Mtandao wa Vikundi vya Wakulima Tanzania (MVIWATA)

And

African Centre for Biodiversity (ACB)

Comments to COSTECH's Application for Confined Field Trial of Stacked Transgenic Maize MON 87460 x MON 810

August 2017

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INTRODUCTION

Mtandao wa Vikundi vya Wakulima Tanzania (MVIWATA) is a national farmers organisation which brings together small holder farmers from all over Tanzania in order to have a common voice to defend interests of smallholder farmers. Founded in 1993 and registered in 1995 under the Society Ordinance Act (Registration number SO 8612). MVIWATA aspires to empower smallholder economically and socially through capacity building, lobbying and advocacy, strengthening groups and networks and facilitating communication and learning in order to enable smallholder farmers defend9(their interests. The national office of MVIWATA is situated in Morogoro town.

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.

Civil society groups in countries participating in the WEMA project have consistently opposed the introduction of genetically modified (GM) maize being marketed as a drought-tolerant variety. In July 2016, the Tanzania Alliance for Biodiversity (TABIO) submitted an objection to the first trial conducted last year at the Makutupora Viticulture Research and Training Centre (VRTC). Similarly, the ACB has been opposing the trials and now general release of MON 87460 in South Africa. 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.

KEY CONCERNS

1. **Claims that MON 87460 X MON 810 improves yield during drought remains 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".
    - Most of the empirical data is removed on the grounds of confidential business information, making any claims by the applicant impossible to verify.
    - Stacking with MON 810 serves to prolong the shelf life of an old, defective variety that is already being phased out in the continent.
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 MON 810 variety. Claims of substantial equivalence are thus unfounded.
- 'History of safe use' cannot be claimed for this crop. Maize is a staple crop in Tanzania 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

4. Environmental Assessment

- Concerns remain regarding potential contamination of conventional maize varieties being grown in the vicinity. Wind dispersal and insect dispersal are potential avenues for contamination. Mitigation measures are not sufficient to guarantee prevention of genetic contamination.
- No risk assessment data exists on potential effects on non-target organisms in Tanzania. The walls protecting the trial site do not prevent the entrance of non-target organisms and therefore potential exposure routes exist.

5. 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.

1. SUMMARY OF APPLICATIONS

The Tanzania Commission for Science and Technology (COSTECH) has applied for a variation of an ongoing field trial release of Monsanto's MON 87460 that was approved in July 2016 (Permit no. CBD.24/202/01/A) taking place at the at the Makutupora Viticulture Research and Training Centre (VRTC).

The current application is a 'stacked variety', where two or more GM lines are combined by traditional cross breeding of two transgenic crops together. In this case the application is for the trialling of MON 87460 maize stacked with MON 810 Bt maize.

MON87460 contains the bacterial cold shock protein B (CspB), derived from the common soil bacterium *Bacillus subtilis*. According to Monsanto "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.

MON87460, or 'Droughtgard', was first commercialised in the US from 2011. Its introduction into Tanzania 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.

MON810 contains an insecticidal Bt protein, Cry1Ab that targets certain members of the Lepidopteran family (moths and butterflies). Bt insecticidal toxins were isolated from the bacterium *Bacillus thuringiensis* subsp. Kurstaki Strain HD-1.

2. BACKGROUND ON WEMA

The water efficient maize project for Africa (WEMA) was officially launched in Kampala, Uganda in 2008. It is a public-private partnership coordinated by the African Agricultural Technology Foundation (AATF). It is a joint collaboration involving the International Maize and Wheat Improvement Centre (CIMMYT), the national agricultural research institutions (NARS) of the five WEMA countries (Kenya, Mozambique, South Africa, Tanzania and Uganda) and Monsanto. It is primarily funded by the Bill and Melinda Gates Foundation and the Howard G. Buffet Foundation. The project has two components: a conventional hybrid breeding programme using maize germplasm donated to WEMA by each of the participating parties, (CIMMYT), the NARS of the five WEMA and Monsanto; and a programme focussing on producing GM drought-tolerant maize varieties. Much of the germplasm donated by CIMMYT will come from an earlier breeding program, called the Drought Tolerant Maize for Africa (DTMA) project, which ran from 2007-2015 with the aim of developing open pollinated and hybrid varieties for drought.

