Objection against Pioneer Hi-Bred's application for field trials of RNAi /gene silencing (DP-566113-9) GM maize

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Key concerns

- The DP-056113-9 maize variety has no proposed benefits for anyone other than seed producers and the applicant itself. It appears that only one single-season trial on this product has been conducted anywhere else in the world. If approved for commercialisation in South Africa, it may well occur before anywhere else. In a country where there are limited capacities and resources to monitor and manage any unforeseen risks at the trial stage or following commercialisation, this is ostensibly a neo-colonial exploitation of our environment and people.

- While the applicant claims that pollen from DP-056113-9 is inviable and thus will not spread, its own study shows that the pollen is not 100 % 'transgene-free'. Containment measures proposed by the applicant are not adequate to prevent pollen escape into surrounding farmers' fields and thus threatens to contaminate local maize varieties from this GM maize that has not been approved for entering the food supply.

- The variety is one of a few GM crops that carries dsRNA molecules that utilise the RNA interference pathway intended to "switch-off" the expression of genes in the plant. Such dsRNAs raise novel biosafety concerns such as unintended off-target silencing of genes within the GM plant, with safety implications. Unintended effects such as activation of gene silencing could also occur and expose humans or other non-target organisms. Safety considerations in regard to the dsRNA have been completely overlooked by the applicant in the data that it has presented to the South African GMO authorities.

- Molecular characterisation is lacking in the application, which is key to determining if any unintended effects of the genetic modification procedure has occurred, which may have implications for food and environmental safety.

- The promotion of hybrid seed further consolidates corporate domination over the South African food system, entrenches farmer dependency, and erodes seed biodiversity at a time when it is vital for us to embrace seed diversity as climate resilience tools. The ACB is on record for calling for an urgent shift away from the focus on a maize monoculture towards embracing a diversity of crops – particularly indigenous African summer grain crops – and diverse agricultural practices that support healthy ecosystems, economies, and societies.

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 ACB wholly objects to the application for environmental release of DP-056113-9 based on a complete lack of information and data to demonstrate safety of this product, which opens up biosafety concerns for both human health and biodiversity. Not a single demonstration of safety tests has been described in the application, while the potential biosafety implications regarding the inclusion of transgenes that activate gene silencing mechanisms were not even mentioned. It is widely acknowledged that GM crops cannot be contained, and thus approving environmental release of DP-056113-9 at this stage risks the health of the South African people and the biodiversity of our environment.

This product is designed to serve the applicant and certified/registered seed producers at the expense of South Africa's small-holder farmers and biodiversity, local seed varieties that are needed to combat deepening inequality, food insecurity, climate change and hunger.

Basing approval of environmental release of a novel food crop with no history of safe use on assumptions and unproven claims goes against the precautionary approach of the Convention of Biological Diversity's Cartagena Biosafety Protocol, to which South Africa is a Party.

Furthermore, there is record of this GM event only being tested in one trial site in the US. The application is thus about using the land, biodiversity and resources of our country to enable the applicant to conduct risky experiments that will benefit only the seed industry. This smacks of neo-colonial exploitation of our environment and people. The ACB refuses to accept that South African people are used as guinea pigs for this untested and unproven technology.

Summary of application

Approval for environmental release of Pioneer Hi-Bred International, Inc's genetically modified (GM) DP-566113-9 for field trials in the following areas: Brits, Delmas, Groblersdal, Leandra, Pretoria, Rustenburg, Nelspruit and Potchefstroom, South Africa. The exact trials sites are unknown under rules of confidential business information (CBI). The crop event is summarised below in Table 1.

Crop Event	Traits of interest	Genes introduced
DP-566113-9	Male sterility in parental female hybrid lines; red fluorescent marker	*Zm-aa1, zm-Ms44 ami-RNA, DsRed2*

The crop is described by the producer to carry three transgenes:

1. *Zm-aa1:* a gene that encodes alpha-amylase protein. Alpha-amylase lowers starch levels in developing pollen, rendering the pollen non-viable. This makes the maize male-sterile.
2. *zm-Ms44 ami-RNA:* Artificial microRNA targeting the Ms44 gene, a novel, synthetic gene that encodes an artificial microRNA molecule (amiRNA), which functions to silence a mutant version of the Ms44 gene in a sterile male maize variety, restoring fertility to this existing variety.
3. *DsRed2:* encodes a fluorescent red protein that originally derives from a sea coral-like anemone. This is designed as a visible marker to allow for identification of transgenic grain.

