Biosafety Advisory Council

25-04-2008

Secretariat

O./ref.: WIV-ISP/BAC/2008_733
Email: bac@sbb.ihe.be

Title: 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

Context

The notification B/BE/07/V2 has been submitted by the VIB to the Belgian Competent Authority (CA) in November 2007 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 for the production of bio-ethanol"**. This release has the purpose to produce enough wood from lignin-modified poplars in order to evaluate its properties for bio-energy production, in particular bio-ethanol. The release can also be seen as a partial repetition of the trial B/FR/07/06/01 at INRA-Orleans in France, providing additional scientific value to the outcomes of this trial and *vice versa*.

The notification has been officially acknowledged by the CA on 30 November 2007 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. Four 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 genetically modified (GM) poplar trees would not raise any problems for the environment, animal or human health.

On 22 January 2008, based on a list of questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. This information was

received on 17 April 2008 by the Biosafety Advisory Council and evaluated by the scientists in charge of evaluating the dossier. The additional information was considered satisfactory.

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

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 shoots or branches can occur (OECD, 2001[1]) in *Populus* species. Vegetative propagation through branches is very unlikely for grey poplar.

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

The small scale and restricted field trial will be designed as a short-rotation poplar coppice. Young rooted GM poplars will be planted during spring 2008. Before the start of the second growing season (2009) the trees will be cut down to stimulate the formation of many stems per plant. At the end of 2011 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

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

harvested at the end of 2014. Occasionaly branches will be cut down to be analysed in the laboratory. At the end of 2014 rootstocks and roots of the trees will be destroyed mechanically**.** Potentially emerging suckers will be destroyed during 2015.

3. Information related to the genetic modification

Two events introduced in female clone 717-1-B4 will be tested in the field experiment: they are identified as CCR-lines WT52-3 and WT52-40.

These CCR-grey poplars have a modified lignin (a major constituent of wood) content due to the decreased activity of an enzyme (cinnamoyl coenzymeA reductase, CCR) involved in the lignin biosynthetic pathway. Lines WT52-3 and WT52-40 were obtained through genetic transformation with *Agrobacterium tumefaciens*. The *ccr* gene is inserted between a duplicated version of the promoter of the cauliflower mosaic virus (CaMV) and a transcription terminator from the gene coding for the CaMV 35S RNA. In addition, the transgenic lines also have a selection gene (hygromycine B phosphotransferase, *hpt*) that confers antibiotic resistance and is controlled by the nopaline-synthase (Pnos) promoter and a transcription terminator from the T7 gene from the T-DNA (tAg7). Absence of vector sequences relevant to human and veterinary therapy has been demonstrated. The information is considered as 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

As the branches of the lignin-modified poplars will be harvested every 3 years, the poplars are not expected to flower. Nevertheless, monitoring will be carried out to check for flowering. If unexpected flower buds occur, they will be removed before seed set. As the grey poplar used in the field trials is a female clone 717-1-B4, there is no possibility of dissemination through pollen.

Spontaneous regeneration from branches is considered unlikely, as clone 717-1-B4 does not easily form rooted scions even under optimal laboratory conditions.

The possibility of horizontal gene transfer between GM plants and bacteria is considered as a rare event under natural conditions.

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 and vertebrates) and humans. The impacts of lignin-modified trees on pathogens and leaf eating insects have shown to be negligible. Also effects on herbivores (e.g. rabbits) are expected to be negligible. The fence surrounding the entire field plot will restrict entrance into the field plot; during the first year the young shoots will be protected by a cylinder of small meshed hardware cloth.

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.

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

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

The management measures proposed were considered as sufficient to prevent potential adverse effects to the environment, animal and human health. However, to minimise the spread of transgenes into the environment, additional measures are proposed. It is recommended to monitor for female flowers more frequently and to extend the monitoring for suckers. All woody material should be chopped inside the fence and machinery used for chopping should be cleaned inside the fence before leaving the trial site. The notifier should register all occasions that branches are taken away to be analysed in the laboratory.

Conclusion

Based on the scientific assessment of the dossier by the Belgian experts, the Biosafety Advisory Council concludes by majority that it is unlikely that this small scale restricted field trial with GM poplar with an altered wood composition will pose any risks for 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:

- Monitoring for flowers should be done twice a week (instead of once a week) during the flowering period and monthly during the growing season (instead of every two months). 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 and 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 suckers should be extended until the moment that two years have past after the last observed outgrowing suckers.

Other considerations:

The Biosafety Advisory Council is of the opinion that the molecular detection protocol alone is not accurate enough to distinguish GM poplars from non-GM poplars. Therefore, the Council proposes that a combination of the phenotypic and genotypic method is used when detection is considered necessary.

