CBD

UNEP

Convention on
Biological Diversity

Distr.
GENERAL

UNEP/CBD/BS/AHTEG-RA&RM/4/4
25 April 2012

ENGLISH ONLY

AD HOC TECHNICAL EXPERT GROUP ON RISK
ASSESSMENT AND RISK MANAGEMENT UNDER
THE CARTAGENA PROTOCOL ON BIOSAFETY
Fourth meeting
Montreal, 4 - 8 June 2011

RISK ASSESSMENT OF LIVING MODIFIED TREES

Version of 2 April 2012

The considerations in this guidance complement the Roadmap for Risk Assessment of Living Modified Organisms and aim at providing additional guidance on the risk assessment of LM trees in accordance with Annex III to the Cartagena Protocol on Biosafety.

BACKGROUND

Forest biodiversity is one of the seven thematic programmes of work under the Convention on Biological Diversity (CBD). During its eighth and ninth meetings, the Conference of the Parties to the CBD recognized "the uncertainties related to the potential environmental and socio-economic impacts, including long-term and transboundary impacts, of genetically modified trees on global forest biological diversity", recommended "Parties to take a precautionary approach when addressing the issue of genetically modified trees" and urged Parties to undertake a number of actions with regard to LM trees, such as "to develop risk-assessment criteria specifically for genetically modified trees".[1]

Given the above decisions and the mandate by the Parties to the Protocol to develop "further guidance on new specific topics of risk assessment, selected on the basis of the priorities and needs by the Parties and taking into account the topics identified in the previous intersessional period",[2] and on the basis of a priority-setting exercise conducted in the Open-ended Online Expert Forum on Risk Assessment and Risk Management,[3] the AHTEG agreed to develop additional guidance on risk assessment of LM trees introduced into the environment.

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[1] See COP decisions VIII/19 paragraphs 2 and 3 (http://www.cbd.int/decision/cop/?id=11033) and IX/5 paragraphs 1(s)-(z) (http://www.cbd.int/decision/cop/?id=11648).

[2] See COP/MOP decision V/12, Annex 3(c).

[3] See http://bch.cbd.int/onlineconferences/forum_ra.shtml.

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INTRODUCTION

Tree species belong to many different taxonomic orders and families of angiosperms (flowering plants; e.g. mahogany, poplar, apple) and gymnosperms (“naked seed” plants; e.g. pine, spruce, cedar). Trees differ from annual crop plants by characteristics such as size, perennial growth habit with a long lifespan, and delayed onset of reproductive maturity.

High fecundity together with seed dormancy, many pathways for dispersal of propagules, and high seed viability are important aspects for the reproductive capacity of many, although not all, tree species.

Because of their perennial growth and long lifespan, trees may develop more complex and multi-level ecological interactions with other organisms as compared to annual crop plants. These interactions can involve, either directly or indirectly, organisms ranging from decomposers to birds, from insect pollinators to large wild animals. The root systems of trees are extensive and usually associated with microorganisms and fungi, such as mycorrhizae (symbiotic associations).

Concerning reproductive maturity and breeding systems, many tree species undergo a distinct juvenile phase which may last for several years to more than a decade before the onset of reproductive maturity. As a result, some commercialized tree species have gone through only a limited number of breeding cycles. Additionally, some trees species are dioecious (i.e. plants that are either male or female) and cannot undergo selfing (i.e. common practice for increasing homogeneity of many crops), leading to greater use of methods for vegetative propagation to ensure uniformity of the propagated trees. By using cuttings from some tree species, in particular some fruit trees, grafting of a desirable selected genotype onto a rootstock of a different genotype may be done. For many forest and fruit tree species, clonal multiplication of identical individuals can be achieved through regeneration of entire trees from vegetative propagules such as cuttings or somatic embryos.

