RESPONSE TO AFRICAN CENTER FOR BIOSAFETY OBJECTIONS ON SYNGENTA's APPLICATION FOR GENERAL RELEASE OF Bt11 x GA21: REFERENCE NUMBER 17/3/1-SYNGENTA-10/109

The objections received from African Center for Biosafety (ACB) reflect those previously received for Syngenta applications related to general release, commodity import and field trials. No new objections have been made.

1. POSSIBLE IRREGULARITIES INCLUDING OPEN READING FRAMES AND A TRUNCATED CONSTRUCTS WHICH COULD GIVE RISE TO UNINTENDED GENE EFFECTS

1.1 Bt11

Bt11 maize contains a synthetic version of the *cryIA(b)* gene derived from *Bacillus thuringiensis kurstaki* strain HD1 under the control of a 35S promoter from Cauliflower Mosaic Virus, and IVS 6 intron from the maize alcohol dehydrogenase gene and the nopaline synthase terminator sequence of *Agrobacterium tumefaciens*, and a synthetic version of the pat gene derived from *Streptomyces viridochromogenes* under the control of a 35S promoter from Cauliflower Mosaic Virus, an IVS intron from the maize alcohol dehydrogenase gene and the nopaline synthase terminator sequence of *Agrobacterium tumefaciens*. Data from **Southern analysis and DNA sequencing** demonstrated that a single copy of the *Not* I insert exists in Bt11 maize. Consistent with the expectation, this single copy contains one copy of the *cry1Ab* gene and one copy of the phosphinothricin acetyltransferase (*pat*) gene. Both of the genes require a promoter to ensure expression and in both cases this is the cauliflower mosaic virus 35S promoter. The transformation plasmid pZO1502 was designed specifically in this way and this is what is present in Bt11 maize. Sequence analysis of the entire insert present in Bt11 maize confirms the overall integrity of the insert and that the contiguousness of the functional elements has been maintained compared to the original plasmid sequence. This sequence data confirmed that the insert in Bt11 maize is stably integrated and inherited and was provided by Syngenta to the **Belgian Council for Biosafety.** As a result, the Belgian Council concluded that the Bt11 maize can be considered as safe having no adverse effects on human and animal health[1].

The EFSA panel has issued a positive recommendation stated that 'the placing of Bt11 maize on the market will not cause an adverse effect on human or animal health or the environment in the context of its proposed use. No data have emerged to indicate that Bt11 maize is less safe than its conventional counterpart"[2]. Commodity Clearance approval in South Africa for Bt11 maize was received in 2002, Bt11 x GA21 maize received provisional Commodity Clearance approval in 2009 and Bt11 maize also has conditional General Release approval since 2003.

The 35S promoter originated from the **Cauliflower mosaic virus**. This type of virus infects a wide range of cabbage family crops, including cauliflowers and Brussels sprouts. Similar virus DNA is found in genomes of many plants that have never been in a genetic engineering laboratory. These viruses always replicate using cell nucleus and occasionally insert virus DNA fragments in plant chromosomes, by accident it seems. Thus many food crops including potato, tomato, banana and rice have

[1] http://www.conseil-biosecurite.be/docs/BAC_2004_SC_165.pdf
[2] The EFSA Journal (2005) 213, 1-33

Cauliflower mosaic virus -like DNA fragments in their genomes[3]. They have not triggered production of viruses that cause harm to humans, and their demonstrable safety is a realistic indication that these hypothetical hazards are unimportant to humans. Their common occurrence in plant material makes it unreliable to use chemical detection of the 35S promoter as a means of identifying genetically modified crops. Genetic recombination is a normal feature of conventional plant breeding and of all natural populations, thus recombination and hotspots for recombination are not unique features of the CaMV 35S promoter. There are various arguments to support the view that the CaMV 35S promoter will not increase the risk over those already existing from the breeding and cultivation of conventional crops, as there would be no more risks arising from potential recombination than there are from existing conventional crops[4]. It is also not a potential site of DNA instability in transgenic plants, as proven recently by Kohli and Christou (2008)[5] with the sequencing of the transgenic papaya genome. They provide evidence of stable transgene integration in the papaya genome as well as intact structurally and functionally transfer from generation to generation. There is thus no evidence in literature that the 35S promoter will have any direct effects even if being consumed in abnormal large quantities.

The L-isomer of phosphinothricin (L-PPT) is the active ingredient of the herbicide glufosinate ammonium. Species of the genera *Streptomyces* and *Kitasatosporia* are the only organisms reported to synthesize the amino acid L-PPT. L-PPT has been reported as a component of only two tripeptides, bialaphos and phosalacine. Although Bialaphos is an antibiotic naturally produced by *Shygroscopicus* and *S. viridochromogenes*[6], it is not used therapeutically in human and animal medicine.

