

# **Objection against the General Release of MON87460 X NK603 X MON89034 in South Africa**

**Submitted by: The African Centre  
for Biodiversity**

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**african centre for biodiversity**

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# INTRODUCTION

The African Centre for Biodiversity (previously 'Biosafety') (ACB) was established in 2003 and registered in 2004. ACB carries out research, analysis, capacity and movement building, and advocacy, and shares information to widen awareness and catalyse collective action and influence decision-making on issues of biosafety, agricultural biodiversity and farmer-managed seed systems (FMSS) in Africa. The ACB's work both informs and amplifies the voices of social movements fighting for food justice and food sovereignty in Africa.

The ACB has played an essential watch-dog role on new GMO permits in South Africa for a decade now, adding substantially to the discourse about the scientific assessment of GMOs as well as issues of socio-economic impacts and democratic decision-making, through lodging substantive comments on at least 30 permit applications.

The African Centre for Biodiversity has consistently opposed the introduction of genetically modified (GM) maize being marketed as a drought-tolerant variety. ON the 7th August 2015, the ACB launched an application to review the decision by the Executive Council to approve commercial release of MON 87460. Although this review was not successful, the ACB is in the process of having the decisions to allow the commercialisation of MON 87460 set aside by a High Court in SA.

In 2016, the ACB also submitted an objection to the extension of field trials, supported by a petition with over 20 00 signatures. In addition, 63 members of the public copied the objections they have submitted to the Registrar: GMO Act regarding these trials. Members of the public have similarly in respect to this application, signed a petition opposing these trials as well as 71 having submitted direct objections to the Registrar: GMO Act which was copied to the ACB. Information with regard to this petition will be handed over to the Registrar in due course.

## **Acknowledgments**

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# KEY CONCERNS

## 1. Claims that MON87460 X NK603 X MON89034 improves yield during drought unsubstantiated.

- Drought tolerance is a highly complex genetic trait that cannot be addressed by single gene insertions, as shown by the lack of data backing up the applicant's claim that this GM variety shows "improvements to yield under drought stress".
- While the field trial data states statistically significant differences in glyphosate-tolerance in the GM crop, it fails to report statistically significant improvements on yield under drought stress.
- Most of the empirical data is removed on the grounds of confidential business information, making any claims by the applicant impossible to verify.

## 2. Molecular concerns

- Lack of information included on the characterisation of the inserted transgenes. These transgenes have been made synthetically and therefore have no history of safe use.
- Introduced genetic elements such as the cauliflower mosaic virus and the nos-3 terminator sequences introduce known hazards that may introduce instability of the transgenes and/or production of novel nucleotide sequences. Such risks have not been tested for.
- Incomplete information is provided to substantiate claims of the insertion of transgenes in terms of integrity of the transgene and copy number of insertions
- Parental lines have been shown to have altered compositional profiles in peer-reviewed independent data. The applicant fails to mention this and provides information on techniques with limited sensitivity to confirm no alterations in transcriptome, proteome or metabolome has occurred.

## 3. Safety Assessment

- The applicant claims substantial equivalence to conventional varieties without data using the latest global profiling techniques that allow for

unbiased and sensitive screening of altered composition of plant constituents. Numerous studies have shown non-equivalence of GM crops including the parental NK603 variety. Claims of substantial equivalence are thus unfounded.

- 'History of safe use' cannot be claimed for this crop. Maize is a staple crop in South Africa and therefore the quantity of transprotein does not compare to those present in other foods such as pro-biotics and cheese. Since there are intended and unintended changes in the transgenes, they are also not equivalent to those that exist in nature.
- Allergenicity studies are limited to predictive analyses that are not thorough enough to assess all potential allergenic properties of the transproteins. Cry toxins have been shown in numerous studies to cause immune reactions, questioning the reliability of these protocols
- Dietary exposure is based on limited data on expression levels of the transproteins in a single trial season in the US. Transgene expression is known to be variable depending on environmental factors. No data on expression levels exist from the South African field trial data to be able to estimate potential exposure levels.

## 4. Environmental Assessment

- Limited information was provided on the potential hazards and risks to non-target organisms. Only a summary description of results obtained from field trials in the US on 8 species was described. This is of limited relevance to the South African context. Risk Assessment should take into account the receiving environment of the crop, as has been performed in Kenya, Vietnam and Brazil.
- The applicant states that no risk management plan is required for monitoring of the crop post-commercialisation. This is a complacent approach that prevents the identification of any effects not identified during the risk assessment process.
- Insect and weed resistance are expected outcomes following widespread cultivation of such GM crops, including South Africa. Weeds resistant to

glyphosate are now spreading at epidemic levels in many GM growing regions. Pests resistant to Bt have also been documented across the world including South Africa. A study on parent MON 89034 indicates that expression of the Cry toxins is only moderate, thus promoting the spread of resistance. A refuge strategy in this case cannot be expected to be successful in delaying resistance onset.

## 5. Socio-economic considerations

- A generalised claim that GM biotechnologies have improved environmental and economic standards goes against many independent studies that contest claims of improved yields, economic gains and reductions in pesticide use. Such claims are not relevant to South Africa and the context of small holder farmers.
- Small holder farmers of GM cotton in South Africa has failed in the last 14 years, serving as an example that GM crops are not a techno-fix that can improve farmer livelihoods.
- A recent study shows that GM maize has been outperformed by non-GM and open pollinated varieties in South Africa. Local varieties are better suited to small-holder agro-ecologies, fluctuations in environmental conditions and maintain seed and food sovereignty for farmers and consumers

## 6. Conclusions

- GM crops sold for drought conditions are a marketing opportunity in an era of unpredictable climate change. Stacking this trait with herbicide tolerant and insecticidal traits is a mechanism to prolong the utility of these traits and the sales of their associated pesticides.
- During times of climate unpredictability, industrialised GM agriculture that promotes genetic uniformity and ecological destruction in the form of chemical use will only serve to exacerbate the problem and leave farmers more vulnerable to climate extremes. A shift towards dietary and agricultural diversity that promotes the cultivation of local crops that are adapted to the local climate is urgently needed.