The origins of the germplasm that Monsanto has used to develop its GM variety is of huge significance to the claims made by Monsanto that their GM trait confers increased drought tolerance, and raises biopiracy, legal and socio-economic concerns over the use of publicly developed conventional drought-tolerant germplasm developed by DMTA and donated by CIMMYT.

3. MON 87460 DROUGHT TOLERANCE CLAIM UNSUBSTANTIATED

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.** As advertised under Monsanto's Genuity brand for stacked traits, DroughGard® Hybrids are developed as part of a systems approach that combines "best agronomic practices, germplasm selected for top-end yield potential and superior drought-tolerance characteristics" (Genuity.com, 2017).

Further, drought is more than just a lack of water for a plant. It is also associated with wider effects that can include fluctuating temperatures; effects on soil processes such as reduced recycling of plant residues into soil organic matter, soil fertility and water holding capacity, high risks of erosion, low availability of nutrients; as well as increased likelihood of floods following a drought period. Again, this highlights the complexity of plant processes required for withstanding drought conditions and the complex networks of genes involved in such quantitative traits. The introduction of the *cspB* gene has not been shown to alleviate any of the wider issues of drought on maize crops.

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.

Finally, the trials were originally supposed to start in April/May when there is a dry season, so water management can be controlled. With delays in the commencement of the trial, what useful information can be gathered during the wetter seasons of Tanzania?

4. STACKING OF MON 87460 WITH OLD, DEFECTIVE GM VARIETIES

The trial being conducted is now testing the stacked event combining MON 87460 with MON 810, a Bt insecticidal crop. The applicant states that MON 8740 is insufficient in ensuring yield protection due to the problem of insect infestation:

"*the problem of stem-borers is a serious production constraint that significantly reduces maize yields in drought-prone areas implying that addressing water use efficiency alone will not suffice as a solution*".

It is frank admission to the limitations of MON 87460. Unfortunately, MON 810 is also not a solution for dealing with production constraints. Indeed, it is currently being phased out of South Africa due to the development of large-scale resistance to the Cry1Ab toxin by the corn-borer species *B. fusca* that was reported only 6 years after the commercialisation of the variety in 1998. One of the three stem borer species present in Tanzania is *B. fusca*, along with the spotted *C. Partellus* and the pink *S. calamistis.* It is only a matter of time before such resistance becomes an issue for Tanzania. MON 810 also came off-patent in 2011, and has been 'donated' to the WEMA project. However, a stacked variety such as the MON 87460 x MON 810 to be trialled in Tanzania, will retain its intellectual property rights.

As stated by Monsanto in 2012, they "*plan to have Genuity® VT Triple PRO®, Genuity® VT Double PRO® and Roundup Ready® Corn 2 technologies serve as the agronomic trait platforms for DroughtGard Hybrids.*" (Monsanto.com, 2012). So, the drought-tolerant trait will be sold only in stacked varieties that can prolong intellectual property and attempts to cover up production constraints of the MON 87460 of surviving drought conditions as a standalone product.

Further complications with 'production constraints' of MON 87460 x MON 810 are also possible given the recent scientific publication showing that drought conditions lead to increased pest resistance to the Cry1Ab Bt toxin. Published by Venugopal and colleagues (2017), the new study found that temperature anomaly buffers and its interaction with elevated selection pressure induced by widespread cultivation of GM sweetcorn varieties in the US, led to accelerated Bt-resistance development in target pests.

The issues pertaining to extending intellectual property of a crop that has been a failure in Africa to date, in a drought situation suggested to accelerate pest resistance even further, cannot be a fitting solution to remedying the effects of climate change for small-holder farmers in Tanzania.

5. 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.

5.1 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, however this information provided in Annex 3 is publicly unavailable due to deletion of Confidential Business Information (CBI).
Description of the parental singe 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 the transgene introduced into MON810 has been shown to be unstable (Hernández et al., 2003).