Background to Pioneer's 'seed production technology' that employs the DP-566113-9 GM 'maintainer' line

The DP-566113-9 crop is designed to speed up the process of producing hybrid maize seed varieties. When hybrid maize varieties are produced, male and female inbred hybrid lines are crossed to make hybrid seed. In order to preserve the traits of the hybrid seed, the female hybrid parental lines are generally de-tasselled either manually or with machinery to prevent the female from self-pollinating and thus reducing the genetic purity of the parental lines.

More recently, genetic engineering approaches have been used to control unwanted self-pollination. GM and other strategies are in continuous development to take advantage of naturally male-sterile plants as parental lines for hybrid seed production, which have the advantage of being unable to self-pollinate, but have the limitation of being difficult to multiply for the continuous production of hybrid seeds.

The 'Seed Production Technology' system developed by Pioneer Her-Bred Inc employs the DP-566133-9 GM variety to be trialled, as a 'maintainer' line that allow for the continuous propagation of male-sterile varieties by self-pollination that would otherwise be difficult.

The DP-566133-9 maintainer line was generated by introducing three transgenes into a naturally male sterile variety. The sterility in this variety is caused by a mutation in a gene called *Ms44* (male sterility 44). By introducing an artificial microRNA targeting the *Ms44* mutant gene, it silences this mutation, making the maintainer DP-566133-9 fertile. It can thus be continuously propagated. The introduction of the red fluorescent protein into the GM variety allows for the sorting of the transgenic grains to be kept for propagation of the maintainer line. The fluorescent grains need to be detected under a sorting process with fluorescent detectors. This is required because only half of the grains produced will be transgenic, while the other half will be non-transgenic and will thus be discarded.

The inclusion of the alpha-amylase zm-aa1 gene functions in theory, to render 50% of the DP-566133-9 pollen completely inviable. The other 50 % pollen produced by the maintainer DP-566133-9 will be viable, but in theory, none of the viable grains will contain the transgenic DNA. So, when this pollen is crossed with a sterile-male plant (carrying the Ms44 mutation), the progeny will be male-sterile, but can be classified as non-GM, though as detailed below, there is still potential risk for transgenic pollen escape. These male-sterile progenies are then to be used as the female parental line for hybrid seed production when crossed with a parental male hybrid variety.

Inadequate contamination measures

A major concern regarding the environmental release of DP-056113-9 for field trials is the potential for transgene spread and subsequent contamination of cultivated maize varieties. Such contamination has health and environmental implications. Though detection of GMO contamination at the trial stage at a global level is lacking, nonetheless contamination from field trials has been documented, including for maize (see Price & Cotter, 2014). Maize is reported to account for a quarter of all contamination incidents globally and is defined by the

EU's Environment Agency as a "medium to high risk" for potential contamination of non-GM varieties, as well as for pollen contamination of honey due to ability to spread on the airflow (Eastham & Sweet, 2002).

The measures put in place noted by the applicant to prevent potential pollen flow are completely inadequate based on established evidence of maize gene flow dynamics. The applications states that 400m isolation distances and a 4-meter buffer zone of non-GM plants designed to capture any escaping pollen will be implemented to contain pollen escape. However, cross-pollination has been detected between different maize varieties at up to 800 meters, making the 400m isolation distance insufficient. It has also been estimated that a small quantity of maize pollen is likely to travel much further under suitable atmospheric conditions. As noted by the applicant in section 9.2 of its application, with regards to the likelihood of spread/dissemination outside of release site: "Possible but unlikely impacts may come from seed and pollen dispersal."

The application further states in section 6.9.4 that: "dissemination of DP-056113-9 maize can only occur by way of seeds since all fertile pollen is non-transgenic." However, as noted in a publication by the applicant in 2016 (Wu et al., 2016) and summarised by Wan et al., 2019 regarding a parallel product produced by the applicant that carries the identical transgenic constructs for pollen lethality *(Zm-aa1)*: *"the rate of transgene transmission through pollen was found to vary from 0% to 0.518% (Wu et al., 2016). Even that the final seed sorting step would reduce the risk of transgenic seed being in the hybrid production field, there is still a potential risk of transgenic pollen flow during the male sterile line propagation phase via the transgenic intermediary"*. **The claims made by the applicant are not in line with their own data, and expose a route of escape and contamination of conventional and local varieties of maize.** Contamination by the GM seeds is also a possible route of escape.

Further information on the surrounding areas to the trial site have been deleted under CBI rules, making it impossible to determine the likelihood of contamination, or to inform those whose crops may be affected.

