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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. 92-087-2]

Interpretive Ruling on Calgene, Inc., Petition for Determination of Regulatory Status of FLAVR SAVR™ Tomato

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice of interpretive ruling and determination.

SUMMARY: The Animal and Plant Health Inspection Service (APHIS) is announcing the issuance of an interpretive ruling that the Calgene, Inc. FLAVR SAVR™ tomato does not present a plant pest risk and is not a regulated article under the regulations contained in 7 CFR part 340. This action is in response to a petition submitted by Calgene, Inc., seeking a determination from APHIS that its FLAVR SAVR™ tomato no longer be deemed a regulated article based on an absence of plant pest risk. The effect of this action is that Calgene's previously field tested lines of the FLAVR SAVR™ tomato and their progeny using one of seven binary vectors and the FLAVR SAVR™ gene with its associated promoter and terminator are no longer subject to regulation under these regulations. This notice also attaches the determination that provides the basis for this ruling.

EFFECTIVE DATE: This ruling is effective October 19, 1992.

ADDRESSES: The determination, the Calgene, Inc. submission, and written comments received in response to our July 14, 1992 notice published in the Federal Register 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.

FOR FURTHER INFORMATION CONTACT: Dr. Michael Schechtman, Senior Microbiologist, or Dr. Sally Van Wert, Biotechnologist, Biotechnology, Biologics, and Environmental Protection, APHIS, USDA, room 850, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, 301-436-7601.

SUPPLEMENTARY INFORMATION: On July 14, 1992 (57 FR 31170, Docket No. 92-087-1) the Animal and Plant Health Inspection Service (APHIS) published a notice requesting comments on a proposed interpretive ruling concerning a "Petition for Determination of Regulatory Status of FLAVR SAVR™ Tomato" from Calgene, Inc., (Calgene) of Davis, CA. The Calgene petition requested a determination from APHIS that its FLAVR SAVR™ tomato no longer be considered a "regulated article" under regulations in 7 CFR part 340 (the regulations).

The FLAVR SAVR™ tomato, as described by Calgene, is a tomato cultivar or progeny of a tomato line genetically engineered using one of seven binary vectors and the FLAVR SAVR™ gene with its associated promoter and terminator. The FLAVR SAVR™ gene is an antisense polygalacturonase gene isolated from tomato, which, when transcribed, results in delayed ripening of the tomato fruit. APHIS had considered the FLAVR SAVR™ tomato a regulated article under the regulations because it was developed through the use of components from plant pathogenic sources.

The APHIS determination is based on data submitted by the petitioner, written comments submitted during the 45-day comment period which ended on August 28, 1992, our review of the scientific literature, and expert opinion from tomato breeders and pathologists. From this review and analysis, APHIS has determined that the FLAVR SAVR™ tomato: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than the non-engineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage of processed agricultural commodities; and (5) is unlikely to harm other organisms that are beneficial to agriculture.

A detailed, point-by-point analysis of each of the above-mentioned 5 topics is set forth in the determination. As a result of the APHIS determination, tomato lines containing the FLAVR SAVR™ gene that were derived using any of the seven above-mentioned binary vectors, and that have already been field tested under the regulations, will no longer be subject to regulation under 7 CFR part 340. This determination also applies to progeny of field tested FLAVR SAVR™ tomato lines.

Summary of Comments

The following discussion summarizes the comments received by APHIS, which are discussed in greater detail in the determination. APHIS received comments from 19 respondents on the Calgene petition. Fifteen respondents affiliated with industry, academia, and State government expressed support for the APHIS proposed interpretive ruling that the FLAVR SAVR™ tomato does not present a present a plant pest risk. Four commenters representing environmental and/or policy organizations either urged caution, delay, or in one case, a disapproval of the Calgene petition. In summary, the cautionary statements were based on the assertions (1) that APHIS has not established a comprehensive regulatory policy for large-scale releases, and (2) that Calgene had not submitted sufficient experimental field test data in the Petition.

APHIS disagrees with these assertions. With reference to the first assertion, APHIS notes that its regulations in 7 CFR part 340 establish a comprehensive regulatory program for certain new plant varieties that is not scale-dependent. As conducted for the past 5 years, the APHIS regulatory program has considered, on a case-by-case basis, the potential for plant pest risk in over 320 field tests at over 650 test sites involving certain new plant varieties developed through the use of genetic engineering techniques. APHIS has also addressed this issue by holding a series of crop-specific workshops discussing appropriate safeguards for planned releases of transgenic derivatives of those crops.

APHIS also believes that it possesses sufficient information to justify approval of the Calgene petition. The FLAVR SAVR™ tomato has been field tested under eight APHIS permits between 1988 and 1992. Field test data contained

in the Calgene petition verifies that the variety exhibits the expected biological properties, and demonstrates that while derived using components from plant pests, the FLAVR SAVR™ tomato does not possess plant pest characteristics. In reaching the determination that the FLAVR SAVR™ tomato does not present a plant pest risk, APHIS analyzed the data presented by Calgene and other scientific data to consider the potential of the variety for plant pathogenicity, weediness, alteration in the weediness of close relatives of cultivated tomato, and the effects on beneficial organisms. This analysis, which is presented in detail in the determination, yields the conclusion that there is no reason to believe that the FLAVR SAVR™ tomato and its progeny will present a plant pest risk.

One respondent expressed the opinion that Calgene should have been required to perform experiments specifically designed to provide additional data on gene flow, weediness, and indirect adverse effects, while conceding that some of these experiments could address only potential "rare occurrences." APHIS believes that the commenter has failed to provide sufficient reason to believe that the FLAVR SAVR™ modification will affect weediness potential of the survival of any other cultivated tomato or tomato relative.

