Notices

Federal Register
Vol. 60, No. 113
Tuesday, June 13, 1995

This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-042-1]

Receipt of Petition for Determination of Nonregulated Status for Genetically Engineered Tomato Line

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public that the Animal and Plant Health Inspection Service has received a petition from the Monsanto Company seeking a determination of nonregulated status for a tomato line designated as 8338 that has been genetically engineered for delayed ripening. The petition has been submitted in accordance with our regulations concerning the introduction of certain genetically engineered organisms and products. In accordance with those regulations, we are soliciting public comments on whether this tomato line presents a plant pest risk.

DATES: Written comments must be received on or before August 14, 1995.

ADDRESSES: Please send an original and three copies of your comments to Docket No. 95-042-1, Regulatory Analysis and Development, PPD, APHIS, Suite 3C03, 4700 River Road Unit 118, Riverdale, MD 20737-1237. Please state that your comments refer to Docket No. 95-042-1. A copy of the petition and any comments received 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 access to that room to inspect the petition or comments are asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. Susan Koehler, Biotechnologist, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612. To obtain a copy of the petition, contact Ms. Kay Peterson at (301) 734-7601.

SUPPLEMENTARY INFORMATION: The regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests," regulate, among other things, the introduction (importation, interstate movement, or release into the environment) of organisms and products altered or produced through genetic engineering that are plant pests or that there is reason to believe are plant pests. Such genetically engineered organisms and products are considered "regulated articles."

The regulations in § 340.6(a) provide that any person may submit a petition to the Animal and Plant Health Inspection Service (APHIS) seeking a determination that an article should not be regulated under 7 CFR part 340. Paragraphs (b) and (c) of § 340.6 describe the form that a petition for determination of nonregulated status must take and the information that must be included in the petition.

On February 22, 1995, APHIS received a petition (APHIS Petition No. 95-053-01p) from the Monsanto Company (Monsanto) of St. Louis, MO, requesting a determination of nonregulated status under 7 CFR part 340 for a tomato line designated as 8338 that has been genetically engineered for delayed ripening. The Monsanto petition states that the subject tomato line shall not be regulated by APHIS because it does not present a plant pest risk.

As described in the petition, tomato line 8338 has been genetically engineered to express the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCd), which catalizes deamination of ACC, an essential precursor for ethylene biosynthesis. Levels of ethylene control the rate of fruit ripening, and removal of ACC in the subject tomato line reduces ethylene production and delays ripening. The *accd* gene, which confers the delayed-ripening trait, was isolated from the soil bacterium *Pseudomonas chloroaphis*, strain 6G5. Tomato line 8338 also contains the neomycin phosphotransferase (*nptII*) selectable marker gene which encodes the enzyme NPTII. The presence of the NPTII protein in the plant genome confers tolerance to the antibiotic kanamycin and allows selection of the transformed cells in the presence of kanamycin. Expression of the *accd* gene and the *nptII* gene is driven by constitutive 35S promoters derived from the plant pathogenic caulimoviruses figwort mosaic virus and cauliflower mosaic virus, respectively. The subject tomato line was transformed through the use of disarmed vectors from a common soil-borne bacterium, the plant pathogen *Agrobacterium tumefaciens*.

Tomato line 8338 is currently considered a regulated article under the regulations in 7 CFR part 340 because it contains the 35S promoters and 3' regulatory gene sequences derived from the plant pathogens mentioned above, and because *A. tumefaciens* was used as the plant transformation vector. Tomato line 8338 was evaluated in field trials conducted under APHIS permits or notifications since 1992. In the process of reviewing the applications for those field trials, APHIS determined that the vectors and other elements were disarmed and that the trials, which were conducted under conditions of reproductive confinement, would not present a risk of plant pest introduction or dissemination.

In the Federal Plant Pest Act, as amended (7 U.S.C. 150aa *et seq.*), "plant pest" is defined as "any living stage of: Any 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 substances, which can directly or indirectly injure or cause disease or damage in any plants or parts thereof, or any processed, manufactured or other products of plants." APHIS views this definition very broadly. The definition covers direct or indirect injury, disease, or damage not just to agricultural crops, but also to plants in general, for example, native species, as well as to organisms that may be beneficial to plants, for example, honeybees, rhizobia, etc.

Food or animal feed uses of the subject tomato line may be subject to

regulation by the Food and Drug Administration (FDA) under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*). The FDA published a statement of policy on foods derived from new plant varieties in the **Federal Register** on May 29, 1992 (57 FR 22984–23005). The FDA statement of policy includes a discussion of the FDA's authority for ensuring food safety under the FFDCA, and provides guidance to industry on the scientific considerations associated with the development of foods derived from new plant varieties, including those plants developed through the techniques of genetic engineering. Monsanto has completed its consultation with the FDA on the food safety of the subject tomato line.

In accordance with § 340.6(d) of the regulations, we are publishing this notice to inform the public that APHIS will accept written comments regarding the Petition for Determination of Nonregulated Status from any interested person for a period of 60 days from the date of this notice. The petition and any comments received are available for public review, and copies of the petition may be ordered (see the **ADDRESSES** section of this notice).

After the comment period closes, APHIS will review the data submitted by the petitioner, all written comments received during the comment period, and any other relevant information. Based on the available information, APHIS will furnish a response to the petitioner, either approving the petition in whole or in part, or denying the petition. APHIS will then publish a notice in the **Federal Register** announcing the regulatory status of Monsanto's tomato line 8338 and the availability of APHIS' written decision.

Authority: 7 U.S.C. 150aa–150jj, 151–167, and 1622n; 31 U.S.C. 9701; 7 CFR 2.17, 2.51, and 371.2(c).

Done in Washington, DC, this 5th day of June 1995.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95–14382 Filed 6–12–95; 8:45 am]

BILLING CODE 3410–34–M

USDA/APHIS Determination on a Petition 95-053-01p of Monsanto Company Seeking Nonregulated Status for Delayed-Ripening Tomato Line 8338

Environmental Assessment and
Finding of No Significant Impact

September 1995

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture (USDA) has prepared an environmental assessment prior to issuing a determination of nonregulated status for a genetically engineered delayed-ripening tomato line 8338. The genetic modification causes expression of the enzyme 1-amino-cyclopropane-1-carboxylic acid deaminase which metabolizes the precursor of ethylene and thereby delays the ripening process of detached tomato fruit. APHIS received a petition from the Monsanto Company regarding the status of line 8338 as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has prepared a finding of no significant impact on the environment from its determination that the genetically modified tomato line 8338 shall no longer be a regulated article. As a result of the preparation of a finding of no significant impact, the preparation of an Environmental Impact Statement is not required.

for John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: SEP 27 1995

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I. SUMMARY

APHIS prepared an Environmental Assessment (EA) prior to making its determination on the regulated status of the genetically engineered delayed-ripening (DR) tomato line 8338. The developer of line 8338, the Monsanto Company, (hereafter referred to as Monsanto), has petitioned APHIS for a determination that line 8338 does not present a plant pest risk and should therefore no longer be a regulated article under the APHIS regulations found at 7 CFR Part 340. As a regulated article under these regulations, interstate movements and field tests of line 8338 have been conducted under permits issued by or notifications acknowledged by APHIS.

