PO BOX 29170, Melville, 2109, South Africa

Objection to Pioneer Hi-Bred's application for trial release of multiple GM maize events

Prepared for the African Centre for Biosafety by Dr. William Stafford, PhD. April, 2012

Introduction

This assessment considers four trial release applications for GMO maize. The proposed trial involves genetically modified (GM) maize and stacked maize from Pioneer transgenic events 59122 TC1507, MON810, NK603. The applications are for approval for trial release of:

1) TC1507
2) TC1507xMON810
3) TC1507xMON810xNK603
4) 59122

The **TC1507xMON810 and TC1507xMON810xNK603 GMO lines containing stacked transgenes** have been generated by conventional breeding using the single GMO parental lines. The stacking of these genes by cross-breeding the parental GMOs results in new GMO lines that express **both the Cry1F and Cry1ab Bt toxins, and both the PAT and EPSPS genes that encode for resistance to the two herbicides, glufosinate and glyphosate.**

The 59122 transgenic maize contains both the Cry34AB1 and Cry35Ab1 genes to incorporate two Bt toxins into the same transgenic line. This GMO event also contains the **PAT** gene for **resistance to the herbicide glufosinate**. The incorporation of the two Cry genes and PAT gene was carried out using a transgenics.

The **proposed benefits** of these GMOs are that they result in **improved crop yields** and **reduced pesticide usage as compared to conventional pest management practices**. The use of several Cry transgenes, expressing different Bt proteins can **protect against several insect pests**, while the herbicide resistance could also lead to **reduce tillage and improved farming practices that help to conserve soil structure.**

The reasons stated by Pioneer for all these trials is to conduct backcrossing and additional evaluations using germplasm of different backgrounds (white and yellow maize) in support of plans for future commercialization in South Africa.

In addition the stated purpose is also to evaluate the efficacy of TC1507xMON810xNK603 maize, with or without applications of glyphosate herbicides. These experiments have to be conducted in an agricultural area to take into account the impact of pedo-climatic conditions and insure full development of the plants, that are intended for general release between 2012 and 2013 (section 3.2.1application). Similarly, for 59122 an additional reason stated for the trial is that Pioneer proposes to add two more locations, and the regulations thus require a new application to be filed (section 2 of application)

Details of GMOs

TC1507 maize was obtained by insertion of a linear DNA fragment (insert PHI8999A) containing the cry1F and pat coding sequences and the necessary regulatory components into maize cells using the particle acceleration method. TC1507 maize expresses the Cry1F protein that confers resistance to certain lepidopteran pests such as the spotted stalk borer (*Chilo partellus*); and the PAT protein, as a selectable marker, that confers tolerance to glufosinate-ammonium herbicides.

MON810 contains the Cry1ab gene, which codes for the expression of the Cry1Ab protein, which confers resistance to certain lepidopteran insect pests such as maize stalk borer (*Busseola fusca*) and maize stem borer (*Chilo partellus*).

NK603 maize was obtained by microprojectile bombardment using particles coated with a purified fragment PV-ZMGT32L obtained from plasmid PV-ZMGT32. The modification is comprised of two copies of a gene element containing a glyphosate tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), from *Agrobacterium* sp. strain CP4 under the control of two separate promoters. The CP4 EPSPS protein is important for aromatic amino acid biosynthesis in plants. Glyphosate inhibits the protein in plants. The CP4 EPSPS protein from *Agrobacterium* is naturally resistant to this inhibition and allows NK603 plants to continue to develop normally in the presence of glyphosate.

59122 maize was generated by the insertion of cry34Ab1 and cry35Ab1 genes, both isolated from Bt (*Bacillus thuringiensis*), and a gene for phosphinothricin acetyltransferase (pat) isolated from *Streptomyces viridochromogenes*. The Cry34Ab1 and Cry35Ab1 proteins, encoded by the cry34Ab1 and cry35Ab1 genes, together comprise an active insecticidal crystal protein that confers resistance to certain coleopteran pests, including western corn rootworm (*Diabrotica virgifera avirgifera*). The PAT protein, as a selectable marker, confers tolerance to the herbicidal active ingredient glufosinate-ammonium.