This advice reflects the opinion of the Biosafety Advisory Council on a this small scale restricted **field trial with GM grey poplars grown as a short-rotation poplar coppice.** It is obvious that any other occasional establishment (e.g. a large scale or commercial plantation), either grown as a short-rotation coppice or as a timber production unit, should be the subject of a new advice, owing to the different conditions.

Prof. D. Reheul
President of the Biosafety Advisory Council

Annex I: Summary Notification Information Format submitted by the notifier in November 2007.
Annex II: Compilation of comments of experts in charge of assessing the dossier B/BE/07/V2 (ref: BAC_2007_PT_661)
Annex III: Amended Summary Notification Information Format submitted by the notifier in April 2008

BIJLAGE 8

Summary Notification Information Format

(as submitted by the notifier in November 2007)

A. General information

A1. Details of notification

Notification Number
B/BE/07/xx/xx

Member State
Belgium

Date of Acknowledgement
xx/xx/xxxx

Title of the Project
Field evaluation of poplars with an altered wood composition for the production of bio-ethanol

Proposed period of release:
01/05/2008 to 31/12/2014

A2. Notifier

Name of the Institute(s) or Company(ies)
VIB

A3. Is the same GMPt release planned elsewhere in the Community?
The same and similar plants have been introduced in France by INRA, see B/FR/07/06/01 and B/FR/99/02/15. One transgenic line WT/52-40 will be introduced into the environment for the first time and will not be released elsewhere.

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*
(c) Species:	*Populus x canescens (Populus alba x Populus tremula)*
(d) Subspecies:	-
(e) Cultivar / breeding line:	717-1B4
(f) Common name:	Grey poplar

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications

The genetically modified poplars exhibit modified lignin (a major constituent of wood) due to the decreased activity of an enzyme of the lignin biosynthetic pathway. Depending on the transgenic line, the altered enzyme is:
- CCR (Cinnamoyl coenzymeA reductase): 2 transgenic lines WT52-3, and WT52-40.
- CCoAOMT (Caffeoyl coenzymeA O-methyl transferase): 2 transgenic lines 101 and 416.
The down-regulation has been obtained either by antisense strategy (101) or by co-suppression (WT52-3, WT/52-40, 416). The enzyme residual activity varies between 3 to 100 % and is not necessarily uniform within the plant. Consequently, the quality or/and quantity of lignin is modified. These modifications and the consequences on some wood properties have been described in several publications (Baucher et al., 1996, van Doorsselaere et al., 1995 ; Meyermans et al., 2000 ; Lapierre et al., 1999 ; Pilate et al., 2002 ; Lapierre et al., 2004).
In addition, all transgenic lines have also integrated a selection gene (hpt) that confers an antibiotic resistance. This antibiotic resistance has been used during in vitro culture steps to select for genetically modified cells.

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 inserted genetic material is the T-DNA from the Ti plasmid of Agrobacterium tumefaciens harbouring the gene of interest (for lignin modification) and the gene for selection (antibiotic resistance). The gene of interest is one among two poplar genes coding for one among two enzymes of the monolignol biosynthetic pathway. Monolignols are the elementary units of the lignin polymer. The coding sequence of any of these 2 genes is inserted in sense or antisense orientation between i) the promoter of the cauliflower mosaic virus (CaMV) in a duplicated version (p70) and ii) a terminator sequence, either from the T7 gene from the T-DNA (pAg7) or from the gene coding for the CaMV 35S RNA (pA35S). The antisense insertion aims to turn off the expression of the corresponding endogenous gene: The mRNA of the antisense gene interferes with the corresponding endogenous mRNA that results in a strong reduction in the production of the endogenous protein. A sense insertion leads in a few transgenic lines (this is the case for the sense transgenic lines included in this application) to a similar effect, i.e. a reduction in the activity of the target enzyme, through another mechanism named co-suppression.
The two poplar genes listed below derive from cDNA sequences isolated from a xylem cDNA library from the Populus trichocarpa “Trichobel“ clone (for CCoAOMT and CCR cDNA).

i) CCR (Cinnamoyl coenzymeA reductase): the full-length cDNA coding for CCR (accession AJ224986 ; Leplé et al., 1998) inserted in sense orientation. The corresponding chimeric gene (p70-S-CCR-pA35S) once introduced in the pBIBHygro binary vector generates the pBIBHygro/S-CCR pBIBHygro transformation vector.

ii) CCoAOMT (Caffeoyl coenzymeA O-methyl transferase): the full-length cDNA coding for CCoAOMT (accession AJ224894 ; Meyermans et al., 2000) inserted in sense or antisense orientation. The corresponding chimeric genes (p70-S-CCoAOMT-pA35S and p70-AS-CCoAOMT-pA35S) once introduced in the pBIBHygro binary vector generate respectively the pBIBHygro/S-CCoAOMT and pBIBHygro/AS-CCoAOMT transformation vectors.