1.2 GA21

Sequence analysis of the entire GA21 insert demonstrates that the insert is comprised of six adjacent regions derived from the 3.49 kb *Not*I restriction fragment from the pDPG434 plasmid employed in the generation of GA21 as in detailed explained on page 22 of the dossier. Northern and Western blot analysis failed to detect evidence for transcrips or truncated mRNA of *mepsps* gene. The maize sequence 5' of copy 1 is homologous to maize chloroplast DNA. BLAST analysis of the maize sequence 3' of copy 6 demonstrated homology to several maize sequences in NCBI nucleotide database. The GA21 insert does not contain any additional OTP, *mepsps,* NOS terminator sequences or rice actin sequences other than those associated with the demonstrated structure of the GA21 transgenic insert. Based on the results obtained from compositional, agronomical as well as 44 day poultry studies conducted with GA21 and Bt11 x GA21, no evidence of any adverse effects on human health or adverse consequences to the food chain was found. All these studies support the conclusion that no unintended biological significant changes (such as production of unintended proteins) were detected in GA21 and Bt11 x GA21.

An *in silico* screen for putative **open reading frames** (ORFs) at the junction between the maize genome and the GA21 insert was performed. This assessment defined an ORF as beginning with an ATG and ending with any of the three stop codons (TAG, TAA or TGA) and with a minimum size of 50 amino acids. Employing these criteria,

---

[3] Harper, G. *et al* (2002). Review. Viral sequences integrated into plant genomes. Annual Review of Phytopathology 40:119–36. Numerous bits of viruses are found inside the chromosomes of plants that we eat.
[4] Hull *et al*. (2000) "Genetically modified plants and the 35S promoter: assessing the risks and enhancing the debate." Microbial Ecology in Health and Disease 12:1-5.
[5] Kohli and Christou (2008) "Stable transgenes bear fruit". Nature Biotechnology 26: 653-654
[6] OECD (1999) ENV/JM/MOMO (1999)11

five putative ORFs were identified. Putative ORF1 (98 amino acids) and putative ORF2 (108 amino acids) are wholly contained within the maize sequence 3' of the Event GA21 insert. While these putative ORFs are comprised entirely of maize sequence, due to their proximity to the truncated actin promoter in Copy 6, they were examined further. Putative ORF3 (101 amino acids) and putative ORF4 (163 amino acids) originate in the maize sequence 5' of the GA21 insert and continue into the GA21 insert. The first 17 amino acids encoded by putative ORF4 correspond to the hypothetical Cytochrome C biogenesis protein found in the maize chloroplast DNA that was disrupted upon insertion of the GA21 insert. The presence of organelle sequences in the nuclear genome is not without precedent as this observation has been made previously in several conventional (non-GM) plant species, including maize (Figueroa *et al*., 1999[7]; Fukuchi *et al*., 1991[8]; Goff *et al*., 2002[9]; Kemble *et al*., 1983[10]). In is highly likely that the presence of a functional cytochrome C biosynthesis gene in the maize chloroplast genome of GA21 would compensate for the disrupted version seen in the nuclear genome. Evidence for this is provided by phenotypic and compositional measurements which could find no evidence for disruption of cytochrome C activity and which suggest that GA21 is substantially equivalent to conventional maize. The remaining 146 amino acids are derived from the GA21 insert. Putative ORF5 (126 amino acids) originates in the maize sequence 3' of the GA21 insert and continues into the GA21 insert. All five of the hypothetical proteins represented by these putative ORFs were examined for sequence homology to known toxin and allergens. None of the five hypothetical proteins represented by the putative ORFs identified in GA21 demonstrate sequence homology to proteins known to be toxins or allergens.

1.3 Bt11 x GA21

Bt11 x GA21 was produced through traditional breeding of Bt11 and GA21 maize, it therefore contains the *cry1ab, pat* and *mepsps* **genes** from Bt11 and GA21 maize. Comparative Southern analysis of Bt11 x GA21 maize with the individual parental Bt11 and GA21 maize was conducted to determined the hybridization patterns following stacking of the genes by traditional breeding methods. Data analysis demonstrate that the maize hybrid developed by conventional breeding to combine GA21 and Bt11 maize (Bt11 x GA21) has stably inherited *cry1Ab* and *pat* genes from the parent Bt11 maize and the *mepsps* gene from the parent GA21 maize, retaining hybridization patterns as predicted. In addition, extensive protein expression analysis of GA21, Bt11 and Bt11xGA21 has been performed which support the conclusion of stably inherited trait expression. Since the stability of each insert in each of the single events has been demonstrated, the combination of these two events through conventional breeding crosses is highly unlikely to result in rearrangements of the inserts. In the unlikely event that rearrangements had occurred in the stack product, this would have been detected in the Southern analysis, which is not the case. It can therefore be concluded that Bt11 x GA21 maize will not result in unintended genetic changes. According to the World Health Organization (1995)[11], the conclusions of the safety assessments conducted for each of the individual traits apply to the combined trait products when the traits are

