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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. 92-087-1)

Proposed Interpretive Ruling in Connection with Calgene, Inc. Petition for Determination of Regulatory Status of FLAVR SAVR™ Tomato

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice of proposed interpretive ruling.

SUMMARY: We are advising the public that the Animal and Plant Health Inspection Service (APHIS) has received a petition from Calgene, Inc., seeking a determination regarding the regulatory status of its FLAVR SAVR™ tomato. APHIS is requesting comments on its proposal to issue an interpretive ruling that the FLAVR SAVR™ tomato does not present a plant pest risk, and therefore, would no longer be considered a regulated article under its regulations.

DATES: Consideration will be given only to written comments that are received on or before August 28, 1992.

ADDRESSES: To help ensure that your written comments are considered, send an original and three copies to Chief, Regulatory Analysis and Development, PPD, APHIS, USDA, room 804, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782. Please state that your comments refer to Docket No. 92-087-1. A copy of the Calgene submission and any written 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. A copy of the Calgene petition may be obtained by contacting Ms. Kay Peterson at 301-436-7601.

FOR FURTHER INFORMATION CONTACT: Michael A. Lidsky, Deputy Director, or Sally L. McCammon, Chief, Domestic Programs Branch, Biotechnology, Biologicals, and Environmental Protection, APHIS, USDA, room 850, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, 301-436-7601.

SUPPLEMENTARY INFORMATION: On June 2, 1992, the Animal and Plant Health Inspection Service (APHIS) received a "Petition for Determination of Regulatory Status" from Calgene, Inc. (Calgene), of Davis, CA. The Calgene petition seeks 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 has been described by Calgene as a tomato cultivar or progeny of a tomato line which contains an antisense copy of the constituent polygalacturonase gene which, when transcribed, results in delayed ripening of the tomato fruit.

The Calgene petition states that the FLAVR SAVR™ tomato should no longer be regulated by APHIS because it does not present a plant pest risk. The FLAVR SAVR™ tomato is currently considered a regulated article under the regulations because it was developed through the use of vectors, promoters, and terminators from plant pathogenic sources. However, as indicated in the petition, the vectors used in producing the FLAVR SAVR™ tomato were disabled, and the other plant pathogen derived elements did not present a risk of plant pest introduction or dissemination. The field testing of the FLAVR SAVR™ tomato indicates that it does not present a plant pest risk.

Under the regulations, a genetically engineered plant or other organism is a regulated article, subject to regulatory oversight by APHIS, if it is a plant pest or it is unclassified or the Deputy Administrator has reason to believe it is a plant pest. Based on reviews for a number of field tests of the FLAVR SAVR™ tomato and the information in the petition submitted by Calgene, APHIS believes that the FLAVR SAVR™ tomato is not a plant pest, and that there is no reason to believe that it may be a plant pest or otherwise presents any plant pest risk. Therefore, APHIS is proposing to issue a ruling that the FLAVR SAVR™ tomato is not a regulated article under its regulations.

APHIS is requesting comments on the petition and the proposed ruling.

After reviewing the data submitted by the petitioner, written comments received during the comment period, as well as other relevant literature, and interpreting the application of statutes and regulations to these data and comments, APHIS will issue its interpretive ruling regarding the regulatory status of the FLAVR SAVR™ tomato. A notice of the ruling and its availability will be published in the Federal Register.

Done at Washington, DC, this 8th day of July 1992.

Robert Melland,

Administration, Animal and Plant Health Inspection Service.

[FR Doc. 92-16348 Filed 7-13-92; 8:45 am]

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CALGENE

May 31, 1992

Director, Biotechnology, Biologics, and
Environmental Protection
c/o Biotechnology Coordination and
Technical Assistance
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Room 850, Federal Building
6505 Belcrest Road
Hyattsville, MD 20782

**PETITION FOR DETERMINATION:
FLAVR SAVR™ Tomato as a Non-Regulated Article under 7 CFR 340**

Dear Sir or Madam:

The undersigned submits this petition under 7 CFR 340 for a determination that the FLAVR SAVR™ tomato does not present a plant pest risk, is not otherwise deleterious to the environment, and is therefore not a regulated article.

The FLAVR SAVR tomato is defined as 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.

Currently, the FLAVR SAVR tomato is considered a regulated article because it contains the following subspecies (DNA sequences) from the list of organisms in § 340.2: the *tml* 3' terminator, the *mas* 5' promoter, the *mas* 3' terminator, and the right and left border regions from *Agrobacterium tumefaciens*; and the 35S promoter region (CaMV35S) from cauliflower mosaic virus.

Other components are not from organisms considered to be plant pests. *Lycopersicon esculentum* Mill. is not a regulated article and the FLAVR SAVR™ gene (an antisense polygalacturonase gene isolated from tomato) is not from a plant pest. The *kan^r* gene encoding APH(3')II (aminoglycoside 3'-phosphotransferase II) was isolated as a component of transposon Tn5 from a Cole1::Tn5 containing strain of *Escherichia coli* K12. The Tn5 gene is from *E. coli*, as is the Lac Z' gene. The *kan^r* and Lac Z' genes are from *E. coli* which is not a plant pest.

The attached Statement of Grounds provides appropriate support and data for this request that the FLAVR SAVR tomato does not present a plant pest risk, is not otherwise deleterious to the environment, and is therefore not a regulated article.

This petition contains no confidential business information.

The undersigned certifies, that to the best of his/her knowledge and belief, this petition includes all data, information and views relevant to the matter, whether favorable or unfavorable to the position of the undersigned, which is the subject of this petition. No known information which might be unfavorable to the petition has been withheld. No data have been produced to date which reflect negatively on this petition.

This request is made with filings with the Food and Drug Administration ("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 "FLAVR SAVR™ Tomato: Status as Food," Request for Advisory Opinion, U.S. Food and Drug Administration, Docket #91A-0330/API, August 12, 1991) for coordinated consideration and response by both the USDA APHIS and FDA.

Sincerely,



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cc Jim Maryanski, FDA

Calgene, Inc.

Petition for Determination:

FLAVR SAVR™ Tomato as a Non-Regulated Article under 7 CFR 340

May 31, 1992

Table of Contents

Volume 1. Petition for Determination: FLAVR SAVR™ Tomato as a Non-Regulated Article under 7 CFR 340

Table of Contents2
Statement of Grounds for Decision4
I. Overview4
Introduction.....4
Rationale for Development.....4
Removal from Regulated Status.....4
Petition to USDA APHIS.....5
II. The Recipient Plant: Tomato6
Tomato as a Crop6
Taxonomy of Tomato6
Natural Range of Tomato.....6
Genetics of Tomato.....7
Weed Characteristics and Environments.....8
Weediness Potential in Tomato.....9
 1. Likelihood of FLAVR SAVR Tomato Becoming a Weed
 Comparison of Transformed Crops with Exotic Species.....10
 2. Potential for FLAVR SAVR Tomato to Become a Weed
 Pest11
 3. Potential for Hybridization Between FLAVR SAVR
 Tomato and Wild Relatives Creating or Enhancing
 Weediness16
Mode of Gene Escape in Tomato.....16
 1. Outcrossing.....16
 2. Potential Plant-to-Microorganism Gene Flow17
Conclusions for Section II.....17
III. The Transformation and Vector System.....18
Conclusions for Section III.....19
IV. Donor Genes from Organisms Considered Regulated Articles.....19
Non-regulated Articles.....20
Sequences from Organisms Considered Regulated Articles20
 Terminator and Promoter21
 Borders21
 CaMV35S.....22
Conclusions for Section IV.....22
V. The Engineered Plant.....23
Conclusions for Section V26
**VI. Environmental Consequences of Introduction of the
Transformed Cultivars.....27**
APH(3')II Toxicity and Degradation.....27
Stability of Gene Products in the Environment.....28
Horizontal Gene Flow28

1. Likelihood that the genes will move from the plants to soil bacteria.....	29
2. Likelihood the soil bacteria might persist or be selected for in the soil.....	30
3. Likelihood that the genes may be transferred to yet other bacteria.....	30
Conclusions for Section VI.....	31
VII. Petition to USDA APHIS.....	31
VIII. Statement of Grounds Unfavorable.....	31
IX. List of Appendices.....	32
X. List of References.....	33

Volume 2: **Appendix 1. FLAVR SAVR™ Tomato: Status as Food. Request for Advisory Opinion, U.S. Food and Drug Administration, Docket #91A-0330/API. August 12, 1991.**

Volumes 3 & 4: **Appendix 2. *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. Two volumes (Vol I and Vol II).**

Volume 5: **Appendices 3 to 7.**

Appendix 3. State of California, Department of Food and Agriculture, Division of Plant Industry, "Pest Ratings of Noxious Weed Species and Noxious Weed Seed," 1/8/90; and Federal Noxious Weed Regulation, CFR 360.

Appendix 4. Letters from experts in tomato breeding and cultivation.

Appendix 5. Field trial reports for FLAVR SAVR tomatoes.

Appendix 6. Germination frequency and rate of FLAVR SAVR tomatoes and controls.