The DR line 8338 has been developed in an effort to allow the harvest of vine-ripened tomatoes with extended market and shelf life to supply fruit of good flavor quality to the consumer. Mature fruit, harvested for fresh market production or at more ripe stages for processing, ripen more slowly and remain firmer longer than the non-modified recipient tomato fruits. The gene conferring this characteristic was introduced via genetic engineering techniques. These techniques enabled the developer to insert two transgenes into the genome of a processing tomato variety: (1) a gene sequence encoding the enzyme 1-amino-cyclopropane-1-carboxylic acid deaminase (ACCd) that metabolizes the precursor of the fruit ripening hormone ethylene, and (2) a gene encoding the selectable marker neomycin phosphotransferase (NPTII). The enzyme NPTII confers resistance to certain antibiotics used to select transformed cells. Line 8338 is a regulated article under APHIS regulations because some DNA regulatory sequences accompanying the introduced genes were derived from known plant pests, and their introduction into the plant genome was mediated via a well-characterized plant pest vector system.

Previous EAs, prepared before granting permits for field trials of line 8338, addressed questions pertinent to plant pest risk issues concerning the conduct of such trials under physical and reproductive confinement. These, however, do not address several issues relevant to the unconfined growth of line 8338. With respect to these new issues, APHIS concludes that line 8338: (1) exhibits no properties of plant pathogens; (2) is no more likely to become a weed than other commercial tomato cultivars developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential or impact the biodiversity of any other cultivated or wild species with which it can interbreed; (4) will not harm threatened or endangered species or organisms that are beneficial to agriculture; and (5) should not cause damage to processed agricultural commodities.

APHIS believes that the DR tomato line 8338 and its progeny will be just as safe to grow as traditionally-bred tomato varieties with similar characteristics that are not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if line 8338 and its progeny are no longer considered regulated articles under 7 CFR Part 340.

II. BACKGROUND

Development of line 8338. Monsanto has submitted a "Petition for Determination of Nonregulated Status" to APHIS for a tomato line designated 8338 that has been genetically engineered to exhibit delayed ripening (DR) of detached fruit. Monsanto requested a determination from APHIS that line 8338, and any progeny derived from hybrid crosses between these lines and other nontransformed tomato varieties, no longer be considered regulated articles under 7 CFR Part 340.

DR tomato line 8338 was developed by introducing into the genome of a processing tomato cultivar UC82B a gene (*accd*) derived from a nonpathogenic soil bacterium (*Pseudomonas chlororaphis*) that encodes the enzyme ACCd. In the plant, this enzyme catalyzes metabolism of 1-amino-cyclopropane-1-carboxylic acid (ACC), an essential precursor for the biosynthesis of the plant hormone ethylene. The initiation and progression of tomato fruit ripening depends on increased levels of ethylene. In line 8338, ACC is sufficiently reduced in detached fruit so that ethylene becomes limiting and the ripening process is delayed. Noncoding DNA sequences that are associated with the introduced *accd* gene to regulate its expression in line 8338 include the constitutively expressed 35S promoter derived from the plant pathogen figwort mosaic virus, a leader sequence from the petunia HSP70 gene, and the 3′ terminator region of a pea ribulose-1,5-bisphosphate carboxylase small subunit gene. Line 8338 has also been transformed with the *nptII* gene derived from *E. coli* that encodes the enzyme NPTII. NPTII confers resistance to certain antibiotics, such as kanamycin, that are used to select transformed cells. Noncoding DNA regulatory sequences associated with the *nptII* gene comprise the 35S promoter derived from the plant pathogen cauliflower mosaic virus and 3′ termination sequences derived from the nopaline synthase gene from the plant pathogen *A. tumefaciens*. These two genes were introduced into the tomato cultivar UC82B to create the original (R_0) transformed DR line via *Agrobacterium*-mediated transformation. This is a well-characterized procedure that has been used widely for over a decade to introduce various genes of interest directly into the plant genome. The DR tomato line 8338 is actually a homozygous R_2 selection from the R_0.

Line 8338 and backcross progeny have been extensively field tested in seven locations in major tomato growing regions of Florida, California, and Illinois under permits and acknowledgments of notifications by APHIS (USDA # 92-049-01r, 92-176-01r, 93-054-01r, 93-063-04r, 93-203-01n, 94-014-01n, 94-234-01n). Line 8338 and backcross progeny have been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and do not pose a plant pest risk. Although the field tests have been conducted in agricultural settings, the permit conditions for the tests have stipulated physical and reproductive confinement from other plants.

APHIS Regulatory Authority. APHIS regulations under 7 CFR Part 340, promulgated pursuant to authority granted by the Federal Plant Pest Act (7 U.S.C. 150aa-150jj as amended), and the Plant Quarantine Act (7 U.S.C. 151-164a, 166-167 as amended), pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. 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 regulations and is also a plant pest or there is reason to believe that it is a plant pest. Monsanto DR tomato line 8338 has been considered a regulated article because some noncoding DNA regulatory sequences and portions of the plasmid vector are derived from plant pathogens and the transformation vector is a plant pathogen.

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 APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency will grant the petition in whole or in part. As a consequence, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination of nonregulated status of DR tomato line 8338 and its progeny under APHIS regulations. Monsanto, the developer of line 8338, submitted a petition requesting that APHIS make a determination that line 8338 and its progeny should no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with: (1) The National Environmental Policy Act of 1969 (NEPA)(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; 60 FR 6000-6005, February 1, 1995).

IV. ALTERNATIVES

A. No Action.

Under the "no action" alternative, APHIS would not come to a determination that line 8338 and its progeny were not regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS

would still be required for the introduction of line 8338 and its progeny. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the uncontained cultivation of line 8338 and its progeny.

B. Proposed Action: Determination of Nonregulated Status.

Under this alternative, line 8338 and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or acknowledged notifications from APHIS would no longer be required for the introduction of line 8338 or its progeny. A basis for this determination would include a "Finding of No Significant Impact" under NEPA.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that line 8338 and its progeny should no longer be considered regulated articles under 7 CFR Part 340. Previous EAs prepared by APHIS for the issuance of permits for field tests of line 8338 and backcross progeny have addressed various properties of these tomatoes. This EA discusses the genetic modification and potential environmental impacts associated with the unconfined cultivation and distribution of line 8338. These environmental impacts are compared to the environmental impacts posed by the unconfined cultivation and distribution of tomatoes not subject to APHIS regulations.

Additional technical information and literature citations included in the determination document (Appendix 1) in this EA are incorporated by reference. These include detailed discussions of the biology of tomato, the genetic components used in the construction of line 8338, the genotypic and phenotypic differences between the recipient and line 8338, and the analyses that lead to the conclusion that line 8338 does not pose a plant pest risk.

A. Potential for line 8338 to exhibit increased weediness relative to other commercially cultivated tomatoes.

Various definitions of the term "weed" have been proposed. The salient point is that a plant can be considered a weed when it is growing where humans do not want it (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) lists common attributes that can be used to assess the likelihood that a plant species will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed these characteristics to develop guidelines to assess the weediness potential of transgenic plants; both authors emphasize the importance of evaluating the parent plant and the nature of the specific genetic changes.

The parent plant of line 8338 is a processing tomato variety that exhibits no appreciable weedy characteristics. Cultivated tomato is

grown as an annual crop in the United States for both fresh market and processed tomato fruit, with the bulk of the commercial production in Florida and California. Tomato has been grown for centuries throughout the world without any report that it is a serious weed pest. It is not classified as a serious, principal, or common weed pest in the U.S. (Holm et al., 1979), and it is not listed under the Federal Noxious Weed Act (7 CFR Part 360). Although tomato volunteers are common, they are easily controlled with herbicides or by mechanical means.

