transformed tissues containing the *epsps* gene construct. This technique using disarmed *Agrobacterium* followed by selection has a history of safe use and has been used for transformation of a variety of plant tissues for over 20 years (Howard, *et al.*, 1990).

DNA sequences inserted into sugar beet event H7-1

Data supplied in the petition and reviewed by APHIS (Section V.A., pp 29-44) support the conclusion that event H7-1 contains the following sequences: 1) a 35S promoter from a modified figwort mosaic virus (P-FMV), 2) coding sequence for a chloroplast transit peptide from *Arabidopsis thaliana*, 3) the 5-enolpyruvylshikamate-3-phosphate synthase gene (*epsps*) from *Agrobacterium* sp. strain CP4, and 4) DNA containing polyadenylation sequences from the 3' non-translated region of the *Pisum sativum* (pea) *rbc*S E9 gene. The non-coding 35S promoter is from the plant pathogen figwort mosaic virus. This sequence, however, cannot cause plant disease and serves a purely regulatory function for the *epsps* gene. The *epsps* gene is from the soil-inhabiting bacterial plant pathogen, *Agrobacterium* sp. strain CP4. It encodes the EPSPS protein which functions to impart tolerance to the broad spectrum herbicide glyphosate. It does not cause disease and has a history of safe use in a number of genetically engineered plants (e.g., corn, cotton and soybean varieties).

Evaluation of intended effects

As expected, as a result of introduction of the *epsps* gene into the sugar beet genome, the resulting plants are tolerant to glyphosate, the active ingredient in the herbicide Roundup®.

Analysis of inheritance: Data was provided and reviewed by APHIS that demonstrates stable integration and inheritance of the *epsps* gene and its associated regulatory sequences over several breeding generations. Statistical analyses show that glyphosate tolerance is inherited as a dominant trait in a typical Mendelian manner (petition Table V-2, pp. 45-46).

Analysis of gene expression: Data on EPSPS (5-enolpyruvylshikamate-3-phosphate synthase) protein concentrations was collected from field trials conducted at several locations. Using standard laboratory ELISA techniques, protein concentrations from beet leaves and processed roots (brei) were determined (petition Table V-3, p. 50). EPSPS protein concentrations averaged 161 µg/gram in the leaves and 181 µg/gram in brei. EPSPS is ubiquitous in plants and microorganisms and has not been associated with hazards from consumption or to the environment. Crops that contain this protein and have been granted non-regulated status have included corn, soybean, cotton, rapeseed and sugar beet (http://www.aphis.usda.gov/brs/not_reg.html). In 2004, significant acreages of corn (10.3 million acres or 11% of the total), upland cotton (4.1 million acres or 30% of the total) and soybean (62.6 million acres or 85% of the total) grown in the U.S. were planted with herbicide tolerant varieties (http://usda.mannlib.cornell.edu/). Although the data include all herbicide tolerant varieties, glyphosate tolerant ones (containing EPSPS) predominate. All have also undergone review by FDA (http://www.cfsan.fda.gov/~lrd/biocon.html).

Analysis of the intended trait: Numerous field trials were conducted in the U.S. (Appendix 2 of this EA) and in Europe (petition Table VI-7) to evaluate event H7-1 in different genetic backgrounds and in different environments. Standard field trials evaluated 1) agronomic performance, 2) disease and pest resistance performance, 3) steckling (seedling) production and 4) seed multiplication. Standard industry farming practices for the various locales was utilized in these trials. These practices would typically include control measures for weeds, diseases and insects. Where Roundup® was used in trials, no negative impacts from application of Roundup® were noted.