Appendix 7. Supplemental Data Submitted to FDA:

A) Fate of APH(3')II and Implications for Kanamycin Efficacy

B) Supplemental Information on Human Toxicity: *kan^r* Selectable Marker Gene and Gene Product

C) *kan^r* Protein Homology - Toxins

D) *kan^r* Protein Homology - Allergens

Volumes 6-9: **References**

Volume 1

Statement of Grounds for Decision

I. Overview

Introduction. The FLAVR SAVR™ tomato is defined as any 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) as described in (McBride and Summerfelt 1990) and the FLAVR SAVR gene with its associated promoter and terminator. The FLAVR SAVR™ gene is an antisense polygalacturonase gene isolated from tomato.

Complete descriptions of these seven binary vectors and the FLAVR SAVR gene with its promoter and terminator regions are contained within this petition. This petition contains a detailed description and data on the safety of the FLAVR SAVR tomato.

Rationale for Development. FLAVR SAVR tomatoes were developed by Calgene to improve the flavor and taste of fresh market tomatoes. The polygalacturonase (PG) gene was isolated from tomato and reintroduced into tomato in the reverse or "antisense" orientation as the FLAVR SAVR™ gene. The PG enzyme is responsible for pectin degradation in tomato fruit and is associated with fruit softening. Reducing the amount of PG in tomatoes slows cell wall breakdown resulting in ripe fruit which remain intact for an extended period. Fresh market tomatoes can then be vine-ripened for enhanced flavor and have a longer shelf life. Processing tomatoes produce a product with improved serum viscosity.

Removal from Regulated Status. Calgene requests that USDA APHIS, based on data and information presented in this document, determine that FLAVR SAVR tomatoes (species *Lycopersicon esculentum* Mill.), defined as a tomato cultivar or progeny of 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, do not present a plant pest risk, are not otherwise deleterious to the environment, and are therefore not a regulated article.

FLAVR SAVR tomatoes (Appendix 1) contain specific gene sequences introduced into the plant genome via the binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578)(Appendix 2 pages 25-34) plus the FLAVR SAVR gene with its associated promoter and terminator (Sheehy et al. 1988; Sheehy et al. 1987; Appendix 1 pages 160-171). The *tml* 3' terminator (Barker et al. 1983), the *mas* 5' promoter (Barker et al. 1983), the *mas* 3' terminator (Barker et al. 1983), and the right and left border regions (Barker et al. 1983) are from *Agrobacterium*

tumefaciens. The 35S promoter region (CaMV35S) is from cauliflower mosaic virus (CaMV)(Gardner et al. 1981).

The FLAVR SAVR gene (an antisense polygalacturonase gene)(Sheehy et al. 1987) was isolated from tomato, which is not considered a plant pest according to 7 CFR 340.2.

The *kan^r* gene encoding APH(3')II (aminoglycoside 3'-phosphotransferase II) was isolated as a component of transposon Tn5 from a ColE1::Tn5 containing strain of *Escherichia coli* K12 (Garfinkel et al. 1981). The Tn5 gene (Auerswald et al. 1981) and the Lac Z' gene (Yanisch-Perron et al. 1985) are from *E. coli* which is not a plant pest according to 7 CFR 340.2.

Although *A. tumefaciens* and CaMV are regulated articles, the FLAVR SAVR tomato containing sequences from these regulated articles should not be classified as a plant pest risk, as deleterious to the environment, nor as a regulated article under 7 CFR 340 for the following reasons:

1. Tomato is not a regulated article.
2. Genetic sequences from regulated articles used to produce FLAVR SAVR tomatoes have been disarmed and do not pose a plant pest risk.
3. Genes from regulated articles, introduced into tomato, do not confer characteristics that would present FLAVR SAVR tomato as a plant pest risk (e.g. cause tomato to become a weed pest risk).
4. No new compounds have been measured in FLAVR SAVR tomato that pose a hazard or are deleterious to the environment.

Petition to USDA APHIS. This petition is for USDA APHIS to determine that FLAVR SAVR tomatoes, 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, do not present a plant pest risk, are not otherwise deleterious to the environment, and are therefore not a regulated article.

This petition is made in conjunction with two filings with the Food and Drug Administration ("*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 FLAVR SAVR™ Tomato: Status as Food, Request for Advisory Opinion, U.S. Food and Drug Administration, Docket #91A-0330/API, August 12, 1991) for coordinated consideration and response by both the USDA APHIS and FDA. Calgene's approach to safety assessment of the FLAVR SAVR tomato is consistent with the FDA Statement of Policy: Foods Derived from New Plant Varieties (Docket No. 92N-0139) and with recommendations by both the International Food Biotechnology Council (International Food Biotechnology Council 1990) and the Joint FAO/WHO Consultation report (Joint FAO/WHO Consultation 1991).

II. The Recipient Plant: Tomato

Tomato as a Crop

Essentially all cultivated forms of tomato belong to the species *Lycopersicon esculentum* Mill. Tomato is grown commercially wherever environmental conditions permit an economic yield to be obtained. The principal growing regions for fresh market tomatoes are Florida (55,000 acres in 1987 valued at \$489 million) and California (29,000 acres valued at \$177 million). Other major states are Alabama, Arkansas, Georgia, Indiana, Louisiana, Maryland, Massachusetts, Michigan, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Virginia (USDA 1990).

The edible portion of tomato is botanically a fruit, although it is commonly considered a vegetable. Tomatoes are consumed fresh or processed. Processing tomatoes are prepared for a variety of uses: canned whole, in salsa, in ketchup, as tomato juice, as spaghetti and pizza sauces, as paste, as soups, etc. Fresh market tomatoes are eaten whole, sliced or diced in a variety of foods. They also are used in many foods, such as pizza and as fresh cooked tomatoes.

A detailed description of tomato and its cultivation and use are detailed in Appendix 1 (pages 185-228).

Taxonomy of Tomato

Tomato (*Lycopersicon esculentum* L) is a member of the Solanaceae family which includes potato and tobacco. Cultivated tomato is one of nine *Lycopersicon* species (Rick 1978), all of which have the same number of chromosomes ($2n = 2x = 24$) and chromosome morphology (Rick 1976).

Gross morphological characteristics of tomato include herbaceous perennial growth, sprawling or prostrate habit, pinnately segmented leaves, stem organization in sequences or 2- or 3-leaved sympodia, cymose inflorescences, yellow corolla and anthers, anthers connate or connivent, and fruit as a soft berry (Rick 1979).

Natural Range of Tomato

Esquinas-Alcazar (1981) describes the natural range of *Lycopersicon*:

The natural distribution of the genus *Lycopersicon* extends from northern Chile to southern Colombia and from the Pacific coast (including the Galapagos islands) to the lower eastern foothills of the Andes. Many species overlap but no evidence of natural introgression has been found, with the exception of *L. pimpinellifolium* and *L. esculentum*. All the species have well-defined ranges of distribution, except *L. esculentum* var.

cerasiforme (cherry tomato) which is the only wild and weedy *Lycopersicon* found outside the area of distribution of the genus. It is also present in the Old World where it might have escaped cultivation.

No wild *Lycopersicon* can be found outside Latin America, except for the very uniform *Lycopersicon esculentum* var. *cerasiforme*.

Genetics of Tomato

The factors (both pre- and post-fertilization barriers) that prevent cross-pollination between *Lycopersicon* species are well documented (Rick 1979; Taylor 1986) and are applicable to the FLAVR SAVR tomato. Tomato can only be crossed by hand-pollination to wild *Lycopersicon* species with varying degrees of success. The genus has been divided into two subgenera, one comprising those easily crossed with commercial tomato (*esculentum* subgenera), and the other those that cannot (*peruvianum* subgenera). The *esculentum* subgenera consists of *L. esculentum*, *L. cheesmanii*, *L. chmielewskii*, *L. hirsutum*, *L. parviflorum*, *L. pimpinellifolium*, and *Solanum pennelli*, which are naturally inter-crossable. The *peruvianum* subgenera consists of *L. chilense* and *L. peruvianum*, which are not naturally inter-crossable with the other subgenera. Wide hybridization between members of the two subgenera usually leads to early embryo breakdown and nonviable seed. Sexual hybridization between the two subgenera can only be accomplished using embryo culture. The closest genetic relatives of *Lycopersicon* are in the genus *Solanum*. *L. esculentum* can also be crossed with *S. lycopersicoides* using controlled pollination techniques, although the hybrids are usually sterile (Stevens and Rick 1986). Attempts to cross *L. esculentum* with *S. rickii* and *S. ochranthum* failed (Rick 1979). Recently, a controlled cross between *L. esculentum* and *S. rickii* was successful using a sesquidiploid bridging hybrid (De Verna et al. 1990), which may provide a means to move genes from *S. rickii* to commercial cultivars. No other member of the genus, including *S. nigrum*, a common weed in tomato fields, has yielded any viable hybrids with tomato (Taylor 1986).

Cultivated tomato and close relatives are self-fertile. Although tomato outcrossed "to a considerable extent in its native region and certain other subtropical areas, ... elsewhere [it] is almost completely self-pollinating" (Rick 1976). This autogamy is a result of transition in cultivated tomato from exerted to inserted stigmas within the anther cone (Rick 1979). Over the past 50 years, the change in style-length has been dramatic, "which further improved self-pollination and consequent fruit set and practically eliminated outcrossing" (Rick 1976). Taylor (1986) reports, "All representatives of *L. esculentum* are self-compatible and exclusively inbreeding."

