NATIONAL BIOSAFETY AUTHORITY

SUMMARY OF THE APPLICATION FOR ENVIRONMENTAL RELEASE, CULTIVATION AND PLACING ON THE MARKET OF MON 89034 EVENT CONFERRING RESISTANCE TO STEM BORERS AND FALL ARMYWORM IN MAIZE VARIETIES IN KENYA

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Name and identity of the genetically modified organism

Genetically modified maize with event MON 89034 contains *cry1A.105* and *cry2Ab2* genes from *Bacillus thuringiensis* (Bt) *subsp. kurstaki* which confer resistance to stem borers and the fall armyworm. The trait MON 89034 is owned by Bayer Company and licensed royalty-free to the TELA Maize Project for maize improvement to benefit resource poor farmers in Kenya, Ethiopia, Nigeria, South Africa and Mozambique.

The unique identifier designation code for MON 89034 maize is MON-89Ø34-3.

Donor organism and Technique used for modification

Genetic modification of the maize was done through *Agrobacterium*-mediated transformation of conventional maize line LH172 by use of a plasmid vector PV - ZMIR245. The transformation process included the use of selectable marker gene *nptII*, to enable selection of the transformed cells and once the transgenic cells were identified, the selectable marker gene was removed through subsequent breeding and its absence was confirmed by both Southern blot and ELISA analyses.

Microbial formulations of Bt have been registered in many parts of the world including Kenya. In their history of widespread and continuous use around the world for over 50 years, Bt microbial pesticides have not caused any known adverse human, animal or environmental effects. Literature has been provided in the application to support safety on use of Bt formulations.

Summary of contained use and confined field trial data

Bioassays were conducted to evaluate efficacy of Cry1A.105 and Cry2Ab2 proteins with representative lepidopterans, two representative coleopterans, boll weevil (*Anthonomus grandis grandis*) and southern corn rootworm (*Diabrotica undecimpunctata howardi*); and two representative hemipterans (western tarnished plant bug (*Lygus hesperus*) and green peach aphid (*Myzus persicae)*). The results showed that Cry1A.105 and Cry2Ab2 proteins had insecticidal activities against all four representative lepidopteran insects. There was no indication of activity of these two proteins against coleopteran or hemipteran species representing beneficial non-target organisms (NTOs). The results confirm that, under expected agricultural use, these proteins have the targeted range of insecticidal activity against lepidopteran insect pests.

Confined field trials (CFT) undertaken with maize containing event MON 89034 at the Institute of Agricultural Research (IAR) in Samaru, Zaria in Nigeria and in South Africa evaluated insect damage, agronomic parameters such as pant and ear heights, anthesis-silking, grain moisture and yield. On average, the Bt maize hybrids gave 40% higher yield than the commercial varieties. The agronomic parameters measured were not significantly influenced by the genotypes. The modified maize had very low damage on grains, leaves and stems. The confined field trials successfully demonstrated the efficacy of the *Bt* genes, *Cry 1A.105* and *Cry2Ab2* in event MON 89034 in the control of stem borers and the fall armyworm. Additional confined field trials are underway in Ethiopia and Kenya to ensure the yield benefits are optimized.

Characteristics of genetic modification

The vector, PV-ZMIR245 used in the transformation is a 2T-DNA which contains two separate T-DNAs. The first T-DNA, designated as T-DNA I, contains two expression cassettes (*Cry 1A.105* and *Cry2Ab2* expression cassettes). The second T-DNA, contains the *npt*II expression cassette. The gene elements in T-DNA I and II are provided in detail in the application.

Genetic and molecular characterization was conducted to confirm the identity and integrity of genetic material present in maize with event MON 89034.

Molecular analysis including, Southern blot, PCR and DNA sequencing analyses were performed using genomic DNA isolated from MON 89034 in order to characterize the integrated DNA and assess the following: 1) number of insertions of the integrated expression cassettes; 2) number of copies of the integrated expression cassettes; 3) intactness of the expression cassettes; 4) the presence or absence of plasmid backbone sequences; 5) the stability of the inserted DNA across multiple generations and during conventional breeding; and 6) organization of the insert in MON 89034. PCR amplification and DNA sequencing were used to confirm the 5' and 3' insert-to-plant junctions, confirm the organization of the elements within the insert, and determine the complete DNA sequence of the integrated DNA.