# SUMMARY OF THE APPLICATION

Approval of commercial release of Monsanto South Africa's MON87460 x NK603 x MON89034 with the intention for cultivation in the entire region of South Africa. The crop event is summarised in Table 1.

**Table 1.**

Crop Event	Traits of interest	Genes introduced
MON87460 x NK603 x MON89034	Drought tolerance, insecticidal activity, Glyphosate tolerance	<i>cspB</i> , <i>Cry2Ab2</i> and <i>Cry1A.105</i> , <i>CS-cp4 epsps</i> (two copies)

This crop is a 'stacked variety' where two or more GM varieties are combined from traditionally cross breeding the GM parental lines MONm87460 with MONm89034 and NKm603.

MON 87460 contains the bacterial cold shock protein B (*CspB*), derived from the common soil bacterium *Bacillus subtilis*. According to Monsanto's general release application for MON87460, the *cspB* gene helps to preserve cellular functions during certain stresses' and 'reduces yield loss, primarily through increasing kernel numbers per ear'. It also contains the antibiotic resistance marker *nptII*, conferring resistance to neomycin and kanamycin antibiotics.

MON 87460, or 'Droughtgard', was first commercialised in the US from 2011. Its introduction into South Africa for trials stems from a Monsanto/Gates Foundation project, Water Efficient Maize for Africa (WEMA). The project is being implemented in South Africa, Kenya, Uganda, Tanzania and Mozambique, and purports to offer the GM drought tolerant maize to smallholder farmers in Africa as a 'Climate Smart' solution to abiotic stresses such as drought.

The first field trials took place in 2007 in Hopetown and Orania in Northern Cape. In 2009, they took place in a further three

locations, Delareyville, Lutzville and Pretoria. The ACB has previously submitted objections to these trials (ACB, 2007; 2010; 2012). On 7th August 2015, the ACB launched an application to review the decision of the Executive Council to allow the general release of MON 87460 (2015). Such approval means that Monsanto can sell the GM maize seed, MON87460, to farmers in South Africa for cultivation.

MON 89034 is a stacked Bt crop, containing two Bt toxins, Cry2Ab2 and Cry1A.105. Cry1A.105 (also known as CS-cry1A.105 3.53) is not one Bt toxin, but a protein comprised of naturally occurring Cry1Ab, Cry1F, and Cry1Ac proteins. The gene cry1A.105 is a chimeric gene comprising of 4 domains from other cry genes previously used in transgenic plants. Bt insecticidal toxins were isolated from the bacterium *Bacillus thuringiensis* subsp. kurstaki Strain HD-1 and *Bacillus thuringiensis* subsp. Kumamotoensis.

NK 603 contains two copies of the CS-cp4 epsps gene from the bacterium *Agrobacterium tumefaciens* CP4, for glyphosate herbicide tolerance.

Field trials for the stacked variety MON 87460 X NK 603 X MON 89034 were conducted during growing seasons 2014/2015 (permit number: 39.4(4/14/396)), and 2015/2016 (permit number: 39.4(4/15/171)) At Hopetown, Orania and Malelane. Trials for a third season are currently underway.

## UNSUBSTANTIATED CLAIMS OF DROUGHT-TOLERANCE

Drought tolerance is a highly complex, quantitative trait involving a network of many finely tuned and interacting genes affecting the entire physiology of a plant. At least 60 genes have been linked to drought-tolerance in plants. Controlling such a network via the introduction of a single gene is therefore not widely considered a successful strategy for generating drought tolerant varieties. The successful manipulation of so many genes

without side effects, to adapt to a number of conditions, is also a very long way off current scientific knowledge.

Based on documents that can be publicly evaluated, the claim of drought tolerance has not been established and remains unpublished. The claim that the integration of the cspB transgene improves tolerance against drought rests entirely on unpublished claims by the producer. The US authority responsible for exempting MON 87460 maize from regulation, the USDA, judges the claimed 'drought tolerance'; to be at best moderate, and comparable or less so than drought tolerance in non-GM, conventionally bred maize varieties that are available also as open pollinated varieties with no intellectual property claims associated with it. How cspB maize performs comparatively to these known and documented maize varieties with tolerance to drought, in particular those that emerged from the Drought Tolerant Maize for Africa (DTMA) project, is also unstudied and undocumented.

The applicant cites two studies to support the claim that a single gene event can increase drought-tolerance. However, even the applicant's own published study reports a disappointing 6 % reduction in yield loss from the 15 % loss observed under water-limited conditions over three seasons in the US, with one season observing a 0 % change in yield in comparison to conventional varieties (Nemali et al., 2015). Though this study was purporting a "yield increase" there was in reality, a 9 % yield loss under water-limited conditions. Further, a recent study also reported that MON 87460 is estimated to increase maize productivity in the US nationwide by a mere 1 % (Union of Concerned Scientists, 2012), questioning any likely benefit of this crop to overall maize production if introduced into South Africa.

### **Trial data do not substantiate claims of drought-tolerance**

It is impossible to substantiate claims of drought-tolerance of MON 87460 x MON 89034 x NK603 since the trial data for both seasons has been deleted on the grounds of 'confidential business information' (CBI).

Nevertheless, there are a few sentences that allude to disappointing data and lack of yield gains.

