OBJECTIONS TO THE APPLICATION MADE BY SYNGENTA SOUTH AFRICA FOR A PERMIT FOR A COMMODITY IMPORT OF GM MAIZE MIR 604XGA21_

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The African Centre for Biosafety submits these scientific objections in respect of he application for commodity import by Syngenta of its GM maize, MIR604xGA21. We have over the years made several submissions on various legal aspects concerning GM applications in South Africa and will not repeat these here. In addition, we note that the *de facto* moratorium on the approval of new GM events is still in place and we ask that the Executive Council continue to keep such moratorium in place, for the reasons we have previously outlined in various objections for commodity import permits.

Independent scientific objection to the commodity import of Syngenta Stacked Maize (MIR604xGA21)

The application is for a stacked GM maize (MIR604xGA21) that was obtained by crossing two GM maize lines: MIR604 maize expresses a modified *Bt* insecticidal protein (mCry3A) for control of certain *Diabrotica* spp. (corn root worm) pests, and a phosphomannose isomerase (PMI) protein which acts as a selectable marker. GA21 maize expresses a modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate. This application is for commodity clearance of MIR604xGA21 maize to be used in food products for human consumption and in animal feed**.**

Unintended genetic effects: mutations multiple insertions, and truncations

The GM cassettes for the two events are:

MIR604 maize

The modified Cry3A, (mCry3A) is from the bacteria *Bacillus thuringiensis* and is effective against western corn rootworm, *Diabrotica virgifera*. The MTL (metallothionein-like gene) promoter derived from *Zea mays* (maize) drives the expression of the mCry3A preferentially at the plant roots. This cassette uses the PMI gene as a selectable marker in contrast to the antibiotic resistance markers commonly used in many other GM food crops on the market. The PMI (phosphomannose isomerase) gene is driven by the ZmUbiIntr promoter from *Zea mays* and enables transformants to grow on mannose as the sole carbon source.

GA21 maize

The mutated EPSPS gene, (3-enoyl pyruvyl shikimate 5-phosphate synthase), which confers resistance to glyphosate is from *Agrobacterium* spp. The expression of this gene is driven by a promoter consisting of the actin 1 gene promoter as well as the first exon of the actin 1 gene from *Oryza sativa* (rice). The mEPSPS gene was fused to an optimized chloroplast transit peptide to allow targeting to the chloroplast (the site of the shikimate pathway and mode of action of the glyphosate herbicides). The chloroplast transit peptide is a synthetic peptide derived from peptide sequences from maize and sunflower RuBisCo genes.

The sequence analysis of the individual above events revealed that unexpected mutations, multiple insertions, and truncations had occurred. Sequence analysis of MIR604 revealed that truncation had occurred: a 44 bp at the right border and 43 bp at the left border of the transgenic cassette. Additionally,

three mutations were present. One within the MTL promoter that controls expression, and the other two mutations within the PMI gene sequence resulting in two amino acid changes; a V61A and Q210H. These changes were unintended, but not considered important since the cassette still functioned. Furthermore, the company states that sequencing and Southern blot data have demonstrated that MIR604 maize contains a single DNA insertion with one copy of both the mCry3A and the PMI genes, however, this data was not provided (Appendix 1 absent).

Sequence analysis of the GA21 event revealed multiple (six) insertions of the transgenic cassette! Additionally, many of these copies contain deletions or truncations. Copy 1 has a 5' deletion of 696 bp; copies 2, 3 and 4 are intact; copy 5 contains a truncated mEPSP gene (only 288 bp of the mepsps gene) while copy 6 contains only the rice actin promoter and a truncated actin first exon; with all other genetic elements truncated.

