Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/20/V1 (maize with altered growth characteristics) from VIB for deliberate release in the environment of genetically modified plants for research and development

10 March 2020
Ref. SC/1510/BAC/2020_0256

The notification B/BE/20/V1 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2020 for a request of deliberate release in the environment of genetically modified higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: *Scientific field evaluation of maize with modified growth characteristics.* The purpose of the release is to confirm the maize's modified growth characteristics under normal field conditions and to measure the effect of the modification on the cob formation and cob filling.

The notification has been officially acknowledged by the CA on 8 January 2020 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB), answered positively to this request.
The experts assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the GM maize lines would not raise any problems for the environment, animal or human health in the context of the intended use.

On 12 February 2020, based on a list of questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. Answers to the questions were provided on 24 February 2020.

For the purpose of the scientific evaluation, the following legislation has been considered: the Royal Decree of 21 February 2005 (Belgian Official Journal of 24.02.2005, p. 7129) modified by the Royal Decree of 19 February 2020 (Belgian Official Journal of 02.03.2020, p. 12666).

In parallel to the scientific evaluation, the CA made the dossier available on its website for a one-month public consultation as required in the abovementioned Royal Decree. The CA forwarded the list of questions to the Biosafety Advisory Council. No questions of the public tackling biosafety issues of the GM maize were identified.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Zea mays is an allogamous plant that propagates through seed produced predominantly by cross-pollination. Maize pollen can be collected by honeybees and other insects, however these pollinating insects play a minor role in the cross-pollination of maize plants which relies mainly on wind for the dispersal of its pollen (OECD, 2003[1]). Data on pollen dispersal in maize demonstrated that the levels of cross-fertilisation drop rapidly over the initial meters around the pollen source and that most of the released pollen is deposited within about 30 m of the source (Devos *et al*., 2005[2]). At distances farther than 30 - 50 m from the source, pollen dispersal is very low but not zero. However, vertical wind movements can lift up pollen and distribute it over distances up to kilometers under suitable climatic conditions. In Belgium (and in Europe) there are no sexually cross-compatible wild relatives with which maize can hybridise and form progeny (OECD, 2003). The only recipient plants that can be cross-fertilised by maize are therefore other cultivated maize varieties.

Seed dispersal of individual kernels of domesticated plants are mainly the result of field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities. Spilled maize seeds can overwinter, germinate and appear in the field as volunteers. However, maize is incapable of sustained reproduction outside the domestic cultivation area as it has lost its ability to survive in the wild due to its long process of domestication (OECD, 2003). Volunteers will only occur after a warm winter period (with no temperatures lower than 0°C for more than 6 to 8 hours) and will be characterised by a low probability of cross-pollination (Grüber *et al*., 2008[3]; Palaudelmàs *et al*., 2009[4]). Given the Belgium weather conditions, volunteers are not likely to occur.

2. Information on the design and management conditions in the field trial

The field trial will be conducted during three growing seasons (from April/May 2020 until October 2022). The surface of the area for cultivation will not exceed 1100 m^2.

Prior to complete formation, tassels from the GM maize will be removed by hand in order to prevent the dispersal of GM pollen. Once the last leave has been formed, monitoring of upcoming tassels will take place every two days until all tassels have been removed and will be maintained until September 15. Removed tassels will be transported in closed bags and inactivated.

During harvest, cobs of the GM maize plants will be collected by hand and transported in closed bags to the lab. Material will be inactivated if no longer needed for research. Stems and leaves, except for a few which will be harvested, will be shredded on the field. Roots and the lowest part of the stem will be left in the ground.

After the field trial, the field will be left fallow and ploughed at the latest during next spring.

[1] OECD, 2003. Consensus Document on the biology of *Zea mays* subsp. *Mays* (maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No. 27:1-49. http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF

[2] Devos *et al.*, 2005. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environmental Biosafety Research 4, 71-87.

[3] Grüber *et al*., 2008. Post-harvest gene escape and approaches for minimizing it. CAB International 2008 (http://www.cababstractsplus.org/cabreviews)

[4] Palaudelmàs *et al.*, 2009. Effect of volunteers on maize gene flow. Transgenic Res. 18, 583-594

3. Information related to the genetic modification

The GM maize lines B104(EF1α::AN3_02), B104(EF1α::AN3_05) and B104(EF1α::AN3_07) were obtained by *Agrobacterium tumefaciens*-mediated transformation, are subject of this field experiment.

