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# ACB'S OBJECTIONS TO SYNGENTA'S APPLICATION FOR FIELD TRIALS OF GM MAIZE (GA21 AND Bt 11xGA21)

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## Description of Application

GA21 and Bt11xGA21 transgenic maize are genetically modified maize events. The Bt11xGA21 stacked maize was produced by the conventional breeding of individual GA21 and Bt11 transgenic events.

GA21 maize produces a modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate.

Bt11xGA21 maize produces modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate; as well as a truncated Cry1Ab protein for control of certain Lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium.

This application is for the continued field trials of GA21 and Bt11xGA21 (Syngenta).

## Problems with identity and integrity of transgenic cassettes

In both GA21 and Bt11 events, we have found a great deal of uncertainties with regard to the nature and integrity of the transgenic cassettes.

GA21: The transgenic cassette GA21 maize is comprised of gene duplications and deletions. There are six contiguous regions derived from the 3.49 kb NotI restriction fragment from pDPG434 employed in the generation of GA21 maize (copies 1-6). Copy 1 contains the rice actin promoter that has a 5' deletion of 696 bp, the actin first exon and intron, the optimized transit peptide, the mepsps gene and the NOS terminator. Copies 2, 3 and 4 are intact versions of the 3.49 kb NotI restriction fragment from pDPG434. Copy 5 contains a complete rice actin promoter, the actin first exon and intron, the optimized transit peptide and the first 288 bp of the mepsps gene which ends in a stop codon and does not contain the 3' end of the mepsps gene nor the NOS terminator. Copy 6 contains the rice actin promoter and a truncated actin first exon only and contains no other elements from pDPG434.

Bt11:Bt11 expresses a synthetic truncated *crylAb* transgene from the soil bacterium *Bacillus thuringiensis kurstaki* that is effective against many Lepidopteran insects and a synthetic *pat* transgene from *Streptomyces viridochromogenes* for resistance to glufosinate herbicides. Each of these is driven by the 35S-CaMV promoter and terminated with the 3' untranslated region of the nopaline synthase (*nos*) sequence- There are therefore in fact, two transgenic cassettes each driven by 35S-CaMV promoter. The company's dossier claimed a single copy insert with the structure:*35S-CaMV-Int II-pat-tnos-35S-CaMV Int VI-crylAb-tnos.* However, analyses by the Belgian Council for Biosafety revealed "primary insert with rearrangements, truncations and unexpected insertions", and "it is not certain if only one copy of the insert is present". Furthermore, 1.1kbp of the plasmid sequence was present at the 5' end of the insert, followed by plant DNA with homology to a 180bp knob specific repeat sequence. The presence of plasmid sequence is of particular concern since

this may contain genetic origins of replication (Col1E1) and the marker gene *pat* that confers resistance to the antibiotic from *Streptomyces viridochromogenes* (phosphinothricin is an antibiotic naturally produced by *Streptomyces viridochromogenes).*The use of the viral CamV 35S promoter also increases biosafety risks. There are risks in the use of 35S-CaMV due to increased rearrangements/ deletions affecting genome integrity and stability and evidence from the laboratory (Koholi et al. 1998 and 2003, Vaden and Melcher 1990) and field studies (Quist and Chapela 2001, Collonier et al. 2000, Ho et al. 2000) that the 35S-CaMV is a recombination hotspot. The increased recombination with other viral elements and may result in the creation of new risk such as the creation of new viruses (Falk et al 1994; Wintermantel et al. 1996, Greene et al. 1994; Ho and Cummins 2000a and b).

The complete biosafety risks of these unintended genetic changes are unknown and uncertain, 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).

There is therefore an urgent need to present a full molecular characterisation of the cassettes of GA21 and BT11xGA21 and to assess the stability and integrity of this transgenic maize in the field. The applicant should analyse 20+ plants in the field; through PCR amplification and DNA sequencing of the transgenic cassette using primers targeting the flanking region of insertion of the transgenic cassettes.

Also of concern is that the application is for “experimental lines of maize event GA21 and BT11xGA21 maize product” (pg5 GA21 application). Specifically, (on page 28 of the GA21xBT11 application) it states that the experimental varieties: X80406, NX74507, X78325” will be imported. There is no description of these experimental varieties or any molecular characterisation presented. What are these experimental varieties?