Biosafety concerns of gene silencing molecules

DP-056113-9 maize, unlike current GM crops widely commercialised, is part of a novel set of crops that utilise the epigenetic mechanism of RNA interference (RNAi). RNAi is a natural biological mechanism that regulates the expression of genes. In short, specific segments of DNA sequences, or genes, carry the information for producing a particular protein. The pathway involves two steps. The first is the transcription, or copying, of a gene into messenger RNA (mRNA). RNA molecules are another type of genetic material, similar in structure to DNA. The mRNA acts as the information bridge between DNA and protein. In the second step, these messenger RNA molecules are translated into proteins. The sequence of the gene and the copied RNA molecule determines what protein is produced. One function of RNAi is to 'interfere' with protein synthesis by either degrading the messenger RNA molecules, or blocking them from being translated into proteins. This interference is caused by certain types of double-stranded RNA (dsRNA) molecules, which are a mirror image in sequence to the messenger RNA to be targeted for interference i.e. there is sequence complementarity between the dsRNAs and the target mRNA.

Through the procedure of genetic engineering, transgenes are introduced into the crop that encode for dsRNAs that are engineered to target and subsequently silence a particular gene of interest. In the case of DP-056113-9 maize, a synthetic transgene was introduced to encode for dsRNA molecules that 'switch off' a male-sterility mutant gene in a maize variety, and thus restoring its fertility. This dsRNA does not exist naturally and thus novel, with no history of safe use.

As far as we are aware, this is the first environmental release of an RNAi crop anywhere in South Africa. South Africa has approved a stacked GM maize variety MON87427 × MON89034 × MIR162 × MON87411 (ACB, 2017), but this GM maize has not been imported into the country and as far as we know, has not entered our food supply.

Potential adverse effects of RNAi crops have implications for both human health and the environment as detailed below.

1. Potential adverse effects of dsRNAs on human health

One of the major biosafety concerns being raised with regards to RNAi inducers, is the potential for unwanted off-target effects. Unwanted off-target activity may take place within the plant, with silencing of additional mRNAs to that of the target, increasing the potential for unexpected effects that by definition are impossible to predict. Knowledge gaps remain with regards to the rules and mechanism of RNAi, such that predicting what off-target effects may occur impossible without confirming it with empirical investigation. Unexpected effects could include altered compositional profile of the plants such as altered levels of nutrients, or increases levels of toxins or allergens.

Off-target effects may also occur in people who consume the plant. Numerous studies have confirmed that unlike other forms of RNA molecules such as messenger RNAs that are very unstable, plant dsRNAs produced are able to survive mammalian digestion and have been shown to also go on to regulate mammalian genes (reviewed by Tome-Carneiro et al., 2018). As described in a new peer-reviewed scientific review on the issue: *"what appears clear from current available evidence is that shRNAs present in food (derived from GM crops or not), or from foliar spray application will, in all likelihood, enter the body of the animal and human consumer"* (Amjad-Nawaz et al., 2019). Recent studies have even shown the potential for dietary-derived dsRNAs such as those from the broccoli vegetable, to be potential anti-cancer agents due to their ability to down-regulate tumour suppressor genes in mice (Mlotshwa et al., 2015), while pharmaceutical companies have patents on oral dsRNA molecules for therapeutic drug development.

Claims made by GM crop developers to suggest that dsRNAs are thus readily digestible and thus present no potential route of exposure without the data in animal models to substantiate such claims is unfounded. In this case, the applicant does not even refer to the presence of the dsRNA in section 8 of the application, which covers human and animal health and pathogenicity. There is thus a complete absence of considering the above exposure risks and potential for unintended effects to human health.

Though dsRNAs such as those from plants are present in the food supply, the dsRNA molecule introduced into DP-056113-9 does not exist naturally and was made synthetically by combining different sequences together. It thus would represent a novel dsRNA molecule, not previously included in maize, the environment or any food supply, and thus has no history of safe use.

The ACB is aware that the maize grown in the proposed field trial is not intended to enter the food supply. However, with the biosafety risks that the synthetic dsRNA present to human health combined with the complete lack of safety tests makes it unjustifiable that a product that may be completely unsuitable for human consumption may be grown in an open air field trial. As detailed above, this presents opportunity for contamination via pollen flow and seed escape. Biosafety experts have researched and proposed various risk assessment measures such as full "omics" profiling to assess for unintended effects on gene expression and composition of the plant, along with chronic, multi-generational animal testing to assess the novel risks presented by dsRNAs.