5.2 The CaMV 35S promoter

Both 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 recent 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 Tanzanian government to seriously consider the public and environmental risks of antibiotic resistance to human and veterinary therapy.**

5.3 T-nos terminator sequence

MON 87460 single event line carries 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.

5.4 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. 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.**

5.4.1 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.

In order to determine single insertions of the transgene, end fragment analysis should be performed. Most multiple integration events occur as tandem repeats, therefore restriction digests performed on the genomic DNA need to cut within the transgene for potential tandem insertions to be detected. The applicant does not provide details on where the restriction enzymes cut the DNA.

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, due to CBI deletions, no access to these types of details are available for independent methodological scrutiny.

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 applicant refers to PCR analysis of MON 810 but not MON 87460.

Independent analysis of MON 810 by various techniques including PCR, have shown complex rearrangements of the transgenes and production of novel RNA nucleotides (Rosati et al., 2008), highlighting the instability of the transgenic lines and the inadequate characterisation of these crops by the producers.

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.

5.5 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 810 as part of a hazard identification procedure. This is of upmost concern considering the already existing data on disturbed protein and metabolite profiles of MON 810 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.

6. SAFETY ASSESSMENT

Establishing the food and feed safety of MON 86460 x MON 810 is essential considering that maize is not only consumed by humans and animals in Tanzania, **but it 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 MON 86460 x MON 810 is compositionally equivalent to conventional varieties of maize; 2) a lack of oral toxicity; 3) the transproteins are rapidly digested in mammalian gastrointestinal systems; 4) the proteins have no structural similarities to known toxins or other biologically active proteins that could cause adverse effects, and 5) has a history of safe use.

It is important to first note that the applicant did not test the whole plant material of MON 86460 x MON 810. **As such, MON 86460 x MON 810 has not yet been through any risk assessment as a whole plant as a stacked event**.

6.1 Issues of Substantial equivalence

Substantial equivalence is a concept that states that if a new food is found to be 'substantially equivalent' to an already existing food product, it can be treated the same way as the existing product with respect to safety. It allows for the comparison of a GM line to any existing variety within the same species, and even to an abstract entity made up of ingredients from a collection of varieties. A GM variety can therefore have the worst traits of many different varieties and still be deemed substantially equivalent. This concept has been widely criticised by biosafety analysts for its crudity and flexibility in interpretation, as well as the procedures which only compare gross measurements of for example total carbohydrates, proteins and sugars, which cannot begin to tackle issues of safety.

The applicant therefore unsubstantiated claims of substantial equivalence of the stacked variety that remains untested for any safety assessment:

"*The stack event of MON 87460 x MON 810 maize is expected to be substantially equivalent to conventional maize, except for the expression of CspB and Cry1Ab proteins"*

"the crossing of single GM events, as in MON 87460 x MON 810 is not likely to result in interactions that may cause compositional, agronomic, or phenotypic changes that would raise safety concerns"

As acknowledged in the applicant for general release of stacked MON 87460 x MON 89034 x NK 603 in South Africa however (ACB,2017), 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).

The substantial equivalence tests performed on MON 87460, consisted of analysing 62 components in total (fibre, minerals, total amino acid, fatty acids and vitamins), which were analysed alongside control, commercial hybrid lines, as cited in the paper referenced (Harrigan et al., 2008), though the application makes a claim of 434 comparisons being made. Even so, there were statistically significant differences in 2 components from material derived from the US field trial, and 3 components that were significantly altered in material deriving from the trial conducted in Chile. However, the applicant goes on to dismiss these differences and makes claims that MON 87460:
"can be considered compositionally equivalent to those derived from conventional maize with a history of safe consumption". Similar claims are later made for MON 810.

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.