Resistance to 14 pests has been bred into commercial cultivars from wild *Lycopersicon* species using controlled crossing techniques. For example, the gene for fusarium resistance (*I*) came from *L. pimpinellifolium* and the gene

for root knot nematode resistance (*Mi*) came from *L. peruvianum* (Rick 1983). Other examples of fungal resistance bred into cultivated tomato are: early blight (*Alternaria solani*), anthracnose (*Colletotrichum phomoides*) and verticillium wilt (*Verticillium albo-atrum*) from *L. esculentum* var. *cerasiforme*; and botrytis mold (*Botrytis cinerea*) from *L. hirsutum* (Esquinas-Alcazar 1981). Resistance to curly top virus came from *L. chilense* (Esquinas-Alcazar 1981). Similarly, improvements were made in high soluble solids in fruit using lines developed from crosses with *L. chmielewskii* (Rick 1983). Genes that prevent easy fruit abscission and retention of pedicels came from *L. chmielewskii* (Esquinas-Alcazar 1981). Additional information on tomato genetics is presented in Appendix 1 (pages 530-548).

Although these reports indicate that crosses between *L. esculentum* with all *Lycopersicon* species within the genus can be achieved, natural interspecific crossing is at least confined within the tomato's natural range in South America and only within the *esculentum* subgenera. There is strong evidence, however, that even in the natural range, interspecific crossing does not occur. Esquinas-Alcazar (1981) states that "many species overlap but no evidence of natural introgression has been found, with the exception of *L. pimpinellifolium* and *L. esculentum*." Raymond Clark (Appendix 4) wrote "we have over 3,000 accessions of tomatoes in our collection here and have never seen a single outcross to wild species." A further barrier to interspecific crossing, as described, is that cultivated tomato with its inserted stigma is almost completely self-crossing (see also USDA APHIS 1991).

Weed Characteristics and Environments

Evaluation of weediness potential requires a careful definition of terms. The term "weed" has been variously defined, depending on the different perspectives of ecologists, agronomists, and the public. In this document, we define a weed as "an unwanted or undesirable plant that persists in natural or human environments." A weed pest is a weed that "is considered a pest." These definitions reflect the concern that genetically engineered plants might become weed pests or cross-pollinate with weedy relatives enhancing their pest characteristics.

Assessment of weediness potential can be done at two levels. The first is a gross determination of whether the target crop species is itself a weed pest under specific conditions and/or environments or is sexually compatible with weedy relatives. If either is true, then a second level of assessment is needed to examine specific properties of the crop, particularly those that are generally attributed to weeds such as seed dormancy, long soil persistence, germination under diverse environmental conditions, rapid vegetative growth, a short life cycle, high seed output, high seed dispersion, and long-distance dispersal of seeds (Baker 1974). As will be seen with FLAVR SAVR tomato, only the first level of assessment is needed in determining its weediness potential except for consideration with *L. esculentum* var. *cerasiforme*.

Environmental conditions are of concern, especially with respect to control mechanisms that hold a plant species in balance with a particular environment, whether it is natural or disturbed. A plant can become a weed if it escapes control by migrating to a new environment that lacks the factors that controlled the plant in its original habitat. In most parts of the world, including the United States, the bulk of the weed pests are exotic plants (Holm et al. 1977; Mack 1985; Mack 1986). The possibility of FLAVR SAVR tomato becoming a weed pest strictly because it is planted in new environments will not be a factor because its growing region is within the existing production range of cultivated tomato. A second method for a plant to become a weed is when it remains in its original habitat but effectively escapes a particular control factor by gaining a trait that imparts to it the ability to overcome the control factor, such as acquiring resistance to disease. This is not the case for the FLAVR SAVR tomato, because the introduced trait has no selective advantage (Appendix 1 and USDA APHIS 1991).

Weeds can be considered according to whether they invade natural or human environments:

- 1) Natural areas
- 2) Rangelands
- 3) Parks and roadsides
- 4) Crop production fields
- 5) Urban and suburban environments (backyard weeds).

Through natural selection, many weed pests have apparently adapted to conditions in disturbed environments, such as being able to escape from biotic control agents (predators, pathogens, and competitors) and persistence, either vegetatively or by high seed production, in repeatedly disturbed areas as is found in cultivated fields (Harper 1965). Perhaps most successful (most widespread, persistent and abundant) are those weeds that have not only immigrated, but also have a long history of close association with human settlement (Baker 1974). Although weediness is primarily an economic concern for disturbed areas, it is also an ecological concern, particularly for natural areas. In determining the weediness potential for tomato, both environments are considered.

Weediness is determined by characteristics of the species and its relationship with biotic control agents (e.g. predators) and environmental conditions. The likelihood that a given species or line becomes a weed is very low (or zero) if at least one of the following is true: the species (and sexually compatible relatives) does not have weediness traits, there are clear and adequate natural control agents, or the environment is not conducive for natural persistence of the particular species.

Weediness Potential in Tomato

The single most commonly voiced concern about the introduction of genetically modified plants is the potential to inadvertently produce a new

weed or increase the aggressiveness of existing weeds (Colwell et al. 1985; Tiedje et al. 1989). Three aspects of weediness are of concern (Keeler 1989):

1) Comparison of transformed crops with exotic species. Is the experience with the introduction of exotic plants into new environments (sometimes with the result that a weed problem is created) a valid analogy for the introduction of genetically modified plants?

2) Potential for transformed, domesticated crops to revert to a weedy state. For a given crop such as tomato, are there examples in which the crop has become weedy, such as due to plant breeding or movement of the species outside its center of origin?

3) Potential for hybridization between domesticated crops and wild relatives creating or enhancing weediness. Will crops such as tomato outcross with wild relatives and, if so, what is the potential for increased weediness?

Manasse and Kareiva (1991) state, "It is the prospect of uncontained spread that underlies many of the worries environmentalists express regarding biotechnology." They affirm "that an organism whose rate of spread is minimal poses negligible risk compared to an organism that can multiply its population and rapidly expand its range." In assessing the possibility of a genetically engineered crop or a sexually compatible weed pest becoming a greater weed problem, it is important to consider that weedy properties usually represent complicated, multigenic traits and generally do not result from single gene traits (Keeler and Turner 1991)

1. Likelihood of FLAVR SAVR Tomato Becoming a Weed: Comparison of Transformed Crops with Exotic Species. The analogy between the introduction of an exotic species into a new environment and the introduction of a genetically modified crop plant is tenuous (Fincham and Ravetz 1991). Introduced exotic plants that have become pests bring with them many traits that enhance weediness and, very importantly, leave behind control organisms (predators) and competitors. Genetically modified plants are altered in only a few, specific characteristics that relate to crop production characteristics (National Research Council 1989). Unlike exotic plant introductions, genetically modified crops will generally not be released into exotic environments, but will be planted in typical growing regions for the specific crop. FLAVR SAVR tomato will be grown in areas currently under tomato cultivation. Tomato has been grown throughout the world without it becoming a weed pest. In addition, tomato does not demonstrate characteristics associated with weed pests (Keeler 1989).

For the most part, introductions of exotic species have been environmentally harmless and economically beneficial; most of North American crop plants are in fact exotic species. On rare occasions, such as kudzu, introductions have resulted in environmentally undesirable consequences. In most cases, careful review of the organism's biology would have predicted the unfavorable consequences (Williams 1980) and the problem of weediness could have been avoided. In like manner, careful

consideration of the biology of a genetically engineered crop (as addressed in this document) should alleviate any concern that the crop might respond like an exotic species that becomes established as a weed pest.

The likelihood of enhanced weediness is low for genetically modified, highly domesticated crop plants, on the basis of our knowledge of their morphology, reproductive systems, growth requirements, and unsuitability for self-perpetuation without human intervention (National Research Council 1989). Tomato has been highly characterized and is a well-defined major food crop. Since tomato is an exotic species in the United States and has not become a weed pest, the model of exotics becoming pests upon introduction into a new environment is inappropriate for FLAVR SAVR tomato.

2. Potential for FLAVR SAVR Tomato to Become a Weed Pest. By using a variety of plant breeding techniques, plant varieties have been continually selected for improved resistance or tolerance to external factors that inhibit their inherent productivity. Plant varieties have been selected for 1) insect, disease and herbicides resistance, 2) better tolerance of environmental constraints to growth, such as heat, cold and drought tolerance, and ability to withstand high moisture, excessive alkalinity, excessive salts, iron deficiency and high aluminum content in soils; and 3) ability to prevail in competition with weeds through quick germination and extremely rapid growth in the seedling stage. In theory, such improved cultivars presumably are better adapted to persist in the presence of disease, insects, herbicides and a number of environmental constraints to growth. However, plant breeders have a long history of incorporating these types of traits into crops without evidence of enhanced weediness (USDA APHIS 1991).

Similarly, it can be expected that crops modified by molecular and cellular methods should present no different risks in regards to weediness potential. Since molecular methods are highly specific in terms of what genes are being added, users of these methods will be more certain about the traits they introduce into plants (USDA APHIS 1991) and the weediness potential may actually be less than using traditional breeding methods. Nevertheless, it is important to consider the effect of new, introduced genes on the potential of a crop to become a weedy pest.

For new genes to be retained in a population, the genes must have at least one of three characteristics (Hauptli et al. 1985):

- 1) They must confer improved fitness to the first and resultant generations of the species.
- 2) They must have no negative effect on fitness.
- 3) The genes must be tightly linked to other genes conferring improved fitness.