It is unjustifiable that multinational seed and agrochemical companies risk the health of people and threaten the biodiversity of our country by being allowed to release their untested products in uncontained environments. **Contained laboratory experiments should be performed to establish safety before plants are approved for environmental release.**

2. Potential adverse effects of dsRNAs on Environment

The dsRNA present in DP-56113-9 may have adverse effects on non-target organisms that may be exposed to the plant tissue, pollen or decaying plant matter. dsRNAs have been shown to be relatively persistent in the environment (Heinemann et al., 2013), thus providing a potential route of exposure. Gene silencing in non-target organisms could thus be triggered following consumption or via direct contact with the skin (for certain species such as nematodes). The applicant did not consider unintended effects such as changes to gene expression that could have adverse effects on the health of non-target organisms. Gene silencing could occur in non-target organisms if the sequence of the dsRNA matches that of a messenger RNA sequence in a non-target organism. Gene silencing has previously been documented in non-target organisms exposed to a GM maize that expressed dsRNA to target a crop pest, even though there was incomplete sequence complementarity (79-83%) to the silenced messenger RNA in the non-target organisms (Baum et al., 2007). The rules and extent of off-target activity is not yet known, and is further exacerbated by the limited data on the messenger RNA sequences of relevant off-target organisms. Of crucial relevance is the omission of sequence information of the dsRNA produced in DP-056113-9, preventing any independent analysis of potential unintended effects of the product DP-056113-9.

dsRNAs may also exert heritable effects (Heinemann, 2018), having long-term biosafety implications that should also be considered and tested prior to environmental release.

Assessment of potential unintended effects on non-target organisms was completely absent from consideration by the applicant. **It is premature to go ahead with an open- air field trial that will expose non-target organisms to the product without the above concerns being addressed under contained conditions first.**

Molecular concerns

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.

1. Molecular data missing on transgenic DNA

The transgenic material has been generated synthetically and therefore have no history of safe use in nature. A detailed description of the sequence of the transgenes should, therefore be provided. As stated in Annex I of Cartagena Biosafety Protocol, to which South Africa is a party:

> *"It is important that a description of the nucleic acid introduced into the recipient organism be available. It provides information about all the genes including control elements that have actually been introduced...if there is introduced nucleic acid, then it will contain a number of elements with functions important to the production of a gene product; to the amount of gene product produced ...These are important in considering how the introduced genetic information may be expressed in the modified organism."*

Description of the event fails to include sequence information, as it is CBI deleted. The applicant should be asked to provide sequence information to confirm integrity of the inserted transgenic material.

2. Molecular data missing on genomic maize DNA

The applicant does not provide any details on the specific location of the transgene. There is no sequence information or description of the flanking genomic DNA provided. This information is essential for showing that no gene function in the maize plant has been disrupted by the insertion of transgenic DNA.

Nor does the applicant provide and sequence information of the genomic DNA, to show that the genetic modification has not disrupted of the integrity of the maize genome as a whole. This is essential because it has become well established that the process of genetic modification introduces widespread unintended effects at the genetic and epigenetic level that can affect gene expression, and thus protein and metabolite levels in GM organisms. The use of *Agrobacterium tumefaciens* as a vector to introduce the transgenes is particularly problematic. A new 2019 (Jupe et al., 2019) study using the latest technology that allows for detailed sequencing of plant DNA, revealed that the vector induces genetic deletions, insertions and chromosomal rearrangements, translocations, scrambling of sequences and epigenetic (chemical modifications of DNA) perturbations.

The applicant also fails to provide a characterisation of the transcriptome, proteome or metabolome to show no alterations to the composition of the product, with potential to increase level of toxins and allergens, or reduce important nutrients in the product. This information is necessary considering the above described unintended effects that can arise as a result of the GM process which are impossible to predict.

As recently revealed by Mesnage et al., (2016), unintended disturbances in plant composition have been observed in GM crops, with negative health implications, using "omics" profiling techniques to analyse proteome and metabolome profiles of a GM maize. Altered levels of proteins and metabolites indicative of oxidative stress, alterations in levels of enzymes involved in glycolysis metabolism, as well as Krebs cycle involved in energy production were observed. 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 of 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).

The above studies highlight that claims made by the applicant that "The DP-056113-9 maize plants are no different from unmodified, conventional maize plants except for the traits conferred by the inserted sequences of interest", are completely unfounded and unscientific assertions.

