Parkway, Norcross, GA 30092–2911. Telephone No. (404) 729–8101. *Underwriting Limitation b/:* $367,000. *Surety Lincenses c/ :* GA. *Incorporated In:* Georgia.

Certificates of Authority expire on June 30 each year, unless revoked prior to that date. The Certificates are subject to subsequent annual renewal as long as the companies remain qualified (31 CFR part 223). A list of qualified companies is published annually as of July 1 in Treasury Department Circular 570, with details as to underwriting limitations, areas in which licensed to transact surety business and other information.

Copies of the Circular may be obtained for the Surety Bond Branch, Funds Management Division, Financial Management Service, Department of the Treasury, Washington, DC 20227, Telephone (202) 874–6696.

Dated: May 16, 1994.

Charles F. Schwan III,

Director, Funds Management Division, Financial Management Services.

[FR Doc. 94–12448 Filed 5–20–94; 8:45 am]

BILLING CODE 4810–35–M

[Dept. Circ. 570, 1993—Rev., Supp. No. 21; 4–00236]

Surety Companies Acceptable on Federal Bonds, Change of Name; Planet Insurance Company

Planet Insurance Company, a Wisconsin corporation, has formally changed its name to Reliance National Indemnity Company, effective March 31, 1994. The Company was last listed as an acceptable surety on Federal bonds at 57 FR 35811, July 1, 1993.

A Certificate of Authority as an acceptable surety on Federal bonds, dated today, is hereby issued under sections 9304 to 9308 of title 31 of the United States Code, to Reliance National Indemnity Company, Philadelphia, PA. This new Certificate replaces the certificate of Authority issued to the Company under its former name. The underwriting limitation of $6,615,000 established for the Company as of July 1, 1993, remains unchanged until June 30, 1994.

Certificates of Authority expire on June 30, each year, unless revoked prior to that dated. The Certificates are subject to subsequent annual renewal as long as the Company remains qualified (31 CFR part 223). A list of qualified companies is published annually as of July 1, in the Department Circular 570, which outlines details as to underwriting limitations, areas in which licensed to transact surety business and other information. Federal bond-approving officers should annotate their reference copies of the Treasury Circular 570, 1993 Revision, at page 35811 to reflect this change.

Questions concerning this notice may be directed to the Department of the Treasury, Financial Management Service, Funds Management Division, Surety Bond Branch, Washington, DC 20227, telephone (202) 874–6696.

Dated: May 16, 1994.

Charles F. Schwan III,

Director, Funds Management Division, Financial Management Service.

[FR Doc. 94–12450 Filed 5–20–94; 8:45 am]

BILLING CODE 4810–35–M

[Dept. Circ. 570, 1993—Rev., Supp. No. 18; 4–00236]

Surety Companies Acceptable on Federal Bonds, Change of Name; Transamerica Insurance Company

Transamerica Insurance Company, a California corporation, has formally changed its name to TIG Insurance Company, effective September 24, 1993. The Company was last listed as an acceptable surety on Federal bonds at 57 FR 35817, July 1, 1993.

A Certificate of Authority as an acceptable surety on Federal bonds, dated today, is hereby issued under sections 9304 to 9308 of title 31 of the United States Code, to TIG Insurance Company, Los Angeles, California. This new Certificate replaces the Certificate of Authority issued to the Company under its former name. The underwriting limitation of $29,500,000 established for the Company as of July 1, 1993, remains unchanged until June 30, 1994.

Certificates of Authority expire on June 30, each year, unless revoked prior to that date. The Certificates are subject to subsequent annual renewal as long as the Company remains qualified (31 CFR part 223). A list of qualified companies is published annually as of July 1, in the Department Circular 570, which outlines details as to underwriting limitations, areas in which licensed to transact surety business and other information. Federal bond-approving officers should annotate their reference copies of the Treasury Circular 570, 1993 Revision, at page 35817 to reflect this change.

Questions concerning this notice may be directed to the Department of the Treasury, Financial Management Service, Funds Management Division, Surety Bond Branch, Washington, DC 20227, telephone (202) 874–6696.

Dated: May 16, 1994.

Charles F. Schwan III,

Director, Funds Management Division, Financial Management Service.

[FR Doc. 94–12447 Filed 5–20–94; 8:45 am]

BILLING CODE 4810–35–M

[Dept. Circ. 570, 1993—Rev., Supp. No. 19; 4–00236]

Surety Companies Acceptable on Federal Bonds, Change of Name; Transamerica Insurance Company of Michigan

Transamerica Insurance Company of Michigan, a Michigan corporation, has formally changed its name to TIG Insurance Company of Michigan, effective August 30, 1993. The Company was last listed as an acceptable surety on Federal bonds at 57 FR 35817, July 1, 1993.

A Certificate of Authority as an acceptable surety on Federal bonds, dated today, is hereby issued under sections 9304 to 9308 of title 31 of the United States Code, to TIG Insurance Company of Michigan, Battle Creek, Michigan. This new Certificate replaces the Certificate of Authority issued to the Company under its former name. The underwriting limitation of $2,037,000 established for the Company as of July 1, 1993, remains unchanged until June 30, 1994.

Certificates of Authority expire on June 30, each year, unless revoked prior to that date. The Certificates are subject to subsequent annual renewal as long as the Company remains qualified (31 CFR part 223). A list of qualified companies is published annually as of July 1, in the Department Circular 570, which outlines details as to underwriting limitations, areas in which licensed to transact surety business and other information. Federal bond-approving officers should annotate their reference copies of the Treasury Circular 570, 1993 Revision, at page 35817 to reflect this change.

Questions concerning this notice may be directed to the Department of the Treasury, Financial Management Service, Funds Management Division, Surety Bond Branch, Washington, DC 20227, telephone (202) 874–6696.

Dated: May 16, 1994.

Charles F. Schwan III,

Director, Funds Management Division, Financial Management Service.

[FR Doc. 94–12449 Filed 5–20–94; 8:45 am]

BILLING CODE 4810–35–M

UNITED STATES INFORMATION AGENCY

Social Science Curriculum Fellowships for Russian University Teachers

ACTION: Notice—request for proposals.

SUMMARY: Prospective recipient will provide no fewer than ten research fellowships for Russian university teachers to pursue individual programs in social science fields in U.S. universities and research institutions in the academic year 1995–1996 in support of strengthened social science curricula in Russian higher education institutions. Participants should be teaching at the university level in social science fields, including but not limited to, political science, sociology, economics, law, and demography. Programs will include subject matter exposure, materials acquisition, scholarly networking, and orientation to current American approaches in these fields, especially empirical methods. Recipient organization is responsible for publicizing the program abroad, selecting the participants, placing participants in appropriate U.S. institutions, and for assuring appropriate supervision. Recipient is also responsible for all administrative arrangements, for program evaluation, and for establishing procedures for follow-up after participants return to their Russian universities.

Overall grant making authority for this program is contained in the Freedom Support Act. The funding authority for the program is appropriated under the Foreign Assistance Act 1994.

Programs and projects must conform with Agency requirements and guidelines outlined in the Application Package. It is expected that recipient will provide cash and/or in-kind cost sharing.

DATES: Deadline for proposals: All copies must be received at the U.S. Information Agency by 5 p.m. Washington, DC time, on Wednesday June 29, 1994. Faxed documents will not be accepted, nor will documents postmarked on June 29, 1994, but received at a later date. It is the responsibility of each assistance award applicant to ensure that the proposals are received by the above deadline. Grants should begin in the Fall of 1994.

ADDRESSES: The original and 8 copies of the completed application, including required forms, should be submitted by the deadline to: U.S. Information Agency, Reference: (E/AAS–94–02), Office of Grants Management, E/XE, room 336, 301 4th Street SW., Washington, DC 20547.

FOR FURTHER INFORMATION CONTACT: Interested organizations/institutions should contact Gretchen Christison at the U.S. Information Agency, Study of the U.S. Branch, E/AAS room 256, 301 4th Street SW., Washington, DC 20547 tel: (202) 619–4557 fax: (202) 619–6790 to request a detailed Application Package, which includes award criteria additional to this announcement, all necessary forms, and guidelines for preparing proposals, including specific criteria for preparation of the proposal budget. Interested applicants should read the complete **Federal Register** announcement before addressing inquiries to the Study of the U.S. Branch or submitting their proposals. Once the RFP deadline has passed, USIA staff may not discuss this competition in any way with applicants until after the Bureau proposal review process has been completed.

SUPPLEMENTARY INFORMATION: Pursuant to the Bureau's authorizing legislation, programs must maintain a non-political character, and should be balanced and representative of the diversity of American political, social, and cultural life. Academic programs under the authority of the Bureau must maintain their scholarly integrity and should meet the highest standards of academic achievement. "Diversity" should be interpreted in the broadest sense and encompass differences including but not limited to ethnicity, gender, religion, geographic location, socio-economic status, and physical challenges.

Applicants are strongly encouraged to adhere to the advancement of this principle.

Overview

The program seeks to increase and improve the quality of social science teaching in Russia. The program's immediate goal is to provide an opportunity for approximately ten qualified Russian university teachers to update and enhance their knowledge of their social science fields in support of revised and strengthened curricula.

Guidelines

The program is designed for Russian scholars who are teaching at the university level in social science fields including but not limited to, political science, sociology, economics, law, and demography. In the early stages of the program, the award recipient is responsible for publicizing the program, recruiting strong applicants and selecting the most qualified candidates. Participants should be chosen through a public, open competition which includes U.S. professional peer review for the final selection of participants. Selections should be made and announced in reasonable time for applicants to make plans for absence from their countries and to undertake departure formalities. USIA, the Study of U.S. Branch (E/AAS), and USIS Moscow should be informed of the final selection. Proposals should demonstrate extensive contacts with and knowledge of Russian universities to ensure that the best possible candidates are recruited and selected.

Award recipient will arrange appropriate placement in U.S. universities and research institutions for participants. To the extent possible, waivers of tuition fees should be procured.

Fellowships should be six to twelve months in duration. Family members may accompany recipients for part of the grant period, but grant monies must not be used to support the maintenance of or travel for dependents. The fellowships will provide for all the costs of the recipients. The dollar amount of the fellowship should be reduced in situations where Russian university salaries, transferable into U.S. dollars, continue to be received by the fellow while in the United States.

Award recipient will make all administrative arrangements, including travel, visa, disbursement of grant funds, insurance and related matters. The recipient should maintain contact with the participants and liaison with university hosts during the course of the grant to offer assistance with participant academic interests and administrative concerns such as housing, travel within the U.S., or emergency matters. It is expected that recipient organization will have substantive contact with university hosts to ensure that participants are able to make maximum use of institutional resources and facilities. To this end, it may prove useful for the recipient organization to encourage host institutions to designate specific faculty members to serve as mentors for the Russian participants.