General Risks and uncertainties regarding molecular characterisation of the transgenics

Characterisation of the hybrid line is assumed to be the equivalent to the sum of the characterisations of the individual parent lines. It assumes that crossing these two GMO maize varieties to combine the individual events will not result in any polygenic or combinatorial effects. 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). The Food and Agriculture Organisation of the United Nations (FAO) have pointed out that the potential risks and benefits of genetically modified organisms (GMOs) need to be carefully **assessed on a case-by-case basis**. **It cannot be assumed that the two inserts or stacked transgenics cassettes act independently and that there are no interactions between the parental transgenes in these new GMO lines.**

The applicant also states that there are single insertion events genome and other changes, but the evidence is not presented, nor are the sites of integration (genome flanking sequence) provided. 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. 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 omission of key supporting data is of serious concern. It is not possible to independently assess claims by the applicant without providing pertinent data that supports these claims. For example section 4.5 and 4.6 of the 59122 Maize dossier states that the event is genetically stable as demonstrated by Southern blots and the introduced genes can be identified by PCR or Southern blots with a reference to annex 1- however none of this data is provided. A similar statement is made in the TC1507xMON810xNK603 application (section 3.6) that no other traits have been introduced or modified but again no molecular data is provided to

support this.

There are additional biosafety risks associated with the transgenic method and components of the transgenic cassette. Transgenic plants are generally produced by two methods:

(1) *Agrobacterium* -Ti system*: Agrobacterium* is a soil bacterium that infects plants to cause crown galls or tumours. It transfers a portion of its DNA into the nuclear genome of the host plant and acquires unusual amino acids (opines) from its host. Most of the machinery for tumour induction is on the tumour-inducing (Ti) plasmid, which has have been developed as a vector for engineering transgenic DNA. The transgene is ligated between the left and right borders of the Ti-plasmid *in vitro*, transformed by electroporation or conjugation into *Agrobacterium* which is then allowed to infect plant cells and mediate the transgenic event. The Ti-DNA insertion occurs at a random place in the host genome and extraneous vector DNA can be integrated. In one study of 112 Ti-DNA insertion events, the correct transgene integration rarely occurred with around 25% of the events having rearrangements and deletions at the insertion site, 10% had insertions from other parts of the plasmid and many of the remaining events having DNA insertions of unknown origin (Forsbach et al.2000; Marton *et al* 2004).

(2) Ballistics: The transgenic DNA is coated onto micro-sphere gold particles (0.4-1.3 um) that are projected into plant cells using a particle gun. Once inside the cell, some of the DNA integrates into the host genome. Ballistics can be used to transfer DNA to a range of cells and tissues including plant cell suspensions, immature embryos, and pollen. The integration events in ballistics are described as complex (Pawlowski and Somers 1996), with multiple copies of delivered DNA often interspersed with small or large fragments of plant genomic DNA (Kohli *et al.* 2003; and Ulker *et al.* 2002). The reasons for the rearrangements, deletions and multiple insertions in both *Agrobacterium*-mediated transformation and ballistics are that the introduction of the DNA into the cell initiates a response that induces plant DNA repair enzymes. During repair of the host genome some of the transgenic DNA is randomly incorporated into the host genome. In addition, there is also evidence of other genome wide changes as a result of the transgenic technology. These may be a result of the transformation event or somaclonal variation (Sala *et al* 2001) during tissue culture and, depending on the extent of backcrossing, some of these mutations will be in the final commercialised GMO. Due to the random integration into the genome, the transgenic cassette also contains a promoter to drive gene expression.

The **promoter used is often also transgenic and is of viral origin** (such as the cauliflower mosaic virus poromoter: 35S-CaMV). There are risks in the use of 35S-CaMV due to increased rearrangements / deletions affecting genome integrity and stability. There is evidence from the laboratory (Koholi *et al.* 1998 and 2003) 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 element may result in the creation of new risks such as the creation of new viruses (Falk *et al* 1994; Wintermantel *et al.* 1996, Vaden and Melcher 1990, Greene *et al.* 1994; Ho and Cummins 2000).

The consequences of these transgenic methods and the use of viral promoters have yet to be fully revealed. However, evidence is emerging that this results in **genome instability,** due to recombinations and arrangements.

For example, in a recent study on five commercially approved transgenic lines carried out by two

French laboratories, all five transgenic inserts were found to have rearranged, not just from the construct used in transformation, but also from the original structure reported by the company. This was clear evidence that all the lines were genetically unstable. Further evidence from the Service of Biosafety and Biotechnology (SBB) of the Scientific Institute of Public Health (IPH) in Brussels (http://biosafety.ihe.be/TP/MGC.html) reports on the molecular characterisation of the genetic map of six transgenic lines, four of which overlap with those analysed by the French laboratories. The Brussels reports are an overview of data presented at a meeting of the Belgian Biosafety Advisory Council. The reports found evidence of genetic instability similar to those described in the French study.