First, in the trial report for the 2015/2016 season, while declaring statistically significant efficacy of both the herbicide-tolerance, it fails to mention any statistically significant reduction in drought-stress or yield. The application goes on to state:

*“Our experience in testing transgenes controlling quantitative traits, is that unpredictable biotic factors, such as excess rain or extreme drought, can impact the testing season and that multiple testing are often required to obtain the ideal conditions under which to assess the drought trait and achieve statistically significant results”*

and goes on to conclude:

*“MON 87460 × MON 89034 × NK603 provides statistically significant improvements to stalk borer attack and tolerance to Roundup herbicide application, and also provides improvements to yield under drought stress.” This conclusion reveals a lack of statistically significant results on improvements under drought stress.*

Even claims of a non-statistically significant improvement cannot be substantiated without information on what controls they were compared to. It also remains undefined how drought-stress was even measured and what it was compared to. Important questions remain with regards to the conventionally bred varieties that the traits were introduced to, and the level of natural drought-tolerance they already display.

Some data was provided on ear and plant height and flowering, while yield and kernel data was removed. Ear height has been shown in to correlate with yield. Without yield data provided, the non-statistically significant differences in ear and plant height also raise doubts over the improvements that MON

87460 × MON 89034 × NK603 is supposed to express.

Claims of drought-tolerance prior to completion of the third season of trials, when there is no significant changes in tolerance to drought-stress makes the claim that the product is for the “benefit of the economy and the environment” highly questionable. Nothing new has been learnt from the trial and gaps in our knowledge on efficacy as well as safety have not been addressed in any way to carry this product forward to the commercialisation stage.

The trial also reports also state that the efficacy of pest resistance and herbicide tolerance were tested under well-watered conditions. Since abiotic factors are known to affect expression of transgenes, and this plant being marketed for cultivation under abiotic stresses, the efficacy of these two traits under these conditions remains unproven in this crop.

## MOLECULAR CHARACTERISATION

Characterising the genetic modification is necessary at the level of the genome to identify the location of the integration site of the transgene, stability of the transgenes as well as the number of copies of the transgene integrated into the maize genome. Any disturbances at the genomic level could have consequences for the transcriptomic, genomic or metabolomic activity of the plant.

### **Description of the recombinant DNA before and after modification**

The transgenic material in the single and stacked events has been generated synthetically and therefore has no history of safe use in nature. A detailed description of the sequence of the transgenes should therefore be provided. Description of the parental single event lines fails to include sequence information. The applicant states

that there is a single amino acid substitution in the N-terminus of the CspB transprotein. Independent analysis of data provided to Indian authorities for NK 603 and MON 89034 found unintended modifications in the inserted transgenic DNA (Then, 2013).

### The CaMV 35S promoter

All three parental event lines use the 35S promoter from the cauliflower mosaic virus (CaMV). Concerns surrounding the use of this promoter include the potential risks associated with the presence of viral gene VI within the promoter sequence, as well as the presence of a recombination hotspot. A 2012 paper entitled “Possible consequences of the overlap between CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants” raised concerns over the sequence overlap of the CaMV 35S promoter and gene VI, with gene VI potentially being expressed into the P6 protein (Latham et al, 2012). A proper retrospective risk assessment on the Gene VI fragment showed that the gene product is toxic to plants probably through, among other things, the inhibition of gene silencing, a necessary function universal to plants and animals (see later); hence it is also likely to be toxic to animals including humans. The applicant has not mentioned this possibility let alone checked for expression of this protein.

The promoter is also documented for carrying a recombination hotspot, which may increase potential for genetic rearrangements and horizontal gene transfer (HGT) (Ho et al., 1999). The promoter, contrary to claims by GM producers, is active in human cells and any horizontal transfer to human cells therefore has the potential to disturb human gene expression (Ho, 2013). The applicant dismisses risks of HGT, stating “negligible risk” particularly with relation to the nptII antibiotic resistance markers present in MON 87460 as almost “null”. However, a study by Heinemann and Traavik (2004) on HGT to antibiotic-resistant bacteria demonstrates that existing scientific data cannot support this claim. Their analysis concludes that environmental impacts of HGT may occur at frequencies approximately a trillion times below current estimates. The insensitivity of current techniques for

monitoring HGT also undermine the claims that HGT is of no significance to human health or the environment. *NptII* encodes for resistance to neomycin and kanamycin antibiotics, both recently classified as critically important antibiotics for humans and animals by the WHO (WHO, 2012). The spread of antibiotic resistance is now acknowledged as a major threat to public health. The GMO panel of the European Food Safety Authority (EFSA) has thus rightly stated that antibiotic resistant marker genes should be restricted to field trial purposes and should not be present in GM plants to be placed on the market. **As such, we urge the South African government to seriously consider the public and environmental risks of antibiotic resistance to human and veterinary therapy.**

### T-nos terminator sequence

All three parental single event lines carry the nos 3' terminator sequence. Terminator sequences mark the end of the gene, the site where transcription of the gene should terminate. Analysis of this terminator in transgenic plants has shown that this terminator does not reliably terminate transcription, leading to the generation of novel RNA variants. There is no mention of assessing for the absence of novel RNA variants. As EFSA says, 2009):

*“(...) the data did demonstrate that an RNA species could be detected that likely initiated in the promoter of the NK603 insert and proceeded through the nos 3' transcriptional termination sequence continuing into the maize genomic DNA flanking the 3' end of the insert.”*

EFSA assumes that only very low levels of proteins are produced from such RNA species, and are thus unlikely to be toxic or allergenic. However, it has since been shown that short RNA species survive digestion and can interact with cell regulation (Zhang et al., 2012), therefore hazard identification can relate to the novel RNA produced, not just novel peptides.