Recipient will develop evaluation instruments and procedures to determine the participants' scholarly activity during the course of the grant, the adequacy of the stipend, and the adequacy of recipient and university administrative arrangements. Participants should also report on their general impressions of the U.S. and how they intend to apply the materials or new information gained during the research in their professional work in their own countries. The recipient will establish procedures for follow-up

communication with grantees to ascertain the application of their fellowship activity to their professional responsibilities, such as new publications, workshop leadership, new positions, or new course offerings stemming from their fellowship experience.

Proposed Budget

Applicants must submit a comprehensive line item budget for which specific details are available in the Application Package. A USIA-funded budget will not exceed $250,000. The recipient organization is expected to provide significant cash and/or in-kind cost-sharing.

Grants awarded to eligible organizations with less than four years experience in conducting international exchange programs will be limited to $60,000.

The line-item budget is divided into administrative and program sections. The line-item budget should include the categories listed in the Budget Guidelines found in the Application Package. An addendum should provide details about the budget.

Review Process

USIA will acknowledge receipt of all proposals and will review them for technical eligibility. Proposals will be deemed ineligible if they do not fully adhere to the guidelines established herein and in the application packet. Eligible proposals will be forwarded to panels of USIA officers for advisory review. All eligible proposals will also be reviewed by the appropriate geographic area office, and the budget and contracts offices. Proposals may also be reviewed by the Agency's Office of General Counsel. Funding decisions are at the discretion of the Associate Director for Educational and Cultural Affairs. Final technical authority for grant awards resides with USIA's grants officer.

Review Criteria

Technically eligible applications will be competitively reviewed according to the following criteria:

1. *Quality:* Proposals should exhibit originality, substance, rigor, and relevance to Agency mission and program goals.

2. *Program Planning:* Detailed agenda and relevant work plan should demonstrate appropriate content and logistical capacity. Agenda and plan should adhere to the program overview and guidelines described above.

3. *Ability to achieve program objectives:* Objectives should be reasonable, feasible, and flexible. Proposals should clearly demonstrate how the institution will meet the program's objectives and plan.

4. *Multiplier effect/impact:* Proposed programs should strengthen long-term mutual understanding, including maximum sharing of information and establishment of long-term institutional and individual linkages.

5. *Institutional Capacity:* Proposed personnel and institutional resources should be adequate and appropriate to achieve the program or project's goals.

6. *Institution's Record/Ability:* Proposals should demonstrate an institutional record of successful exchange programs, including responsible fiscal management and full compliance with all reporting requirements for past Agency grants as determined by USIA's Office of Contracts (M/KG). The Agency will consider past performance of prior grantees and the demonstrated potential of new applicants.

7. *Follow-on Activities:* Proposals should provide a plan for continued cost-effective follow-on activity which insures that USIA-supported programs are not isolated venues.

8. *Evaluation Plan:* Proposals should provide a plan for evaluation by the recipient institution.

9. *Cost-Effectiveness:* The overhead and administrative components of grants, as well as salaries and honoraria, should be kept as low as possible. All other items should be necessary and appropriate.

10. *Cost-sharing:* Proposals should maximize cost-sharing through other private sector support as well as institutional direct funding contributions.

11. *Support of Diversity:* Proposals should demonstrate the recipient's commitment to promoting the awareness and understanding of diversity throughout the program. This can be accomplished through documentation (such as a written statement or account) summarizing past and/or on-going activities and efforts that further the principle of diversity within both the organization and the program activities.

Notice

The terms and conditions published in this RFP are binding and may not be modified by any USIA representative. Explanatory information provided by the Agency that contradicts published language will not be binding. Issuance of the RFP does not constitute an award commitment on the part of the Government. Final award cannot be made until funds have been fully appropriated by Congress, allocated and committed through internal USIA procedures.

Notification

All applicants will be notified of the results of the review process on or about August 15, 1994. Awarded grants will be subject to periodic reporting and evaluation requirements.

Dated: May 16, 1994

Barry Fulton,

Deputy Associate Director, Bureau of Educational and Cultural Affairs.

[FR Doc. 94–12470 Filed 5–20–94; 8:45 am]

BILLING CODE 8230–01–M

Sunshine Act Meetings

Federal Register
Vol. 59 No. 98
Monday, May 23, 1994

This section of the FEDERAL REGISTER contains notices of meetings published under the "Government in the Sunshine Act" (Pub. L. 94-409) 5 U.S.C. 552b(e)(3).

UNITED STATES INTERNATIONAL TRADE COMMISSION

[USITC SE-94-17]

TIME AND DATE: May 26, 1994 at 2:30 p.m.

PLACE: Room 101, 500 E Street SW., Washington, DC 20436.

STATUS: Open to the public

1. Agenda for future meeting
2. Minutes
3. Ratification List
4. Inv. No. 731-TA-651 (Final) (Silicon Carbide from China)—briefing and vote
5. Outstanding action jacket:
   1. ID-94-010; Inv. No. 332-350 (Monitoring of U.S. Imports of Tomatoes).

In accordance with Commission policy, subject matter listed above, not disposed of at the scheduled meeting, may be carried over to the agenda of the following meeting.

CONTACT PERSON FOR MORE INFORMATION: Donna R. Koehnke, Secretary, (202) 205-2000.

Issued: May 18, 1994.

Donna R. Koehnke,

Secretary.

[FR Doc. 94-12641 Filed 5-19-94; 2:28 pm]

BILLING CODE 7020-02-P

NATIONAL WOMEN'S BUSINESS COUNCIL

ACTION: Notice of meeting.

SUMMARY: In accordance with the Women's Business Ownership Act, Public Law 100-533 as amended, the National Women's Business Council announces a forthcoming Council Meeting. The meeting will cover action items to be taken by the National Women's Business Council in Fiscal Year 1994 including but not limited to increasing procurement opportunities and access to capital for women business owners.

DATE: June 2, 1994, 4 p.m. to 5 p.m.

ADDRESS: Hilton Hotel and Towers, 720 S. Michigan Avenue, Chicago, Illinois.

STATUS: Open to the public.

CONTACT: For further information contact Amy Millman, Executive Director or Juliette Tracey, Deputy Director, National Women's Business Council, 409 Third Street, SW., suite 5850, Washington, DC 20024, (202) 205-3850.

Gilda Washington,

Administrative Officer, National Women's Business Council.

[FR Doc. 94-12619 Filed 5-19-94; 11:49 am]

BILLING CODE 6820-AB-M

TENNESSEE VALLEY AUTHORITY

Meeting No. 1466

TIME AND DATE: 10 a.m. (EDT), May 25, 1994.

PLACE: TVA Knoxville Office Complex, 400 West Summit Hill Drive, Knoxville, Tennessee.

STATUS: Open.

Agenda

Approval of minutes of meeting held on April 26, 1994.

Discussion item

1. Integrated Resource Planning.

Action Items

New Business

E—Real Property

E1. Release of a Restrictive Covenant Affecting Approximately 39 Acres of Land in Jefferson County, Illinois, to the State of Illinois, Department of Conservation.

E2. Release of a Restrictive Covenant Affecting Approximately 20.99 Acres of Land on Wheeler Reservoir in Morgan County, Alabama, to the City of Decatur.

E3. Amendment to the Kentucky Reservoir Plan to grant a 25-Year Easement Affecting Approximately 6.8 Acres of Land in Marshall County, Kentucky, to the Kentucky Department of Fish and Wildlife Resources.

E4. Sales of Noncommercial, Nonexclusive Permanent Easements Affecting 0.32 Acre of Tellico Lake Shoreline in Loudon and Monroe Counties, Tennessee.

F—Unclassified

F1. Revisions in Organizational Responsibilities for TVA's Security Clearance and Classified Information Program.

F2. Contract with Babcock and Wilcox for the Cumberland Fossil Plant, Units 1 and 2, Subject to Final Review Prior to Execution.

F3. Contract with F.E. Moran, Inc., Special Hazard Systems for a System-Wide Fire Protection Upgrade, Subject to Final Review Prior to Execution.

F4. Filing of Condemnation Cases.

Information Items

1. Public Auction Sale of Beaver Creek Reservoir Land.
2. Public Auction Sale of Clear Creek Reservoir Land.

CONTACT PERSON FOR MORE INFORMATION: Ron Loving Vice President, Governmental Relations, or a member of his staff can respond to requests for information about this meeting. Call (615) 632-6000, Knoxville, Tennessee. Information is also available at TVA's Washington Office (202) 898-2999.

Dated: May 18, 1994.

William L. Osteen,

Associate General Counsel and Assistant Secretary.

[FR Doc. 94-12595 Filed 5-19-94; 9:13 am]

BILLING CODE 8120-08-M

federal register

Monday
May 23, 1994

Part II

Department of Health and Human Services

Food and Drug Administration

21 CFR Parts 173 and 573

Secondary Direct Food Additives Permitted in Food for Human Consumption; Food Additives Permitted in Feed and Drinking Water of Animals; Aminoglycoside 3′-Phosphotransferase II; Final Rule

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 173 and 573

[Docket No. 93F-0232]

Secondary Direct Food Additives Permitted in Food for Human Consumption; Food Additives Permitted in Feed and Drinking Water of Animals; Aminoglycoside 3'-Phosphotransferase II

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of aminoglycoside 3'-phosphotransferase II (APH(3')II) as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton. APH(3')II is a protein encoded by the kanamycin resistance (*kan*^{r}) gene. This action is in response to a petition filed by Calgene, Inc.

DATES: Effective May 23, 1994; written objections and requests for a hearing by June 22, 1994.

ADDRESSES: Submit written objections to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Nega Beru, Center for Food Safety and Applied Nutrition (HFS-206), Food and Drug Administration, 200 C St., SW., Washington, DC 20204, 202-254-9523.

SUPPLEMENTARY INFORMATION:

Table of Contents

I. Introduction

A. Regulatory History

In accordance with 21 CFR 10.85, Calgene, Inc., submitted to FDA on November 26, 1990, a request for advisory opinion regarding whether the *kan*^{r} gene, a selectable marker, may be used in the production of genetically engineered tomato, cotton, and oilseed rape plants intended for human food and animal feed uses (*kan*^{r} Gene: Safety and use in the production of genetically engineered plants, Docket Number 90A-0416). In the **Federal Register** of May 1, 1991 (56 FR 20004), FDA announced that the request had been received and solicited comments from interested persons. The data submitted to the agency with the request for advisory opinion and the comments received were made available to the public at the Dockets Management Branch.

Subsequent to the submission of the request for advisory opinion, FDA published its "Statement of Policy: Foods Derived From New Plant Varieties" (the 1992 policy statement) in the **Federal Register** of May 29, 1992 (57 FR 22984). This policy statement clarified FDA's interpretation of the Federal Food, Drug, and Cosmetic Act (the act) with respect to human foods and animal feeds derived from new plant varieties, including plants developed by new methods of genetic modification such as recombinant deoxyribonucleic acid (DNA) techniques.