Tree species and genotypes are highly diverse and exhibit a wide range of distribution and complex associations with other organisms, as well as significant ecological, economic, environmental, climatic and socio-economic values. Fruit, ornamental, and forest tree species of economic interest grow in various regions of the world from temperate to tropical climates. Thirty one per cent of the total global land area or more than 4 billion ha are covered by forests. Minimally managed forest habitats and non-managed forests like tropical rainforests or boreal forests in the northern hemisphere are of high conservation value. Accordingly they represent important protection goals which should be taken into account when assessing the possible adverse effects of LM trees and emphasis should be given to the precautionary approach.

Fruit and forest trees, especially those suited for plantations, are the focus of advanced breeding strategies including genetic modification through modern biotechnology as defined by the Cartagena Protocol on Biosafety. Currently about 30 to 40 different tree species have been modified through modern biotechnology through the insertion of transgenes, and have been introduced into the environment for small scale releases (FAO 2005, Verwer et al. 2010, IUFRO 2011[4]). The majority of these LM trees are species of economic interest used in managed orchards, forests and plantations. The genetic modification has focused on traits related to herbicide tolerance, wood composition (e.g. lignin), growth rates and phenology (including flowering and fruiting), resistance to pests and diseases, or abiotic stress tolerance. By far, poplars make up most of the LM trees that were developed and subjected to field trials to date (Canada Norway Workshop 2007), followed by eucalypts, pines and spruce. LM apples, plum and papaya make up most of the fruit trees approved for field trials (Gessler & Patocchi, 2007; Hanke & Flachowski 2010). Both papaya and plum have been approved for commercial cultivation in the USA[5], papaya also in one province in China (James 2011). Poplars are the only transgenic forest trees planted for commercial

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[4] IUFRO Tree Biotechnology 2011 - http://www.treebiotech2011.com/. Full proceedings available at http://www.biomedcentral.com/1753-6561/5?issue=S7.

[5] See http://www.isb.vt.edu/search-petition-data.aspx.

cultivation, although, to date, only on small scale in China (Ewald et al. 2006). Examples of risk assessments in LM trees or other woody perennials including small and large scale experimental releases are available on-line from a number of sources (Australia, New Zealand and the USA, EU, Canada).[6] Other countries have approved field trials, including Brazil, China, Malaysia, Mexico and Japan, but only limited information is available.

The OECD Working Group on Harmonization of Regulatory Oversight has published consensus documents on the biology of many tree species of economic interest that have been modified through modern biotechnology.[7]

SCOPE OF THIS GUIDANCE

According to the Food and Agriculture Organisation of the United Nations (FAO), a tree is: "a woody perennial with a single main stem, or, in the case of coppice, with several stems, having a more or less definite crown".[8] This guidance focuses on true botanical trees and does not cover any additional species such as palms, bamboos and shrubs[9]. Although not addressed specifically in this guidance, where some of the characteristics of trees are shared by other plant species, such as perennial growth or vegetative propagation, this guidance may provide some insights useful for the evaluation of LMOs of those species.

OVERARCHING ISSUES IN THE RISK ASSESSMENT PROCESS (*see "Overarching issues in the risk assessment process" in the Roadmap)*

Transboundary movements of LM trees and the Cartagena Protocol

According to the Protocol, risks associated with LMOs or products thereof[10] should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment. Therefore, when characterizing the likely potential receiving environment of an LM tree as part of its risk assessment, not only the movement of seeds for intentional introduction into the environment should be taken into account, but also the movement of vegetative propagules since for many tree species (e.g. some poplars and eucalypts) this is the most common way of propagating them. Issues related to unintentional transboundary movements may also be taken into account in cases where LM trees could cross national boundaries through, for example, pollen or seed dispersal by physical and biological vectors, including the international trade of fruits with seeds.

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[6] Australia: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1 (papaya, plus sugarcane, rose and banana). New Zealand: http://www.epa.govt.nz/new-organisms/Pages/default.aspx (Radiata pine). USA: Commercial releases - http://www1.usgs.gov/usbiotechreg/ (papaya (2), plum); field trials - http://www.isb.vt.edu/search-release-data.aspx (Eucalyptus, poplar, apple, sugarcane, sweetgum, cranberry, poplar/white spruce, plum, papaya, Amelanchier laevis, walnut). EU: http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx. Canada: http://www.inspection.gc.ca/english/plaveg/bio/dt/term/2010/2010e.shtml.