**The applicant should be asked to provide data proving complete absence of novel RNA variants.**

## Characterisation of the indel

The applicant does not provide any details on the specific location of the transgenes in any of the individual or stacked events beyond a restriction map with the flanking genomic regions illustrated. There is no sequence information or description of the flanking genomic DNA provided. The applicant therefore does not provide information to confirm a lack of disruption to endogenous maize genes or regulatory sequences. **The applicant should provide details showing a lack of disruption to the endogenous maize genome.**

### Southern blot analysis

Southern blotting is used to assess the integrity of the transgene insertion and how many copies have been inserted.

The applicant does not provide a description of the stringency or sensitivity of the probes. This information should be made available by the applicant for each of the blots for each probe.

A family of probes should be used to characterise a GMO, with each probe corresponding to a part of the full-length recombinant DNA molecule used and the sum of probes comprehensively covering the entire recombinant molecule, so that transgene rearrangements, or the undesired integration of additional partial transgene fragments into the genome can be detected. However, for characterisation of the individual events MON89034, MON87460 and NK603, only 2 probes were used for the first event, while only 1 probe was used for the latter two, respectively.

Further, numerous studies recommend the combined use of both Southern blotting and polymerase chain reaction (PCR) techniques for the analysis of small/complex products of insertion sites. Some transgene rearrangements are documented to be too subtle to be detected by Southern blotting and thus require PCR for the detection of potentially common minor rearrangements in transgenic organisms (Kohli *et al.*, 2003). **The data provided does not confirm the integrity of the transgene sequence, not does it substantiate claims made by the applicant that**

the integrated DNA is stable, and that only a single copy of the transgene is present in each parental line.

### Description and characterisation of changes to the transcriptome, proteome and metabolome

The application fails to mention any profiling techniques that are now routinely employed to assess global changes in gene, protein and metabolite expression.

The latest studies in relation to GM crops reveal that the genetic modification process has the potential to disrupt endogenous gene expression in the plant, that can introduce human and environmental hazards as well as agronomic disturbances. Mesnage *et al.*, (2016) used such techniques to analyse proteome and metabolome profiles of NK 603, detecting altered levels of proteins and metabolites indicative of oxidative stress, alterations in levels of enzymes involved in glycolysis metabolism, as well as TCA cycle involved in energy production. Metabolome alterations also included a 28-fold rise in polyamines, which play multiple roles in cell growth, survival and proliferation; they can be either toxic or protective depending on the context.

A study on golden rice also revealed that outcrossing of the GM rice engineered to have increased beta-carotene content to a local Indian rice variety revealed stunted growth related to disruption of growth hormone and photosynthesis levels ascribed to the genetic modification process by the researchers (Bollinedi *et al.*, 2017). Such a disruption could have far reaching socio-economic consequences for farmers in the event of genetic contamination from neighbouring GM fields.

**The applicant should be asked to provide profiling data for MON 87460 × MON 89034 × NK603 as part of a hazard identification procedure.** This is of upmost concern considering the already existing data on disturbed protein and metabolite profiles of NK603 and independent data on unintended changes to the insert, lack of reliable data on

copy number and transgene stability, as well as the associated issues in relation to promoter and terminator regulatory elements included in the transgenic cassettes.

## SAFETY ASSESSMENT

Establishing the food and feed safety of MON 86460 x MON 89034 x NK 603 is essential considering that maize is not only consumed by humans and animals in South Africa, **and is an important staple crop consumed on a daily basis.**

A number of claims made in the safety assessment are questionable. For example, the applicant concludes that MON86460 x MON89034 x NK603: 1) MON86460 x MON89034 x NK603 is compositionally equivalent to conventional varieties of maize; 2) has a history of safe use; 3) the proteins have no structural similarities to known toxins or other biologically active proteins that could cause adverse effects, and 4) the transproteins are rapidly digested in mammalian gastrointestinal systems.

### Issues of Substantial equivalence

It is important to first note that the applicant did not test the whole plant material of MON 86460 x MON 89034 x NK 603. As such, MON86460 x MON89034 x NK603 has not yet been through any risk assessment as a whole plant. The applicant states:

*“The risk assessment of each of the singles used to produce MON 87460 x MON 89034 x NK603 consisted of comprehensive analyses that demonstrated the substantial equivalence, and therefore safety, of each single to its conventional counterpart. The only factor that the individual assessments did not address, is that of the potential for interactions between the gene products of the transgenes, when combined in a breeding stack.” [emphasis added]*

As acknowledged by the applicant above, combinatorial effects may occur due to

interactions between the novel transproteins and metabolites produced in the stacked variety. For example, having multiple Bt toxins may have cumulative or synergistic effects on non-target organisms. This is the basis for the EU regulation that requires risk assessment of stacked traits which defines a stacked event derived from conventional breeding of existing single event GM varieties as a “new entity” (Regulation (EC) No 1829/2003). It takes into account the possibility of stacked varieties showing disturbances in transgene and host genome stability, expression of novel proteins, and potential synergistic/combinatorial interactions between the individual modifications. Such interactions in stacked events have been documented in stacked maize that carries both Bt toxins and glyphosate tolerance, showing alterations of transgene expression in the stacked versus single event lines (Vilberte et al., 2016).

Instead, the applicant describes acute mammalian toxicity tests performed on mice for each transprotein tested individually: the CspB, CP4 EPSPS, CP4 EPSPS L214P, Cry1A.105, Cry2Ab2 and NptII proteins. Monsanto claim that no testing of the whole plant is necessary:

*“...MON 87460 x MON 89034 x NK603 grain and forage are compositionally equivalent to conventional maize grain and forage. Therefore, no testing of any constituent other than the newly expressed proteins is required.”*

However, these claims on substantial equivalence are now outdated. NK603 has since been shown to have altered protein and metabolome profiles (Mesnage et al., 2016) and other GM crops have also been shown to be substantially ‘non-equivalent’ (Abdo et al., 2013; Bøhn et al., 2014; Agapito-Tenfen et al., 2013). The comparative tests included in the application do not allow for such detailed and unbiased detection of compositional differences. The principle of ‘substantial equivalence’ for risk assessment is not a risk assessment but an analytical exercise that compares arbitrary comparators of GM crops to any variety or composite of varieties of conventional crops. As highlighted by Mesnage et al., (2016), the techniques used for determining substantial equivalence and thus

considering a GMO as Generally Recognised as Safe (GRAS) are not sensitive to pick up differences caused by the genetic modification process.