In the 1992 policy statement, FDA stated that the postmarket authority under section 402(a)(1) of the act (21 U.S.C. 342(a)(1)) would continue to be the primary legal tool for ensuring the safety of whole foods derived from genetically modified plants. FDA also noted that under the statutory definition of "food additive" in section 201(s) of the act (21 U.S.C. 321(s)), the transferred genetic material and the intended expression products could be subject to regulation as food additives, if such material or expression products were not generally recognized as safe (GRAS) (57 FR 22984 at 22990). FDA further stated that the agency would use its food additive authority to the extent necessary to ensure public health protection (such as when an intended expression product in a food differs significantly in structure, function, or composition from substances found currently in food) (57 FR 22984 at 22990).

The 1992 policy statement specifically discussed selectable markers that provide antibiotic resistance in product selection and development. With such markers, both the antibiotic resistance gene and the gene product, unless removed, are expected to be present in foods derived from such plants. FDA stated:

> Selectable marker genes that produce enzymes that inactivate clinically useful antibiotics theoretically may reduce the therapeutic efficacy of the antibiotic when taken orally if the enzyme in the food inactivates the antibiotic. FDA believes that it will be important to evaluate such concerns with respect to commercial use of antibiotic resistance marker genes in food, especially those that will be widely used. (See 57 FR 22984 at 22988.)

Subsequently, in January 1993, Calgene requested that FDA convert its request for advisory opinion to a food additive petition under section 409 of the act. FDA then announced in the **Federal Register** of July 16, 1993 (58 FR 38429), that a food additive petition (FAP 3A4364) had been filed by Calgene, Inc., 1920 Fifth St., Davis, CA 95616, proposing that the food additive regulations be amended to provide for the safe use of APH(3')II as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton.

After completing its review of the data submitted by Calgene, FDA convened a public meeting of its Food Advisory Committee on April 6 through 8, 1994, to undertake a scientific discussion of

the agency's approach to evaluating the safety of whole foods produced by new biotechnologies; a genetically modified tomato developed by Calgene containing the *kan*^{r} gene served as an example and focus of the discussion. The membership of the standing committee was supplemented with temporary members and consultants to the committee, representing scientific disciplines appropriate to the evaluation of foods derived from new plant varieties developed using recombinant DNA techniques.

At the meeting, Calgene presented a summary of the data they considered adequate to show safety of the tomato, and FDA presented its evaluation of the data. The committee was asked to comment on the approach used by FDA to evaluate whole foods and specifically, on the approach used for the Calgene tomato (Ref. 1). During committee discussion of the Calgene and FDA presentations, the committee members generally expressed the view that the approach used by FDA to evaluate the safety of the tomato, including the safety of the *kan*^{r} gene, was appropriate and that all relevant scientific questions had been adequately addressed.

In regard to the use of the *kan*^{r} gene, Calgene and the agency presented, and the committee discussed, such issues as the potential allergenicity of APH(3′)II and the potential for ingested APH(3′)II to inactivate orally administered antibiotics. Most of the discussion concerning the *kan*^{r} gene focused on the potential transfer of the gene to microorganisms in the gastrointestinal (GI) tract or in the environment. In evaluating Calgene's food additive petition for the use of the *kan*^{r} gene product, APH(3′)II, in the development of new varieties of tomato, oilseed rape, and cotton, FDA has considered the committee's discussions and recommendations on this subject, which are summarized in section III.B.3. of this document.

B. Scope of the Regulation

Having completed its evaluation and having considered the deliberations of the Food Advisory Committee, the agency is amending the food additive regulations to permit the use of APH(3′)II in the development of genetically modified tomatoes, oilseed rape, and cotton intended for food use. Only the translation product of the *kan*^{r} gene, APH(3′)II, and not the gene itself, is being regulated as a food additive. As the 1992 policy statement indicated, FDA does not anticipate that transferred genetic material (deoxyribonucleic acid (DNA)) would itself be regulated as a food additive (57 FR 22984 at 22990). DNA is present in the cells of all living organisms, including every plant and animal used for food by humans or animals, and is efficiently digested (Ref. 2). In this respect, the DNA that makes up the *kan*^{r} gene does not differ from any other DNA and does not itself pose a safety concern as a component of food.

This final rule is being promulgated after consideration of the issues relating to the safety of the use of APH(3′)II in the selection of transgenic plants. In addition, as noted above, because of the property of the *kan*^{r} gene to confer antibiotic resistance, the agency has considered the possibility that the gene might be transferred to other organisms (discussed in section III.B. of this document).

Potential safety issues specific to particular food products that contain the *kan*^{r} gene are not addressed by the agency in this document because such issues are beyond the scope of this rulemaking. For example, issues associated with other co-transferred DNA sequences, including other genes intended to impart specific traits, and issues related to potential genetic instability are not addressed because such issues will vary with specific products.

Developers of new plant varieties are responsible for addressing potential safety issues associated with specific food products resulting from the transfer of genetic materials and for ensuring the safety of the food products that they market. The policy statement contains a "Guidance to Industry" section (57 FR 22984 at 22991) that outlines an approach for the safety evaluation of foods derived from transgenic plants and suggests that the agency be consulted, as needed, to resolve critical issues.

As noted, issues related to genetic instability are not addressed because such issues are not unique to the *kan*^{r} gene but apply to any transferred genetic material irrespective of the transfer techniques used. Genetic instability could arise as a result of insertion of multiple copies of a given construct, especially if insertion occurs at multiple loci. Recombinations of the transferred DNA could cause deletions, duplications, or rearrangements within the plant genome (Ref. 3). Hence, in the 1992 policy statement, the agency noted that the genetic stability of a new plant variety is an important safety consideration and further stated that, "Factors that favor stability include a minimum number of copies of the introduced genetic material, and insertion at a single site." (57 FR 22984 at 23004).

In developing new plant varieties, developers are therefore responsible for following good manufacturing and good agricultural practices to ensure that they have developed a genetically stable transgenic plant. As a practical matter, this would ordinarily include using such techniques as segregation and Southern blot analysis to ensure that new plant varieties chosen for development have the new genetic material inserted into a single locus and that the number of copies of inserted DNA at a given site is limited to the minimum sufficient to achieve the intended effect.

C. Determination of Safety

Under section 409(c)(3)(A) of the act, a food additive cannot be approved for a particular use unless a fair evaluation of the data available to FDA establishes that the additive is safe for that use. The concept of safety embodied in the Food Additives Amendment of 1958 is explained in the legislative history of the provision: "Safety requires proof of a reasonable certainty that no harm will result from the proposed use of an additive. It does not—and cannot—require proof beyond any possible doubt that no harm will result under any conceivable circumstance." (H. Rept. 2284, 85th Cong., 2d sess. (1958)). FDA has incorporated this concept of safety into its food additive regulations. Under 21 CFR 170.3(i), a food additive is "safe" if "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."

The agency has reviewed the data and studies submitted in the request for advisory opinion, material that was submitted subsequent to the conversion of the request for advisory opinion to a food additive petition, the deliberations of the Food Advisory Committee that took place at the April 1994 meeting, as well as other information in its files. In addition, the agency has considered the comments that were received in response to the **Federal Register** notice announcing receipt of the request for advisory opinion. The comments are addressed in section IV. of this document. As discussed below, FDA has concluded, based upon its review, that the use of aminoglycoside 3′-phosphotransferase II is safe for use as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton intended for food use.

II. Use of the *kan*^{r} Gene As a Selectable Marker in Transgenic Plants

A Background

Developers have for many years used plant breeding techniques to introduce desirable genetic traits into new varieties that can be used in agriculture. Traditionally, breeders have relied on selection of mutants and on hybridization between different varieties of the same species to achieve this goal. More recently, recombinant DNA techniques (commonly referred to as "genetic engineering" techniques) have come into use to generate new plant varieties with desirable characteristics. Recombinant DNA techniques involve the isolation, and subsequent introduction into a host plant, of discrete DNA segments containing the gene(s) of interest. This introduction of exogenous DNA into a cell, resulting in its acquisition of a new phenotype, is commonly referred to as "transformation," and transformed plants that contain genetic material derived from sources other than the host plant itself are called transgenic.

The desired gene(s) may be introduced into a host plant by one of several methods, including: (1) Direct DNA uptake by the plant cells mediated by chemical or electrical treatments; (2) microinjection of DNA directly into plant cells; (3) biolistics, or firing tiny particles coated with the DNA of interest into plant cells; and (4) the use of a bacterium, such as the soil bacterium *Agrobacterium tumefaciens*, as a vehicle to carry the DNA into plant cells. (For a discussion of these processes, see Ref. 4).

B. Need for a Selectable Marker

Transformation of plant cells by introducing exogenous DNA is an inefficient process and, in general, only a small proportion of cells will successfully take up, integrate, and express the new genetic material (Ref. 5). Further, the few cells that do so are not readily distinguishable from the vast majority of cells that do not. Therefore, developers of transgenic plants need a means to distinguish cells that are successfully transformed from those that are not. Selectable markers, such as the *kan*^{r} gene, perform this function.

The *kan*^{r} gene is linked to the gene (or genes) of interest and then this genetic material is inserted into plant cells. Because plant cells are sensitive to the antibiotic kanamycin, incorporation of the *kan*^{r} gene into cells and subsequent expression of APH(3')II provides a convenient method for selecting successfully transformed cells. *Kan*^{r} works as a marker because only successfully transformed cells (which contain both the *kan*^{r} and the desired genetic material) survive when grown in a kanamycin-containing medium. These cells are subsequently regenerated into transgenic plants.

C. Identity of the Additive

APH(3')II [1] (CAS Reg. No. 58943–39–8) is encoded by the *kan*^{r} gene, which was originally isolated as a component of transposon Tn5 [2] from the bacterium *Escherichia coli* (Refs. 6 and 7). APH(3')II is an enzyme with an apparent molecular weight of 25,000 that catalyzes the transfer of a phosphate group from adenosine 5'-triphosphate (ATP) to a hydroxyl group of aminoglycoside antibiotics (see below), thereby inactivating the antibiotics.

APH(3')II inactivates the aminoglycoside antibiotics neomycin, kanamycin, paromomycin, ribostamycin, gentamicins A and B, as well as butirosins (Refs. 8 and 9). Of the antibiotics that are inactivated by APH(3')II, only neomycin and kanamycin are currently approved for use in humans or animals in the United States (Refs. 10 and 11).[3]

The APH(3')II evaluated in this document is the enzyme whose synthesis is directed by the *kan*^{r} gene derived from transposon Tn5. This enzyme is not to be confused with enzymes that may be similarly named (e.g., a type I aminoglycoside phosphotransferase encoded by a gene isolated from transposon Tn601) or other bacterial enzymes (including acetyltransferases, nucleotidyltransferases, and phosphotransferases) that inactivate kanamycin and neomycin (Refs. 8 and 12).

D. Use and Intended Technical Effects

Aminoglycoside antibiotics exert their effect on bacteria by binding to bacterial ribosomes and inhibiting protein synthesis. Phosphorylation of the antibiotics by APH(3')II interferes with this binding and thus prevents the antibiotics from inhibiting protein synthesis (Ref. 13). In this way, cells that contain the *kan*^{r} gene and that express APH(3')II are rendered resistant to the action of the antibiotics. In plant cells, the antibiotics exert their effect on mitochondria and chloroplasts where protein synthesis takes place on ribosomes that resemble bacterial ribosomes (Ref. 14).

