Biosafety Advisory Council

02-10-2013

Secretariat

O./ref.: WIV-ISP/BAC/2013_0580

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/13/V1** of the VIB, Flanders Institute for Biotechnology, for deliberate release into the environment of genetically modified poplars with an altered wood composition

Context

The notification B/BE/13/V1 has been submitted by the VIB to the Belgian Competent Authority (CA) in January 2013 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 an altered wood composition"**. This release has the purpose to check whether the genetically modified (GM) poplars, adapted in the production of lignin, produce a biomass that can be more efficiently converted to glucose under factual production conditions.

The notification has been officially acknowledged by the CA on 15 January 2013 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 Division of Biosafety and Biotechnology (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 GM poplar trees would not raise any problems for the environment, animal or human health.

On 8 March 2013 and 3 June 2013, the Biosafety Advisory Council sent a list of requests for additional information to be provided by the notifier to the CA. Additional information was received on 14 May 2013 and 26 September 2013, and evaluated by the scientists in charge of evaluating the dossier.

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.

Note that one member of the Biosafety Council, René Custers, had an interest in this dossier and was, therefore, denied from taking part in the process that led to the finalisation of this advice. The advice was approved by written procedure. Four members (Marc De Loose, Vincent Demoulin, Dirk Reheul and Alfred Volckaerts) supported the advice, two members (Philippe Baret and Lucette Flandroy) did not support the advice for the reasons mentioned in Annex I and II of this advice, and the other members did not react.

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 small scale field trial will be designed as a short-rotation poplar coppice. Young rooted GM poplars will be planted during spring 2014. Before the start of the second growing season (2015) the trees will be cut down to stimulate the formation of many stems per plant. At the

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

end of 2018 all biomass will be cut down and chopped to be processed into bio-ethanol. The regrowth will be allowed to grow for another 3 growing seasons and will be harvested at the end of 2021. Occasionaly branches will be cut down to be analysed in the laboratory. At the end of 2021 rootstocks and roots of the trees will be destroyed mechanically**.** Potentially emerging suckers will be destroyed.

3. Information related to the genetic modification

Three lines of the female clone 717-1-B4, transformed in their expression of CAD (Cinnamyl Alcohol Dehydrogenase), will be tested in the field experiment: they are identified as lines pHG8-CAD4, pHG8-CAD19 and pHG8-CAD24.

These three lines, obtained through genetic transformation with *Agrobacterium tumefaciens,* have a modified lignin (a major constituent of wood) content due to the decreased activity of the CAD enzyme involved in the lignin biosynthetic pathway. In the GM poplar lines, the *cad* gene from *Populus* is partly inserted in sense, partly in anti-sense orientation with both parts being separated by an intron. The expression leads to the production of RNA in a hairpin turn. The *cad* sense-antisense construct is located between the promoter of the gene coding for the 35S RNA of the cauliflower mosaic virus and the transcription terminator of the gene coding for the octopine synthase gene of *A. tumefaciens*. In addition, the transgenic lines contain a selection gene (neomycine phosphotransferase, *nptII*) that confers resistance to the antibiotics neomycine and kanamycine. The *nptII* gene is controlled by the nopaline-synthase (Pnos) promoter and a transcription terminator from the T7 gene from the T-DNA (tAg7). Absence of vector backbone sequences relevant to human and veterinary therapy, i.e. the *aadA* gene which confers resistance to spectinomycin and streptomycin, has been demonstrated in all three lines.

Upon request of additional experimental data proving the absence of the *aadA* gene, the information related to the genetic modification was considered sufficient and in accordance with the guidelines of the SBB (SBB, 2002)[2].

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

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:

- The GM poplars are not expected to flower, as the branches of the lignin-modified poplars will be harvested every 3 years. Nevertheless, monitoring will be carried out each year 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.

[2] SBB, 2002. http://www.biosafety.be/gmcropff/EN/TP/partC/GuideMGC_PartB_C.htm

- Root suckers observed during the trial period will be removed, as well as root suckers that might emerge after the field trial.

The possibility of horizontal gene transfer between plants and micro-organisms is considered as a rare event under natural conditions. In case transfer of GM material (i.e. *nptII*) 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; Halpin et al., 2007[4]; Bradley *et al*., 2007[5]; Danielsen *et al*., 2012[6]). 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 Biosafety Advisory 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 the fence before leaving the trial site, and the branches taken away to be analysed in the laboratory need to be registered.

[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] Halpin *et al*., 2007. Ecological impacts of trees with modified lignin. Tree Genetics & Genomics 3, 101-110.
[5] 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.
[6] 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

Conclusion

Based on the scientific assessment of the dossier by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that this small scale field trial with GM poplar with an altered wood composition 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 past after the last observed outgrowing suckers.

p.o. Philippe Herman

Prof. D. Reheul
President of the Biosafety Advisory Council

Annex I: Opinion of Philippe Baret on notification B/BE/13/V1
Annex II: Opinion of Lucette Flandroy on notification B/BE/13/V1
Annex III: Summary Notification Information Format submitted by the notifier in January 2013.
Annex IV: Compilation of comments of experts in charge of assessing the dossier B/BE/13/V1 (ref: BAC_2013_0206)

Divergent opinion (3/10/2013) of Professor Philippe Baret on the file B/BE/13/V1 (GM poplars, modified lignin)

1. Considering the point 2 of article 4 of 2001/18 EU Directive, genes of resistance to antibiotics have to be eliminated of plants under the part B (trials) after the 31 December 2008. The proposed trial violates these official regulations and may endanger environment and human health despite the existence of alternative to the antibiotic resistance genes.

2. The assessment of the impact on non-target organism is very partial and not substantiated by quantitative data. Vague affirmations on the "negligible" nature of the risk or on "unlikely effect" are inconsistent with a science based risk assessment.

Divergent opinion (3/10/2013) of Dr. Lucette Flandroy on the file B/BE/13/V1 (GM poplars, modified lignin)

Following data provided to the BAC members, this file is lacking the rigor required for a science-based risk assessment. Essential scientific proves of declarations presented as facts in the file and arguing for no important risks are missing, as well as relevant and sufficient monitoring plan during the trial. I did not get any answer to my various precise questions (see Annex) raised in written comments before the finalization of the advice and aimed at clarifying these issues that without adequate treatment may place the environment at risk.

