Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/24/V7 from Corteva Agriscience for deliberate release in the environment of genetically modified plants for research and development

12 March 2025
Ref. SC/1510/BAC/2025_0354

The notification B/BE/24/V7 has been submitted by Corteva Agriscience Belgium B.V. to the Belgian Competent Authority (CA) in January 2025 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: *Field trial request for three CRISPR-Cas9 maize concepts.* The purpose of the release is for educational purposes and to show the potential of gene-edited maize.

The notification has been officially acknowledged by the CA on 2 January 2025 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. Five 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 edited maize lines would not raise any problems for the environment, animal or human health in the context of the intended use. See Annex I for an overview of all comments received.

On 19 February 2025, based on questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. An answer to the questions was received by the Secretariat on 26 February 2025.

For the purpose of the scientific evaluation, the following legislation has been considered:
- 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. One question of the public tackling biosafety issues of the GM maize was received.

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 indigenous wild relatives with which maize can hybridise and form progeny (OECD, 2003; EFSA, 2016[3]). Teosinte, regarded as an invasive weed in Europe since its first occurrence in France (1990) and Spain (2009), has so far not been reported in Belgium. The only recipient plants that can be cross-fertilised by maize in Belgium 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 can only occur after a warm winter period (with no temperatures lower than 0°C for more than 6 to 8 hours) and are characterised by a low probability of cross-pollination (Grüber *et al.*, 2008[4]; Palaudelmàs *et al.*, 2009[5]). In the prevailing Belgian climate, volunteers are unlikely to occur.

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

The field trial will be conducted during one growing season in 2025. The surface of the area for cultivation will not exceed 1000 m^2.

Prior to complete formation, tassels from the modified maize will be removed by hand to prevent the dispersal of pollen. Once the last leaf has been formed, monitoring of emerging tassels will take place three times a week until all tassels have been removed and will continue for at least two weeks or longer until all male flowers are removed. Removed tassels are stored in a closed container on the field till the end of the trial. The container will be transported to the composting facility for destruction.

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[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] EFSA (European Food Safety Authority), 2016. Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094. 13 pp.

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

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

During harvest, maize cobs will be collected manually, chopped on the field and transported in closed bags to the composting facility. Stems and leaves 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.

3. Information related to the genetic modification

Three maize concepts created through the use of CRISPR-Cas9 technology are the subject of this field experiment.

3.1 CRISPR-Cas9 waxy maize

The CRISPR-Cas9 waxy maize, obtained through the deletion of the endogenous *wx1* gene, was shown to result in a low amylose/high amylopectin phenotype of the kernels, also known as waxy maize.

The waxy maize was obtained via particle bombardment of immature maize embryos with six plasmids. Plasmid 1 is the donor of Cas9 endonuclease; plasmids 2 and 3 are the donors of 5' guide RNA and 3' guide RNA, respectively; plasmids 4 and 5 are donors of the two helper genes to improve embryoid response and plant regeneration frequencies; and plasmid 6 is the donor of NPTII selectable marker. The NPTII protein results in resistance to the antibiotic neomycin and kanamycin.

Transformed plants, selected based on NPTII, with confirmed *wx1* gene deletion were subsequently backcrossed with the wild-type parent to obtain plants solely containing the mutation (and no plasmid DNA). The resultant plants were tested for the lack of vector sequences via Southern-by-Sequencing technology. These tests indicated the absence of plasmid DNA. A selected plant, confirmed for *wx1* gene deletion and absence of unintended plasmid DNA sequences from all transformation plasmids, was advanced towards development of inbred lines. Subsequently, using conventional crossing of two inbred lines, a maize hybrid was obtained. This hybrid is subject of this field trial.

3.2 CRISPR-Cas9 NLB18 maize

The CRISPR-Cas9 NLB maize was obtained via allele replacement of the endogenous disease susceptible allele of the *nlb18* gene, *nlb18*-S, with the disease resistant allele of the same gene, *nlb18*-R, derived from inbred PH26N. CRISPR-Cas9 NLB18 maize was shown to result in an improved resistance to maize Northern Leaf Blight (NLB).