The proposed use of the *kan*^{r} gene and gene product APH(3')II is as a processing aid in the development of new varieties of tomato, cotton, and oilseed rape intended for food use. As discussed above, because transformation of plant cells is an inefficient process, the presence of APH(3')II and the consequent ability of the plant cells to grow in the presence of antibiotics is used to distinguish between transformed and nontransformed cells. Therefore, the intended technical effect of APH(3')II is to permit, in the early phases of development of genetically modified plants, the selection of transformants carrying the *kan*^{r} gene along with the genetic material of interest. However, APH(3')II has no intended technical effect in the final plant or final crop product.

III. Safety Evaluation

A. APH(3')II

Safety issues associated with APH(3')II can be divided into two areas: (1) Those associated with the direct effects of ingestion of the protein, including the possibility of allergenicity; and (2) those associated with the biological activity of APH(3')II (i.e., the effect of the enzyme on the therapeutic efficacy of orally administered antibiotics).

1. Direct Effects of Ingestion

Calgene provided evidence that APH(3')II is rapidly inactivated by stomach acid, is degraded by digestive enzymes, and is not modified by glycosylation (i.e., does not contain sugar molecules attached to the protein) when produced in the transgenic plants under consideration. In addition, Calgene noted that enzymes such as APH(3')II are heat labile. Thus, Calgene concluded that APH(3')II does not possess any of the characteristics associated with allergenic proteins such as proteolytic stability, glycosylation, or heat stability (Ref. 15). In April 1992, Calgene also conducted protein and DNA sequence comparisons using sequences in four separate databases (GenBank, EMBL, PIR 29, and Swiss-Prot) and established that APH(3')II does

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[1] Other names for this enzyme include neomycin phosphotransferase II (NPT II), neomycin phosphotransferase, and kanamycin phosphotransferase II.

[2] A transposon is a segment of DNA that is mobile and has the capacity to move from one site in the genome to another. Transposons vary in size and frequently contain, as does Tn5, antibiotic resistance genes in addition to genes coding for functions concerned with movement of the transposon.

[3] Gentamicin, which is used therapeutically, is composed of a complex mixture of the antibiotic substances produced by *Micromonospora purpurea* that contain primarily gentamicin C_1 (25 to 50 percent), gentamicin C_{1a} (10 to 35 percent), and gentamicins C_{2a} and C_2 (25 to 55 percent) (Ref. 10). Gentamicins A and B are at most minor components of the commercial drug. Thus, APH(3')II does not confer resistance to gentamicin that is used therapeutically (Ref. 12).

not have significant homology to any proteins listed as food allergens or toxins in these databases.

FDA agrees with Calgene that the characteristics of APH(3')II do not raise a safety concern. First, each whole food, on average, contains several thousands of different proteins (Ref. 16). As a class, proteins are rarely toxic (Ref. 17) and APH(3')II is not known to be toxic. Second, APH(3')II is a phosphorylating enzyme, and all plants and animals that are part of the food supply contain such phosphorylating enzymes without adverse consequences. Third, APH(3')II has been shown to be rapidly degraded under simulated gastric conditions (Refs. 18 through 21). Finally, the estimated dietary exposure to APH(3')II is very low (480 µg APH(3')II per person per day,[4] or 0.16 part per million in the diet, based on a 100-percent market share for tomatoes containing APH(3')II (Ref. 18)).

Based upon the available evidence, the agency believes that this protein does not possess any properties that would distinguish it toxicologically from other phosphorylating enzymes in the food supply. Further, because of the low exposure levels and normal digestibility of APH(3')II, the agency concludes that no limits other than good manufacturing practice are needed to ensure the safety of the petitioned use of APH(3')II (Ref. 20).[5]

2. Effects on the Therapeutic Efficacy of Orally Administered Antibiotics

a. *APH(3')II in human foods.* i. *Relevant source of APH(3')II.* Calgene considered whether APH(3')II could affect the therapeutic efficacy of orally administered aminoglycoside antibiotics. In doing so, Calgene stated that only APH(3')II from fresh tomatoes is relevant because it is the only form that is enzymatically active. Processed tomato products (such as processed whole tomatoes, chili, juice, pulp, paste, catsup, and soup) are subjected to temperatures in the range of 82 to 100 °C; these temperatures would be expected to inactivate the APH(3')II enzyme. For edible oils extracted from cottonseed and rapeseed, high temperature treatment, solvent extraction, and subsequent purification steps generally included in the processing of such oils would also be expected to inactivate APH(3')II.

FDA agrees that high temperature treatment denatures proteins and inactivates enzymes and therefore, processed products that contain tomatoes with the *kan*^{r} gene are unlikely to contain any enzymatically active APH(3')II. In addition, purified oils essentially do not contain protein; therefore, oils derived from transgenic cottonseed and rapeseed modified using the *kan*^{r} gene would not be expected to contain active or inactive APH(3')II (Refs. 18 and 23). Thus, FDA agrees that fresh tomatoes from plants developed using the *kan*^{r} gene are the only source of active APH(3')II.

ii. *Effect of APH(3')II in fresh tomatoes on the therapeutic efficacy of orally administered antibiotics.* Calgene performed several experiments intended to address whether APH(3')II consumed as a component of fresh tomatoes could render orally-administered kanamycin ineffective. These experiments were performed under simulated gastric and intestinal conditions (i.e., appropriate pH, reagent concentrations, temperature, and reaction times) chosen to reflect conditions expected in vivo. In some studies both tomato extract and nonfat milk were added to determine whether the presence of additional food-source proteins in the simulated gastric and intestinal fluids might slow the proteolytic degradation of APH(3')II by competition. After evaluating the loss of immunologically detectable APH(3')II, Calgene concluded that, under normal gastric and intestinal conditions, APH(3')II would be effectively degraded before the enzyme could inactivate kanamycin or neomycin and therefore, APH(3')II would not interfere with orally administered kanamycin or neomycin therapy. The results of Calgene's experiments were the same whether done in the presence or the absence of tomato extract and nonfat milk.

In addition, Calgene presented the results of in vitro degradation studies performed under simulated abnormal gastric conditions, such as may exist in patients treated with drugs that reduce stomach acidity. Calgene stated that these studies demonstrated that APH(3')II is not degraded in neutralized (pH 7.0) simulated gastric fluid and thus, APH(3')II may remain active in such abnormal gastric conditions. However, Calgene pointed out that, even under those conditions, APH(3')II would not be expected to inactivate orally administered kanamycin or neomycin because the concentration of ATP, which the enzyme requires to inactivate kanamycin and neomycin, would be limiting. In support of this contention, Calgene presented data from the published literature on ATP levels in fresh fruits and vegetables. Calgene then estimated ATP intake and calculated the fraction of neomycin that would be phosphorylated assuming that all of the available ATP reacted with the antibiotic. Under the worst-case situation (high intake of ATP-containing food, low dose of antibiotic) Calgene's calculations showed that only a small fraction (no more than 1.5 percent) of the antibiotic would be inactivated. Moreover, Calgene presented data that showed that no significant inactivation of kanamycin was observed during in vitro studies conducted with tomato extract containing APH(3')II and kanamycin over a 4-hour incubation period.

iii. *Agency conclusions.* The agency has evaluated the data and other information presented by Calgene (Refs. 18 through 21 and 24). FDA agrees that Calgene's in vitro digestion studies show that, as is the case for dietary protein in general, the biological activity of APH(3')II is destroyed during gastric and intestinal phases of digestion. Further, the agency has determined that any active APH(3')II that might remain would not significantly inactivate kanamycin or neomycin in the gut because the small amount of ATP in fruits and vegetables would limit the amount of antibiotic that could be phosphorylated. ATP is an extremely labile molecule that is susceptible to inactivation both by heat (e.g., cooking) and by enzymes, such as alkaline phosphatases (Ref. 25), that are found in the intestine. Because the ATP in meat, poultry, fish, and cooked vegetables would be broken down by cooking, the primary source of ATP in the gastrointestinal (GI) tract of patients would be uncooked fruits and vegetables. However, the amount of ATP in a variety of fruits and vegetables would provide enough ATP to inactivate only a small percentage of kanamycin or neomycin, even if one makes the conservative assumption that

[4] Because oils produced from transgenic cottonseed and rapeseed would not contribute APH(3')II to the human diet (see also section 2 below), the exposure estimate was derived exclusively for tomatoes. The agency made several conservative assumptions in arriving at the probable per capita exposure to APH(3')II of 480 µg/person/day. For example, FDA assumed that all tomatoes contain APH(3')II at a level of 0.1 percent of total protein although, of the two lines intended for commercialization by Calgene, one contains less than 0.01 percent and the other less than 0.002 percent of APH(3')II (as a percentage of total protein). Second, FDA included APH(3')II in processed products in its estimate although high temperature treatment used in the production of processed products would be expected to result in loss of enzymatic activity of APH(3')II. In summary, the exposure estimate represents a theoretical maximum rather than a realistic estimate of exposure to APH(3')II.

[5] A recently published study (Ref. 22) also showed that APH(3')II is rapidly degraded under simulated mammalian digestive conditions. In addition, in an acute mouse feeding study, the investigations showed that feeding highly exaggerated doses of purified APH(3')II caused no deleterious effects.

all of the ATP in these fruits and vegetables would survive the alkaline phosphatases in the intestines and would be available for catalytic phosphorylation of kanamycin or neomycin.

In addition, the agency has considered the patient population likely to be exposed to aminoglycoside antibiotics. Oral aminoglycosides are most commonly administered to either pre-operative patients (prior to bowel surgery) or patients with hepatic encephalopathy. Neither patient population would be expected to be ingesting tomatoes or any other fresh fruits and vegetables; therefore there is little or no risk of inactivating the oral antibiotic in these patients (Refs. 24 and 26). For these reasons, FDA concludes that the presence of APH(3')II in food will not compromise the therapeutic use of orally administered kanamycin or neomycin.

b. *APH(3')II in animal feed.* Calgene also considered the potential inactivation of neomycin that is used in animal feeds manufactured using cottonseed meal and rapeseed meal obtained from transgenic plants. The transgenic tomato was not considered because only small amounts of tomato and tomato byproducts are used in the animal feed industry. Further, neomycin is primarily used to treat calves and swine whereas tomato byproducts, to the extent that they are used in animal feed, are primarily used as ingredients in cattle diets (Ref. 27).

Calgene analyzed neomycin levels both in nontransgenic medicated cottonseed and rapeseed meals and in transgenic medicated cottonseed and rapeseed meals over a storage period of 56 days (considered a worst-case situation) and concluded that there was no significant inactivation of neomycin.

