



NATIONAL TECHNICAL COMMISSION OF BIOSECURITY
TECHNICAL OPINION No. 192/2023/SEI-CTNBio - Members

TECHNICAL OPINION: 8405/2023

Process: 01245.013892/2022-12

Subject: Commercial release of maize (*Zea mays* L.), genetically modified Bt11 x MIR162 x NK603.

Applicant: Syngenta Seeds Ltda.

CQB:001/96

Protocol Date: 08/25/2022

Address: BR 452 Km 142 Uberlândia/MG.

Title: Commercial release of genetically modified corn (*Zea mays* L.), Bt11 x MIR162 x NK603.

Previous Statement: 8482/2022

Decision: Deferred

Meeting: 259th Ordinary Meeting held on 03/02/2023

GMO identification

GMO designation: Maize MIR162, and Maize NK603.

Species: *Zea mays* L.

Feature Inserted: The combined product provides control to certain lepidopteran insect pests through the expression of two insecticidal proteins (Cry1Ab and Vip3Aa20) and tolerance to herbicides containing the active principle of glyphosate, through the expression of the CP4 EPSPS protein and glufosinate ammonium, through the expression of the PAT protein.

Trait introduction method: Corn Bt11 x MIR162 x NK603, developed through classic genetic improvement, through the

cross between lines containing the individual transformation events Corn Bt11.

Proposed use: cultivation, production, handling, transport, transfer, commercialization, import, export, storage, release and disposal of this GMO, its possible combinations and derivatives, as well as its progenies.

Summary of Technical Rationale:

Syngenta has developed corn (*Zea mays* L.), Bt11 x MIR162 x NK603 by combining three individual transformation events using classical genetic improvement. The combined product provides control of certain lepidopteran insect pests through the expression of two insecticidal proteins (Cry1Ab and Vip3Aa20) and tolerance to herbicides containing the active principle of glyphosate, through the expression of the CP4 EPSPS protein and glufosinate ammonium, through the expression of the PAT protein. The individual events present in this combined product have already been previously evaluated and approved by CTNBio for commercial use in Brazil, whether with individual events or in several combined products, since 2008 and information about their transformation events is well known.

Based on Article 14, Annex IV, Section B, of CTNBio Normative Resolution No. 32, of June 15, 2021, Syngenta Seeds Ltda, through its Internal Biosafety Commission - CIBio, submits for consideration by the National Technical Commission de Biossegurança – CTNBio, information on the combined product Corn Bt11 x MIR162 x NK603, aiming at its commercial release, including cultivation, manipulation, transport, commercialization, consumption, release and disposal of this product and its derivatives, including its progenies.

The information regarding the Biosafety Assessment for Human and Animal Health, as well as the Biosafety Assessment for the Environment for the individual transformation events that make up the combined product Corn Bt11 x MIR162 x NK603 were previously presented to CTNBio for their respective risk analysis, according to the respective normative resolution, in force at the time of their submissions. Studies carried out with the individual transformation events present in the combined product Corn Bt11 x MIR162 x NK603, combined by classical genetic improvement, demonstrated that the individual transformation events are as safe for the environment and for human and animal health as corn conventional, not genetically modified, and are summarized below, according to each Process and respective CTNBio Technical Opinion, to support the risk assessment of the combined product Corn

Bt11 x MIR162 x NK603, emphasizing that there were no other genetic modifications, besides the introgression of these events in the maize lines, through classic genetic improvement.

Bt11 Maize contains the *cry1Ab* gene and the *pat* gene. The genetically modified Bt11 maize was obtained by direct transfer of DNA into protoplasts of the H8540 maize lineage, derived from embryogenic cells in suspension culture, treated with enzymes to degrade the cell wall. It contains the synthetic gene *cry1Ab*, from *Bacillus thuringiensis* var. *kurstaki*, which encodes the insecticidal protein Cry1Ab, which gives Bt11 corn resistance to pest lepidopteran insects, and the *pat* gene, derived from the soil microorganism *Streptomyces viridochromogenes* strain Tu494, which encodes the enzyme phosphinothricin N-acetyltransferase (PAT), which confers tolerance to herbicides containing glufosinate ammonium.