For the p70-S-CCoAOMT-pA35S, p70-AS-CCoAOMT-pA35S, p70-S-CCR-pA35S, the selection gene is the hygromycine B phosphotransferase (hpt (or hph) gene fused to the promoter of the nopaline synthase gene (pNOS) from Tn7 and to the terminator of the gene 7 from the T-DNA (pAg7).

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 for the content and/or quality of lignin. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. These different transgenic lines of poplars have been already evaluated in a previous field trial in France, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purpose to produce enough wood from lignin modified poplars in order to evaluate its properties for bio-energy production, in particular bio-ethanol. Both lignin/cellulose ratio and the accessibility to cellulose are critical for the production of bioethanol from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture on a low-grade soil (marginal land) 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 the start-up of a bioprocess pilot plant for bio-energy production. The release can also be seen as a partial repetition of the trial B/FR/07/06/01 (the current release only involves 4 lines, where the FR trial includes more lines) at INRA-Orleans in France, providing additional scientific value to the outcomes of this trial and vice-versa.

C2. Geographical location of the site
The University of Ghent Science and Industry park in Zwijnaarde, Belgium.

C3. Size of the site (m2)
The site is in total 6500 m2 of which a maximum of 2400 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
There has been one previous release involving four of the five same CCR and CCoAOMT lignin modified poplars (notification number B/FR/99.02.15).

During this previous field trial, no significant differences between GM and wild type poplars with regards to reproductive aspects were observed. Lignin modified poplar flowering time and intensity did not appear affected by the genetic modifications.
However, lignin is involved in major biological functions for tree growth and development such as mechanical support, water conduction and pathogen defense. Field trials with lignin modified trees over more than 12 years have shown that important lignin modifications are very rapidly translated into changes in the function of conduction and/or support. It has also come out that some lines that were shown to grow normally in the greenhouse (i.e. in optimal growth conditions), where unable to do so in a nursery. Some transgenic lines were even unable to survive. Apparently there has to be a balance between the lignin modification that can be of

interest for certain applications on the one hand and the impact of the modification on tree growth and development on the other hand. The lines in this application have been shown to grow almost normal (CCR down-regulated) to normal (CCoAOMT down-regulated), and have been shown to release up to twice the amount of glucose in biochemical breakdown experiments, when compared to conventional poplars.
The experiences with lignin modified poplar appear to suggest that lignin modified poplars will have a fitness that is less or at the maximum comparable to their wild type counterparts.

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

Note especially if the introduced traits could directly or indirectly confer an increased selective advantage in natural environments; also explain any significant expected environmental benefits

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. 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 targeted at non target species. In former trials no effects on non target species were identified. One could speculate on the effect of lignin modification on the degradation of leaves and wood under natural circumstances. But there are currently no data available on that.

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.

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 are not expected to flower. Suckers are also regularly monitored and destroyed once a year using a contact herbicide.
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

In this field trial there will be no data collection of new data on the environmental and human health impact of the release. However, in the similar field trial B/FR/07/06/01 there will be data collection on the effects on biodiversity.

G. Final report

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H. European Commission administrative information

I. Consent given by the Competent Authority:

Not known

Bioveiligheidsraad
Conseil de Biosécurité

14 January 2008

Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC_2008_732[1]
Email: bac@sbb.ihe.be

Compilation of comments of experts in charge of assessing the dossier B/BE/07/V2

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 22 November 2007
Coordinator: Prof. Dr. ir. D. Reheul
Experts: Philippe Baret (UCL), Patrick du Jardin (FUSAGx), SBB (WIV/ISP), Lieve Gheysen (UGent)
Domains of expertise of experts involved: Molecular characterisation, genetic engineering, transgene expression, Population genetics, outcrossing to wild relatives, biodiversity, risk analysis
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **B/BE/07/BV2** 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 November 2007 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.

[1] revised version of document BAC_2008_PT_661

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 22 January 2008 to the notifier with a request to provide additional information. This information was received on 17 April 2008 by the Biosafety Advisory Council and evaluated by the experts. The additional information was considered satisfactory by the experts .

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

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

Comment 1

The information on the viability of seed (2 to 4 weeks) is not referenced. In a context of risk assessment, information on viability shouldn't be restricted to average values or a normal range but should also refer to the maximum values.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Section B7 should describe potential allergenicity of the pollen of *Populus* (despite the fact that the clone used is this study is female, as this section is intended to provide some general background information on the recipient species).

Comment 4

It is mentioned that fallen branches might shoot. What is the likelihood that and the conditions under which shooting of branches occurs? This should be included as background information on the recipient species.

2. INFORMATION RELATED TO THE GENETIC MODIFICATION

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

Comment 1

The polyadenylation signal of nos or of gene 7 are abbreviated as pA but are explained as terminator. This is not really exact and may also cause confusion for lay people who interpret this sometimes as 'terminator technology' being present. It would be better to change this in the text and table on pages 6 and 7 and also in the SNIF document. The Pnos promoter is (3 times) incorrectly explained as terminator in the table.