Comments on Scope of Interpretive Ruling

Two of the four commenters urging caution provided specific queries regarding the breadth of APHIS' ruling on Calgene's petition. These queries will be paraphrased for clarity and addressed (1) Does this determination apply to a FLAVR SAVR™ gene introduced into any plant other than tomato? Response: No. (2) Does APHIS' finding that these FLAVR SAVR™ tomatoes present no plant pest risk mean that the tomatoes are no longer subject to the Federal Plant Pest Act? Response: The tomatoes covered by this determination are no longer considered to be resulted articles under 7 CFR part 340, but, as for any other plant, this authority could be reasserted if a new plant pest risk should ever be uncovered in the future. (3) Does this determination regarding plant pest risk extend to other tomatoes independently made by other individuals using the same genes, or to other tomatoes engineered using a different antibiotic resistance marker? Response: No. This determination covers FLAVR SAVR™ tomato lines that have been field tested under permit to Calgene plus all their genetic descendants. New tomato lines carrying different marker genes would not be within the scope of this determination. (4) Are the plant pest-derived sequences in FLAVR SAVR™ tomatoes derived from *Agrobacterium* and CaMV no longer regulated under 7 CFR part 340? Response: No. These sequences will continue to be regulated. Calgene has provided data to APHIS that pertains specifically to the lack of plant pest risk for its vector constructs in FLAVR SAVR™ tomatoes.

After reviewing the data submitted by the petitioner, written comments received during the comment period, as well as other relevant literature, and after interpreting the application of statutes and regulations to these data and comments, APHIS is issuing this interpretive ruling regarding the regulatory status of FLAVR SAVR™ tomatoes.

A copy of the determination is attached to this notice. Done at Washington, DC, this 9th day of October 1992.

Robert Melland,

Administrator, Animal and Plant Health Inspection Service.

Response to Calgene Petition for Determination of Regulatory Status

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

I. Determination

The Animal and Plant Health Inspection Service (APHIS) has determined, based on a review of scientific data, that a trademarked tomato, called the FLAVR SAVR™ tomato, does not present a plant pest risk and is therefore not a regulated article under its regulations at 7 CFR Part 340.

The FLAVR SAVR™ tomato, as defined by its developer (Calgene, Inc., of Davis, California), is "a tomato cultivar or progeny of a tomato line genetically engineered using one of the following binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578) and the FLAVR SAVR™ gene with its associated promoter and terminator. The FLAVR SAVR™ gene is an antisense polygalacturonase gene isolated from tomato." The associated promoters and terminators used by Calgene to direct expression of the FLAVR SAVR™ gene are, respectively, the promoter from the *35S* gene derived from cauliflower mosaic virus, or two copies of that promoter in tandem, and terminators from the transcript 7 and *tml* genes from the octopine-type Ti plasmid pTiA6.

The effect of this determination is that all those tomato lines containing the FLAVR SAVR™ gene, that were derived using any of the above-mentioned binary vectors and that have previously been field tested under permit, will no longer be considered regulated articles under APHIS regulations at 7 CFR part 340. Permits under those regulations will no longer be required from APHIS for release into the environment, importation, or interstate movement of those tomatoes or their progeny. Agronomic practices involving these FLAVR SAVR™ tomato lines, e.g., cultivation, propagation, movement, and crossbreeding with other non-regulated tomato lines, can now be conducted without APHIS permit. (Importation of FLAVR SAVR™ tomatoes [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR part 319). Variety registration and/or seed certification for individual tomato lines carrying the FLAVR SAVR™ gene may involve future actions by the U.S. Plant Variety Protection Office and State Seed Certification officials.

Based on its authority (under the Federal Plant Pest Act and the Plant Quarantine Act) for protecting American agriculture against diseases injury, or damage, APHIS regulates plant pest organisms, organisms whose plant pest status is unknown, and organisms containing components derived from plant pests. The regulations have the premise that when plants are developed using biological vectors from pathogenic sources, use material from pathogenic sources, or pathogens are used as vector agents, that they should be evaluated to assure that there is not a plant pest risk. APHIS does a review that allows a verification of the biology of the organism: assesses the degree of uncertainty and familiarity; and allows the identification of any risks, should they be present and predictable. The FLAVR SAVR™ tomato contains components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. APHIS' determination that the FLAVR SAVR™ tomato does not present a plant pest risk is based on an analysis of data provided to APHIS by Calgene and other relevant published scientific data obtained by APHIS concerning the components of the FLAVR SAVR™ tomatoes and observable properties of the tomatoes themselves.

From this review, we have determined that these FLAVR SAVR™ tomatoes: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than its non-engineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) do not cause damage to processed agricultural commodities; and (5) are unlikely to harm other organisms, such as bees, that are beneficial to agriculture. In addition, we have determined that there is no reason to believe that new progeny FLAVR SAVR™ tomato varieties bred from these lines will present a pant pest risk, i.e., have properties substantially different from any observed for the FLAVR SAVR™ tomato lines already field tested, or those observed for tomatoes in tradional breeding programs.

APHIS' determination has been made in response to a petition received from Calgene, Inc., of Davis, California, dated may 31, 1992. The petition seeks a determination from APHIS that the FLAVR SAVR™ tomato does not present a plant pest risk and is therefore not a regulated article. On July 14, APHIS announced receipt of the Calgene petition in the Federal Register (57 FR 31170) and stated that the petition was available for public view. In that notice, APHIS also announced its intent to issue an interpretive ruling that the FLAVR SAVR™ tomato does not present a plant pest risk and would therefore no longer be considered a regulated article under its regulations. APHIS invited written comments on this proposed action, to be submitted on or before August 28, 1992.