Earlier work on MON 810 varieties from Egypt have also highlighted the substantial non-equivalence in nutritional content when compared to conventional, near isogenic varieties (Abdo et al., 2013). In 2010 SANBI published the results of a joint research project, carried out with the Norwegian government on the environmental impact of MON810. The study found MON810 to be not substantially equivalent to conventional varieties, finding that – "GM plants grown in the same environment as the near isogenic-parent (non-GM counterpart), respond differently to the same environmental conditions, as shown by the

differences in protein expression, for a number of proteins". The study showed that some proteins have different expression levels (i.e. they are present at different amounts) in the GM and the non-GM comparator, even though both plant types are grown in the same field. The researchers recommended that further research is needed to identify what effects these have on the environment and if these differences also are present in other growing environments in South Africa (SANBI. 2011).

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.

6.2 Claims of 'lack of acute oral toxicity'

As stated earlier, no toxicity tests referred to in the application have been performed on the stacked event, or even on whole plant material derived from the stacked event. Instead, the transproteins used for toxicity tests 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 810.

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

Second, no long-term tests on MON 87460 have been performed. The only test referred to in the application is a 14-day test on mice. This means that the safe levels of exposure, which are based on calculations using the dose at which no effect is observed, called the 'no observable adverse effect level' (NOAEL) that has been calculated in the application refers only to an acute safe dose, not a chronic dose which needs to be derived from long-term studies. No safe levels of exposure have therefore been calculated for MON87460. Further, only gross measurements in the acute toxicity tests were performed, which is wholly inadequate for determining safety. Measurements such as body weight, food consumption and survival over a 14-day period are highly limited in detecting potential toxicity. Further, no data is provided for independent scrutiny of the toxicity results.

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).

Lack of hazard identification for all potential exposure routes is another biosafety concern. Exposure via pollen for example, has not been mentioned in the application.

The applicant should be asked to provide long-term safety tests of the stacked event MON 87460 x MON 810 that can allow for analysis of long-term effects of consuming a staple crop on a daily basis.

6.3 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 great 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 Biosafety Act and the Cartagena Protocol on Biosafety, to which Tanzania is a Party.

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. For example, Cry1Ac is known to enhance immune reactions and able to bind to epithelial cells in the intestine of mice (Vázquez-Padrón *et al.*,1999), Vásquez-Padrón *et al.*, 2000), despite bioinformatics analysis by the producer showing lack of similarity to known allergens. 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, an unintended change of a single amino acid in the CSPB protein is described. Whether these alterations were included in the bioinformatics analysis is not clarified.

The applicant should provide experimental evidence of lack of allergenicity of the whole plant material for MON87460 x MON 810.

6.4 '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 states that consumption of bacterial species such as *Lactobacillus* in live bacterial cultures used in dairy products shows that there is a 'history of safe use' of CspB proteins. However, as they state, the proteins in Lactobacillus only share 49-79 % sequence homology to the CspB protein found in MON 87460. Such assumptions go against scientific understanding of allergenicity and toxicity of proteins as described above.

Further, 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 810 based on a history of exposure to naturally produced CspB proteins. Finally, this claim does not consider the high maize consumption patterns in Tanzania that would go beyond history of human exposure to *B. thuringiensis* consumption in other foods such as probiotics and cheese.

7. ENVIRONMENTAL RISK ASSESSMENT

7.1 Gene Flow

Gene flow is one of the most important biosafety hazards surrounding GM foods, especially in Tanzania, a completely GM-free country where farmers practice predominantly agro-ecological and organic techniques. The introduction of GM food trials into the country jeopardises the integrity of Tanzania's food system, an issue that is likely irreversible.

Current evidence suggests that containment of transgenic DNA is impossible to guarantee. This is corroborated by the documentation of over 396 incidents of GM contamination across the globe (1997-2013) (Price et al., 2014). These findings come in spite of a chronic lack of monitoring by regulatory agencies and industry as a whole.

Genetic contamination has not only occurred with commercialised crops, but also un-approved varieties, highlighting the failures of containment measures used in previous GM trials (Price et al., 2014). This is exemplified by the fact that the highest numbers of contamination have been recorded in rice, despite there never having been a commercialised GM rice product anywhere in the world.

Genetic contamination is already an issue on the continent, where a South African study recently showed that small farmers' maize fields were contaminated with MON810 maize, while 25 % of seed stocks were positive for transgenic DNA (Iversen et al., 2014).

