Bioveiligheidsraad
Conseil de Biosécurité

16-03-2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2012_0313

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/11/V4** of VIB for deliberate release in the environment of genetically modified maize with altered growth characteristics

Context

The notification B/BE/11/V4 has been submitted by VIB to the Belgian Competent Authority (CA) in December 2011 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 research using maize that has altered growth characteristics**. The specific purpose of the trial is to verify the altered growth characteristics of the maize under agricultural conditions and to test the suitability of this maize for a higher plant density.

The notification has been officially acknowledged by the CA on 16 December 2011 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 SBB also took part in the evaluation of the dossier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified (GM) maize lines would not raise any problems for the environment, animal or human health in the context of the intended use.

On 02 February 2012, based on a list of questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. The list of questions is further on called LQ. Answers to the questions were provided on 29 February 2012; this document is called ATQ further on.

For the purpose of the scientific evaluation, the following legislation has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

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. The questions of the public

tackling biosafety issues of the GMOs under consideration are taken in consideration in the opinion of the Biosafety Advisory Council. Answers to the questions of the public have been sent to the CA.

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 on wind for the dispersal of its pollen (OECD, 2003; Eastham and Sweet, 2002; Malone and Burgess, 2009). Various parameters, such as size, shape and orientation of the pollen source and recipient, wind characteristics, rain, local environment, pollen viability, water status of pollen play a role in the dispersal of viable pollen (reviewed in Devos *et al*., 2005). Data on pollen dispersal in maize demonstrated that the levels of cross-fertilization 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. 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 (Hofmann *et al*., 2010).In Belgium (and in Europe) there are no sexually cross-compatible wild relatives with which maize can hybridise and form progeny (Eastham and Sweet, 2002; OECD, 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize varieties.

Maize is an annual plant and has lost the ability to survive in the wild due to its long process of domestication. Maize cannot survive temperatures below 0°C for more than 6 to 8 hours after the growing point is above ground (5 to 7 leaf stage) (OECD, 2003). Seed dispersal of individual kernels of domesticated plants does not occur naturally and dispersal of individual kernels of corn are mainly the result of field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities. Although seeds from the previous crop year can overwinter and germinate the following year, the plant cannot persist as a weed. Moreover, based on the observations in central Europe (Grüber *et al*., 2008), one can expect that volunteers may only occur after a warm winter period and will be characterized by a low probability of cross-pollination (Palaudelmàs *et al*., 2008).

The parental line considered here is the late flowering American B104 inbred line. According to the notifier, the parental line has been chosen on the ground of its relative ease with which it can be modified and not for its agricultural properties. The unmodified B104 line apparently has been tested at ILVO in 2001 (p 2 in ATQ). Although requested in LQ, the notifier does not provide data on flowering in ATQ. Provided data on maturity in ATQ show that the untransformed B104 is an extremely late maturing maize in Flanders.

2. Information on the purpose of the field trial

The notifier mentions in the dossier (Section D1, p 8) that in previous experiments in a glasshouse, stems were up to 40-50% longer than the non transformed line. Stems were thinner and leaves were longer, but biomass was not affected. As indicated in Section F1 (p19) the purpose of the trial is a) to confirm the stem elongation in real agronomic conditions and to study the effect on biomass; b) to study if the increased length affects lodging and c) to find out if the plants allow to be planted at higher densities in order to increase yield. In Section F5 (p19), plant densities varying from 8-15 plant per m^2 are mentioned.

Answering Q7, in which the agronomic relevance of very long plants with thin stems (which are usually prone to enhanced lodging) was questioned, the notifier writes to be aware that long plants with long internodes usually are prone to enhanced lodging, particularly at higher

plant densities (ATQ, p 8). The notifier further states that he has no indications (positive nor negative) from the greenhouse experiments on the standing power. The higher plant density should provide an enhanced leaf area index, indicated by the notifier as an important factor for yield potential. He further writes that, depending on the genotype, enhanced lodging does not automatically mean a loss of agronomic value. The interest of the notifier is "mainly scientific (ATQ, p9) and the field trial is part of research investigating the molecular mechanisms that determine the effect of the GA20oxidase enzyme". Data from field trials are said to be necessary for publications in high impact international journals since "data obtained in *Arabidopsis*" do no longer suffice.