If the genes do not confer improved fitness to the species, then individuals containing the genes will not have a selective advantage over those that do not. "For example, if the gene improves nutritional quality or prevents early

ripening, then it is unlikely to assist weedy relatives in surviving in an agricultural field" (Keeler and Turner 1991).

Tomato. Tomato is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1977; Muenscher 1980), nor is it present on the lists of noxious weed species distributed by the State of California and the Federal Government (Appendix 3).

Twenty crop plants have been analyzed for weedy characteristics (Keeler 1989). Tomato was listed as having the following traits that affect a species propensity to become weedy (15 traits total were analyzed):

- 1) Broad germination breadth - ability to germinate in a variety of environments.
- 2) Rapid growth to flowering.
- 3) Continuous seed production as long as growing conditions permit.
- 4) Facultatively self-compatible.
- 5) Outcrossing pollination via wind.
- 6) Seed production can occur in many environments.
- 7) Seeds adapted for both short and long distance dispersal.

However, item #5 does not accurately reflect tomato pollination. Rick (Appendix 4) writes that there is "no evidence whatever for wind transport of pollen under field conditions."

According to Keeler (1989), tomato does not have the following traits that characterized weedy species:

- 1) Internally controlled, discontinuous germination.
- 2) Long-lived seed.
- 3) Very high seed output.
- 4) Perennial species with vegetative propagation.
- 5) Difficult to uproot.
- 6) Good competitor.
- 7) Polyploid.
- 8) Reported as a weed.

In general, tomato lacks many of the traits characteristic of weed pests and has not been considered a weed pest in the United States. The USDA has concluded in environmental assessments of transgenic field trial applications that tomato does not display significant potential to develop into a weed itself (USDA APHIS 1991).

L. esculentum var. *cerasiforme*. One *L. esculentum* variety, the cherry tomato, requires special consideration. According to Rick (1983), "the wild source [of cultivated tomato] must have been *L. esculentum* var. *cerasiforme*, which previously had migrated from the Andean center of origin of the genus through northern South America, across the Panamanian Isthmus to Central America and southern Mexico." Rick describes this ancestor as a "weedy self-fertile annual" (Rick 1983) and states, "some biotypes of *cerasiforme* are so successful as weeds that they have spread throughout all of tropical America as far north as southern Texas and Florida and to most of the tropical regions of the world" (Rick 1973). However, cherry tomato is not considered a weed pest.

Although *cerasiforme* has become established in south Florida and southernmost Texas, there is almost no probability of FLAVR SAVR tomato naturally introgressing into *cerasiforme*. Rick's view is "that the risk of such introgression is nil or almost nil" (Appendix 4). The only intercrossable wild species are limited to Latin America. Natural outcrossing between *cerasiforme* and cultivated tomatoes is unidirectional, with *cerasiforme* serving as the pollinator whenever such crossings do occur. Other tomato experts have a similar opinion that cultivated tomato will not cross with weedy species (Appendix 4). Therefore, it is highly unlikely for introgression to occur from FLAVR SAVR tomatoes into *L. esculentum* var. *cerasiforme*, even in south Florida or southernmost Texas.

Field trials. Extensive field trials have been conducted by Calgene with tomatoes containing the FLAVR SAVR gene in the principal tomato producing states in the U.S., California and Florida (Table 1). In addition, one tomato trial was conducted in Mexico. These trials have been conducted, in part, to determine the effect of the FLAVR SAVR gene and transformation process on agronomic and horticultural traits. In general, these trials have shown that there were no changes that might affect weediness potential and that the tomatoes grew normally. Greenhouse and field observations conducted during all trials have shown that FLAVR SAVR tomatoes had similar horticultural traits as traditionally bred tomatoes. No unpredicted changes occurred, as documented in the field trial reports (Appendix 5), subsequent publications (Kramer et al. 1992; Kramer et al. 1990), and Calgene's Request for Advisory Opinion filing with the FDA (Appendix 1). Rick (Appendix 4) concluded that he doubted "that any of the altered traits of your ASPG [FLAVR SAVR] tomato would cause an enhanced propensity for it to become a weed." FLAVR SAVR tomato should have no increased tendency to revert to a weedy state since it possesses similar agronomic characteristics to non-engineered tomato.

Table 1. Calgene's Field Trials with Tomatoes Containing the FLAVR SAVR Gene.

Permit #	Site	Date Issued	Trial Completed?	Report Enclosed*
92-022-04	Central Valley, California	5/20/92	no	n/a
91-268-01	Indio, California	12/17/91	no	n/a
91-107-04	Manteca, California	7/11/91	yes	yes
91-050-01	Solano Co., California	5/22/91	yes	yes
90-249-01	Indio, California	10/16/90	yes	yes
90-019-01	Winters, California	3/19/90	yes	yes
89-320-01	Ruskin, Florida	2/12/90	yes	yes
88-344-07	Yolo Co., California	4/6/89	yes	yes
n/a	Mexico	Fall 1988	yes	n/a

*Appendix 5

n/a = not applicable

Introduced genes. The FLAVR SAVR gene does not confer any selective advantage which would enhance survival in the field. The FLAVR SAVR gene affects only the composition of pectin in the fruit (Appendix 1; Kramer et al. 1992; Kramer et al. 1990). The gene has no effect on levels of vitamins and nutrients, on production of potential toxins (tomatine), on taste, on non-pectin related processing traits, on horticultural traits (growth form, time to flowering, time to fruit set, etc.), fruit pH and acidity, and fruit color and size. In general, there were no unintended effects (Appendix 1). In its environmental assessments, the USDA concludes, "The antisense PG [FLAVR SAVR] gene does not provide the transformed tomato plants with any measurable selective advantage over nontransformed tomato plants in their ability to be disseminated or to become established in the environment" (USDA APHIS 1991).

The FLAVR SAVR gene does cause intended increase in serum viscosity and consistency, decrease in fruit softening rate, and an increase in fungal resistance (Appendix 1, Section A & C). The increase in fungal resistance is probably due 1) to the presence of a more intact barrier (the middle lamella and cell wall) to fungal invasion, 2) to less available substrate for fungal attack, and/or 3) to inactivation of fungal-produced polygalacturonase. The increased resistance provides only a temporary delay in fungal degradation. The tomato fruit will still rot, although the onset of rot may be delayed (Appendix 1, pages 235-236; Kramer et al. 1992). None of these intended traits affect the weediness potential of cultivated tomato.

The other introduced gene sequences (*kan^r* gene and specific regulatory sequences) in the FLAVR SAVR tomato also do not confer any selective advantage which would enhance survival in the field. No characteristics of these sequences (Appendix 2) give any indication that these genes would increase fitness of the tomato and none of the sequences have any relationship to the traits that characterize weed pests (Keeler 1989). Therefore, even if transfer of genes among *L. esculentum* species (including *L. esculentum* var. *cerasiforme*) did occur, there would be no change in weediness potential.

Seed germination. To test the hypothesis that genetically engineered lines were unchanged in regards to seed germination, rate and frequency of germination were measured in three field trials, comparing FLAVR SAVR tomatoes with control lines (Appendix 6, Part I). These germination measurements were conducted in the greenhouse prior to transplanting into the field. If the engineered lines were different than the controls, it would be expected that all lines would be in one class separate from the controls. However, this was not the case and no meaningful differences were measured.

Eleven separate tests were conducted consisting of transgenic lines and the control lines from which they were derived. For four of the comparisons, the transgenic line had a higher germination rate and frequency than the control. For five of the comparisons, the controls were higher. For two, the final germination frequencies were the same. An analysis of variance was done

using the SAS procedure CATMOD (Appendix 6, Part II). These results have high chi-square values for differences among all lines ("Line"), between transformed and control lines ("Trans"), among the three different time-points ("Day"), among different transgenic lines ("Line*Trans"), and among all lines in regards to rate ("Line*Day"). These differences are a result of very large sample sizes (22,816 transgenic seeds and 5,532 controls) and need to be considered as such, since very large sample sizes allow minor differences to appear significant. Examination of the raw data (Appendix 6) suggests that the large chi-square values are based on variation in rank among the lines: sometimes the transgenics are higher and sometimes lower. For example, in the San Joaquin trial, the transgenic line 501 had a final, average germination frequency of 66.6% as compared to a higher frequency for the 501 control, 85.9%. This ranking changed in the Indio trail, with a frequency of 97.6% for 501 and 71.9% for the control. Also, some of the transgenic lines, such as 501-1019-4 and 501-1035-4 (San Joaquin trial), had very low germination frequencies which resulted in a very high chi-square value (2277.25) for line differences in the analysis of variance (Appendix 6, Part II, Table B). Because of the changes in rank among lines, there is no evidence that the transgenic lines have an increase (or decrease) in germination frequency.

The chi-square value was low for differences in rate between transgenic and controls lines ("Trans*Day"), which provides strong support that germination rate is unaffected by transformation.

Strict quality control (QC) standards are implemented for seed production of commercial lines, since uniform, high germination frequency is essential for commercial cultivars. Tomato lines under product development are not finished varieties and therefore are not subject to strict QC measures such as high quality field production and screening/gravity table or size sorting. Seed production for FLAVR SAVR tomato lines has generally been in the greenhouse which results in lower quality seed at times. The plant breeding and product development process will eliminate or improve lines with slow germination rate and low frequency. Under commercial settings, all seed must have fast germination and a high frequency. Thus, as expected the germination frequency means of the commercial lines were higher than the means of the transgenic lines under development.