The genetically engineered *accd* gene in line 8338 results, in part, in a reduction in the biosynthesis of the plant hormone ethylene. Because ethylene can effect many growth, developmental, and disease processes in plants, Monsanto submitted results of an extensive field study of line 8338 and its parental line that was designed to examine parameters that have been proposed as indicators of weediness potential. Some differences were noted in the number of seeds produced per plant and in the average number of days to flowering. These differences, however, were within the normal range of variation for cultivated tomatoes. Thus, there is no indication that the genetic modification will convert line 8338 into a weed.

The introduction and expression of the selectable marker gene (*nptII*) into tomato plants also does not affect their weediness characteristics. This gene facilitate the selection of transformed cells in the laboratory, but provides no practical selective advantage in agricultural environments. This gene is not involved in plant disease or damage. Also, its use does not result in the presence of the antibiotics in tomatoes and does not imply that antibiotics will be used in the cultivation of the tomatoes.

No other characteristics of line 8338 reported by Monsanto, including disease and pest susceptibilities, fruit ripening characteristics or the number of volunteers in field trials, suggest that line 8338 will be any more "weedy" than present tomato cultivars derived from traditional breeding.

B. Potential impacts associated with gene transfer into sexually-compatible relatives of tomato and with horizontal gene transfer into other organisms.

APHIS considered the potential for genes to be transferred from line 8338 to other sexually-compatible relatives of cultivated tomato (*Lycopersicon esculentum* var *esculentum* Mill.), and the impact that such introgression would have on the weediness potential and biodiversity of progeny. Tomato generally does not cross-pollinate with other plants in the U.S. without the intervention of man. Cultivated tomato is self-fertile and almost exclusively self-pollinating, due, in part, to the presence of an inserted stigma (Rick, 1976). Tomato is not wind-pollinated and insect pollination is limited (Rick, 1976). Monsanto reported no differences in flower morphology between line 8338 and the nonmodified recipient, therefore

outcrossing in line 8338 is naturally limited by its reproductive biology.

The cherry tomato, *L. esculentum* var. *cerasiforme* is the direct ancestor of the modern cultivated tomato and is the only naturally sexually-compatible wild or weedy relative found in the United States. The cherry tomato is used for human consumption and has been transported around the world. It can occur as a weed in tropical America, southern Texas, and Florida (Rick, 1973), Mexico, Africa, and parts of Southeast Asia (Taylor, 1986; Rick, 1976; Rick and Fobes, 1975), but it is not considered a serious or principal weed pest (Holm et al, 1979). The probability of the introduced genes in tomato line 8338 naturally introgressing into var. *cerasiforme* in the United States is extremely low, due to (1) the low rate of outcrossing in var. *esculentum* (Rick, 1949), (2) low population densities in areas of the United States that are devoted to large scale cultivation of tomatoes (Dr. C. Rick, tomato geneticist, University of California, Davis, personal communication), and (3) the fact that cultivation of *L. esculentum* requires the maintenance of genetic purity as a standard breeding practice and for many commercial purposes.

Because tomato has no relatives other than itself with which it can naturally cross in the United States, and because commercial tomatoes are almost exclusively self-pollinating, there is little possibility of a cross unaided by man between the line 8338 tomato and another plant. Therefore, there is no likelihood that line 8338 will impact the biodiversity of other plant species in the United States. Although limited by high self-pollination rates, outcrossing of line 8338 to primitive tomato cultivars and wild or weedy relatives (particularly those in the *esculentum*-complex) is possible in Mexico, Central America, and northwestern South America. This is discussed in further detail in the determination. Our analysis of the biology of cultivated tomato and its relatives leads us to predict that the environmental impacts of cultivation of line 8338 anywhere in the world would be no different from such impacts attributable to similar varieties produced with traditional breeding techniques. Our decision in no way prejudices regulatory action in any other country. We note also that any international traffic in line 8338 would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC).

Even if an outcrossing event involving pollen from tomato line 8338 did occur, there is no reason to believe that the progeny would be any more weedy than progeny from crosses resulting from pollination by other traditionally-bred tomato cultivars. The minor genotypic and phenotypic differences observed in line 8338 are not expected to confer a selective advantage that would increase their weediness potential relative to other commercial tomato cultivars, nor would they be expected to increase the weediness potential if introgressed into other sexually-compatible plants.

Horizontal gene transfer of transgenes from genetically engineered plants to other organisms is not well documented; however, horizontal gene transfer of transgenes from higher transgenic plants to a soil microorganism has been reported (Hoffmann et al., 1994). Because ACCd activity and kanamycin resistance have already been characterized in many soil microorganisms (Tran and Kretzmer, 1993; Henschke and Schmidt, 1990), APHIS anticipates that horizontal gene transfer into soil microorganisms of the plant transgenes conferring these traits should pose no greater plant pest risk or risk to biodiversity than would direct or horizontal gene transfer among soil microorganisms.

C. Potential impacts on organisms that are threatened or endangered or that are beneficial to agriculture, such as bees and earthworms.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for line 8338, and plant products derived from it, to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those which are recognized as threatened or endangered in the United States.

Monsanto analysis of biochemical components of line 8338 tomato fruit identified no toxic components that are present in concentrations significantly different from the concentrations in nontransgenic tomatoes. The genetic modification in line 8338 does not result in the production of new proteins, enzymes or metabolites in the plant that are known to have toxic properties. The plants also do not exhibit any pathogenic properties.

APHIS concludes that the unconfined growth of line 8338, and products derived from it, will have no deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honey bees) or on other organisms, including any species recognized as threatened or endangered in the United States.

D. Potential impacts on agricultural practices.

DR tomato line 8338 exhibits the typical agronomic characteristics of the parent tomato line UC82B with a few exceptions, those being the delayed ripening phenotype, a 2-3 day decrease in the average number of days to flowering, and a slight increase in both seed number and susceptibility to the fungal *Fusarium* crown rot disease. APHIS believes that the slight increase in crown rot-susceptibility in line 8338 does not pose a plant pest risk nor will it have a significant impact on agricultural practices because: (1) as demonstrated by Monsanto, susceptibility of line 8338 to this disease can be eliminated during development of commercial lines by crossing into available crown rot-resistant germplasm, and (2) crown rot-susceptible varieties are commonly grown in major tomato producing states. As a result of the DR phenotype, fruit from fresh market tomato varieties developed from line 8338 could be harvested at a slightly more mature stage (breaker stage) than they might be otherwise. This

may slightly broaden or delay the harvesting period. Tomatoes from line 8338 are expected to have a longer shelf life, and may not need to be refrigerated during storage. The DR trait is also expected to delay over-ripening of processing tomatoes that may be developed from line 8338. This could provide growers with more flexibility in harvest dates and potentially reduce losses caused by ripe-rot diseases.

APHIS concludes that the DR tomato line 8338 should not have any major potential impacts on agricultural and cultivation practices.

E. Potential impact on raw or processed agricultural commodities.

A determination of nonregulated status by APHIS under Section 340.6 includes an evaluation of whether the regulated article causes disease, injury, or damage to raw or processed agricultural products. Safety concerns for human and animal consumption of products of new plant varieties, however, are addressed by the FDA. Their 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, at 57 FR 22984-23005. Monsanto has satisfactorily completed a voluntary food safety consultation with the FDA consistent with this policy statement. Monsanto provided to the FDA extensive composition studies of tomato fruits and processed tomato products derived from line 8338 which revealed that they are not materially different from those of the parental cultivar, and that they meet processing standards.