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

With the dossier, a permission is asked for a field trial to be conducted during three consecutive years (from May 2012 until October 2014). The field, harbouring the trial is well indicated. According to the dossier, the surface of the area for cultivation of the parental line and the GM maize GA20OXI will not exceed 500 m^2. Weeds will be destroyed by conventional herbicides. No glufosinate treatment will be applied. Harvesting will occur in October of each year. According to the ATQ (p 10), the seeds will be sown approximately at 20 April and at a depth of 4 cm; bird nets will be used to prevent birds from removing seeds or seedlings. Early sowing is scheduled for two reasons i) to take into account the late maturity of B104 and ii) to test the cold tolerance during early growth (the latter aspect mentioned in the ATQ and not in the dossier).

Prior complete formation, tassels will be removed by hand in order to prevent the dispersal of pollen. Monitoring of upcoming tassels will start on July, 1st and will take place three times a week. Monitoring will be enforced and occur on a daily basis from the moment the formation of a tassel has been observed. This extended monitoring will be maintained until the last tassel has been removed by hand. The collected tassels will be transported in closed bags and will be inactivated by incineration.

During harvesting, kernels are said to be collected by hand and transported in closed and labelled bags to the lab. Seeds will be stored for research or will be inactivated as GMO (incineration). Stems and leaves will be collected in closed bags. Roots and the lowest part of the stem will be left in the ground.

According to the dossier, the year following the trial, the field plot will be used again for the cultivation of maize. No measures will *a priori* be taken for post field trial monitoring of volunteers based on the low probability that volunteers will arise: pollen dispersal will be prevented by removal of the tassels, natural dispersal of seeds will be prevented and cobs with seeds will be carefully removed upon manual harvest in October. The notifier writes in the ATQ that he is willing to install a non maize crop the year of the trial, should the BAC ask for this. Such a crop would make it easier to spot and destroy potential volunteers.

The dossier mentions that the trial will be performed with the GA20OX1 line. ATQ mentions a homozygous and an azygous version of this line.

Since the dossier did not provide a clear field design it was asked for in the LQ. ATQ provides two field designs, one for the year 2012 and a larger one which will be used both in 2013 and in 2014. The smaller size in 2012 is due to the restricted seed availability. Planned plant densities are 9, 10 and 11 plants per m^2.

In all designs transformed maize plants will be detasselled, the field trial is surrounded by a border of 3 m of the non-transformed isoline, and the whole area is situated within a maize field.

The area of the trial exceeds 500 m^2 in 2013 and 2014.

4. Information related to the genetic modification

The GM maize has an altered growth characteristic resulting from the introduction of a *Ga20Oxidase-1* gene originating from *Arabidopsis thaliana*, which is under the control of ubiquinine promoter not functional in bacteria. The gene encodes for GA20oxidase, which has its natural homologue in maize and which catalyzes one of the steps of the feedback regulated biosynthetic pathway of the bioactive gibberelline GA1. In the leaves of the GM plant, a 300-fold increase of the *Ga20oxidase-1* gene transcript has been observed corresponding with a 3-fold increase only of the bioactive gibberellins GA1 in the leaves. This is due to a natural negative feedback mechanism present in the plant suppressing biosynthesis and activating catabolism of active gibberellins. The GM plants are phenotypically distinguishable by their increased length without an expected increase of biomass.

The *bar*-gene from *Streptomyces hygroscopicus*, that served as a selection marker during the *Agrobacterium tumefaciens* based transformation, is still present in the plant. This *bar*-gene produces the Phosphinotricin Acetyl Transferase enzyme (PAT), which acetylates phosphinotricin, also known as glufosinate, the active ingredient of the broad spectrum herbicides Basta and Liberty.