The Calgene petition to USDA was made in conjunction with the following two filings made to the U.S. Food and Drug Administration: (1) "*kan*^{r} Gene: Safety and Use in the Production of Genetically Engineered Plants," Request for Advisory Opinion, U.S. Food and Drug Administration Docket 90A-0416, November 26, 1990; and (2) "FLAVR SAVR™ Tomato: Status as Food," Request for Advisory Opinion, U.S. Food and Drug Administration Docket 91A-0330/API, August 12, 1991. The U.S. Food and Drug Administration has authority over the safety of foods offered for sale, and has presented its policy regarding the safety of new plant varieties, including those produced through biotechnology, in a notice "Statement of Policy' Foods Derived From New Plant Varieties" (57 FR 22984-23005).

The body of this document consists of the following two parts: (1) background information which provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of FLAVR SAVR™ tomatoes and a summary and response to comments provided to APHIS on its proposed action during the public comment period; and (2) analysis of the key factors relevant to APHIS' decision that the FLAVR SAVR™ tomato does not present a plant pest risk.

II. Background

APHIS regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under Section 340.0 of the regulations, a person is required to obtain a permit prior to introducing a regulated article. A genetically engineered organism is deemed a regulated article either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. Permission to conduct a field trial with an article regulated under 7 CFR part 340 is granted when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

The FPPA gives USDA authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants which may harbor injurious pests or diseases, and required that they be grown under certain conditions after importation. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties, and to demonstrate that although derived using components from plant pests, they do not possess plant pest characteristics. However, an organism is not subject to the permitting requirements of 7 part 340, whether or not it has previously been treated as a regulated article, when it is demonstrated not to present a plant pest risk.

FLAVR SAVR™ tomatoes have been considered "regulated articles" for field testing under Part 340.0 of the regulations in part because of the vector system used to transfer the antisense polygalacturonase (PG) gene into the recipient tomato. The vector system was derived from *A. tumefociens*, which is on the list of organisms in the regulation and is widely recognized as a plant pathogen. In addition, certain noncoding regulatory sequences were derived from plant pathogens, i.e., from *A. tumefociens* and from cauliflower mosaic virus.

Under existing regulations, APHIS considers whether organisms, such as the FLAVR SAVR™ tomato, that are derived at least in part from plant pests, pose any potential plant pest risk, before they enter any commercial use. Such consideration may aid the entry of new plant varieties into commerce or into breeding and development programs. The Calgene petition is the first request received by APHIS for a determination that an organism, for which field trials have been conducted under permit under the 7 CFR part 340 regulations, does not present a plant pest risk and is hence not a regulated article. The decision by APHIS that the FLAVR SAVR™ tomato is not a regulated article is based in part on evidence provided by Calgene concerning the biological properties of the FLAVR SAVR™ tomato and its similarity to other varieties of tomato grown using standard agricultural practices for commercial sale or private use. The FLAVR SAVR™ tomato has been field tested under eight APHIS permits (88-344-07, 89-320-01, 90-019-01, 90-249-01, 91-050-01, 91-107-04, 91-268-01, and 92-022-04) in California and Florida with two of the trials involving more than one field site (91-050-01, 2 sites; 92-022-04, 4 sites). Calgene, in appendix 5 of its petition request, has provided field data reports from all of the field trials completed before 1992.

When 7 CFR part 340 was published as a Final Rule on June 16, 1987, APHIS anticipated that, at an appropriate time, individuals might seek to exempt organisms from the regulations by means of a petition, as described in § 340.4, to amend the list found in § 340.2, "Groups of organisms which are or contain plant pests." To date, APHIS has not received any requests of this type. APHIS believes that the Calgene petition for determination of the regulatory status of the FLAVR SAVR™ tomato is representative of the type of petitions that APHIS may receive

regarding other organisms in the future. In order to facilitate future petitions analogous to the Calgene petition, APHIS is preparing a proposal to add a provision to 7 CFR part 340 formalizing this petition process, and expects that this proposal will be completed in the near future. Until such a system is in place, applicants may nonetheless continue to petition APHIS for determinations of regulatory status of particular organisms.

The certification 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' approach to plant pest risk is considerably broader than a narrow definition which encompasses only plant pathogens. Rather, 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 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 plant pathogenic properties; (2) is no more likely to become a weed than its nonengineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can be interbred; (4) does not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. Evidence has been presented by Calgene that bears on all of these topics. In addition, inasmuch as the Calgene petition seeks a determination regarding new tomato varieties containing the FLAVR SAVR™ gene, it should be established that there is no reason to believe that any new tomato varieties bred from FLAVR SAVR™ tomato lines addressed here will present a plant pest risk, i.e., have properties substantially different from any observed for tomatoes in traditional breeding programs or as seen in the development of the FLAVR SAVR™ tomato lines already field tested.

Public Comments: Analysis and Response

During its 45-day comment period, APHIS received 19 comments regarding its proposed interpretive ruling in response to Calgene's petition. Of the 19 comments, 15 were supportive of APHIS' proposed action and 4 expressed serious reservations or disapproval of it.