We urge the Executive Council and the Advisory Committee to request that the applicant provide detailed molecular characterisation to show the integrity of the transgenic, flanking genomic DNA and the wider genomic DNA as a whole, and to show that no unintended effects arising from the genetic modification procedure have occurred in the plant. Techniques such as "omics" profiling that is now routinely performed in research laboratories should be adopted to provide profiling data.

Concerns regarding complete lack of safety data

Establishing the food and feed safety of DP-056113-9 is essential, considering it will eventually be used for production of hybrid seeds destined for human and animal consumption. There is no history of safe use for the transgenes or the transgene products in the food supply.

The applicant claims that "DP-056113-9 maize has no anticipated adverse effects on human health. Therefore, there are no health or safety implications anticipated from these small-scale trials and trained personnel will be available for regular monitoring during the duration of the trials."

However, as evident from this statement, this is an assumption of "anticipated" effects that have not been corroborated by scientific toxicological analyses. The application completely fails to provide any evidence of human health risk assessments. It merely provides one sentence eluding to safety: that "proteins expressed in DP-056113-9 maize have been shown to lack acute toxicity." It is unclear what this claim is based on, as a scientific literature search performed by the ACB failed to find any safety assessments of the dsRNA expressed in the plant, or the DsRed2 protein. DsRed2 is not included in any crops apart from DuPont's current line and the previous version of this same technology, event DP-32138-1 that according to the CBD, is only approved by one country but is yet to be commercially grown anywhere in the world. Some data from peer-reviewed studies suggest that DsRed2 is toxic when expressed in human cell lines (Zhou et a., 2011), though data on toxicity following oral exposure remains lacking.

The risks of DP-05113-9 are completely untested and thus unknown. As such, it is entirely premature to allow the crop to be approved for environmental release that may risk contamination of South Africa's food supply. **We urge the government to ask the applicant to provide thorough safety tests including chronic toxicological studies.**

Socio-economic concerns

1. **Beneficiaries of the 'Seed Production Technology' are certified/registered seed producers and multinational hybrid seed companies**

The purpose of this proprietary technology as described by Pioneer Hi-Bred is primarily to reduce production costs of hybrid seed production that can be applied to all maize germplasm. Second, Pioneer Hi-Bred Inc suggest it can be used to further promote the use of hybrid seed adoption across the African region where in general maize hybrid seed cultivation is very low. In the case of Southern Africa where farmer input support programs use hybrid maize, the benefits of such a system will lie with certified seed producers who will licence the product from the developer. As such, this technology does nothing to assist small- and medium-scale farmers in the country nor the region, but instead, consolidates the multinational agribusiness's stranglehold over the South African food systems, entrenching the corporate domination over seed.

2. **Shifting away from hybrid seed urgently need to protect biodiversity, climate resilience, food security**

Hybrid seeds, first developed by the founder of HiBred Corn company (now Pioneer Hi-Bred Inc) Henry A. Wallace, were generated by manually detasseling plants, until male-sterile plants were later introduced. The early development of male-sterile lines led to disaster, when in the 1970s in a damp summer in the US, the primary line used for male-sterile plants, which was susceptible to fungal disease due to the effects of the male-sterility gene, resulted in disastrous fungal disease across the corn belt (see Ho, 2005).

This early example of hybrid seed technology serves as a cautionary tale that is even more relevant now in an era of unpredictable climate change- that the absence of seed diversity is bound to lead to potentially lethal epidemics in a country that is already food insecure. Seeds are tools for adaptation that have been used by farmers for thousands of years to cope with changing conditions. The Food and Agriculture Organisation (FAO) estimates that 75 % of crop diversity has already been lost in the 20th century, and recognises the need for the role of genetic diversity for coping with climate change (Jarvis et al, 2015).

We urge the government to support a shift away from the continued corporate erosion of South Africa's genetic diversity that occurs at the hands of multinational corporations seeking profits gained from hybrid and GM seed.

To conclude

The applicant has failed dismally in providing adequate information to demonstrate the safety of DP-056113-9 for human health and the environment. Instead, it makes unsubstantiated, unscientific claims to dismiss biosafety concerns, rendering field trials premature and irresponsible at this stage. Measures to prevent escape of the transgenes into the environment are inadequate, threatening farmer managed seed and food systems. Lastly, there are no benefits for the proposed technology for South African citizens and small-holder farmers. Multinational and certified seed producers are the sole potential beneficiaries of a technology in an ever increasingly unsustainable food system entirely unfit to cope with today's critical challenges of climate change, food insecurity and rising inequality.

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