The GM plants bear recombination sites attB4, attB1 and attB2, which are sequences that originate from phage lambda but are present as mutated sequences in the vector construct used to modify the plants.

The dossier does not provide data on potential interactions between the PAT and the GA20oxidase enzyme.

The backbone sequence of the vector used for *A. tumefaciens* transformation harbours the *aadA* gene. Data were provided suggesting the absence of the aadA gene in the GM maize. Furthermore, like other streptomycin resistance genes, the *ant(3")*-Ia gene is demonstrated to be distributed in bacteria from a range of European environmental habitats (EFSA,2009).

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

According to the dossier, laboratory observations with the GA20OXI line indicate no alteration in the inflorescence, formation of pollen, seed formation or seed dispersal. The increased amount of plant bio-active gibberellin (i.e. 3-fold increase of GA1 in leaves) is not expected to present harmful effects such as toxic effects for organisms or allergic effects for human health. According to the notifier, potential unintended effects due to the insertion of the gene of interest in the plant genome cannot be ruled out. However, due to i) the prevention of pollen dispersal by removal of the tassels ii) the unlikely natural dispersal of seeds iii) the low winter tolerance of rare spilled seed, iv) the low probability of appearance of volunteers; gene flow (if any occurs) is not expected to cause harm to the environment. Based on these elements, the notifier mentions that no particular measures will be taken for post trial monitoring.

Current scientific evidence indicates that the transfer of genes from GM plants into bacteria and their stable integration, either does not occur or, if it occurred, has been below the limit of detection in the studies performed. The possibility of horizontal gene transfer between the GA20OX1 maize plants and bacteria has been given particular attention due to the presence of the recombination sites attB4, attB1 and attB2. However, the occurrence of an active integrase-excisionase complex in an environment where attP4, attP1 and attP2 are present is estimated low. In addition, if any horizontal gene transfer of *Ga20Oxidase*-1 gene and its expression would occur (in GA20OX1, *the GA20oxidase-1* gene is under the control of a ubiquinine promoter which is a promoter that is not functional in bacteria), no apparent selective advantage would be conferred to bacteria. The same holds for the presence of the

bar-gene. As regards to the *aadh* gene, adverse effects on human health and the environment resulting from a transfer from the GM plants to bacteria is judged unlikely, mainly due to the distribution of genes conferring streptomycine resistance in bacteria from a range of European environmental habitats (EFSA,2009).

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

The early removal of any appearing tassel in the transformed line will prevent gene flow by pollen spread, since there will be no pollen spread. Careful manual harvesting of the cobs and storing them in closed recipients will prevent seed dispersal. The notifier announces that all material will be destroyed after analysis.

The notifier mentions that no particular measures will be taken for post-trial monitoring

Conclusion

1. Provided that the notifier assesses the risk of a potential interaction between the introduced PAT and the overexpressed GA20oxidase enzyme, and provided that the trials are conducted as described in the dossier and in ATQ,

2. The Biosafety Advisory Council concludes that it is very unlikely that this proposed small scale field trials (Chapter II of the Royal Decree of 21 February 2005) with a late maturing maize inbred line will harm human health, animals or the environment.

3. In case the field trial is installed, the Biosafety Advisory Council demands the trials to be harvested at the latest by the end of September and to install a low growing non-maize crop the year after the trial and to destroy any spotted volunteer well before flowering. Acting like this will facilitate all monitoring work.

In addition to the advice on biosafety, the Biosafety Advisory Council has several reflexions on the real aims of the trial and on the scientific methodology to reach the mentioned goals. Details are given in Annex I of this advice.

Philippe Baret, Lucette Flandroy, Jean-Claude Grégoire and Damien Winandy do not completely accept the advice written here above.
According to their opinion, the dossiers should as a matter of principle be presented in such a way that the Biosafety Advisory Council should not have to undertake the risk assessment by itself. The scientific quality of the dossier presented here does not allow a proper risk analysis.