**Instead of relying on substantial equivalence analysis, the applicant should incorporate “omics” global profiling techniques now being used routinely as exemplified by Mesnage et al., 2016.**

Second, the transproteins are derived from bacterial and not from the GM plant itself. The use of bacterially derived toxins is of limited relevance to mammalian toxicity as post-translational modifications of proteins that occur in plants do not occur in bacteria. Such tests cannot therefore prove safety of the given transproteins produced in MON 87460 x MON 89034 x NK603.

**The applicant should be asked to provide safety tests based on the whole plant material, not individual bacterially derived toxins.**

### **‘history of safe use’**

Alterations in the transgene sequences as described in the above section on molecular characterisation confirm that these novel transproteins have no ‘history of safe use’ and have never existed in nature. The applicant cites studies that assess safety of bacterial species *B.thuringiensis* that contains the CspB protein. However, with unintended alterations in the amino acid sequence such comparisons are of limited relevance to the CspB protein expressed by MON87460. It is therefore not possible to claim safety of MON 87460 x MON 89034 x NK603 based on a history of exposure to naturally produced CspB proteins. Further, this claim does not consider the high maize consumption patterns in South Africa that would go beyond history of human exposure to *B. thuringiensis* consumption in other foods such as probiotics and cheese.

### **Bioinformatics analysis of allergenicity**

Two bioinformatics tools were used in the assessment of allergenicity. The second tool, an eight-amino acid sliding window search, was used by the applicant to specifically identify

short linear polypeptide matches to known or suspected allergens. The applicant notes that the Codex Alimentarius Commission (2003) recommends that the size of the contiguous amino acid searched should be based on a scientifically justified rationale, and chooses to use eight amino acids in its analysis (Codex, 2003).

The 2001 FAO/WHO consultation on the assessment of possible allergenicity due to GM foods however had suggested moving from eight to six identical amino acid segment searches. Codex (2004) notes: “The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives, inversely the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of comparison”. Using six amino acids for comparison would therefore be more precautionary, and in line with the thrust of the GMO Act and the Cartagena Protocol on Biosafety, to which South Africa is a Party.

In addition, a research paper comparing six amino acids, found that Cry2Ab show sequence similarity with known allergenic proteins (Kleter et al., 2002).

It should also be noted that bioinformatics should not be the only or major data for assuring safety. Spök et al. (2005) describe that it is well known that non-allergenic isoforms of allergens exist which differ by only a few amino acids compared to their allergenic counterparts. Moreau et al. (2006) have highlighted that allergenicity can be sometimes better predicted based on non-contiguous stretches of amino acids.

Limitations in the allergenicity analyses is highlighted by studies that have now linked Cry toxins to immunogenic reactions in mammals. Especially Cry1Ac (which shows some similarity to Cry1A.105) is known to enhance immune reactions and able to bind to epithelial cells in the intestine of mice (Vázquez-Padrón et al., 1999), Vázquez-Padrón et al., 2000). The applicant should therefore provide further detailed experimental data to rule out the potential for the transproteins to induce allergenic responses.

As described in the molecular characterisation, unintended changes in transgene sequences for MON89034 and NK603 have been documented, as well as a single amino acid change in the CSPB protein. Whether these alterations were included in the bioinformatics analysis is not clarified.

## Human Dietary Exposure

Estimation of human dietary exposure is based on a number of assumptions of limited relevance to the South African context. Estimations of transprotein concentration in the crop was based on field trial data from a single season in Chile under well-watered conditions. However, climatic conditions or varietal effects in the plants can play a significant role in the level of expression of the DNA constructs, as exemplified by field data forwarded to EFSA (2009) on the parental NK 603 variety:

*In maize forage, the mean CP4 EPSPS protein levels from the four different field sites in Europe were as follows: 44.2 µg/g fw (fresh weight) (site 1, Southern France), 45.7 µg/g fw (site 2, Southern France), (43.6 µg/g fw (site 3, Northern France), and 60.9 µg/g fw (site 4, Italy). The overall mean CP4 EPSPS protein level in maize forage across all four sites was 48.6 µg/g fw. In maize grain, the respective values for the (sites were 13.2 µg/g fw, 12.7 µg/g fw, 2.2 µg/g fw and 5.5 µg/g fw. "the overall mean CP4 EPSPS protein level in maize grain across all four sites was 8.4 µg/g fw."*

**Much more investigation is necessary to assess the impact of environmental factors, including drought, on the expression of the transgenes within the South African context.**

The applicant also claims that exposure is limited based on data from *in vitro* digestibility assays with all the transproteins that show them to be rapidly digested by gastrointestinal enzymes. However, these protocols prescribed by the WHO/FAO are limited as they do not test a range of pHs despite variability in human stomach pH, with infants generally having a higher pHs. Simulation experiments are of limited relevance to the physiology of

the mammalian gut and do not prove a lack of survival of proteins in the digestive tract. Indeed, analysis of human blood samples of pregnant women and their foetal blood supply found 90 % of women consuming a standard Canadian diet tested positive for Bt toxins, despite the toxin having been shown in digestibility assays to be rapidly digested in regulatory testing (Aris *et al.*, 2011).