Traditional breeding was used to isolate plants that contain the cry1A.105 and the cry2Ab2 expression cassettes (T-DNA I) but do not contain the *npt*II expression cassette (T-DNA II), therefore producing marker-free, lepidopteran-protected maize with event MON 89034.

Analysis of the stability of the integrated DNA demonstrated that a unique Southern blot fingerprint of MON 89034 was maintained in seven generations during conventional breeding, thereby confirming the stability of the insert. Additionally, T-DNA II analysis of multiple generations in the MON 89034 breeding history indicated that there were no detectable T-DNA II elements other than those which are common to T-DNA I, i.e., *35S* promoter, *nos* 3´ end sequence, Left Border sequence.

Furthermore, these generations were shown not to contain any detectable backbone sequence from plasmid PV – ZMIR245. Further, PCR and DNA sequencing analysis of the complete DNA insert and adjacent genomic DNA confirmed the organization of the genetic element within the insert as confirmed by SDS - gel electrophoresis. The results demonstrated that event MON 89034 contains a single functional copy of *cry1A.105* and *cry2Ab2* expression cassettes.

Description of phenotypic characteristics

Results from the phenotypic and agronomic characteristics assessments indicate that maize with event MON 89034 does not possess characteristics that would confer an increased plant pest risk compared to conventional maize.

Expression Levels of Cry1A.105 and Cry2Ab2 Proteins in MON 89034

The expression of introduced proteins was determined using standard methods, including ELISA and PCR analysis. Results of the measurements demonstrated that both Cry1A.105 and Cry2Ab2 proteins were expressed in all tissues collected, including leaf, root, forage, silk, pollen, grain and stover. Detailed information is provided in the main application submitted to the National Biosafety Authority.

Activity of the expressed protein (s)

The mode of action of *Bacillus thuringiensis* delta-endotoxins is well understood and has been studied extensively. The bacterially produced crystal proteins are first solubilized in the insect midgut, followed by activation of the protoxins (full-length proteins) to active toxins (proteolytic resistant cores) by midgut proteases. The activated proteins bind to midgut membrane receptors in susceptible insects, insert into the apical membrane and form pores that cause loss of osmotic regulation, and eventually insect death.

Taxonomic name/status and origin of recipient organism or parental organisms

Zea mays L. Maize, corn or *mahindi* in Kiswahili.

Maize is believed to have been domesticated in central America but is widely cultivated throughout the world over a wide range of climates.

Description of the proposed deliberate release, including the purpose (s) and foreseen products

It is proposed in this application that Bt maize with event MON 89034 be considered for commercial release in Kenya as an additional tool to mitigate the damaging effects of stem borers and the fall armyworm. If approved for environmental release, the event MON89034 will be introgressed into maize adapted to Kenya's agro-ecologies. This technology, with highly selective mode of action only to certain target lepidopteran insect pests, will also reduce over-reliance on chemical insecticides that are costly and which also impact negatively on humans, animals and the environment, including the beneficial non-target organisms.

Upon approval for environmental release, the insect protected maize hybrids will be evaluated as required under the Seeds and Plant Varieties Act of Kenya which requires a variety to be released officially. The applicants will comply with post release requirements.

Suggested method(s) for safe handling, transport and storage during release

Except for the labelling obligations outlined in the Biosafety Act, 2009, no specific methods are considered necessary for handling maize with event MON 89034 since upon approval for environmental release and placement on the market, the maize with event MON 89034 is not different from conventional maize except for the presence of Bt genes that confers protection against target stem borers and the fall armyworms. This maize has been shown to be as safe and nutritious as conventional maize. Therefore, upon release, maize with event MON 89034 and derived products from it will be handled, transported, stored, packaged and used in the same manner as current commercial maize varieties grown in Kenya while considering the approval conditions.