Prof. D. Reheul
President of the Biosafety Advisory Council

Annex I: Additional considerations of the Biosafety Advisory Council on this field trial
Annex II: References
Annex III: Compilation of comments of experts in charge of assessing the dossier B/BE/11/V4 (ref: BAC_2012_0184)

Annex I: Additional considerations of the Biosafety Advisory Council on this field trial

(a) As can be verified in the Section 'Summary of the scientific evaluation', it is unclear what the objectives of the notifier are.

- In the dossier the emphasis is on (i) the confirmation of the stem elongation "in real agronomic conditions", and (ii) the evaluation of the biomass production and the interaction between enhanced length and the standing power of the maize in real agronomic conditions. Plant densities are scheduled to vary between 8-15 plants per $m^{2.}$.

- In the answers to the questions (ATQ) the notifier says that his interest is "mainly scientific (ATQ, p9); the field trial is part of research looking into the molecular mechanisms that determine the effect of the GA20oxidase enzyme". Data from field trials are said to be necessary for publications in high impact international journals since "data obtained in *Arabidopsis*" do no longer suffice. Proposed plant densities in ATQ vary between 9 and 11 plants per m^2.

The Biosafety Advisory Council considers the objectives formulated in ATQ mainly as a 'proof of concept' to verify if the results found in the glasshouse experiments are confirmed in the open air.

(b) Whatever the real aim of the trial might be, the Biosafety Advisory Council wants to make some comments on the intrinsic and scientific value of the planned field trial.

(b1) The notifier used the American B104 maize material because of its ease of transformation. This maize is maturing extremely late in our circumstances: hence its agronomic relevance in Belgium is questionable.

(b2) The transformed B104 material is an inbred line. Inbred lines have no intrinsic agronomic value: their value lies in their combining ability with other lines in order to produce hybrids which are the plants that are grown in farmers' fields. The performance of an inbred line is not necessarily reflected in the derived hybrids. If the notifier wants information on potential yield performance and on the interaction with standing power of the transformed material, it is recommended to test hybrids instead of an inbred line.

(b3) Given both the expected architecture of the transformed line (considerably longer than the non-transformed isoline) as well as the proposed distances[1] between the short non-inbred pollinator circumventing the detasseled transformed line, the proposed trial design does not allow a uniform formation of cobs in the trial; hence it is very unlikely that the trial will produce sound, reliable and repeatable scientific information on the biomass formation.

(b4) The proposition to increase plant densities to enhance yields with long phenotypes (scheduled increments in plant density are much lower in ATQ than in the dossier) is an odd reasoning. Although one might bring in some theoretical grounds to do so, experience tells the opposite: higher plant densities usually are used with shorter varieties. To confirm this: in spring 2012 a new maize variety (code number VG/A/080 /01198 will be added to the Belgian national list of varieties with a specific remark that it was accepted under the conditions of a plant density of 10 plants per m^2 instead of the standard density of 9 plants per m^2. Its performance was insufficient with 9 plants per m^2, but sufficient when tested at 10 plants per m^2 .This variety is considerably shorter than the standard varieties: 227 cm versus 238 cm at 9 plants per m^2.

[1] Measured perpendicular to the longest axis of the trial area, the longest distance between the non-transformed and non-detasseled isoline and the centre of the detasseled transgene area is 3 m maize + the width of 1 path (width non specified) in 2012, but 4,5 m + the width of 2 paths (width non specified) in the design for 2013 and 2014.
It is common practice in seed production fields to use 1 row of pollinator for each 4-5 rows of seed parents in order to have a good and uniform cob filling and seed production. This means that the seed parent never is further than 3 m away from the pollinator.

Annex II: References

Devos Y, Reheul D, De Schrijver A, 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.

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

Palaudelmàs et al. (2008) Effect of volunteers on maize gene flow. Transgenic Res. 18, 583-594

Eastham K, Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency, http://reports.eea.eu.int/environmental_issue_report_2002_28/en/GMOs%20for%20www.pdf

EFSA (2009). Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ) on a request from the European Commission on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal* (2009) 1034, 1-81.