Comment 2

In appendix 1, figure 2 is not clear and the genetic elements mentioned in the above table can hardly be located on the map of figure 2. There is also a discrepancy between figures 3 and 4 of the same appendix, regarding the orientation of the pAnos-EcoRI fragment, that should be clarified. Abbreviations (*e.g.* of the T-DNA borders) should be coherent form one figure to the other.

Comment 3

p. 6/22: in the tables the function of Pnos should be "Transcriptie promoter" and not "Transcriptie terminator".

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Some information on the *A. tumefaciens* gene 7 promoter should be provided.

Comment 3

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

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

Comment 1

The use of antibiotic marker is questionable.

I am aware of the EFSA advice EFSA-Q-2003-109 “The GMO Panel considers the frequency of horizontal gene transfer from GM plants to other organisms as very low for all ARMGs considered. This, in itself, is an important consideration with regard to any risk posed by the use of ARMGs. However, with respect to clinical importance the Panel has categorised ARMGs into three groups with different potentials for compromising human health and the environment. ARMGs in the first group include genes conferring resistance to kanamycin and hygromycin. In this group the nptII gene, which confers kanamycin resistance, has a 13-year history of safe use in food crops and resistance to this group of antibiotics is widespread in naturally occurring microbes in humans and the environment. The Panel is of the opinion that with regard to safety there is no rationale for inhibiting or restricting

the use of genes in this category, either for field experimentation or for the purpose of placing on the market. But I express doubt on the utility of increasing the diffusion of antibiotics in the environment especially when producing biomass.

The argument of EFSA seems weak to me as it may be used to justify the presence of any pollutant. For example, "As there is a widespread diffusion of CFC in the atmosphere, there is no need to restrict its usage". I expect that the notifier will justify why he made use of an antibiotic marker instead of using another approach as recommended by EFSA for a long time.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Genomic site of integration : The mere evidence of bands on a Southern blot can not be regarded as a proof of nuclear integration, it the absence of segregation data. Appropriate controls should be provided (like spiked DNA using plasmid vector at different dilutions for a valid analysis of band intensities). If the DNA submitted to Southern analysis is purified nuclear DNA instead of total genomic DNA, experimental arguments should be provided.

Number of copies : The Southern blot on page 7 of Appendix 1 is not acceptable : no indication is provided on the DNA amounts loaded on the gel, the exposure time is not mentioned, and no molecular size markers were run in parallel with the samples, preventing any estimation of the size of the restriction fragments. This Southern blot analysis should be repeated with an improved protocol.

Absence of vector sequences (oriV and npt-III) in the final GMP : the PCR analysis of the vector sequences potentially present in the final GMP (appendix 1 page 12) lacks appropriate controls : the absence of DNA bands in the lanes corresponding to the GMP samples can be interpreted as the absence of the target sequence only if it is proven that the DNA was competent for PCR amplification (absence of inhibitors) and this should be proved by using primers corresponding to endogenous genes or by spiking the test sample with plasmid sequences containing the target genes. The PCR analysis should thus be repeated with appropriate positive controls.

Comment 4

Annex 1 p. 7 : Southern blot of CCoAOMT-transgenic lines is not very clear! Therefore, we cannot verify if the predictions on copy number are correct. It is recommended to include a molecular marker in the blot.
Note: The lack of a detailed map of the vectors used for transformation, including their genetic elements and relevant restriction sites (as asked for in the Belgian guidelines on molecular data for Part B releases), makes it less easy to verify the results of the Southern Blot analysis.

Annex 1 p. 12 : The PCR-test used, gives a good indication of the absence of *npt*III and oriV. However, a Southern blot (using the complete amplification products as a probe) should be included to prove the absence of a functional *npt*III. This request is justified by Art. 4 of the Directive, which envisages the phasing out of antibiotic resistance markers in GMOs which may have adverse effects

on human health and the environment. Amikacin is considered as an antibiotic highly relevant for human therapy by EFSA (EFSA, 2004). Therefore, the *npt*III gene should not be present in the transgenic lines of this field trial which will be conducted for several years.

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

Has evaluated this item and has no questions/comments.

Comment 2

Information is very limited (as acknowledeged by the applicant : "*Er is geen informatie voorhanden over de precieze mate van expressie van het donormateriaal"*) and restricted to indication on associated phenotype (wood coloring), hence on secondary xylem only, with no clue on expression in other tissues. Papers are quoted, as well as field releases in France (Cornu et al 1999), but it is impossible to draw clear conclusions on what is available on the precise lines under study. The applicant should be requested to present a summary of the data available on these lines, *e.g.* from previous greenhouse experiments and field trials, and to detail the protocol that will be used for examining the expression of the inserts over the testing period. The expert is of the opinion that field trials give the opportunity to collect expression data which are not possible or pertinent when collected in the greenhouse, hence the experimental protocols for the expression studies should be included in the application dossier.
Regarding the expression of the antibiotic resistance marker *hpt* under the control of the *A. tumefaciens* gene7 promoter, no data or comments are provided in the dossier : this should be included by the applicant.