Most of the comments (11 of the 15) in support of APHIS' proposed action based their support on scientific data concerning the lack of plant pest risk presented by some or all of the components of the FLAVR SAVR™ tomato, i.e., the parent organism, the disarmed *A. tumefaciens* vector, and/or the pant pathogen-derived regulatory DNA sequences. Two comments voiced general approval for APHIS' actions, one of which also indicated that passive movement via pollen of the introduced traits from FLAVR SAVR™ tomatoes into other cultivated tomatoes should be acknowledged. APHIS concurs in this observation, but has identified no implications of low level movement of the FLAVR SAVR™ gene that would differ from that for any other tomato gene. The final comment expressed support for the legal basis for APHIS' use of the interpretive ruling mechanism to arrive at its determination. Among the 11 comments that addressed the lack of plant pest risk posed by components of the FLAVR SAVR™ tomato, one also expressed the opinion that FLAVR SAVR™ tomatoes would be grown mostly on larger commercial farms where there are well-established environmental management practices. This comment noted further that longer ripening times on the vine for FLAVR SAVR™ tomatoes were not likely to result in increased pesticide use in cultivation, because few pesticides are approved for use on tomatoes close to harvest time, and that Calgene has in any event been a strong proponent of the use of Integrated Pest Management techniques for tomato cultivation. Another comment, while supportive of APHIS' action, expressed some concern about the slim possibility that horizontal gene transfer could take place from FLAVR SAVR™ tomatoes to microorganisms. APHIS believes that this topic has been adequately addressed by Calgene and in this determination. APHIS has not identified any potential effects arising from any rare gene transfer from FLAVR SAVR™ tomatoes to microorganisms, should any occur.

Four commenters opposed APHIS approving the Calgene petition at this time. The commenters requested that APHIS delay approval or deny the petition at least until such time as new regulations are promulgated that are intended to address "large scale releases." The commenters noted that the current regulations are not intended to address large scale releases *per se*. APHIS does not believe that the absence of "large scale" regulations should preclude the Agency from approving the Calgene petition. APHIS notes that its current regulations are not scale-dependent. Furthermore, APHIS believes that it possesses sufficient information to justify approval of the Calgene petition.

One of the four commenters expressed the opinion that the environmental and health risks of genetically engineered organisms remain undetermined and unpredictable. APHIS believes that there is no support for this general supposition and that Calgene has provided adequate data for evaluation of the relevant properties of the FLAVR SAVR™ tomato.

Three of the four commenters questioned the adequacy of data collected in small scale field trials to address environmental issues arising upon large scale cultivation or commercial use of FLAVR SAVR™ tomatoes. APHIS believes that these comments raised no new pest-related issues which have not been considered by APHIS in this determination. To address this question further and in a more general way, however, APHIS convened, on August 19 and 20, 1992, a panel of experts in tomato biology and cultivation to discuss safeguards for planned releases of transgenic tomato. The workshop, held at and cosponsored by the University of California at Davis, was run in conjunction with an international meeting on the Molecular Biology of Tomato. Workshop participants identified no unique risks associated with genetic engineering of tomato, and indicated that traits currently being introduced into tomato via genetic engineering and other methods seem to pose little threat in themselves. A summary of the discussions at the workshop is currently in preparation. Similar crop-specific workshops have been or are being held for rapeseed, potato, corn, wheat, and rice. In addition, as discussed in Section II above, APHIS is preparing a proposed modification to 7 CFR Part 340 which will codify a petition process for determination of regulatory status. That

proposed modification will address the questions that pertain to releases of transgenic crops regardless of scale.

Three of the four commenters expressed concern that this determination will have broad and undesirable policy implications. APHIS disagrees. Although Calgene's petition is the first to APHIS concerning an organism which has been field tested under permit under 7 CFR part 340 regulations, the petition request is quite specific. It asks that the Agency determine that the FLAVR SAVR™ tomato should no longer be considered a regulated article based on information presented on that tomato. Our ruling is based on the information provided on a specific organism and is a ruling, based on scientific data, on the regulatory status of that organism.

Two of the four commenters expressed the opinion that Calgene has not adequately addressed the potential for transfer of genetic material to cultivated tomatoes and wild relatives including cherry tomato, and the potential for weediness in FLAVR SAVR™ tomatoes. These comments indicated that additional experiments were necessary to provide data on gene movement and weediness. APHIS disagrees. The comments have failed to provide any reason for believing that the FLAVR SAVR™ modification will in any way modify weediness or affect the survival of any other cultivated tomato or tomato relative. APHIS further believes that it has considered these issues adequately in its determination. (See Section III, Point (2).)

One of the four commenters questioned Calgene's assertion that the FLAVR SAVR™ gene would provide no selective advantage to organisms containing it, based on the fact that it appears to confer some additional resistance to damage by certain types of fungal infection. APHIS believes that this type of fungal resistance is not significantly different from resistance phenotypes seen in certain other commercially available tomato varieties having altered ripening properties. This question is addressed further in Section III, Point (2).

One of the four commenters indicated that Calgene has not adequately addressed the potential for indirect effects from cultivation of the FLAVR SAVR™ tomato. In APHIS' judgment, no potential indirect effects were identified by the commenter to warrant concern.

One of the four commenters suggested that APHIS should consider in its decision the implications of export of FLAVR SAVR™ tomatoes to other nations where other sexually compatible tomato relatives are present. APHIS authority to regulate plant pests and other organisms does not extend beyond the borders of the U.S. Rather, there are phytosanitary regulations governing the international movement of individual plants and commodities throughout the world. APHIS will, however, consult with regulatory officials of other nations upon request, regarding scientific data on gene movement and other factors which may be relevant to their decisions regarding their importation and cultivation of these organisms.