Commentaires de L. Flandroy (27-09-2013) sur le dossier B/BE/13/V1 et sur le projet d' avis du CCB (daté du 23-09-2013) sur ce dossier

D' une façon générale, je déplore que ce dossier n' ait pu être discuté en séance et que les membres du groupe n'aient pas eu l' occasion plus tôt de poser également des questions de clarification au notifiant. Cela aurait accéléré le processus et évité une série de questions posées ici.

Je suis surprise que les 2 experts qui ont analysé le dossier complet soient tous 2 satisfaits du dossier et du projet d' avis dans leur état, vu les questions/remarques qui ont été faites au notifiant et les réponses reçues.

Sur base des éléments du dossier auxquels j' ai eu accès , je suis d' accord avec des remarques d' un expert signifiant que le notifiant fait différentes assomptions sans base scientifique suffisante.

A ce sujet, je ne suis pas d' accord avec le recours abusif au principe de « familiarité », invoqué tout le long du dossier et également dans les réponses au public, pour argumenter l'absence de risque ou la faible probabilité de risque de ce nouveau peuplier GM sans avoir fait d' expérience à ce sujet ou sans en présenter les résultats de façon scientifiquement pertinente.

En effet, le recours au principe de familiarité permettrait surtout de conclure que, si des anomalies similaires sont observées de façon répétitive dans d' autres lignées également modifiées au niveau de la lignine , cela augmenterait la probabilité que les lignées concernées ici pourraient poser les mêmes problèmes. L' absence de caractéristique problématique liée à des modifications de la lignine, démontrée de façon probante, dans toutes autres lignées déjà transformées ne ferait que diminuer les probabilités d' anomalies dans les lignées concernées ici. Mais l' absence d' anomalies dans d' autres lignées GM à lignine modifiée n' exclut pas des effets involontaires adverses liés directement au site d' insertion dans les lignées concernées ici et/ou à des effets pleiotropiques. Le contenu de cette dernière phrase est du reste reconnu par le notifiant (et dans le projet de réponse au public)

Plus précisément, on peut admettre que toutes les évaluations de risques sur l' environnement potentiellement étudiables en confiné (en particulier sur des NTO – *non target organisms*) n' aient pas été réalisées avec l' ensemble des lignées développées initialement dans ce dossier .

Cependant, arrivé à ce stade de développement où seules 3 lignées ont été sélectionnées pour essai en champ, des évaluations scientifiques de risque en confiné auraient déjà dû être réalisés avant tout essai dans l' environnement, leurs protocoles et leurs résultats présentés, surtout 1) si une caractérisation moléculaire précise des sites d' insertion n' a pas été faite (ce qui semble être le cas à ce stade) et qu' il est donc totalement impossible de conclure qu' il n' y a pas d' effet involontaire néfaste qui serait dû à l' interruption d' un gène ou la création d' un ORF dans l' ADN hôte.- ; 2) si un ensemble d'articles de la littérature sur des peupliers GM transformés au niveau de la lignine n' est pas présenté dans le dossier et n' informe pas sur l' absence d' effet corrolaire potentiellement adverse de cette transformation.

Par ailleurs, cette notion de « familiarité » ne devrait pas permettre d' exclure une surveillance spécifique (en particulier durant un premier essai dans un environnement spécifique) d' anomalies potentielles qui résulteraient non seulement d' effets involontaires du processus de transgénèse mais également de l' interaction avec l' environnement local (en particulier, surveillance de la « fitness » , et d' impacts éventuels sur des *NTO – non-target organisms*) (Et ce plan de surveillance devrait être d' autant plus complet que des anomalies auraient été observées dans d' autres lignées.et/ou que certaines « anomalies « auraient été observées en confiné ave les lignées concernées ici)

Par conséquent, j' ai les remarques suivantes à faire et les questions suivantes à poser dans le cadre de ce dossier:

A.Concernant des tests sur des lignées dites « comparables « dans le dossier :

1) Un expert signale qu' il n' y a aucune donnée ni d' essai dans ce dossier ni dans la littérature concernant des impacts éventuels sur des petits animaux, tels oiseaux ou mammifères qui pourraient entrer en contact avec les peupliers sur le terrain d' essai, mais que le notifiant déclare néanmoins qu' il n' y a pas de risque. Peut-on avoir plus d' information à ce sujet ?

2) Je souhaiterais avoir accès (ainsi que les membres du BAC qui le souhaitent) aux protocoles de tests pour l'évaluation des risques et à leurs résultats et conclusions , qui ont dû être réalisés dans le cadre de l' essai en champ de peuplier GM antérieur du VIB, à savoir le dossier B/BE/07/V2 réalisé avec d' autres lignées à lignine modifiée. (L' autorisation de cet essai n' avait en effet été donnée qu' avec des conditions supplémentaires aux données du dossier présentées par le notifiant, et en l' occurrence moyennant des études d' impacts, durant l' essai, sur des endophytes, sur la microfaune du sol, et sur des insectes phytophages et xylophages spécifiques, dont la pertinence du choix avait dû être justifiée. Dans la réponse du notifiant à une question d' un expert au sujet du rapport de surveillance de cet essai antérieur, seuls des mesures sur des endophytes sont mentionnées, sans information précise sur ces mesures.

3) De la même manière, pourrait-on avoir accès aux tests réalisés en serre dans cet dossier ~~essai~~-ci, qui sont mentionnés dans les réponses au public comme ayant été réalisés et n'ayant pas montré d' anomalies phénotypiques. ?

4) Concernant un article de la littérature (Kleeman et al. -2011) relatant des « anomalies « éventuelles : selon la réponse du notifiant à la question d' un de nos experts, cet article indique un effet important du changement en composés phénoliques des feuilles (? de peupliers GM ?) sur la quantité d' insectes herbivores présents sur les arbres. . Voilà donc un impact important qui peut être induit sur la biodiversité (et + particulièrement sur l' écosystème arbres /insectes) par des changements de biosynthèse de la lignine telle qu' opérée dans ces peupliers GM. Or, le projet de réponse du SBB au public (réponse 4 , concernant effets sur NTO) signale « *Studies …..in serre wijzen in de richting dat GG populieren fenotypisch geen abnormaliteiten vertonen* ».