Based on these germination results and observations made during eight field trials, FLAVR SAVR tomato seed is not different from seed produced through traditional breeding and is unaffected by the FLAVR SAVR gene and other inserted genes. There is no indication that the transgenic seed will be dispersed differently, last longer or be more competitive in new environments. Therefore, there is no greater potential for FLAVR SAVR tomato or any other tomato cultivar crossed with it to become a weed pest risk, than for traditionally bred tomatoes.

3. Potential for Hybridization Between FLAVR SAVR Tomato and Wild Relatives Creating or Enhancing Weediness. Tomato has never been considered a weed pest itself, so any possible weediness problems would have to be a result of outcrossing with weed pest relatives. However, "some crop plants, although open-pollinated to produce the crop, are in fact, largely selfing, so the frequency of gene transfer to wild relatives, should they be present, is low. Tomatoes (*Lycopersicon esculentum* Miller) and peas (*Pisum sativum* L.) fall in this category" (Keeler and Turner 1991). As discussed, *L. esculentum* does not outcross with weed pest relatives and cultivated tomato is almost completely self-fertile due, in part, to the inserted stigma developed through breeding over the past 50 years (Rick 1976).

Of significance, is the lack of weed pest relatives of tomato. *Solanum nigrum* (black nightshade or yocoyoco) is the only major weed pest related to tomato. It is a principal weed of lima bean and a weed in sugar beets and tomatoes in the United States. It is a principal weed of tomatoes and vegetables and a weed in soybeans in Canada. It is a weed in bananas, citrus, coffee, corn, and sugarcane in Mexico (Holm et al. 1977; Lange et al. 1986). Other members of the nightshade family which are weeds in tomato fields are: *S. sarrachoides* (hairy nightshade), the groundcherries (*Physalis heterophylla* Nees., *P. lanceifolia*, *P. ixocarpa*, and *P. acutifolia*), *Nicotiana bigelovii* (Indian tobacco), and jimsonweeds (*Datura stramonium* L., *D. meteloides*, and *D. ferox*) (University of California 1985). Other weedy Solanaceae species are: *Hyoscyamus niger* (black henbane), *Lycium ferocissimum* (African boxthorn), *P. virginiana* var. *sonorae* (smooth groundcherry), *P. viscosa* (grape groundcherry), *S. cardiophyllum* (heartleaf nightshade), *S. carolinense* L. (horsenettle), *S. dimidiatum* (Torrey's nightshade), *S. dulcamara* L. (bitter nightshade) (Lorenzi and Jeffery 1987), *S. elaeagnifolium* (white horsenettle), *S. lanceolatum* (lanceleaf nightshade), *S. marginatum* (white-margined nightshade), and *S. torvum* (turkeyberry) (Appendix 3).

L. esculentum is sexually incompatible with all of these weedy relatives. Only through specific, controlled crosses is it even possible to cross *L. esculentum* with *S. lycopersicoides* (Rick 1979) or with *S. rickii* (De Verna et al. 1990). These two *Solanum* species are not weed pests in the United States, however, and are not listed as pests by other references (Holm et al. 1977). Furthermore, *L. esculentum* will not naturally cross with these two *Solanum* species. Because tomato has no weed pest relatives, there is no possibility of a cross between FLAVR SAVR tomato and wild species which would enhance weediness. In addition, because there are no threatened or endangered sexually compatible tomato relatives in the United States, there is no threat of interbreeding causing a loss of genetic resources.

Mode of Gene Escape in Tomato

1. Outcrossing. Genes in tomato can move via pollination from one individual to another within the species *L. esculentum*. As described above,

there are no wild relatives in the United States that will cross with cultivated tomato. Therefore, any outcrossing that occurs will be with other *L. esculentum* varieties. As with any other tomato line, seed purity will be maintained using standard breeding practices, such as for maintenance of pure seed stock. Because the FLAVR SAVR gene affects only fruit characteristics, it is not expected to have any effect on managed ecosystems (i.e. tomato production areas).

There will be no impact of transfer and expression of foreign genes in wild and weedy relatives of tomatoes, because no sexually-compatible wild relatives are present in the United States. There is no likelihood of gene transfer to endangered species, since no sexually compatible relatives are endangered. There are no native species in the United States that could outcross with *L. esculentum*. Consequently, it is extremely unlikely that FLAVR SAVR tomatoes will have any impact on unmanaged ecosystems.

Pollen movement and range of outcrossing are characteristics that are highly unlikely to be affected by the presence of the FLAVR SAVR gene or the other gene sequences inserted using the binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578) and the FLAVR SAVR gene with its associated promoter and terminator. Because the FLAVR SAVR gene confers no selective advantage to tomatoes, there will be no danger of a decrease in variability should the unlikely event of outcrossing with a related species occur. If such an event did occur, the delay in fruit softening would not affect seed persistence.

2. Potential Plant-to-Microorganism Gene Flow. Another potential mode of gene escape from tomato is the possibility that a gene would not remain immobile (stably integrated in the tomato genome), but would migrate from its chromosomal location in the tomato cultivar, and take up residence in some other organism, such as a microorganism. Such movement is termed "horizontal transfer." The possibility of horizontal transfer is of concern when addressing antibiotic resistance genes, because of the potential to expand the population of antibiotic resistant pathogens.

This issue is discussed thoroughly in Section VI of this document and in Appendix 2 (Vol I, Section E; and Vol II, Section G). In summary, horizontal gene flow does not represent a risk because there is no known mechanism for such transfer and even if it could occur, the probability of such a transfer posing a risk has an extremely low probability.

Conclusions for Section II

1. The tomato genus *Lycopersicon* is not a weed pest risk. There is a no likelihood that FLAVR SAVR tomato will have enhanced weediness traits compared to non-transformed tomato.
2. There is little risk of genetic transfer to other *Lycopersicon* species because of natural outcrossing barriers.

3. Commercial tomato is not compatible with weed species, nor are wild *Lycopersicon* species considered weed pests in the United States. Therefore, there is no potential for exchange of weedy traits by cross-pollination with weeds or weedy relatives.
4. The FLAVR SAVR gene will not confer weediness to tomato. There is no selective advantage to possession of this trait which temporarily delays fruit softening. Normal crop practices will control any persistence (which will be no different than any other tomato cultivar) that might occur in tomato fields, since the plant is grown as an annual.

III. The Transformation and Vector System

The vector system used to transfer the FLAVR SAVR gene to plants is based on the Ti plasmid from *Agrobacterium tumefaciens*. The vector system is "disarmed" or non-pathogenic, i.e. all the genes responsible for crown gall disease normally found in the T-DNA have been deleted. This system is also "binary" with the genes to be transferred on one plasmid and the genes encoding necessary functions for transfer, the *vir* genes, on a second plasmid. Genes on the second plasmid are not transferred to the engineered plant. A regulatory sequence, the 35S promoter (CaMV35S), was isolated from cauliflower mosaic virus (CaMV).

Specific genes from *A. tumefaciens* are the *tml* 3' terminator, the *mas* 5' promoter, the *mas* 3' terminator, the *overdrive* T-strand, and the right and left border regions. Although the *overdrive* T-strand is on the same plasmid as these other sequences, it is in a part of the right border region that is not transferred into the plant host. Depending on the specific binary, either the *mas* 5' or CaMV35S promoter is used for the *kan^r* gene. A region of the CaMV35S promoter was duplicated (double CaMV35S) to enhance activity of the promoter for expression of the FLAVR SAVR gene. Seven binary vectors (McBride and Summerfelt 1990) are used for production of FLAVR SAVR tomatoes (Appendix 2, pages 25-34):

Binary vectors pCGN1547, pCGN1548, and pCGN1549 (the 1547 series) have the *mas* regulatory signals driving the *kan^r* gene.

Binary vectors pCGN1557, pCGN1558, pCGN1559, and pCGN1578 (the 1557 series) have the CaMV35S promoter and *tml* 3' terminator for the *kan^r* gene.

Vectors within a series differ from one another only in orientation of the genes. Although each vector contains the right and left border regions, only part of the right and left border regions (from the right border and left border nicks, respectively) are transferred into the tomato plant genome (Appendix 2, pages 25-34).

The binary vector, pCGN1547, and the FLAVR SAVR gene with its associated promoter and terminator were used to produce the pCGN1436 construct. pCGN1436 is one of several constructs, based on the binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578) and the FLAVR SAVR gene with its associated promoter and terminator, that is used to produce FLAVR SAVR tomatoes. The pCGN1436 construct has been used to produce a number of FLAVR SAVR tomato varieties. Additional varieties will also be produced using the pCGN1436 construct and other constructs based on the binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578) and the FLAVR SAVR gene with its associated promoter and terminator. Varieties will also be developed by traditional breeding using current FLAVR SAVR tomato varieties.

The USDA has written in environmental assessments that, "the vector used to transfer the antisense polygalacturonase [FLAVR SAVR] gene to tomato plants has been evaluated for its use in this specific experiment [field trial] and does not pose a plant pest risk. The vector, although derived from a DNA sequence with known plant pathogenic potential, has been disarmed: that is, the genes that are required for pathogenicity have been removed ... These DNA sequences transferred by *A. tumefaciens* were modified such that they no longer incite plant tumors or any other disease symptoms on a susceptible plant" (USDA APHIS 1991).