Two of the same four commenters provided specific queries regarding the breadth of APHIS' ruling on Calgene's petition. These queries will be paraphrased for clarity and addressed. (1) Does this determination apply to a FLAVR SAVR™ gene introduced into any plant other than tomato? Response: No. (2) Does APHIS' finding that these FLAVR SAVR™ tomatoes present no plant pest risk mean that the tomatoes are no longer subject to the Federal Plant Pest Act? Response: The tomatoes covered by this determination are no longer considered to be regulated articles under 7 CFR part 340, but, as for any other plant, this authority could be reasserted if a new plant pest risk should ever be uncovered in the future. (3) Does this determination regarding plant pest risk extend to other tomatoes independently made by other individuals using the same genes, or to other tomatoes engineered using a different antibiotic resistance marker? Response: No. This determination covers FLAVR SAVR™ tomato lines that have been field tested under permit to Calgene plus all their genetic descendants. New tomato lines carrying different marker genes would not be within the scope of this determination. (4) Are the plant pest-derived sequences in FLAVR SAVR™ tomatoes derived from *Agrobacterium* and CaMV no longer regulated under 7 CFR part 340? Response: No. Calgene has provided data to APHIS that pertains specifically to the lack of plant pest risk for its vector constructs in FLAVR SAVR™ tomatoes.

III. Analysis of the Properties of the FLAVR SAVR™ Tomato

A brief discussion of the biology of tomato follows in the next paragraph as background information for the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular issues with respect to the FLAVR SAVR™ tomato.

The tomato, *Lycopersicon esculentum* var. *esculentum*, is distributed worldwide and is grown commercially wherever agronomic conditions will permit an economic yield to be obtained. *Lycopersicon* is a genus of the large and diverse family Solanaceas, which also includes peppers, tobacco, and eggplant. The genus has been divided into two subgenera, the *esculentum* complex which contains those species which are easily crossed with commercial tomato, and the *peruvianum* complex which contains those species which are crossed with considerable difficulty (Stevens and Rick, 1986; Taylor, 1986). *Lycopersicon* species are native to Ecuador, Peru, and the Galapagos Islands; however, most evidence suggests that the site of domestication of *L. esculentum* was Mexico (Taylor, 1986). The cultivated tomato is self-fertile and almost exclusively self-pollinating, generally requiring the intervention of man for cross-pollination. The only relative of *L. esculentum* var. *esculentum* that is found in the U.S. and with which var. *esculentum* is sexually compatible is *L. esculentum* var. *cerasiforme*. The cultivated tomato is a highly inbred perennial that is grown almost exclusively as an annual in the U.S. Of the over 500,000 acres of tomatoes that are grown annually in the U.S., approximately 40% are grown for fresh market consumption; the balance are grown for processing.

To reach its determination that the FLAVR SAVR™ tomato does not present a plant pest risk, APHIS has analyzed not only public comments and basic information on the biology of tomato, but also data presented by Calgene and scientific data on other topics relevant to each of the considerations previously listed as relevant to a discussion of plant pest risk. Based on the data described, APHIS has arrived at a series of conclusions regarding the properties of the FLAVR SAVR™ tomato.

(1) Neither the introduced genes, their products, nor the added regulatory sequences controlling their expression presents a plant pest risk in these FLAVR SAVR™ tomatoes.

The disarmed *Agrobacterium tumefaciens* transformation vector does not present a plant pest risk in FLAVR SAVR™ tomatoes. The vector system used to transfer the FLAVR SAVR™ gene into the tomato nuclear genome is based on the natural tumor-inducing (Ti) plasmid system used by the plant pathogenic bacterium *A. tumefaciens* for plant infection and gene transfer (Zambryski, 1988). (*A. tumefaciens* is the causal agent of a plant disease called crown gall.) Calgene has presented evidence that the Ti-plasmids used in the construction of FLAVR SAVR™

tomatoes (pCGN1547, pCGN1548, PCGn1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578; McBride and Summerfelt, 1990) have been *disarmed*, i.e., the natural pathogenicity genes which result in the characteristic symptoms of crown gall (e.g., overproduction of phytohormones in the plant resulting in unusual cell and organ overgrowth and the formation of galls, and synthesis of unusual, tumor-specific amino acids) in an infected plant have been removed from the transferred or T-DNA. The natural gene sequences between the T-DNA border sequences can be deleted and replaced by DNA from other sources without affecting the ability of *A. tumefaciens* to transfer the T-DNA to plants (Caplan et al., 1983). Only the border sequences of the T-DNA are required for transfer into the plant nuclear genome and only DNA located between the border sequences is efficiently transferred and integrated (Wang et al., 1984); therefore, other genes inserted into the T-DNA region by conventional cloning techniques will be transferred and integrated into the plant nuclear genome (Hernalsteens et al., 1980). The vector system used by Calgene is said to be "binary," i.e., the genes to be transferred are found on one plasmid and the genes encoding functions necessary for transfer are found on a second plasmid.

The scientific literature, reviewed by Calgene and previously evaluated by APHIS in environmental assessments relative to field trials for FLAVR SAVR™ tomatoes under permit, supports the finding that only the T-DNA region is transferred into the plant genome and only the sequences contained between the border DNA sequences are integrated (Fraley et al., 1986). Briefly, it has been established that the border sequences do not remain intact during the process of insertion of T-DNA into the plant cell genome, and therefore the inserted DNA is no longer a functional T-DNA. In other words, the transferred T-DNA segment cannot be transferred a second time to a new recipient using the same mechanism that originally inserted it into the plant genome (Zambryski et al., 1982). The plasmid vector by itself is not viable and can only replicate inside bacterial cells.