The NLB18 maize was obtained following a two-step approach: first, the *nlb18*-S gene was deleted via particle bombardment of immature maize embryos with two guide RNAs homologous to genomic sequences flanking the *nlb18*-S allele. The elements for the *nlb18*-S gene deletion were provided on six separate plasmids. Subsequently, in immature maize embryos homozygous for the *nlb18*-S deletion the *nlb18*-R allele - provided on another plasmid - was integrated. The resultant plants were tested for the lack of vector sequences via Southern-by-Sequencing technology. These tests indicated the absence of plasmid DNA. A final NLB18 inbred line was crossed with a conventional inbred line to generate hybrid maize. This hybrid is subject to this field trial.

3.3 CRISPR-Cas9 DL maize

The CRISPR-Cas9 DL maize, obtained via incorporation of two native maize genes (*nlb18*-R and *ht1*) for improved resistance genes against Northern Leaf Blight (NLB) and one native maize gene (*rppK*), for an improved resistance to southern corn rust.

The DL maize was obtained via *Agrobacterium*-mediated transformation of a maize inbred line with a single plasmid containing a single sequence locus, making up the disease locus (DL) of the three native

genes. The resultant plants were tested for the lack of vector sequences via Southern-by-Sequencing technology. These tests indicated the absence of plasmid DNA. This maize inbred is the subject to this field trial.

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 CRISPR-Cas9 waxy maize compared to non-GM maize is expected, as the reduced amylose content is not known to confer a selective advantage to survivability. The changed characteristics of CRISPR-Cas9 NLB18 maize and CRISPR-Cas9 DL maize, namely improved resistance to pathogens, may confer a selective advantage to survivability. However, due to the (a)biotic factors limiting maize's survival, it is also not expected that changes in these characteristics will increase the ability of maize to survive. Moreover, the measures taken (removal of tassels and manual collection and chopping of cobs) rule out the development and survival of the modified maize in the year(s) after the field trial.

Vertical gene transfer to cultivated maize in the surroundings through pollen can virtually be ruled out due to tassel removal.

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[6]). In case gene transfer from the modified maize to micro-organisms would take place and gene expression would occur, negative effects on the environment and humans are not expected. The mutated genes, expressing proteins involved in starch production or plant defense systems, are unlikely to confer a selective advantage to micro-organisms.

Further, it is not expected that the modified maize would have significant effects on organisms other than micro-organisms, as no trait that would affect the behaviour or development of vertebrates and invertebrates via contact or feeding has been integrated. Given the restricted scale of the field trial, any potential adverse effect on micro-organisms other than the envisaged pathogens and on biogeochemical processes - if these would occur - will be of a local and temporal nature.

As the release of 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[7]) 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 monitoring and removal of any appearing tassel in the transformed lines will prevent gene flow by pollen spread. Careful manual harvesting of the cobs, followed by chopping and transport of the chopped material in closed bags will prevent seed dispersal. The chopped seeds will be composted, first at the ILVO facility and subsequently at the VLACO facility.

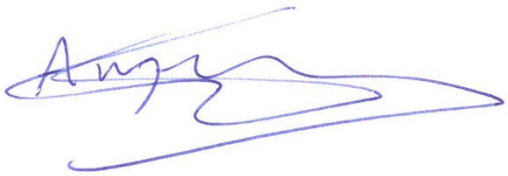
Years of experience have shown that no volunteer plants appear in the year following maize field trials. The field will be left fallow and ploughed at the latest during next spring.

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

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

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 trial with modified maize lines 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/24/V7 (ref: BAC_2025_0257)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the comments of the experts in charge of evaluating notification B/BE/24/V7

Ref. SC/1510/BAC/25_0257

Coordinator: Geert Angenon
Experts: Philippe Baret (UCLouvain), Henri Batoko (UCLouvain), Jacques Dommes (ULiege), Nina Papazova (Sciensano), Frank Van Breusegem (UGent), Nikolas Van Larebeke (KULeuven),
SBB coordinator: Adinda De Schrijver

INTRODUCTION

Dossier **B/BE/24/V7** concerns a notification of Corteva Agriscience Belgium B.V., 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 2 January 2025 and concerns a field trial request for three CRISPR-Cas9 maize concepts.

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 consolidated version of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005

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. The comments encompass biosafety questions relevant for risk assessment and to come to a risk/safety conclusion.
Comments received that do not fall within the remit of the work of the Biosafety Advisory Council (e.g. socio-economic considerations) are listed in an annex.

