PART 1 GENERAL INFORMATION

EXECUTIVE SUMMARY

Pioneer Hi-Bred International, Inc. (Pioneer) is submitting an application to amend the *Australia New Zealand Food Standards Code* to allow for the inclusion of insect-resistant and herbicide-tolerant maize event DP-ØØ4114-3, hereafter referred to as 4114 maize, in Standard 1.5.2 – Food Produced Using Gene Technology.

Pioneer has developed maize line 4114 as a "molecular stack" containing the same exact traits as the "breeding stack" of the combined traits from maize lines 1507 (DAS-Ø15Ø7-1) and 59122 (DAS-59122-7). Maize line 4114 expresses the same insect-resistance and herbicide-tolerance traits as present in currently commercialized hybrids containing 1507 × 59122, which are marketed under the trade name Herculex®[1] XTRA. Just like Herculex® XTRA hybrids, 4114 maize expresses the Cry1F protein, providing protection against certain lepidopteran insect pests (e.g., European corn borer), the Cry34Ab1 and Cry35Ab1 proteins, which together form an active binary insecticidal protein providing protection against corn rootworm, and the PAT enzyme, which provides tolerance to glufosinate herbicides.

Unlike the 1507 × 59122 breeding stack, where the DNA insertions carrying traits derived from events 1507 and 59122, respectively, are located at two unlinked genetic loci, the genes encoding the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins in 4114 maize are present at a single locus. This allows event 4114 to be bred more efficiently into new product offerings and to serve as a more efficient platform for the production of larger stack combinations through conventional breeding. Efficient breeding of multiple traits is becoming more important as growers demand more complex products, including multiple modes of action for insect control and herbicide-tolerance.

Maize line 4114 was produced via *Agrobacterium*-mediated transformation of PHWWE maize embryos with plasmid PHP27118, resulting in the incorporation of the same *cry*1F gene cassette as present in authorized maize line 1507, the same *cry*34Ab1 and *cry*35Ab1 gene cassettes as present in authorized maize line 59122, and the same *pat* gene cassette as present in both 1507 and 59122 maize. Detailed Southern blot analysis of 4114 maize genomic DNA confirmed that the introduced DNA (T-DNA) was incorporated intact into a single site within the maize genome, without truncation or deletion of nucleotide sequences within any of the gene expression cassettes. In addition, the organization and integrity of the inserted DNA, including the potential to create new novel open reading frames, was evaluated based on nucleotide sequencing of the entire inserted DNA and flanking host genomic sequences in maize 4114. Based on a combination of genotypic and phenotypic testing, it was determined that the introduced *cry*1F, *cry*34Ab1, *cry*35Ab1, and *pat* genes were stably inherited across multiple generations, and segregated as a single genetic locus in 4114 maize according to Mendelian rules of inheritance.

[1] Herculex is a registered trademark of Dow AgroSciences LLC.

Based on the method of genetic modification and nucleotide sequencing of the introduced DNA, the deduced amino acid sequence of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins present in 4114 maize are identical to the corresponding proteins in events 1507 and 59122, and by extension, the conventional breeding stack of 1507 × 59122 maize. Protein equivalence was further demonstrated via western immunoblot analysis comparing the molecular weight and immunoreactivity of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins produced either in 4114 maize or in 1507 × 59122 maize. Therefore, previous safety assessments of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins conducted during the reviews of 1507 and 59122 maize are also applicable for 4114 maize. Furthermore, the concentrations of each of these proteins in maize grain samples were similar between maize lines 4114 and 1507 (Cry1F, PAT), between 4114 and 59122 (Cry34Ab1, Cry35Ab1, PAT), or between 4114 and 1507 × 59122 (Cry1F, Cry34Ab1, Cry35Ab1, PAT). Thus, there is no anticipated change in potential dietary exposure to the Cry1F, Cry34Ab1, Cry35Ab1, or PAT proteins as a consequence of commercial introduction of maize hybrids containing event 4114.

Grain from 4114 maize, which would be the source of all maize-derived food products, was analyzed for 82 compositional components, including protein, fat, fibre, ash, carbohydrates, minerals, vitamins, amino acids, fatty acids, key antinutrients and secondary metabolites. In comparisons between 4114 and control maize, only six statistically significant differences were noted (ash, phosphorus, potassium, oleic acid, eicosenoic acid, and inositol), The magnitudes of the differences in mean concentrations were small, less than 15 percent, and in every case the mean values from 4114 maize grain samples were within each respective tolerance interval and the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically meaningful changes in composition or nutritive value of the grain had occurred as a consequence of the genetic modification, or expression of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins in 4114 maize. It was concluded that grain from 4114 maize was compositionally equivalent to grain from unmodified near-isogenic control maize, and to other commercial maize hybrids. Processing is unlikely to alter the compositional components of maize grain, thus, products derived from 4114 maize grain will also be compositionally equivalent to their conventional counterparts.

Maize hybrids derived from the 1507 × 59122 breeding stack, expressing the same insect-resistance and herbicide-tolerance traits as present in 4114 maize, have been commercially cultivated on significant acreages in both the United States and Canada, and harvested grain from these hybrids is present in grain exports to many countries, including Australia and New Zealand. Hence, there has been a history of safe use and exposure to the introduced Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins present in 4114 maize. As confirmed through measurements of protein expression and by extensive compositional analysis, the genetic modification resulting in 4114 maize did not introduce any new characteristics relative to commercial 1507 × 59122 maize, nor did it result in the loss of any existing characteristic or in one or more characteristics falling outside the normal range of variation for maize. Relative to 1507 × 59122, maize line 4114 does not produce any other new novel proteins, nor does it produce any new secondary metabolites.