Analysis of possible unintended effects: Expression of EPSPS in event H7-1 sugar beet is not expected to cause plant disease or influence susceptibility of H7-1 or its progeny to diseases or other pests. Both qualitative and quantitative data addressing disease susceptibility and overall agronomic performance were collected in order to assess possible effects from introduction of the *epsps* gene and its associated regulatory sequences. The petitioner has described these trials, conducted over several years in a variety of locations, and presented this data in Section VI of the petition (starting on p. 53). Out of 98 trials conducted in the U.S. from 1998-2001, differences in disease susceptibility of some H7-1 progeny were noted in six trials. At three trial sites increased susceptibility to powdery mildew was noted while at three other sites decreased susceptibility was noted. Given the interactions between the environment, the genetic backgrounds of the cultivars used and some inherent genetic variability within sugar beet varieties, APHIS believes that these results are not unexpected and do not indicate an increased pest risk. A similar likely insignificant difference was noted in a greenhouse trial using different *Fusarium* fungus isolates. Other researchers have suggested that it may be difficult to predict field results from greenhouse/

laboratory experiments (Estok *et al*., 1989; Wan *et al*., 1998). European trials conducted in Germany and France over 2 years, using regionally adapted conventional sugar beet lines and genetically similar lines to H7-1, noted no differences in susceptibility to 10 different sugar beet pests (Table VI-7, p. 70). Other phenotypic characterizations comparing H7-1 lines with conventional and control lines were also completed. Data was provided and assessed by APHIS on leaf color, leaf size, hypocotyl color, seed germination and dormancy, vernalization, bolting, flowering onset and seed harvest date. No qualitative or quantitative observations indicated any biologically meaningful differences from control lines or differences outside the range of conventional sugar beet norms.

Al-Kaff, et al.(1998) have noted gene silencing effects when transgenic plants have been infected by a virus with DNA sequence homology to a portion of the introduced genes. None of the viral diseases of beet is related to figwort mosaic virus (http://image.fs.uidaho.edu/vide/refs.htm and Whitney and Duffus, 1986) (a *caulimovirus* and from which the promoter for the *epsps* gene originates) so silencing of the *epsps* gene should not occur.

In addition to field studies on agronomic parameters, Monsanto/KWS analyzed sugar beets for compositional changes as part of their submission to FDA in the consultation process. While FDA uses these data as indicators of possible nutritional changes, APHIS views them as a general indicator of possible unintended changes. Compositional analyses evaluating carbohydrates, proteins, fiber, fat, sugars and eighteen amino acids (a total of 55 statistical comparisons) identified seven statistically different values compared with the near isogenic control line. All analyses fell within the range of values observed for both the near isogenic control line and conventional sugar beet varieties, providing additional evidence that event H7-1 sugar beet does not exhibit unexpected or unintended effects.

Potential impacts on the relative weediness of event H7-1 compared to traditionally bred sugar beets

APHIS assessed whether H7-1 sugar beet is any more likely to become a weed than the non-transgenic recipient sugar beet line or other currently cultivated sugar beet. The assessment considers the basic biology of sugar beet and an evaluation of unique characteristics of H7-1 sugar beet.

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). The parent plant in this petition, *Beta vulgaris* L. ssp. *vulgaris*, is not listed as a weed by the Weed Science Society of America (1992) nor is it listed as a noxious weed species by the U.S. Federal Government (7 CFR Part 360). Occasionally, sugar beets volunteer in fields the year after harvesting. These plants can be controlled by mechanical means or several other registered herbicides beside glyphosate that can be used on sugar beet volunteers (Crop Protection Chemical Reference, 1996). Sugar beets possess few of the characteristics of plants that are notable of successful weeds (Baker, 1965; Keeler, 1989).

As part of a bilateral agreement between the United States and Canada, USDA/APHIS and the Canadian Food Inspection Agency (CFIA) have generated documents that outline basic data requirements for developers of genetically engineered plants. One of these documents, Appendix II, outlines the environmental characterization data requirements for unconfined releases. As a part of the entire package requesting a determination of non-regulated status, these data are designed to address characteristics that influence reproductive and survival biology of the transgenic plant compared to its non-transgenic counterpart.