The new EU Directive 2001/18/EC on deliberate release of GMOs also requires information documenting *genetic stability* (Annex IIIB) as a condition for market approval. Genetic stability can only be demonstrated by 'event specific' molecular data of the kind carried out in the two studies. In view of the finding that practically every transgenic insert has rearranged from that reported in the company's original dossier, it would indicate that the transgenic lines have failed the test of genetic stability, and are no longer the same lines that were risk assessed, and in some cases, placed on the market. This has important safety implications. Rearrangements and deletions are signs of structural instability, which enhances horizontal gene transfer and recombination, with all the attendant risks.

For Mon 810, Company data showed that the insert has a P35S driving a *crylAb* synthetic gene with terminator T-nos. Maize heat shock protein intron is located between P35S and *crylAb*. Analysis revealed however, that T-nos and part of the 3' (tail) end of the *crylAb* gene have been deleted. T-nos is detected elsewhere in the genome, indicating that it may have moved from its original position. The 5' (head) end of the insertion site shows homology to the long terminal repeats (LTR) of the maize alpha Zein gene cluster, but no homology to the maize genome was detected at the 3' site, indicating that there had been scrambling of the maize genome at the insertion site. The strong P35S promoter could therefore be driving the transcription of an unknown gene downstream.

Researchers from the Institute of Molecular Biology in Barcelona, Spain, analysed MON810 maize Certified Reference Material (CRM) obtained from the European Commission's Institute for Reference Materials and Measurements (IRMM) and commercialised by Fluka (Buchs, Switzerland). They found that the transgene insert had rearranged and probably moved, yet again, from its whereabouts reported a year ago, when MON810 maize, along with at least 5 other lines, were found to have rearranged, and no longer matched the genetic maps provided by the companies.

Recently, researchers in the Industrial Toxicology Research Centre in Marg Lucknow, India, have also analysed the MON810 insert using multiple PCR primers, and came to the same conclusion. Their finding "confirms the structural instability of MON810 transgene cassettes." **Contrary to Monsanto's claim that *nptII* is absent in MON810, they consistently found the presence of *nptII* as well as *Tnos* in their sample.** This inconsistency has been noted previously (ISIS). [1]

Risks of using Antibiotic resistance marker genes

Current evidence shows that horizontal gene transfer (HGT) to bacteria does occur and is

1 ISIS website: http://www.i-sis.org.uk/MON810gmMaizeMiceImmuneSystem.php
http://www.i-sis.org.uk/UTLI.php

significant and occurs at a high frequency when sequence homology is present. Horizontal transfer of DNA occurs at very low frequency under laboratory conditions. For example *Acinetobacter,* a soil- and water-borne bacterium (Gebhard and Smalla, 1998), *Streptococcus gordonii,* a cause of dental cavities and heart valve infection (Mercer et al., 1999), *Aspergillus niger*, a fungus harnessed to produce citric acid for soft drinks (Hoffmann et al., 1994). Crucial to the detection of HGT is the use of assay systems that are sensitive enough to detect even very rare events. The detection limits of some culture-based methods (typical detection limit of 10^{-8}–10^{-11} HGT events per bacterium) can exceed expected rates of HGT (10^{-16}–10^{-17}) by several orders of magnitude. Harbouring a plasmid with an nptII gene containing a small deletion (hence non-functional) was used to detect the frequency of HGT from plants containing transgenic DNA. The nptII gene in transgenic potato plants coding for kanamycin resistance, transforms naturally competent cells of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* BD413 with the same high efficiency as nptII genes on plasmid DNA ($3x10^{-5}$ -$1x10^{-4}$) despite the presence of a more than 10^6 fold excess of plant DNA. However, in the absence of homologous sequences in the recipient cells the transformation dropped by at least about 10^8 fold - 10^9 fold. This indicates that recombination in bacteria is most efficient where sequence homolgy is present (de Vries J, Wackernagel W 1998). The npt11 gene has many gene homolgs 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).

