

Executive Summary

Foods derived from insect-protected corn containing the *eCry3.1Ab* & *pmi* genes – 5307 corn.

Section A – General information on the application

1. Syngenta has developed a corn line 5307 which produces the eCry3.1Ab and PMI proteins. The gene *ecry3.1Ab* encodes the eCry3.1Ab protein, a chimeric protein engineered from portions of modified Cry3A (mCry3A) and Cry1Ab proteins which are derived from *Bacillus thuringiensis*. The eCry3.1Ab protein is insecticidally active against the larvae of Western corn rootworm (*Diabrotica virgifera virgifera* Le Conte), Northern corn rootworm (*D. Longicornis barberi* Smith and Lawrence) and Mexican corn rootworm (*D. Vigifera zea* Krysan and Smith). These important coleopteran pests cause significant damage to U.S. corn crops annually. The *pmi* gene, also known as *manA*, is derived from *Escherichia coli* strain K-12 and encodes the PMI protein, which was utilised as a selectable marker during the development of 5307 corn. GM corn 5307 in breeding stack combinations with other insect-resistant corn products will allow growers to have optimal broad-spectrum insect control.
2. 5307 corn is not intended for commercialisation in Australia or New Zealand, therefore, it is not expected that there will be any undue prejudice to trade with other countries as a result of approval of 5307 corn in Australia or New Zealand.
3. Authorisation of 5307 corn for food and feed uses, import and processing will be sort in all major importing corn/corn countries.

Section B – Technical information on the genetically modified food

4. Corn (*Zea mays* L.) is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 cm long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and long styles (silks) protrude from the tip of the ear as a mass of silky threads. Pollen is produced entirely in the staminate inflorescence and eggs, entirely in the pistillate inflorescence. Corn is wind pollinated and both self and cross-pollination are usually possible. Cultivated corn is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Corn is cultivated worldwide and represents a staple food for a significant proportion of the world's population.
5. 5307 corn was created via *Agrobacterium*-mediated transformation.

6. Plasmid pSYN12274, which contained the *pmi* gene from *E. coli* and the coding sequence for *ecry3.1Ab* between the right and left borders of the T-DNA, was placed into *A. tumefaciens* strain LBA4404.
7. Southern blot analyses demonstrate that 5307 corn (1) contains a single copy each of the gene *ecry3.1Ab*, its CMP promoter sequence, the marker gene *pmi* (also known as *manA*), its ZmUbi1nt promoter sequence, and the two expected copies of the NOS polyadenylation sequence, one NOS polyadenylation sequence regulating *ecry3.1Ab* and one NOS polyadenylation sequence regulating *pmi*; (2) does not contain any extraneous deoxyribonucleic acid (DNA) fragments of the functional elements inserted elsewhere in the corn genome; (3) and does not contain backbone sequence from the transformation plasmid, pSYN12274.
8. The transgenic locus is stably inherited over several generations during conventional breeding.
9. eCry3.1Ab and PMI proteins were present at quantifiable levels in flour and germ when produced by standard dry-mill processing of 5307 corn grain. In contrast, the eCry3.1Ab and PMI proteins were below the limit of detection in the dried germ, starch, and gluten when produced by standard wet-mill processing of 5307 corn grain.
10. An event specific polymerase chain reaction (PCR) method is available to detect 5307 deoxyribonucleic acid (DNA) extracted from grain and seed samples.

Section C – Information related to the safety of the genetically-modified food

11. Intact eCry3.1Ab produced in recombinant *E. coli* is readily degraded under simulated mammalian intestinal conditions. The eCry3.1Ab protein is degraded so rapidly that no intact eCry3.1Ab can be detected upon sampling of the reaction mixture at 1 minute. However, immunoreactive fragments of eCry3.1Ab protein (molecular weights ca. 56, 40 and 5 kDa) are detected over a 48-hour time course. Based on this, it is evident that the eCry3.1Ab protein is sensitive to proteolysis by pancreatin and is rapidly degraded to constituent peptides.
12. PMI is readily degraded under simulated mammalian intestinal conditions.
13. eCry3.1Ab is inactivated, and denatured upon heating at temperatures of 95°C and above.
14. PMI is inactivated, and denatured upon heating at temperatures of 65°C and greater.
14. Mammalian safety assessments were conducted for both eCry3.1Ab and PMI proteins as expressed in 5307 corn. No adverse effects were observed for either protein. The estimated LD₅₀ value for pure eCry3.1Ab protein in male and female

mice is >2000 mg/kg body weight, the single dose tested. For the pmi protein, the estimated LD₅₀ value is > 2000 mg/kg body weight, the single dose tested.

15. Amino acid sequence comparisons between novel proteins and known protein toxins and allergens are part of the weight-of-evidence approach to assessing potential mammalian toxicity and allergenicity. eCry3.1Ab shows no biologically relevant amino acid sequence similarity to any known or putative protein allergens.

16. PMI amino acid sequence shows no significant similarity with any known or putative toxins.

17. The eCry3.1Ab amino acid sequence shows no significant similarity with any known or putative toxins other than known or putative delta-endotoxin proteins (also known as Cry proteins or insecticidal crystal proteins).

18. Grain and forage from 5307 corn were substantially equivalent in composition to both the non-transgenic control hybrid and to other commercial corn with an established history of safe use.

Section D – Information related to the nutritional impact of the genetically-modified food

19. There were no adverse dietary effects on broiler chickens consuming diets prepared with 5307 corn grain when compared with those consuming diets prepared with non-transgenic corn grain.