B. INFORMATION RELATING TO THE PLANT RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANT

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

Comment:
4.A "*Maize has been domesticated for thousands of years and as a result maize kernel dispersal does not generally occur naturally*". The link here is not evident to me.

Note coordinator & SBB: This is meant to explain that maize has been selected to retain kernels in a cob and therefore seed dispersal is limited (see also B.2.a.ii).

C. INFORMATION ON THE GENETIC MODIFICATION

(A) CRISPR-Cas9 waxy maize

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

Comment:
I do not understand the use of the NPTII selectable marker in this procedure. How can this marker, which is not a plant DNA sequence, be of help in this process without being incorporated in the plant genome.

Note coordinator & SBB: The NPTII marker is used to select those maize embryos that have obtained the plasmids through particle bombardment.

(B) CRISPR-Cas9 NLB18 maize

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

Comment:
I wonder whether, in the Southern-by-Sequence analysis, the improved removal of endogenous junctions featuring in the SbS technology developed by Zastrow-Hayes et al. (2015) could mask the detection of the insertion of non-plant sequences in fact belonging to a plasmid used in the genetic construction.

(C) CRISPR-Cas9 DL maize

Have evaluated this section and had no comments/questions: 4 experts

D. INFORMATION RELATING TO THE GENETICALLY MODIFIED PLANT

(A) CRISPR-Cas9 waxy maize

D.1. Description of the trait(s) and characteristics that have been introduced or modified

Have evaluated this section and had no comments/questions: 4 experts

D.2. Information on the sequences actually inserted/deleted

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

Comment:
It would be interesting to have data about the relative frequency with which plants containing unintended plasmid sequences are recovered.

Note coordinator & SBB: Usually, plants that do not contain unintended sequences are selected for further research and development. As this is not a question on the maize under evaluation, we do not see the need to ask for more information on this issue.

D.3. Information on the expression of the insert

Have evaluated this section and had no comments/questions: 4 experts

D.4. Information on how the GM plant differs from the recipient plant

Have evaluated this section and had no comments/questions: 4 experts

D.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 4 experts

D.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: 3 experts

Comment:
Still it seems possible to me that the presence of plants deleted for an enzyme involved in the production of amylose might increase the prevalence, in the surroundings, of maize poor in amylose.

Note coordinator & SBB: As measures, including removal of male flowers, are taken to prevent gene flow (see G.2), the spread of the waxy maize trait will be prevented.

D.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: 3 experts

D.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: 3 experts

D.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

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

Comment:
Not applicable

D.10. Possible variations 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

D.11. Possible interactions with the abiotic environment

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

D.12. Description of detection and identification techniques for the GM plant

Have evaluated this section and had no comments/questions: 4 experts

Comment 1:
In general, the protocol is well-described. However, a better description of the positive and the negative controls (named standard samples in the protocol) is needed, in particular whether this is the CRISPR-Cas waxy maize (homozygote or heterozygote) or WT maize.

Comment 2:
Figure on page 21 : Is the left image a non-waxy maize or a waxy maize?

Note coordinator & SBB: Waxy maize is high in amylopectin (nearly 100%) and very low in amylose content. Amylose forms an insoluble complex with iodine resulting in a deep blue colour, while amylopectin has reduced affinity for iodine absorption which results in a reddish brown colour. Iodine staining of waxy maize therefore results in a lighter red-brownish colour, while staining of non-waxy maize in a dark blue colour. The figure is therefore correct.

D.13. Information about previous releases of the GM plant, if applicable

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

(B) CRISPR-Cas9 NLB18 maize

D.1. Description of the traits and characteristics, which have been introduced or modified

Have evaluated this section and had no comments/questions: 4 experts

D.2. Information about actually inserted/deleted sequences

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

Comment:
The statement “Thus, the final plant line produced by the CRISPR-Cas9 technology is indistinguishable from the plants that could result from the native genome variability or developed in conventional breeding program” is correct, but does not imply that these plants are identical.

D.3. Information on the expression of the insert

Have evaluated this section and had no comments/questions: 4 experts

D.4. Information on how the GM plant differs from the recipient plant

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

Comment:
The resistance to disease could give the CRISPR-Cas9 NLB18 a competitive advantage and an advantage in dissemination and survival. However, this is unlikely to be a problem.