FDA reviewed the data submitted by Calgene and concludes that there was no significant difference with respect to neomycin stability between medicated cottonseed and rapeseed meals prepared from transgenic cottonseed and rapeseed containing APH(3')II, and appropriate controls (Ref. 28). Therefore, the agency concludes that transgenic strains of cottonseed and rapeseed containing APH(3')II have no apparent untoward effect regarding the stability of neomycin and that the therapeutic efficacy of neomycin in animal feed will not be affected. The agency also considers this conclusion applicable to other aminoglycoside antibiotics, e.g., gentamicin, when orally administered.

B. The Kan^r Gene

The agency also evaluated issues relevant specifically to the safety of the use of the *kan^r* gene in tomato, oilseed rape, and cotton. In particular, FDA evaluated the potential for horizontal transfer of the gene and subsequent expansion of the population of antibiotic-resistant pathogens. The agency evaluated whether efficacy of oral antibiotic treatment of humans or animals could be compromised by consumption of food containing the *kan^r* gene either because of the development of resistant intestinal microflora in humans and animals or because the cells lining the intestinal lumen might become transformed. In addition, the agency considered the possible transfer of the *kan^r* gene from transgenic plants to soil microorganisms and expansion of the antibiotic-resistant bacterial population.

1. Potential Transfer of the *kan^r* Gene to Intestinal Microorganisms and Cells Lining the Intestinal Lumen

Calgene presented theoretical and experimental evidence to demonstrate that the potential for compromise of antibiotic therapy by horizontal transfer of the *kan^r* gene to gut microorganisms or intestinal epithelial cells is not of significant concern. Calgene considered the sources of the *kan^r* gene, the role digestion plays in degrading DNA, and possible DNA transfer mechanisms.

a. *Relevant source of the kan^r gene available for transformation.* Calgene considered potential transfer of the *kan^r* gene only from fresh tomatoes because processing is expected to inactivate the *kan^r* gene in processed tomato products and in food products derived from cotton and oilseed rape. The *kan^r* gene is not expected to survive procedures used to process tomatoes because heating processes, such as those used in commercial processing, can directly degrade DNA or can damage DNA by releasing cellular DNA-degrading enzymes.

The *kan^r* gene is also not expected to survive the process of oil production from cottonseed and rapeseed. Mechanical grinding or flaking of oilseeds during the production of oils and meals from oilseeds is expected to liberate degradative enzymes normally present within the cell that would degrade the *kan^r* gene. In addition, oil processing also includes high temperatures and solvent extractions, both of which would be expected to inactivate the *kan^r* gene. Moreover, because DNA is hydrophilic, it is unlikely to fractionate into oil, which is hydrophobic, during the extraction of oil from cottonseed and rapeseed. Therefore, intact DNA, including the *kan^r* gene, is not expected to survive the production of oils and animal feeds from cottonseed and rapeseed.

b. *Effect of digestion on the availability of the kan^r gene for possible transformation.* Calgene demonstrated that most if not all of the DNA comprising the *kan^r* gene ingested by humans will be degraded in the stomach and upper small intestine before it reaches the lower small intestine, cecum, and colon, and would be unavailable for potential transformation of gut microorganisms. Calgene estimated that 99.9 percent of fresh tomato DNA would be digested to fragments smaller than 1,000 base pairs. This estimate was based on in vitro studies that found that only 0.1 percent of DNA could be detected as fragments of 1,000 base pairs or longer after exposure to stomach-simulating fluids for 10 minutes and to intestinal-simulating fluids for another 10 minutes. Thus most of the DNA remaining after digestion would be smaller than the *kan^r* gene which is about 1,000 base pairs long.

Regarding animal feed, food-producing animals consume primarily processed forms of cottonseed and rapeseed, in which, as discussed above, the *kan^r* gene is not expected to remain intact. In addition, researchers have shown that nucleic acids introduced into the rumens of calves, or incubated with calf, sheep, or cow rumen contents in vitro, were rapidly and completely degraded to nucleotides and nucleosides (Ref. 29).

c. *Calculation of worst-case transformation frequencies.* In its submission, Calgene addressed the potential for horizontal transfer of the *kan^r* gene. Natural transformation, i.e., the uptake and incorporation into the genome of free DNA, is known to occur in some bacterial species. This is the only possible mechanism by which intestinal microflora could take up free DNA (Ref. 30). However, none of the species known to be present in the GI tract has been found capable of acquiring exogenous DNA by natural transformation. Nonetheless, to consider the worst-case scenario, Calgene assumed that all microbes in the intestine would be able to take up and incorporate exogenous DNA at a frequency found for certain species of the genus *Streptococcus*. Calgene noted that although the firm developed its transformation model for certain *Streptococcus* species, they are not aware of any information indicating that *Streptococcus* species found in the GI tract can be naturally transformed.

To undergo natural transformation, the recipient bacterium must be transformation-competent, i.e., ready to take up DNA. As noted, none of the bacterial species that occur in the GI tract is known to be capable of becoming transformation-competent. In addition, the genome of a recipient bacterium should contain DNA homologous to the incoming DNA (Refs. 31 and 32). Because the genomes of intestinal *Streptococci* or other intestinal bacteria are not expected to exhibit homology to the DNA constructs containing the *kan*^{r} gene[6], Calgene assumed that the *kan*^{r} gene could only undergo "illegitimate" recombination, a process that does not require significant DNA homology. Calgene noted that illegitimate recombination occurs in microorganisms at a much lower rate than homologous recombination.

Under the foregoing worst-case assumptions, Calgene estimated that if a person consumes fresh tomatoes at the 90th percentile level (i.e., eats more tomatoes than 89 percent of the individuals in the population), the transformation frequency of the intestinal microorganisms with the *kan*^{r} gene will be approximately 3×10^{-15} transformants per day. This transformation frequency is more than 5 orders of magnitude less than the frequency of mutation to kanamycin resistance per bacterial replication, i.e., 10^{-9} (Ref. 12). Thus, Calgene showed that for every 300,000 bacteria that mutate to kanamycin resistance per replication (generally a matter of hours), there would be, at most, under worst-case conditions, one kanamycin-resistant bacterium per day added to that number due to transformation.

Calgene stated that the potential for food-producing animals to experience decreased efficacy of antibiotic therapy as a result of pathogenic intestinal microflora incorporating and expressing the *kan*^{r} gene would be similar to that described for humans, i.e., equally improbable. In reaching this conclusion, Calgene relied on the finding that DNA is rapidly and completely digested in the gut of food animals (Ref. 29) and on the contention that the worst-case transformation scenario described above for human gut microorganisms also applies to microorganisms found in the gut of food-producing animals.

[6]One population that does contain DNA segments homologous with part of the *kan*^{r} construct is *E. coli*, because the *kan*^{r} construct contains part of an *E. coli* gene. Although *E. coli* constitutes one of the predominant species of aerobic GI tract bacteria, *E. coli* is not transformation-competent under conditions that prevail in the GI tract (Ref. 33). Thus, transformation of *E. coli* due to homologous recombination is not an issue.

With respect to epithelial cells lining the intestinal lumen, Calgene provided information that no transformation of human epithelial cells has been demonstrated in vivo (Ref. 2). In addition, even if transformed, intestinal epithelial cells are terminally differentiated (i.e., do not divide) and have a relatively short life span (Ref. 34), and thus would continually be shed and replaced by nontransformed cells.

2. Potential Transfer of the *kan*^{r} Gene to Soil Microorganisms

Calgene also considered the possibility that the *kan*^{r} gene might be transferred to soil microorganisms, thereby increasing the level of antibiotic-resistant organisms in the environment. Calgene pointed out that the only plausible mechanism by which gene transfer could occur between plants and bacteria is through natural transformation. Taking this mechanism into consideration and using worst-case assumptions similar to those discussed above for intestinal microorganisms, Calgene calculated that, at worst, kanamycin-resistant transformants resulting from plant DNA left in the fields would represent not more than one in 10 million of the existing kanamycin-resistant soil population.

3. Food Advisory Committee Discussions Regarding Potential Horizontal Transfer of the *Kan*^{r} Gene

As part of its discussion of the scientific issues related to the evaluation of Calgene's genetically engineered tomato, the Food Advisory Committee discussed the possibility that the *kan*^{r} gene might be transferred to microorganisms in the GI tract and in the environment (Ref. 1).

The committee members concluded that transfer of the *kan*^{r} gene consumed as a component of tomatoes to microorganisms in the GI tract was highly unlikely based on published data in the scientific literature. Similarly, the committee members judged that the potential for transfer of the *kan*^{r} gene from plants to microorganisms in the environment is highly unlikely based on the members' knowledge of mechanisms of gene transfer. In addition, members of the committee pointed out that the rate at which such transfer could take place, if at all, was of so small a magnitude that, coupled with the high prevalence of kanamycin resistant organisms already present in the environment, it would not cause a significant environmental impact.

Some members of the committee, while convinced by the information presented at the meeting that the transfer of the *kan*^{r} gene from tomato plants to microorganisms in the soil was improbable, expressed concern regarding the use of the *kan*^{r} gene in other crops that may be grown on a wide scale. In addition, some committee members were concerned that a determination of safety with regard to the use of *kan*^{r} gene in Calgene's tomato might signal to producers that it is now permissible to use the *kan*^{r} gene in other crops. In light of such concerns, these committee members advised that use of the *kan*^{r} gene in other crops should be evaluated on a case-by-case basis.

4. Agency Conclusions

The agency has considered the recommendations of the members of the Food Advisory Committee. The agency agrees that the potential transfer of the *kan*^{r} gene, as well as other antibiotic resistance marker genes, from crops to microorganisms should be evaluated on a case-by-case basis. As noted, Calgene petitioned for the use of the *kan*^{r} gene product, APH(3')II, in the development of genetically engineered cotton and oilseed rape in addition to tomato. As discussed below, the agency has evaluated data and information concerning horizontal transfer of the *kan*^{r} gene from its use in all three crops. This is consistent with the committee's advice that safety of the use of the *kan*^{r} gene be evaluated on a case-by-case basis. In addition, Calgene's petition seeks to amend the food additive regulations to permit the use of APH(3')II only in tomato, cotton, and oilseed rape; approval of Calgene's petition would not mean that developers could use the *kan*^{r} gene in crops other than those identified in the petition.

FDA has also evaluated the information submitted by Calgene and has determined that the probability of transfer of the *kan*^{r} gene to gut microflora is remote and that even under worst-case conditions, the number of microorganisms that would be converted to kanamycin resistance is negligible when compared to the reported prevalence of gut microflora that are already resistant to kanamycin (Ref. 35). This conclusion applies to both humans and animals. The agency has determined that exposure to foods that contain the *kan*^{r} gene will not compromise the efficacy of antibiotic treatment because the likelihood of increasing the number of antibiotic resistant microorganisms is extremely low. Further, the agency has determined that there is no evidence that free DNA containing the *kan*^{r} gene, even if present, can transform cells lining the GI tract (Ref. 2).