In trials conducted in both the U.S. and Europe, no differences were observed between H7-1 lines and non-transgenic lines with respect to the plants ability to persist or compete as a weed. APHIS considered data relating to plant vigor, bolting, seedling emergence, seed germination, seed dormancy and other characteristics that might relate to increased weediness. No unusual characteristics were noted that would suggest increased weediness of H7-1 plants. Additionally, no characteristics relating to disease or insect resistance that might effect weediness were noted that were consistent over all trial locations. H7-1 sugar beet is still susceptible to the typical insect and disease pests of sugar beet.

Potential Impacts from Outcrossing of Line H7-1 to Wild Relatives

APHIS evaluated the potential for gene introgression to occur from H7-1 to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Sugar beets are sexually compatible with several other *Beta* species (OECD, 2001). The centers of origin for *Beta vulgaris* is generally believed to be in the Mediterranean or Near East region and no *Beta* species are known to be native to the U.S. (OECD, 2001).

Although sugar beets have escaped cultivation and their progeny have persisted in the environment for many years (especially in California), these plants are not serious weed problems (Johnson and Burtch, 1958; Panella, 2003). Some of these plants are found in the San Francisco Bay area where sugar beets are no longer cultivated. Another population of sexually compatible plants is in the Imperial Valley of California. The movement of the glyphosate tolerance trait from H7-1 to any other sexually compatible *Beta vulgaris* should not have a significant impact especially if glyphosate is not applied to these plants. APHIS cannot find any evidence that herbicides are applied routinely to these plants living outside cultivated areas. Even if these plants become tolerant to glyphosate there are other registered herbicides that can be used to kill them and other methods of control can still be used (OECD, 2001). Further consideration and discussion of the potential for introgression of genes from sugar beet into related species can be found in Appendix I.

Potential Impact on Threatened or Endangered Species or Non-target Organisms Including Beneficial Organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target organisms, including those on the U.S. Fish and Wildlife Service (FWS) Threatened and Endangered Species (TES) list, from cultivation of H7-1 sugar beet and its progeny. The enzyme EPSPS that confers glyphosate tolerance is from the bacterium *Agrobacterium* sp. strain CP4. This gene is similar to the gene that is normally present in sugar beets and is not known to have any toxic property. Field observations of event H7-1 revealed no negative effects on non-target 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.

Even though the likelihood of toxicity is low for the CP4 EPSPS protein, a number of researchers have conducted laboratory investigations with different types of arthropods exposed to genetically engineered crops containing the CP4 EPSPS protein (Goldstein, 2003; Boongird et al., 2003; Jamornman, et al., 2003; Harvey et al., 2003). Representative pollinators, soil organisms, beneficial arthropods and pest species were exposed to tissues (pollen, seed, and foliage) from GE crops that contain the CP4 EPSPS protein. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to these crops (Nahas et al., 2001; Dunfield and Germida, 2003, Siciliano and Germida 1999).

EPSPS has received an exemption from tolerance requirement from the EPA on all raw agricultural commodities (http://www.epa.gov/fedrgstr/EPA-PEST/1996/August/Day-02/pr-840.html). APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms. The petitioners have addressed potential impacts on non-target species particularly looking at use of event H7-1 sugar beet in comparison to current weed control practices using multiple herbicides (p. 95, Table VII-7, Table VII-8, Table VII-9).

From the above analysis of both qualitative and quantitative information from the petition and published data, APHIS concludes that the unconfined release of H7-1 and its progeny would not harm any non-target or threatened or endangered species in the United States. Consistent with APHIS' Fish and Wildlife Service TES assessment requirements, this is a "no harm" decision.

Potential Impacts on Agricultural and Cultivation Practices.