ACCd is not expected to cause disease, injury, or damage to agricultural commodities because it not associated with plant pathogenicity. The enzymatic reaction it catalyzes and the products of that reaction are not expected to cause damage or injury to tomato fruit. APHIS has previously determined that expression of NPTII in certain genetically-engineered tomato lines does not have an adverse impact on agricultural commodities (see APHIS EA and Determination documents for petitions 92-196-01p and 94-290-01p). The DR phenotype is expected to increase the quality of fruit harvested from commercial fresh market and processing cultivars to be developed from line 8338. The slight increase in susceptibility to *Fusarium* crown rot disease in line 8338 should not have an adverse impact on agricultural commodities because this is not a post-harvest disease.

APHIS concludes that the DR tomato line 8338 does not have any unique characteristics, compared to commercially cultivated tomatoes, that would pose a direct or an indirect plant pest risk or have an adverse impact on any raw or processed agricultural plant commodity.

VI. CONCLUSION

APHIS has evaluated the scientific literature as well as data submitted by Monsanto relevant to line 8338. APHIS has considered the foreseeable consequences of a determination of nonregulated status for line 8338 and its progeny, and has reached the following conclusions:

1. Tomato line 8338 exhibits no properties of plant pathogens. Although pathogenic organisms were used in the development of this line, these tomato plants are not infected, nor can they cause disease in other plants.

2. Tomato line 8338 is no more likely to become a weed than other tomato lines developed by traditional breeding techniques. Tomato is not a weed pest, and there is no reason to believe nor data to indicate that the introduced genetic constructs will provide a selective advantage sufficient to enable tomato lines expressing these genes to become weeds.

3. The potential for unintended gene introgression from tomato line 8338 into sexually-compatible weedy or wild relatives by outcrossing or into other organisms by horizontal gene transfer is limited. Furthermore, gene introgression from line 8338 into sexually-compatible relatives would be no more likely to increase their weediness potential than would gene introgression from other cultivated tomatoes developed by traditional breeding methods.

4. Tomato line 8338 will not have deleterious effects on threatened or endangered species or other organisms which are beneficial to agriculture.

5. Tomato line 8338 should not cause disease, damage, or injury to raw or processed agricultural commodities.

Therefore, APHIS concludes that line 8338, and any progeny derived from hybrid crosses between these lines and other nontransformed tomato varieties, will be just as safe to grow as traditionally-bred tomato lines that are not regulated under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if line 8338 and its progeny were no longer considered regulated articles under 7 CFR Part 340.

VII. PREPARERS AND REVIEWERS

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Animal and Plant Health Inspection Service

Biotechnology, Biologics, and Environmental Protection

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RESPONSE TO THE MONSANTO COMPANY PETITION FOR DETERMINATION OF NONREGULATED STATUS FOR DELAYED-RIPENING TOMATO LINE 8338.

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology, Biologics, and Environmental Protection

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I. SUMMARY

Based on a review of scientific data and literature, the Animal and Plant Health Inspection Service (APHIS) has determined that the delayed-ripening (DR) tomato line 8338 does not present a plant pest risk, and therefore this line, and any progeny derived from crosses involving this line, will no longer be considered regulated articles under 7 CFR Part 340. Consequently, oversight under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of tomato line 8338 or its progeny. Importation of tomatoes derived from line 8338 and nursery stock or seeds capable of propagation, however, remains subject to restrictions found in the foreign quarantine notices in 7 CFR Part 319.

This determination by APHIS has been made in response to a petition received from the Monsanto Company (hereafter, Monsanto) dated February 22, 1994 (and amended on April 26, 1995), that requested a determination from APHIS that DR tomato line 8338 does not present a plant pest risk and therefore should no longer be considered a regulated article. On June 13, 1995, APHIS announced receipt of this petition in the Federal Register (60 FR 31139-31140) and stated that the petition was available for public view. APHIS invited written comments on this proposed action to be submitted by August 14, 1995.

DR tomato line 8338, as defined by its developer, Monsanto, was developed by introducing into the genome of a processing tomato cultivar a gene that encodes an enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCd), whose expression results in delayed fruit ripening. This enzyme catalyzes the metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor for ethylene biosynthesis. The initiation and progression of tomato fruit ripening depends on increased levels of ethylene. In line 8338, ACC is sufficiently reduced in detached fruit that ethylene becomes limiting and the ripening process is delayed. Because the delay in fruit ripening in line 8338 occurs after the fruit are removed from the plant, the fruit can be harvested for fresh market use at a later stage of ripening than they would be otherwise; and fruit harvested for either fresh market or processing will have an extended market life, resulting in potential benefits to growers, packers, shippers, and consumers. Line 8338 was also genetically engineered to express a selectable marker gene (*nptII*), encoding the enzyme neomycin phosphotransferase II (NPTII), that confers resistance of transformed tissue to the antibiotic kanamycin. The introduced DNA also has regulatory sequences that modulate expression of the introduced genes.

APHIS regulations at 7 CFR Part 340, 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), pertain to 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 provisions of 7 CFR Part 340 when it is

demonstrated not to present a plant pest risk. Section 340.6 of the regulations 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 is granted, thereby allowing unregulated introduction of the article in question.

Tomato line 8338 has been considered a "regulated article" under Part 340 of the regulations, in part, because it has been engineered using components from known plant pests. Some of the DNA regulatory sequences were derived from the following plant pathogens: the bacterium *Agrobacterium tumefaciens* and the caulimoviruses, cauliflower mosaic virus (CaMV) and figwort mosaic virus (FMV). In addition, the vector system used to transfer the two genes into the recipient tomato was derived from *A. tumefaciens*. Field testing of line 8338 has been conducted since 1992 under APHIS permit or notification using conditions of reproductive confinement as stipulated.

This determination has been made based on an analysis that reveals that line 8338: (1) exhibits no properties of plant pathogens; (2) is no more likely to become a weed than the nonmodified parental variety or other tomatoes developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential or adversely impact the biodiversity of any other cultivated plant or native wild species with which the organism can interbreed; (4) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture; and (5) will not cause damage to processed agricultural commodities. APHIS has also concluded that there is no reason to believe that new progeny tomato varieties derived from line 8338 will exhibit new plant pest properties, i.e., properties substantially different from those observed for DR tomato line 8338 already field tested, or those observed for tomatoes derived from traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA)(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; 60 FR 6000-6005, February 1, 1995). An Environmental Assessment (EA) and Finding of No Significant Impact (FONSI) was prepared by APHIS for its determination that line 8338 and its progeny are no longer considered regulated articles under 7 CFR Part 340.

The body of this document consists of three parts: (1) background information that provides the legal framework under which APHIS has regulated the introduction of line 8338, (2) a summary of comments provided to APHIS during the public comment period and APHIS' response

to those comments; and (3) an analysis of the key factors relevant to APHIS' decision that line 8338 does not present a plant pest risk.

II. BACKGROUND

Regulatory Authority. APHIS regulations at 7 CFR Part 340, promulgated pursuant to 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), pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. The FPPA provides the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. The PQA also enables USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases.

Under § 340.0 of the regulations, a person is required to obtain a permit before introducing a regulated article. 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 the regulations and is also a plant pest; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. Permission to conduct a field trial of an article regulated under 7 CFR Part 340 is granted when APHIS has determined that the conduct of the field trial does not pose a plant pest risk.

Before the introduction of a regulated article, a person is required under § 340.0 of the regulations to (1) notify APHIS in accordance with § 340.3, or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 340.3) requires that specified eligibility criteria and performance standards be met. The eligibility criteria set 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 § 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.

An organism is no longer subject to 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 is granted, thereby allowing for unregulated introduction of the article. A petition may be granted in whole or in part.