Comment expliquer cette différence avec l' article de Kleeman et al. ? Ne s' attendrait-on pas (si l' article de Kleeman et al. est pertinent à citer comme référence dans ce dossier et donc si des changements analogues de la biosynthèse de la lignine existent dans le cas de ce dossier-ci) à voir également, dans le cas du dossier que nous traitons, une différence du nombre d' insectes herbivores sur les peupliers GM par rapport au comparateur (? et donc peut-être une différence de prolifération de ces insectes dans l' environnement et une attaque plus importante de ces peupliers GM, qui peut être préoccupante ?)

NB :

- Le fait que des arbres ont *naturellement* des compositions « différentes » en lignine et en composés phénoliques , et qu' il existe à ce sujet une grande diversité n' est pas un argument pour justifier qu' on ne devrait pas évaluer les risques de cette lignée-ci. La diversité naturelle concernée fait partie d'un équilibre écologique qui n' existerait pas dans des monocultures de lignée(s) mise(s)au point ici, dont les impacts doivent donc être mesurés.
- La volonté (qui revient en force) de se réorienter vers un processus d' évaluation des risques « product-based » (et *trait-based*) plutôt que « process-based » (*technology-based*) mérite en soi un débat que nous n' avons pas à épuiser ici.. La législation OGM à laquelle nous avons à nous référer demande bien d' évaluer les risques potentiels des OGM au cas par cas, donc non seulement pour des transgènes différents mais événement par événement, et ceci pour tenir compte d'effets involontaires résultant précisément du processus de transgénèse et d' interactions avec l' environnement.

B.Concernant la surveillance de l' essai proposé dans ce dossier-ci :

En dehors des protocoles et résultats demandés ci-dessus de tests antérieurs sur des lignées ressemblantes, je souhaiterais obtenir plus de détails sur le plan de surveillance d' impacts sur NTO prévu durant cet essai-ci. (types et variétés de NTO – terrestres et du sol - choisis, et explication de la pertinence de ce choix ; protocole des tests), ainsi que sur la surveillance de la *fitness* des lignées GM par rapport au comparateur. Ceci pour les raisons évoquées ci-dessus , qui ne permettent pas de se fier uniquement à des conclusions d' absence d' impacts négatifs importants d' essais antérieurs en Belgique ou autres. -Même s' il ne s' agit que d' un essai à petite échelle, une modification de la sensibilité des peupliers à certaines pestes pourrait notamment constituer des foyers d' infection qui se répercutent sur l' environnement proche. De + , il faudrait être certain que le terrain de l' essai sera abandonné par la suite (pas utilisé à d' autres essais ou à d' autres plantations) pour considérer que les impacts sur la composition du sol local sont sans importance. -Enfin, il serait bon d' avoir dès ce niveau des informations sur des impacts éventuels sur des vertébrés (dont le décès sur place ou des impacts quelconques sur la santé pourraient avoir des conséquences plus vastes sur l' environnement.).

C.Concernant impacts sur la santé humaine

Vu la taille de l' essai et la suppression des fleurs avant floraison et l' existence d'une barrière entourant l' essai, on peut admettre qu' il n' y ait pas eu de test de toxicité ou allergénicité pour la santé humaine dans cet essai.

Je suis par contre d' accord avec la remarque de l' expert disant qu' il n' est pas scientifique de la part du notifiant de déclarer sans justification ni références que « *Er zijn geen redenen om te veronderstellen date en gewijzigde houtsamenstelling een toxisch, allergeen of ander schadelijk effect zou hebben op de menselijke gezondheid*. « Il est donc évident que des tests expérimentaux devraient être faits à ce sujet en cas de dépôt du dossier pour mise sur le marché.

D.Concernant la présence de gène de résistance aux antibiotiques

Les opinions de l' EFSA (de 2004 et 2009) concernant le non danger d' utilisation de certains gènes de résistance aux antibiotiques, dont le gène npt-II , constituent un raccourci qui fait fi de différents éléments, qui ont du reste été résumés dans un document du secrétariat du Conseil de biosécurité le 6/05/2011.

Ces opinons ne tiennent notamment pas compte d' avis minoritaires émis par des membres du Panel BIOHAZ qui a émis une opinion conjointe sur ce sujet avec le Panel OGM de l' EFSA, ni d' une série d' autres considérations (dont l' usage différent d' antibiotiques dans différents Etats membres de l' UE outre dans le reste du monde, dont des différences locales d' incidence de résistance dans la population bactérienne, dont des différences de probabilité de transfert horizontal de gènes selon différents paramètres de l' environnement local,…..) sur lesquels existent des incertitudes et manques de données.(en particulier sur les effets cumulés et à long terme d' événements sporadiques de transfert)

D' autre part, s' il est possible que l' utilisation de ces gènes de résistance dans les plantes GM ne représente qu' un fait mineur jusqu' ici à côté des résistances induites par l' utilisation massive d' antibiotiques dans l' élevage et l' agriculture, l' OMS a spécifiquement fait remarqué, lors du World Health Day de 2011, que « Everyone can make a contribution » dans l' action concertée des gouvernements et autres acteurs qu' elle a lancé à cette occasion sous le thème « Combat Drug Resistance » , reconnaissant le problème sanitaire mondial crucial que représente l' augmentation des résistances aux antibiotiques.