Calgene has presented evidence (See Appendix 1) that the transferred genetic material in FLAVR SAVR™ tomatoes is genetically stable and segregates in a Mendelian fashion, i.e., in a fashion consistent with integration of the added genetic material into nuclear chromosomal DNA. Calgene has also analyzed the physical structure of integrated FLAVR SAVR™ genetic material in several transformant lines (See Appendix 1, Subsection Appendix D–2). In addition to these direct analysis, there is a wealth of data in the scientific literature, some of which is presented by Calgene, showing that *A. tumefaciens* T-DNA with or without genes for tumorigenicity becomes integrated into nuclear chromosomal DNA as part of the gene transfer process. A single unconfirmed report has shown that T-DNA can insert into choloroplast DNA (de Block et al., 1985). As integrated pieces of plant chromosomes, T-DNAs are subject to the same rules governing chromosomal rearrangements and gene stability as other plant genes. Once integrated into plant chromosomes (as no other type of T-DNA maintenance in transformed cell lines has been demonstrated), T-DNA becomes no different than naturally occurring plant genes in terms of stability, or potential ability to persist in the environment outside of direct progeny of transformed plants. The T-DNA containing the FLAVR SAVR™ gene is transmitted through mitosis and meiosis as a new and novel locus that is an inherent part of the plant genome.

Following the use of the disarmed *Agrobacterium* vector system for tomato transformation, the bacterium has been killed with the antibiotic carbenicillin to eliminate the possibility of subsequent infection or transformation (Fillatti et al., 1987). Calgene has further indicated in its field reports that none of the transgenic tomatoes show disease symptoms indicative of infection by *A. tumefaciens*.

The introduced coding regions do not confer a pest risk. Tomato plants have been transformed with the FLAVR SAVR™ gene, an antisense polygalacturonase gene isolated from tomato. Tomato, *Lycopersicon esculentum* var. *esculentum*, is not a regulated article. FLAVR SAVR™ tomatoes have reduced levels of polygalacturonase (PG), a pectin degrading enzyme, which results in slowed cell wall breakdown and associated fruit softening. There is no reason to believe that this antisense gene, essentially a reverse copy of part of the native tomato PG gene, could impart any capability to the FLAVR SAVR™ to cause disease or damage to any other plant. The FLAVR SAVR™ tomato plants have also been transformed with a kanamycin resistance (*kan*^{r}) gene. The *kan*^{r} gene encodes the enzyme aminoglycoside 3'-phosphotransferase II, which confers resistance to the antibiotic kanamycin. (The *kan*^{r} gene is also frequently referred to in the literature as *nptII*, which encodes neomycin phosphotransferase.) This gene was introduced as a marker, i.e., as a tag enabling identification of tomato cells that had concomitantly taken up the antisense PG gene. the *kan*^{r} gene was isolated from a transposon contained in a strain of *Escherichia coli* K12 (Beck et al., 1982; Jorgensen et al., 1979). *E. coli*, a common enteric bacterium found in the human gut, is not a regulated article. The *kan*^{r} gene has no involvement in plant disease or damage. Also, its use does not result in the presence of the antibiotic kanamycin in FLAVR SAVR™ tomatoes and does not imply that kanamycin will be used in the cultivation of the tomatoes.

The introduced regulatory sequences do not confer a pest risk. Some of the regulatory sequences fused to the FLAVR SAVR™ and *kan*^{r} genes were derived from organisms that are on the list of regulated articles. Specifically, 3' transcription termination and polyadenylation sequences from the *tml* gene and the transcript 7 gene from the octopine-type Ti plasmid pTiA6 (Barker et al., 1983), and 5' promoter and 3' transcription termination and polyadenylation sequences from the *mas* gene (Velten et al., 1984), are derived from *A. tumefaciens*; and the *35S* promoter region is derived from the cauliflower mosaic virus (CaMV) (Odell et al., 1985). In addition, as a consequence of the transformation process, portions of the T=DNA border sequences were transferred to the tomato genome. None of these sequences has any direct involvement with pathogenicity in the pathogenic organism from which it was derived. Despite the presence of pathogen-derived sequences in the FLAVR SAVR™ genome, no crown gall or CaMV disease symptoms were observed by Calgene in any FLAVR SAVR™ tomato plants during greenhouse or field studies. Calgene further provides evidence that expression of any of the introduced genes does not result in disease symptoms (See Appendix 5) or the synthesis of products toxic to other organisms (See Appendices 1 and 7). None of the regulatory sequences encodes any polypeptide product.

There is no published evidence for the existence of any mechanism, other than sexual crossing of compatible tomatoes, by which the *kan*^{r} and FLAVR SAVR™ genes can be transferred to other organisms (appendix 2). Comparative analyses of numerous gene sequences from microorganisms and plants have never to our knowledge yielded any published evidence of strong inter-kingdom gene homologies that would be

indicative of recent or frequent gene homologies that would be indicative of recent or frequent gene exchanges between plants and microorganisms, except for *Agrobacterium* mediated gene transfers. A certain amount of information can be found in the scientific literature (e.g., Carlson and Chelm, 1986; Wakabayashi et al., 1986; Doolittle et al., 1990) that provides a suggestion that transfer of genes from plants to microorganisms may have occurred, but only over evolutionary time, i.e., in the millennia since the various times of divergence between the kingdoms. A single report (Bryngelsson et al., 1988) has suggested that plant DNA can be taken up by a parasitic fungus, but no further evidence has ever been forthcoming that such DNA uptake has resulted in the transfer of a functional DNA sequence. Even if a rare plant-to-microbe gene transfer were to take place, there is no reason to believe that transfer of either the *kan*^{r} gene or FLAVR SAVR™ gene would pose any plant pest risk. Also, in its petition to APHIS, Calgene has presented a calculation of the potential contribution of kanamycin resistant bacteria derived by horizontal gene movement from the genome of the genetically engineered tomato based on a worst case scenario which starts with the premise that gene transfer will undoubtedly occur. One conclusion they present based on these calculations is that kanamycin resistant soil bacteria arising from transformation from plant debris would represent no more than 1.4×10^{-16}% of the kanamycin resistant microbes already present. Based on Calgene's calculations as well as the data present in the scientific literature, we conclude that concerns regarding DNA transfer from FLAVR SAVR™ tomatoes to microorganisms are at best speculative.