## ENVIRONMENTAL RISK ASSESSMENT

### Assessment of impacts to non-target organisms

The applicant provides very limited information on potential hazards to non-target organisms. Instead the environmental risk assessment is based on claims of substantial equivalence to conventional maize in terms of compositional, phenotypic and environmental characteristics. The applicant has therefore failed to assess for potential adverse effects; the likelihood of such adverse effects; the consequences of such adverse effects, and the overall risk posed by such genetic modifications introduced into MON 87460 x MON 89034 x NK603.

The only information on testing the effects on non-target arthropod species derive from a summary of a single season of field trials in the US on six species. However, there has been no risk assessment on the potential affected species in the receiving environment of South Africa. A scientifically environmental risk assessment has been proposed and adopted by Kenya (for Bt maize), Brazil for (Bt cotton) and Vietnam (for Bt cotton) to incorporate testing of organisms local to the receiving environment, including those that have important ecological function. Hence, observed biological effects would constitute a biologically and meaningful result of concern that merits further investigation or surveillance. The crop is also put at the centre of the testing program to be able to detect all possible direct and indirect effects including cumulative and interaction effects.

The combinatorial effects of Bt toxins and glyphosate herbicides to be used on the crop have also not been tested.

Existing data shows that many Cry toxins are not as specific as previously thought and have detrimental effects on a variety of beneficial organisms such as pollinators (Ramirez-Romero *et al.*, 2008), pest predators (Hilbeck *et al.*, 1998) and soil fungi (Castadini *et al.*, 2016). Such hazards were not detected in initial risk assessments, raising the concern that further detailed tests in this area should be performed.

**The applicant should be asked to provide meaningful data that provides information on the potential hazards and risks to the local South African environmental.**

### **Lack of risk management and monitoring**

The applicant claims that there is no need for a risk management plan beyond insect resistance:

*“The comprehensive data package provided with this application, demonstrates that MON 87460 × MON 89034 × NK603 is equivalent to conventional maize, except for the intentionally introduced drought tolerant, insect resistant and herbicide tolerant traits, and has no negative impacts on human and animal health. There are therefore no risk management measures that users of MON 87460 × MON 89034 × NK603 would have to adhere to and MON 87460 × MON 89034 × NK603 maize would be handled in the same way as conventional maize”.*

However, this is a very complacent approach that will remove chances of observing any unanticipated effects that were not picked up during the risk assessment process. Such an approach to monitoring is recognised in the “Guidance on Risk Assessment of Living Modified Organisms” (UNEP/CBD/BS/COP-MOP/6/13/Add.1) developed under the Cartagena Protocol on Biosafety, to which South Africa is a Party. In case changes that could lead to an adverse effect are detected through general monitoring, possible causes for the observed changes are examined and,

where appropriate, a more specific hypothesis is developed and tested to establish whether or not a causal relationship exists between a GMO and the adverse effect, and can be followed up by case-specific monitoring or further research.

**The applicant should be asked to submit a detailed monitoring plan and information on the monitoring techniques (beyond insect resistance monitoring and management) to be employed, in accordance with the requirements of the GMO Act, NEMA and the relevant regulations.**

## **INSECT AND WEED RESISTANCE**

Field resistance of weeds to glyphosate-based herbicides is rapidly rendering glyphosate-tolerant GM crops redundant in regions such as the US. There has been a rapid proliferation of resistant weeds since their introduction, with over 260 independent cases of resistance documented across the world in 36 species as of February, 2017. Three of these species were documented in South Africa, including Conyza, an economically important weed for maize farming. The epidemic of resistant weeds forced Monsanto to declare any crop losses due to resistant weeds to be excluded from their warranty. This issue has led to the commercialisation of crops tolerant to additional herbicides to delay futility of the herbicide tolerant trait and prolong sales of associated pesticides.

A similar situation is developing with regards to pest resistance to Bt toxins, also documented in various parts of the world. Indeed, South Africa was one of the first places where resistance in the field was documented, and new research suggests that the country has the necessary environmental conditions for the spread of Bt pesticide resistance in corn borers, a major pest for South African farmers (Campagne, 2016). Field resistance has also been now been documented in the US to Cry1A.105+Cry2Ab2 included in MON

89034 (Dively *et al.*, 2016) which the authors concluded was due to factors such as high adoption rates and moderate expression of the Cry toxins that allowed for organisms with minor heterozygote resistance alleles to survive. It is only a matter of time before resistance is documented in South Africa. The applicant's claim that compliance with a 5 % refuge strategy is "*the core element of an insect management system*" is therefore brought into doubt by the fact that refuge strategies rely on high expression of the Bt toxins in the plants to delay the spread of dominant resistance allele heterozygosity.

As noted below, this has important socio-economic implications for farmers with the technology at best, having short term lifespan with regards to the efficacy of these two traits.

## SOCIO-ECONOMIC CONSIDERATIONS

The applicant states that "*Monsanto fully anticipates that drought tolerant maize combined with other biotechnology-derived traits, as found in MON 87460 × MON 89034 × NK603, will add to the positive cumulative impact of biotechnology products on the economy and the environment.*"

This conclusion is based on generalised claims of the benefits of biotechnologies to crop production, including increased yields, reduced chemical use and non-tillage agriculture along with increased farmer income that are of limited relevance to South Africa.