In assessing the genetic integrity of MIR604xGA21 it appears that only limited molecular studies have been done. A limited set of gene probes were used in Southern blots to determine the correct insertion of the genes, but this cannot determine rearrangement and fragmentation in the genome. Syngenta also states that there are single insertion events in the genome but the evidence to this effect has not been presented. The Southern hybridisation data or ELISA to detect protein together with the phenotypic characterisation after several generations only establishes if the transgenic cassettes are still present and functioning. The integrity of the cassette and other unintended genetic effects has not been studied. It is therefore not known if there are any other genome changes. Appropriate experiments would include quantitative Southern blots or quantitative PCR with several probes or primers (spanning the cassette and including flanking regions) on plants in field trials with the DNA sequencing of the amplified cassette from these plants in the field over 3-6 generations. Furthermore it is assumed that no other genetic changes were introduced during the construction of MIR604xGA21. In order to prove this assumption techniques such as repPCR, RAPD and comparative genome hybridization (CGH) have been shown to be effective in establishing genome similarity (Bao *et al.* 1993, Pinkel and Albertson 2005) and will help establish if additional, unintended genetic changes were introduced .

The biosafety risks of these unintended genetic changes are unknown but may include the production of novel allergenic or toxic proteins, changes in cellular gene expression and metabolism as well as increased recombination and horizontal gene transfer (HGT).

Risks to human and animal health

High concentrations of mCry3A protein were detected in leaves, roots and whole plants (3-94 g/g dry weight) of MIR604 x GA21 maize. Quantifiable concentrations of mEPSPS protein were detected in all MIR604 x GA21 maize derived plant tissues while PMI protein was detected (ND to 2.1 g/g dry weight) in most of the MIR604 maize-derived plant tissues analyzed. The dietary exposure of South Africans is very different from the European and American situation (maize makes up a small percentage of the diet and usually in a highly processed form such as corn oil or syrup in Europe and the US) with maize and maize meal forming a staple of the majority of South Africans. Therefore assumptions used for the approval of this GM maize in other countries (low exposure, absence or protein from highly processed maize products) do not apply to South Africa. Equally maize (kernels, cobs, leaves, stalks) are a popular animal feed and South African cattle surely consume more maize that European counterparts. The increased exposure raise increased risks of any toxic or allergenic effects.

Allergenicity

There are several aspects to consider when determining whether a novel protein is likely to be an allergen:- sequence similarity to known allergens, ability to simulate gastric fluid, and immune response in feeding studies.

- mCry3A protein: The mCry3A has been shown to contain to have significant sequence similarity to the known allergen beta-lactoglobulin (a milk allergen) (Gendel, 1998). There are therefore biosafety risks associated with this novel insecticidal protein particularly since evidence to date attributes a large percentage of allergies to milk (http://www.ourfood.com/Food_Allergies.html).
- EPSP protein: The wild-type maize EPSPS enzyme has not been associated with any allergic effects, nor is its amino acid sequence homologous with any know protein allergens. However, the modified EPSP is 99.3% identical to EPSP and therefore may present a novel antigenicity.
- PMI protein: There is limited sequence homology between the PMI protein and two allergens, Hev b 13, from the latex of the rubber tree (Havea brasiliensis) and alpha-parvalbumin from the frog (*Rana* spp) that warrant further *in vivo* studies to determine allergenicity and biosafety.

The studies of the allergenic potential have been limited to establishing whether the protein heat labile (95°C for 30min) and was degraded by acid and/or enzymatic hydrolysis when exposed to *simulated* gastric or intestinal fluids in the laboratory. The allergenic potential has not been studied in animal models nor have antibody levels been determined in controlled feeding studies in order to monitor immune response.

The compositional analysis of MIR604 x GA21 maize is stated as equivalent to conventional maize but the data was not presented or publicly available to support this statement.

Environmental effects: Herbicide resistance, horizontal gene transfer (HGT) and gene escape.

There are risks associated with the consumption of maize and maize products by animals and humans as well as threats to biodiversity. In South Africa many of the maize derived food products are not highly processed maize cobs, samp. maizemeal) and both the transgenic proteins and the DNA will be present. It is now well known that DNA can persist in soil, and many processed food products. Furthermore, current evidence shows that horizontal gene transfer (HGT) to bacteria does occur and is significant and occurs at a high frequency when sequence homology is present (de Vries and Wackernagel 1998). The EPSPS, cry3a and PMI genes all have many gene homologs in soil bacteria indicating an increased risk for horizontal gene transfer. Furthermore, a study carried out by the British Food Standards Agency, to determine if transgenic DNA transferred to bacteria of the human gut by HGT, found that this did indeed occur (Netherwood 1990). No animal feeding studies have been established but there are reports where beneficial predators have been harmed because they have eaten pests that have fed on Bt GM plants (Bigler & Keller, 1997; Hawkes, 1997) indicating accumulating toxic tier effects up the food chain or induced allergenicity.