DR tomato line 8338 has been considered a "regulated article" under Part 340 of the regulations, in part, because some of the noncoding DNA regulatory sequences were derived from plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and the caulimoviruses, figwort mosaic virus and cauliflower mosaic virus (CaMV). In addition, the vector system used to transfer the two genes into the recipient tomato was derived from *A. tumefaciens*. APHIS believes it prudent to provide assurance before commercialization that organisms such as DR tomato line 8338, that are derived in part from plant pests, do not pose a 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 line 8338 is no longer a regulated article is based, in part, on evidence provided by Monsanto concerning the biological properties of tomato line 8338 and its similarity to other varieties of tomato grown under standard agricultural practices for commercial sale or private use.

A determination that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage, either when grown in the field, or when stored, sold, or processed. APHIS' definition of plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within 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."

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: (1) exhibits no properties of plant pathogens; (2) is no more likely to become a weed than the non-modified parental variety; (3) is unlikely to increase the weediness potential of any other cultivated plant; (4) is unlikely to harm other organisms that are beneficial to agriculture; and (5) does not cause damage to processed agricultural commodities. Evidence has been presented by Monsanto that bears on these topics. In addition, because the petition also seeks a determination on any progeny derived from crosses between DR tomato line 8338 and traditional tomato varieties, it should be established that such progeny will not exhibit plant pest properties substantially different from those observed for

tomatoes in traditional breeding programs or as seen in the development of line 8338.

III. RESPONSE TO COMMENTS

APHIS received two comments on the subject Petition. Both were from State departments of agriculture and expressed support for the petition of nonregulated status for DR tomato line 8338. APHIS appreciates these comments, and concurs with the conclusion that line 8338 tomatoes should no longer be subject to regulation under 7 CFR Part 340.

IV. ANALYSIS OF THE PROPERTIES OF DR TOMATO LINE 8338

In order to establish that DR tomato line 8338 or varieties derived from it do not pose a plant pest risk greater than or different from that presented by traditionally-bred tomatoes grown in the United States, APHIS considered, among other things, the biology, cultivation, storage, and processing of the nonmodified recipient tomato, and the plant pest risks associated with the modified organism and these activities. A brief discussion follows of these issues and the biology, development, and intended use of DR tomatoes. This information provides background for the plant pest issues and is expanded in subsequent sections, when relevant to address particular issues related to line 8338.

DR line 8338 was developed by Monsanto by genetically engineering a tomato (*Lycopersicon esculentum* var *esculentum* Mill.) cultivar UC82B to express a gene conferring the DR phenotype and a kanamycin resistance gene as a selectable marker for transformation. UC82B is a processing variety of commercially cultivated tomato which has been grown extensively in California (Stevens et al., 1976). Monsanto commercialization strategy for DR tomatoes is to use backcross breeding methods to transfer from this cultivar the introduced gene controlling delayed ripening to a wide variety of both processing and fresh market tomatoes.

Cultivated tomato, *L. esculentum* var *esculentum* Mill., is distributed worldwide and is grown commercially for tomato fruit wherever agronomic conditions permit an economic yield. The cultivated tomato is a highly inbred perennial in its native tropical habitat, but is grown almost exclusively as an annual in the United States. Of the over 500,000 acres of tomatoes grown annually in the United States, approximately 40% is grown for fresh market consumption; the balance is grown for processing. In the United States, nearly 90% of the total production of processing tomatoes occurs in California, with the remainder in parts of the Midwest (Petition pg. 15). Commercial fresh market tomato production occurs in more than 20 states, primarily for local markets, but production in California and Florida (primarily for distribution to more distant markets) accounts for nearly 67% of the total. The varieties and cultural practices used for fresh market and

processing tomatoes are significantly different, and the fruit are harvested at different stages of maturity.

The tomato fruit is a berry composed of flesh (pericarp walls and skin) and pulp (placenta and locular tissue including seeds) (Ho and Hewitt, 1986). Of the compositional changes that occur during tomato fruit ripening, one of the most obvious is the progressive change in color of the flesh and skin of the fruit from green, to light pink, to pink, to red. This color change is the basis of the six classes of tomato ripeness included in the U.S. Standards (USDA, 1976). Production systems for fresh market tomatoes are based on harvesting fruit at the mature green stage. Fruit are then shipped to local packing houses for distribution. In contrast, processing tomatoes are generally mechanically harvested at a fully-ripened stage, and shipped directly to a processing plant.

Fruit harvested at the mature green stage can be stimulated to ripen by the exogenous application of ethylene gas or ethylene-generating compounds; however, immature green fruit do not ripen readily in response to ethylene (Grierson and Kader, 1986). During the normal ripening process, increased ethylene production beginning in the mature green fruit stimulates the physiological and biochemical changes required for ripening. The immediate precursor to ethylene is ACC. During ripening, there is a large increase in the concentration of ACC and in the activity of the enzyme ACC synthase which synthesizes it. The DR tomato line 8338 has been engineered to express the enzyme ACC deaminase which can metabolize ACC. The concentration of ACC and ethylene become limiting in detached fruit, thereby delaying the ripening process.

Quality is determined, in large part, by the occasionally conflicting demands, needs, and expectations of producers, shippers, marketers, and consumers. The maturity of tomato fruit at harvest is an important determinant of the composition and quality of tomatoes. Immature green fruit contaminating mature green fruit harvested for fresh market use reduces market quality. To avoid this problem, growers may harvest fruit when it just begins to show color (breaker stage); however, these tomatoes (considered vine-ripened) have a shorter market life. Reduced temperatures and controlled atmospheres used to retard or slow ripening of mature green or breaker tomatoes can have an adverse effect on quality attributes, such as color and flavor, and may cause chilling injury. Ripened and over-ripened fresh market tomatoes or processing tomato fruit are subject to ripe-rot diseases caused by bacterial and fungal pathogens. Introduction of the DR trait could enable fresh market tomatoes to be harvested at a more mature stage (breaker stage) than they might be otherwise, and the DR tomatoes are expected to have a longer shelf life. The DR trait is also expected to delay over-ripening of processing tomatoes. This may provide growers with more flexibility in harvest dates and potentially reduce losses caused by ripe-rot diseases.

The introduced genes, their products, and the added regulatory sequences controlling their expression do not present a plant pest risk in line 8338.

Line 8338 was produced by using *Agrobacterium*-meditated transformation to introduce into the genome of the processing tomato cultivar UC82B a gene (*accd*) encoding the enzyme ACCd, that confers the DR phenotype, and a selectable marker gene (*nptII*), encoding the enzyme NPTII, that confers resistance to the antibiotic kanamycin. The enzyme ACCd catalyzes the deamination of ACC to ammonia and α-ketobutyrate. This reduces the concentration of ACC available as a precursor for ethylene production in the tomato fruit and delays ripening of detached fruit. The *accd* gene was derived from the soil bacterium, *Pseudomonas chlororaphis* strain 6G5 (Klee et al., 1991; Bruce Hemming, Monsanto Co., personal communication). *P. chlororaphis* is a saprophyte which is not associated with either plant, animal or human pathogenicity (Doudoroff and Palleroni, 1974). ACCd is found in several other common soil bacteria, and in some filamentous fungi and yeasts. Other types of deaminase activity have been associated with the development of late stages of disease symptoms in tomato caused by the plant pathogen *P. syringae*, but this activity is specific only to certain amino acids and results in the release of large amounts of ammonia (Bashan et al., 1986). ACCd enzymes purified from saprophytic soil microorganisms (*Pseudomonas sp.* ACP and the yeast *Hansenula saturnus*), however, exhibit high substrate specificity for ACC and are not active against amino acids tested (Honma and Shimomura, 1978). Ammonia and α-ketobutyrate are natural metabolic intermediates in plant amino acid biosynthesis (Goodwin and Mercer, 1990), and since ACC concentrations in tomato fruit are low (0.1 to 10 nmol/g fresh weight) during ripening, the metabolite concentrations should also be low and the metabolites rapidly assimilated. Therefore, the products of ACC metabolism by ACCd in line 8338 will not cause a plant pest risk.