Hofmann F, Epp R, Kruse L, Kalchschmied A, Maisch B, Müller E, Kuhn U, Kratz W, Ober S, Radtke J, Schlechtriemen U, Schmidt G, Schröder W, van den Ohe W, Vögel R, Wedl N, Wosniok W, 2010. Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008. Umweltwissenschaften und Schadstoff-Forschung 22, 229-251.

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

Bioveiligheidsraad
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Secretariaat
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Compilation of comments of experts in charge of assessing the dossier B/BE/11/V4

Coordinator: Prof. Dr. ir. Dirk Reheul
Experts: Adinda De Schrijver (WIV-ISP), Jan Van Doorsselaere (KATHO Roeselaere) and Michel Van Koninckxloo (HEP Hainaut-Condorcet)
SBB: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/11/V4** 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 16 December 2011 and concerns a field trial with a genetically modified maize that has altered growth characteristics.

Depending on their expertise, the experts were invited to evaluate the genetically modified organisms 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
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

LIST OF COMMENTS RECEIVED FROM THE EXPERTS

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 02-02-2012 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

Items left blank have been evaluated by the experts but they had no comments or questions.

1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

(e.g. reproduction, survivability, dissemination, geographic distribution,...)

Comment 1

Based on the observations in Central Europe reported by Grüber et al. (2008), one can expect that in Belgium volunteers will only occur after a warm winter period. Further, Palaudelmàs et al. (2009) reported that in Spain the vigour of volunteer plants is low: they are much shorter than normal plants and rarely have cobs (if produced normally without grains). Tassels are frequently produced, but cross-pollination was estimated to be low, most probably due to loss of hybrid vigour and uniformity in plant size, flowering times, amount of fertile pollen etc..From the information in the dossier, it is not clear if tassels are formed (or have been observed) under Belgian climate conditions.

2. INFORMATION RELATED TO THE GENETIC MODIFICATION

(e.g. methods used for the modification, description of the vector,...)

Comment 1

C1

P5 het is wenselijk om meer informatie te geven over de lijn B104: is dit een commerciële lijn die ook in Vlaanderen wordt gekweekt? Wordt deze uitsluitend gebruikt om F1 hybriden te maken?

P7 Het is wenselijk om bij de sequentie het desbetreffende Accessienummer te vermelden. Is dit AK221496 of NM_118674?

Additional comment Coordinator:

Additional information on the parental line B104 is needed. How this parental line has been obtained, what the genome characteristics are, the flowering time and maturation rate in Flanders

Additional comment SBB:

As it is not clear for which gene the accession number is requested by the expert, this information could be asked for each gene.

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

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

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes,...)

Comment 1

The presence of the backbone sequences coding for the antibiotic resistance gene *aadA* was checked via PCR analyses and found to be absent in the GM maize line. According to EFSA (2004), the *aadA* gene belongs to the group of antibiotic resistance marker genes (Group II) that should be restricted to field trial purposes. For this particular field trial, the potential presence of the *aadA* gene does therefore not raise a safety issue.

One remark concerning the PCR testing the absence of the backbone sequences, including *aadA*: it is not clearly described in the dossier which controls were used in the PCR for testing the absence of the backbone sequences. Can it be clarified whether the EF-1α has been included in this particular PCR as genomic control? This information would reveal whether the maize line GA20OX1 DNA extracts were fit for PCR

Can it be clarified whether the same probes as mentioned on p.11 (and resulting in an amplicon of 184 bp) have been used in the PCR to estimate the copy number (see p.12-13), but resulting in an amplicon of 200 bp.

Comment 2

D2 P9

Uit de PCR experimenten wordt geconcludeerd dat er geen backbone sequenties aanwezig zijn in de GA20OX1 lijn maar er kan echter niet volledig worden uitgesloten dat dit zo is. Hiervoor dienen Southern experimenten met de volledige backbone probe te worden uitgevoerd. Waar bevinden zich de aadA primers?