Corn NK603 contains the *cp4 epsps* gene, which encodes a glyphosate-tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was isolated from the bacteria *Agrobacterium tumefaciens* strain CP4 and inserted into the genome of corn through the biolistics method (acceleration of particles). Glyphosate causes the death of plants because it has the ability to block the activity of the target enzyme (EPSPS) belonging to the biosynthetic pathway of the aromatic amino acids tyrosine, phenylalanine and tryptophan. However, plant cells that express the CP4 EPSPS protein continue to produce aromatic amino acids essential to their metabolism even in the presence of glyphosate. The CP4 EPSPS protein is one of many EPSPS proteins found in nature, which are produced by plants, bacteria and fungi, but not by animals, since they lack the metabolic pathway for its synthesis. Therefore, different versions of the EPSPS protein are normally present in all plant and microorganism-derived foods. The gene donor organism, *A. tumefaciens* strain CP4 is a commonly found soil bacterium that causes galls on susceptible plants and there is no scientific evidence to indicate that it can cause adverse effects in humans or animals.

MIR162 corn contains the *vip3Aa19* gene that encodes a protein from *Bacillus thuringiensis*, toxic to pest lepidopteran insects, such as those of the species *Spodoptora frugiperda*. Transformation was mediated by *Agrobacterium tumefaciens* in immature maize embryos. The T-DNA transfer did not disrupt any genes in the maize genome and no new ORFs were created by the insertion. The inserted genes segregate in a Mendelian and stable manner in successive generations, which were analyzed. Maize MIR162 also expresses the *pmi* gene obtained from the bacteria *Escherichia coli* strain K-12 that encodes the phosphomannose isomerase enzyme that interconverts mannose-6-phosphate/fructose-6-phosphate allowing the bacteria to use mannose as a carbon source. This gene, introduced into

plants, it prevents the depletion of phosphate sequestered as mannose-6-phosphate accumulated when mannose is added to the culture medium, being a versatile and safe marker to identify transformed plant cells, allowing the selection of plant cells that express it in medium containing mannose as substrate.

For the combined product, Corn Bt11 x MIR162 x NK603 the activities of the transgenic proteins present, do not share any metabolic pathway, have different modes of action and are synthesized by the plant independently.

CP4 EPSPS and PAT proteins confer tolerance to glyphosate and glufosinate ammonium herbicides, respectively. They are distinct enzymes with specific modes of action that act in different metabolic pathways of the plant and in different cell compartments, both with a history of safe use.

The insecticidal proteins Cry1Ab and Vip3Aa20 confer tolerance to Lepidoptera insects, using different mechanisms of action and receptors as reported in specific studies of interactions, also with a history of safe use, either in genetically modified plants, or through the use in biological insecticides.

All proteins present in the combined product have been approved by CTNBio, individually or in combinations as shown in Table 1 or by other regulatory agencies around the world.

Additionally, the proteins present in corn Bt11 x MIR162 x NK603 are present in other combined products and were evaluated by CTNBio.

Final Opinion

Considering that the CTNBio norms are based on internationally accepted technical criteria, that the biosafety assessment of Corn Bt11 x MIR162 x NK603 concludes on its similarity to conventional corn in terms of biosafety for the environment and human and animal health, CTNBio decided at least GRANTING.

In view of the above and considering the internationally accepted criteria in the risk analysis process of genetically modified maize, it is possible to conclude that the Bt11 x MIR162 x NK603 event in the commercial release process is safe. The data presented in the majority request for Bt11 x MIR162 x NK603 corn meet current standards and legislation aimed at ensuring biosafety for the environment, agriculture, human and animal health, and allow the conclusion that Bt11 x MIR162 x NK603 corn is substantially equivalent to conventional corn, and its consumption

Safe for human and animal health. With regard to the environment, it can be concluded that the genetically modified subcombinations are not potentially the cause of significant degradation of the environment, maintaining a relationship with the biota identical to that of conventional corn.

As established in art. 1 of Law 11,460, of March 21, 2007, "research and cultivation of genetically modified organisms in indigenous lands and areas of conservation units are prohibited".

Within the scope of the powers conferred on it by art. 14 of Law 11.105/05, as well as the provisions of Normative Resolution 32, CTNBio considered that the request complies with the norms and legislation in force that aim to guarantee the biosafety of the environment, agriculture, human and animal health, and this activity does not have significant impacts on the environment.

Post commercial release monitoring:

CTNBio did not identify a non-negligible risk, therefore the company is exempt from the post-commercial release monitoring plan, as determined by Art. 18, first paragraph of CTNBio's RN32.

Data: 03/03/2023

Dr Paulo Augusto Vianna Barroso
President of CTNBio