The application cites studies that are contested by independent data. For example, a recent study comparing yield of staple crops in the US Midwest to the EU where GM crops are largely banned, showed that yield improvement with less variability were higher in Europe since the 1960s (Heinemann *et al.*, 2013). Socio-economic studies of Indian farmer indebtedness directly linked farmer indebtedness and suicide to GM crop cultivation in rain-fed areas (. Herbicide use in the US has risen sharply with GM crop cultivation. Two-thirds of all

glyphosate usage applied in the US from 1974 onwards, was applied in the last 10 years alone, corresponding to 74 % of the world's usage (Benbrook, 2012). Argentina has documented an 858 % rise in pesticide use from 1994 to 2010, with glyphosate consisting of 64 % of total sales, partly due to weed resistance to glyphosate. In US, an estimated 1 billion dollars lost by 2014 in crop damages, while soy farmers estimated to spend 88 % more on crop protection than six years ago. Increased farmer inputs is partially a result of weed and pest resistance, a natural phenomenon that is expected to spread with increased cultivation of Bt and herbicide-tolerant crops including MON 87460 × MON 89034 × NK603.

Small holder farmer experiences in South Africa with regards to GM cotton also disproves the generalised claim that GM technologies are cost-effective, particularly for small holders. After 14 years of concerted efforts to cultivate GM cotton varieties there has been a complete failure to introduce it to small-scale farmers despite government subsidies, the implementation of irrigation and Monsanto financial and technical support. The end result was the closure of the local credit institution following crippling debt due to millions of unpaid loans; about 80 % of farmers defaulted.

These claims lack a cost-benefit analysis relevant to South Africa and to MON 87460 × MON 89034 × NK603 cultivation. Contrary to the generalised claims made by the applicant on socio-economic benefits of GM crops is a recent study on South African GM experiences by Fischer, Van den Berg and Mutenga (2012) entitled "Is Bt maize effective in improving South African smallholder agriculture?". It concludes that commercial varieties into which the Bt trait is introduced, are outperformed by locally used non-GM hybrids and open pollinated varieties, which are better adapted to smallholder agro-ecologies, fluctuations in rainfall and suboptimal storage conditions.

This study is complimented by comments submitted in July 2010 by a group of smallholder farmers under the auspices of the Right to Food Sovereignty and Agrarian Reform Campaign, to the Council on field trials. In that comment they stated that "(t)he introduction of bio-technologies like drought resistant

maize for South Africa and Sub Saharan Africa undermines the seed and food sovereignty of the countries people and farmers. Seed saving is an important component of farmers in Sub Saharan Africa and South Africa in particular. This technology would further deskill and destroy the farming practices of poor black farmers.”

**The applicant should be asked for independent verification of its claims that introduction of drought-tolerant maize will have positive socio-economic impact on small-holder farmers in South Africa specifically.**

## CONCLUSIONS

There is currently no data to back up claims by the applicant that MON 87460 x MON 890334 x NK603 will alleviate yield loss due to drought stress. On the other hand, conventional breeding has recently seen increased yields of drought-tolerant varieties by as much as 30 % (La Rovere *et al.*, 2014). As stated in a *Nature* piece in 2014 (Gilbert, 2014), the race to develop drought-tolerant varieties has been clearly won by conventional breeding over GM techniques to date. The development of hybrid varieties has its own socio-economic and sustainability problems, but these results offer the proof-of-principle concept that developing genetically complex traits can be achieved much more efficiently through cross-breeding than single-gene transgenic insertions. Indeed, drought-tolerance is not a new innovation and there are already established open-pollinated and farmer seed varieties on the continent.

With the unsubstantiated efficacy of the drought-tolerance trait in MON 87460 x MON 890334 x NK603, it raises the question that this variety is merely another means to maintain the sales of their glyphosate herbicides and Bt traits under the guise of improving food security during an era of drought and climate change. A 2008 comment piece published in *Nature* reveals that using drought and rising food prices as a business opportunity:

*“Our first products were all about weeds and bugs; we really believe that the next decade is going to be about yield,” says Steve Padgett, Monsanto’s vice-president for biotechnology research. He adds that although drought tolerance is indubitably more complex than the traits the industry has worked with before, research is catching up with the complexity. “The science is more tractable and the market is pulling,” he says. William Niebur, vice-president for Crop Genetics Research and Development at Pioneer Hi-Bred, says that the company sees a market for drought-tolerant crops across all regions and at all scales, but the products, and the profits, may be long in coming. “This is much more complex than identifying a protein that will kill an insect or make a plant withstand a herbicide,” says Niebur. “We see this as an area where we will spend our entire careers and there will still be room for improvement.”*

During an era of climate unpredictability, the widespread cultivation of genetically uniform varieties increases vulnerability to abiotic and biotic stresses such as drought and disease. Over-reliance on genetically modified (GM) maize to provide a staple food and animal feed has resulted in huge production losses: the last (2015-16) maize crop is the smallest harvested since 2006/7 (7.125 million tonnes). Food prices have burgeoned at a 40% year on year increase. Despite South Africa being the 8th largest cultivator of GM crops, around 46 % of South African households go hungry every day, a testament to the failure of these technologies to improve food security and lives of the South African people.

The ACB urges a transition away from industrialised agriculture towards agroecological methods that support dietary diversity and agricultural diversity that is sustainable in the face of a changing climate, and reverses the corporate concentration in seed and agrochemical markets that infringe on the rights of small holder farmers and the people of South Africa to their right to food sovereignty.