D.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 4 experts

D.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: 4 experts

D.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:
As toxic and allergenic effects are unlikely, further investigation of toxicity/allergenicity is not considered needed.

D.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

Comment:
As toxic and allergenic effects are unlikely, further investigation of toxicity/allergenicity is not considered needed.

D.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

D.10. Possible variations 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

D.11. Possible interactions with the abiotic environment

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

D.12. Description of detection and identification techniques for the GM plant

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

Comment:
Not applicable

D.13. Information about previous releases of the GM plant, if applicable

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

(C) CRISPR-Cas9 DL maize

D.1. Description of the traits and characteristics, which have been introduced or modified

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

Comment:
More information should be provided on the mechanisms by which the Ht1, NLB18 and RppK proteins act.

Note coordinator & SBB: *NLB18* encodes a Pattern Recognition Receptor (PRR) and can trigger a PTI response (Pattern Triggered Immunity). *Ht1* and *RppK* encode Nucleotide Binding Site/Leucine Rich Repeat (NBS/LRR) receptors that recognise pathogen effectors and can trigger ETI responses (Effector Triggered Immunity). These types of immune receptors and immune responses have been extensively studied in plants. Genes encoding PRRs and NBS/LRR receptors have been extensively used in classical plant breeding to enhance disease resistance. Further, we do not consider more information on the mechanism of action is needed to conduct the risk assessment, given that these proteins occur in a number of commercialised maize hybrids and the scale of this field trial.

D.2. Information about actually inserted/deleted sequences

Have evaluated this section and had no comments/questions: 4 experts

D.3. Information on the expression of the insert

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

Comment:
CRISPR-Cas9 DL maize was generated by inserting maize genomic sequences (cisgenes) encoding the NLB18-R, HT1, and RPPK proteins - each conferring resistance to different fungal pathogens - into a single locus, namely the NLB18 locus. According to the application (p. 28, point 3), the expression of NLB18-R, HT1, and RPPK in this CRISPR-Cas9 DL maize is expected to be similar, both spatially and temporally, to that in conventional maize because these are native, unmodified sequences complete with their endogenous regulatory elements.
However, inserting these cisgenes into the NLB18 locus alters their genomic context compared to that in conventional maize. This change not only affects the context of the inserted NLB18-R but also that of the HT1 and RppK alleles. Such a reorganization may modify their regulation - for instance, through interactions with enhancers that are not part of their intrinsic cis-regulatory elements.
Although the release and cultivation of CRISPR-Cas9 DL maize are intended for educational purposes (and not for food or feed), it is anticipated that this line could serve as a genetic resource for conventional breeding programs aimed at enhancing maize resistance to the specified pathogens. Therefore, it would be prudent to experimentally confirm that the genomic reorganization of these three cisgenes via CRISPR-Cas9 does not adversely affect their expression, ensuring that it remains comparable to that observed in conventional maize.

Note coordinator & SBB: 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 field trial stage, it is considered sufficient to give information on the expected expression, rather than the real expression.

D.4. Information on how the GM plant differs from the recipient plant

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

Comment:
The resistance to disease could give the CRISPR-Cas9 DL a competitive advantage and an advantage in dissemination and survival. However, this is unlikely to be a problem.

D.5. Genetic stability of the insert and phenotypic stability of the GMHP

Have evaluated this section and had no comments/questions: 4 experts

D.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: 4 experts

D.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:
As toxic and allergenic effects are unlikely, further investigation of toxicity/allergenicity is not considered needed.

D.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

Comment:
As toxic and allergenic effects are unlikely, further investigation of toxicity/allergenicity is not considered needed.

D.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

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

Comment:
To my opinion insufficient information is provided on the mechanisms of action of the proteins coded for by the introduced genes.

Note coordinator & SBB: see feedback on D.1.

D.10. Possible variations 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

D.11. Possible interactions with the abiotic environment

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

D.12. Description of detection and identification techniques for the GM plant

Have evaluated this section and had no comments/questions: 4 experts

Comment:
In general, the protocol is well-described. However, a better description of the positive and the negative controls (named standard samples in the protocol) is needed, in particular whether this is the CRISPR-Cas DL maize (homozygote or heterozygote) or WT maize.