The transferred genetic material in FLAVR SAVR tomatoes was shown to be genetically stable and segregate in a Mendelian fashion (Appendix 1, specifically Section C). None of the transgenic tomatoes have shown any pest characteristics, such as gall formation, even after several generations (Appendix 1, Section C; and Appendix 5). All data generated on FLAVR SAVR tomatoes suggest that the transgenic sequences and resultant selected plants are not a pest risk, nor have any negative results been produced on the safety of these tomatoes.

Conclusions for Section III

1. Vectors used in production of FLAVR SAVR tomatoes are derived from two series: pCGN1547, pCGN1548, and pCGN1549 (the 1547 series) and pCGN1557, pCGN1558, pCGN1559, and pCGN1578 (the 1557 series).
2. Vectors used in production of FLAVR SAVR tomatoes have been disarmed and do not pose a plant pest risk.
3. FLAVR SAVR tomatoes are genetically stable and do not exhibit any pest risk characteristics, such as gall formation as caused by *A. tumefaciens*.

IV. Donor Genes from Organisms Considered Regulated Articles

The FLAVR SAVR tomato has been considered a regulated article because it contains the following subspecies (sequences) from the list of organisms in

7 CFR 340.2: the *tml* 3' terminator, the *mas* 5' promoter, the *mas* 3' terminator, and the right and left border regions from *Agrobacterium tumefaciens*; and the 35S promoter region (CaMV35S) from cauliflower mosaic virus (CaMV). Both *A. tumefaciens* and CaMV are considered plant pests.

Non-regulated Articles

Lycopersicon esculentum Mill. is not a regulated article. The FLAVR SAVR gene (an antisense polygalacturonase gene isolated from tomato) is not a regulated article. The *kan^r* gene encoding APH(3')II was isolated as a component of transposon Tn5 from a ColE1::Tn5 containing strain of *Escherichia coli* K12. The Tn5 gene and the Lac Z' gene are from *E. coli* which is not considered a plant pest. These genes and gene sequences are contained in the binary vectors (pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, or pCGN1578) as well as the construct identified as pCGN1436.

A description of the function of the genetic modification in the FLAVR SAVR tomatoes is provided in Appendices 1 and 2. Polygalacturonase, a tomato gene, was identified, cloned, and reinserted into the tomato genome in the reverse or antisense orientation (the FLAVR SAVR™ gene). Expression of the FLAVR SAVR gene interferes with normal expression of the endogenous polygalacturonase gene by dramatically reducing levels of the polygalacturonase mRNA available for translation (Sheehy et al. 1988). The result is a reduction of active polygalacturonase enzyme in ripening tomato fruit which is the direct intended technical effect of the FLAVR SAVR gene (Fincham and Ravetz 1991; Kramer et al. 1992; Kramer et al. 1990).

Descriptions of the *kan^r* and Lac Z' genes are also described in Appendix 2 (pages 25-34). Specific references for these genes are Beck *et al.* (1982) and Yanisch-Perron *et al.* (1985). These genes are important for the genetic engineering and transformation processes, allowing for selection of desired material.

The USDA has concluded that the FLAVR SAVR gene, its expression product (the antisense RNA), the *kan^r* gene, and its expression product (APH(3')II) do not confer "on tomato any plant pest characteristic" (USDA APHIS 1991).

Sequences from Organisms Considered Regulated Articles

A description of the *tml* 3' terminator, the *mas* 5' promoter, the *mas* 3' terminator, the right and left border regions, and the CaMV35S promoter are provided in Appendix 2 (pages 25-34). These sequences, isolated from their source organisms, do not present a plant pest risk.

A. tumefaciens has a broad host range, generally defined within dicotyledonous plant species, but not strictly limited as such (Houck et al. 1990; White 1989).

The Ti plasmid used in the production of FLAVR SAVR tomatoes was "disarmed" so that the plasmid no longer could re-direct plant cells into biosynthesis of phytohormones leading to tumor (or gall) formation. This was done by constructing a plasmid that did not contain the phytohormone (*onc*) genes. The Ti plasmid contains the T-DNA (transferred DNA) which is the piece of the Ti plasmid that is stably integrated into the plant nuclear genome. Inserted into the T-DNA and transferred into the tomato genome is the FLAVR SAVR gene. Because none of the T-DNA genes are involved in transfer and integration (Zambryski 1988), this integrated material (T-DNA containing the FLAVR SAVR gene) does not contain the necessary *A. tumefaciens* genes, such as the *vir* genes needed for transfer and infection (Fincham and Ravetz 1991).

Functions of a native (fully armed) Ti plasmid that are not transferred to the genetically engineered tomato are the *vir* and *onc* genes, the nopaline or octopine catabolism genes (*nos* and *oct*), the ability for conjugal transfer of the Ti plasmid between bacteria (*tra* functions), and origin of replication and other replication functions (Hohn and Schell 1987; Koukolíková-Nicola et al. 1987).

Following the use of *Agrobacterium* (which contains the Ti plasmid) for plant transformation, the *Agrobacterium* are killed with carbenicillin so no subsequent infection or transformation can occur (Fillatti et al. 1987). The transformed plants are then grown to flowering, and seed is collected and used for future generations of plants before the final generation is selected for field production. Because of these procedures, the original plant transformation vector (Ti plasmid) does not remain associated with the plants, and any further transfer of genes from such plasmids to humans, animals or the environment could not occur.

Segregation data demonstrating stability, Southern analyses to identify gene copy number and demonstrate lack of gene movement, and complete nucleotide sequences of these regulated articles are described in detail in Appendices 1 and 2.

Terminator and Promoter. The *tml* 3' terminator, *mas* 5' promoter, and *mas* 3' terminator (Barker et al. 1983) function only in expression of the *kan^r* and FLAVR SAVR genes. Expression of these two genes is detailed in Appendices 1 and 2. No *A. tumefaciens* disease symptoms were observed in any plants in any of the field trials (Appendix 5). These sequences, as used in producing FLAVR SAVR tomatoes, no longer function as regulated sequences since they do not make FLAVR SAVR tomatoes a plant pest risk.

Borders. The right and left border regions (Barker et al. 1983) are the only necessary *cis*-acting elements in T-DNA (Klee and Rogers 1989) for T-DNA transfer. The use of a binary vector system allows for other necessary elements to act in *trans* so that only the border regions are required to be integrated into the plant host genome (Zambryski 1988). These regions are only partially transferred to the tomato genome. During the transformation

process, the left border is cut between nucleotides 293 and 294 (left border nick) while the right border is cut between nucleotides 7603 and 7604 (right border nick) as detailed in Appendix 1 (pages 160-171). In addition, the *overdrive* T-strand remains in the portion of the right border outside the right border nick and therefore is not integrated into the tomato genome.

As described in Appendix 1 (Section A) and Appendix 2 (Vol I, Section A), these border regions functioned as predicted in facilitating integration of the specific gene sequences into the tomato genome. No *A. tumefaciens* disease symptoms were observed in any plants in any of the field trials (Appendix 5). These sequences, as used in producing FLAVR SAVR tomatoes, no longer function as regulated sequences since they do not make FLAVR SAVR tomatoes a plant pest.

CaMV35S. The 35S promoter region (CaMV35S) is derived from cauliflower mosaic virus (Gardner et al. 1981). Cauliflower mosaic virus is a double-stranded DNA caulimovirus with a restricted host range, primarily to cruciferous plants. Genome size is about 8 kb. CaMV35S has a very high constitutive strength as compared to other plant promoters, allowing it to be widely used as a promoter for high expression of genes (Gronenborn and Matzeit 1989).

CaMV35S has not been shown to be a plant pest risk in plants. Palukaitis (1991) concludes that, "while some of these plants [containing CaMV35S promoter] may have shown either unusual or abnormal responses, it has in every case been possible to delimit these host abnormalities to the expression of the gene and not to the presence of a promoter of viral origin. There is no evidence that the sequences of the CaMV promoters are in themselves inducers of pathogenicity. Thus, the major gene product rather than the well-characterized regulatory signals on the CaMV DNA are involved in the induction of pathogenicity in plants."

CaMV35S is the promoter region that drives the FLAVR SAVR gene (and the *kan^r* gene for some of the binaries). Expression of the FLAVR SAVR gene is described in detail in Appendix 1. No cauliflower mosaic virus symptoms were observed in any plants transformed using this promoter. This sequence, as used in producing FLAVR SAVR tomatoes, does not cause these tomatoes to become a plant pest risk.

Conclusions for Section IV

1. Data were generated to show that the regulated articles (7 CFR 340.2), *tml* 3' terminator, *mas* 5' promoter, *mas* 3' terminator, the right and left border regions from *A. tumefaciens*; and the double 35S promoter region from cauliflower mosaic virus, do not make the FLAVR SAVR tomato a plant pest risk. These sequences, isolated from their source organisms, do not present a plant pest risk in and of themselves.
2. Components of the Ti plasmid of *A. tumefaciens* that are considered a plant pest risk are not present in the FLAVR SAVR tomato.