(2) FLAVR SAVR™ tomatoes have no significant potential to become successful weeds.

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."

A weed pest is a plant that grows persistently in locations where it is unwanted. As indicated in the Calgene petition, tomato has been grown for centuries throughout the world without any reports that it is a serious weed pest. In the U.S., it is not listed under the Federal Noxious Weed Act. In fact, tomato, though an exotic species introduced into the United States, is not classified as a serious, principal, or common weed pest (Holm et al., 1979). Although tomato volunteers are not uncommon, they are easily controlled using herbicides or by mechanical means. Tomato possesses few of the characteristics of plants that are notably successful weeds, e.g., it does not produce abundant, long-lived seed (Keeler, 1989). It is a perennial crop which is grown almost exclusively as an annual crop in the U.S. Tomato is considered to be a highly domesticated, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. The FLAVR SAVR™ tomato is likely to be grown mostly in areas that are currently under tomato cultivation, i.e., in typical growing regions for the crop.

Calgene has designed experiments and collected data from greenhouse and field trials that support the contention that the FLAVR SAVR™ tomato has little potential to become a serious or successful weed. These observations have shown that the FLAVR SAVR™ tomatoes have: (1) Agronomic and horticultural traits (e.g., fruit size, shape, and pigmentation) similar to those of traditionally bred tomatoes (See Appendix 5); (2) a range of seed germination rates and frequencies comparable to those of nontransformed tomatoes (See appendix 6); and (3) no alterations in traits such as seed germination or dispersal that could confer a selective advantage and could enhance survival in the wild (See appendices 1 and 2).

There are no morphological, physiological, or disease resistance characteristics of the FLAVR SAVR™ tomato that would entail the use of agricultural practices which vary from the traditional practices used today for the cultivation and propagation of tomatoes. To achieve optimal flavor, however, FLAVR SAVR™ tomatoes may be left to ripen in the field longer than other tomatoes.

FLAVR SAVR™ tomato fruits do show a decreased rate of lesion expansion when wounds are inoculated with opportunistic pathogens of stressed fruit, the fungi *Geotrichum candidum* and *Rhizopus stolonifer*, causal agents of sour rot and Rhizopus rot, respectively (See Appendices 1 and 5; Kramer et al., 1992); they do, however, still rot. Sour rot and Rhizopus rot are two of the most common postharvest diseases of ripening tomato fruits (Jones et al., 1991). The fungi rot the flesh of the fruit, but are not thought to destroy the seeds (T.A. Zitter, personal communication; J.A. Bartz, personal communication). There is no reason to believe that the increased resistance to degradation by these fungi will lead to an increase in the persistence of the FLAVR SAVR™ tomato fruits or seeds. Several naturally occurring mutants of tomato exist that produce lower levels of PG and display slowed ripening of tomato fruit (Tigchelaar et al., 1978; DellaPenna et al., 1987). Cultivars which contain one or more of these mutations, specified by single genes, are grown and marketed today in the U.S. (J.W. Scott, personal communication; E.C. Tigchelaar, personal communication). Their fruits also show increased resistance to postharvest pathogens (Barkai-Golan and Kopeliovitch, 1980, 1981; Lavy-Meir et al., 1989). Based on these observations, and based on the fact that the intended modification in FLAVR SAVR™ tomatoes is unrelated to any trait which could affect weediness, there is no likelihood that FLAVR SAVR™ tomatoes will have enhanced weediness traits compared to non-transformed tomatoes.

(3) The FLAVR SAVR™ tomato will not increase the weediness potential of any other plant with which it can interbreed.

Tomato is not considered a weed pest itself and breeding of cultivated tomatoes has never produced a weed pest. Tomato does not cross-pollinate with other plants in the United States without the intervention of man. Cultivated tomato is self-fertile and also 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).

Many other members of the nightshade family are found as weeds in tomato fields. *Lycopersicon esculentum* is sexually incompatible with all these weedy relatives (Rick, 1979). Two *Solanum* species, *S. lycopersicoides* and *S. rickii*, can be crossed with commercial tomato under specific, controlled conditions, but they do not naturally cross with *L. esculentum* (Stevens and Rick, 1986; De Verna et al., 1990). Neither of these *Solanum* species is a weed pest in the United States.

The cherry tomato, *L. esculentum* var. *cerasiforme*, was most likely the wild progenitor of the cultivated tomato (Rick, 1963). Some biotypes of *L. esculentum* var. *cerasiforme* are successful weeds that have spread throughout tropical America and into southern Texas and Florida (Rick, 1973).