D.13. Information about previous releases of the GM plant, if applicable

Have evaluated this section and had no comments/questions: 4 experts

E. INFORMATION RELATING TO THE PLACE OF RELEASE

Have evaluated this section and had no comments/questions: 4 experts

F. INFORMATION RELATING TO THE RELEASE

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

Note SBB: Removed tassels will be stored in closed containers on the field. Can it be clarified how long they will be stored in those containers and what will be done with the tassels once the field trial is finalised?

G. INFORMATION RELATED TO CONTROL, MONITORING, POST-RELEASE AND WASTE TREATMENT PLANS

G.1. Any precautions taken

Have evaluated this section and had no comments/questions: 4 experts

G.2. Description of methods for post-release treatment of the site

Have evaluated this section and had no comments/questions: 4 experts

G.3. Description of post-release treatment methods for the GM plant material, including wastes

Have evaluated this section and had no comments/questions: 4 experts

G.4 Description of monitoring plans and techniques

Have evaluated this section and had no comments/questions: 4 experts

G.5. Description of any emergency plans

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

Comment:
Mechanical destruction is to be preferred above use of a herbicide.

G.6. Methods and procedures to protect the site

Have evaluated this section and had no comments/questions: 4 experts

ENVIRONMENTAL RISK ASSESSMENT IN LINE WITH ANNEX II D.2

Information relevant for different parts of the environmental risk assessment

(A) CRISPR-Cas9 waxy maize

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

(B) CRISPR-Cas9 NLB maize

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

(C) CRISPR-Cas9 DL maize

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

Comment:
I think that insufficient information is provided concerning the mechanisms of action of Htl and RPPK to assess Environmental risk. The one argument that is available is the fact that these proteins also occur in natural non-GMO plants.

Note coordinator & SBB: see feedback on D.1 for CRISPR-Cas9 DL maize

1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats

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

2. Any selective advantage or disadvantage conferred to the GMHP

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

Comment 1:
I think that resistance to disease can confer a selective advantage and an advantage in dissemination and survival. However, this is unlikely to be a problem.

Comment 2:

1. "The CRISPR-Cas9 waxy maize is not expected to differ from conventional Wx1 maize or non-waxy maize lines in relation to mode(s) and/or rate of reproduction, dissemination and survivability."
   This affirmation is not substantiated. No data is provided on the comparison between the CRISPR-Cas9 maize and conventional Wx1.
2. "The CRISPR-Cas 9 NLB18 maize is not expected to differ from conventional maize in relation to mode(s) and/or rate of reproduction, dissemination and survivability."
   This statement is not substantiated. No data is provided on the comparison between the CRISPR-Cas 9 NLB18 maize and conventional maize.
3. "The CRISPR-Cas 9 DL maize is not expected to differ from conventional maize in relation to mode(s) and/or rate of reproduction, dissemination and survivability."
   This statement is not substantiated. No data is provided on the comparison between the CRISPR-

Cas 9 DL maize and conventional maize is provided.

Note coordinator & SBB: Given (1) the well-known biology of maize (see OECD, 2003; CFIA, 2021 and references therein provided in the dossier) that it is highly domesticated to the point that it cannot establish itself as a feral plant outside the agricultural habitat under European conditions; and (2) (a)biotic factors will limit maize's survival, regardless of the introduced changes, we consider the anticipations made by the notifier correct and sufficient. Moreover, the measures taken (removal of tassels and manual collection and chopping of cobs) rule out the dissemination of pollen, and the reproduction and survival of the modified maize in the year(s) after the field trial.

A key element with the CRISPR-Cas 9 method is the effect of off-target sites. Both documents (summary and full document) claim equivalence of transgenic lines and conventional lines. It overlooks unintended changes in the CRISPR_Cas9 process (Koller and Cieslak 2023).

Note coordinator & SBB: We want to note that the search for/verification of off-target changes is and has not been considered an issue to be considered for Part B field trial notifications (due to the restricted environmental release and use).

3. **Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species**

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

4. **Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable)**

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

5. **Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organism, including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens**

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

   Comment:
   Insufficient information is provided on the mechanisms of action of HtI and RPPK to assess environmental risk.