[7] Figueroa, P. *et al* (1999). Transfer of *rps*14 from the mitochondrion to the nucleus in maize implied integration within a gene encoding the iron-sulphur subunit of succinate dehydrogenase and expression by alternative splicing. Plant Journal, 18, 601-609.
[8] Fukuchi, M. *et al* (1991) Analysis of nuclear sequences homologous to the B4 plasmid-like DNA of rice mitochondria; evidence for sequence transfer from mitochondria to nuclei. Current Genetics, 20, 487-494.
[9] Goff, S.A. *et al*. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science, 296, 92-100.
[10] Kemble, R.J. *et al* (1983) Sequences homolgous to episomal mitochondrial DNAs in the maize nuclear genome. Nature, 304, 744-747.
[11] World Health Organization. (1995). Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. WHO Workshop, pp. 1-80, 1995

unrelated and do not affect the same metabolic pathway. This also applies to the double herbicide-tolerance present in maize hybrids Bt11 x GA21, as the tolerance mechanisms rely on completely different mechanisms (detoxification of glufosinate ammonium by the PAT enzyme in the case of the tolerance to glufosinate ammonium; no inhibition of mutated EPSPS protein by glyphosate in the case of tolerance to glyphosate). Potential synergism between transgenic proteins has been considered by previous authorities. Recently, the EFSA panel has concluded that Bt11 x GA21 maize is equivalent in composition and agronomic characteristics to its non-GM maize counterpart. Furthermore, the nutritional properties of Bt11 x GA21 maize do not differ from those of its non-GM maize counterpart[12].

2. GENE FLOW

2.1 The **possibility of introgression of Bt11 x GA21 maize into small farmers maize lines** will be similar than the introgression of non GM maize into the varieties. The percentage of cross pollination with other maize crops in the vicinity will depend on factors such as separation distance, local barriers to pollen movement and local climate and topography. In order for pollen-mediated gene flow and introgression to occur, a number of conditions must be satisfied. Under natural conditions, the two plants must be sexually compatible, fecundity must coincide, a pollen vector must be available, and the progeny plants must be fertile and able to persist in the environment. Pollination by insects is not common, as the insects visit the tassel but rarely the silk. A study under South African conditions determined that although maize pollen of a specific maize genotype can be detected at 400 m from its source, out-crossing declines rapidly from 1% observed at 25m to an out-crossing of 0.36% at 81.6m (Chetty, 2005)[13].

2.2 The **horizontal intact gene transfer** from genetic modified (GM) plants to bacteria with subsequent expression of the transgene is regarded as a highly unlikely event under natural conditions, especially in the absence of selective pressure in the intestinal tract and/or the environment as in detailed explained in the dossier on page 41 and 42.

Bt11 x GA21 maize was produced by combining Bt11 maize and GA21 maize through conventional breeding. Novel DNA sequences in Bt11 maize came from soil organisms and comprise only a minute fraction of the total DNA in the plants. Therefore the cultivation of Bt11 maize will unlikely pose any additional risk compared with the large amount of DNA from the donor microorganisms naturally present in all soils. The *cry1Ab* gene and the *pat* gene expressed in the Bt11 maize are under the control of eukaryotic promoters with limited if any activity in prokaryotic organisms. If such genes would be transferred they would not be functional. The *mepsps* gene expressed in the GA21 maize is under the control of a rice actin promoter and *epsps* genes are ubiquitous in nature. This eukaryotic promoter is necessary for expression of the new protein, mEPSPS. However, this element is not functional in prokaryotic gut microorganisms, as critical DNA sequences are not recognized by the protein expression machinery of bacteria, including those normally present in mammalian intestines. Furthermore, microorganisms including bacteria and fungi contain an endogenous *epsps* gene and corresponding EPSPS protein product. Due to the variety of naturally occurring *epsps* sequences across species in the environment, there are organisms exhibiting a potential for glyphosate tolerance. It is possible that if intact gene transfer did occur and if the *mepsps* was positioned

[12] http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902900450.htm
[13] Chetty (2005). http://etd.uovs.ac.za/cgi-bin/ETD-browse/view_etd?URN=etd-09062005-080914

next to a bacterial promoter, expression of mEPSPS could enhance the tolerance of a microbe to glyphosate. However, native microbial EPSPS's are already ubiquitous and many are relatively insensitive to glyphosate. Thus, in the unlikely event of intact gene horizontal transfer, a selective advantage would not be conferred since many microorganisms already contain a glyphosate tolerant *epsps* gene.

Neither the *amp* gene nor any other antibiotic resistance gene is present in Bt11 x GA21 maize. The *bla* gene coding for ampicillin resistance was only used as a bacterial selection marker during the construction of plasmids pDPG434 and pZO1502 and was not inserted in the GA21 or Bt11 maize genomes. Therefore there is no risk of transfer of the *bla* gene to microorganisms. Consequently, no extraneous DNA sequences intended as marker genes were ever introduced into these plant lines, thus there is no case of any risk of transfer of such genes.

The NOS terminator sequences present in Bt11 x GA21 are of bacterial origin and arecommonly used in the production of genetically modified plants. However, there is no evidence to suggest that the presence of this sequence enhances the potential of intact horizontal gene transfer from GM plants to bacteria. Therefore no change in the ability of the Bt11 x GA21 maize to transfer genetic material to other organism is perceived compared to conventional maize. It should be noted that if intact gene transfer to bacteria were to occur and resulted in successful gene expression, no selective advantage is envisaged since the c*ry1Ab, pat and mepsps* genes are ubiquitous in nature. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in micro-organisms in natural environments.