The maize lines contain the AN3 gene from *Zea mays* that encodes for a transcriptional co-activator that is involved in the regulation of cell proliferation. As a result of the modification the duration of growth is elongated which results in the formation of larger plant organs such as larger leaves. It is also results in the formation of more biomass.

In addition, the transgenic lines contain the *bar* gene from *Streptomyces hygroscopicus* that served as a marker for the selection of transformants after *Agrobacterium tumefaciens*-mediated transformation. The *bar* gene produces the phosphinotricin acetyl transferase (PAT) enzyme, which acetylates phosphinotricin (also known as glufosinate, the active ingredient of the broad spectrum herbicides), thereby rendering it inactive.

The backbone sequence of the vector used for transformation harbours the *aadA* gene conferring resistance to the antibiotics spectinomycin and streptomycin. Absence of the *aadA* gene has been demonstrated in the transgenic line B104(EF1α::AN3_02), but is present in the other two lines as the result of integration of the transformation vector.

4. Potential risks for the environment, animal or human health associated with the release of the GM maize

No increased persistence in the field or invasiveness into natural habitats of the GM maize lines compared to non-GM maize is expected, as the intended changed characteristic (larger plant organs) is not expected to confer a selective advantage to survivability. Other (unintended) changed characteristics observed in the glass house are a shorter anthesis silking interval and a better drought tolerance compared to the non-GM lines. These characteristics may result in a selective advantage. However, the measures taken (removal of tassels and manual collection of cobs) rule out the development and survival of the GM maize in the year(s) after the field trial.

Vertical gene transfer through pollen can virtually be ruled out due to the removal of the tassels.

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[5]). The possibility of horizontal gene transfer between the GM maize plants and bacteria has been given particular attention due to the presence of (modified) recombination sites *att*B1, *att*B2 and *att*B4 of *E.coli* for phage λ in the GM maize lines. It was questioned whether the presence of these *att*B sites could increase the uptake of plant DNA by lysogene bacteria. The occurrence of such an event in an environment where phage *att*P1, *att*P2 and *att*P4 sites are present that could recognise the modified *att*B sites was considered unlikely. In case gene transfer from the GM maize to micro-organisms would take place and gene expression would occur, negative effects on the environment and humans are not expected. The resistance genes (i.e. *bar* and *aadA*) occur naturally in microbes and not expected to cause hazardous effects (for bar, see OECD, 1999[6]; for *aadA*, see

[5] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.

[6] OECD, 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to phoshinothricin herbicide. Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 11, Organisation for Economic Corporation and Development, Paris.

EFSA, 2004[7], 2009[8]), and the AN3 gene, expressing a protein involved in plant cell proliferation, will not confer a selective advantage to bacteria.

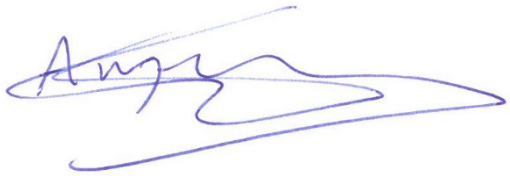
Further, it is not expected that the GM maize would have significant effects on organisms (invertebrates, vertebrates and soil micro-organisms) and humans, as no trait that could affect the behaviour or development of organisms via contact or feeding has been integrated. Given the restricted scale of the field trial, any potential effect to organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. As the release of GM pollen in the environment is prevented, a possible altered allergenicity potential of the transgenic pollen (allergy from maize pollen may occur in case of occupational exposure to high amounts of pollen grains, see *e.g.* Oldenburg *et al*., 2011[9]) does not form a concern for human health.

5. Information related to the control, monitoring, post-release and waste treatment

The management measures proposed are considered as sufficient to prevent potential adverse effects to the environment, animal and human health during and after the field trial. The removal (or covering) of any appearing tassel in the transformed line will prevent gene flow by pollen spread. Careful manual harvesting of the cobs and storing them in closed bags will prevent seed dispersal. The seeds and the few collected plants will be destroyed after analysis.

Conclusion

Provided that the trials are conducted as described in the dossier, the Biosafety Advisory Council concludes that it is very unlikely that this proposed small scale field trials with GM maize will harm human health, animals or the environment.



Prof. Dr. ir. Geert Angenon
President of the Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of assessing the dossier B/BE/20/V1 (ref: BAC_2020_0205)

[7] EFSA, 2004. Opinion of the Scientific Panel on GMOs on the use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA Journal 48, 1-18.