Cherry tomato, however, is not considered a weed pest. Although *L. esculentum* var. *esculentum* and var. *cerasiforme* can cross with either plant as male or female parent (Rick, 1979), the probability of FLAVR SAVR™ tomato naturally introgressing into var. *cerasiforme* in the United States is almost nil since the rate of outcrossing in var. *esculentum* is low (Rick, 1949; C. M. Rick, personal communication), and var. *cerasiforme* is not present in areas of the U.S. that are devoted to large scale cultivation of tomatoes (J. W. Scott, personal communication; Appendix 4). There are no published reports that visible traits of cultivated tomato have introgressed into var. *cerasiforme* from cultivated tomatoes in areas where the wild cherry tomato commonly grows.

Because tomato has no relatives other than itself with which it can naturally cross in the United States, and because commercial tomatoes are virtually exclusively self-pollinating, there is little possibility of a cross unaided by man between the FLAVR SAVR™ tomato and another plant. Therefore, there is no likelihood that the FLAVR SAVR™ tomato will increase the weedy potential of another plant. Cultivation of *L. esculentum* requires maintenance of genetic purity as a standard breeding practice. Regulations specifying procedures for the maintenance of genetic purity have been codified (See 7 CFR Part 201). Even if an outcrossing event involving pollen from a FLAVR SAVR™ tomato did occur, there is no reason to believe that the delay in fruit softening brought about by the antisense modification could affect seed persistence or weediness potential in progeny. Expression of the FLAVR SAVR™ gene in any of the lines thus far tested has not changed any morphological or physiological characteristics which might affect pollination (See Appendix 1), and there is also no reason to believe that this characteristic could be affected by the introduced genes.

(4) The FLAVR SAVR™ tomato will not cause damage to processed agricultural commodities.

In APHIS' opinion, the components and processing characteristics of FLAVR SAVR™ tomatoes reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. Although the expression of the antisense PG gene decreased the rate of the pectin breakdown and thereby increased the solid content of the tomato and the viscosity of processed products, such as paste, derived from it, these effects should have no bearing on the susceptibility of any processed plant commodity to disease or damage. The petition notes that FLAVR SAVR™ tomatoes, in fact, exhibit increased resistance to certain fungal pathogens (See Appendices 1 and 5; Kramer et al., 1992), perhaps because of increased integrity of tomato cell walls in the ripening FLAVR SAVR™ tomato. In addition, Calgene has presented both evidence on the inability of FLAVR SAVR™ tomatoes and the FLAVR SAVR™ gene to cause disease as well as evidence concerning the improbability of gene transfer to bacteria (including plant pathogens which could affect processed plant products). The evidence as to the components and processing characteristics of FLAVR SAVR™ tomatoes was provided by Calgene to the FDA in its Request for Advisory Opinion, "FLAVR SAVR™ Tomato: Status as Food," included in the petition to APHIS as Appendix I. The information submitted included a biochemical characterization of tomato components, nutrients, and potential toxins in tomatoes selected for low PG activity and fresh market tomato characteristics.

(5) The FLAVR SAVR™ tomato will not be harmful to beneficial organisms, including bees.

There is no reason to believe that deleterious effects on beneficial organisms could result specifically from the cultivation of FLAVR SAVR™ tomatoes, based on two lines of reasoning: (1) Analysis of biochemical components of FLAVR SAVR™ tomatoes (provided by Calgene in appendix 1) identified no toxic components of these tomatoes which are present in concentrations significantly different from the concentrations in nontransgenic tomatoes; and (2) no direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis towards beneficial organisms such as bees and earthworms, were identified by Calgene for FLAVR SAVR™ tomatoes. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenetic effect. It should be noted that, although rotting of FLAVOR SAVR™ tomatoes is delayed relative to that of nontransgenic tomatoes, they will in fact decay into components which are virtually identical to the decay components of nontransgenic tomatoes.

The definition of FLAVR SAVR™ tomatoes encompasses not only tomato lines that already have been field tested, but also new tomato lines produced through breeding using FLAVR SAVR™ tomatoes as one or both parents. APHIS believes that the analysis applied to FLAVR SAVR™ tomatoes already field tested will apply equally well to these new tomato lines, and that the data provided by Calgene justify the conclusion that such new FLAVR SAVR™ tomatoes will not present a plant pest risk. The variation in agronomic characteristics among the FLAVR SAVR™ tomato lines that have been field tested does not differ significantly from that seen in commercial cultivars of tomato which have never been considered regulated articles. While it is impossible to predict the exact agronomic characteristics of the progeny of a cross between a FLAVR SAVR™ tomato and a non-regulated tomato cultivar, cross-breeding between well-characterized tomato varieties is the traditional means by which new and improved tomato varieties are created. Thus, APHIS has concluded that there is no reason to believe that these progeny of the FLAVR SAVR™ tomato will present a plant pest risk, i.e., have properties substantially different from any observed for the FLAVR SAVR™ lines already tested or those observed for tomatoes in traditional breeding programs.

IV. Conclusion

APHIS has determined that tomato plants containing the FLAVR SAVR™ gene, that were derived using any of the seven above-mentioned binary vectors and that have previously been field tested under permit, will no longer be considered regulated articles under APHIS regulations at 7 CFR part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those tomatoes or their progeny. (Importation of FLAVR SAVR™ tomatoes [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR part 319.) This determination has been made based on an analysis which revealed that those tomatoes: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their non-engineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) do not cause damage to processed agricultural commodities; and (5) are unlikely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is no reason to

believe that new progeny FLAVR SAVR™ tomato varieties bred from these lines will present a plant pest risk, i.e., have properties substantially different from any observed for the FLAVR SAVR™ tomato lines already field tested, or those observed for tomatoes in traditional breeding programs.

Terry L. Medley,

Director, Biotechnology, Biologics, and Environmental Protection.

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