

## FINDING OF NO SIGNIFICANT IMPACT

### FOR

Food Additive Petition 3A4364, submitted by Calgene, Inc., to amend the food additive regulations to provide for the safe use of aminoglycoside 3'-phosphotransferase II (APH(3')II), encoded by the kanamycin resistance gene (*kan<sup>r</sup>*), as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton plants.

The Environmental Impact Staff, Center for Food Safety and Applied Nutrition, has determined that the approval of this petition will not significantly affect the quality of the human environment and therefore will not require the preparation of an environmental impact statement. This finding is based on information submitted by the petitioner in an environmental assessment prepared using the format described in 21 *CFR* 25.31a(a) and on the following analysis:

- I. Adverse environmental effects are not expected to result from APH(3')II being constitutively expressed in cotton, tomato and oilseed rape containing *kan<sup>r</sup>*. The reasons for this conclusion are as follows:
  - A. APH(3')II in terrestrial and aquatic environments will be subject to rapid physical and chemical degradation. In addition, the enzyme is very substrate specific and requires free ATP for activity (see Section G.2 of the EA).
  - B. Specific ecotoxicity testing was not performed by Calgene. However, the Division of Health Effects Evaluation (DHEE) evaluated oral toxicity studies conducted in rats submitted by Calgene and found no acute toxic effects from ingestion of tomato homogenate containing the protein. DHEE additionally concluded from *in vitro* digestion studies that APH(3')II is destroyed during digestion. The protein does not appear to have sequence homology with known toxins or allergens. Based on this information, we do not expect that native fauna would be adversely affected if they were to consume cotton, tomato or oilseed rape plants containing APH(3')II.
  - C. The commercial use of cotton, tomato, and oilseed rape plants containing APH(3')II will not have an effect on agricultural or food-processing practices, or on the disposal of plant debris or food-processing byproducts containing the protein (see Sections F and I of the EA).
- II. Adverse environmental effects are not expected to result from *kan<sup>r</sup>* being used as a marker in genetic transformations of tomato, cotton, and oilseed rape plants. The reasons for this conclusion are as follows:
  - A. No adverse environmental effects have been associated with the development and production of cotton, tomato, or oilseed rape plants containing *kan<sup>r</sup>*.
    1. Transforming vectors will not be released to the environment. Calgene has provided information on physical containment procedures and disposal

practices established to prevent the escape of plant vectors or source organisms containing *kan<sup>r</sup>* (see Sections F.2 and G.1.3 of the EA). These standards are mandated by the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) for laboratory, greenhouse, and field containment and apply to any other companies developing genetically engineered varieties of cotton, tomato and oilseed rape.

2. The introduced constructs containing *kan<sup>r</sup>* have been well characterized by Calgene. Testing and genetic analysis have been performed to confirm the presence of the gene and the level of its expression product (see Section E of the EA). *Kan<sup>r</sup>*, once incorporated in the plant genome, has been shown to transfer in Mendelian fashion (see Section G of the EA). Any undesirable phenotypic traits resulting from the genetic modification were culled out during development and field testing for commercial production (see Sections F.1 and G.1.1 of the EA). FDA is not aware of information indicating that other companies developing *kan<sup>r</sup>*-containing cotton, tomato and oilseed rape crops will not maintain similar product quality standards.
  3. Calgene has stated that it is in compliance with all applicable requirements covering chemical emissions at the site(s) of development and production of crop plants expressing APH(3')II (see Section F of the EA).
- B. Adverse environmental effects are not expected to result from the commercial use of cotton, tomato, or oilseed rape plants modified to contain *kan<sup>r</sup>* as a selectable marker. The reasons for this conclusion are as follows:
1. Calgene has documented that insertion of *kan<sup>r</sup>* into cotton, tomatoes, and oilseed rape does not lead to increased weediness, differences in host/pest interactions, or changes in the occurrence of plant diseases (see Section G of the EA).
  2. The introduced *kan<sup>r</sup>* gene is not expected to have adverse effects on individual species populations or the structure and function of ecological communities because the gene will not confer a competitive advantage upon plants containing it (see Section G of the EA).
  3. It is unlikely that *kan<sup>r</sup>* could move from the plant genome into microorganisms via horizontal gene transfer since no such mechanism of transfer has been demonstrated. In order for *kan<sup>r</sup>* to be taken up, integrated, and expressed in a recipient microorganism, the gene would have to remain intact (not be sheared, degraded, or sequestered) in soil; the microorganism would have to be capable of transformation; the recipient organism would require sequence homology with the transforming construct; and *kan<sup>r</sup>* would have to be inserted in the genome after a bacterial promoter (the construct is currently under the control of a plant promoter). Even if transfer could occur, calculations of a worst-case scenario performed by Calgene demonstrate that exposure to *kan<sup>r</sup>* from agricultural residues would not result in a significant increase in kanamycin resistant soil microorganisms over the existing background population of kanamycin resistant organisms in the soil (see Section G.1.2 and Appendix EA-9 of the EA).

- C. No adverse environmental effects are expected to result from the transfer of *kan<sup>r</sup>* to other plants as a consequence of commercial plantings of cotton, tomato, and oilseed rape plants containing the selectable marker. The reasons for this conclusion are as follows:**
- 1. It is possible for cotton, tomato and oilseed rape plants to transfer *kan<sup>r</sup>* 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. Outcrossing is not expected to have an adverse effect on agriculture because the presence of *kan<sup>r</sup>* in cotton and tomatoes, and oilseed rape does not lead to increased weediness, differences in host/pest interactions, or changes in the occurrence of plant diseases (see Section G.1.1 of the EA).**
  - 2. Introduction of this trait into other plants by hybridization is not expected to have adverse effects on individual species populations or disrupt the structure and function of ecological communities because it will not confer a competitive advantage upon plants containing it (see Section G.1.1 of the EA).**
- D. No changes in agricultural or food-processing practices will result from the commercial production of crops containing *kan<sup>r</sup>*.**
- 1. The commercial use of cotton, tomatoes, and oilseed rape containing *kan<sup>r</sup>* will not have an effect on crop use patterns or agricultural practices. Cotton, tomato, and oilseed rape plants containing *kan<sup>r</sup>* will simply substitute for crops not containing *kan<sup>r</sup>*. There will be no changes in agronomic practices that could lead to changes in water quality, tillage practices, or agrochemical, energy, or other natural resource use (see Section I of the petition).**
  - 2. There will be no changes in the processing of plant products or in the potential uses for food-processing byproducts (see Section I of the petition).**
  - 3. No potential health effects have been identified from environmental exposure of agricultural or processing plant workers to *kan<sup>r</sup>* in plant debris or food-processing products (see Section G of the EA).**
  - 4. There will be no changes in waste disposal of plant debris or food-processing byproducts containing *kan<sup>r</sup>* (see Sections F and I of the EA).**

As part of the submission in support of the environmental safety of the *kan<sup>r</sup>* gene and gene product, Calgene has provided information that is specific to their proprietary FLAVR SAVR tomato, bromoxynil resistant cotton, and high stearate canola varieties (all of which contain *kan<sup>r</sup>* as a marker gene). This Finding of No Significant Impact applies only to the environmental safety of the *kan<sup>r</sup>* gene and its gene product. We did not evaluate the information provided on additional characteristics engineered into Calgene's cotton, tomato, and oilseed rape varieties; therefore, this finding does not imply an acceptance of conclusions drawn by Calgene on the safety of these other characteristics.

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**Date: December 16, 1993**

Approved by: \_\_\_\_\_/s/\_\_\_\_\_  
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**Date: December 16, 1993**