[8] EFSA, 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants' and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in GMPs on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 1108, 1-8.

[9] Oldenburg 2011. Maize pollen is an important allergen in occupationally exposed workers. Journal of Occupational Medicine and Toxicology 6: 32.

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Compilation of comments of the experts in charge of evaluating notification B/BE/20/V1

12 February 2020
Ref. SC/1510/BAC/2000_0205

Coordinator: Bart Panis
Experts: Jan Van Doorsselaere (VIVES), Patrick du Jardin (ULiège), Nina Papazova (Sciensano, GMOLAB), Michel Van Koninckxloo (CARAH), Nicolas Van Larebeke (UGent)
SBB: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/20/V1** concerns a notification of the VIB, for deliberate release in the environment of genetically modified higher plants (GMHP) according to Chapter II of the Royal Decree of 21 February 2005.

The notification has been officially acknowledged on 08 January 2020 and concerns a field trial transgenic maize with altered growth characteristics

Experts were invited to evaluate the GMHP considered in the notification as regards their potential impacts on the environment, including human and animal health, and information relating to pre- and post-release treatment of the site.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005

EVALUATION

The comments below served as basis for a list of questions that the competent authority forwarded to the notifier with a request to provide additional information. The comments highlighted in grey correspond to the questions/comments selected and sent to the notifier.

1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS
(e.g. reproduction, survivability, dissemination, geographic distribution,...)

Have evaluated this section and had no comments/questions: 3 experts

2. INFORMATION RELATED TO THE GENETIC MODIFICATION
(e.g. methods used for the modification, description of the vector,...)

Have evaluated this section and had no comments/questions: 2 experts

Comment 1
C3 page 7: description of the table contains some errors; table is not as figure of the plasmid shows; it should be: attB4, pEF1a, attB1, AN3, attB2; EF1a and AN3 should be switched in the table
Size of pVS1 rep, pBR322: size on plasmid does not correspond with size in the table; needs to be checked

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

3.1. Information related to the traits and characteristics, which have been introduced or modified

Have evaluated this section and had no comments/questions: 3 experts

3.2. Information on the molecular characteristics of the final GMO
(e.g. number of copies of the transgenes,...)

Comment 1
D2 Page 9: it is concluded that for line 05 and 07 the complete vector is integrated; based on the PCR results one can only say that this is likely but not 100% sure
page 10: is it correct to assume that line 05 contains 16 copies on 1 locus?

Comment 2
As reported in the dossier, two of the GM lines contain the Streptomycin/Spectinomycine resistance gene *aadA* from the vector backbone, which was used in the transformation process but not intended to be present and expressed in the field-grown plants. The consequences for the safety assessment will be discussed in later sections.
The Directive 2001/18/EC on the deliberate release of GMOs has decided the phasing out of antibiotic resistance markers, for both Parts C and B dossiers, in the following terms:
Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. ***This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to part C and by 31 December 2008 in the case of GMOs authorised under part B.***

Note SBB: An important issue to consider on the phasing out, is that the Directive clearly states that ARMs which may have adverse effects on human health and the environment need to be phased out, and not every ARM.

The antibiotic Spectinomycine is included by WHO in the list of “Essential Medicines” (version 2019 in Excel table in annex, downloaded from https://www.who.int/medicines/publications/essentialmedicines/en/). The remaining question is whether the *aad* gene in GM maize ‘*may have adverse effects on human health and the environment”* and the current dossier does not address this issue. In line with the provision of the Directive, I consider that the lines B104(pEF1a::AN3_05) and B104(pEF1a::AN3_07) should not be authorized for field trialling before a risk assessment concludes that the likelihood of adverse effects is negligible. Such assessment should be asked to the notifier.

Note SBB and coordinator: To our understanding the issue ‘adverse effects on human health’ is addressed in D.7 (gezondheid op de mens) and in the Technical Dossier ‘Selectieve voordelen of nadelen die op de maïsplanten zijn overgedragen’.

3.3. Information on the expression (of the insert)

(e.g. parts of plants where the insert is expressed, (expected) expression of the insert during the lifecycle of the plant,...)

Have evaluated this section and had no comments/questions: 1 expert

Comment 1

D3 page 10: Please provide some qPCR data concerning expression of the AN3 gene; now merely literature on the promotor activity of EF1a and 35S are mentioned; is AN3 expressed and what is the difference between the lines?