The *nptII* gene that encodes the enzyme NPTII (also called aminoglycoside 3'-phosphotransferase II) was derived from the *E. coli* transposon Tn5 (Beck et al., 1982). *E. coli*, a common enteric bacterium found in the human gut, is not a regulated article. The gene has no involvement in plant disease or damage. This gene was introduced as a marker enabling identification of tomato cells that had concomitantly taken up the *accd* gene. Following transformation, plant cells expressing the enzyme NPTII can survive laboratory selection by the antibiotic kanamycin because NPTII deactivates, by phosphorylation, aminoglycoside antibiotics such as kanamycin. Its use does not result in the presence of the antibiotic kanamycin in line 8338 tomato plants, and its presence does not imply that kanamycin will be used in the cultivation of these tomatoes.

The introduced gene sequences also have accompanying nontranslated DNA regulatory sequences, such as promoters and 3' terminator sequences. Specifically, the DNA regulatory sequences associated with the *accd* gene comprise the constitutively expressed 35S promoter derived from the plant pathogen figwort mosaic virus (Shepherd et al, 1987; Richins

et al., 1987), followed by the transcribed, nontranslated leader sequence from the petunia HSP70 gene (Winter et al., 1988) and the 3' terminator region of a pea ribulose-1,5-bisphosphate carboxylase, small subunit gene (Coruzzi et al., 1984). The DNA regulatory sequences associated with the *nptII* gene comprise the 35S promoter derived from the plant pathogen cauliflower mosaic virus and 3' termination sequences derived from the nopaline synthase gene from the plant pathogen *A. tumefaciens*. Although some of these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate.

The genes and their regulatory sequences were inserted between the T-DNA borders of an *A. tumefaciens* plasmid vector for plant transformation. This vector was used to transform UC82B via a well-characterized technique by which DNA sequences inserted between the T-DNA borders are introduced into the chromosome of the recipient plant (see reviews by Klee and Rogers, 1989; and Zambryski, 1988). Although some DNA sequences used in the transformation process were derived from the plant pathogen, *A. tumefaciens* (the causal agent of crown gall disease), the genes that cause disease were removed so that the transformed plant does not develop crown gall disease.

Monsanto has demonstrated that the introduced genes in the transformed plant are maintained and sexually-transmitted in the same manner as other genes. The original transformant (R_0) was self-pollinated to obtain R_1 seed, which segregated 3:1 for ACCd expression as expected for a single locus insert. The DR tomato line 8338 is actually a homozygous R_2 selection from the R_0. Monsanto demonstrated that in six generations of backcrossing to seven commercial lines, the T-DNA insert is stably integrated and behaves as a single dominant locus inherited in a Mendelian fashion (Petition, Table V.1., pg. 30). Southern blot data confirms the inheritance data and demonstrates that DNA contained outside the T-DNA borders in the transformation vector PV-LERP07 (ie., bacterial genes and origins of replication) was not transferred to the plant.

Line 8338 and backcross progeny have been extensively field tested in seven locations in the U.S. under APHIS oversight since 1992. Field test reports submitted by Monsanto include observations of these plants by experienced tomato breeders or agronomists for general appearance, growth, vigor, bushiness, leaf morphology, plant height, germination rates, days to reach reproductive maturity, number of flowers or fruits produced, number of volunteers, disease (bacterial spot, *Alternaria* target spot, *Fusarium*, and viruses) and insect susceptibility (loopers, armyworms, fruitworms, pinworms, whiteflies, aphids, leafminers, and thrips). These plants exhibited the typical agronomic characteristics of the parent tomato line UC82B with a few exceptions, those being the DR phenotype, a 2-3 day decrease in the average number of days to flowering, and a slight increase in both seed number and susceptibility to the fungal *Fusarium* crown rot disease (*Fusarium oxysporum*, f.sp. *radici-lycopersici*).

With regard to the latter, Monsanto submitted results from field and growth chamber studies (Petition, pp. 93-102 and 111-113) that further demonstrated the following: (1) another transgenic DR tomato line expressing the *accd* gene was not more susceptible to *Fusarium* crown rot, (2) when line 8338 was backcrossed to tomato lines containing the *Fusarium* crown rot resistance gene *fcr*, the resulting progeny were resistant to crown rot regardless of whether or not they were expressing the *accd* gene, and (3) when line 8338 was backcrossed to crown rot-susceptible tomato lines, those progeny that expressed *accd* were more susceptible than those that did not. These segregation data do not conclusively demonstrate that expression of the *accd* gene in line 8338 is responsible for the increase in susceptibility to *Fusarium* crown rot. It is possible that another tomato gene, chromosomally linked to the inserted gene, could have been affected during development and inbreeding of line 8338. Regardless of the cause, APHIS believes that the slight increase in crown rot-susceptibility in line 8338 compared to the parental line UC82B does not pose a plant pest risk because: (1) susceptibility of line 8338 to this disease can be eliminated during development of commercial lines by crossing into available crown rot-resistant germplasm, and (2) crown rot-susceptible varieties are commonly grown in major tomato producing states (Dr. J.W. Scott, vegetable breeder, University of Florida, personal communication to Dr. Glen Austin, Monsanto, Petition, pg. 117).

It is unclear whether the slight decrease in the average number of days to flowering or the slight increase in seed number is due to expression of the introduced genes, however, as discussed in the following section, this should not pose a plant pest risk. In APHIS' opinion, the components and processing characteristics of line 8338 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. Line 8338 exhibits no plant pest characteristics.

Line 8338 has no significant potential to become a successful weed.

A study (National Research Council, 1989) produced for the National Academy of Sciences, entitled "Field Testing Genetically Modified Organisms: Framework for Decisions", identified the potential to inadvertently produce a new weed or increase the aggressiveness of existing weeds as "perhaps the single most commonly voiced concern about the introduction of genetically modified plants."

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.

Tomato has been grown for centuries throughout the world without any report that it is a serious weed pest. It is not classified as a serious, principal, or common weed pest in the United States (Holm et al., 1979), and it is not listed under the Federal Noxious Weed Act (7 CFR Part 360). Although tomato volunteers are common, they are easily controlled using herbicides or by mechanical means. Tomato does not possess a significant number of the characteristics of plants that are notably successful weeds (Keeler, 1989). Tomato is considered a highly-domesticated, well-characterized crop plant that is not persistent in the environment without human intervention. Line 8338 and commercial processing and fresh market tomato varieties developed from it will most likely be grown in commercial production areas as discussed above.