Si le développement d' OGM augmente dans le monde, il est dès lors inadmissible que ces gènes marqueurs persistent en cas de mise sur le marché. On pourrait admettre qu' ils soient encore autorisés

pour la sélection des clones en confiné. Mais à ce stade d' essai en champ, il ne reste que 3 lignées spécifiques qui ont déjà été sélectionnées en confiné . Ce gène devrait être retiré avant l' essai en champ, s'il n' y a pas de bonne raison pour le conserver à ce stade. Je souhaiterais une explication précise à ce sujet. Si la raison est un problème technique et qu' il n' y a pas d' autre alternative adéquate actuellement aux gènes de résistance aux antibiotiques, cela pose un problème général à résoudre car on ne peut poursuivre dans cette voie. Le fait que ces gènes marqueurs de résistance aux antibiotiques continuent à être majoritairement utilisés (parmi des dizaines de gènes marqueurs potentiels) ne résulte-t-il pas d' une facilité que la recherche n' a pas cherché suffisamment à dépasser vu la relative tolérance des législations à ce sujet ? C' est ce que suggère certaines de mes lectures , tout en reconnaissant que je ne suis pas spécialiste pointue du domaine. La demande de *phase out* de ces gènes marqueurs de résistance aux antibiotiques date de 2001 dans l' UE ; le début des recherches sur ce dossier-ci est bien plus tardif ; le notifiant aurait donc pu utiliser une autre technique de marquage. Je souhaiterais aussi une explication à ce sujet.

Je ne peux en tout cas admettre que l' on considère anodine l' utilisation de ces gènes de résistance, comme suggéré dans le projet d' avis du Conseil et la réponse à la question 2 du public.

En résumé :

- Je souhaite recevoir réponse à mes questions surlignées en jaune dans ce texte, et ne pourrais donner un avis favorable à ce dossier sans avoir reçu réponses à ces questions et si les réponses sont satisfaisantes (càd notamment, si les réponses ne justifient pas d' exiger des évaluations de risques en confiné, non réalisées jusqu' ici, avant essai en champ, ou plus généralement, ne suggèrent pas des risques à ne pas prendre en champ dans l' état).
- Je ne peux de toute façon accepter en l' état le § de l' avis du Conseil évoquant le transfert horizontal de gènes et l' absence d' effet attendu sur l' environnement et la santé humaine des gènes de résistance aux antibiotiques.
- Dans l' avis, on devrait ajouter qu' il est acceptable que des tests d' impacts sur la santé humaine n' ont pas été réalisés et ne sont pas prévus vu la taille de l' essai et vu des précautions évitant le contact entre les peupliers GM et la population, mais que de tels tests devraient être réalisés en cas de mise sur le marché.
- Enfin, je m' étonne que des mesures additionnelles à celles présentées par le notifiant doivent être proposées par le BAC lui-même dans le plan de surveillance. Certaines de ces mesures sont déjà proposées dans le projet d' avis. Et je demande ci-dessus d' autres informations concernant la surveillance, qui n'ont pas été présentées spontanément par le notifiant. Cela me laisse songeuse sur la façon dont la surveillance a été opérée dans le cas de l' essai du dossier B/BE/07/V2.

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number
B/BE/13/V1

Member State
Belgium

Date of Acknowledgement
……

Title of the Project
Field evaluation of poplars with a modified wood composition

Proposed period of release:
01/05/2014 to 31/04/2021

A2. Notifier

Name of the Institute
VIB

A3. Is the same GMPt release planned elsewhere in the Community?
No.

A4. Has the same GMPt been notified elsewhere by the same notifier?
No

B. Information on the genetically modified plant

B1. Identity of the recipient or parental plant

a) family name:	Salicaceae
b) genus:	*Populus,* sectie *Populus*. Subsectie *Albidea*
c) species:	*Populus tremula x Populus alba* (*Populus x canescens)*
d) subspecies:	-
e) cultivar / breeding line:	717-1B4, vrouwelijke kloon
f) gangbare naam:	Grey poplar

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications
The genetically modified trees have an altered lignin composition resulting from the

downregulation of the Cinnamyl Alcohol Dehydrogenase (CAD) enzyme through RNAi. CAD catalyzes the last step in the monolignol synthesis. The remaining CAD activity in the modified trees is about 15% of that in wildtype trees. The altered lignin composition has a positive effect on the ease with which the lignin can be broken down to gain access to the valuable sugar content in the cellulose and hemicellulose in the wood of the trees.
A lowered CAD activity has been found to naturally occur in the U.S. in loblolly pine (*Pinus taeda*) and in wild black poplar in Europe with comparable effects on the wood composition.
The modified trees also carry the NPT-II selection marker gene allowing an easy selection of transformed plants.

B3. Type of genetic modification
Insertion of genetic material.

B4. In case of insertion of genetic material, give the source and intended function of each constituent fragment of the region to be inserted
The region which has been inserted, and which is flanked by the T-DNA borders from the Ti-plasmid of Agrobacterium tumefaciens contains the following elements:

Element	Function	Origin
Right T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*
CaMV 35S	Transcription promotor	cauliflowermosaicvirus
attB1	Recombination site*	E.coli
scad (sense CAD)	Coding sequence of part of the enzyme cinnamyl alcohol dehydrogenase	Poplar
attB2	Recombination site*	E.coli
intron	Leads, together with the (A)SCAD sequences, to the formation of a hairpin RNA molecule	
attB2	Recombination site*	E.coli
scad (in opposite direction)	Coding sequence of part of the enzyme cinnamyl alcohol dehydrogenase	Poplar
attB1	Recombination site*	E.coli
OCS terminator	Transcription terminator of the octopine synthase gene	*Agrobacterium tumefaciens*
NOS promotor	Transcripton promotor of the nopaline synthase gene	*Agrobacterium tumefaciens*
nptII	Neomycine phosphotransferase	Tn5
NOS terminator	Transcription terminator of the nopaline synthase gene	*Agrobacterium tumefaciens*
Left T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*

*the AttB1 and -2 recombination sites are synthetically altered versions of a recombination site originally isolated from E.coli.

B6. Brief description of the method used for the genetic modification
The method used for the genetic transformation is based on Agrobacterium tumefaciens cocultivation of excised internodes from in vitro grown poplar plantlets (Leplé et al., 1992). After this cocultivation step where the gene transfer takes place, the transformed cells are selected using a positive screen (based on antibiotic resistance) and induced to regenerate a whole plant.