Note SBB and coordinator: The Biosafety Advisory Council guidelines for Molecular Characterisation of GM Plants for a Standard Part B Consent do not require determination of the level of expression at protein level. At this first field trial stage (where several lines are tested), it is sufficient to give information on the expected expression, rather than the real expression.

Comment 2

"*De elementen die op deze backbone zijn gelegen zijn immers van bacteriële origine en zijn enkel functioneel wanneer zij in een bepaalde bacteriële achtergronden zijn opgenomen*".
Hoe kan dit met zekerheid gesteld worden?

"*Het aadA-gen dat aanwezig is in de lijnen B104(pEF1a::AN3_05) en B104(pEF1a::AN3_07) staat onder controle van een bacteriële promoter en komt om die reden normaal gesproken niet tot expressie in de planten. Dit geldt ook voor de overige elementen aanwezig op de vector backbone aanwezig in deze lijnen."*
Hoe kan dit met zekerheid gesteld worden?

Note SBB and coordinator: Men kan niet met 100% zekerheid zeggen dat genen onder controle van een bacteriële promoter niet tot expressie komen in planten, maar ervaring heeft aangetoond dat bepaalde bacteriële promoteren niet actief zijn in planten. Terwijl de eerste quote wat te straf is geformuleerd, is de tweede meer genuanceerd.

Comment 3

Bibliographic information is provided on the expected expression in the plant, but not experimental data are given. Although I see no safety issue here based on the nature of the inserted genes, this seems not in line with the “Guidelines for MC of GMP for a standard Part B Consent (according to Anne III B of directive 2001/18/EC)”, requesting experimental data.

Note SBB: See feedback to comment 1. We will consider to make this more clear in the guidelines.

3.4. Information on how the GM plant differs from the recipient plant

Have evaluated this section and had no comments/questions: 3 experts

3.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
Several generations of these lines have been tested in the greenhouse and stability is maintained.

3.6. Any change to the ability of the GMHP to transfer genetic material to other organisms

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
Site-specific recombinogenic sequences *attB*1, 2 and 4 derived from the Gateway vector system are present in the plants having integrated the whole backbone of the transformation vector. Bibliographic arguments indicate no safety issue, but do not exclude the theoretical possibility of horizontal gene transfer to environmental bacteria mediated by these site-specific recombinogenic sequences. This is relevant for the risk assessment of the presence of the antibiotic resistance marker *aadA* in two of the transgenic lines.

Note SBB and coordinator: We could point out to the VIB that not all bullet points on page 11-12 of the 'Technisch Dossier', in particular, '*als er al horizontale genoverdracht zou zijn, dan geeft het promoter-gen construct geen selectief voordeel, waardoor de kans bijzonder klein is dat het er zich zou handhaven*', apply to all genes present in the GM maize (i.e. the *aad* gene). We could ask the VIB to reconsider their evaluation taking the *aad* gene into account and to argue if the presence of recombinogenic sequences *attB* on the vector backbone is potentially involved in the promotion of gene transfer to recipient *E. coli* bacteria.

3.7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification

Have evaluated this section and had no comments/questions: 2 experts

Comment 1
As reported in the dossier, two of the GM lines - B104(pEF1a::AN3_05) and B104(pEF1a::AN3_07) - contain the Streptomycin/Spectinomycine resistance gene *aadA* from the vector backbone, which was used in the transformation process but not intended to be present and expressed in the field-grown plants.
The Directive 2001/18/EC on the deliberate release of GMOs has adopted a precautionary approach by deciding to phase out of antibiotic resistance markers for both Parts C and B dossiers, in the following terms:
Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. ***This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to part C and by 31 December 2008 in the case of GMOs authorised under part B.***

The antibiotic Spectinomycine is included by WHO in its list of "Essential Medicines" (version 2019 which can be downloaded from https://www.who.int/medicines/publications/essentialmedicines/en/). The next question to be answered by the risk assessment is whether the *aadA* gene in GM maize '*may have adverse effects on human health and the environment".* However, in my view, the words *'may have'* can be interpreted in different ways, either considering a theoretical scenario or concluding on the basis of experimental data.

