

**List of Subjects**

Environmental protection, Agricultural commodities, Feed additives, Food additives, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: November 17, 2003.

**Janet L. Andersen,**

*Director, Biopesticides and Pollution Prevention Division, Office of Pesticide Programs.*

**Summary of Petition**

The petitioner's summary of the pesticide petition is printed below as required by FFDCA section 408(d)(3). The summary of the petition was prepared by the petitioner and represents the views of the petitioner. The petition summary announces the availability of a description of the analytical methods available to EPA for the detection and measurement of the pesticide chemical residues or an explanation of why no such method is needed.

**Syngenta Seeds, Incorporated**

*PP 3F6761*

EPA has received a pesticide petition (3F6761) from Syngenta Seeds, Incorporated, P.O. Box 12257, 3054 Cornwallis Road, Research Triangle Park, NC 27709-2257, proposing pursuant to section 408(d) of the FFDCA, 21 U.S.C. 346a(d), to amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for the plant-pesticide inert ingredient hygromycin B phosphotransferase (APH4) marker protein and the genetic material necessary for its production, proposed for use as a plant-incorporated protectant formulation. APH4 protein is an aminocyclitol phosphotransferase that catalyzes the phosphorylation of hygromycin and closely related aminoglycoside antibiotics. Expression of the APH4 gene in plant cells allows for growth and selection of transformed cells in the presence of hygromycin B. APH4 has no insecticidal activity.

Pursuant to section 408(d)(2)(A)(i) of the FFDCA, as amended, Syngenta Seeds, Incorporated has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by Syngenta Seeds, Incorporated and EPA has not fully evaluated the merits of the pesticide petition. The summary may have been edited by EPA if the terminology used was unclear, the summary contained extraneous material, or the summary unintentionally made the reader conclude that the findings reflected

EPA's position and not the position of the petitioner.

**A. Product Name and Proposed Use Practices**

Hygromycin B phosphotransferase (APH4) marker protein is proposed for use as a plant-incorporated protectant formulation inert ingredient. APH4 protein is an aminocyclitol phosphotransferase that catalyzes the phosphorylation of hygromycin and closely related aminoglycoside antibiotics. Expression of the APH4 gene in plant cells allows for growth and selection of transformed cells in the presence of hygromycin B. APH4 has no insecticidal activity.

**B. Product Identity/Chemistry**

1. *Identity of the pesticide and corresponding residues.* The APH4 gene in event COT102 cotton plants was derived from a plasmid harbored by a hygromycin-resistant isolate of *E. coli*, and encodes a 341 amino-acid enzyme, hygromycin B phosphotransferase APH4. Hygromycin B phosphotransferases with significant homology to the APH4 protein in event COT102 plants have also been identified in other microbes including *Streptomyces hygroscopicus*, the source of hygromycin B.

APH4 has a molecular weight of *ca.* 42,000 and catalyzes the phosphorylation of the 4-hydroxyl group on the hyosamine moiety of hygromycin B, thereby inactivating it. The enzyme has a narrow range of substrates, in that it phosphorylates hygromycin B, hygromycin B<sub>2</sub> and the closely-related antibiotics destomycin A and destomycin B, but does not phosphorylate other aminocyclitol or aminoglycoside antibiotics including neomycin, streptomycin, gentamycin, kanamycin, spectinomycin, tobramycin, and amikacin. Hygromycin B is not used in human clinical therapy, but is principally used as an antihelminthic agent in swine and poultry feeds.

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* A determination of the magnitude of residue at harvest is not required for residues exempt from tolerances. However, the petitioner has provided data on the quantity of APH4 protein measured in various plant parts including seeds of VIP3A cotton, as measured by enzyme linked immunosorbent assay (ELISA). APH4 was either not detectable in most COT102 plant tissues or the levels were too low to quantify. Pollen was the only tissue in which quantifiable levels were measured.

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* An analytical method is not required because this petition requests an exemption from tolerances. However, the petitioner has submitted an analytical method for detection of the APH4 protein by ELISA analysis.

**C. Mammalian Toxicological Profile**

Syngenta Seeds is providing the results of a mammalian toxicology study, *in vitro* digestibility study, and bioinformatics evaluations conducted on the selectable marker protein APH4. These studies, summarized herein, demonstrate the lack of toxicity of the APH4 protein following acute oral exposure to mice, rapid degradation of APH4 upon exposure to simulated gastric intestinal fluids, and the lack of amino acid sequence similarity of the APH4 protein to proteins known to be mammalian toxins or human allergens.

When proteins are toxic, they are known to act *via* acute mechanisms and at very low doses (Sjogblad, R.D., J.T. McClintock and R. Engler (1992) "Toxicological Considerations for Protein Components of Biological Pesticide Products." *Regulatory Toxicol. Pharmacol.* 15: 3-9). Therefore, when a protein demonstrates no acute oral toxicity in high-dose testing using a standard laboratory mammalian test species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

Because it is not possible to extract sufficient APH4 protein from transformed plants for toxicology studies, APH4 protein was produced in recombinant *E. coli* by over-expressing the same APH4 gene that was introduced into VIP3A cotton event COT102. The APH4 gene was cloned into the inducible, over-expression pET-3a® vector (Novagen, Madison, WI) in *E. coli* BL21DE3pLysS. The APH4 protein, as encoded in this vector, was identical in amino acid sequence to that encoded by the plant transformation vector, pCOT1, except for an additional 11 amino acids from the T7 Tag™ and 3 amino acids from the vector polylinker. Following purification from *E. coli*, dialysis and lyophilization, the resulting sample, designated Test Substance APH4-0102, was estimated by ELISA to contain *ca.* 42.6% APH4 protein by weight. The test material was confirmed to be enzymatically active.

An acute mouse oral toxicity study was conducted at the Syngenta Central Toxicology Laboratory (Alderley Park,