In relation to the transfer of **novel intact genes** from genetically modified food to bacterial cells via the digestive tract, this is extremely unlikely to occur. In considering the potential impact on human health, it is important to note that humans have always consumed large amounts of maize DNA as a normal component of food and there is no evidence that this consumption has had any adverse effect on human health. Novel DNA sequences in genetically modified foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods. Maize is consumed for thousands of years by humans and animals and no report of intact gene transfer was reported yet. In the very unlikely event that such horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced or expressed in microbial communities. In 1991, the World Health Organization (WHO) issued a report of a Joint FAO/WHO Expert Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO, 1991[14]). It was concluded by that consultation that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

Taking into account the origin of the transgenes in Bt11 x GA21 maize, their naturally occurring related genes, the inherent rapid digestibility of DNA and the lack of selective pressure in the intestinal tract and the environment, the likelihood that intact horizontal gene transfer of the genes would occur is extremely low. In the highly unlikely event that intact gene transfer did occur the possibility to confer selective advantage or increased fitness to microorganisms is very limited. For this reason it is very unlikely that genes from conventional maize or Bt11 x GA21 maize would

[14] FAO/WHO (1991). Strategies for assessing the safety of foods produced by biotechnology. Report of a Joint FAO/WHO Consultation. Geneva, Switzerland, World Health Organization.

become transferred and established in the genome of microorganisms in the environment or human and animal digestive tract. A large number of studies have been performed to date and there are no reports of intact gene transfer from transgenic plants to soil micro-organism in natural systems (Nielsen *et al.*, 1997)[15].

3 HERBICIDE TOLERANCE, USE AND EFFECTS

3.1 Herbicide tolerance and effect on non target species

There is no credible evidence that GM crops would become more difficult to manage than conventional bred crops or create a **super weed.** Bt11 x GA21 will not be more likely to invade other habitats, spread into other crop varieties or create a super weed than any other maize plant. Crop plants bear little resemblance to their wild ancestors, having been selected and bred over many centuries to secure food yields. Since there are no wild relatives in South Africa, potential for development of glyphosate resistant superweeds as a result of cross fertilisation with Bt11 x GA21 maize plants is negligible. Superweeds hybrids are very rare in nature as random cross-breeding almost never yields offspring that can reproduce, let alone flourish.

Selective herbicides in conventional maize, and glyphosate in Bt11 x GA21 maize, provide effective weed control. Selective herbicide and glyphosate application regimes do not necessarily eliminate all weeds, as this may be uneconomic, unachievable, or both. In general terms, therefore, introduction of Bt11 x GA21 maize cultivation to South Africa does not represent a change in management in terms of the use of post-emergence herbicides to control weeds in maize. Herbicides differ in their effectiveness at controlling certain weeds; this leads to the phenomenon of **weed shifts**, whereby continuous use of a particular herbicide regime leads to a change in the species abundance of the weed flora. Weed shifts are unlikely in rotations, but may occur in continuous maize following a change from selective herbicides to glyphosate (or *vice versa*). All weed control systems have strengths and weaknesses, such that if the grower continues with the same system, year on year, natural succession dictates that the least susceptible species will become more troublesome. Growers have always known this and as part of good agricultural practice are advised to use both crop rotation and herbicide rotation to manage (or pre-empt) such problems. Considering the needs of weed control in maize and the characteristics of glyphosate, this product will be widely used in mixture or in sequence with other herbicides with different mode of action with the effect to limit the potential weed shifts. Syngenta's recommendations to counteract resistance build-up can be found in Section 3.3.

Several of the confirmed **glyphosate resistant weed species** have been found in areas where no genetically modified herbicide tolerant crops have been grown, although populations of *Conyza bonariensis*, *Lolium rigidum* and *Plantago lanceolata* have already been identified and reported with known resistance against glyphosate containing herbicides. It should be avoided to spray such populations with glyphosate. Control of glyphosate resistant weeds is achieved in the same way as other herbicide resistant weeds, via the use of other herbicides in mixtures or sequences. Glyphosate use will be sustainable if there is diversity in weed control practices such as using including alternative herbicides, mechanical tools (tillage, mowing, hand-weeding), and biological factors (grazing animals, crop competition)[16].

---

[15] Nielsen, K M *et. al*. (1997). Evaluation of Possible Horizontal Gene Transfer from Transgenic Plants to the Soil Bacterium *Acinetobacter Calcoaceticus* Bd413. Theoretical and Applied Genetics (5Oct)(N-5-6): 815-821.

[16] Duke S.O. & Powles S.B. (2009), Glyphosate-Resistant Crops and Weeds: Now and in the Future. *AgBioForum, 12(3&4): 346-357*

Glyphosate still delivers significant benefits to farmers, given it provides effective control to over 300 weeds, has a history of crop safety in biotech herbicide tolerant crops and has a good environmental profile[17].