The applicant addresses this issue by referring in vague terms to the EFSA scientific opinion (EFSA, 2009). This article indicates that the prevalence of the *aadA* gene in most environments makes it unlikely that *aadA* genes from the DNA of GM plants cause additional adverse effects. In this part B dossier, the presence of recombinogenic sequences attB on the vector backbone is relevant as it is potentially involved in the promotion of gene transfer to recipient *E. coli* bacteria, but such transfer of the *aadA* from the GM plant is likely to proceed at a much lower frequencies compared with the transfers occurring from the natural soil gene reservoirs.

In conclusion, considering (i) the low exposure of environmental bacteria to the GM maize DNA in the context of this part B dossier and (ii) the expected presence of *aadA* and other spectinomycin-resistance genetic determinants in the receiving environment, the likelihood of adverse effects to human and animal health and the environment is negligible. However, considering that (i) this gene is dispensable for the scientific purpose of this experiment, (ii) other GM lines without the antibiotic-resistance marker, like the B104(pEF1a::AN3_02) might be available, (iii) taking also into account the precautionary approach laid down by the directive aiming at phasing out the antibiotic-resistance markers under defined conditions, I personally regret that the applicant did not take the initiative to exclude these lines from the dossier.

3.8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects from the genetic modification, where the GMHP is intended to be used in animal feedstuffs

Have evaluated this section and had no comments/questions: 2 experts

3.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

Have evaluated this section and had no comments/questions: 2 experts

3.10. Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification

Have evaluated this section and had no comments/questions: 3 experts

3.11. Potential interactions with the abiotic environment

Have evaluated this section and had no comments/questions: 3 experts

3.12. Description of detection and identification techniques for the GM plant

Have evaluated this section and had no comments/questions: 3 experts

Comment 1:
The notifier is asked to verify why 2,5 µl of a 10X buffer will be used on a total volume of 20 µl. Please also provide information on the amount of grind tissue to be dissolved in 500 µl of CTAB buffer for the isolation of genomic DNA.
Further, the primer attachment sites need to be presented schematically (as requested in the Detection Protocol).

3.13. Information about previous releases of the GM plant, if applicable

Have evaluated this section and had no comments/questions: 3 experts

4. INFORMATION RELATING TO THE SITE OF RELEASE
(e.g. description of the site ecosystem, presence sexually compatible species, proximity of protected areas, ...)

Have evaluated this section and had no comments/questions: 2 experts

Comment 1

The footnote of annex 3 mentions another Part B application : “REG/rc-bvl/17-00659 GA20OX1 x GA2ox-PLA1 140-01 _ dossier bijlagen 1”. This a mistake. The applicant should confirm that this annex is the correct one for this application!

5. INFORMATION RELATING TO THE RELEASE

(e.g. purpose of release, dates and duration of the release, methods for preparing and managing the release site, number of plants, ...)

Have evaluated this section and had no comments/questions: 2 experts

Comment 1

In parts B4 and G1 of the dossier, the applicant indicates that the male inflorescences will be removed in order to prevent pollen dissemination. However, in part D4 the applicant indicated that the anthesis-to-silking interval is an agronomically relevant phenotype which will be assessed during the experiment and, in part F, measurements of grain setting and grain filling are said to be performed during the trial, which necessitate pollination of the female inflorescences.

My conclusion is that the non-GM lines used as control will be used as pollinators of the GM lines. Is this correct? Removal of the male inflorescences will be achieved at the onset of anthesis, i.e. at stamen maturity, but before pollen shed. Is this correct?

The applicant proposes to visit the field trial every second day. Whether this interval is appropriate in order to detect the onset of anthesis while preventing any pollen shed is a question which is not answered by the information on maize biology provided in the dossier. How long is an anther mature before it opens and sheds pollen? The applicant should answer this question to support his claim.

6. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

6.1. Information on the likelihood for the GMHP to become more persistent than the recipient or parental plants or more invasive

Have evaluated this section and had no comments/questions: 2 experts

Comment 1

"Waarschijnlijkheid dat de genetisch gewijzigde maïsplanten persistenter worden dan de recipiënte of de ouderplanten in landbouwgebieden of invasiever in natuurlijke habitats."

Er dient opgemerkt dat de planten een soort groei-gen geïncorporeerd kregen, een transcriptionele co-activator betrokken bij de celproliferatie onder controle van een actieve promotor, en dat het niet uitgesloten is dat dit een selectief voordeel oplevert in de natuur.