There is also a more obvious manner in which transgenic genes may escape into the environment. MIR604xGA21 maize kernels that are approved for food, feed or industrial use will inevitably be planted by farmers and enter the environment. The distinction between seed and feed is unclear in the eyes of many farmers where part of the maize harvest is always saved as seed. A poor harvest may necessitate the purchase of seed for consumption but naturally some will be saved for planting in the hope of a better crop the next year. Since the MIR604xGA21 will be indistinguishable in the field from many other varieties, this type of contamination will be unnoticed and may spread following years of seed saving and planting.

There may be several consequences of gene escape and hybridisation with other maize varieties or landraces. These include the spread of herbicide resistance, and non-target effects on other plant and animals (Cui and Xia 1999, Hillbeck 1999) and soil microorganisms (Benbrook 1999 and Kowalchuk 2003, Koskella and Stotzky 1999, Tapp and Stotzky, 1998). After almost three decades of world wide use, confirmed resistance to glyphosate exists in *Lolium rigidum* (annual ryegrass) in Australia, South Africa, and California; *Lolium multiflorum* (Italian ryegrass) in Chile, *Eleusine ind*ica (goosegrass) in Malaysia; and *Conyza canadensis* (marestail) in certain states of the eastern US. http://www.cropscience.org.au/icsc2004/symposia/2/5/2166_killmer.htm

South Africa had a unique biodiversity with a high level of endemism and the close interface of natural areas with agricultural lands therefore also increases the risks to biodiversity and requires monitoring to take place where GM genes or gene product have entered the wider environment.

Lack of monitoring and compliance

The detection methods developed for the single events should be appropriate for use on the stacked event. A real time PCR method for detection of Event MIR604 and a method for detection of Event GA21 have been developed by Syngenta.

However, a specific and sensitive DNA method is required so that MIR604xGA21 can be distinguished from the single events MIR604 and GA21; PCR with primers flanking the insertion site would easily enable the events to be distinguished, but this has not been carried out.

There is no proposal to monitor any of possible unintended effects (mentioned above) which, contravenes the Convention on Biological Diversity (CBD), the Biosafety Protocol and various environmental laws in South Africa where the monitoring of changes in biodiversity as well as environmental impacts resulting from the transboundary movements of GM seeds. Essentially, the monitoring has been left to country legislation that determines if tracking and labeling is effective and in place. Syngenta state: "The provisions concerning traceability and labeling for placing on the market of MIR604xGA21 maize will allow the prompt identification of products containing or consisting of this maize, and thus enable any unanticipated adverse effects to be effectively traced". However, since South Africa does not have a mandatory labeling and tracking process in place a contamination will only be realised if/when exports of maize arrive in a country of destination where mandatory testing will take place (http://www.asdmas.com/documentos/ICGEB%20Lima%20Bt%20Corn%20Syn%20OK.pdf).

In essence, the company requests a commodity permit for a new GM event, MIR604xGA21, but relies on a large proportion of biosafety assessment from the parent GM lines. This contravenes the very precept of biosafety risk assessment that each GM event must be assessed on a case-by-case basis. It also assumes that crossing these two GM maize to combine the individual events will not result in any polygenic, combinatorial, synergistic or antagonistic effects of these genetic cassettes. This assumption is severely flawed since the interactions of genetic elements are well known and widely studied in plant breeding and molecular biology (e.g. Xu 2003)

In summary, the importation of MIR604xGA21 for human food and animal and feed carries additional biosafety risks in South African (compared to the EU or North America) since unprocessed maize is a staple for many Africans and may also provide the bulk of animal feed resulting in a greater exposure to transgenic proteins and DNA. The importation of maize kernels also carries unacceptable risks of gene escape since maize bought for feed is saved for seed

by farmers and planted. In light of these facts, we request that the Executive Council reject the application.

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