Comment 3

Has evaluated this item and has no questions/comments.

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

Comment 1

The point D4c is insufficiently documented : no data, no reference. More precision should be asked before authorisation of the field trial.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

D4(a) A reference to earlier conducted trials is lacking: this should be mentioned.

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

Comment 1

Evidences on the genetic stability are not provided. The only soft evidences (non scientifically documented) are on phenotypic stability.
Note from the SBB: The information on genetic stability of the insert was not found relevant for risk assessment of Part B dossiers (see guidelines molecular characterisation)

Comment 2

Although the genetic stability has not been analysed at the molecular level, it is clear from the persistent phenotype of the lines that the inserts are stable.

Comment 3

The applicant should be asked to be more precise on the number of vegetative generations tested in the greenhouse or in the field, and whether seeds and seed progenies were also obtained and tested in the greenhouse experiments. Whether the studied epigenetic effects are stably transmitted after meiosis (sexual reproduction) is a question that may be of interest from a biosafety perspective.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

Comment 1

The allergenicity and the toxicity were not tested. I think important that this point be clearly communicated to the public as it is not stated in the SNIF that the toxicological and allergenicity assessments were not achieved.

Comment 2

"dt"-fout: omdat het npt-III-gen in de natuurlijke microflora nog niet heel erg **verspreid** is

Comment 3

Considering : (i) the remark under item 3.2 above, claiming that the absence of the *nptIII* gene in the final GMOs was not proven in a convincing way, (ii) that the *nptIII* gene is classified in the highest risk category of antibiotic resistance markers by EFSA (The EFSA Journal, 2004, 48 : 1-18), the expert estimates the no consent should be granted to this application before the clear-cut demonstration of the absence of the *nptIII* gene in any of the transgenic lines.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

This item is not relevant

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

Comment 1

The impact and potential toxicity for wild animals was not evaluated.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

Description of the impact on non-target organism is insufficient. A single reference is quoted (and the reference list is incomplete). It is an important issue for two reasons : on the one hand, a correct risk assessment require a comprehensive study of the literature; on the other hand, an identification of the uncertainties concerning ecological risks will help to plan specific measurements during the field trial in order to contribute to our knowledge of the risk. As an expert, I cannot accept a field trial without a correct preparation by a thorough study of the scientific literature. Moreover, if this trial doesn't' contributed to a learning process on risk, it leads to a potential paradoxical lock in as eventual positive technological results will be impaired by insufficient knowledge on risk issues. Both learning processes should be parallel and, if not, our knowledge on risk issues should precede technicological assessment.

Comment 2

I have evaluated this item and I have no questions/comments

Comment 3

The unique reference quoted for discussing that issue on page 11 of the technical dossier (Halpin et al.) is incomplete, both in the text and in the bibliography. The applicant should give the complete reference and explain to what extent the conclusions of this work (review ?) may be extended to their own biological materials and trials.

Comment 4

Has evaluated this item and has no questions/comments.

3.11. Potential interactions with the abiotic environment

Comment 1

The notifier doesn't anticipate any interaction with abiotic environment but this statement is neither justified nor documented. I ask that the authorisation be postponed waiting for further details about this issue.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

I have evaluated this item and I have no questions/comments

Comment 2

I have evaluated this item and I have no questions/comments
For clarity, the appendix 11 should be referenced in the technical dossier under item D12.
In addition, the protocol targets the *hpt* marker gene, which makes sense for detecting the transgenic materials, but primers amplifying some endogenous poplar sequence that could be used as an internal positive control should also be included.

Comment 3

A detection protocol has been provided that allows to identify the transgenic lines on the basis of the presence of the *hpt* gene. A negative control (endogenous bacterial sequence) should be included to eliminate that positive signals are the result of bacterial contamination.

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

Comment 1

Previous releases are mentioned but no results are provided on the outcomes of these releases. Key publications resulting from these releases are omitted. It denotes some carelessness in the building of the dossier. If the results of previous releases are not thoroughly exploited, new trials are irrelevant and risky. A full assessment of the previous data is a minimal requirement.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

As the paper describing the data obtained from the previous field trials in France (Leplé et al) is in press, but not yet available, its inclusion in the dossier (as an annex) would be welcome.

Comment 4

Reference is made to another Part B dossier covering 3 of the lines which are considered in the current notification, namely B/FR/99.02.15. As notification B/FR/99.02.15 contains information on biosafety issues - as mentioned in the current notification - information on this notification (at least the SNIF - which is not publicly available - and/or a review of the biosafety studies done in B/FR/99.02.15) should be included in the current notification.