3.2. Herbicide use and GM crops

Farmers need to use the best technologies and management techniques to control pests and produce consistent yields. Biotechnology crops with built in resistance provide one more tool for the farmer's toolbox. Different studies come to the same conclusion: lower **herbicide use** in herbicide-resistant crops is possible[18,19]. The use of glyphosate containing herbicides is not new in South Africa, as it has been registered since 1975 under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) and proven safe. Products currently registered are, amongst others, Roundup® (L0407), TOUCHDOWN forte HIGH-TECH® (L7305) and HALEX® (L84150)[20].

The prescribed way of agrochemical usage for biotechnology crops is similar to conventional crops. It is being used and evaluated since 1975 in South Africa. The prescribed amount of glyphosate containing product to be used will be regulated on Bt11 x GA21 maize as on any other crops. The prescribed way of using these products is highly regulated and clearly described on the label of the herbicide, and the farmer in South Africa is obliged under act 36 of 1946 to follow the recommendations. An additional benefit of planting of Bt11 x GA21 maize, the farmer can reduce the frequency of activity required to remove weeds, which will in turn reduce soil erosion and reduce the use of fossil fuels. Also, by enabling more food to be grown on limited land, biotechnology crops can reduce habitat destruction and maintain biodiversity.

3.3. Impact of glyphosate use

Syngenta provides guidance to the farmers on the safe use of herbicide products as well as possible development of weed resistance. Weed resistance is a recognised problem, as it can also occur with conventional crops. In order to counteract a **build-up of resistance to glyphosate**, Syngenta urges the sensible use of TOUCH DOWN forte HITECH and HALEX by posting the following best practice information on their labels:

> For resistance management, TOUCHDOWN Forte HITECH is a group code G herbicide. Any weed population may contain individuals naturally resistant to TOUCHDOWN Forte HITECH and other group code G herbicides. The resistant individuals can eventually dominate the weed population if these herbicides are used repeatedly and exclusively in programs. These resistant weeds may not be controlled by TOUCHDOWN Forte HITECH or any other group code G herbicides.
>
> To delay herbicide resistance:
>
> 1. Avoid exclusive repeated use of herbicides from the same herbicide group code.

---

[17] PG Economics (2009) Briefing note: 19 November 2009. http://www.pgeconomics.co.uk/pdf/OCreportcritiqueNov2009.pdf

[18] Fernandez Cornejo J & Caswell M (2006). The First Decade of Genetically Engineered Crops in the United States. United States Department of Agriculture, Economic Research Service, Washington, DC Available: http://www.ers.usda.gov/publications/EIB11

[19] Sankula S *et.al*. (2005). Biotechnology-Derived Crops Planted in 2004 –Impacts on US Agriculture. [Online]. National Center for Food and Agricultural Policy,Washington, DC. Available: http://www.ncfap.org/whatwedo/pdf /2004biotechimpacts.pdf

[20] http://www.nda.agric.za/doaDev/sideMenu/ActNo36_1947/AR/AR%20Lists.htm

2. Alternate or tank mix with products from different herbicide group codes. Refer to individual product labels when alternating products or when using tank mixtures.
3. Integrate other control methods (chemical, cultural, biological) into weed control programs.
4. Follow a sound crop rotation system wherein different herbicides from different herbicide mode of action classes can be used e.g. follow glyphosate tolerant maize with conventional soya beans in order to satisfy the requirement for crop rotation and the avoidance of repeated use of glyphosate.
5. All cultivation of fields should be done to prevent weeds from flowering and seeding
6. Maintain herbicide use records for each field.
7. Prevent movement of resistant weed seeds and vegetative material to other fields by cleaning harvesting and tillage equipment and planting clean seed.
8. Inspect each land annually in order to identify the development of resistance early.
As populations of *Conyza bonariensis*, *Lolium rigidum* and *Plantago lanceolata* have already been identified and reported with known resistance against glyphosate containing herbicides, it should be avoided to spray such populations with TOUCHDOWN Forte HITECH. Due to the fact that these resistance populations vary in size and localities and are difficult to ascertain it is essential that each land must be inspected annually to identify possible resistance early. If the preventative measures discussed above are not strictly adhered to SYNGENTA cannot be held responsible for the failure of TOUCHDOWN Forte HITECH to control resistant weeds.
1. Always use TOUCHDOWN FORTE HITECH as part of an integrated crop and resistance management program (strategy) in order to prevent weed resistance.
2. This crop and resistance management strategy should always include sequences with herbicides of alternative modes of action.
3. TOUCHDOWN FORTE HITECH should not be used more than twice a year per field. If it becomes necessary to spray escapee target plants, use herbicides from another chemical class.
4. To control and eliminate resistant or possible resistant weeds the aim should be a total prevention of seeding by these biotypes.
5. TOUCHDOWN FORTE HITECH should be used as a tool to manage weed populations in order to • prevent or delay resistance to products of various chemical classes • control various levels of resistance to different products and chemical classes

Weed scientists worldwide are carefully monitoring populations of weeds that are developing resistance to a specific herbicide[21].