Current weed control practices in sugar beet can be somewhat complicated and are considered by many growers to be their most significant problem (Dexter and Luecke, 2003). Current practices include mechanical tillage, hand weeding and both pre-and post-planting application of broadleaf and grass herbicides. Each of these practices has its limitations and can be significantly impacted by growing conditions, soil pH, target weed size, crop size, etc. Some of the current herbicides can also leave significant soil residues that can limit subsequent crop rotation options (http://www.uidaho.edu/sugarbeet/weed/herbicides%20for%20sugar%20beets.pdf and in the petition Table VII-6). Roundup® (glyphosate), a non-selective herbicide, would provide post-planting control of most annual grass and broadleaf weeds in glyphosate resistant sugar beet under a wide range of growing conditions. Glyphosate would control larger broadleaf weeds than currently available herbicides and allow more application flexibility when environmental conditions prevent the timely application required by some currently used herbicides. In addition, glyphosate would provide a different herbicide mode of action in the growers' crop rotation, which is important in preventing the development of herbicide resistant weeds. Glyphosate is applied like any other post emergent herbicide used in any other crop. Glyphosate tolerant sugar beet may 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.

Potential impacts on organic farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods.

The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic sugar beets, will be significantly impacted by the expected commercial use of this product since: (a) non-transgenic sugar beet will likely still be sold and will be available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed and labeled as glyphosate tolerant.

No transgenic varieties of sugar beet are currently in commercial production. Varieties derived from event H7-1 should not present new and different issues with respect to impacts on organic farmers. With the exception of seed production fields, sugar beets do not typically flower in their one year production cycle, therefore, the likelihood of cross pollination to organic fields is unlikely. Current seed certification standards (http://www.oscs.orst.edu/standards/Sugar%20Beets%20Standards.pdf) are sufficient to address this issue.

Potential Damage to Raw or Processed Agricultural Commodities.

APHIS review of the information provided by the applicant regarding the components and processing characteristics of these plants revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity. APHIS believes that the modifications for herbicide tolerance should not affect this commodity in any significant manner.

Other Environmental Statutes and Considerations

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects. Each alternative was analyzed in its ability to affect minority and low-income populations. None of the alternatives were found to pose disproportionately high or adverse human health or environmental effects to any specific minority or low-income group.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. None of the alternatives is expected to have disproportionately high or adverse human health or environmental effects to children.

EO 13112, "Invasive Species", requires that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. The non-engineered plant is grown in the U.S. and based on the data submitted by the applicant and reviewed by APHIS, the engineered plant is not significantly different in any fitness characteristics from its parent that might increase its invasive potential.

In 1992, world leaders agreed on a strategy for "sustainable development". One feature of this agreement was the Convention on Biological Diversity (CBD). The U.S. government has not ratified the CBD. One part of the CBD is the Cartegena Protocol on Biosafety which became effective on September 11, 2003. The Biosafety Protocol is designed to ensure an adequate level of safety in the transfer, handling and use of "living modified organisms" addressing the potential adverse effects on conservation, sustainable use of biological diversity, taking into account risks to human health. Signatory countries are required to implement a system to address these issues. (The CEQ report on incorporating biodiversity considerations into environmental impact analysis under the National Environmental Policy Act are similar to those addressed by the Biosafety Protocol).

Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that event H7-1 sugar beets exhibit no traits that would cause increased weediness, its cultivation should not lead to increased weediness of other cultivated sugar beet or other sexually compatible relatives, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it is unlikely that event H7-1 sugar beet or its progeny will pose a significant impact on biodiversity.