Uncertainty of the purity of Bt11

There is additional uncertainty as to the molecular characterization of Bt11 because Bt11 was contaminated with an unapproved GMO, Bt10. From 2001- 2004 165 000 tons of maize was sold on international markets (including to South Africa who approved Bt11 for commercial release in 2003- http://cat.inist.fr/?aModele=afficheN&cpsidt=18553781; and http://www.i-sis.org.uk/BT10DMA.php; and http://gmo-crl.jrc.it/doc/Bt10_Executive%20summary.pdf). Therefore, many batches of Bt11 seed may contain the unapproved and significantly different Bt10 GMO. Importantly, Bt10 contains the ampicillin resistance gene that could be transferred to bacteria, thereby compromising the ability to treat diseases. The ampicillin resistance gene should not be present in GMOs since it will spread antibiotic resistance to pathogens thereby compromising our ability to treat present and future disease. This statement is supported by every organization, including the WHO and EFSA and it is for this reason that the EU decided not to approve GMOs with ampicillin resistance genes from 2004. (http://www.gmo-compass.org/pdf/documents/efsa_marker.pdf).

The company requests a permit application for field release of a new GMO event, Bt11xGA21, but relies on a large proportion of biosafety assessment from the parent GMO lines. This contravenes the very precept of biosafety risk assessment that each GMO event must be assessed on a case-by-case basis. It also assumes that crossing these two GM maize events 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 light of these facts, there is even greater need to fully characterize the Bt11 and Bt11xGA21 events (using Southern blotting and PCR) and also to actively demonstrate that Bt10 is not present, but this has not been carried out.

## Spreading of transgenes through pollen flow, seed dispersal and horizontal gene transfer (HGT)

There may be several consequences of gene escape and out-crossing or hybridization with other maize varieties or landraces. Maize has undergone many generations of breeding and natural selection to create numerous varieties suited to South Africa (adapted fror increased resistance to soils, drought, pests etc.)- this forms part of the indigenous knowledge systems and unique seed banks of maize varieties and landraces. Since GMO maize will freely cross-pollinate with non-GMO maize, there are risks of contamination of South Africa's landraces and loss of South Africa's unique maize seed diversity. Contamination of non GM maize by GM maize can result in rejection of maize exported from South Africa by importing countries that have not approved the GM maize. The proposed isolation for containment (pg8) is that there is a 15m fallow area and 12m conventional maize buffer around the field. Also, "The trials will be further planted with a time isolation of at least 4 weeks to any other maize that may be planted within 500m of the trial site". This level of uncertainty provides little confidence that the Bt11xGA21 and GA21 transgenic plants will be isolated from other maize growing in the vicinity for two main reasons. Out-crossing was <0.01% at 500m and no out-crossing was detected at 750m and 2 week of temporal separation (Halsey *et al*. 2005). From this data the isolation should be at least 750m, because a level of 0.01% out-crossing is still unacceptable. There is no evidence is provided of the required survey of farmers in the area (50km2) that will be planting maize at this time. This will establish who will be planting maize and also the time at which they will plant so that confidence in the statement for temporal isolation of transgene pollen-flow is substantiated.

The transgenes may also escape by other means. The dispersal of seed from the trial is also a possibility: by water, bird, mammals (mice, deer, and cattle). In fact, the Greytown GA21 trials (pg3) were destroyed by cattle. The dissemination of the seed by these means has not been considered. Maize plants may also escape into the environment accidentally through the spillage of seeds on transport routes so that feral plants are established. This has been clearly observed in other countries

where people have looked for feral GMOs in the environment. For example, the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea (Kee Woong Park *et al.* 2009). These effects will be exacerbated if the Bt11xGA21 has increased seed dormancy and germination, but the company produces no data to explore this possibility nor does it plan to measure these in the field trial.

The Bt11xGA21 may also have increased weediness compared to its parent non-GMO maize, but there appears to be no aims to address these points or to measure this in the field trial. These problems with feral Bt11xGA21 will only be detected as part of a monitoring program or when maize is shipped to other countries (South Africa's obligations under the Cartagena Protocol on Biosafety- a transboundary movement requires monitoring and prior notification to the affected party). A specific PCR detection method for GA21 and GA21xBt11 is required (see below)

It is now well known that DNA can persist in soil, and in many processed food products. Furthermore, 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 genes for EPSPS, Cry1Ab and pat have homologs in soil bacteria indicating an increased risk for recombination and horizontal gene transfer (HGT) compared to the non-GM parental maize line. Furthermore, a study carried out to determine if transgenic DNA transferred to bacteria of the human gut by HGT, found that this did indeed occur (Netherwood 2004).

There may be several consequences of gene escape and hybridization 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 indi*ca (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

Syngenta has stacked GA21 with BT11 to combine the traits of the Bt insecticide with the dual herbicide resistance (glyphosate and glufosinate) in order to stay ahead this emerging resistance.