B7. If the recipient or parental plant is a forest tree species, describe ways and extent of dissemination and specific factors affecting dissemination
Grey poplar (*P. x canescens*) can disseminate vegetatively through the production of suckers from superficial roots. Pollen and seed are disseminated by the wind, possibly on rather long distance. The seed is very small and devoid of albumen: for this reason the seed viability in the wild is rather short (between 2 and 4 weeks). In fact, seed regeneration is not often observed as ecological conditions necessary to seed germination and plantlet development are seldom met:

naked soil, no competition at all with any other species, full light, permanent humidity, but not in excess…

C. Experimental Release

C1. Purpose of the release
As already specified, the genetically modified poplars are modified in their lignin content. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. Different transgenic lines of poplars with a modified lignin content have already been evaluated in previous field trials in the UK and France, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purposes to test the performance of these new CAD-downregulated poplar lines under real life conditions and to produce enough wood from lignin modified poplars in order to evaluate its properties to serve as a good biomass source for extracting sugars and other valuable compounds. Lignin composition, lignin/cellulose ratio and the accessibility to cellulose are critical for the extraction of sugars from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture on a low-grade soil using sustainable low-input conditions. The release also intends to take advantage of the developments in the Ghent-BioEnergy-Valley, where a number of bio-energy initiatives have taken ground, including a bioprocess pilot plant for bio-energy production.

C2. Geographical location of the site
On grounds belonging to the ILVO research institute on the border of the municipalities of Wetteren and Melle.

C3. Size of the site (m2)
The trial site is in total about 1300 m2, of which about 810 m2 will be planted with transgenic poplars.

C4. Relevant data regarding previous releases carried out with the same GM-plant, if any, specifically related to the potential environmental and human health impacts from the release
The genetically modified plants have not been released before.

D. Summary of the potential environmental impact from the release of the GMPts

The environmental impact from the release is expected to be zero, since the GM poplars are not going to flower and any suckers from superficial roots will be destroyed. Spontaneous regrowing of trees from fallen branches is considered to be extremely unlikely, as it is known that *P.x canescens* and the clone 717-1-B4 does not easily shoot. Only under ideal conditions in the laboratory with the application of shooting powder, *P. x canescens* is able to shoot. This means that there will be no transfer of transgenes to native or cultivated poplars, or spread of the GM poplars themselves. When poplar is grown in short rotation intensive culture the trunks and branches will not become older than three years, and therefore they will not flower. Grey poplar normally starts to flower between 5 – 8 years of age, only in some cases after 4 years. But anyhow, if monitoring would reveal any flowering, these flowers will be removed. For information: The clone used as a recipient is a female clone, unable to produce male flowers and therefore also unable to produce pollen.

The modification of the trees is not expected to have significant effects on non target species. In former trials no effects on non target species were identified. From scientific literature it can be

deduced that lignin modified trees do not have an effect on the interaction with pathogens, that there is no or very limited effect on leaf-eating insects, and that for the decay of lignin-modified wood other factors like environmental conditions, the chosen poplar species and clone have more significant effects than the lignin modification.

And as outlined above, there is no expected selective advantage of the GM poplar. It is more likely that the GM poplar will have a selective disadvantage.

With regard to possible toxic and allergenic effects we state that any possible toxic effects of these specific lines has not been tested. With regard to allergenicity it can be stated that for these transgenic lines there is not a concern for an altered allergenicity of the transgenic pollen (pollen form poplar is known as a moderate allergen), as we are working with a female clone that does not produce pollen.

It is also known that trees with comparable alterations in the lignin content already exist in nature (in loblolly pine in the U.S. and in black poplar in Europe). If there would be any alteration of the way the modified trees interact with nature and in particular with non-target organisms, this altered interaction would be comparable with the interactions of those wilde type trees. Also, there are no indications from the loblolly pine and black poplar mutants that the modified wood, would have any negative impact on the health of humans or animals.

E. Brief description of any measures taken for the management of risks

Grey poplar (P. x canescens) is dioecious (every tree is either male or female). The 717-1B4 clone is female. In consequence, there is no risk of dissemination through pollen. Moreover, as flower development occurs before vegetative bud burst and leaf development, it is very easy to identify and eliminate female catkins, before their full development. But as the modified poplars will be grown as short rotation intensive culture with a harvest of all trunks and branches after 3 years of growing, the GM poplars will not flower. Suckers are also regularly monitored and destroyed once a year using a contact herbicide. After a storm the site will be inspected for possible fallen branches and these will be removed. The site is designed in such a manner that fallen branches will not disperse by wind from the plot and will remain within the boundaries of a fence surrounding the trial.
At the end of the trial, the rootstock will be mechanically removed and the soil will be worked with a rotary cultivator. The plot will be monitored for at least two years for suckers, which will be destroyed using a suitable contact herbicide. If necessary monitoring will be extended until there has been one year without any suckers.
The field trial plot will be surrounded by a 1.80 m high wire fence to prevent accidental trespassing and accidental removal or spread of GM material.

F. Summary of foreseen field trial studies focused to gain new data on environmental and human health impact from the release

There will be monitoring of certain insects and of endofytes.

G. Final report

-

H. European Commission administrative information

I. Consent given by the Competent Authority:

Not know

Bioveiligheidsraad
Conseil de Biosécurité

26-03-2013

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2013_0206
Email: BAC@wiv-isp.be

Compilation of comments of experts in charge of assessing the dossier B/BE/13/V1

Coordinator: Prof. Dr. ir. Dirk Reheul
Experts: Philippe Baret (UCL), Patrick du Jardin (ULg-Gembloux Agro BioTech) and Katia Pauwels (WIV-ISP)
SBB: Didier Breyer, Fanny Collard, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/13/V1** concerns a notification of the VIB, Flanders Institute for Biotechnology 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 30 January 2013 and concerns a field trial with poplars with an altered wood composition for the production of bio-ethanol.

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 08-03-2013 to the notifier with a request to provide additional information. The comments 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.

Please note that questions on measures to be taken have not been sent to the notifier, as finally it is not up to the notifier which measures need to be implemented. These questions have been taken into account in the discussions of the Biosafety Advisory Council.

The answers of the notifier were received on 14-05-2013 by the Biosafety Advisory Council and evaluated by the experts.