Additional comment SBB:

Since the potential presence of the aadA gene does not raise a safety issue in this particular case (field trial purposes), it could be argued that Southern analysis is not requested to further substantiate the absence of backbone sequences in the GM maize line.

Beside the fact that primers of aadA were not visualized (neither on the map of vector PPZP200 nor on that of pBbm42GW7-pUBIL-Ga20ox), it would have been preferable to indicate the location of all primers used for PCR-analyses on the map of pBbm42GW7-pUBIL-Ga20ox.

P11. Het zou wenselijk zijn om meer informatie te geven over de GA20OX2 lijn (waarvan sprake op p 11). Blijkbaar bevat deze lijn wel backbone DNA en slechts 1 T-DNA kopij?

P12. Er wordt geen verklaring gegeven waarom op de Southern blot slechts 1 HindIII fragment te zien is in GA20OX1. Hoe moet dit worden verklaard?

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,...)

Comment 1

D3
P 13
Er is geen informatie ter beschikking over de aanwezigheid van GA20oxidase eiwit of GA20oxidase activiteit. Op pagina 13 veronderstelt men dat er een sterk verhoogde GA20 oxidase activiteit is.
Er zou beter worden gesteld dat: "… er is niets gekend over de abundantie van het GA20 oxidase eiwit. Het opgemerkte fenotype doet vermoeden dat er verhoogde GA20oxidase activiteit is en als gevolg verhoogde GA niveau's"

Additional comment Coordinator :
The spatial expression of the *Ga20 oxidase-1* gene and the *bar*-gene in the GM maize has been assumed on the basis of the characteristics of the respective promoters. The notifier should present experimental data to substantiate these assumptions.

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

Comment 1

D4 c
P 14
Er staat in het dossier: "…Er zijn ook geen redenen om te veronderstellen dat een langere plant beter bestand zou zijn tegen afbraak of tegen winterse omstandigheden":
Waarschijnlijk wordt hiermee bedoeld: "… zaden afkomstig van een langere plant"; moet gecorrigeerd worden.
Is getest of de zaden een snellere kieming vertonen ivm zaden van de ouderlijn (aangezien gekend is dat GA3 een rol speelt bij zaadkieming)?

Additional comment Coordinator :
From the observation that there are no differences in the formation of seed, pollen or inflorescences (D4a and D4b: geen invloed op bloeiwijzen, pollenvorming, zaadvorming of -verspreiding), it is assumed that the survivability of pollen and seed from the GM maize will not differ from the parental line. The notifier is requested to present experimental data to substantiate these assumptions.

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

Comment 1

D5
P 14
Het is niet duidelijk welke planten in de veldproef zullen worden aangeplant: zijn dit T2 zaden? Gaat het om homozygote planten? Hoeveel kopijen bevatten deze planten en hoe is het gesteld met de segregatie van de beide T-DNA kopijen?

Dit is onduidelijk en het is wenselijk dat toch ten minste wordt gezegd over welk plantenmateriaal zal worden aangeplant.

Er wordt gesteld dat er PCR werd uitgevoerd op materiaal van de 2de generatie maar hierover zijn geen gegevens getoond. Dit zou wenselijk zijn en bovendien kan een schema met gedane ZB(?)-en informatief zijn (cfr ook bovenstaande vraag: welke generatie van planten wordt aangeplant?)

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

Comment 1

I agree with the analysis that in the unlikely event of recombination, the transferred trait (GA20oxidase) would not result in adverse effects for the human/animal health and the environment (see also item 7 in bijlage gezondheids- en leefmilieurisicobeoordeling).
Note: Ow, 2007 is not in reference list. Most likely, the correct reference here is Katzen (2007)?

Comment 2

D6
P 16
Betreft horizontale overdracht: er kan ook worden gesteld dat indien er horizontale overdracht zou geschieden dat dit niet zal leiden tot genexpressie.