History and results of previous environmental releases, as well as uses of the genetically modified organism

Maize with event MON 89034 has been approved for cultivation and use in more than fifteen countries as shown below;

MON 89034 GLOBAL APPROVALS			
Country	**Competent Authority**	**Purpose**	**Year**
Argentina	Secretariat of Agriculture, Livestock, Fisheries and Food (SAGPyA)	Food, Feed, Environment release	07-Oct-10
Australia/New Zealand	Food Standards Australia New Zealand (FSANZ)	Food	04-Dec-08
Brazil	National Technical Biosafety Committee	Food, Feed, Environment release	16-Oct-09
Canada	Canadian Food Inspection Agency (CFIA)	Environment release	21-Oct-11
Canada	Canadian Food Inspection Agency (CFIA)	Food, Feed, Environment release	19-Jun-08
China	Ministry of Agriculture	Food, Feed	30-Dec-10
Colombia	CTNbio - Instituto Colombiano Agropecuario (ICA)/ CTN-Health; Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA)	Food, Feed	28-Aug-07
European Union	European Commission	Food, Feed	30-Oct-09
Honduras	Secretaria de Agricultura y Ganaderia (SAG)	Environment release	24-Nov-10
Indonesia	Ministry of Agriculture/ National Agency of Drug and Food [(BPOM)Badan Pengawas Obat Dan Makanan]	Food, Feed	16-Apr-13
Japan	Ministry of Agriculture, Forestry and Fisheries / Ministry of the Environment/ Ministry of Health, Labor and Welfare (MHLW)	Food, Feed, Environment release	31-Jan-08
Korea	Korea Food and Drug Administration	Food	02-Apr-09
Korea	Rural Development Administration	Feed	03-Apr-09
Malaysia	Ministry of Natural Resources and Environment	Food, Feed	30-Apr-15
Mexico	Ministry of Health - Federal Commission for Protection from Sanitary Risks	Food, Feed	22-Jul-08

Paraguay	Ministry for Agriculture and Livestock	Food, Feed, Environment release	04-Dec-13
Philippines	Department of Agriculture-Bureau of Plant Industry	Food, Feed, Environment	19-Nov-10
Russian Federation	Federal Services for Supervision of Consumer Rights/ Federal Service for Veterinary and Phytosanitary Surveillance	Food, Feed	11-Dec-14
Singapore	Agri-Food and Veterinary Authority (AVA)	Food, Feed	21-Sep-11
South Africa	Department of Agriculture, Forestry and Fisheries	Environment release	19-Oct-10
Taiwan	Council of Agriculture, Department of Health	Food, Feed	3-Feb-17
USA	Environmental Protection Agency	Environment release	12-Feb-14
USA	Food and Drug Administration	Food, Feed	08-Aug-07
USA	United States Department of Agriculture	Environment release	24-Jul-08
Vietnam	Ministry of Agriculture and Rural Development Ministry of Natural Resources and Environment	Food, Feed, Environment release	27-Aug-14

Intended use of the Bt Maize

Upon environmental release, maize with event MON 89034 is expected to be used in the same manner as any other maize that is cultivated in Kenya and elsewhere. This will cover seed production, grain production for food, feed and for industrial processing.

Receiving environment

Maize is not indigenous to Kenya, having been introduced in the 16th century. Kenya is neither a center of origin nor a center of diversity (OECD, 2003). The first point of environmental release of maize with event MON 89034 will be at least the 7 trial sites designated for National Performance Trials in each of the three maize-growing agro-ecologies in Kenya to generate data for national performance trials (NPT) and Distinctiveness, Uniformity and Stability (DUS) observations which are mandatory for variety release and registration. Thereafter, seed will be multiplied in designated seed multiplication sites. Maize with event MON 89034 will then be availed through the existing seed marketing and distribution channels for cultivation in all the stem borer and fall armyworm-infested maize growing areas of Kenya across various agro-ecologies in Kenya.