[7] Up to now for 13 tree species consensus documents on their biology have been developed to support an environmental risk assessment. These documents can be found at http://www.oecd.org/document/15/0,3746,en_2649_34385_37336335_1_1_1_1,00.html.

[8] http://www.fao.forestry/site/24690/en.

[9] Some experts representing the Parties to the Protocol in the Open-ended Online Forum and AHTEG are of the view that fruit trees should not be included in this guidance.

[10] ..."namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology" (see Protocol, Annex III, paragraph 5).

PLANNING PHASE OF A RISK ASSESSMENT OF TRANSGENIC TREES

The comparative approach *(see "Planning Phase of the Risk Assessment", "The choice of comparators" in the Roadmap)*

Rationale

As with the risk assessments of any other type of LMO, a comprehensive planning phase is needed in order to define, among other things, how a comparative approach can be carried out in the risk assessment of an LM tree.

For both annual and perennial plants the characteristics of the receiving environment should be considered in the comparative approach as it often changes over time, including interactions and interactive networks with other organisms as well as biotic and abiotic conditions. Annual plants, which germinate and re-establish each year, are likely to be more sensitive to such variations than perennial plants, including trees Indeed, to survive for many years, trees must be robust to a wide range of environmental factors, including those resulting from human activities (Roloff 2004).

In all forms of forestry, the use of well adapted provenances (i.e. trees that have evolved or been bred within the region where they will be grown commercially[11]) is of great importance because they may show better adaptive capabilities and consequently better performance than unselected germplasm (Hubert & Cundall 2006).[12] These regional provenances and their management, whether part of the local flora, domesticated species or introduced but bred and adapted varieties, may provide appropriate comparators for LM trees in accordance with national protection goals and good forest management practices.

For those tree species for which there is little or no information the comparative approach may be challenging. In such situations the use of closely related lines may provide a good alternative for the comparative risk assessment.

Due to the large physical size of trees only limited data may be available from glasshouse experiments. Not only can glasshouses be limiting with respect to the height of the tree, but the area or footprint required for each individual tree can quickly fill the available space thus limiting the practicality of replicated samples. This may be particularly challenging when obtaining data over a number of growing seasons to address the perennial growth nature of trees.

In instances where LM tree species have a long lifespan and a high potential for dispersal, outcrossing and establishment beyond the intended receiving environment (e.g. into natural or less managed ecosystems) should be taken into account when considering any limitations in the predictive power of the comparative environmental risk assessment.

Points to consider

(a) Availability of information and knowledge of the biology of the species and/or genotype (including regional provenances or ecotypes as appropriate) to be used as a comparator;
(b) Whether one or more suitable comparators are available and the possibility of their use in the appropriate experimental design;

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[11] A comparable concept for crop plants would be regionally adapted crop varieties.

[12] For example the Ministerial Conference on the Protection of Forests in Europe recommended "Native species and local provenances should be preferred where appropriate. The use of species, provenances, varieties or ecotypes outside their natural range should be discouraged where their introduction would endanger important/valuable indigenous ecosystems, flora and fauna".

(c) Design of field trials in relation to established methodologies for the non-modified trees, including for example the length of the period before flowering, the length/age of trials, testing in different environments and exposure to multiple biotic and abiotic stresses.

(d) Availability of data from glasshouse experimentation (including exposure to abiotic and biotic stresses);

CONDUCTING THE RISK ASSESSMENT

The information provided in this section aims at covering different tree species and management practices and may be taken into account on a case-by-case basis.