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

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

Comment: see Q1 and Q8

My comments relate to the risk of dissemination.

As only female plants are used in the trial, the dissemination by pollen is impossible.

The only risk is related to the development of suckers. The data presented in the dossier are insufficient to assess this risk and, in consequence, I consider that, in the present configuration of the dossier, a possible dissemination by suckers cannot be excluded. The following information has to be provided in order to achieve a proper assessment of dissemination risk:

What is the maximum distance between the parent tree and observed suckers?

How many suckers are expected in the trial?

Data on the observation of suckers in the previous trial are only qualitative. More precise and quantitative data are required to assess the risk.

Note coordinator/SBB: Quantitative data on root suckers were not considered necessary to conclude on the risk. To determine the overall risk, the management measures need to be taken into account: we know there is a risk of spread via root suckers, but the measures (removal of all root suckers observed) will reduce this risk to about 0.

On basis of trial and according to (M Fladung and Hoenicka 2012) : « the occurrence of root suckers putatively developed from roots of transgenic and non-transgenic trees was studied in the years 4 and 5 after establishment of the field trial. An increasing number of root suckers was found. » This important paper is not quoted in the dossier. See also (Matthias Fladung et al. 2003).

Note coordinator/SBB (see Q8): This remark raises the question if recent scientific knowledge has been taken into account when defining the monitoring period.

2. INFORMATION RELATED TO THE GENETIC MODIFICATION

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

Comment 1:

Mistakes to be corrected in Annex 2:

In Table 1, footnote: attR1 and -2, and attR should be replaced by attB1 and -2, and attB, respectively

Comment 2:

Annex 2 B.2. Table: the presence of a cytotoxin protein B gene element ccdB is mentioned. The functionality and the fate of this element has not been further described in the annex nor in the technical dossier. The applicant could give a short description similar to what has been done for the attB1, -2 and attB sequences.

Note coordinator/SBB: As the *ccdB* gene is not in the vector used for transformation, this information will not help to conclude on the ERA and is therefore not asked for.

Comment coordinator: see Q3

p 9/32 D1, second paragraph, The notifier states "*er niet zozeer minder lignine wordt gemaakt, maar dat de lignine anders van samenstelling is*" and that (on p. 22/32) the purpose of the transformation, which is "*na transformatie van de biomassa een grotere glucose-opbrengst halen*". Which indications are there that the observed changed lignin composition result in this objective?

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

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

No comments received

3.2. Information on the molecular characteristics of the final GMO

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

Comment 1: see Q2

In annex 2, section C2 :

- Editorial comment: on page 5/7 : line 2,"Table 2" should be replaced by "Table 3" in the text.
- pages 4-5/7 : In line with the BAC Guidelines for MC of GMPs for a standard Part B consent, the experimental data must be provided by the applicant for the description of the transgene loci, not only the conclusions of the experiment. In sections C1 and C2, only the conclusions are indicated by the applicant for the qPCR analysis, preventing the external evaluator to check the conclusions. The applicant should provide the experimental data supporting his claims.
- page 5/7 : about the Southern blot analysis : the applicant forgot to mention the probe used in this experiment, hence no interpretation of the blot is possible by the external evaluator. The applicant should be requested to indicate which probe was used, allowing in particular to discriminate between internal fragments of the insert and insert-genomic DNA junction fragments. It would also be appropriate to display (e.g. in a table format) the expected and measured sizes of the restriction fragments hybridized by the probe.

In conclusion, either the lack of communicated data or the lack of details on the experimental procedure prevents from drawing conclusions on the localisation and copy number of the insert in the three GM clones.

Note coordinator/SBB: Information on localisation of the insert is not a requirement for a Standard Part B notification according to the BAC guidelines for Molecular Characterisation of GM Plants and therefore not asked for.

Section C3:
page 6/7: The applicant provides the conclusions of the experiment, but not the experimental data supporting the conclusions. In particular, the experimental data supporting the absence of backbone sequences (including the antibiotic resistance marker aadA) should be displayed. Based on the Table 6 summarizing the PCR results, it is not clear whether and which controls (e.g. spiked DNA) were included to support the conclusions, although the general PCR procedure described in annex 11 prompts to use such controls.

In conclusion, the absence of backbone sequences in the GM trees cannot be concluded on the basis of the data provided in the dossier.

Comment 2: see Q2

- The figure on page 5 of Annex 2 has no legend and is of poor quality. Based on the vector map and the position of the HindIII restriction sites, one could expect the appearance of a 800 bp band, however results are not verifiable since it is not clear whether the figure represents a Southern blot and, if this is the case, which probe has been used.
- Annex 2 C.2. describes the results obtained by Southern blot analysis, however no indication is given on the probe that has been used. Hence, the conclusion made by the applicant as regards the copy number and the loci are hardly verifiable.
- In Annex 2 C3 (p 7) it is mentioned that CAD activity is lowered to 15% of the normal WT activity. How the activity has been determined? Could the applicant provide data to substantiate this statement?

Note coordinator/SBB: Information on how CAD activity has been determined will not aid us to conclude on the risks and is therefore not asked for.

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:

Although few expression data are included in the dossier – limited to some indication on CAD residual activity and on lignin colour as associated phenotype – this may be considered as sufficient for the risk assessment of the field trials.

Comment 2:

See comment 3.2 on results related to the CAD activity.

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

No comments received

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

Comment 1:
Limited information is available on this point, but the GM trait can be easily followed by its associated wood colour phenotype and no indication of instability is mentioned by the applicant. Moreover, loss of the trait is not regarded as a biosafety issue *per se*.

Comment 2:
Phenotypic observations indicate a phenotypic stability. Information on genetic stability was not found a requirement for risk assessment of Part B dossiers (see Guidelines for molecular characterisation for a standard Part B consent).

Comment 3:
« De genetische stabiliteit van het donormateriaal is niet direct getest. Echter, de observaties van de kleurwijziging van het actieve xyleem over verschillende jaren heen suggereert een goede fenotypische stabiliteit van de transgene bomen »

A proper test of genetic stability is a requirement of the risk assessment procedure. In absence of this test without any justification, are we in the conditions for an authorization?

