Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on notification B/BE/24/V1 from VIB for deliberate release in the environment of genetically modified plants for research and development

27 March 2024
Ref. SC/1510/BAC/2024_0452

The notification B/BE/24/V1 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2024 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 evaluation of poplars with a decreased lignin content***. The purpose of the release is to test the performance of the mutated poplar lines under real life conditions and to produce wood to evaluate its properties to serve as a good biomass source for extracting sugars and other valuable compounds.

The notification has been officially acknowledged by the CA on 25 January 2024 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. Two 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 genetically modified poplar 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 29 February 2024, based on a question prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. Answers to the questions were received by the Secretariat on 6 March 2024.

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. No questions of the public concerning biosafety issues of the GM poplar were received.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Grey poplar (*Populus x canescens*) is dioecious (every tree is either male or female) and an obligatory outcrosser. Grey poplars begin flowering between the age of 5 and 8 years. Male and female flowers are borne in catkins. Male flowers ripen and shed pollen a few days before females, ensuring that pollen is in the air when the first females are receptive. Seeds can be dispersed over great distances, resulting in high rates of migration.

Grey poplar is sexually compatible with a few other *Populus* species present in Belgium, namely *Populus alba*, *Populus tremula*, hybrids of *Populus canescens* and *Populus tremuloides*.

The grey poplar used in the field trials is a female clone 717-1-B4. Hence, there is no production of pollen.

Besides sexual reproduction, also vegetative propagation through root suckers or branches can occur (OECD, 2001[1]) in *Populus* species. Vegetative propagation through branches is however very unlikely for grey poplar under natural conditions.

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

The field trial will be conducted during four growing seasons, from May 2024 until March 2028. The small-scale field trial (no more than 500 m²) will be designed as a short-rotation intensive culture. The trees will be planted manually during spring 2024. Occasionally branches will be cut down manually to be analysed in the laboratory. The harvested parts will never be more than three years old at the time of harvest. At the end of the trial, in spring 2028, all biomass will be cut down and chopped to be composted *in situ*. The rootstocks will be mechanically removed and destroyed, and the plot will be tilled with a rotary cultivator. Subsequently, the plot will be left vacant for a year and monitored for suckers and it is proposed this will be done until there has been one year without any suckers. Potentially emerging suckers will be destroyed.

3. Information related to the genetic modification

Two lines of the female clone 717-1-B4 were obtained through *Agrobacterium tumefaciens*-mediated transformation with a vector containing a gene coding for CAS9 and sequences coding for guideRNA's, targeting either the *tra2a* or the *tra2b* gene. The resulting mutation leads to a knock-down of *tra2*, which has the downstream effect that about 15% less lignin and 8% more cellulose is formed. This altered composition of the wood results in the easier extraction of sugars. The transformation vector also contains the *hph* gene for resistance to antibiotics, used as a selection marker during the transformation process. Both the *hph* and the *cas9* genes are not functional in the final plant.

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

No increase in persistence in the field or invasiveness into natural habitats compared to non-GM grey poplars is expected, as the modified lignin content is not known to confer a selective advantage to survivability. Due to the characteristics of the poplar cultivar used for transformation and through the measures taken during the release, vertical gene transfer through seed, pollen, branches or root suckers can virtually be ruled out:

[1] OECD, 2001. Consensus document on the biology of *Populus* L. (poplars), ENV/JM/MONO(2000)10

- The GM poplars are not expected to flower, as the branches of the lignin-modified poplars will never be older than 3 years at the time of harvest, and poplar usually first blooms after 5 to 8 years. Nevertheless, monitoring will be carried out each year during the flowering season to check for flowering. If unexpected flower buds occur, they will be removed before seed set.
- There is no possibility of dissemination through pollen, as the grey poplar used in the field trials is a female clone 717-1-B4.
- Spontaneous regeneration from branches is considered unlikely, as clone 717-1-B4 does not easily form rooted scions even under optimal laboratory conditions.
- Root suckers observed during the trial period will be removed, as well as root suckers that might emerge after the field trial.

Horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions (Keese, 2008[2]). In case transfer of GM material (i.e. *hph* gene for resistance to the antibiotic hygromycin) from the GM poplars to micro-organisms would take place, negative effects on environment and humans are not expected, as this resistance gene is widespread in naturally occurring microbes in humans and the environment (EFSA, 2004[3]).

From data from former trials and literature, it can be concluded that the GM poplars are not expected to have significant effects on non-target organisms (invertebrates, vertebrates and soil micro-organisms) and humans. The impacts of lignin-modified trees on microbial pathogens, leaf eating insects and microbial soil composition have been shown to be negligible (see e.g. Brodeur-Campbell *et al*., 2006[4]; Halpin et al., 2007[5]; Bradley *et al*., 2007[6]; Danielsen *et al*., 2012[7] & 2013[8]). Also effects on mammalian herbivores (e.g. rabbits) are expected to be negligible. The fence surrounding the entire field plot will restrict – although not entirely – entrance of mammals into the field plot, reducing their contact with the GM poplars. Given the restricted scale of the field trial, any potential effect to non-target organisms and biogeochemical processes - if these would occur - will be of a local and temporal nature. As clone 717-1-B4 does not produce pollen, a possible altered allergenicity of the transgenic pollen (pollen from poplar is known as a moderate allergen) does not form a concern for human health.