## REFERENCES

- Abdo EM, Barbary OM, Shaltout OE. Chemical Analysis of BT corn “Mon-810: Ajeeb-YG ®” and its counterpart non-Bt corn “Ajeeb”. *IOSR Journal of Applied Chemistry* 2013, 4, 55-60
- Agapito-Tenfen SZ, Guerra MP, Wikmark OG, Nodari RO. Comparative proteomic analysis of genetically modified maize grown under different agroecosystems conditions in Brazil. *Proteome Science* 2013, 11, 46. doi: 10.1186/1477-5956-11-46.
- Aris A, Leblanc S. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol.* 2011, 31, 528-33.
- Bollinedi H, S. GK, Prabhu KV, Singh NK, Mishra S, Khurana JP, *et al.* Molecular and Functional Characterization of GR2-R1 Event Based Backcross Derived Lines of Golden Rice in the Genetic Background of a Mega Rice Variety Swarna. *PLoS ONE* 2017, 12(1): e0169600. doi:10.1371/journal.pone.0169600
- Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. *Food Chemistry* 2013, 153, 207-215 <http://dx.doi.org/10.1016/j.foodchem.2013.12.054>
- Campagne P, Capdevielle-Dulac C, Pasquet R, Cornell SJ, Kruger M, Silvain JF, LeRü B, Van den Berg J. Genetic hitchhiking and resistance evolution to transgenic Bt toxins: insights from the African stalk borer *Busseola fusca* (Noctuidae). *Heredity (Edinb)*. 2017, 118, 330-339
- Codex Alimentarius Commission. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Codex CAC/GL 45-2003:1-13.
- Codex Alimentarius Commission. 2004. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants (CAC/GL 45-2003). Rome: Food and Agriculture Organization of the United Nations and World Health Organization.
- Dively GP, Venugopal PD, Finkenbinder C. Field-Evolved Resistance in Corn Earworm to Cry Proteins Expressed by Transgenic Sweet Corn. *PLoS ONE* 2016, 11(12): e0169115. doi:10.1371/journal.pone.0169115
- EFSA (2009) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSAGMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal 2009, 1137 Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)
- Fischer K, van den Berg J, Mutengwa C. Is Bt maize effective in improving South African smallholder agriculture? *South African Journal of Science* 2015, 111, Number 1/2
- Gilbert N. Cross-bred crops get fit faster. *Nature* 2014, 513, 292. doi:10.1038/513292a.
- Gutierrez AP, Ponti L, Herren HR, Baumgärtner J, Kenmore PE. Deconstructing Indian cotton: weather, yields, and suicides. *Environmental Sciences Europe* 2015, 27, 12 DOI: 10.1186/s12302-015-0043-8
- Heinemann JA, Massaro M, Coray DS, Agapito-Tenfen SZ, Wen JD. Sustainability and innovation in staple crop production in the US Midwest. *International Journal of Agricultural Sustainability* 2013, <http://dx.doi.org/10.1080/14735903.2013.806408>
- Heinemann JA and Traavik T. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. *Nat Biotech.* 2004, 23, 488
- Ho MW. Natural versus Artificial Genetic Modification. *Entropy* 2013, 15, 4748-4781
- Ho MW, Ryan A, Cummins J. Cauliflower Mosaic Viral Promoter – A recipe for Disaster? *Microbial Health and Disease* 1999, 11, 194-197
- Kleter GA & Peijnenburg AACM. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE – binding linear epitopes of allergens. *BMC Structural Biology* 2002, 2, 8. doi:10.1186/1472-6807-2-8.
- Kohli, A, Twyman RM, Abranches R, Wegel E, Stoger E, Christou P. 2003. Transgene integration, organization and interaction in plants. *Plant Mol. Biol.* 2003, 52, 247–258.
- La Rovere R, Abdoulaye T, Kostandini G, Guo Z, Mwangi W, MacRobert J, Dixon J. 2014. Economic, Production and Poverty Impacts of Investing in Maize Tolerant to Drought in Africa: An Ex ante Assessment. *Journal of Developing Areas* 2014, 48, 199-225.
- Latham J & Wilson A. Potentially dangerous virus gene hidden in commercial GM crops. *Sci. Soc.* 2013, 57, 4–5.
- Moreau V, Granier C, Villard S, Laune D, & Molina F (2006). Discontinuous epitope prediction based on mimotope analysis. *Bioinformatics* 22, 1088-95.
- Spök A, Gaugitsch A, Laffer S, Pauli G, Saito H, Sampson H, Sibanda E, Thomas W, van Hage M, & Valenta R. Suggestions for the assessment of the allergenic potential of genetically modified organisms. *International Archives of Allergy and Immunology* 2005, 137, 167-80. DOI: 10.1159/000086315.
- Then C. Analysis of the data submitted by Monsanto to the Indian authorities on genetically engineered maize MON89034 x NK603. *Testbiotech*, 2013.
- Union of Concerned Scientists. High and Dry Why Genetic Engineering Is Not Solving Agriculture’s Drought Problem in a Thirsty World. 2012 [http://www.ucsusa.org/food\\_and\\_agriculture/our-failing-food-system/genetic-engineering/high-and-dry.html](http://www.ucsusa.org/food_and_agriculture/our-failing-food-system/genetic-engineering/high-and-dry.html)
- Vazquez-Padron RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA, Lopez-Revilla R. Intra-gastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. *Life Sciences* 1999, 64, 1897-1912

Vazquez-Padron RI, Gonzales-Cabrera J, Garcia-Tovar C, Neri-Bazan L, Lopez-Revilla R, Hernandez M, Morena-Fierra L, de la Riva G.A. Cry1Ac Protoxin from *Bacillus thuringiensis* sp. Kurstaki HD73 binds to surface proteins in the mouse small intestine. *Biochem. and Biophys. Research Comm.* 2000, 271,54-58

Vilperte V, Agapito-Tenfen SZ, Wikmark OG, Nodari RO. Levels of DNA methylation and transcript accumulation in leaves of transgenic maize varieties. *Environ Sci Eur.* 2016, 28, :29. Epub 2016

WHO. Critically Important Antimicrobials for Human Medicine. 3rd Revision 2011 WHO Press, World Health Organization, Geneva (2012) [http://www.who.int/foodborne\\_disease/resistance/cia/en/](http://www.who.int/foodborne_disease/resistance/cia/en/)

Zhang L et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Research* 2012, 22, 107-26. doi: 10.1038/cr.2011.158. Epub 2011 Sep 20.