

National Biosafety Committee opinion on an application by CHI Farm Limited **for the placing on the market of genetically modified soybean for food or feed import and processing** for the import and Export of GM Maize containing events, MON 863 (insects resistance) DAS-40278-9 (to contain detoxifies 2, 4 –D herbicide) MON 863 X MON810 X NK603 ( Cry 1Ab confers resistance to lepidopteran) (cry 3Bb1: confers resistance to coleopteran and cp4 epsps (glyphosate herbicide tolerance), MON 8934 X NK603 (cp4 epsps (glyphosate herbicide tolerance), ( Cry 1A confers resistance to lepidopteran insect) and ( Cry 1Ab2 (confers resistance to lepidopteran insect)

### **Policy Framework**

- To ensure safety to human health and the environment taking into consideration food security

### **General Assumptions and Monitoring plans for all products**

- The NBC in expressing these opinions has relied heavily on previous positive reviews of risk assessment for these products in the USA and Canada and especially from European Food Safety Authority (EFSA) as well as on the long history of safe use.
- The scientific assessment of this product included molecular characterization of the inserted DNA and expression of the new protein. A comparative analysis of agronomic traits and composition was undertaken and the safety of the newly expressed protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.
- The monitoring plan and reporting intervals were in line with the intended uses.

## **MON 863 (insect resistance)**

### Molecular Characterization

MON 863 maize was developed to provide protection against certain coleopteran pests, principally corn rootworm (*Diabrotica* spp.) by the introduction of a variant *Bacillus thuringiensis* cry3Bb1 gene expressing an insecticidal protein.

Molecular analysis of MON 863 maize demonstrated that only the two expected full-length proteins, Cry3Bb1 and NptII, would be encoded by the insert with respect to the presence of an intact nptII gene,

The NBC concluded that the use of nptII as a selection marker did not pose a risk to the environment nor to human and animal health. DNA sequences at the junctions between the insert and parent genome were determined revealing the presence of mitochondrial DNA at both flanks.

The molecular analysis does not differentiate between the integration of insert DNA within a region of mitochondrial DNA that is already present in the nuclear genome and the acquisition of this organellar DNA as part of the primary integration during transformation.

### **Compositional Analysis**

- Compositional analyses of MON 863 macro-nutrients, micro-nutrients, and anti-nutrients, as well as secondary metabolites were measured. Although some statistically significant differences were observed for palmitic acid between MON 863 and its control, these differences were small and within the historical background range and thus of no biological significance.
- The NBC is of the opinion that soybean MON 863 is as safe as its conventional counterpart and non-GM soybean reference varieties in the context of the intended uses as proposed by the applicant. The altered fatty acid profile did not raise concerns regarding toxicity.

## **Environmental effects: Post Market Monitoring**

- Considering the scope of the application, not for cultivation, the NBC is of the opinion that the likelihood of the spread and establishment of soybean MON 863 is very low and that unintended environmental effects due to this soybean will be no different from that of conventional soybean varieties.
- The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of soybean MON 863. The monitoring plan provided by the applicant is in line with NBC guidance document and the Opinion of the NBC on post-market environmental monitoring.

## **Conclusions**

- In conclusion, the NBC considers that GM Maize MON 863 is as safe as its non-genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore, the NBC concludes that soybean MON 863 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses.

## **DAS- 40278-9**

### **Molecular Characteristics**

- Maize DAS- 40278-9 was developed by direct whisker-mediated transformation to express the aryloalkanoate dioxygenase-1 (AAD-1) protein, conferring tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxy-phenoxypropionate (AOPP) herbicides. The molecular characterization of DAS – 40278-9 did not raise any safety issues.

### **Compositional Analysis, Toxicity Allergenicity**

- The agronomic, phenotypic and compositional characteristics of comparative analysis of Maize DAS- 40278-9 tested under field conditions revealed no difference with respect to its non - genetically modified counterpart.

- DAS- 40278-9 Maize is as nutritious as its non GM conventional counterpart reference varieties.
- The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced proteins.
- There are no indications that the genetic modification might significantly change the overall allergenicity of Maize DAS- 40278-9 when compared with that of its conventional counterpart.

### **Feeding Studies; Horizontal Gene Transfer**

- Potential interactions of DAS- 40278- 9 Maize with the biotic and abiotic environment were not considered as issue due to the low level of exposure.
- The post market environmental monitoring plan and reporting intervals Maize DAS- 40278-9 are in line with the intended uses.
- Based on the results of studies of 28- day oral repeated dose in mice, it is concluded that feeding stuffs derived from Maize DAS- 40278- 9 are as safe and nutritious as those derived from other non-GM soybean varieties
- The NBC considers that the Maize DAS- 40278-9, as described in this application, is as safe as the non- GM conventional counterpart with respect to no potential effects on human and animal health and the environment in the context of its intended uses.

### **MON 863 X MON810 X NK603**

#### **Molecular Characterization**

- Traditional breeding methods were used to produce MON 863 × MON 810 × NK603 maize and no new genetic manipulation was involved. The three transgenic inserts that are present in MON 863 x MON 810 x NK603 maize were derived from three independent single trait GM maize lines: MON 863, MON 810 and NK603.

