

Macclesfield, Cheshire, UK) according to EPA Test Guideline OPPTS 870.1100. The test substance APH4-0102 (see above description of test substance) was administered to 5 male and 5 female mice (strain Alderely 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 lethal dose (LD)₅₀ 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 mammalian toxins or human allergens; is not derived from a source known to produce allergens; is not targeted to a cellular pathway for glycosylation in the plant; and is rapidly degraded upon exposure to simulated gastric and intestinal fluids.

The genetic material necessary for the production of APH4 as an inert ingredient are the nucleic acids, deoxyribonucleic acid (DNA) which comprise genetic material encoding this

protein and its regulatory regions. “Regulatory regions” are the genetic material that control the expression of the genetic material encoding the protein, such as promoters, terminators, and enhancers. DNA is common to all forms of plant and animal life and the Agency has previously stated that they are not aware of an instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food. These ubiquitous nucleic acids, as they appear in the subject inert ingredient, have been adequately characterized. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the subject inert plant pesticidal ingredient.

D. Aggregate Exposure

1. *Dietary exposure—i. Food.* Derivatives of cottonseed (*e.g.*, refined cottonseed oil) and fiber (*e.g.*, linters, which are essentially 100% cellulose) are used in some food products. However, APH4 was not detected in most of the samples of COT102-derived cottonseed analyzed or any of the cotton fiber samples analyzed. In the few cottonseed samples in which APH4 was detectable, the quantities were below the limit of quantification (LOQ) (<137 nanogram (ng) APH4/grams (g) fresh weight (wt); <150 ng APH4/g dry wt.). It is expected that any trace quantities of APH4 in cottonseed will be eliminated by standard seed processing methods. As demonstrated by the analysis of cottonseed products for VIP3A protein, no VIP3A was detected in refined cottonseed oil from COT102-derived plants, despite the presence of *ca.* 3 micrograms VIP3A/g seed (fresh or dry wt.). Additionally, no protein of any kind was detected in the same sample of refined cottonseed oil. It can be concluded that APH4, as produced in COT102-derived cotton plants, does not pose a risk of becoming allergenic *via* food, because there will be no exposure *via* food. Additionally, the APH4 protein shows no amino acid sequence homology to known allergens; is not derived from a source known to produce allergens; is not targeted to a cellular pathway for glycosylation in the plant; and is rapidly degraded upon exposure to simulated gastric and intestinal fluids.

ii. *Drinking water.* No exposure to APH4 and the genetic material necessary for its production as an inert ingredient *via* drinking water are expected. The protein is incorporated into the plant and will therefore not be available to drinking water sources.

2. *Non-dietary exposure.* Non-dietary exposure is not anticipated, due to the proposed use pattern of the product. Exposure *via* dermal or inhalation routes is unlikely because the inert ingredient is contained within plant cells. However, if exposure were to occur by non-dietary routes, no risk would be expected because the APH4 protein is not toxic to mammals.

E. Cumulative Exposure

Because there is no indication of mammalian toxicity to the APH4 protein, it is reasonable to conclude that there are no cumulative effects for this inert ingredient.

F. Safety Determination

1. *U.S. population.* The lack of mammalian toxicity at high levels of exposure to the APH4 protein demonstrates the safety of the product at levels well above possible maximum exposure levels anticipated *via* consumption of processed food products produced from VIP3A cotton. Moreover, little to no human dietary exposure to APH4 protein is expected to occur *via* VIP3A cotton. Due to the lack of toxicity of the APH4 protein and its very low potential for allergenicity, dietary exposure is not anticipated to pose any harm for the U.S. population. No special safety provisions are applicable for consumption patterns or for any population sub-groups.

2. *Infants and children.* Syngenta has evaluated the acute toxicity data generated on APH4, the lack of homology to known allergens or toxins, and the limited exposure to this protein based on the residue profile and limited number of food/feed products resulting from cotton and has determined that there is ample evidence to indicate a reasonable certainty of no harm to infants and children as a result of the use of this product.

G. Effects on the Immune and Endocrine Systems

The inert ingredient APH4 is a protein, derived from sources that are not known to exert an influence on the endocrine or immune systems.

H. Existing Tolerances

The registrant is not aware of any known existing tolerances or exemptions for APH4 and the genetic material necessary for its production as an inert ingredient.

I. International Tolerances

The registrant is not aware that any codex maximum residue levels exist for