EXECUTIVE SUMMARY

Syngenta Crop Protection, LLC. has developed a genetically modified maize (*Zea mays* L.) using the techniques of modern molecular biology, to produce Event MZIR260 (OECD ID - SYN-ØØ26Ø-3) to provide control of fall armyworm (FAW, *Spodoptera frugiperda*).

Insect pests cause immense agronomic losses worldwide. One of the most destructive pests of maize crops is FAW, *Spodoptera frugiperda*. The ability to migrate long distances, a prodigious appetite, and a demonstrated ability to develop resistance to insecticides, makes it a difficult target to control. Insecticidal proteins, for example those produced by the bacterium *Bacillus thuringiensis* (*B. thuringiensis* or *Bt*), are among the safest and most effective insect control agents. GM crops expressing such proteins are a key part of a successful integrated pest management (IPM) program for FAW, *Spodoptera frugiperda*. However, the rise of FAW, *Spodoptera frugiperda*. populations that are resistant to these GM interventions highlights an urgent need for the development and commercialisation of new GM traits to effectively manage this pest (Fabrick and Wu, 2023; Zwack *et al*., 2024).

Maize plants derived from transformation Event MZIR260 produce the insecticidal protein eCry1Gb.1Ig encoded by the gene *eCry1Gb.1Ig-03*. The protein eCry1Gb.1Ig was engineered to have improved insecticidal activity against FAW, *Spodoptera frugiperda*. MZIR260 maize plants also produce the enzyme phosphomannose isomerase (PMI), encoded by the gene *pmi-15*. PMI enables transformed plant cells to utilize mannose as a primary carbon source; it was used as a selectable marker in the development of MZIR260 maize.

Transformation of *Zea mays* to produce Event MZIR260 maize was accomplished using *Agrobacterium tumefaciens*-mediated transformation of immature embryos, as described by Zhong *et al*., 2018). The transformation plasmid pSYN24795 used to generate Event MZIR260 consists of two expression cassettes and backbone sequences.

Genetic analysis confirmed the integrity of a single MZIR260 insert. The integration of the MZIR260 insert did not disrupt any known maize endogenous genes or generate any new ORFs with risk of allergenicity or toxicity to human or animal consumers. No unintended changes leading to safety concerns were identified during the molecular characterization of the MZIR260 maize. The transgenes *eCry1Gb.1Ig-03* and *pmi-15* were shown to be stably inherited and expressed over multiple generations.

The Newly Expressed Proteins (NEPs) in Event MZIR260 maize were assessed for toxicity and allergenicity risks using a weight-of-evidence approach. This approach incorporates information on the source organism, history of safe use (HOSU), *in silico* comparison to known and putative allergens and toxins, and/or stability under heat and digestive condition.

Neither eCry1Gb.1Ig or PMI proteins were found to share biologically relevant amino acid similarity to known or putative protein toxins or allergens of mammalian significance. Both proteins readily degrade and are inactivated under simulated mammalian gastric condition, and when exposed to heat. Cry and PMI proteins have a demonstrated HOSU in commercial GM crops (ISSAA, 2024). In combination with well characterised modes of action, both eCry1Gb.1Ig and

PMI were considered unlikely to be food toxins or allergens. Additionally, their low expression levels in maize tissues result in minimal anticipated exposure. All these factors support the prediction that no adverse health effects will result from the exposure to the eCry1Gb.1Ig and PMI proteins expressed in MZIR260 maize. Therefore, a reasonable certainty exists that exposure to MZIR260 maize and the NEPs eCry1Gb.1Ig and PAT, will not result in harm to consumers.

Furthermore, the compositional analysis established nutritional adequacy. No biologically relevant impact was observed on the nutritional status of forage and grain derived from MZIR260 maize as a result of the transformation process.

Overall, the data and information presented in this application support the conclusion that MZIR260 maize is comparable to, and as safe as, conventional maize, and that no adverse health effects will result from the consumption of MZIR260 maize or maize products.

REFERENCES

Fabrick JA, and Wu Y. (2023). Mechanisms and molecular genetics of insect resistance to insecticidal proteins from Bacillus thuringiensis. In *Advances in Insect Physiology* (Vol. 65, pp. 123-183).

ISSAA. (2024). International Service for the Acquisition of Agri-biotech Application. Retrieved from https://www.isaaa.org/gmapprovaldatabase

Zhong H, Elumalai S, Nalapalli S, Richbourg L, Prairie A, Bradley D, Dong S, Su XJ, Gu W, Strebe T, Shi L and Que Q. (2018). Advances in Agrobacterium-mediated Maize Transformation. *Methods Mol Biol, 1676*, 41-59. doi:10.1007/978-1-4939-7315-6_3. PMID: 28986903

Zwack PJ, Wu Y, Leininger C, Williams J, Richards EE, Wood C, Wong S and Bramlett M. (2024). Characterization of the mode of action of eCry1Gb.1Ig, a fall armyworm (Spodoptera frugiperda) active protein, with a novel site of action. *Pesticide Biochemistry and Physiology, 201*(105881), 1-6.