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

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

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

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

3.11. Potential interactions with the abiotic environment

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

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

Comment 1

The question seems to be misunderstood by the applicant. But it appears clearly that the question is not applicable (no previous releases).

4. INFORMATION RELATING TO THE SITE OF RELEASE

(e.g. description of the site ecosystem, presence sexually compatible species, proximity of protected areas,...)

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,...)

Comment 1

F2
P 19 Er wordt gesteld dat de proef zal lopen van 2012 – 2014. In de bijlage (proefprotocol p 6 is er sprake van de teelt van niet-GGO mais in 2013 (als vervolg op de proef). Echter in dezelfde bijlage op p8 is er sprake van … verdere observaties van de GGO in het 2de en 3de jaar.
Het moet verduidelijkt worden of de GGO proef verschillende malen zal worden herhaald of niet.

Additional comment Coordinator (see also comment under 7.4) :

The notifier should present a detailed and transparent trial design, indicating the number and specification of objects, number of replicates, dimension of individual plots and the overall field trial and the surrounding barriers and crops.
If the nofitier wants a consent for three years, he should provide this information for the three growing seasons to come.

In all cases the notifier should provide a detailed monitoring plan to check for and to eliminate potential volunteers even if their presence is expected to be very low.

The proposed field trial does not allow to measure the biomass of the crop reliably. The notifier should present a sound trial design with appropriate management in order to reach the presented goals.

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

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

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

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

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

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

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

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)

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

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

7.1. Precautions taken

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

Comment 1
The precautions taken: the removal of tassels and a buffer zone of 3 m of non-transgenic maize around the GM maize, I consider sufficient to avoid gene flow by pollen and seeds, respectively.

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

Comment 1
How will tassels and cobs/seeds not used for further investigation, be inactivated?

Additional comment Coordinator :
The notifier mentions that stalks and leaves are not reproductive and for this reason no more can be considered as a GMO (……..stengels en bladeren zijn niet reproductief en als zodanig om die reden geen GGO meer).The notifier should comment on this.

7.4 Information related to monitoring plans and the detection techniques

Comment 1

In the year following the trial, non-GM maize will be cultivated on the site in spring. Given the uncertainty if maize volunteers in Belgium produce tassels (see item 1), it seems relevant to monitor for volunteers in the year following the trial in case of an extreme warm winter. Further (also in case of an extreme warm winter), to prevent pollen flow from these (potentially pollen-producing) volunteers to conventional maize, it would be advisable to destroy the volunteers before tassel production and not to cultivate maize on the trial site. Setting the land aside or cultivation of another crop would be more recommendable.

Comment 2:
Not really applicable to this application.

Aditional comment Coordinator:
The notifier should present a detailed and transparent trial design, indicating the number and specification of objects, number of replicates, dimension of individual plots and the overall field trial and the surrounding barriers and crops.

If the nofitier wants a consent for three years, he should provide this information for the three growing seasons to come.

In all cases the notifier should provide a detailed monitoring plan to check for and to eliminate potential volunteers even if their presence is expected to be very low.

The proposed field trial does not allow to measure the biomass of the crop reliably. The notifier should present a sound trial design with appropriate management in order to reach the presented goals.

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

7.6. Information on methods and procedures to protect the site

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

Taalfouten (dt) op p3, 15, 19 en 21

Aditional comment Coordinator :

The notifier mentions that no biomass increase is to be expected while it is expected that plants are much longer than the non-transformed material. The notifier wants to test higher plant densities. It is well known that in many plant species, plants with long internodes usually are more prone to lodging than short plants, particularly when they are grown at higher plant densities. Hence one may expect the transformed material to be more lodging susceptible than the non transformed material in the proposed field trial. If this is the case, the field trial has no agricultural significance. Unless the notifier has indications that the standing power of the plants are conserved or improved. The notifier should inform on this.

References (not present in notification)

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

Palaudelmàs et al. (2008) Effect of volunteers on maize gene flow. Transgenic Res. 18, 583-594.