Consideration of Potential Environmental Impacts Associated With the Cultivation of Event H7-1 outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of non-regulated status for H7-1 sugar beet. *Beta vulgaris* ssp. *maritima* (wild sea beet) is a problem weed in coastal regions of the Mediterranean Sea and North Sea in Europe and in Asia. In other examples, natural hybrids between cultivated sugar beet and resident species have occurred to produce weed beets in commercial operations. For example, hybrids between *Beta macrocarpa* and commercial sugar beets are a weed problem in production fields (Hultén and Fries, 1986) and natural hybrids have also occurred between cultivated sugar beets (*Beta vulgaris* ssp. *vulgaris*) and wild beets (*Beta vulgaris* ssp. *maritima*) in Europe. This has resulted in a hybrid form of "weed beet" that can bolt in a single season, while growing among biennial sugar beet varieties (Parker and Bartsch, 1996). Weed beet populations are described as possessing domesticated characteristics such as wider leaves and an annual growth habit (Lange *et al*., 1999). Weed beets cause yield losses and can delay harvest (Bartsch *et al*., 2003).

Any international traffic in sugar beet subsequent to this determination would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed 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. In addition, issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations, including within 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 also participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development (OECD). APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico, and has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology. APHIS has sponsored several workshops on safeguards for planned introductions of transgenic crops most of which have included consideration of international biosafety issues. It should also be noted that all the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new sugar beet cultivars internationally, apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR Part 340.

C. Determination that H7-1 Sugar Beets are No Longer Regulated Articles, in Part

If APHIS were to grant the petition for non-regulated status in part, sugar beet event H7-1 and its progeny would no longer be considered regulated articles, with some restriction. Given that the request from the petitioners is for a single event, H7-1, an in-part determination would be limited to a geographic restriction. All of the environmental considerations under Part B would be applicable to such a determination.

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VII. CONSULTATIONS

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Appendix A: Sugar Beet Biology and the potential for introgression into related species

Sugar beets, *B. vulgaris* L. ssp. *vulgaris*, are a member of the family *Chenopodiaceae* which also includes the leaf beet (Swiss chard), and the red table beet (fodder beet), from which the sugar beet was derived (Cooke and Scott, 1993; OECD, 2001). Members of this family are dicotyledonous and usually herbaceous in nature. Sugar beets are grown world wide. Sugar beet is largely wind pollinated and is normally a biennial that develops a large succulent root the first year and a seed stalk the second. Since it is normally harvested during the first year while still in the vegetative phase, flowers rarely develop. 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 *et al*., 1983; Durrant and Jaggard, 1988).

The genus *Beta*, including wild relatives, is divided into four sections with various species and subspecies (Lange *et al*., 1999; Frese, 2001): Section Beta with *B. vulgaris* ssp. *vulgaris*, ssp. *maritima*, and ssp. *adanensis*, *B. macrocarpa*, and *B. patula*: Section Corollinae with *B. corolliflora, B. macrochiza, B. lomatogona*, *B. intermedia* and *B. trigyna*, and Section Procumbentes with *B. patellaris, B. procumbens*, *B. webbiana*, and Section Nanae with *B. nana*. Sugar beet hybridizes freely with all members of the section *Beta* and the resulting progeny are fully fertile. Hybrids between sugar beet and members of the other three sections do not naturally occur without human intervention. Artificial hybrids can be produced with difficulty with species in Section *Corollinae*; however, these hybrids are highly sterile and set few seeds when backcrossed to sugar beet. Hybrids between sugar beet and Section *Procumbentes* members normally die at the seedling stage. No hybrids between cultivated beets and *B. nana* have been reported. Therefore, natural crosses between cultivated sugar beet and species from Sections other than Beta are highly unlikely.

In the genus *Beta* there are different examples of weediness. For example, the species itself can be a serious weed. According to Holm *et al*. (1979; 1991), *B. vulgaris* (subspecies not given) is classified as an occasional to serious weed in the following countries: Afghanistan, Australia, Mexico, Morocco, the United States, Iraq, Israel, Portugal, and Egypt. Subspecies *maritima* (wild sea beet) is a problem weed in coastal regions of the Mediterranean Sea and North Sea in Europe and in Asia. In other examples, natural hybrids between cultivated sugar beet and resident species have occurred to produce weed beets in commercial operations. For example, hybrids between *Beta macrocarpa* and commercial sugar beets are a weed problem in production fields (Hultén and Fries, 1986) and natural hybrids have also occurred between cultivated sugar beets (*Beta vulgaris* ssp. *vulgaris*) and wild beets *(Beta vulgaris* ssp. *maritima*) in Europe. This has resulted in a hybrid form of "weed beet" that can bolt in a single season, while growing among biennial sugar beet varieties (Parker and Bartsch, 1996). Weed beet populations are described as possessing domesticated characteristics such as wider leaves and an annual growth habit (Lange *et al*., 1999). Weed beets cause yield losses and can delay harvest (Bartsch *et al*., 2003).