4. INFORMATION RELATING TO THE SITE OF RELEASE

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Appendix13 should be referenced in the technical dossier, for the sake of clarity.

Comment 3

Has evaluated this item and has no questions/comments.

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

The purpose of release is insufficiently documented : it is difficult to situate the proposed trial vs. previous releases. No contribution to risk assessment is planned in the trial : it may be justified if our knowledge on the risks is comprehensive but this point is not demonstrated.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Appendix 5 ("*Plan van de proef*") does not correspond to either the 2008 or the 2009 plot – if I understand correctly. The applicant should make it clearer.

Comment 4

Note: The plan of the field experiment still includes 5 lines in stead of 4. Therefore, the plan needs to be adapted. However, we do not consider this plan as relevant information for risk assessment.

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

Comment 1

References should be provided in the B.5. section

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

None study on fitness is provided

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

The introduced trait (changed lignin content) is not expected to change the selective advantage of the GMHP.

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

Comment 1

The information on the viability of seed (2 to 4 weeks) is not referenced. In a context of risk assessment, information on viability shouldn't be restricted to average values or a normal range but should also refer to the maximum values.
A strict control of shoots is mentioned but no information is provided on the frequence and the duration of this control

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

Not relevant : no target organism

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

No target organisms

Comment 4

Not applicable as there are no target organisms in this particular case

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 1

The B7 section is outdated and partly irrelevant. Recent studies on the impact of low lignin poplar on insects are not quoted (and more generally recent studies on interactions between insects and poplars are overlooked (for example, Tomescu, 2007). From my point of view, this lack of knowledge on the interactions between poplars and insects and other non target organisms is an important issue. A classic prerequisite of any field trial implying risk or ethical issues is a full and critical review of the literature. If the notifier is not able to compile the relevant literature on ecological aspects of the system he works on, I have doubt on his ability to manage any risk related to an ecological issue.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

See comment under item D3.10, however, not a risk issue regarding the exposure level of the environment to the transgenic poplars.

Comment 4

Non-target effects on invertebrates and vertebrates are not assessed. The introduced trait (changed lignin content) is not expected to have impact on non-target organisms, such as invertebrates and vertebrates (Pilate *et al*., 2002). If any adverse effect would occur, the environmental impact would be negligible given the size of the field trial.

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

Comment 1

As stated (3.7), the allergenicity and the toxicity were not tested. I think important that this point be clearly communicated to the public as it is not stated in the SNIF that the toxicological and allergenicity assessments were not achieved.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Poplar with a change in lignin content is not expected to result in other interactions with persons compared to conventional poplars.

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

Comment 1

This point is not relevant.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Not relevant question, as poplars are not intended for 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)

Comment 1

see 3.10.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

See comment under item 3.10, however, not a risk issue regarding the exposure level of the environment to the transgenic poplars.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

This point is not document. Indeed, the way this technique will be implemented in the "real world" is not described. It seems to me impossible to avoid persistence of sucklers or flowering in a routine cultivation of this kind of crop.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

As this is a small scale field trial, the impact of agronomic practices are not relevant to consider.

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

7.1. Precautions taken

Comment 1

See 6.9.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

The applicant indicates that the site will be monitored for the possible outgrowing of root suckers for two years after the end of the trial, extending the duration till one year without suckers (appendix 7). Is that enough? The available knowledge on the biology of *Populus x canescens* should be reviewed on this aspect, in the absence of clear answer in the OECD consensus document on poplar biology (http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/NT00002EC2/$FILE/JT00103743.PDF), and considering that active vegetative propagation can not be ruled out, which clearly represents the major possible dissemination route of the transgenic clones. The expert also suggests that at least two years without outgrowing suckers, instead of one single year, is demanded in the post-release monitoring plan.

Comment 4

Has evaluated this item and has no questions/comments.

7.2. Information on methods for post release treatment of site

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Same remark as under 7.1.

Comment 3

Has evaluated this item and has no questions/comments.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 1

Although no perfect cleaning of the plot is feasible, fallen autumn leaves should be collected instead of being left to wind dispersal (page 18 of the technical dossier).

Comment 3

We propose an additional condition:
The machineries that are used to harvest and chop the wood, should be cleaned at the trial site to prevent dispersal of plant material.

Concerning Annex 6.2:
- "cleaning of machinery" should be included into the activities to be mentioned in the "Logboek".
- We would like to ask the notifier to keep records of dates and numbers of inflorescences removed from each genetic line. This information is useful to check the adequacy of monitoring frequency for inflorescences. We want to note that according to the conditions of the Canadian Food Inspection Agency for research field trials of poplar, monitoring is required minimum twice a week during the flowering period and monthly during the growing season (http://www.inspection.gc.ca/english/plaveg/bio/isole.shtml).