Ethylene is known to have many effects on plant growth and development, including effects on seed germination and dormancy, seedling growth, flowering, and fruit ripening (Taiz and Zeiger, 1991). Since ethylene synthesis is reduced significantly in line 8338 (97 to 77% for fruit tissue (Reed et al., 1994) and greater than 90% for leaf tissue) compared to control line UC82B, it is not unreasonable to expect some changes in these plant growth and development characteristics in line 8338, which in turn might effect its weediness potential. Monsanto submitted a report (MSL-13805, Petition pp. 155-175) of a study designed to examine the weediness potential of DR line 8338 compared to the control parental line UC82B when grown using typical fresh market tomato production practices in Bonita Springs, Florida. Percent seed germination under controlled environmental greenhouse conditions was slightly higher for line 8338, but in the uncontrolled field environment, there was no significant difference. Tomato seeds experience no natural dormancy, and there was no increase in seed dormancy in line 8338. Under field conditions, 95% of seed germination occurred within two weeks of planting. There was also no increase or significant difference in the rate of vegetative growth or seedling vigor measured as height, weight, and stem width of one-month-old seedlings. The average seed number per fruit was not significantly different between line 8338 and UC82B; however, seed number per kg of fruit was significantly higher (16%) in line 8338, and fruit yield was also higher (Petition, Table V.3, pg. 35, field site 3). Therefore, the total seed yield per plant (calculated as the seed number/kg fruit times the kg of fruit harvested per plant) was actually 32% higher in line 8338 compared to line UC82B at this site. Even so, this is well within the range of normal seed production for commercial tomatoes, and when line 8338 is backcrossed to commercial fresh market lines, differences in fruit set between the transgenic line and control are minimal (Andrew Reed, plant biochemist, Monsanto, personal communication). The average time to flowering of line 8338 was 2.8 days shorter than that of line UC82B, but fruit maturation occurred at approximately the same time.

The fruit ripening characteristics of other DR tomato lines developed by Monsanto have been described (Klee et al., 1991 and Klee, 1993). In detached mature fruit of DR tomato lines, ethylene is limited, and the fruit exhibit delayed ripening. But when fruit are allowed to ripen on the vine, ethylene concentration in the fruit is sufficient to allow fruit ripening up to the fully red stage in DR tomato lines to proceed at approximately the same rate as in the control nontransgenic parental line. DR fruit, however, do not overripen, and they stay firmer longer. These characteristics are qualitatively similar in DR tomato line 8338 (Andrew Reed, personal communication).

Aside from a slightly increased susceptibility to *Fusarium* crown rot disease, no differences in disease or insect susceptibility were observed between line 8338 and UC82B. In field trials conducted in Illinois, California, and Florida under APHIS permit or notification, no difference in the number of volunteers between line 8338 and UC82B was observed following termination by disking the plants. There are no morphological, physiological, or disease resistance characteristics of the line 8338 that would entail the use of agricultural practices that vary from the traditional practices used today for the cultivation and propagation of tomatoes. To achieve optimal flavor, however, line 8338 tomatoes may be left to ripen in the field longer than conventional tomatoes. APHIS concludes that the phenotypic differences observed in line 8338 are minor and will not increase the weediness potential of line 8338, relative to other commercial tomato cultivars, in unmanaged ecosystems.

Line 8338 will not increase the weediness potential of any other plant with which it can interbreed, and is unlikely to adversely impact biodiversity.

Cultivated tomato (*Lycopersicon esculentum* var *esculentum* Mill.) is self-fertile and is almost exclusively self-pollinating, due, in part, to the presence of an inserted stigma developed through over 50 years of breeding (Rick, 1976). Cultivated tomato is not wind-pollinated and insect pollination is limited (Rick, 1976). Monsanto reported no differences in flower morphology between line 8338 and the nonmodified recipient; therefore, outcrossing in line 8338 is naturally limited by its reproductive biology.

Tomato does not cross-pollinate with other plant species in the United States without the intervention of man. *Lycopersicon* is a genus of the large and diverse nightshade family (Solanaceae), which also includes many crops (potatoes, peppers, tobacco, and eggplant) and weed species. The genus has been divided into two subgenera based on their ability to be crossed to commercial tomato; the *esculentum*-complex contains those species that are easily crossed, and the *peruvianum*-complex contains those species that are crossed with considerable difficulty (Stevens and Rick, 1986; Taylor, 1986). The cherry tomato, *L. esculentum* var. *cerasiforme* of the *esculentum* complex, is the direct ancestor of the modern cultivated tomato and is

the only sexually-compatible relative of *L. esculentum* var. *esculentum* found in the United States. *L. esculentum* var. *cerasiforme* is used for human consumption and has been transported around the world. It can occur as a weed in tropical America, southern Texas, Florida (Rick, 1973), Mexico, Africa, and parts of Southeast Asia (Taylor, 1986; Rick, 1976; Rick and Fobes, 1975). Cherry tomato, however, is not considered a serious or principal weed pest (Holm et al, 1979). Although *L. esculentum* var. *esculentum* and var. *cerasiforme* can cross with either plant as male or female parent (Rick, 1979), the probability of tomato line 8338 naturally introgressing into var. *cerasiforme* in the United States is extremely low due to the low rate of outcrossing in var. *esculentum* (Rick, 1949), and var. *cerasiforme* is not present in large numbers in areas of the United States that are devoted to large scale cultivation of tomatoes (Dr. C. Rick, tomato geneticist, University of California, Davis, personal communication). Introgression into cherry tomato is further limited by the fact that cultivation of *L. esculentum* requires maintenance of genetic purity as a standard breeding practice and for many commercial purposes. Regulations specifying procedures for the maintenance of genetic purity have been codified (See 7 CFR Part 201). Many other members of the nightshade family are found as weeds in tomato fields, but *L. esculentum* is not naturally sexually-compatible with any of these weedy relatives occurring in the United States (Rick, 1979).

Because commercial tomatoes are virtually exclusively self-pollinating and standard breeding practices in the United States ensure genetic purity, and because tomato has no relatives other than itself with which it can naturally cross in the United States, there is little possibility that unintended gene introgression from line 8338 into another plant will occur in the United States.

Although limited by high self-pollination rates, outcrossing of line 8338 to primitive tomato cultivars and wild or weedy relatives (particularly those in the *esculentum*-complex) is possible in Mexico, Central America, and northwestern South America. *Lycopersicon* species are native to the Andean region of Ecuador, Peru, and Chili; and one species *L. cheesmanii* is found exclusively on the Galapagos Islands. But most evidence suggests that the site of domestication of *L. esculentum* is Mexico (Taylor, 1986). Five species, in addition to *L. esculentum*, comprise the *esculentum*-complex, and two species comprise the *peruvianum*-complex (Taylor, 1986). *L. pimpinellifolium* is the only other *Lycopersicon* species for which there is good evidence for natural hybridization with cultivated tomato (Rick, 1958). This species can be found as a weed in commercial tomato fields and is occasionally harvested from the wild for human consumption. Its range is restricted to certain regions of Latin America (predominantly Peru and Ecuador). In addition to *Lycopersicon* species, two *Solanum* species, *S. lycopersicoides* and *S. rickii*, found only in restricted habitats of Peru and Chile, can be crossed with commercial tomato under specific, controlled conditions. But they do not naturally cross with *L. esculentum*, and the hybrids are generally highly sterile (Stevens and Rick, 1986; De Verna et al., 1990).

Even if an outcrossing event involving pollen from tomato line 8338 did occur, there is no reason to believe that the progeny would be any more weedy than progeny from crosses resulting from pollination by the nonmodified parental tomato line UC82B. As discussed above, the minor phenotypic differences observed in line 8338 and backcross progeny derived from this line will not confer a selective advantage that would increase their weediness potential relative to other commercial tomato cultivars; nor would they be expected to increase the weediness potential if introgressed into other sexually-compatible plants.