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

The management measures proposed (e.g. removal of root suckers, monitoring for flowers, chopping of wood inside the fence) were considered as sufficient to prevent potential adverse effects to the environment, animal and human health during the field trial. To minimise the spread of transgenes into the environment after termination of the field trial, monitoring for root suckers will occur. The Council recommends to extend monitoring for root suckers until the moment that two years have passed after the last observed root suckers. In addition, the machinery used for chopping should be cleaned inside

[2] Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.
[3] EFSA, 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA Journal 48, 1-18.
[4] Brodeur-Campbell et al., 2006. Insect Herbivory on Low-Lignin Transgenic Aspen. https://doi.org/10.1093/ee/35.6.1696
[5] Halpin *et al*., 2007. Ecological impacts of trees with modified lignin. Tree Genetics & Genomics 3, 101-110.
[6] Bradley *et al*., 2007. Soil microbial community responses to altered lignin biosynthesis in *Populus tremuloides* vary among three distinct soils. Plant and Soil 294,185-201.
[7] Danielsen *et al.*, 2012. Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. Ecology and Evolution 2, 1935-1948
[8] Danielsen *et al*., 2013. Ectomycorrhizal Colonization and Diversity in Relation to Tree Biomass and Nutrition in a Plantation of Transgenic Poplars with Modified Lignin Biosynthesis. PLoS ONE 8(3): e59207.

the fence before leaving the trial site, and the branches taken away to be analysed in the laboratory need to be registered.

Conclusion

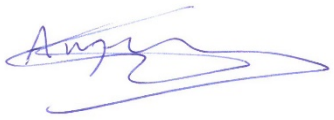
Based on the scientific assessment of the dossier by the experts, the Biosafety Advisory Council concludes that it is unlikely that this small-scale field trial with GM poplar with a decreased lignin content will pose any risks to the environment, animal or human health.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the protocol, the monitoring plan and, if necessary, the emergency measures as described in the dossier.
- Additional conditions should be taken up in the monitoring plan:

1. Monitoring measures taken during the trial:
   - The notifier should keep records of dates and numbers of inflorescences removed from each transgenic line. This information is useful to check the adequacy of the monitoring frequency for inflorescences. Also dates, numbers and identity of branches taken away to be analysed in the laboratory should be recorded.
   - All harvested woody material should be chopped inside the fence and the machineries that are used to harvest and chop the wood should be cleaned at the trial site to prevent dispersal of plant material.

2. Monitoring measures taken after the trial:
   - The period to monitor the occurrence of root suckers should be extended until the moment that two years have passed after the last observed outgrowing suckers.



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/V1 (ref: BAC_2024_0294)

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Compilation of comments of the experts in charge of evaluating notification B/BE/24/V1

Ref. SC/1510/BAC/24_0294

Coordinator: Philippe Baret
Experts: Nina Papazova (Sciensano), Jan Van Doorsselaere (VIVES), An Vanden Broeck (INBO)
SBB coordinator: Fanny Coppens

INTRODUCTION

Dossier **B/BE/24/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 24 January 2024 and concerns a field trial to evaluate poplar with decreased lignin content.

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

EVALUATION FORM

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.

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

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

C. INFORMATION RELATED TO THE GENETIC MODIFICATION

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

D. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

D.1. Information related to the traits and characteristics, which have been introduced or modified

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

D.2. Information on the molecular characteristics of the final GMO

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

Comment:

In Bijlage 2: beschrijving genetische modificatie; page 6: the 4 bp deletion in the cas9 gene; this is not indicated correctly and should be revised.

SBB comment: As the dossier has already been made publicly available, this comment will not be forwarded to the notifier.

D.3. Information on the expression (of the insert)

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

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

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

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

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

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

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. 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: 2 experts

D.11. Potential interactions with the abiotic environment

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

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

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

Comment:

De volgende informatie ontbreekt in het detectieprotocol:

- Schema primer attachment sites
- Finale concentratie van de primers in de PCR-mix, niet enkel de concentratie van de stock
- Condities agarose gel electrophorese
- Uitleg over *Ptlf*-gen (71 bp), komt ook niet in de interpretatie van de resultaten, dus absoluut niet duidelijk waarom dit vermeld wordt

(Voor de vereisten van het detectieprotocol, zie het document https://www.biosafety.be/sites/default/files/partb_protocole_gmo_detection.pdf, beschikbaar via https://www.biosafety.be/content/environmental-release-gmos-experimental-purpose-tools-risk-assessment-and-risk-management)

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

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

E. INFORMATION RELATING TO THE SITE OF RELEASE

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

F. INFORMATION RELATING TO THE RELEASE

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

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

G.1. Any measures taken

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

G.2. Information on methods for post-release treatment of site

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

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

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

G.4 Information on monitoring plans and techniques

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

G.5. Information on any emergency plans

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

G.6. Information on methods and procedures to protect the site

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

ANNEX 1. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

1. Persistence and invasiveness of the GM plant

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

2. Selective advantage / disadvantage

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

3. Gene transfer to plants

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

4. Gene transfer to micro-organisms

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

5. Effects on target organisms

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

6. Effects on non-target organisms

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

7. Effects on human health

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

8. Effects on animal health

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

9. Effects on biogeochemical processes

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

10. Effects of the specific cultivation, management and harvest techniques

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

11. Summary and conclusion

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