Note SBB and coordinator: In het dossier is beschreven dat de aanwezigheid van het AN3-gen dat codeert voor een transcriptionele co-activator leidt tot grotere bladeren, en dat de planten een versterkte groei hebben onder droogte-omstandigheden (in het lab) in vergelijking met wild-type planten. Men kan stellen dat dit een selectief voordeel zou kunnen opleveren voor de GG-maïs. Het is echter onwaarschijnlijk dat dit potentieel selectief voordeel, de GG-plant zal toelaten om de biologische en niet-biologische factoren te overwinnen die de persistentie en invasief karakter van maïs beperkt. Maïs heeft door domesticatie zijn vermogen verloren om in de natuur te overleven, is vorstgevoelig en niet gekend te overleven in de koudere regio’s van Europa (met temperaturen lager dan 0 °C voor meer dan 6 tot 8 uur).

Comment 2

This aspect was briefly addressed in part D4, raising no safety concern.

6.2. Information on the selective advantage or disadvantage conferred to the GMHP

Have evaluated this section and had no comments/questions: 2 experts

Comment 1
"*Selectieve voordelen of nadelen die op de maïsplanten zijn overgedragen.*"
Vermoedelijk is het niet mogelijk volledig uit te sluiten dat de resistentie tegen de antibiotica streptomycine en spectinomycine overgedragen wordt op bacteria. Misschien is het nuttig de expressie van het aadA gen na te gaan in de planten en in bacteriën langdurig in contact met de planten. Is de aanwezigheid van het aadA gen eigenlijk wel nodig?

Note SBB and coordinator: Wat betreft de vraag of het nuttig is om de expressie van het *aadA* gen na te gaan in de planten en in bacteriën langdurig in contact met de planten, zijn wij van mening dat dit niet nodig is, om de volgende redenen:
- de transfer van antibiotica resistentie genen (ARG) van GG-planten naar bacteriën werd enkel aangetoond in optimale lab-condities en niet onder natuurlijke condities;
- indien DNA-transfer tussen de GG-planten en bacteriën zou plaatsvinden, zal dit aan een veel lagere frequentie gebeuren dan tussen bacteriën;
- resistente tegen streptomycine/spectinomycine is wijd-verspreid, en genen die zulke resistentie geven kunnen dus 'geselecteerd' worden uit de natuur en verspreid worden onder bacteriën.

Verder, verwijzen we naar de opinie van EFSA (2004) over het gebruik van ARG als merkers in GG-planten. O.b.v. een aantal criteria werden de ARG ingedeeld in drie 'veiligheidsklassen'. Het *aadA*-gen valt in klasse 2 die ARG omvat die aanwezig mogen zijn in GG-planten die getest worden in veldproeven, maar niet in GG-planten voor commercieel gebruik.

"*Een eventuele verspreiding van het gen naar bacteriën in de omgeving zal, gezien de reeds bestaande wijde verspreiding van het gen, een te verwaarlozen risico op het vergroten van de resistentiepool opleveren*."
Daaraan kan aan getwijfeld worden.

Note SBB and coordinator: Deze formulering is in lijn met die van EFSA (2004). Verder dient men er mee rekening te houden dat de veldproef beperkt is in oppervlak en in tijd.

Comment 2
This aspect was briefly addressed in part D4, raising no safety concern.

6.3. Information on potential of gene transfer to other sexually compatible plant species under conditions of planting and its consequences

Have evaluated this section and had no comments/questions: 2 experts

Comment 1
"*Kans op genoverdracht op dezelfde of andere seksueel compatibele plantensoorten onder de omstandigheden van het planten van de genetisch gewijzigde maïsplanten, en selectieve voordelen of nadelen die op die plantensoorten kunnen worden overgedragen."*
Vermoedelijk is het niet mogelijk om absoluut uit te sluiten dat genoverdracht plaats vindt, onder meer omdat pollen zich over een grote afstand kunnen verspreiden. Stellen dat alle pollen precipiteren binnen enkele tientallen meter is ongeloofwaardig. Dit betekent dat er een (vermoedelijk zeer kleine maar niet onbestaande) probabiliteit is dat de transcriptionele co-activator betrokken bij de celproliferatie onder controle van een actieve promotor wordt overgedragen op andere soorten. Eenmaal een kruisbestuiving met GGO pollen tot een genetisch gewijzigde plant aanleiding heeft gegeven, is men de controle over de verspreiding van in de GGO plant aangebrachte vreemde DNA sequenties kwijt.