Gene introgression from line 8338 into other plants is also unlikely to adversely impact biodiversity. There is already considerable cultivation throughout the centers of diversity for tomato of improved tomato varieties produced through crop breeding. The impact of cultivation of line 8338 on the genetic diversity of wild relatives or primitive cultivars of tomato is likely to be comparable to that from these other nontransgenic improved varieties.

We note also that any international traffic in DR tomato line 8338 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 (98 countries as of December 1992). The treaty, administered through the United Nations Food and Agriculture Organization, came into force on April 3, 1952. It 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. Mexico in particular has in place a regulatory process that would require a full evaluation of the transgenic tomatoes before they could be introduced into their environment. Our decision in no way prejudices regulatory action in any country. The IPPC has also led to the creation of regional plant protection organizations such as the North American Plant Protection Organization (NAPPO) whose member countries are the U.S., Canada, and Mexico. Our trading partners will be kept informed of our regulatory decisions through NAPPO, and other fora. It should also be noted that all the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new tomato varieties internationally apply equally to the transgenic tomatoes covered by this analysis.

Nonsexual, horizontal transfer of transgenes from genetically engineered plants into other organisms is not well documented and is difficult to measure. Horizontal gene transfer of transgenes from higher transgenic plants via the soil to a soil microorganism (the filamentous fungus *Aspergillus niger*), however, has been reported (Hoffmann et al., 1994). The efficiency, the occurrence in natural habitats, and the gene transfer mechanism associated with this

horizontal gene transfer remain unclear. Because ACCd activity and kanamycin resistance have already been characterized in many soil microorganisms (Tran and Kretzmer, 1993; Henschke and Schmidt, 1990), horizontal gene transfer into soil microorganisms of the plant transgenes conferring these traits should pose no greater plant pest risk or risk to biodiversity than would direct or possible horizontal gene transfer among soil microorganisms.

Line 8338 should not be harmful to beneficial organisms.

There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of DR tomato line 8338. Some *Lycopersicon* species contain concentrations of specific glycoalkaloids, particularly tomatine, that are sufficient to produce toxic effects on certain foliar-feeding insect pests. But Monsanto analysis of biochemical components of line 8338 tomato fruit identified no toxic components that are present in concentrations significantly different from the concentrations in nontransgenic tomatoes. Any expression of ACCd in pollen of line 8338 should not be harmful to pollinating insects or other organisms for two reasons: (1) ACCd has a high substrate specificity, and (2) tomatoes are self-pollinated and do not produce copious amounts of pollen. ACCd released from decomposing line 8338 plant tissue should not have an adverse effect on soil- and detritus-dwelling organisms because ACCd activity has been characterized in several soil microorganisms. The biosafety of kanamycin-resistant transgenic plants has been reviewed (Nap et al., 1992). There is also no reason to believe that the NPTII protein conferring kanamycin resistance in line 8338 as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organism. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing this selectable marker. No direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis towards beneficial organisms such as bees and earthworms, were identified by Monsanto for line 8338. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenic effect.

Line 8338 will not cause damage to raw or processed agricultural commodities.

There is no reason to believe that fruit derived from line 8338 would cause disease, damage, or injury to raw or processed agricultural commodities. The delayed ripening trait could increase fruit quality because fruit could be harvested at breaker stage for fresh market use, thereby avoiding contamination by green immature fruit that reduces flavor quality. Physical injury and/or chilling injury during harvest and storage can make fruits more susceptible to post-harvest decay by such fungal plant pathogens as *Alternaria alternata* (black mould), *A. alternata f. lycopersici*, *Botrytis cinerea* (grey mould), *Rhizopus stolonifer* (*Rhizopus* rot), *Rhizoctonia solani* (soil rot) and *Geotrichum candida* and by bacterial soft rot pathogens (Grierson and

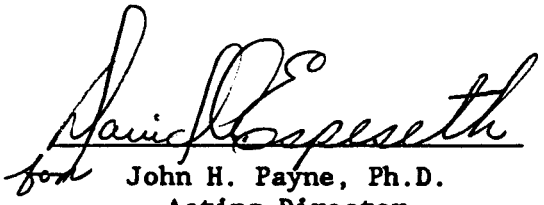
Kader, 1986). Because line 8338 fruit harvested for fresh market will have a longer shelf life and can be shipped and stored at higher temperatures, the chance for physical and chilling injury and associated losses due to plant pathogens could be reduced. The delay in overripening of tomatoes harvested for both fresh market and processing should also reduce the incidence or severity of ripe-rot diseases, thereby improving quality. The only difference in disease susceptibility observed between line 8338 and the nonmodified line UC82B was a slight increase in susceptibility to *Fusarium* crown rot disease. Although this disease can dramatically reduce yields, it is not a post-harvest disease of tomato fruit.

ACCd is not expected to cause disease, injury, or damage to agricultural commodities because it not associated with plant pathogenicity, and the enzymatic reaction it catalyzes and the products of that reaction are not expected to cause damage or injury to tomato fruit. APHIS has previously determined that expression of NPTII in genetically-engineered tomatoes does not have an adverse impact on agricultural commodities (see APHIS EA and determination documents for petitions numbered 92-196-01p and 94-290-01p). Monsanto provided to the FDA extensive composition studies of tomato fruits and processed tomato products (juice, paste, and pomace) derived from line 8338 which revealed that they are not materially different from the parental control, and that they meet processing standards. The FDA concluded the Monsanto consultation with them concerning the animal and human food safety of DR tomato line 8338 and accepted their conclusion that this tomato line is not altered significantly when compared to other tomato varieties with a history of safe use (FDA memorandum of conference, September 19, 1994). Therefore, APHIS concludes that line 8338 should not have a direct or indirect plant pest effect on any raw or processed plant commodity.

APHIS believes that the analysis of the properties of line 8338 already field tested will apply equally well to new tomato lines derived from crosses between line 8338 and traditional tomato varieties, and that the data provided by Monsanto justify the conclusion that such new lines derived from line 8338 will not present a plant pest risk. The variation in agronomic characteristics observed in line 8338 does not differ significantly from that seen in commercial cultivars of tomato that have never been considered regulated articles. APHIS has also determined that another delayed ripening tomato line, similarly developed by genetic engineering to express lower levels of ACC and ethylene during fruit ripening, does not represent a plant pest risk (see APHIS EA and Determination for petition number 94-228-01p). Therefore, there is no reason to believe that progeny of line 8338 will possess plant pest properties.

V. CONCLUSION

APHIS has determined that the DR tomato line 8338, and any progeny derived from crosses involving line 8338 and other nonregulated articles, will no longer be considered regulated articles under 7 CFR Part 340. Cultivation, importation, or interstate movement of line 8338 or its progeny will no longer be subject to permit or notification requirements stipulated by those regulations. Importation of line 8338 and its progeny (including nursery stock or seeds capable of propagation) will, however, remain subject to the restrictions found in foreign quarantine notices in 7 CFR Part 319. This determination has been made based on an analysis of data collected from field trials, laboratory analyses, and literature references included herein that demonstrate that line 8338: (1) exhibits no properties of plant pathogens; (2) is no more likely to become a weed than other commercial tomato cultivars developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential or adversely impact the biodiversity of other cultivated or wild species with which it can interbreed; (4) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture; and (5) should not cause damage to raw or processed agricultural commodities. APHIS has also determined that there is a reasonable certainty that new progeny derived from crosses involving line 8338 and other nonregulated articles, or varieties bred from these lines, will not exhibit new plant pest properties, i.e., those substantially different from any observed for line 8338 already field tested, or those observed for tomatoes derived from traditional breeding programs.



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Date: SEP 27 1995

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