FDA has also evaluated the information submitted by Calgene concerning soil microorganisms and agrees with Calgene that there would be no increase in kanamycin-resistant soil microorganisms because it is highly unlikely that the *kan*^{r} gene could move from the plant genome into soil microorganisms via horizontal gene transfer. Further, the agency has determined that, even if such transfer could occur, the rate at which it could occur is such that it would not result in a detectable increase over the existing background population of kanamycin-resistant bacteria (Ref. 36). Based on the foregoing, FDA has concluded that the use of the *kan*^{r} gene does not pose safety concerns in terms of increase in the population of antibiotic-resistant pathogens due to the potential for horizontal transfer of the gene.

IV. Response to Comments

FDA received 47 comments on Calgene's request for an advisory opinion on the use of the *kan*^{r} gene in the development of new varieties of tomato, oilseed rape, and cotton plants. Comments were received from members of academia, industry and industry-related organizations, State and Federal agencies, environmental groups and other nonprofit organizations, and individual consumers. Additionally, several comments on the agency's 1992 policy statement addressed the use of the *kan*^{r} gene.

Most of the comments supported the use of the *kan*^{r} gene in crop development, stating that there were no health or environmental issues precluding its use. Several comments expressed opinions on a wide range of issues including regulatory approaches for genetically engineered foods, concerns relating to human and animal food safety, and to the environmental effects of the *kan*^{r} gene, and whether foods containing the *kan*^{r} gene and APH(3')II should be specially labeled.

A. Regulatory Issues

Some comments stated that it was not appropriate for FDA to evaluate the safety of the *kan*^{r} gene and APH(3')II under an advisory opinion and that the *kan*^{r} gene and APH(3')II should be treated as food additives by FDA. FDA has discussed above the basis for its decision not to regulate the DNA that makes up the *kan*^{r} gene itself as a food additive. Further, in light of Calgene's conversion of its request for advisory opinion on the use of the *kan*^{r} gene to a food additive petition, the comment concerning the regulation of APH(3')II as a food additive no longer requires a response

B. Food Safety

Several comments stated that the presence in food of APH(3')II raised no food safety concerns whatsoever. Others questioned whether Calgene had supplied adequate data to ensure the safety of the *kan*^{r} gene and gene product, APH(3')II, when present in food. The substantive questions raised are discussed in sections IV.B.1 through 5 of this document

1. Glycosylation

Two comments stated that APH(3')II might be glycosylated (i.e., might contain sugar molecules attached to the protein via the amino acid asparagine (N-linked) or via the amino acids serine, threonine, or hydroxyproline (O-linked)) when produced in tomatoes or other plants and, therefore, might become a food allergen. One of the comments asserted that for this reason, Calgene should be required to test whether APH(3')II is glycosylated. The comments, however, did not provide any information showing that glycosylated APH(3')II is likely to be, or is, allergenic.

At this time, FDA is unaware of any practical method to predict or assess the potential for new proteins in food to induce allergenicity. Although many food allergens that have been characterized at a structural level are glycosylated (Ref. 37), the agency is not aware of any information on structural or other properties of glycosylated proteins that would be predictive of their allergenicity. As noted, the comments did not provide such information. Moreover, glycosylated proteins are widespread in food. For these reasons, glycosylation is not a useful positive predictor of a potential allergenic effect. Accordingly, FDA did not request that Calgene determine whether APH(3')II is glycosylated.

Nevertheless, in a submission dated October 24, 1991, entitled "Response to Public Comments," Calgene addressed whether APH(3')II is likely to be glycosylated and concluded that it is not. Calgene noted that APH(3')II lacks the amino terminal sequence of amino acids (commonly referred to as a "signal peptide") that is necessary to direct the protein into the cellular compartments where glycosylation occurs. Calgene also asserted that the unchanged molecular weight of APH(3')II in plants (relative to the molecular weight of bacterial APH(3')II, which is not glycosylated) supports the conclusion that APH(3')II is not glycosylated in plants. Finally, Calgene stated that the amino acid sequence (asparagine-X-serine/threonine) that is required to direct N-linked glycosylation to specific asparagine moieties is not present in APH(3')II. (Calgene noted that a corresponding argument for the lack of the appropriate amino acid sequence to direct O-linked glycosylation cannot be made because the sequences that direct O-linked glycosylation have not been defined.)

FDA has considered the information and arguments submitted in the comments and Calgene's response and has concluded that the available evidence indicates that APH(3')II is not glycosylated in plants. However, even if glycosylation had been demonstrated, FDA emphasizes that glycosylation alone does not necessarily establish that APH(3')II is likely to produce an allergenic response because the positive predictive value of glycosylation with respect to the potential for inducing allergenicity has not been demonstrated

2. In Vitro Digestibility Studies

In its original submission, Calgene presented the results of in vitro digestibility studies that demonstrated that APH(3')II enzymatic activity is rapidly decreased in simulated gastric fluid and in simulated intestinal fluid.

One comment asserted that Calgene should provide a more thorough study of degradation of APH(3')II in the digestive tract because the conditions of the in vitro digestibility study submitted by Calgene did not fully mimic the complex environments of the human gut. The comment further asserted that it was not clear whether the digestibility data also apply to neonates and to people with coeliac disorders or ulcers who can absorb peptides and intact proteins through their intestines. The comment noted that the applicability of the data to neonates would be of special importance should *kan*^{r} be used in soybeans because soy protein is a major component of some infant formulas. Importantly, however, the comment presented no information to provide a basis for concluding that the absorption of APH(3')II occurs, or that if it does, such absorption presents a health concern greater than that posed by the absorption of any other protein in the diet.

As discussed above, FDA has evaluated the studies presented by Calgene to demonstrate the normal digestibility of the enzyme and concurs with Calgene's conclusion that APH(3')II is rapidly degraded under normal conditions in the GI tract. Therefore, FDA believes that the intestinal transfer of intact or large fragments of APH(3')II is not likely to occur in individuals with normal GI tracts.

In regard to the possibility of increased intestinal absorption of proteins in neonates and individuals with special conditions (e.g., ulcers), FDA has concluded that there is no reason to expect that absorption of the intact or partially digested APH(3′)II protein would present a safety problem different from absorption of any other protein in the diet. As discussed above, proteins, as a class, are rarely toxic. Furthermore, APH(3′)II is a phosphorylating enzyme and does not contain any properties that would distinguish it toxicologically from any other phosphorylating enzymes that historically have been part of the food supply without adverse consequences. Finally, because Calgene did not petition FDA for the use of APH(3′)II in soybeans, it is not necessary to address the comment concerning the applicability of Calgene's digestibility data to neonates fed soybean-derived formulas.

3. Copy Number of the *kan*^{r} Gene and Expression Level of APH(3′)II

In its submission of November 26, 1990, Calgene stated that it did not intend to commercialize lines that contained more than 10 copies of the *kan*^{r} gene. In addition, Calgene also declared that, in tomatoes, the APH(3′)II level would be no more than 0.1 percent of the total protein of the tomato and that processing procedures would destroy APH(3′)II in processed tomatoes and edible oils extracted from cottonseed and rapeseed.

One comment asserted that Calgene inadequately described the methods by which it would ensure that no lines with greater than 10 copies of the *kan*^{r} gene would be marketed. The comment further asserted that many of the analyses offered by Calgene to prove the safety of the *kan*^{r} gene depend on estimates of the number of genes per cell and that, if the company cannot ensure this relatively low level of gene incorporation, many of its safety arguments are undermined. The comment, however, did not identify which of Calgene's safety analyses depended on estimates of the numbers of genes per cell.

The comment may have been referring to Calgene's assumption that each plant cell would contain 10 copies of the gene when it calculated a worst-case frequency of transformation of microorganisms with the *kan*^{r} gene that would result from use of the gene in transgenic plants. However, the agency notes that the outcome of those calculations, i.e., Calgene's conclusion that the transformation frequency of microorganisms with the *kan*^{r} gene is insignificant, would not change had Calgene assumed much higher gene copy numbers in its calculations. Therefore, FDA's safety assessment does not depend on precise estimates of gene copy number. Nor does the comment provide a basis for concluding that it is necessary to have precise methods for ensuring that no plants with more than 10 copies of the gene will be marketed.

A second comment maintained that Calgene provided an inadequate description of the quality control and assurance procedures the company would use to ensure that APH(3′)II would be kept to no more than 0.1 percent of total protein of the tomato, and that a number of the company's safety analyses rely on the amount of APH(3′)II in the food. The comment, however, did not identify which of Calgene's safety analyses relied on estimates of the concentration of APH(3′)II in the food.

FDA has determined that there is no need to set a tolerance for the amount of APH(3′)II that will be consumed because the agency knows of no reason why this protein would have any properties that would distinguish it toxicologically from any other phosphorylating enzymes in the food supply. Also, as discussed above, APH(3′)II will not affect efficacy of orally administered antibiotics because APH(3′)II is rapidly digested under normal conditions in the GI tract, and even in abnormal gastric conditions where APH(3′)II may not be rapidly digested, the amount of ATP available in food would allow only a small proportion of kanamycin and neomycin to be inactivated. Therefore, the agency concludes that there is no need to require quality control and assurance procedures to ensure that the APH(3′)II level will be no more than 0.1 percent of the total protein in commercial tomato varieties.

A third comment argued that Calgene did not provide data to establish that APH(3′)II would not be present after tomato processing and after extraction of edible oils.

The agency's exposure estimates included an assumption that APH(3′)II would be present in both processed tomatoes and fresh tomatoes even though the high temperatures involved in processing inactivate enzymes and therefore, processed tomato products are unlikely to contain enzymatically active APH(3′)II (Ref. 18). In addition, well-established processing procedures used to extract edible oils from oilseed crops do not extract significant amounts of protein (Ref. 23). Therefore, exposure to APH(3′)II obtained from rapeseed oil and cottonseed oil would be negligible (Ref. 18). The comment did not present any information to contradict FDA's analysis and conclusion on this point.

4. The Potential for Side Effects From Consumption of Genetically Engineered Foods

One comment asked whether there might be side effects from consumption of genetically engineered foods, and if so, whether these side effects would be short term or long term. Another comment noted that food plants and humans exhibit complex and unpredictable behavior and that therefore, the safety of a food substance should be based on thoughtfully gathered empirical evidence.

The comments did not point to any specific side effects of genetically engineered foods. FDA has evaluated the safety of APH(3′)II and has determined that it is safe for its proposed use. This safety assessment is in fact based on empirical evidence, such as the structure and function of APH(3′)II, the low level at which APH(3′)II occurs in foods, the digestibility of APH(3′)II, and the inability of APH(3′)II to interfere with clinically useful antibiotics under usual conditions of use for the antibiotics.

5. Relevance of Clinical Studies

Several comments noted that a National Institutes of Health (NIH) gene therapy trial in which cancer patients were infused with cells containing the *kan*^{r} gene, and which was cited by Calgene as strong evidence for the safety of the *kan*^{r} gene, provides little information concerning the safety of the *kan*^{r} gene and APH(3′)II in food. One comment also noted that the combination of data from the in vitro studies and the gene therapy study was an inadequate basis for a safety determination of the *kan*^{r} gene and APH(3′)II in food that millions of people might eat.