Note SBB and coordinator: De mannelijke bloeivorm van de maïs zullen verwijderd worden, zodat er geen pollen verspreid kunnen worden.

Comment 2
The question of the risks of pollen release related with the critical timing allowing both the measurement of the time of anthesis and the prevention of pollen shed was raised in section 5 before.

6.4. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with target organisms

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
This aspect was briefly addressed in part D4, raising no safety concern.

6.5. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, including herbivores, parasites, symbionts...

Have evaluated this section and had no comments/questions: Jan Van Doorsselaere

Comment 1
"*Mogelijke onmiddellijke en/of vertraagde leefmilieueffecten van de directe en indirecte interacties tussen de genetisch gewijzigde maïsplanten en niet-doelwitorganismen, inclusief de effecten op de populatieniveaus van concurrenten, planteneters, symbionten, parasieten en ziekteverwekkers."*
Men kan zich afvragen of het eten van GGO mais door insecten, duiven of andere vogels niet kan leiden tot een verspreiding van in de GGO plant aangebrachte vreemde DNA sequenties.
Note SBB and coordinator: De maïs in de veldproef zal zaad vormen. Hoewel maïs zijn zaad niet spontaan laat vallen (daar het vervat zit in kolven) is een mogelijk verspreiding van dit zaad door vogels niet uitgesloten. Gezien de Belgische weersomstandigheden en de biologische karakteristieken van maïs is het zeer onwaarschijnlijk dat zaad dat verspreid wordt door vogels in de natuur overleeft (we verwijzen hiervoor naar de informatie in 6.1).

Comment 2
Conclusion on this aspect needs to refer to section 3.10 before, where no changes in the interaction of the GM maize as compared to non-GM maize is indicated.

6.6. Information on possible effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or living in the vicinity of the GMHP release

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
"*Mogelijke onmiddellijke en/of vertraagde effecten op de menselijke gezondheid van mogelijke directe en indirecte interacties tussen de genetisch gewijzigde maïsplanten en personen die werken met, in contact komen met of in de nabijheid komen van de introductie."*
Zeer waarschijnlijk zijn er geen onmiddellijke schadelijke effecten op de mens te vrezen. Of er op lange termijn kans bestaat dat door een zeldzame gebeurtenis mensen in contact komen met bacterien die antibiotica resistentie verworven hebben of die agressievere eigenschappen hebben door het dragen van een transcriptionele co-activator betrokken bij de celproliferatie onder controle van een actieve promotor is weinig waarschijnlijk maar moeilijk uit te sluiten.
Indien DNA van genetisch gewijzigde planten door omstandigheden peroraal wordt opgenomen door de mens kunnen kleine hoeveelheden van in het maagdarm kanaal aanwezig DNA, die niet noodzakelijk afgebroken worden door de spijsvertering, opgenomen worden in de bloedstroom, vooral dan in individuen met spijsverteringsstoornissen of immunodeficiency. De vraag rijst of dergelijk DNA ook in menselijke cellen kan terecht komen.

De ingevoerde genetische wijziging berust op het aanwenden van recombinatiesequenties die mogelijk ook actief kunnen zijn in menselijke cellen. Men kan zich afvragen of dit een effect zou kunnen hebben op de recombinatie activiteit of op de genexpressie in menselijke cellen.

Verhoogde recombinatie activiteit kan leiden tot een verhoogde frequentie van clonen van recombinante cellen, een fenomeen dat zich onder meer in de pancreas voordoet en, zoals aangetoond bij muizen, toeneemt met de leeftijd en onder invloed van mutagene agentia (Wiktor-Brown et al., 2006).

Het is ook onzeker of een combinatie van een transcriptionele co-activator betrokken bij de celproliferatie onder controle van een actieve promotor zou kunnen geintegreerd worden in een menselijke cel (uiteraard zou dit slechts een zeer uitzonderlijk gebeuren kunnen zijn) en kunnen leiden tot een tumorpromotor effect, met een tumor als gevolg. Er dient opgemerkt dat kanker steeds ontstaat uit één enkele tumoraal getransformeerde cel.

Een andere bron van zorg is de introductie in de mens van sequenties die recombinatie of genexpressie, onder meer door disruptie van micro-RNA's, zouden beinvloeden.

Note SBB and coordinator: De maïs afkomstig uit deze veldproef zal noch geconsumeerd worden door dier, noch door de mens. Om die reden dienen mogelijke effecten van de orale opname van de maïs niet geëvalueerd worden.