Note coordinator/SBB: Information on genetic stability of the insert is not a requirement for a Standard Part B notification according to the BAC guidelines for Molecular Characterisation of GM Plants and therefore not asked for.

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

Comment 1:
The risk assessment of the attB1and 2 sequences in the T-DNA (possible impact on horizontal gene transfer), as presented by the applicant, is appreciated and considered sufficient.

Comment 2:
Sentence such as “Eerdere veldproeven met CAD-gemodificeerde populieren lijken te suggereren dat er geen wijziging is opgetreden in het vermogen om te overleven. » is too vague for a proper risk assessment. From previous trial, information should be available on the fitness of the transgenic trees. Comparative quantitative data are required for an evaluation of fitness.

Note coordinator/SBB: We would consider this a correct formulation if fitness was not the objective of the former field trials.

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

Comment 1:
As indicated previously (under section 3.2.), no firm conclusion on the absence of the aadA antibiotic resistance marker gene in the GM plants can be drawn from the data presented in the dossier.

Comment 2:
Er zijn geen redenen om te veronderstellen dat een gewijzigde houtsamenstelling een toxisch, allergeen of ander schadelijk effect zou hebben op de menselijke gezondheid.
This sentence is unscientific. No justification, no references are provided. As the evaluation has to be « science based », scientific evidence of the absence of toxicity or allergenicity are required. They are not provided by the notifier.

Deze mutant komt van nature voor in de Verenigde Staten en er zijn geen aanwijzingen dat deze mutant in vergelijking met niet-mutante dennen meer toxisch of allergeen zou zijn.

Was the mutant tested for toxicity or allergenicity? If not, this sentence is senseless.

Note coordinator/SBB: As pointed out by the notifier, trees with altered lignin are found in nature. What is in the natural environment can be used as a baseline for risk assessment. This is a common principle in risk assessment.

Het is natuurlijk niet volledig uit te sluiten dat als gevolg van insertie van het donormateriaal op een ongunstige locatie in het genoom van de plant, effecten zijn opgetreden die op dit moment nog niet gekend zijn. De kans hierop is relatief klein doordat de huidige lijnen geselecteerd zijn geweest uit een reeks transformanten op basis van gewenste fenotypische eigenschappen

A proper risk assesment required a science-based evaluation of risk . If « the probability is relatively small », could the notifier provide a value for this « small effect ». How small?

Note coordinator/SBB: This is a too strong requirement for a Part B application and not in line what has been asked before for Part B notifications.

Comment coordinator: *see Q4*:
p15/30: Eerste paragraaf: explain S/G ratio

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

Not relevant as no feed uses in the scope of the application.

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

Not relevant

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

Comment 1: see Q5
As regard the presence of phenolic compounds and the impact on the interaction with non-target organisms the applicant could refer to a more recent publication: Kleemann et al. (2011).

Comment 2: see Q5
The use of antibiotic resistance genes is in contradiction with the 2001/18 directive: “Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to Part C and by 31 December 2008 in the case of GMOs authorised under Part B.”.

Five years after the end of the phasing out period, why is this technique still in use ? The only justification can be the absence of alternative as selection marker or the impossibility of removing the markers but none of these option are discussed in the dossier. The justification of nptII on the basis of the EFSA guidelines is procedural. As already noted, a minority opinion on this EFSA guideline is emphasizing the risk related to antibiotics whatever the category is. Are they no alternative to antibiotic resistance gene as selection markers?

The presence of antibiotic resistance gene in other GMO is not a justification and has nothing to do with risk assessment. Moreover, techniques for antibiotic resistance gene removal exist and are not quoted (Lamtham and Day 2000).

Er zijn in de wetenschappelijke literatuur geen gegevens gevonden over de mogelijke effecten van lignine-modificatie op vertebraten zoals hazen, konijnen of herten. → So, no information in the scientific litterature means no risk. With that kind of rationale, is it possible to consider that the evaluation is « science-based » ?

Comment coordinator: *see Q5*
Why did the notifier use *ARM* genes, knowing well that this attitude provokes reactions?

3.11. Potential interactions with the abiotic environment

Comment:
The applicant claims no change in the interaction with the abiotic environment. This seems in contradiction with the scientific report quoted under the previous section (Axelsson 2010), indicating changes in leaf litter quality and decomposition in aquatic environments when CAD down-regulated poplars are compared with the wild type. The possible impact on biogeochemical cycles should have been discussed here, though no safety concerns may arise from such small-scale field trials.

Note coordinator/SBB: Given that this article is on changes in biogeochemical processes in aquatic environments and not in field, we do not see the need to include this in the RA of a field trial.

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

Comment 1:
The PCR primers mentioned in the dossier allow the detection and identification of the trait, which seems to be in line with the BAC *Guidelines for MC of GMPs for a standard Part B consent.* However, is is noticed by the external evaluator that no detection/identification tools specific to each of the transformation events (lines) are described.

Comment 2: see Q6
- Please note that we consider the detection on basis of the *nptII* gene too unspecific as *nptII* is widely spread in other organisms, such as bacteria. In order to detect the GM poplar lines a primer pair covering the junction between the *nptII* gene and an adjacent sequence could be chosen.
- The position of the primers to detect the GM poplar lines should be illustrated.
- In the PCR protocol (point 2), PCR mix volumes are incorrect: total should be 25 µl and not 21 µl. Further, it is not clear if the concentrations mentioned are start or end concentrations.

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

Comment: see Q5

"In de veldproef B/BE/07/V2 met bomen waarin het CCR (Cinnamoyl CoA Reductase) enzym neergeregeld is en die als gevolg daarvan ook een verlaagd ligninegehalte is in twee seizoenen een endofytenbepaling uitgevoerd. »

The description of results is very vague. No figures, no statistical analysis. Could the notifier provide the report of that study?

Comment coordinator: see Q7

p20/32, D13, 4th paragraph: "*De lijnen zijn gezond gebleven gedurende de gehele periode van de proef.*" How long did the experiment last?