In determining that APH(3′)II is safe for its proposed food additive use, FDA did not rely on the NIH gene therapy trial. However, FDA does believe that the in vitro degradation data provide important information that should be and was considered by the agency as part of its overall safety assessment of the *kan*^{r} gene and APH(3′)II, as discussed earlier in this document.

C. Possible Effect on Clinical Efficacy of Orally Administered Kanamycin or Neomycin

Several comments questioned whether the presence of APH(3′)II in tomatoes or other foods might compromise the clinical efficacy of orally administered kanamycin or

neomycin. One comment noted that Calgene claimed that at most only 76,800 people annually were administered kanamycin or neomycin orally, and argued that those people deserved not to be put at risk. The comment further requested that Calgene be required to perform animal studies on the effects of ingestion of APH(3')II on the efficacy of orally administered kanamycin and neomycin. The comment asserted that if APH(3')II were shown to compromise clinical efficacy of kanamycin or neomycin, food containing APH(3')II should be appropriately labeled.

Other comments observed that ingested APH(3')II would not impair the efficacy of orally administered kanamycin and neomycin, that these antibiotics are rarely administered orally, and that the *kan*^{r} gene is therefore a good choice as a selectable marker gene.

FDA agrees with Calgene that kanamycin and neomycin are rarely administered orally. The primary clinical role for orally administered neomycin, and to a lesser extent kanamycin, is cleansing the bowel of microbes prior to bowel surgery. This use is relatively minor because of severe side effects (auditory nerve damage and kidney damage) that may result from the antibiotic that is absorbed from the GI tract (Ref. 38).

As discussed above, for most individuals receiving oral kanamycin or neomycin, APH(3')II will be inactivated by the acidic environment of the stomach and degraded by the digestive enzymes present in the GI tract. More important, even for patients receiving simultaneous treatment to reduce stomach acidity, the amount of ATP available from food would allow, at most, only a small fraction of kanamycin or neomycin to be inactivated. The comment advocating animal studies did not contradict directly or indirectly FDA's analysis concerning the inactivation and degradation of APH(3')II or the information concerning ATP levels. FDA has therefore determined that the presence of APH(3')II in food will not compromise therapy with orally administered kanamycin or neomycin. On this basis, FDA has concluded that neither animal studies on the effects of ingestion of APH(3')II on the efficacy of the antibiotics, nor special labeling of foods containing APH(3')II for patients receiving orally administered kanamycin or neomycin, are necessary.

D. Fate of the kan^r *Gene in the Environment*

1. Potential Transfer of the *kan*^{r} Gene From Crops to Microorganisms

One comment posited a connection between "the prophylactic use of antibiotics [resulting] in antibiotic-resistant bacteria reaching the human population" with a health risk from the possible addition of up to "10 antibiotic genes [sic] in most of the cells of major crops." The comment agreed with Calgene's documentation that the widespread use of antibiotics has led to an increase in antibiotic-resistant bacteria in the environment, but went on to postulate that this was evidence that introducing antibiotic-resistance genes into plants has human health implications.

The comment further asserted that the "scientific question is whether the resistance genes in the crops can be transferred by any mechanism [to] organisms that might be human pathogens," and that the company should be required experimentally to "determine the rates of gene transfer to soil bacteria from plant debris, the persistence or selection of organisms containing such genes in soil ecosystems, and other important factors in the assessment of the likelihood of releases compromising the use of antibiotics." The comment noted that Calgene analyzed these issues "in some detail," but with "arm chair calculations, most based on extrapolations from experiments done with other organisms under other circumstances."

A second comment noted that Calgene had supplied information that three kinds of bacteria, with and without plasmids [7] carrying antibiotic resistance genes, had little effect on several measures of soil ecosystems, but wrote that the "relevance of experiments on bacteria to releases of plants is marginal, at best." A third comment asserted, without any supporting evidence, that "genetic resistance to antibiotics in these plants could be transferred by plasmids to microorganisms in the soil and elsewhere in the food chain."

FDA agrees that increasing the number and prevalence of antibiotic-resistant microbes may have serious human health implications if those microbes are themselves pathogens of humans or domesticated animals, or share the same microenvironment as such pathogens. FDA considers the relevant scientific question to be whether there would be a meaningful increase in antibiotic-resistant pathogenic microbes in the human environment due to transfer of the *kan*^{r} gene from plants to microbes. This issue was also the subject of considerable discussion at the April 1994 Food Advisory Committee meeting. As discussed in detail above, FDA has determined, based on the body of evidence presented by Calgene and based on the discussions of the Food Advisory Committee (Ref. 1), that the transfer of the *kan*^{r} gene from plants to microbes will not occur at a detectable frequency and overall will result in no significant increase in the numbers of antibiotic-resistant microbes. Regarding whether Calgene should be required to determine experimentally the rate of transfer, the agency notes that Calgene's calculations represent worst-case scenarios, and the agency believes it would not be useful to do experiments to attempt to measure that which is too small to measure.

Regarding the relevance of experiments on bacterial releases to the environment, FDA finds that information concerning the lack of an environmental effect from the release of microbes with and without antibiotic resistance genes is of limited direct relevance to the environmental effects of plants with antibiotic resistance genes. The agency did not rely on this information in reaching its determination that there will be no significant increase in the antibiotic-resistant microorganism population of the soil.

Finally the claim that the *kan*^{r} gene could be transferred from plants to bacteria by plasmids is without basis because there is no evidence that plasmids exist in plants.

2. Potential Transfer of the *kan*^{r} Gene to Other Crops and to Wild Relatives

Comments were also received on the potential transfer of the *kan*^{r} gene to other crops and wild relatives. These comments address environmental issues and do not bear on the safety of APH(3')II for its proposed food additive use and are therefore addressed in section VII. of this document.

E. Possible Effects of Consumption of Animal Feeds Containing APH(3')II on Animals and Their Gut Microflora

One comment argued that empirical evidence should be gathered to assess the potential effects of modified foods on animals and their gut microflora.

The agency is aware of no information that APH(3')II would affect animals or their gut microflora any differently than any other protein in the diet, nor did the

[7] Plasmids are self-replicating units of DNA commonly found in bacteria and are responsible for transfer of antibiotic resistance between bacteria.

comment provide such information. The comment may have been referring to the theoretical potential for APH(3')II in animal feed to affect efficacy of neomycin administered to animals, and the theoretical potential for the gut microflora to take up the *kan*^{r} gene and become resistant to neomycin. As discussed above, the likelihood of transfer of the *kan*^{r} gene to gut microflora of food animals is extremely remote. Also, as discussed above, FDA has evaluated the study presented by Calgene addressing the possibility of inactivation of neomycin by APH(3')II in animal feed and has concluded that the therapeutic efficacy of neomycin in animals would not be affected by consumption of feed containing transgenic cottonseed and rapeseed modified through the use of the *kan*^{r} gene.

F. Labeling of Foods Containing the Kan^r *Gene and APH(3')II*

One comment asserted that APH(3')II should be labeled as an ingredient. The comment further stated that, if FDA exempted APH(3')II from ingredient labeling requirements (based on its classification as a processing aid that is present at insignificant levels in a finished food and has no technical or functional effect in that food), FDA should require special labeling if the ingestion of food containing APH(3')II could compromise the clinical efficacy of orally administered kanamycin or neomycin.

FDA's authority over food labeling is based on section 403 of the act (21 U.S.C. 343). Section 403(i) of the act requires that, in the case of foods fabricated from two or more ingredients, a food product bear on the label the common or usual name of each ingredient, unless compliance with the requirement for labeling is impracticable or results in deception or unfair competition. FDA considers an "ingredient" to be a substance used to fabricate (i.e., manufacture or produce) a food. FDA does not consider those substances that are inherent components of food to be ingredients that must be disclosed in the food's label.

A genetic substance introduced into a plant by breeding becomes an inherent part of the plant as well as of all foods derived from the plant. Consistent with FDA's general approach on ingredient labeling, the agency has not treated as an ingredient a new constituent of a plant introduced by breeding, regardless of the method used to develop the new plant variety. The comment provides no basis for FDA to deviate from its current practice in the case of APH(3')II.[8] Accordingly, FDA has determined that neither the *kan*^{r} gene nor APH(3')II is an ingredient that, under section 403(i) of the act, must be individually identified in labels of foods containing them.

FDA has also determined that the presence of APH(3')II is not a material fact that must be disclosed in the labeling of foods that contain the enzyme. Under section 403(a)(1) of the act (21 U.S.C. 343(a)(1)), a food is misbranded if its labeling is false or misleading. Under section 201(n) of the act (21 U.S.C. 321(n)), labeling is misleading if it fails to reveal all facts that are "* * * material with respect to consequences which may result from the use of the article * * *." As discussed at length above, FDA has determined that the ingestion of food containing APH(3')II will not compromise the clinical efficacy of orally administered kanamycin or neomycin. Because the consequences alleged in the comment—compromise of clinical efficacy—will not occur, the presence of APH(3')II is not a material fact requiring disclosure.

V. Conclusions

FDA has evaluated data in the petition and other relevant material and concludes that the proposed use of APH(3')II as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton is safe, and that 21 CFR parts 173 and 573 should be amended as set forth below.

VI. Inspection of Documents

In accordance with §§ 171.1(h) and 571.1(h) (21 CFR 171.1(h) and 571.1(h)), the petition and the documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person listed above. As provided in 21 CFR 171.1(h) and 571.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

VII. Environmental Impact

Calgene's initial submission requesting an advisory opinion regarding whether the *kan*^{r} gene may be used in the production of genetically engineered tomato, cotton, and oilseed rape plants included an environmental assessment (EA). The agency received comments on this EA. As noted earlier, the request for advisory opinion was later converted to a food additive petition at Calgene's request at which time Calgene submitted an updated EA. At the time the notice of filing was published in the **Federal Register**, FDA announced that the petitioner's EA was being made available to the public at the Dockets Management Branch (address above) and expressly solicited comments on the EA. No additional comments were received in response to this request for comments. The comments received on the original EA are discussed below.

One comment asserted that the *kan*^{r} gene could spread from tomato, cotton, and oilseed rape plants to other crops and related weeds by pollen flow when the *kan*^{r} gene-containing crops are grown near nontransgenic crops, and in locations where the *kan*^{r}-gene containing crops have wild relatives. The comment noted that transfer of the *kan*^{r} gene would create a problem if it were to make wild and weedy relatives more difficult to control.

The comment also criticized the Calgene submission for not addressing whether it is "wise to contribute foreign genes to the gene pools of wild plants even where the plants do not become weeds or manifest other obviously harmful traits" and stated that Calgene's submission "too easily dismissed the problem of outcrossing from the engineered oilseed rape." The comment noted that oilseed rape has wild and weedy relatives with which it can breed, and that "it is not sufficient to rely on traditional commercial control practices to control gene flow," but that the rate of gene flow must be experimentally determined and then "controlled by procedures that are demonstrated, not assumed, to work."