V. The Engineered Plant

A detailed description of FLAVR SAVR tomatoes, characterization of their modifications in molecular, physical, genetic, and agronomic terms, and a description all altered characteristics, both intended and unintended are thoroughly detailed in Appendices 1 and 2 and in two references (Kramer et al. 1992; Kramer et al. 1990). The following summary Tables 2-8 from Appendix 1 demonstrate that the FLAVR SAVR tomato differs from traditionally bred tomatoes only for intended effects:

Table 2. Molecular Characterization of Eight Lines of FLAVR SAVR Tomatoes.

Component	Quantitation
Number of <i>kan^r</i> genes (haploid)	≤3
Levels of APH(3')II	<0.08% of total protein
Number of FLAVR SAVR genes (haploid)	≤3
Level of PG mRNA	<10% of control lines
Level of PG enzyme activity	<1% of control lines
Gene linkage between <i>kan^r</i> and FLAVR SAVR genes	yes
Number of insertion sites	one

Table 3. Comparison of Selected Tomatoes Containing the FLAVR SAVR Gene with Nontransformed Controls.

Component	Changed	Unchanged
Recommended Daily Allowances		+
Potential toxins		+
Taste		+
Serum viscosity	+	
Other processing traits		+
Horticultural traits		+
Fungal resistance	+	
Color (pigmentation)		+
Softening rate	+	

Table 4. Nutritional Components (RDAs) for Eight pCGN1436 Lines, Five Controls as Compared to Normal Ranges for Tomato.

Constituent	Normal range	Measured range for pCGN1436 lines	Measured range for control lines
Protein	0.85 g (.015 se)	0.75 - 1.14	0.53 - 1.05
Vitamin A	192 - 1667 IU	330 - 1600	420 - 2200
Vit. B ₁ (Thiamin)	16 - 80 µg	38 - 72	39 - 64
Vit. B ₂ (Riboflavin)	20 - 78 µg	24 - 36	24 - 36
Vitamin B ₆	50 - 150 µg	86 - 150	10 - 140
Vitamin C	8.4 - 59 mg	15.3 - 29.2	12.3 - 29.2
Nicotinic acid (Niacin)	0.3 - 0.85 mg	0.43 - 0.70	0.43 - 0.76
Calcium	4.0 - 21 mg	9 - 13 mg	10 - 12
Magnesium	5.2 - 20.4 mg	7 - 12	9 - 13
Phosphorus	7.7 - 53 mg	25 - 37	29 - 38
Sodium	1.2 - 32.7 mg	2 - 5	2 - 3
Iron	0.2 - 0.95 mg	0.2 - 0.41	0.26 - 0.42

Table 5. Morphological Components of FLAVR SAVR Tomatoes as Compared to Controls.

Component	Changed	Unchanged
Fruit color		+
Fruit size		+
Fruit shape		+
Fruit firmness after harvest	+	
Other morphological characters		+

Table 6. Processing Components of FLAVR SAVR Tomatoes as Compared to Controls.

Component	Changed	Unchanged
Total solids	+	
Soluble solids	+	
pH		+
Acidity		+
Color (lycopene)		+
Serum viscosity	+	
Consistency	+	

Table 7. Disease Resistance of FLAVR SAVR Tomatoes as Compared to Controls.

Component	Changed	Unchanged
Fruit weight loss	+	
Fruit area with lesions	+	
Lesion size	+	

Table 8. Tomatine Levels in Green and Red Fruit of FLAVR SAVR Tomatoes (Construct pCGN1436).

Fruit Stage	FLAVR SAVR Range	Control Range
Green	124.7-860.8µg/g dwt	58.6-999.2 µg/g dwt
Red	1.54-7.59	0.70-7.17

These data demonstrate that FLAVR SAVR tomatoes were altered for very specific traits that are predictable from the function of the polygalacturonase gene and the effect of decreasing its activity by 99%. In all areas measured, unintended effects were not found. These data and observations suggest that breeding and development of FLAVR SAVR tomatoes are directly analogous to traditional tomato breeding methods, and that FLAVR SAVR tomatoes pose no greater risk than other tomato cultivars.

The use of FLAVR SAVR tomatoes will likely not affect current agricultural practices for tomatoes. The only change is that the fruit will be harvested later in the ripening process than current practice for fresh market tomatoes (harvest time for processing tomatoes will probably be unchanged). This change should have no impact on floral communities, faunal communities, endangered or threatened species, health of plants or animals, or genetic resources of tomatoes. The principle effect of use of FLAVR SAVR

tomatoes is expected to be the availability of better tasting fruit for human consumption.

Because the genetic modification used to produce the FLAVR SAVR tomato was the isolation of the FLAVR SAVR gene from *L. esculentum* and re-insertion of the gene into the same species, it is generally considered a class of modification that is inherently safe (Keeler 1988). This becomes even a stronger argument when the nature and function of the FLAVR SAVR gene are considered (Appendix 1, Sections A and C), since the gene slows pectin degradation and fruit softening, effects that have no selective advantage for persistence in the environment.

All greenhouse and field observations to date show that there are no deleterious effects on humans involved in cultivation, post-harvest production, or consumption of FLAVR SAVR tomatoes. A toxicity study conducted with rats showed no adverse effects (Appendix 1, pages 549-573). These studies (Appendices 1, 2, 5, 6, & 7, plus other data presented in this document) have led to the following conclusion: the issue of risk resulting from consumption of the FLAVR SAVR tomato is not of significant concern.

Conclusions for Section V

1. The FLAVR SAVR tomato differs from other tomato cultivars only in terms of characteristics related to pectin and the presence of the novel *kan^r* gene and APH(3')II gene product. Nutritional levels, taste (for tomatoes picked at the same stage), processing characteristics, horticultural and developmental traits, and potential toxins (solanine and tomatine) are unchanged, except for those related to pectin.
2. The number of *kan^r* genes is less than 10 and the level of APH(3')II is less than 0.1% of total protein in representative plants.
3. Composition of FLAVR SAVR tomatoes is essentially unchanged. The amount of DNA added to the tomato genome is insignificant. The FLAVR SAVR tomatoes contains between 7.5 to 75.0 kb of additional DNA inserted into the tomato genome which has approximately 10⁶ kb DNA. This is a 0.0075% increase in total genomic DNA.
4. No adverse pleiotropic traits (i.e. insertional mutagenesis, the inactivation of host genes into which the incoming DNA is inserted) have been detected to date in FLAVR SAVR tomatoes selected for low PG activity and typical fresh market tomato characteristics. The probability of incurring pleiotropic effects relevant to food safety as a result of the genetic engineering process is low and likely not significantly different than the probability of pleiotropic effects resulting from other plant breeding techniques.
5. FLAVR SAVR tomatoes were shown to be genetically stable. The inserted genes were shown to be immobile and segregated according to Mendelian predictions.
6. No *Agrobacterium* or cauliflower mosaic virus disease symptoms were observed in any plants in any of the field trials (Appendix 5). The

- regulated articles (sequences), as used in producing FLAVR SAVR tomatoes, no longer functioned as regulated sequences since they were isolated from their source organisms that are considered plant pests and since they did not change FLAVR SAVR tomatoes, making them a plant pest risk.
7. Seed germination rate and frequency of FLAVR SAVR tomatoes is equivalent to traditionally bred varieties. FLAVR SAVR tomatoes have been used for breeding purposes; no changes in flowering time, no improved outcrossing characteristics, no changes in seed production, and no changes in controlled pollination were measured. Yield of FLAVR SAVR tomatoes is equivalent to traditionally bred controls. Current agricultural practices will not be affected by cultivation of the FLAVR SAVR tomato.
 8. We conclude that FLAVR SAVR tomatoes are food and no unintended changes were detected in FLAVR SAVR tomatoes to affect their status as tomatoes. It is further concluded that traditional plant breeding and selection processes used to develop future commercial FLAVR SAVR tomato varieties will be adequate to insure there are no unintended changes in these tomatoes that will affect their status as food.

VI. Environmental Consequences of Introduction of the Transformed Cultivars

APH(3')II Toxicity and Degradation

APH(3')II protein (aminoglycoside 3'-phosphotransferase II) is not toxic to humans. The protein occurs naturally, being produced by bacteria in the human gut. An acute toxicity study demonstrated no toxicity, mortality or gross necropsy in rats fed FLAVR SAVR tomatoes which contain the *kan^r* gene (Appendix 1, pages 549-573). Experiments have shown that the enzyme is inactivated (degraded) by pepsin in simulated gastric fluids and by simulated intestinal fluids, as is the case for any other typical protein (Appendix 7). Even if not degraded, APH(3')II will be inactive in the absence of the energy producing cofactor ATP and under the low pH conditions of the gut. Glycosylation and subsequent increase in the antigenic capacity of APH(3')II will not occur because APH(3')II does not contain the necessary sequence information for transport to the subcellular locations at which glycosylation reactions take place.

APH(3')II was shown not to have significant homology with known toxins and allergens (Appendix 7). An environmental assessment (Appendix 2, Vol II) was conducted to demonstrate that the risk of using the *kan^r* gene for tomato variety development was insignificant.

Therefore, use of the *kan^r* gene in FLAVR SAVR tomatoes will not compromise efficacy of use of kanamycin and will not affect allergenicity of

FLAVR SAVR tomatoes (Appendix 7). There is no increased risk to the environment from the gene product, APH(3')II (Appendix 2, Vol II).

Stability of Gene Products in the Environment

Use of the FLAVR SAVR tomato will not affect the weediness status of tomato or any related species (see section II). The FLAVR SAVR gene (an antisense polygalacturonase gene) does not encode a protein, producing only transient RNA, and therefore does not pose an environmental risk.