The main concerns are that GMOs containing antibiotic resistance maker genes will spread antibiotic resistance genes amongst pathogens via HGT. As regards to selection or selective pressure, the use of antibiotics such as kanamycin (and related B-aminoglycoside antibiotics such as spectromycin since there is cross- resistance ; Onaolapo J. 1994, and Mikkelson et al 1999) will place a selective pressure on selection of transgenic contructs that have transferred to the intestinal bacteria. Kanamycin is still used in operative procedures of colon and rectum and to treat ear infections and has also been found to be effective against E coli 0157 (Ishikawa et al., 1999, 5, 86-90, Hehl et al. 1999, Yelon J, et al. 1996, Ito et al. 1997). The selective pressures that would confer advantage to soil bacteria are poorly studied but may confer an advantage *per se* since many antibiotics are produced by Actinomycete bacteria to kill competing bacteria in the soil; acquiring antibiotic resistance may acquire a selective advantage resulting in a change in soil biodiversity and functioning.

Selective pressures may also include several stresses such as soil tilling or application of agrochemicals since current evidence suggests that a stress response facilitates the HGT and spread of antibiotic resistance genes. For example, the SOS response—induction of specific genes in response to DNA damage—alleviates the repression of genes necessary for horizontal transfer of the mobile integrating conjugative element SXT. This is a ~100 kb plasmid derived from *Vibrio cholerae* that confers resistance to the antibiotics chloramphenicol, trimethoprim, streptomycin, and methoxazole. (Beaber et al., 2003).

The emergence of bacterial antibiotic resistances as a consequence of the wide-scale use of antibiotics has resulted in a rapid evolution of bacterial genomes. Mobile genetic elements have played a key role in the spreading antibiotic resistance genes amongst bacterial populations and contribute to multiple antibiotic resistance by bacterial pathogens (Salyers and Shoemaker, 1994; and Witte., 1997). Therefore there are risks associated with the spread of antibiotics-resistance genes amongst soil bacteria or to the human gut bacteria, even when there is no selection for the transgenic construct per se (such as selection for kanamycin resistance).

Practically every medical organization that has looked at GMO crop safety has expressed concern, including the American Medical Association, World Health Organization, UK Royal Society, United Nations Food and Agriculture Organization, Pasteur Institute, European Food Safety Authority, and the British Medical Association. Alerted to these risks, the European Union decided to prohibit and phase out GMOs with antibiotic resistance genes after the 31st December 2004 (directive 2001/18EC and Revising Directive 90/220/CEE). The EU has taken a pragmatic approach where antibiotic resistance genes are classed into three categories based upon how useful the respective antibiotics are in medicine (to treat disease). The nptII is class1 that may still be used (class 2 and 3 to phase out and not allowed).

The presence of antibiotic resistance amongst microorganisms compromises our ability to treat disease and therefore it is in our interest to limit the spread of antibiotic resistance. The fact that there is already a problem with the spread of antibiotic resistance amongst microorganism and the development of multiple resistant bacteria does not mean that this situation cannot be exacerbated by the further spread of antibiotic resistance genes! Although the B-aminoglycoside antibiotics (such as kanamycin) have limited use in the West for treating diseases, they are important in developing countries such as South Africa because they are used to treat diseases such as TB. Therefore the use of npt11 carries different Biosafety risks in South Africa. With the cross-resistance of B-aminoglycoside antibiotics and the spread of multiple resistance genes between microorganisms it can be expected that the use of npt11 will compromise our ability to treat disease. This is very relevant in South Africa (but not so much in the West) since cross resistance of B-aminoglycoside antibiotics is well recognized and sprectromycin is used to treat widespread diseases such as TB (Heifets, L. B. 1991, Onaolapo J. (1994) and WHO 1997).

These GMOs are reported not to contain antibiotic resistant marker genes, as the herbicide resistance gene was used as a selectable marker during construction of the transgenic. However, the evidence relating to the identification of the nptII gene in MON810 raises these concerns, and the current field trial should incorporate determinations of the stability of the transgenic cassette in the field. There are no details in the applications as to whether sensitive methods such as PCR will be used to determine gene known integrity and stability. Given the implications for risk assessment, and the consequent huge uncertainties in terms of assessing negative impacts when the genetic nature of the GMO is unknown, it is pertinent that the molecular characterisation be carried out using a comprehensive survey of several individuals; at the outset (ie testing seed for planting), during growth in the field and the characterisation of the F1 progeny generated during the field trials.