   Note coordinator & SBB: See feedback on D.1 CRISPR-Cas9 DL maize

6. **Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s)**

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

   Comment:
   Insufficient information is provided on the mechanisms of action of HtI and RPPK to assess possible risk to human health. But these proteins are also present in natural plants to which humans are exposed since a long time.

   Note coordinator & SBB: See feedback on D.1 CRISPR-Cas9 DL maize

7. **Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products 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 :
   Insufficient information is provided on the mechanisms of action of HtI and RPPK to assess possible risk to animal health. But these proteins are also present in natural plants to which animals are exposed since a long time.

   Note coordinator & SBB: See feedback on (C) CRISPR-Cas9 DL maize

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: 2 experts

9. **Possible immediate and/or delayed effects, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHP**

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

OTHER INFORMATION

Comment :
I have one general comment concerning this kind of dossier. To me, as long as the modifications yield only native DNA sequences or sequences that could arise by traditional breeding techniques, these edited plants should not be considered as GMO. I don't mean that some kind of evaluation is not necessary. But among the different aspects of the biosafety evaluation, I think that only two are really relevant :

- the integrity of the edited sequence (or of neighboring sequences in case of deletion)
- the absence of any sequence from the plasmids used in the process.

REFERENCES

Koller, F., and M. Cieslak. 2023. "A Perspective from the EU: Unintended Genetic Changes in Plants Caused by NGT - Their Relevance for a Comprehensive Molecular Characterisation and Risk Assessment." Frontiers in Bioengineering and Biotechnology 11 (October). https://doi.org/10.3389/fbioe.2023.1276226.

Koller, F., M. Schulz, M. Juhas, A. Bauer-Panskus, and C. Then. 2023. "The Need for Assessment of Risks Arising from Interactions between NGT Organisms from an EU Perspective." Environmental Sciences Europe 35 (1): 27. https://doi.org/10.1186/s12302-023-00734-3.

Annex - List of other comments/questions received from the experts

GENERAL COMMENTS

Comment 1:

The dossier is made of many unsubstantiated statements. It doesn't imply specific risk but it raises concern about the scientific integrity of the Corteva team.

Comment 2:

The trial is justified by "educational purposes". This motivation is very vague and is antinomic with the concept of field trial. Field trials of GM crops are exceptions to the rules of deliberate release of GM. They should be justified by scientific motivations.

Note coordinator: This dossier concerns a deliberate release in the environment according to Chapter II of the Royal Decree of 21 February 2005, which does not limit the purpose of the release, as is evident from the definition of a Chapter II release: "Dissémination volontaire d'OGM à toute autre fin que leur mise sur le marché" / "Doelbewuste introductie van GGO's voor andere doeleinden dan het in de handel brengen" (cfr. Directive 2001/18/EC part B "Deliberate release of GMOs for any other purpose than for placing on the market").

Comment 3:

The coexistence of different lines in the same trial may be an opportunity of testing risk of interaction between different lines (Koller et al. 2023). As plants will be castrated the opportunity of testing this issue will be missed.

D.13. Information about previous releases of the GM plant (CRISPR-Cas9 NLB18 maize), if applicable

Comment:

The text here reflects the differences in regulatory approaches between Europe and other regions

F. INFORMATION RELATING TO THE RELEASE

Comment 1:

In view of possible controversy it would be interesting to establish a contact with NGO's active in the field of genetic modification (which should be considered stakeholders).

Comment 2:

The purpose of the release is not clearly described. On page 32 of the technical dossier, it is stated "for educational purposes and to show the potential of a CRISPR-Cas9 maize". Is it just a demonstration field trial (as stated in the document describing the field trial)? The applicant should give more precise information on the actual purposes of this release. I was expecting some monitoring of the modified traits.

Note coordinator: This dossier concerns a deliberate release in the environment according to Chapter II of the Royal Decree of 21 February 2005, which does not limit the purpose of the release, as is evident from the definition of a Chapter II release: "Dissémination volontaire d'OGM à toute autre fin que leur mise sur le marché" / "Doelbewuste introductie van GGO's voor andere doeleinden dan het in de handel brengen" (cfr. Directive 2001/18/EC part B "Deliberate release of GMOs for any other purpose than for placing on the market").

9. Possible immediate and/or delayed effects, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHP

Comment:
Disease resistance might contribute to lower use of plant protection pesticides