

necessary for its production in all plants in or on all food commodities.

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

Phosphomannose isomerase (PMI) and the genetic material necessary for its production is proposed for use as an inert ingredient in plants producing a plant-incorporated protectant active ingredient. Production of PMI in plant cells allows for selection and growth of genetically transformed plant cells in the presence of mannose as the sole or primary carbon source. PMI has no pesticidal activity. Its use allows the identification of plant cells that have successfully acquired the genetic material necessary to produce a plant-incorporated protectant.

B. Product Identity/Chemistry

1. *Identity of the pesticide and corresponding residues.* PMI is a ubiquitous enzyme that catalyzes the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. No other natural substrates for PMI are known. The *pmi* gene (also known as the *manA* gene) that encodes the PMI enzyme in transformed plants was derived from *E. coli* strain K-12. The gene encodes a 391-amino acid protein with an apparent molecular weight of *ca.* 45,000. Functionally equivalent PMI enzymes with significant amino acid homology to this PMI protein have been identified among many diverse organisms including other bacteria, plants, fungi, insects, nematodes, mammals, and including humans. Unlike the traditional selectable markers used in plant cell transformation, PMI does not confer resistance to an antibiotic or herbicide.

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 PMI

protein measured in various plant parts representing an initial line of transformed corn plants. PMI was detected in grain from these corn plants at *ca.* 1–2 parts per million (ppm) on a dry- or fresh-weight basis, as measured by enzyme-linked immunosorbent assay (ELISA). Average PMI levels measured in chopped whole transformed corn plants were less than or equal to *ca.* 5 ppm on a dry-weight basis and less than or equal to *ca.* 1 ppm on a fresh-weight basis. In silage prepared from the same line of transformed corn plants, no PMI was detectable after 29 days.

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 PMI protein by ELISA.

C. Mammalian Toxicological Profile

Syngenta Seeds, Inc. is providing the results of a mammalian toxicology study, *in vitro* digestibility study, heat stability study and bioinformatics evaluations conducted on the selectable marker protein PMI. These studies, summarized herein, demonstrate the lack of toxicity of the PMI protein following acute oral exposure to mice, rapid degradation of PMI upon exposure to simulated gastric and intestinal fluids, instability of the PMI protein upon heating, and the lack of significant amino acid sequence homology of the PMI 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 (Sjoblad, 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 feasible to extract sufficient PMI protein from transformed plants for toxicology studies, PMI protein was produced in recombinant *E. coli* by over-expressing the same *pmi* gene that was introduced into transformed corn plants. The PMI protein encoded in this *E. coli* system was identical in amino acid sequence to that encoded in the transformed plants,

except for additional N-terminal amino acids representing 13 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 PMI-0198, was estimated by ELISA to contain *ca.* 61% PMI protein by weight. PMI as contained in this test substance was enzymatically active, had the predicted apparent molecular weight, and immunoreacted with anti-PMI antibody. Side-by-side comparisons of PMI in test substance PMI-0198 with PMI extracted from transformed corn plants indicated that the proteins are substantially equivalent, as measured by enzymatic activity, apparent molecular weight, and immuno-crossreactivity with anti-PMI antibody. This justified the use of test substance PMI-0198 in safety studies as a surrogate for PMI as produced in transformed plants.

An acute mouse oral toxicity study was conducted according to EPA Harmonized Test Guideline OPPTS 870.1100. Test substance PMI-0198 was administered to seven male and six female mice *via* a gavage dose of 5,050 milligrams/kilogram body weight (mg/kg bwt), representing *ca.* 3,080 mg of pure PMI protein/kg bwt. A negative control group (six males and five females) concurrently received the dosing vehicle alone, a suspension of 0.5% carboxymethylcellulose, at the same dosing volume used for the test substance mixture. No test substance-related mortalities or clinical signs of toxicity occurred during the study. One male in the control group and two males in the test group died as a result of a perforated esophagus due to dosing error. Gross necropsy of the remaining mice at study termination revealed no observable abnormalities. Body weight, body weight gain, and organ weights (brain, liver, kidneys, and spleen) were comparable in the control and test groups. There was no evidence of toxicity. Accordingly, the lethal dose (LD)₅₀ value for PMI-0198 in male and female mice is greater than 5,050 mg/kg bwt, and the LD₅₀ value for pure PMI protein is greater than 3,080 mg/kg bwt, the single dose tested.

Extensive bioinformatics searches of public protein data bases revealed that the PMI protein shows no significant amino acid homology to proteins known to be mammalian toxins or known or suspected to be human allergens. Additional information and testing indicate that the PMI protein does not have properties that would suggest it has the potential to become a food allergen. The source of PMI (*E. coli*) is not known to produce allergens. Unlike