Comment 2
This aspect was briefly addressed in part D7, raising no safety concern taking into account the nature of the proteins expressed from the transgenes.

6.7. Information on possible effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
"Mogelijke onmiddellijke en/of vertraagde effecten op de gezondheid van dieren en effecten op de voeder/voedselketen van consumptie van de genetisch gewijzigde maïsplanten en alle daarvan afgeleide producten indien deze voor diervoeder bestemd zijn."
Zelensky et al. (2017) toonden aan dat inactivatie van "A-family DNA polymerase theta (Pol theta)" en "canonical non-homologous DNA end joining" de off-target integratie van exogeen DNA elimineert. Deze enzymsystemen zijn waarschijnlijk actief in plantencellen en zijn actief in dierlijke en menselijke cellen. De kans bestaat dus dat off-target integratie is opgetreden in de genetisch gewijzigde mais. De consumptie van genetisch gewijzigde mais door een dier kan gezondheidsproblemen veroorzaken mocht de mais, behalve de gewenste wijziging, ook een andere genetische wijziging ondergaan hebben die tot wijzigingen in de expressie van eiwitten, in de aanwezigheid van kleine moleculen of tot de productie van een toxische stof leidt. Relatief eenvoudige tests kunnen wellicht toelaten om de productie van een toxische stof op te sporen zodanig dat dit probleem kan vermeden worden.

De ingevoerde genetische wijziging berust echter op het aanwenden van recombinatiesequenties die mogelijk ook actief kunnen zijn in dierlijke cellen. Het is ook onzeker of een combinatie van een transcriptionele co-activator betrokken bij de celproliferatie onder controle van een actieve promotor zou kunnen geintegreerd worden in een dierlijke cel (uiteraard zou dit slechts een zeer uitzonderlijk gebeuren kunnen zijn) en kunnen leiden tot een tumorpromotor effect, met een tumor als gevolg. Er dient opgemerkt dat kanker steeds ontstaat uit één enkele tumoraal getransformeerde cel.

Een andere bron van zorg is de ongewilde disruptie van micro-RNA's, wat wijzigingen in genexpressie kan veroorzaken.

Note SBB and coordinator: De maïs afkomstig uit deze veldproef zal noch geconsumeerd worden door dier, noch door de mens. Om die reden dienen mogelijke effecten van de orale opname van de maïs niet geëvalueerd worden.

Comment 2
Not relevant.

6.8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s)

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
Not relevant, considering low exposure of part B dossier. NB Not included in the applicant's risk assessment.

6.9. Information on environmental impact of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs

Have evaluated this section and had no comments/questions: 1 expert

Comment 1
Not relevant, considering low exposure of part B dossier. NB Not included in the applicant's risk assessment.

7. INFORMATION RELATED TO CONTROL, MONITORING, POSTRELEASE AND WASTE TREATMENT

7.1. Precautions taken

Have evaluated this section and had no comments/questions: 3 experts

7.2. Information on methods for post-release treatment of site

Have evaluated this section and had no comments/questions: 3 experts

7.3. Information on postrelease treatment methods for the GM plant material, including wastes

Have evaluated this section and had no comments/questions: 2 experts

Comment 1
G4 De method van inactivering van mannelijke bloemen wordt niet beschreven

7.4 Information related to monitoring plans and the detection techniques

Have evaluated this section and had no comments/questions: 3 experts

7.5. Information on the emergency plan(s) proposed by the notifier

Have evaluated this section and had no comments/questions: 3 experts

7.6. Information on methods and procedures to protect the site

Have evaluated this section and had no comments/questions: 3 experts

8. OTHER INFORMATION

8.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1
VIB PSB has experience in the organisation of field trials. To my knowledge, the previous trials have been conducted according to biosafety practices.

References

Wiktor-Brown, D. M., C. A. Hendricks, W. Olipitz, and B. P. Engelward. Age-dependent accumulation of recombinant cells in the mouse pancreas revealed by in situ fluorescence imaging. *Proc.Natl.Acad.Sci.U.S.A* 103 (32):11862-11867, 2006.

Zelensky, A. N., J. Schimmel, H. Kool, R. Kanaar, and M. Tijsterman. Inactivation of Pol theta and C-NHEJ eliminates off-target integration of exogenous DNA. *Nat.Commun.* 8 (1):66, 2017.