<u>The spread of Herbicide resistance and resistance of insects to Bt</u>

These GMOs, expressing herbicide resistance and producing Bt-insecticidal toxins may have impacts in terms of non-target effects, the generation of multiple herbicide-resistant weeds and changes in soil biodiversity and function (Kowalchuk *et al.* 2003.; Doeffler *et al.* 1997 and 1998; Snow *et al.* . 2004; Doolittle *et al.* 1998; Hillbeck *et al.* 1998; Benbrook *et al.* 1998). The over-reliance on glyphosate herbicide in genetically modified (GM) glyphosate-resistant cropping systems has created an outbreak of glyphosate-resistant weeds (Duke and Powles 2009, NRC 2010). Over recent growing seasons, the situation became severe enough to motivate hearings in the US Congress to assess whether additional government oversight is needed to address the problem of herbicide-resistant weeds (US House Committee on Oversight and Government Reform 2010). Biotechnology companies are currently promoting second generation GMO crops resistant to additional herbicides as a solution to glyphosate-resistant weed problems. We believe

that this approach will create new resistant-weed challenges, will increase risks to environmental quality, and will lead to a decline in the science and practice of integrated weed management.[2]

There is a dramatic rise in the number and extent of weed species resistant to glyphosate (Heap 2011), and a concomitant decline in the effectiveness of glyphosate as a weed management tool (Duke and Powles 2009, NRC 2010). The number and extent of weed species resistant to glyphosate has increased rapidly since 1996, with 21 species now confirmed globally (Heap 2011). Although several of these species first appeared in cropping systems where glyphosate was being used without a resistant cultivar, the most severe outbreaks have occurred in regions where glyphosate-resistant crops have facilitated the continued overuse of this herbicide. The list includes many of the most problematic agronomic weeds, such as Palmer amaranth (*Amaranthus palmeri*), horseweed (*Conyza canadensis*), and Johnsongrass (*Sorghum halepense*), several of which infest millions of hectares (Heap 2011).

The result of the extensive use of these herbicides over vastly expanded areas will likely create 3 interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management.

A similar problem has been reported with growing Bt resistance. Resistance monitoring data from five continents, reported in 41 studies that evaluate responses of field populations of 11 lepidopteran pests to four *Bt* toxins produced by *Bt* corn and cotton. After more than a decade since initial commercialization of *Bt* crops, most target pest populations remain susceptible, whereas field-evolved resistance has been documented in some populations of three noctuid moth species: *Spodoptera frugiperda* (J. E. Smith) to *Bt* corn in Puerto Rico, *Busseola fusca* (Fuller) to Cry1Ab in *Bt* corn in South Africa, and *Helicoverpa zea* (Boddie) to Cry1Ac and Cry2Ab in *Bt* cotton in the southeastern United States (Tabashnik et al 2009). Bt toxins kill by binding to target sites in cell membranes of the mid-gut and disrupt the membranes. One prominent mutation in resistant bollworm involves cadherin, an adhesion protein that binds together cells in solid tissue, thereby preventing disruption of the gut cells [10]. Recently, incomplete recessive alleles of Cry1Ac and Cry2Aa have been identified in bollworm during screening of Bt-cotton crops [11]. Apparently, the finding was not considered an "outbreak", even though it could be the start of one.

To stave off the impending threat of resistance outbreaks, regulators have introduced the ‘refuge’ strategy; the planting of non-Bt crops to prevent or slow the evolution of resistance. The refuge strategy is based on the assumption that resistance will be recessive, so sensitive heterozygotes will die from consuming the Bt crop. If the mutation is dominant or incompletely recessive, resistance will spread despite the refuge. Greenhouse tests showed that the refuge could prevent the spread of resistant mutants if it was maintained as a block of non-Bt crop, rather than as a mixed crop of Bt and non Bt plants. Regulators in North America have set a minimum of 20% non-Bt crop in block-planting.