3.4. Effect on health and environment

In genetically modified glufosinate-tolerant plants, the L-isomer of glufosinate is rapidly metabolized by the action of the enzyme phosphinothricin acetyltransferase (PAT) into the non-phytotoxic stable metabolite N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinico-butanoic acid). N-acetyl-Lglufosinate does not inhibit glutamine synthetase. Therefore, no phytotoxic physiological effects are observed in genetically modified glufosinate-tolerant plants. Glufosinate is a contact herbicide and is taken

[21] www.weedscience.org

up by the plant primarily through the leaves. There is no uptake from the soil through the roots, presumably because glufosinate is rapidly broken down in soil due to microbial degradation. At 20°C, the soil half-life is less than 10 days.[22] Products containing glyphosate are already in South African agricultural use since 1975, and are registered under Act 36 of 1946. In some cases glyphosate is widely used in conventional maize fields, as it is the only herbicide effective for some weeds. A thorough study on **toxicology on environment, animal and human health** was submitted for the registration of these products to Department of Agriculture (now DAFF). The possible increase in glyphosate containing herbicides as a result of planting of Bt11 x GA21 will not increase the potential risk. Currently farmers are using other herbicides to kill the weeds in maize fields. The use of Bt11 x GA21 may allow the use of a different herbicide which must be approved as safe before use. Syngenta provide guidance to farmers on safe use of the herbicide.

A farmer's aim is to destroy plants in the maize field seen as weeds, if he/she does not use a herbicide, they will use cultural methods. Thus, using a registered herbicide containing glyphosate will not **destroy harmless plants**, as all plants in a farmer's field will be regarded as weeds.

The potential impact of glyphosate on **groundwater** under the South African conditions cannot be compared to the European conditions, as our groundwater depth is much more than the generally shallow conditions found in Europe. South Africa's aquifers are mainly also fractured aquifers, where the groundwater moves through a variety of joints, cracks, fractures and faults. Glyphosate does not meet toxicological criteria to be designated a Priority Substance and its use should not lead to a detrimental effect on water quality status. The proposed environmental quality standard for measure surface water residues for glyphosate is 65 µg/L[23] and the resource protection value of glyphosate according to SEPA[24] is 0.1 mg/L,

The highest risk of water contamination in all sectors comes from point source contamination during mixing, filling and container disposal, and can be almost entirely eliminated by Best Practice during use as explained in Section 3.3. Glyphosate is classed as a non-leacher, and application to soil represents a very low risk of water contamination. Glyphosate is absorbed by clay particles in the soil and is broken down by naturally occurring microbes in both soil and water to harmless substances. This means that agricultural applications of glyphosate in field situations present a very low risk of water contamination[25].

Compositional analysis, agronomical equivalence and broiler feeding studies have confirmed that the Bt11 x GA21 maize is equivalent in composition to conventional maize and is as safe and nutritious as conventional maize. The consumption of poultry diets containing Bt11 x GA21 maize grain did not cause any adverse effects on broiler chickens. All diets supported rapid broiler chicken growth at low mortality rates and excellent feed conversion ratios without significant impact on overall carcass yield or quality.

[22] OECD (2002) Series on harmonization of regulatory oversight in biotechnology, no. 25 Module II: Phosphinothricin. **ENV/JM/MOMO (2002) 14**
[23] http://www.environ.ie/en/Legislation/Environment/Water/FileDownLoad,18282,en.pdf
[24] WAT-PS.-10(1), (2010). Assigning groundwater assessment criteria for pollutant inputs. SEPA
[25] Cerdeira, A.L., & Duke, S.O. (2006). The current status and environmental impacts of glyphosate-resistant crops: A review. *Journal of Environmental Quality*, *35*(5), 1633-1658

3.6. Argentina experience

It is not perceived that **deforestation** in South Africa will be a problem, as Bt11 x GA21 maize will be planted in maize production areas. Production of Bt11 x GA21 maize in South Africa cannot be (directly) correlated to production of **roundup ready soybean** in Argentina.

3.7 Management practises

We believe that management practices requested by other countries cannot be directly extrapolated to South Africa. We also believe the South African Regulatory process should focus on the safety assessment of the GM crop as stipulated by the GMO Act and not duplicate regulation of other technologies such as use of crop protection products that are regulated under Act 36.

An Environmental Risk Assessment (ERA) covering both the GMO concerned and the potential receiving environment have been performed (Appendix 11). The assessment process includes evaluation of the characteristics of the GMO and its effect and stability in the environment, combined with ecological characteristics of the environment in which the introduction will take place. The outcome of the ERA performed for the use of Bt11 x GA21 maize has shown that the risk for **potential adverse effects on human and animal health or the environment** is negligible.