4. INFORMATION RELATING TO THE SITE OF RELEASE

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

No comments received

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:

- Careful monitoring of flower formation is requested. The monitoring plan proposed by the applicant looks adequate, taking into account the experience gained from the ongoing field trial (with the same recipient clone).
- Monitoring of the root suckers after the trial is proposed but limited to one year without suckers. This is minimal and extension to based on 2 years in total should be envisaged. This would be in line with the previous opinion of the Belgian Biosafety Advisory Council on lignin-modified poplars, asking for 2 years ("Advice of the Belgian Biosafety Advisory Council on the notification B/BE/07/V2 of the VIB, Flanders Institute for Biotechnology, for deliberate release in the environment of genetically modified poplars with an altered wood composition for research and development", 25/04/2008).

Note coordinator/SBB (see Q8): This remark raises questions concerning the monitoring period for suckers: Can the notifier confirm that the idea is to monitor for 2 years (= minimum monitoring period) and if no suckers will be found in the second year, monitoring will be terminated; but if suckers are found monitoring will be continued for an extra year until there is one year without observed suckers.

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

No comments received

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

No comments received

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

Comment:
"Op grond va de huidig beschikbare kennis …" What do we know? No references are provided, no data. If there is no evaluation of the potential gene transfer, a proper assessment of the risk is unfeasible.
The element of a calculation of a probability of an horizontal transfer are provided but no quantitative data is made available and the risk is not quantified.

Note coordinator/SBB: The chance to horizontal gene transfer is very unlikely. This is common knowledge. We do not think these quantitative data are needed to come to a RA conclusion.

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

Not relevant

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

Comment:
The reference of Axelsson et al 2010 on leaf litter decomposition and possible interaction with decomposers (see section 3.11) should have been mentioned and discussed here. No harm to the environment can be expected from the field trials considering the scale of the deliberate release, however.

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

No comments received

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

Not relevant as no feed uses in the scope of the application.

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)

Comment:
See remarks under sections 3.11 and 6.5.

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

Not relevant in case of field trial

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

7.1. Precautions taken

Comment:
Flower monitoring will be performed once a week during the flowering period and once a month during the growing season. Considering that the applicants proposal has been based on monitoring data (number and dates of flower formation) from a previous transgenic poplar field trial, the proposed monitoring frequency of flower formation is considered acceptable.

The applicant states that the site will be monitored for possible outgrowing of root suckers three times during the period from march until august. After a period of one year, monitoring will be ceased provided no suckers are observed during 1 year.

However a longer post release monitoring period for suckers than a period of one year with no suckers observed, as proposed by the applicant, is considered necessary, especially taking into account that root material will be chopped and part of it will be decomposed within the site of release (p25). Therefore a period of two consecutive years without outgrowing suckers (instead of 1 year) is considered more appropriate for the post-release monitoring plan.

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

Comment:
As mentioned before (section 5), control of root suckers should be extended to up to 2 years with no detectable suckers after the release period. DR Dit lijkt mij inderdaad het meest verantwoorde advies.

Comment coordinator: see Q9

- What is meant with "… *met voor de eerste plot*…" in 4th bullet point of G.2.?
- In G.2 it is stated "*Het gehakselde materiaal zal in zakken, plastic tonnen, of een andere houder worden opgevangen en vervoerd*.", while on p.22 it is said "... *takken... worden verhakseld en in tonnen of containers verzameld*". Can it be clarified how the branches will be transported?

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

Comment: see Q8
Taking into account the advice of the Belgian Biosafety Advisory Council on a previous field trial with lignin-modified poplars, an additional condition should be demanded:
"The machineries that are used to harvest and chop the wood, should be cleaned at the trial site to prevent dispersal of plant material. This activity should also be included into the activities to be mentioned in the "Logboek".
- the notifier is asked to keep records of dates and numbers of inflorescences removed from each genetic line.

- It should be noted that according to the conditions of the Canadian Food Inspection Agency for research field trials of poplar (http://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/field-trials/2011/poplar/eng/1327012806004/1327012886546):

i) the trial site and a minimum 15 meter around the trial site, must not be used to grow poplar trees from the date of termination of the trial until no suckers are observed for three consecutive years.

ii) two guard rows composed of non-transformed poplar producing no or very few suckers should be planted in order to prevent root suckers or root grafting.

Comment coordinator: see Q9

What will be done with the material after analysis in the lab? The text mentions that field material will be destroyed or removed, but no info about the final destination after analysis.

7.4 Information related to monitoring plans and the detection techniques

Comment:

As mentioned before (section 3.12), the detection tools allow the tracing of the trait, but not of the specific events, which seems to be in line with the BAC guidance document.

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

No comments received

7.6. Information on methods and procedures to protect the site

No comments received

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:

Consistency with the experience gained with the ongoing field trial (B/BE/07/V2) should be seeked. This external advice is based on the elements included in this dossier only.

Comment 2:

I have a general comment: the document we received is a description of what we know and think on the biosafety issues related to transgenic poplar. It is not a risk assessment document as it is not based on a science-based and quantitative approach of the question. For most of the items, the rationale is “there is no data indicating a risk so there is no risk”.

Comment coordinator: see Q2 & Q10

- Bijlage 1, punt 10. Is the experimental design really a randomized design?
- Bijlage 2. Explain in clear words the difference between the 3 transgenic lines.
- Bijlage 2. Give the reference of figure on lignin synthesis

References (not present in notification)

Fladung, M, and H Hoenicka. 2012. “Fifteen Years of Forest Tree Biosafety Research in Germany.” iForest - Biogeosciences and Forestry 5 (3) (June 13): 126–130. doi:10.3832/ifor0619-005.

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Kleemann, F., M. von Fragstein, B. Vornam, A. Müller, C.Leuschner, A. Holzschuh, et al. 2011. Relating ecologically important tree traits to associated organisms in full-sib aspen families. Eur. J. Forest Res. 130:707–716.

Lamtham, S, and A Day. 2000. “Removal of Antibiotic Resistance Genes from Transgenic Tobacco Plastids.” Nature Biotechnology 18 (11) (November): 1172–1176. doi:10.1038/81161.