The introduction of the refuge has meant that farmers would have to deal with the potential of 20% of their crops becoming infested, so regulators allowed the refuge to be sprayed with pesticide. In a

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2 Navigating a Critical Juncture for Sustainable Weed Management. David A. Mortensen, J. Franklin Egan, Bruce D. Maxwell, Matthew R. Ryan, Richard G. Smith. BioScience, Vol. 62, No. 1 (January 2012), pp. 75-84. American Institute of Biological Sciences. http://www.jstor.org/stable/10.1525/bio.2012.62.1.12

position paper produced by the Environment Protection Agency and the United States Department of Agriculture, it states that, "In corn growing areas (no cotton), growers should plant a minimum of 20% non-Bt corn to serve as a refuge. In areas where European corn borer (ECB), southwestern corn borer (SWCB), corn earworm (CEW), or other target lepidopteran pests have historically been high, insecticide treatment of the refuge is anticipated. (Morin et al 2003, Burd et al 2003, Tang et al 2001)

A monitoring system must be in place to observe unpredictable effects that may occur in the field trial. The monitoring system requires a sensitive and specific method (such as PCR) to quantify the GMO and observe changes in integrity, stability and HGT of the transgenic cassette. Additionally, it should be designed to observe changes in morbidity and mortality of non-target organisms, and measure typical parameters important for effective agriculture (soil fertility, water and air quality). It is also important to establish aspects of traceability so that observed changes can be tracked to a particular causative agent (Regattieri *et al.* 2005). **Despite the opportunity to study the problem of herbicide-resistance and resistance to Bt-insecticides, these field trials have not assessed the level of insect resistance to Bt with and without herbicide treatments (ie selective pressures), which would be an important finding to derive from these trials. Furthermore, it is a concern that a specific PCR method has not been identified to distinguish the TC1507xMON810 and TC1507xMON810xNK603 from the individual GMOs TC1507, MON810 and NK603. These could be present in a sample of mixed seeds, which could have potentially important consequences for traceability and liability.**

Horizontal gene transfer (HGT) and gene escape: Implications for maize varieties, landraces and liability

It is now well known that DNA can persist in soil, and 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 EPSPS, PAT and genes all have gene homologs in soil bacteria indicating an increased risk for horizontal gene transfer. 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 1990).

There may be several consequences of gene escape and hybridisation 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 for increased resistance to soils, drought, pests etc.). This forms part of the indigenous knowledge systems and unique seed banks. Since GM maize will freely cross-pollinate with non-GM maize, **there is a huge risk of contamination of South Africa's landraces via out-crossing, and the loss of South Africa's unique maize seed diversity.** The issue of co-existence has not been addressed, perhaps because it is assumed by the applicant that the GMO will escape during the field trial. However, the methods used to address this and monitor escape have not been adequately dealt with. Only the emergence of volunteers in the field after the trial has been completed has been addressed in the dossiers.

The details of proximity to other maize plantations, timing of harvest and the geographical and physical methods employed to reduce seed and pollen dispersal have not been described. It is therefore not possible to assess the risks are of gene flow and gene escape. In the TC1507xMON810xNK603 dossier the isolation distance is described as being 400m (section 4.9, however the monitoring of risks that should be detailed in section 4.9.3 refers to section 15.1 which is not present in the dossier obtained). From relevant scientific

literature, maize out-crossing was <0.01% at 500m and no out-crossing was detected at 750m and a 2 week temporal separation (Halsey *et al*. 2005). Thus, the **isolation should be at least 750m, because a level of 0.01% out-crossing is still unacceptable.** There is no evidence provided of the required survey of farmers in the area (50km^2) 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 trans-gene pollen-flow is substantiated.

A lack of co-existence of GMO with non-GMO maize can result in rejection of maize from importing countries that have not approved this transgenic, as well as the spread of herbicide resistance, and non-target effects on other plants animals (Cui and Xia 1999, Hillbeck 1999) and soil microorganisms (Benbrook 1999 and Kowalchuk 2003, Koskella and Stotzky 1999, Tapp and Stotzky, 1998). There are also liability issues when transgenic maize contaminates (cross-breeds or co-mingles) with non-transgenic maize such as other maize varieties or maize land races. Though it has yet to happen in South Africa, in the United States thousands of farmers have been prosecuted on behalf of Monsanto, or forced into out of court settlements, as a result of HGT from transgenic to non-transgenic field.[3]

In summary, these field trials of stacked herbicide-resistant and insecticidal genes represent new GMOs. These should be assessed independently on a case-by-case basis, as opposed to relying on evidence from the single events and supposing that the stack transgenics behave in a similar way to the combination of the individual parental GMOs. The molecular evidence to support the claims of gene stability and integrity in the stacked transgenics are unfounded. The current field trials are claimed to be needed to test agronomic performance in both white and yellow maize backgrounds, but failed to address problems that might emerge with insect resistance, herbicide resistance and gene escape that can lead to loss of maize varieties landraces and impacts to biodiversity.

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