4. PERSISTANCE OF BT TOXIN IN ENVIRONMENT AND EFFECT ON NTO's

Bt11 x GA21 maize was produced by combining Bt11 maize and GA21 maize through conventional breeding. The expression levels of Cry1Ab in pollen of Bt11 x GA21 were similar to those in Bt11 maize and are very low. Apart from the introduced traits (insect resistance and herbicide tolerance), the general characteristics of the crop have not been changed and no adverse effects to human and animal health or to the environment are anticipated, and none have occurred during several years of commercial use of Bt11 and/or field trials of Bt11, GA21 and Bt11 x GA21 in South Africa and other countries. No biologically significant unintended changes in seed dispersal or other traits that might affect the ability of maize to survive without human intervention were observed in Bt11, GA21 or Bt11 x GA21 maize hybrids when evaluated over various years in agronomic field trials in South Africa. The studies demonstrated that both Bt11 maize and GA21 maize were equivalent in agronomic parameters to their near-isogenic non-GM counterparts and that no statistically significant differences were observed as a result of combining the Bt11 and GA21 traits in the stacked Bt11 x GA21 product. The hypothesis of no impact of Bt11 x GA21 maize on non-target organisms was further corroborated in a 44-day feeding study with poultry. The studies showed no adverse effects of consumption of Bt11 x GA21 maize grain compared with consumption of grain of a nontransgenic near-isogenic line. Therefore the potential adverse effects of Bt11 x GA21 maize on non-target organisms will be no different to those for Bt11, which was already concluded safe for general release in South Africa and no reports of adverse effects on the environment have ever been reported in any of the countries where it has been commercialized for years. Thus, Bt11x GA21 maize is highly unlikely to have any direct environmental effects on non-target organisms; that is to say, the effects of Bt11 x GA21 maize on non-target organisms are unlikely to be different from those of non-transgenic maize.

Potential adverse effects on non-target organisms resulting from the general release and cultivation of Bt11 maize were previously assessed as part of the Bt11

application for cultivation in South Africa. The conclusion from these assessments was that Bt11 cultivation is unlikely to result in adverse effects on non-target organisms. Although it is known that Cry1Ab is specific to insects of the order Lepidoptera and an effect on the herbivore stage of these insects could occur, in maize fields herbivore Lepidoptera are considered pests. Outside maize fields exposure to Cry1Ab resulting from the cultivation of Bt11x GA21 maize will be minimal due to the low expression of this protein in pollen. Therefore no adverse effects on non-target non-pest Lepidoptera as a result of cultivation of Bt11 x GA21 maize in South Africa can be expected. The substantial weight of evidence provides a rigorous test of the risk hypothesis of no impact of Bt11 x GA21 maize on non-target organisms at Tier 0, and further testing is unnecessary (Garcia-Alonso *et al*., 2006[26], Garcia-Alonso, 2009[27]; Raybould, 2006[28]).Therefore no adverse effects on non-target non-pest Lepidoptera as a result of cultivation of Bt11 x GA21 maize in South Africa can be expected.

Cry1Ab has a long history of safe use in insecticide products and has been repeatedly shown to be non-toxic to humans and other vertebrates. The use of *Bacillus thuringiensis* in agriculture is not new or restricted to GM crops. *Bacillus thuringiensis* ssp. *kurstaki* is also a registered insecticide in South Africa a.o. Dipel 2xWP, Dipel B^2 WP, Dipel DF WP, Dipel Es SC, Thuricide WP, Biobit HP WP, Rokur WP, Glider WP and exposure to the Cry1Ab protein therefore also occurs through the use of these products. There is no evidence from the history of long use that there is any associated toxicity to humans. The toxicity of this protein is very specific to certain Lepidoptera insects. The lack of activity against many non-target species appears to be due to a number of factors including physical differences in the gut environment and an absence of Cry1Ab-specific gut receptors in other organisms (Frick, 1995). Additionally, there is evidence to demonstrate that the mammalian gut receptors are not comparable to those found in the gut of susceptible insects. *In vivo* studies with rats given Cry1Ab orally, and *in vitro* binding studies with gut tissue isolated from rats, mice, rhesus monkeys and humans did not reveal receptors for the protein (Noteborn *et al.,* 1994[29]).

It was concluded by EFSA (2005[30], 2008[31]) that the influence of Bt11 maize on the variability of non-target Lepidoptera could be expected to be minimal compared with other impact factors (general agricultural management; insecticide use on neighbouring fields, weed abundance; climate). The GMO Panel of EFSA has assessed the study of Rosi-Marshall *et al.* (2007) at its 37th plenary meeting held on 22-23 November 2007 (EFSA, 2007[32,33]), and concluded that based on the available information in the paper, the study shows some weaknesses and that their results do not support their speculative conclusions. It was recently again confirmed that

---

[26]Garcia-Alonso M *et. al.* , (2006). A tiered system for assessing the risk of genetically modified plants to nontarget organisms. *Environmental Biosafety Research* 5, 57-65.

[27] Garcia-Alonso M. ,(2009). Current challenges in environmental risk assessment: The assessment of unintended effects of GM crops on non-target organisms. *IOBC/WPRS Bulletin* 52, 57-63

[28]Raybould A. (2006) Problem formulation and hypothesis testing for environmental risk assessment of genetically modified crops. *Environmental Biosafety Research* 5, 119-125.