In the context of Tanzanian farming systems where seed saving and exchange is still practiced, the possibility of contaminated seed spreading is a major concern. In light of the fact that MON87460 is being promoted through the public/private WEMA project, we must ask what mechanisms will be put in place to ensure that beneficiaries are aware of the special precautions and prohibitions related to genetically modified seeds and what safeguards are implemented to prevent the contamination of farmers' varieties?

The application nevertheless, claims that they have adequate containment measures for from the trial:

"*a number of measures will be implemented to prevent gene flow including maintaining a reasonable isolation distance (400m) between the trial site and the nearest maize fields, destruction of all maize grains harvested at the end of the trial and destroy any volunteer plants during post-harvest monitoring*."

However, the application fails to address additional mitigation measures that a recommended by the Tanzanian Practical Manual for Safe Conduct of Confined Trials (2010). For maize, it is recommended that the flowers are removed and/or bagged to prevent the escape of viable pollen. Further, the application claims that the trials will be performed off-season, as a method of temporal isolation. However, the trials were scheduled to start in April/May and have been delayed making it impossible to substantiate this claim. Further, the surrounding region, is a maize region. Staff living within the trial vicinity also cultivate private maize crops.

Though the majority of cross-pollination occurs at short distances, distances as far as 300 meters are predicted to be insufficient to ensure 0 % contamination. A study from South Africa, performed by testing field trials of GM maize surrounded by non-GM maize concluded that isolation distances of above 135 m are needed to ensure contamination below 1 %, 503 m for below 0.1 % and 1.8 km for ensuring contamination below 0.01 %. Maximum isolation

distances proposed in the trials are only 400 m which is inadequate to effectively exclude the risk of contamination.

Comprehensive analysis of maize pollination also reveals huge variation in the degree of cross pollination, dependent on many factors including wind speed, wind direction and the presence of swirling winds (Sciencedaily, 2010). Being downwind of a GM-trial was shown to significantly increase cross pollination. Current guidelines do not consider wind-speed or direction when calculating isolation distances. The application fails to include environmental data that is necessary to estimate the levels of gene flow. Information on climactic factors such as prevailing winds are not mentioned. This is important as local wind speeds in the Dodoma area typically reach 3-5 metres / sec (Level 2-3 on the Beaufort Scale). There is no scientific data on the extent of gene flow in local conditions. The trial site is surrounded by a walled fence that will fail to ensure that insects can be kept away from the trial site, and appears to be more of a security fence against people than a mitigation strategy against gene flow.

Though maize pollen is known to be dispersed by wind, there is also limited evidence to suggest that insects can also disperse maize pollen (Vaissière & Vinson, 1994), adding further doubts to the effectiveness of the mitigation strategies outlined in the application.

Gene flow is also of relevance to human health, considering the presence of the NptII antibiotic marker in MON 87460. Any transfer via horizontal gene transfer to soil or gut bacteria could compromise its therapeutic effects.

The mitigation measures in place such as the buffer zones are not adequate to prevent gene flow as evidenced by independent data. The surrounding fence also does not guarantee the exclusion of animals that could disperse seed/pollen outside of the vicinity.

The application also fails to include any plans to collect biosafety data. There is a complete omission of data to be collected on gene flow, despite the trial application stating that an objective of the trial is:

"*To generate biosafety data and development of efficacy and safety data for application dossier compilation essential for general product release*."

These claims have been made before, for example in South Africa, though the application for general release failed to incorporate any biosafety data from their previous trials (ACB, 2017).

It remains unclear what type of biosafety data they will produce considering they offer no plans to collect any biosafety data whatsoever. The applicant should be monitoring for potential gene flow that could impact the maize of surrounding local farmers. This is of particular concern when taking into consideration the inadequate proof of safety to human and environmental health as described above.

7.2 Effects on non-target organisms

The fence surrounding the trial site does not prevent the ability of insects and other non-target organisms to be exposed to the GM maize. It remains completely unknown what the effects of MON 87460 x MON 810 has on non-target organisms, in the context of the Tanzanian environment, nor are there described plans to test such potential effects during the trial. There is also no mention of risk assessment for non-target organisms mentioned in the application.