Possible movement of the transgenes via pollen from event H7-1 to other members of the Beta section would be species and geographically specific. Movement of the transgenes to *B. vulgaris* ssp. *adanensis*, ssp. *maritima*, and *B. patula* is not likely in the United States since these plants are not found in the Americas. Based on a search for wild *Beta* populations in the United States (http://www.natureserve.org/explorer) two species are know to occur. *Beta procumbens*, occurs only in Pennsylvania and *Beta vulgaris* is known to have escaped from cultivation in the following states: Alabama, California, Connecticut, Maine, Massachusetts, Michigan, Missouri, Montana, New Hampshire, New York, Oregon, Pennsylvania, Texas and Utah. The largest significant populations occur in California. Wild beets are found from the San Francisco Bay area to the Mexican border (Bartsch and Ellstrand, 1999), concentrated mainly in the Bay area, and in the Imperial valley. The Californian wild or weed beets belong to two different taxa, *B. vulgaris* and *B. macrocarpa,* and have at least three different origins (Bartsch *et al.*, 2002). They evolved from escaped Swiss chard or red beet, from *B. macrocarpa*, or from hybridization of *B. vulgaris* with introduced *B. macrocarpa*.

Hybridization of H7-1 with the wild *B. procumbens* in Pennsylvania is unlikely to occur due to species incompatibility (see above). In California, sugar beet plants, *B. vulgaris,* that escaped from past commercial cultivation in the San Francisco Bay area are unlikely to cross with H7-1 since sugar beets are no longer in commercial production in the Bay area. Thus transgene movement via pollen to these plants is highly unlikely.

The situation in the Imperial Valley of California is more complex. Sugar beet production continues in the Imperial Valley and is a major center of production. There are free living sugar beets that have escaped cultivation and have persisted (Johnson and Burtch, 1958; McFarlane, 1975) and these plants are a minor weed problem in this area. Movement of the transgenes from H7-1 to these plants is likely. In addition *B. macrocarpa* species grows as a weed beet in sugar beet fields in this location (Bartsch *et al.*, 2002) and even though *B. macrocarpa* usually flowers earlier than sugar beet, it can cross with sugar beet bolters when flowering times overlap (Bartsch *et al.*, 2002). In the Imperial Valley, sugar beet is grown in winter culture and vernalization (bolting) of sugar beet is a common phenomenon due to moderately cold winter weather. Bartsch *et al.* (2002) have documented an introgression rate of 2% between from *B. vulgaris* to *B. macrocarpa* indicating past gene flow between these two species. Therefore escape of the engineered trait into weed beet populations is possible.

A number of scientists (Boudry *et al.*, 1993; Bartsch and Pohl-Orf, 1996; Desplanque *et al.*, 2002; Bartsch *et al.*, 2003) have studied the potential movement of herbicide tolerance genes from commercial sugar beets to sexually compatible relatives. Desplanque *et al.* (2002) have shown that in France, when weed beets were present in variable densities in sugarbeet fields, that the transfer of a herbicide resistance trait would be possible into the weed beet population, and suggested ways that this could be mitigated, for example by only incorporating the transgene for herbicide tolerance into tetraploid pollinator breeding lines. Additionally selecting cultivars that have a reduced tendency to bolt would reduce the likelihood of introgression of the trait into weed beet populations.