Risk assessment summary

Maize with event MON 89034 has been approved and used in more than fifteen countries since 2006. This was after review by various Regulatory Agencies in those countries. More recently, four maize varieties with event MON 89034 was released in Nigeria in January 2024. MON 89034 maize hybrids are currently under commercial cultivation by Nigerian farmers. No adverse effects on human, animal or the environment health have been observed.

The potential adverse effects that could occur during environmental release and cultivation of maize with event MON 89034 in Kenya are described in within the clusters of; i) molecular characterization of the inserted gene; ii) the safety of the expressed proteins; iii) food and feed safety assessment of products derived from maize with event MON 89034; and iv) environmental safety of event MON 89034. Further assessment of the likelihood of occurrence of the said adverse effects was carried out and based on weight of evidence, during environmental release and cultivation of MON 89034. The review led to the conclusion that:

i) *Bacillus thuringiensis* (*Bt*) has been used commercially as liquid sprays for the control of insect pests in many parts of the world for decades and based on the available scientific data from regulatory agencies across the world, the use of registered Bt products poses no significant risks to human health, non-target organisms or the environment (US EPA, 1998). Indeed, there exists long history of safety for Cry proteins, further supported by the extensive approval commercial use of event MON 89034 as shown in Part A.

ii) The Cry1A.105 and Cry2Ab2 proteins do not show amino acid sequence homology compared to known toxins or allergens in protein databases and hence event MON 89034 is unlikely to show toxicity to human and animal health following environmental release, cultivation and placing on the market in Kenya. In addition, the Cry proteins are unlikely to have any allergenic potential, and maize with event MON 89034 is as safe as conventional maize regarding the risk for allergenicity.

iii) There were no biologically relevant differences in the parameters measured between broiler chicken fed on maize with event MON 89034 diet and those fed on the control diet (Taylor *et al.*, 2003). The diet of maize with event MON 89034 was as wholesome as the control and commercial reference maize diets based on its ability to support the rapid growth of broiler chicken. These data support the conclusion that maize with event MON 89034 is as safe and nutritious as conventional maize.

iv) There is negligible risk of harm from maize with event MON 89034 on non-target organisms (vertebrates and invertebrates), either through direct or indirect interactions or contact with the newly expressed Cry proteins in the field. Higher trophic interactions between non-target organisms would also not be negatively

affected.

v) The risk of the insect-protection trait introduced into maize with *Bt* event MON 89034 causing any meaningful competitive advantage or disadvantage that could impact the receiving environment is negligible.

vi) Pollen mediated gene-transfer could occur following environmental release and placing on the market and cultivation of maize with event MON 89034 in Kenya but there is no potential for gene transfer to wild plant species in the Kenyan environment.

From the foregoing, environmental release, cultivation and placing on the market of maize with event MON 89034 in Kenya is unlikely to have any adverse consequences to human and animal health as well as the receiving environment. The host plant, maize, has a long history of safe use. The results of extensive compositional analyses of this maize demonstrate that the levels of the important nutritional and non-nutritional components in maize with event MON 89034 are comparable to the parental variety and are within established ranges for commercial maize varieties. The insecticidal proteins expressed in maize with event MON 89034 are present at very low levels and are unlikely to remain in processed maize food and feed products. The safety of the introduced proteins has been assessed through; i) history of safe use as food and feed; ii) determination of no allergenic potential of the introduced proteins and iii) determination of no toxic potential of the introduced proteins. The maize is not different in composition, safety, or any relevant parameter from maize currently grown, marketed and consumed. Sale and consumption of food and feed derived from this maize are expected be consistent with current practices for the development and introduction of new maize varieties.

Recommendation on risk management

Based on the conclusions of molecular characterization of the insert and its genetic elements, the expressed proteins, the safety assessment of the food, feed and the environmental risk assessment the results presented in the application, no risk management strategies are considered by the applicant as necessary.

