

necessary for its production in or on cotton.

Pursuant to section 408(d)(2)(A)(i) of the FFDCA, as amended, Syngenta Seeds, Inc. has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by Syngenta Seeds, Inc. 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 B₂ and the closely related antibiotics destomycin A and destomycin B, but does not phosphorylate other aminocyclitol or aminoglycoside antibiotics including neomycin, streptomycin, gentamicin, 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 and 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 (Ref. 1). 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 three 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, Macclesfield, Cheshire, UK) according to OPPTS Harmonized Guideline 870.1100. Test substance APH4-0102 (see above description of test substance) was administered to five male and five female mice strain Alderley Park albino mouse (APfCD-1); 8–9 weeks old via a gavage dose of 1,828 milligrams/kilogram (mg/kg) body weight. The test substance contained ca. 42.6% APH4 protein by weight. Therefore, the mice received ca. 779 mg APH4/kg body weight. A negative control group (5 mice/sex) concurrently received the dosing vehicle alone, a suspension of 1% methylcellulose, at the same dosing volume as used for the test material mixture. Food was provided *ad libitum*, except during the ca. 1-hour prior to dosing, when the animals were fasted. Water was provided *ad libitum* throughout the study. Observations for mortality and clinical/behavioral signs of toxicity were made at least twice on the day of dosing, and at least once daily thereafter for 14 days. Detailed clinical observations were made for each animal at each observation time. Body weights were recorded daily and food consumption was recorded weekly. Surviving animals were euthanized 14 days post dosing and subjected to gross necropsy. Organ weights (brain, liver with gall bladder, kidneys and spleen) were recorded and principal tissues were processed for microscopic examination.

No mortalities occurred during the study, and no clinical signs of toxicity were observed in either the test or control groups. There were no treatment-related effects on body weight, food consumption, or organ weights, nor were any treatment-related effects observed following macroscopic or microscopic examination. APH4-0102 is not acutely toxic to mice. There is no evidence of toxicity of the test substance at 1,828 mg APH4-0102/kg body weight, representing ca. 779 mg APH4 protein/kg body weight. The estimated LD₅₀ value for pure APH4 protein in male and female mice is >779 mg/kg body weight, the single dose tested.

The APH4 protein shows no homology to proteins known to be