APHIS believes that if and when the glyphosate tolerance trait moves from H7-1 to other sexually compatible *Beta sp.* this will not have a significant impact in the United States. Since the wild or weed beet is regarded as a weed, there will be no impact on the genetic resources of this species, and if glyphosate tolerant individuals did arise through interspecific or intergeneric hybridization, the tolerance would not confer any competitive advantage to these plants unless challenged by glyphosate. This would only occur in managed ecosystems where glyphosate is applied for broad spectrum weed control, or in plant varieties developed to exhibit glyphosate tolerance and in which glyphosate is used to control weeds. As with glyphosate tolerant sugar beet volunteers, these individuals, should they arise, would be controlled using other available chemical and/or mechanical means. Hybrids, if they developed, could potentially result in the loss of glyphosate as a tool to control these species. However, this can be avoided by the use of sound crop management practices.

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Appendix B: APHIS authorizations for field tests of event H7-1 sugar beet

1998 Field Trials	98-065-06n
	98-219-06n
1999 Field Trials	99-110-21n
	99-060-10n
	99-152-01n
2000 Field Trials	00-034-01n
	00-049-09n
	00-053-20n
	00-061-06n
	00-061-09n
	00-062-10n
	00-067-19n
	00-067-21n
	00-180-06n
	00-187-02n
	00-224-05n
2001 Field Trials	01-008-06n
	01-054-08n
	01-054-09n
	01-066-01n
	01-074-17n
	01-074-19n
	01-078-12n
	01-093-10n
	01-115-02n
	01-192-07n
	01-219-06n
	01-250-06n
2002 Field Trials	02-008-01n
	02-057-02n
	02-063-12n
	02-063-14n
	02-070-01n
	02-144-10n
	02-210-02n

Appendix C: Summary table of critical data submitted with petition 03-323-01p for sugar beet event H7-1

Molecular genetic characterization data	**Figure/ table number and page in petition**
Plasmid map of PV-BVGT08	Fig. IV-1, p. 23
DNA insert diagram with restriction sites and predicted fragment sizes	Fig. V-1, p. 32
Southern blots verifying intactness of insert, promoter, coding region, polyadenylation signal and gene copy number	Fig. V-2, p.33, Fig. V-3, p.35, Fig. V-4, p.36 and Fig. V-5, p.37
Southern blots verifying stability of inheritance of the *epsps* gene over multiple generations	Fig. V-11, p. 48
Western blot characterization of EPSPS protein in event H7-1	Fig. V-12, p. 49
Statistical analysis of genetic segregation pattern of multiple generations of event H7-1	Table V-2, pp. 45-46
Agronomic characterization data	
Comparative disease analysis trials	Table VI-1, p. 54, Table VI-2, p. 56, Table VI-3, p. 57
Pest and disease observations for event H7-1	Table VI-5, pp.63-65, Table VI-6, pp. 66-68
Agronomic comparisons with conventional sugar beet varieties	Table VI-9, p. 73
Flowering properties comparison with conventional sugar beet varieties	Table VI-11, p. 76
Plant tissue compositional analyses	Table VI-12, p. 78, Table VI-13, p. 79, Table VI-14, p. 79, Table VI-15, p. 80, Table VI-16, p. 81, Table VI-17, p. 82, Table VI-18, p. 83
Comparisons of Roundup® with other herbicides used in sugar beet production	
Relative efficacy on a variety of weed species	Table VII-4, p. 90
Herbicides used in production	Table VII-5, pp. 92-93, Table VIII-6, pp. 97-101
Herbicide toxicity comparisons	Tables VII-7, VII-8, VII-9, VII-10, VII-11, pp. 102-106
Miscellaneous information	
Herbicide resistant weeds identified in primary sugar beet-producing states	Table VII-12, pp. 108-109, Response to letter of completeness
List of likely crops used in rotation with Roundup Ready® sugar beets	Table VII-13, pp. 111-113