[29] Noteborn H.P. *et. al*. (1995). Safety assessment of the Bacillus *thuringiensis* insecticidal crystal protein Cry1Ab expressed in transgenic tomatoes. In: Genetically modified foods. American Chemical Society Symposium Series 605. Engal K-H, Takeoka GR and Teranishi R (eds) American Chemical Society, Washington, DC

[30] EFSA (2005). Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect resistant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. *The EFSA Journal* **213:** 1-33

[31] EFSA (2008). Request from the European Commission to review scientific studies related to the impact on the environment of the cultivation of maize Bt11 and 1507. *The EFSA Journal* **851 :** 1-27

[32] http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej851_review_of_publications_for_risk_assessment_of_maize_Bt11_and_1507_en.pdf?ssbinary=true

[33] http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/GMO_Minutes_37th_plenmeet.pdf?ssbinary=true

earthworms are not affected by genetically modified Bt maize even after several years of cultivation[34].

Maize does not colonise and rarely survives outside the cultivated environment. It has no cross-compatible wild relatives in SA. Therefore, no unintended environmental effects due to the establishment and spread are anticipated. The likelihood of adverse effects on non-target organisms or on soil functions due to the expression of the *cry*1Ab, *pat and mepsps* gene is considered to be very low. The presence of the *pat* gene and the use of glyphosate and glufosinate ammonium are not likely to give an additional botanical diversity effect compared to other herbicides. The possible development of resistance of target organisms to Bt toxin has been identified as a potential risk due to large scale cultivation and/or long term exposure. Resistance to glyfosinate and glyphosate containing herbicides will not, in itself, render maize weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.

Bt11 x GA21 maize was produced through conventional breeding of Bt11 and GA21 maize. Therefore the **detection methods** developed and validated on each single event are applicable to the stack product. For specific detection of Bt11 maize genomic DNA and GA21 maize genomic DNA, real-time quantitative TaqMan® PCR methods have been developed by Syngenta. For each event-specific method, one of the oligonucleotide primers is located within the maize specific flanking sequence and the other is located in the insert. Quantitative event-specific detection methods to detect and quantify maize event Bt11 and GA21 have been validated by the European Commission Joint Research Centre (JRC) and can be found on the DG-JRC CRL website[35]. Detection of the single events could also confirm presence of the stacked product. The use of the Bt11 and GA21 event-specific detection methods have been evaluated and verified for use on Bt11 x GA21 by the JRC[36]. The detection methods provided for maize events Bt11 and GA21 will unambiguously detect the single events, but also the stacked product in a mixture of seed by using single seed analysis and the detection methods for each of the single events.

5. GENETIC MODIFICATION: DEGREE OF CERTAINTY/ RISK

No activity can be said to be without risk and no amount of experimental data can prove unequivocally that an activity will be risk free. The ACB objection implies that Syngenta has very little knowledge of the risk introduced by genetic engineering, compared to the history of safe use of traditional breeding. This is definitely not the case, the assessments and vigorous testing in GM products are much more than in traditional breeding. Many traditional foods, despite their general history of safe use, have not been systematically evaluated for chemical safety, even though they are known to contain potentially harmful components such as allergens and toxins[37]. In the case of Bt11 x GA21 Syngenta performed a very precise safety assessment and we know precisely where the gene been inserted in the genome. The purpose of a risk assessment is to quantify the risk and assess the likelihood of the occurrence as result of this insertion. In the case of Bt11 x GA21, we have concluded that the risk

[34] http://www.gmo-safety.eu/en/news/743.docu.html
[35] http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Validated_Method%20doc.pdf
http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Val_Report.pdf.
http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syng_validated_Method.pdf
http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syng_val_report.pdf
[36] http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11xGA21_val_report.pdf

[37] Knudsen I, *et al*. (2008). Risk management and risk assessment of novel plant foods: Concepts and principles. Food and Chemical Toxicology 46:1681-1

will be no different to that of conventional maize. The safety of Bt11 x GA21 has been thoroughly assessed according to guidelines set out by the South African Authorities. This involves a detailed examination of the where the insert has been incorporated into the maize genome and on how stable the integration is. A hugely detailed comparison of the compositional and agronomic characteristics of Bt11 x GA21 compared to the conventional comparator has been carried out. The expression has been characterised and many mammalian safety studies have been performed. Using this information, a detailed analysis of the impact of the Bt11 x GA21 maize on human health and the environment has been carried out. All the studies performed have confirmed that Bt11 x GA21 maize is as safe and nutritious as conventional maize, with no potential concerns regarding allergens or toxins. Bt11 x GA21 maize is highly unlikely to have any negative impact on human, animal or environment.

6. CONCLUSION

It is our view that the data and citations in our application are true, not misleading and without inaccuracies. All the studies performed have confirmed that Bt11 x GA21 maize is as safe and nutritious as conventional maize, with no potential concerns regarding allergens or toxins. Bt11 x GA21 maize is highly unlikely to have any negative impact on human, animal or environment.