Information on post release monitoring and emergency response plans

The applicants are cognisant of the monitoring requirements and obligations in the Biosafety Act, the Seeds and Plant varieties Act and the Audit requirements in the EMC Act in the Republic of Kenya. The guideline provided by the National Biosafety Authority (NBA) will be critical in the post release monitoring of maize with event MON 89034. The post control procedure prescribed for monitoring variety seeds will serve as key data points to supplement the proposed plans outlined herewith.

Insect Resistance Management

A critical component for the long-term use of biotechnology - derived Bt crops containing insecticidal proteins is to implement Insect Resistance Management (IRM) programs to prevent or delay the onset of resistance in the target insect pest species. Research by industry as well as academia over the past decade has improved understanding and gained broad agreement for the major elements of IRM plans for Bt crops. The core element of an IRM plan is the use of a refuge to ensure an adequate population of susceptible insects of the target species is available to mate with any rare resistant insects that may survive exposure to the Cry protein produced by the crop. This refuge may include wild host plants, other crops, or non-Bt plantings of the crop in question. Bayer has developed an IRM plan for deployment of MON 89034. A summary of the plan is as described below.

The combination of Cry1A.105 and Cry2Ab2 insecticidal proteins in a single plant of MON 89034 maize provides both better insect control than single Bt maize products and a more effective approach for IRM. Mathematical modeling conducted by Bayer indicates that biotechnology-derived plants expressing two Cry proteins will have significantly greater durability than plants producing either of the single proteins if: (1) cross-resistance between the Cry proteins is low; and (2) if the mortality of susceptible insects caused by each of the individual proteins is at least 90%, and preferably >95%. Cry1A.105 and Cry2Ab2 proteins have important differences in the way in which they bind to the lepidopteran midgut. Therefore, the probability of cross-resistance between these proteins is very low. Comparable biophysical studies also indicate that Cry1A.105 differs from Cry1Ab in the mode of action with regard to binding to the insect midgut. Therefore, Cry1A.105 and Cry2Ab2 have distinct modes of action regarding receptor binding. Furthermore, in vitro and in planta studies with Cry1A.105 and Cry2Ab2 demonstrate that both proteins are highly active against the primary lepidopteran pests of maize (*Busseola fusca, Chilo partellus, Sesamia calamistis* and *Spodoptera frugiperda* - FAW), achieving close to or greater than the critical 95% level of control in all cases. Based on the data and appropriate mathematical models, a percentage of refuge in the bag (RiB) or seed mixes is recommended to preserve the durability of MON 89034 trait.

The specific requirements of the IRM plan for MON 89034 are described below;

a. A Refuge in the bag of non-Bt maize to provide sufficient susceptible population of susceptible target pests (sufficient Bt-susceptible insect pest).

b. Monitoring for any potential development of resistance through the sentinel trial program.

c. Remedial action plan in case of any confirmed development of resistance.

d. IRM plan implementation through farmer education, managing expectations of control, awareness of Bt maize cultivation and proper stewardship.

Maize with event MON 89034 will also be commercialised alongside quality control and monitoring measures encompassing an Insect Resistance Management Plan (IRM) and a Product Stewardship programme in order to secure the valuable agronomic and other benefits of insect-protected maize on a longer-term basis.

Additional notes

Maize containing *cry* genes has been tested in Kenya both at contained and confined field trials. The National Biosafety Authority evaluated the safety of MON 810 in the applications that led to the approval and commencement of the trials and also environmental release in Kenya. The similarity of the Cry 1A.105 and Cry2Ab2 proteins expressed in maize with event MON 89034 is demonstrated in the main application.

Maize with event MON 89034 is substantially equivalent to conventional maize (except for the introduced trait) and therefore the foreseen products as a result of its environmental release (cultivation) are similar to those of other conventional maize varieties which include use of grains for food and feed purposes, use of plants for feed and for industrial and processing purposes.

Maize with event MON 89034 is also approved in Nigeria, which is participating country in the TELA Maize project and four varieties with event MON 89034 have been released officially and are now under cultivation by Nigerian farmers.

The Full Application is available with the NBA