Calculations were made for potential release of APH(3')II into the soil from tomato debris (Appendix 2, Vol II, pages 312-314). It is not expected that such release of APH(3')II will pose an environmental risk, since soil bacteria naturally produce this protein and much of it will likely be sequestered or degraded in the soil (Appendix 2, Vol II, Sections E & G).

Horizontal Gene Flow

Movement of transgenes from the engineered crop plant to microorganisms has been suggested as a risk if such crops are released into the environment. Arguments have been made concerning this potential risk, but no data have been published to support such a concern. There are several reasons why horizontal gene transfer from FLAVR SAVR tomatoes does not represent a risk.

First, no mechanism for transfer of genes from plants to microorganisms is known and no cases of such transfer have been reported. Carlson and Chelm (1986) argued for an eukaryotic (plant) origin of glutamine synthetase II in bacteria, albeit over an evolutionary time period. They suggested that this was evidence that horizontal gene flow from plants to microorganisms had occurred at one point in evolution. However, their paper was directly refuted by Shatters and Kahn (1989) who concluded that "the GS [glutamine synthetase] proteins are highly conserved and the divergence of these proteins is proportional to the phylogenetic divergence of the organisms from which the sequences were determined. No transfer of genes across large taxonomic gaps is needed to explain the presence of GSII in these bacteria." Other "evidence" that horizontal gene flow occurs from plants to microorganisms involves transient changes (non-heritable) such as transencapsidation of chloroplast DNA (Rochon and Siegel 1984) or possibly endocytosis (Bryngelsson et al. 1988), neither of which have been shown to result in actual transfer of genes from plants to microorganisms. No mechanism by which plant DNA could be incorporated into the genomes of the microorganisms has been proposed. In addition, Zambryski et al. (1982) provide evidence that once inserted DNA is integrated into the plant host genome, it cannot be remobilized even if acted on again by *vir* genes. To date, such horizontal gene flow remains speculative with no actual examples.

Second, in regards to the *kan^r* gene, people and animals are already exposed to kanamycin resistance genes due to their widespread natural

occurrence in microbial populations. Although it is theoretically possible for genes to move from plants to microorganisms in the human/animal gut and the environment, no cases have ever been reported, no known mechanisms for such transfer have been discovered, and if there were such a mechanism, such transfer would have an insignificant impact on the extensive kanamycin resistant flora already present. For example, 75% of naturally occurring *Streptococcus faecalis* bacteria and 5 to 92% of strains of 17 other bacterial species isolated from humans were resistant to kanamycin (Atkinson 1986).

Third, using worst case probability estimates for hypothetical gene transfer, the additive effect of a *kan^r* gene entering the microbial flora from genetically engineered plants is insignificant when compared to the population of kanamycin resistant microorganisms naturally present. For worst case calculations, Calgene's assumptions were that free plant DNA containing an intact *kan^r* gene could become disassociated from a plant cell, exist long enough in the soil to be taken up by a soil bacterium, incorporated into the bacterial genome (including bacterial plasmids), and be expressed by the bacteria (even though the kanamycin resistance gene in the plant does not contain bacterial promoter sequences). Even using these unrealistic assumptions, the impact of increased numbers of kanamycin resistant bacteria in humans, animals, or environment is insignificant compared to the population of naturally occurring resistant bacteria.

Calgene's worst case calculations assume that soil transformation rates would be as high as those achieved under ideal laboratory conditions. These calculations suggest that, at worse, only 9×10^5 bacteria per hectare per year would become resistant compared to 7.2×10^{12} kanamycin resistant bacteria per hectare already present naturally in the environment (Henschke and Schmidt 1990). Under the worst case scenario, the increase in background population would be an infinitesimal $1.25 \times 10^{-5}\%$.

For risk assessment, we assigned probabilities to such a scientifically unjustifiable event and generated calculations for worst case scenarios based on existing scientific literature and data. When data were unavailable, the worst case probability (usually 1) was used. To generate a worst case risk assessment for horizontal transfer of genes from plants to microorganisms, we assumed that such transfer could occur and used ideal laboratory conditions as a basis for probability calculations. Consequently, the probability estimates are greatly exaggerated on the side of safety and the actual risk, if any, is much less. The following risks were considered (Appendix 2, Vol II):

1. Likelihood that the genes will move from the plants to soil bacteria: The worst case estimate, assuming DNA could be transferred from plant debris to soil microorganisms, would result in a contribution of 1/10,000,000 to the *kan^r* microorganisms already present in soil.

These are significantly low probabilities as compared to the natural levels of kanamycin resistant microorganisms in the soil, estimated at 7.2×10^{12} per

hectare (Henschke and Schmidt 1990). A more realistic estimate is that *kan^r* bacteria resulting from transformation from plant debris (if such transformation were even possible) would represent no more than 1.4×10^{-11} % of the *kan^r* soil microorganisms.

2. Likelihood the soil bacteria might persist or be selected for in the soil: This possibility is no greater than that for any other naturally occurring *kan^r* bacteria. Because kanamycin is not used for controlling microorganisms in the soil, there would be no selective advantage to any specific *kan^r* bacteria which result from transformation from plant debris compared to those normally resistant.

3. Likelihood that the genes may be transferred to yet other bacteria: This possibility would be no greater than that for genes from any other *kan^r* bacteria. Because the frequency of natural *kan^r* bacteria is far greater than estimated levels of *kan^r* bacteria resulting from transformation from plant debris, there would be no substantial increase in the population of *kan^r* bacteria.

No mechanisms of natural transformation have been demonstrated for transfer of DNA sequences from eukaryotic cells eaten as food to microorganisms found in the human gut. However, to assess risk, Calgene calculated a worst case scenario for transformation of human gut bacteria with the *kan^r* gene from fresh transgenic tomatoes. This calculation resulted in a frequency of 2.6×10^{-3} transformed bacteria/person. This represents 10^{-13} % of susceptible gut bacteria in a human. The population of *kan^r* bacteria which normally inhabit the human gut is substantial (e.g. 75% of *Streptococcus faecalis* bacteria, Atkinson 1986), and the impact, if any, of transfer of the *kan^r* gene from transgenic tomatoes to human gut bacteria would be insignificant.

Experiments were conducted to demonstrate that DNA transfer to human gut microbes would not occur, that APH(3')II is degraded in the human gut and that APH(3')II would not compromise oral dosage of kanamycin (Appendix 7).

The calculations described above assume that transfer of DNA from a plant source is possible, although no mechanisms for such transformation are known. However, even with worst case calculations, the probability of a plant source of the *kan^r* gene impacting human, animal, and the environment is insignificant compared to the naturally occurring reservoir of kanamycin resistance genes.

Finally, for FLAVR SAVR tomato, none of the field trials have shown any evidence of horizontal gene movement (Appendix 5).

Conclusions for Section VI

1. Use of the *kan^r* gene in FLAVR SAVR tomatoes will not compromise efficacy of use of kanamycin or affect allergenicity.
2. There is no increased risk to the environment from the gene product, APH(3')II. The FLAVR SAVR gene (an antisense polygalacturonase gene) does not code a protein, producing only transient RNA.
3. Observations to date from field trials of FLAVR SAVR tomatoes show no evidence of horizontal gene movement. There is no scientific basis to suggest that horizontal gene movement could occur from plants to microorganisms.
4. If there were a mechanism for horizontal gene flow, the effect would be insignificant, a contribution of less than 1/10,000,000 to the *kan^r* microorganisms already present in soil.
5. Experiments were conducted to demonstrate that DNA transfer to human gut microbes would not occur, that APH(3')II is degraded under human gut conditions and that APH(3')II would not compromise oral dosage of kanamycin. Increase in exposure to kanamycin-resistant bacteria from food consumption, therefore, is not theoretically possible.
6. Based on data presented in this document, there are no negative environmental consequences of introduction of FLAVR SAVR tomatoes into agricultural production.

VII. Petition to USDA APHIS

Calgene, Inc. requests that the USDA APHIS determine that the FLAVR SAVR™ tomato does not present a plant pest risk, is not otherwise deleterious to the environment, and is therefore not a regulated article.

VIII. Statement of Grounds Unfavorable

No negative aspects have been determined for the FLAVR SAVR tomato.

IX. List of Appendices

1. FLAVR SAVR™ Tomato: Status as Food. Request for Advisory Opinion, U.S. Food and Drug Administration, Docket #91A-0330/API. August 12, 1991.
2. *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. Two volumes (Vol I and Vol II).
3. State of California, Department of Food and Agriculture, Division of Plant Industry, "Pest Ratings of Noxious Weed Species and Noxious Weed Seed," 1/8/90; and Federal Noxious Weed Regulation, CFR 360.
4. Letters from experts in tomato breeding and cultivation.
5. Field trial reports for FLAVR SAVR tomatoes.
6. Germination frequency and rate of FLAVR SAVR tomatoes and controls.
7. Supplemental Data Submitted to FDA:
 - A) Fate of APH(3')II and Implications for Kanamycin Efficacy
 - B) Supplemental Information on Human Toxicity: *kan^r* Selectable Marker Gene and Gene Product
 - C) *kan^r* Protein Homology - Toxins
 - D) *kan^r* Protein Homology - Allergens

X. List of References

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