The agency has considered the potential for adverse environmental effects from the commercial use of cotton, tomato, and oilseed rape plants modified to contain the *kan*^{r} gene. The agency notes that it is possible for cotton and tomato plants to transfer the *kan*^{r} gene to neighboring plants of the same species via cross-pollination, although commercially grown cotton and tomatoes are primarily self-pollinating. Oilseed rape plants are also capable of pollinating sexually compatible wild relatives, although not all crosses with wild relatives prove fertile. Importantly, however, introduction of the *kan*^{r} gene will not

[8] Furthermore, APH(3')II satisfies the definition of "processing aid" in § 101.100(a)(3)(ii)(c) (21 CFR 101.100(a)(3)(ii)(c)) and will be regulated as such by this final rule. As the comment acknowledges, FDA's labeling regulations exempt processing aids like APH(3')II from the labeling requirements of section 403(i)(2) of the act. Thus, even if APH(3')II were properly considered an ingredient, its presence in a food would not be required to be disclosed in the food's labeling.

confer a competit've advantage upon a plant receiving it That is, the gene will not enhance the plant's capacity to compete with other plants for available resources. In particular, there will be no selective pressure on plants containing the *kan*^{r} gene because kanamycin will not be present in the environment in sufficient concentrations to create such pressure. First, there are no specific therapeutic uses of kanamycin that would result in its widespread application to agricultural crops. Also, kanamycin does not accumulate in the environment from production by soil microbes or by land application of animal wastes (Ref. 36). Accordingly, FDA has concluded that transfer of the *kan*^{r} gene to other crops or related weeds will have no significant adverse environmental effects.

With regard to the comment about outcrossing from engineered oilseed rape, the comment provided no information to show that the transfer of the *kan*^{r} gene to wild or weedy relatives of oilseed rape will be any more frequent or have any greater significance than the transfer of other genes from cultivated oilseed rape. FDA is aware of no human health or environmental concern associated with such transfer. Therefore, the agency does not agree that the cultivation of *kan*^{r}-containing oilseed rape should be subject to control practices any different from those used traditionally.

The agency has carefully considered the potential environmental effects of this action, including those described in the comments discussed in this document. FDA has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

VIII. Objections

Any person who will be adversely affected by this regulation may at any time on or before June 22, 1994, file with the Dockets Management Branch (address above) written objections thereto. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents shall be submitted and shall be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

IX. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Transcript of meeting of the Food Advisory Committee, FDA, Herndon, VA, April 6 through 8, 1994.

2. Hoskins, L.C., "Host and Microbial DNA in the Gut Lumen," The Journal of Infectious Diseases, 137:694–698, 1978.

3. Memorandum from T.A. Cebula, FDA, to N. Beru, FDA, November 2, 1993.

4. Potrykus, I., "Gene Transfer to Plants: Assessment of Published Approaches and Results," in "Annual Review of Plant Physiology and Plant Molecular Biology," Briggs, W.R., R.L. Jones, and V. Walbot, 42:205–225, 1991.

5. Fraley, R.T. et al., "Genetic Transformation in Higher Plants," Critical Reviews in Plant Sciences, 4:1–46, 1985.

6. Beck, E. et al., "Nucleotide Sequence and Exact Localization of the Neomycin Phosphotransferase Gene From Transposon Tn^5," *Gene*, 19:327–336, 1982.

7. Jorgensen, R.A. et al., "A Restriction Enzyme Cleavage Map of Tn^5 and Location of a Region Encoding Neomycin Resistance," Molecular and General Genetics, 177:65–72, 1979.

8. Davies, J. et al., "Plasmid-determined Resistance to Antimicrobial Agents," Annual Review of Microbiology, 32:469–518, 1978.

9. Goldman, P.R. et al., "Purification and Spectrophotometric Assay of Neomycin Phosphotransferase II," Biochemical and Biophysical Research Communications, 69:230–236, 1976.

10. U.S. Pharmacopeia (U.S.P.), The National Formulary (NF) 1990, U.S.P. XXII, NF XVII, U.S. Pharmacopeial Convention, Inc., Mack Printing Co., Easton, PA.

11. Prescott, J.F., and J.D. Baggot, "Aminoglycosides and Aminocyclitols," in Antimicrobial Therapy in Veterinary Medicine, Blackwell Scientific Publications, Boston, MA, pp. 121–152, 1988.

12. Davies, J.E., "Aminoglycoside-aminocyclitol Antibiotics and Their Modifying Enzymes," in "Antibiotics in Laboratory Medicine," 2d ed., Lorian, V., editor, pp. 790–809, 1986.

13. Dickie, P. et al., "Effect of Enzymatic Adenylation on Dihydrostreptomycin Accumulation in *Escherichia coli* Carrying the R-factor: Model Explaining Aminoglycoside Resistance by Inactivating Mechanisms," Antimicrobial Agents and Chemotherapy, 14:569–580, 1978.

14. Nap, J.P et al., "Biosafety of Kanamycin-resistant Transgenic Plants," Transgenic Research, 1:239–249, 1992.

15. Taylor, S.L. et al.,"Food Allergens: Structure and Immunologic Properties," Annals of Allergy, 59:93–99, 1987.

16. Darnel, J. et al., "Molecular Cell Biology," 2d ed., p. 116, Scientific American Books, Inc.

17. Pariza, M.W. et al., "Determining the Safety of Enzymes Used in Food Processing," Journal of Food Protection, 46:453–468, 1988.

18. Memorandum from Z. Olempska-Beer, FDA, to N. Beru, FDA, August 10, 1993.

19. Memorandum from Z. Olempska-Beer, FDA, to J. Maryanski, FDA, July 14, 1992.

20. Memorandum from C.B. Johnson, FDA, to V. Zenger, FDA, September 7, 1993.

21. Memorandum from C.B. Johnson, FDA, to J. Maryanski, FDA, July 14, 1992.

22. Fuchs, R.L. et al., "Safety Assessment of the Neomycin Phosphotransferase II (NPTII) Protein," Biotechnology, 11:1543–1547, 1993.

23. USDA Agricultural Handbook No. 8, Table I, Item 1401.

24. Memorandum from Z. Olempska-Beer, FDA, to N. Beru, FDA, August 9, 1993.

25. Orten, J.M. and O.W. Neuhaus, Human Biochemistry, 10th ed., pp. 537–538, C.V. Mosby Co., St. Louis, MO, 1982.

26. Memorandum from A.T. Sheldon, FDA, to J. Maryanski, FDA, March 30, 1993.

27. Memorandum from S.A. Giduck, FDA, to V. Zenger, FDA, July 21, 1992.

28. Memorandum from J.D. McCurdy, FDA, to V. Zenger, FDA, October 13, 1993.

29. McAllan, A.B. et al., "Degradation of Nucleic Acids in the Rumen," British Journal of Nutrition, 29:467–474, 1973.

30. Stewart, G.J. et al., "The Biology of Natural Transformation," Annual Review of Microbiology, 40:211–235, 1986.

31. Taylor, D.E., "Genetics of Campylobacter and Helicobacter," Annual Review of Microbiology, 46:35–64, 1992.

32. Stewart, G.J., "The Mechanism of Natural Transformation," in "Gene Transfer in the Environment," Levy, S. B. and R. V. Miller, editors, pp. 139–164, McGraw-Hill, New York, 1989.

33. Bergmans, H.E.N. et al., "Transformation in *Escherichia coli*: Stages in the Process," Journal of Bacteriology, 146:564–570, 1981.

34. Lapkin, M., "Proliferation and Differentiation of Normal and Diseased Gastrointestinal Cells," in "Physiology of the Gastrointestinal Tract," 2d ed., Johnson, L.R. et al., editors, Raven Press, New York, NY, 1987.

35. Levy, S.B. et al., "High Frequency of Antimicrobial Resistance in Human Fecal Flora," Antimicrobial Agents and Chemotherapy, 32: 1801–1806, 1988.

36. Memorandum from J. Glover-Glew, FDA, to N. Beru, FDA, December 15, 1993.

37. Sweeney, M.J. et al., "Immunology of Food Allergens," in "Handbook of Food Allergens," pp. 13–35, Breneman, J.C., editor, Marcel Dekker, Inc., New York, 1987.

38. Goodman, L.S. and Gilman, A., "The Pharmacological Basis of Therapeutics," 7th ed., pp. 1157–1160, MacMillan Publishing Co., New York, 1980.

List of Subjects

21 CFR Part 173

Food additives.

21 CFR Part 573

Animal feeds, Food additives.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 173 and 573 are amended as follows:

PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 173 continues to read as follows:

Authority: Secs. 201, 402, 409 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 342, 348).

2. New § 173.170 is added to subpart B to read as follows:

§ 173.170 Aminoglycoside 3'-phosphotransferase II.

The food additive aminoglycoside 3'-phosphotransferase II may be safely used in the development of genetically modified cotton, oilseed rape, and tomatoes in accordance with the following prescribed conditions:

(a) The food additive is the enzyme aminoglycoside 3'-phosphotransferase II (CAS Reg. No. 58943–39–8) which catalyzes the phosphorylation of certain aminoglycoside antibiotics, including kanamycin, neomycin, and gentamicin.

(b) Aminoglycoside 3'-phosphotransferase II is encoded by the *kan*^{r} gene originally isolated from transposon Tn^{5} of the bacterium *Escherichia coli.*

(c) The level of the additive does not exceed the amount reasonably required for selection of plant cells carrying the *kan*^{r} gene along with the genetic material of interest.

PART 573—FOOD ADDITIVES PERMITTED IN FEED AND DRINKING WATER OF ANIMALS

3. The authority citation for 21 CFR part 573 continues to read as follows:

Authority: Secs. 201, 402, 409 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 342, 348).

4. New § 573.130 is added to subpart B to read as follows:

§ 573.130 Aminoglycoside 3'-phosphotransferase II.

The food additive aminoglycoside 3'-phosphotransferase II may be safely used in the development of genetically modified cotton, oilseed rape, and tomatoes in accordance with the following prescribed conditions:

(a) The food additive is the enzyme aminoglycoside 3'-phosphotransferase II (CAS Reg. No. 58943–39–8) which catalyzes the phosphorylation of certain aminoglycoside antibiotics, including kanamycin, neomycin, and gentamicin.

(b) Aminoglycoside 3'-phosphotransferase II is encoded by the *kan*^{r} gene originally isolated from transposon Tn5 of the bacterium *Escherichia coli.*

(c) The level of the additive does not exceed the amount reasonably required for selection of plant cells carrying the *kan*^{r} gene along with the genetic material of interest.

Dated: May 17, 1994.

Fred R. Shank,

Director, Center for Food Safety and Applied Nutrition.

Linda A. Suydam,

Interim Deputy Commissioner for Operations.

David A. Kessler,

Commissioner of Food and Drugs.

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