7.4 Information related to monitoring plans and the detection techniques

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Same remark as under 7.1.

Comment 3

Same remark as 3.12

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

7.6. Information on methods and procedures to protect the site

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

The formal and scientific quality of the dossier is poor. I tried to do my best but it is impossible to assess a dossier with lacunar information, bold statements without references to any scientific support, incomplete reference list and missing references to major recent papers.

In previous dossiers I assessed, a copy of publication was always provided, it is not the case here.

Based on these elements, I have a limited confidence in the scientific dimension of the trial. I formally ask that a new dossier be required in order to conduct a proper risk assessment.

I would be glad to participate to a meeting between experts if it may be helpful.

Some of the missing references :

Seppanen SK, Pasonen HL, Vauramo S, Vahala J, Toikka M, Kilpelainen I, Setala H, Teeri TH, Timonen S, Pappinen A: Decomposition of the leaf litter and mycorrhiza forming ability of silver birch with a genetically modified lignin biosynthesis pathway. *Applied Soil Ecology* 2007, 36(2-3):100-106.
Bradley KL, Hancock JE, Giardina CP, Pregitzer KS: Soil microbial community responses to altered

lignin biosynthesis in Populus tremuloides vary among three distinct soils. *Plant and Soil* 2007, 294(1-2):185-201.
Halpin C, Thain SC, Tilston EL, Guiney E, Lapierre C, Hopkins DW: Ecological impacts of trees with modified lignin. *Tree Genetics & Genomes* 2007, 3(2):101-110.
Pilate G, Guiney E, Holt K, Petit-Conil M, Lapierre C, Leple JC, Pollet B, Mila I, Webster EA, Marstorp HG *et al*: Field and pulping performances of transgenic trees with altered lignification. *Nature Biotechnology* 2002, 20(6):607-612.
Tomescu R, Nef L: Leaf eating insect damage on different poplar clones and sites. *Ann For Sci* 2007, 64(1):99-108.

Comment 2

The SNIF document mentions on page 4: "The modification of the trees is not targeted at non target species." I would assume that non target species are never targeted, that is the definition of non-target. A better wording should be chosen.

Comment 3

No

Comment 4

Biosafety issues of the transgenic lines have already been evaluated in other field trials. A report reviewing the biosafety issues of these field trials should be added.

References

Depicker A, Stachel S, Dhaese P, Zambryski P, Goodman HM. (1982) Nopaline synthase: transcript mapping and DNA sequence.J Mol Appl Genet. 1982;1(6):561-73.

Hunt (1994). Messenger RNA 3' End Formation in Plants. Annual review of plant physiology and plant molecular biology. 45: 47-60 1994.

Mogen, B.D., M. H. MacDonald, R. Graybosch and A. G. Hunt (1990). Upstream Sequences Other than AAUAAA Are Required for Efficient Messenger RNA 3'-End Formation in Plants. The Plant Cell Vol 2, Issue 12 1261-1272.

Pilate *et al*. (2002) Nature Biotechnology 20, 607-612.

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, The EFSA Journal 48, 1-18.

BIJLAGE 8

Summary Notification Information Format

(as amended by the notifier in April 2008)

A. General information

A1. Details of notification

Notification Number
B/BE/07/V2

Member State
Belgium

Date of Acknowledgement
30 November 2007

Title of the Project
Field evaluation of poplars with an altered wood composition for the production of bio-ethanol

Proposed period of release:
01/05/2008 to 31/12/2014

A2. Notifier

Name of the Institute(s) or Company(ies)
VIB

A3. Is the same GMPt release planned elsewhere in the Community?

The same (transgenic event WT/52-3) and similar plants have been introduced in France by INRA, see SNIF B/FR/07/06/01 and SNIF B/FR/99/02/15. Transgenic line WT/52-40 will be introduced into the environment for the first time and will not be released elsewhere.

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*
(c) Species:	*Populus x canescens (Populus alba x Populus tremula)*
(d) Subspecies:	-
(e) Cultivar / breeding line:	717-1B4
(f) Common name:	Grey poplar

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications

The genetically modified poplars exhibit modified lignin (a major constituent of wood) due to the decreased activity of an enzyme of the lignin biosynthetic pathway. Depending on the transgenic line, the altered enzyme is:
- CCR (Cinnamoyl coenzymeA reductase): 2 transgenic lines WT52-3, and WT52-40.
The down-regulation has been obtained by co-suppression (WT52-3, WT/52-40). The enzyme residual activity varies between 3 to 100 % and is not necessarily uniform within the plant. Consequently, the quality or/and quantity of lignin is modified. These modifications and the consequences on some wood properties have been described in several publications (Baucher et al., 1996, van Doorsselaere et al., 1995 ; Meyermans et al., 2000 ; Lapierre et al., 1999 ; Pilate et al., 2002 ; Lapierre et al., 2004).
In addition, all transgenic lines have also integrated a selection gene (hpt) that confers an antibiotic resistance. This antibiotic resistance has been used during in vitro culture steps to select for genetically modified cells.