- MON 863 x MON 810 x NK603 maize was produced by crosses between maize inbred lines containing MON 863, MON 810 and NK603 events to combine the rootworm resistance trait.
- MON 863 with the trait present in MON 810 protecting against lepidopteran pests and the tolerance to the herbicide glyphosate in NK603.
- Molecular analysis of the DNA inserts present in the MON 863 x MON 810 x NK603 maize confirmed that the insert structures of the single events were retained.
- MON 863 maize was developed to provide protection against certain coleopteran pests principally corn rootworm (*Diabrotica* spp) by the introduction of variant bacillus thuringiensis of cry3Bb1 gene.
- Mon 810 maize produces the protein Cry 1Ab, which confers protection against certain lepidopteran insect pests (*Ostrinia nubilalis* and *Sesamia* spp).
- NK603 was developed to be tolerance to the herbicide glyphosate by the introduction of a gene coding for 3-phosphosate synthase from *Agrobacterium* spp. and Cp4 (cp4 Epsps).
- MON 863 molecular analysis demonstrated that only two full length protein, Cry3Bb1 and NpII will be encoded by the insert.
- The bioinformatics analysis revealed the presence of mitochondrial DNA at both 5' and 3' flanks, and the integration of organellar DNA in the nuclear plant genome transformation is established. Bioinformatic analyses did not raise safety issues.
- Molecular characterization demonstrated sufficient sequences of Cry 12Ab coding region to encode an insecticidal Cry1Ab protein.
- No differences were identified in the seed composition that would require further assessment with regard to safety.
- Regarding agronomic and phenotypic characteristics, MON 863 X MON 810 X NK 603 showed no relevant alteration in composition.

- NBC therefore conclude that MON 863 X MON 810X NK603 maize is compositionally and phenotypically equivalence to its parental single traits GM lines and non- genetically modified maize except for the introduced traits.
- Safety assessment identified no concerns regarding the potential toxicity and allergenicity
- MON863 X MON810 X NK603 has been proved to be substantially equivalent to conventional maize, except for the introduced traits.

### **Feeding studies, Horizontal gene transfer**

- MON 863 X MON 810 X NK 603 nutrition studies with boiler showed no adverse effects and nutrition properties of maize MON 863 X MON 810 X NK 603 indicated no different from those of conventional maize.
- The NBC considers that MON 863 X MON 810 X NK 603 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

### **MON89034 X NK603 herbicide-tolerant soybean**

#### **Molecular Characterization**

- Traditional breeding methods were used to produce maize MON89034 × NK603 and no new genetic modification was involved. The two inserts that are present in maize MON89034 × NK603 were derived from maize lines containing two independent single events:
- Maize MON89034 × NK603 combines the lepidopteran protection trait from maize MON89034 and the glyphosate tolerance trait from maize NK603.
- Maize MON89034 was developed through *Agrobacterium*-mediated transformation of immature maize embryos using the binary plasmid vector PV-ZMIR245. PV-ZMIR245 contains two separate T-DNAs. The first T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes.

- Maize NK603 was developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).
- The molecular characterization expression level of the Cry 1A.105, Cry2Ab2 and CP4 EPSPS protein in maize MON 89034 X NK 603 were demonstrated to be comparable to those single events.

### **Toxicity and Allergenicity: Non-target organisms**

- Bioinformatic analyses of 3' and 5' demonstrated unlikely event to cause any potential toxic, allergenic or have other health implications to humans.
- The expression of the newly introduced proteins in MON89034 x NK603 has been sufficiently analysed and proved to be comparable to the expression in the single events.
- The stability of the genetic modifications has been demonstrated over several generations in the single events, and analysis confirmed that the structural integrity of the inserts was maintained in the hybrid.
- The nutritional value of maize MON89034 x NK603 has been studied in a feeding study with broiler chickens and this study indicated that the nutritional properties of maize MON89034 x NK603 is not different from those of its non-GM counterpart.
- The NBC considers that the molecular characterization does not indicate any safety concern.

- No new constituent other than the Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214P proteins are expressed in maize MON89034 NK603 and no relevant changes in the composition of maize MON89034 NK603 were detected by the compositional analysis.
- No specific environmental impact of this GM maize was indicated by the environmental risk assessment and thus no case-specific monitoring is required.

### **Conclusion:**

- The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x NK603 since the environmental risk assessment excluded cultivation and identified no potential adverse environmental effects.
- The NBC is of the opinion that the molecular characterisation provided for maize MON89034 x NK603 produced by conventional breeding is sufficient for the safety assessment.
- The results of the comparative analysis indicates that maize MON89034 x NK603 is compositionally, agronomically and phenotypically equivalent to its non-GM counterpart except for the presence of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in maize MON89034 x NK603.

### **OBSERVATIONS**

1. Adequate preventive measures should be put in place to prevent accidental release of viable seeds into the environment.
2. In the implementation of the recommendations, strict compliance to NBMA's regulatory framework on importation, transportation, handling and use should be adhered to.
3. The intended use of this application is for feed processing; it is not to be cultivated in Nigeria.



4. In the implementation of the recommendations, strict compliance to NBMA's regulatory framework on importation, transportation, handling and use should be adhered to;
5. Future applicants should be required to indicate methods for testing pre-production raw material nutritional values; and
6. Terms and conditions set by NBMA should be strictly adhered to.

### **Recommendations**

- The NBC having critically looked through the dossiers recommends to the National Biosafety Management Agency (NBMA), the approval of the application for importation of four (4) GM events maize MON 863, DAS-40278-9, MON 863 X MON 810 X NK 603 **STRICTLY** for feeds production by CHI Farms LTD.