## Lack of risk assessment to biodiversity, monitoring and compliance with legislation

There should be a risk assessment process and active monitoring program in place to observe changes to biodiversity. The application seems to deal with assessing agronomic performance without considering biodiversity. There are several issues that have not been addressed by the applicant:

Toxic and allergenic effects of transgenic proteins

The Bt toxin genes (cry) are synthetic versions of the natural genes in the soil bacterium, *Bacillus thuringiensis var. kurstaki*, with coding sequences modified to improve expression in plants. The synthetic genes have not been subject to evolution and their properties relevant to biosafety are unknown and untested. These synthetic Bt genes are usually mutants of those found naturally in *Bacillus thuringiensis*. *Bacillus thuringiensis* that can infect Lepidopteran insects causing cell death and then feed and live heterotrophically from the insects remain (http://topics.scirus.com/Bacillus_thuringiensis_Cry_toxins.html). The Cry proteins are synthesised by *Bacillus thuringiensis* as pro-toxins that are inactive. They only become inactive when they have been ingested by certain insects that have alkaline guts and a protease to cleave the pro-toxin into its active toxin form. In this way the *Bacillus thuringiensis* has co-evolved with its host specificity so that its toxin is inactive until it is ingested by its chosen host. The ecological effects of the transgenic *active* toxin expressed in plants is therefore different to that produced by the *Bacillus thuringiensis* pro-toxin in nature or that of *Bacillus thuringiensis* applied topically by organic farmers. These biosafety risks include the lifetime of activity of the toxin in the environment and also unintended effects on non-target organisms (target and non-target toxic and allergenic effects).

Humans have reported allergy to Bt GM plants in India when that country began commercial planting of Bt cotton in 2002/03 in the Nimad region in Western Madhya Pradesh. Workers began complaining of health hazards after Bt cotton was planted. This prompted a three-member team representing a coalition of non-government organizations to carry out a preliminary survey in six villages in Nimad region which concluded. “All the evidence gathered during the investigation shows that Bt cotton has been causing skin, upper respiratory tract and eye allergy among persons exposed to cotton....” Furthermore, a study funded by the US Environment Protection Agency found that exposure to the Bt “may lead to allergic skin sensitization and induction of IgE and IgG antibodies or both” (1999) and other evidence also indicates that Bt can cause allergy (Koskella and Stotzky. 1997, Benbrook 1999; Venkateswerlu and Stotzky. 1992 and http://www.epa.gov/scipoly/sap/2000/index.htm#october.

## Persistence of Bt toxin in the environment and effects on non target organisms

All living organisms that interact with the transgenic plant (bacteria-birds and human beings) are exposed to high levels of the expressed transgene which is new to their physiology so adverse immunological or allergic responses are possible. For example, non-target organisms may be harmed either directly or indirectly from feeding of insects pests that have consumed the Bt maize plant. Earthworms have been shown to be affected (Birch, *et al.* 1997, Marvier, 2001.) and significant reductions in populations of the beneficial parasites *Microplitis sp*. (88.9% reduction) and *Campoletis chloridae* (79.2% reduction) in *Bt* GMO plants fields have also been recorded (Marvier, M. 2001.) Since the Bt toxins are expressed

continuously at high levels throughout the growing season in the GM maize plant, the levels of Bt toxin can accumulate. It is of concern that Bt can persist in certain soil types for up to 234 days (Koskella, and Stotzky. 1997). There is no evidence to address the degradation of Bt toxins from these events in the environment (soil degradation data from trial field study) nor are these plans included in post-release monitoring. Recent evidence indicate that toxins in transgenic crop by-products affect headwater stream ecosystems by causing mortality on non-target stream insects (Rosi-Marshall, et al. 2007)

This contravenes South Africa's obligations under the Biosafety Protocol on Biosafety, National Environmental Management Act (NEMA, 1998) and the and Biosafety Bill (#1576) in failing to monitor changes in biodiversity as well as monitor GMO transboundary movements. A specific and sensitive method is required so that Bt11xGA21 can be distinguished from the single events Bt11 and GA21. PCR with primers flanking or over-lapping the insertion site would easily enable the events to be distinguished, but this has not been carried out. There is also no proposed method for the specific and sensitive detection of GA21 and Bt11xGA21 so that transboundary movements as well as contamination or co-mingling with other maize in the field as well as the food and feed chain can be monitored.

In summary, there is a total lack of molecular characterization of the GA21 and stacked BT11xGA21 maize. The proposed field trials are to assess agronomic performance and do not address risks to biodiversity and are not accompanied by an adequate monitoring program in order to detect transgene escape. This is required under local (NEMA) and international (Biosafety Protocol) legislation. It is our respectful submission that the application should not be approved.

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