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 inserted genetic material is the T-DNA from the Ti plasmid of Agrobacterium tumefaciens harbouring the gene of interest (for lignin modification) and the gene for selection (antibiotic resistance). The gene of interest is one among two poplar genes coding for one among two enzymes of the monolignol biosynthetic pathway. Monolignols are the elementary units of the lignin polymer. The coding sequence of any of these 2 genes is inserted in sense or antisense orientation between i) the promoter of the cauliflower mosaic virus (CaMV) in a duplicated version (p70) and ii) a terminator sequence, either from the T7 gene from the T-DNA (pAg7) or from the gene coding for the CaMV 35S RNA (pA35S). The antisense insertion aims to turn off the expression of the corresponding endogenous gene: The mRNA of the antisense gene interferes with the corresponding endogenous mRNA that results in a strong reduction in the production of the endogenous protein. A sense insertion leads in a few transgenic lines (this is the case for the sense transgenic lines included in this application) to a similar effect, i.e. a reduction in the activity of the target enzyme, through another mechanism named co-suppression.
The two poplar genes listed below derive from cDNA sequences isolated from a xylem cDNA library from the Populus trichocarpa “Trichobel“ clone (for CCR cDNA).

i) CCR (Cinnamoyl coenzymeA reductase): the full-length cDNA coding for CCR (accession AJ224986 ; Leplé et al., 1998) inserted in sense orientation. The corresponding chimeric gene (p70-S-CCR-pA35S) once introduced in the pBIBHygro binary vector generates the pBIBHygro/S-CCR pBIBHygro transformation vector.

For the p70-S-CCR-pA35S, the selection gene is the hygromycine B phosphotransferase (hpt (or hph) gene fused to the promoter of the nopaline synthase gene (pNOS) from Tn7 and to the terminator of the gene 7 from the T-DNA (pAg7).

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 for the content and/or quality of lignin. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. These different transgenic lines of poplars have been already evaluated in a previous field trial in France, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purpose to produce enough wood from lignin modified poplars in order to evaluate its properties for bio-energy production, in particular bio-ethanol. Both lignin/cellulose ratio and the accessibility to cellulose are critical for the production of bioethanol from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture on a low-grade soil (marginal land) 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 the start-up of a bioprocess pilot plant for bio-energy production. The release can also be seen as a partial repetition of the trial B/FR/07/06/01 (the current release only involves 2 lines, where the FR trial includes more lines) at INRA-Orleans in France, providing additional scientific value to the outcomes of this trial and vice-versa.

C2. Geographical location of the site
The University of Ghent Science and Industry park in Zwijnaarde, Belgium.

C3. Size of the site (m2)
The site is in total 6500 m2 of which a maximum of 2400 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
There has been one previous release involving one (the line WT/52-3) of the two CCR lignin modified poplars (notification number B/FR/99.02.15).

During this previous field trial, no significant differences between GM and wild type poplars with regards to reproductive aspects were observed. Lignin modified poplar flowering time and intensity did not appear affected by the genetic modifications.
However, lignin is involved in major biological functions for tree growth and development such as mechanical support, water conduction and pathogen defense. Field trials with lignin modified trees over more than 12 years have shown that important lignin modifications are very rapidly translated into changes in the function of conduction and/or support. It has also come out that some lines that were shown to grow normally in the greenhouse (i.e. in optimal growth conditions), where unable to do so in a nursery. Some transgenic lines were even unable to survive. Apparently there has to be a balance between the lignin modification that can be of interest for certain applications on the one hand and the impact of the modification on tree growth and development on the other hand. The lines in this application have been shown to grow almost normal and have been shown to release up to twice the amount of glucose in biochemical breakdown experiments, when compared to conventional poplars.
The experiences with lignin modified poplar appear to suggest that lignin modified poplars will have a fitness that is less or at the maximum comparable to their wild type counterparts.

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

Note especially if the introduced traits could directly or indirectly confer an increased selective advantage in natural environments; also explain any significant expected environmental benefits

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 at all, not even under optimal conditions using rooting powder, and in nature the environmental circumstances necessary for shooting are seldomly met (bare soil, no or very limited competition, plenty but no too much humidity, and enough but not too much sunlight). 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 leat-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.

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 are not expected to 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

In this field trial there will be data collection concerning the presence/absense of flowering on the short rotation grown poplar, and there will be data collection on the growth characteristics and morphology of the transgenic lines grown in short rotation culture when compared to wild type short rotation trees. In the similar field trial B/FR/07/06/01 there will be data collection on the effects on biodiversity.

G. Final report

-

H. European Commission administrative information

I. Consent given by the Competent Authority:

Not known