To date, the only information on testing the effects of MON 87460 on non-target arthropod species derive from a summary of a single season of field trials in the US on six species. 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 Tanzanian environment.

8. CONCLUSIONS

The applicant fails to back up its claims that MON 87460 x MON 810 will alleviate yield loss due to drought stress. Further, the stacking with MON 810, a failed crop on the continent, seems to be a cynical attempt to prolong the shelf life of this crop that is now off-patent. Conversely, drought-tolerant varieties developed via conventional breeding have been shown to increase yields by 30 %. 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 810, it raises the question that this variety is merely another means to maintain the sales of their Bt traits under the guise of improving food security during an era of drought and climate change, and get access to a huge diversity of germplasm via the collaboration with CIMMYT. 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 Padgette, 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."

Though it is obviously a clear business opportunity, the question remains as to how much the cspB protein confers additional drought-tolerance above the elite germplasm used for generating the MON 87460 varieties. This raises the issue of the consolidation of germplasm biodiversity in the hands of private companies through such projects as WEMA. The transfer of such resources to the hands of multinationals under the guise of philanthropy raises huge socio-economic concerns surrounding biopiracy and the ownership of seed that is required to combat such climactic issues and those surrounding self-sufficiency and food sovereignty in the country. Further, 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.

A transition away from industrialised agriculture towards agroecological methods are recommended by many recent reports including the UN Intnernational Panel on Sustainable Food Systems (IPES-FOOD) to be the most efficient and sustainable way to improve food security, nutrition and climate change including water retention during drought. Independent studies have also shown that organic agriculture can cope better with extreme weather events than industrial systems following hurricanes (Holt-Giménez et al., 2002 and Anon, 1994) and drought (Seufert et al., 2017). The MVIWATA and ACB fully support such policies to deal with climate change, and also to reverse the corporate concentration in seed and agrochemical markets that infringe on the rights of small holder farmers and the people of Tanzania to their right to food sovereignty and healthy food and environment.

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

ACB. 2017. Objection against General Release of MON 87460 x MON 89034x MON 810 in South Africa. African Centre for Biodiversity, Johannesburg.

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.

Anon. Nature farming rice crop succeeds despite record cold summer: Story is front page news in Japan. *World Sustainable Agriculture Association Newsletter* 1994, 3, 1.

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

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.

Heinemann JA and Traavik T. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. Nat Biotech. 2004, 23, 488

Hernández M, Pla M, Esteve T, Prat S Puigdomènech P and Ferrando A. A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3'-transgene integration sequence. Transgenic Research 2003, 170-89

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

Holt-Giménez E. Measuring farmers' agroecological resistance after Hurricane Mitch in Nicaragua: a case study in participatory, sustainable land management impact monitoring. *Agriculture, Ecosystems & Environment* 2002, 93, 87-105.

Iversen M, Grønsberg IM, van den Berg J, Fischer K, Aheto DW, Bøhn T) Detection of Transgenes in Local Maize Varieties of Small-Scale Farmers in Eastern Cape, South Africa. PLoS ONE 2014, 9(12): e116147. doi:10.1371/journal.pone.0116147

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.

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.

Rosati A, Bogani P, Santarlasci A, Buiatti M (2008) Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. Plant Mol Biol 67(3): 271-281.

Sciencedaily. “GM Field Trials 'Underestimate Potential For Cross-pollination.” 2007 https://www.sciencedaily.com/releases/2007/05/070531191126.htm

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.

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

Vaissiere B and Vinson SB. 1994. Pollen morphology and its effect on pollen collection by honey bees, *Apis mellifera L.* (*Hymenoptera: Apidae*), with special reference to upland cotton, *Gossypium hirsutum L.* (*Malvacaece*). *Grana*. 33 : 128-138.

Vazquez-Padron RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA, Lopez-Revilla R. Intragastric 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, Hernndez 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 Biophy.s 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/

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.