Transformation and propagation methods *(see "Step 1", "Point to consider (b)" in the Roadmap)*

Rationale

The cross-breeding process of LMOs (including back-crossing) may be an option to reduce, if appropriate, the presence of marker genes and other genetic elements (e.g. antibiotic resistance genes) that are not desirable.[13] Crossing may not be a viable option for many species of LM trees which have a long juvenile period. Consequently the multiplication of trees is likely to be done through clonal and vegetative propagation, which does not allow for simple removal of such undesirable genetic elements.

In many cases human intervention is required for successful vegetative propagation:

i. Rooted cuttings. In some tree species mass propagation of selected genotypes is accomplished through the preparation of rooted cuttings from stocks maintained as hedges or in tissue culture.

ii. Grafting. Notably in fruit trees a selected variety with desirable traits can be propagated by grafting material, the scion, on to rootstocks of a different genotype. In such cases the scion, the rootstock, or both may be transgenic.

iii. Somatic embryogenesis. In several conifers and other species methods have been developed to mass produce selected genotypes in tissue culture through somatic embryogenesis.

Points to consider

(a) Transformation methods used which may possibly lead to the presence of vector fragments or marker genes;

(b) Propagation method(s) used – cross-breeding (including degree of back-crossing if possible in that species) and/or vegetative propagation;

Long life spans, genetic and phenotypic characterisation and stability of the modified genetic elements *(see "Step 1", "Point to consider (d) and (e)" in the Roadmap)*

Rationale

For tree species, lifespan can range from several decades to several hundred years or longer (Matyssek et al. 2010, Roloff 2004) and therefore they may need an extended time period of observation[14.] They have the capability (like other perennial plants) to adapt to the different abiotic and biotic conditions they encounter during their lives. Phenotypic characterization during risk assessment should consider the developmental stage of the LM trees and environmental conditions, as well as, to the extent possible,

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[13] See Roadmap *"Step 1", "Point to consider (b)".*

[14] See Article 16, paragraph 4 of the Cartagena Protocol.

anticipate whether and how management practices would change over time and if the changes would affect the characterization.

In consideration of the long lifespan of trees, transgene instability including those causing gene silencing and variable expression levels should be considered (Ahuja 2009; Harfouche et al. 2011). On the same basis, gene/environment interactions, that may play a role in the expression level of the transgenes (Strauss et al. 2004), should be duly considered. Consequently, an assessment of the stability of the transgenes and their levels of expression at different points during the lifespan of the LM tree may be important considerations, in particular where transgenic approaches are used for containment strategies (e.g. male sterility or ablation of floral organs).

Confirming the stability of the modified genetic elements over successive generations may be an important issue in the risk assessment, *inter alia*, when bio-containment strategies will be used in the risk management (e.g. see the section on Risk Assessment of LM Mosquitoes). However, verification of stability of the modified elements through successive crosses may not be feasible for vegetatively propagated species (see above).

Points to consider

(a) Changes in the interaction with other organisms, and changes in the ability to maintain role and function in ecosystems;
(b) Phenotypic changes over time in response to different stressors and different developmental stages;
(c) Potential for variability of transgene expression levels, including gene silencing over time.

Dispersal mechanisms *(see “Step 1”, and “Step 2”, “Point to consider (e) and (f)” in the Roadmap)*

Rationale

Trees, like other plants, have developed a variety of ways to reproduce and disseminate via seeds, pollen and/or vegetative propagules. Trees often produce large amounts of pollen and seed per individual and propagules may be designed to spread over long distances (e.g. by wind, water, or animals including insects) (e.g. Williams 2010). The potential for vegetative propagation in certain trees raises consideration of the possibility of establishing new individuals from branches or root parts. Seeds inside fruits may travel as commodities around the globe and be released at the place of consumption such as road margins, railways or touristic areas, as well as in farmers’ fields and local gardens.

Points to consider

(a) Available information on the mechanisms and viability of pollen and seed dispersal for the non-LMO and LM tree species;
(b) Potential for and mechanisms of vegetative propagation in the non-LMO and LM species;
(c) Climatic conditions, or management practices that affect reproductive biology.
(d) Potential for dispersal mechanisms from anthropogenic activities (e.g. trade and consumption of fruits).

The likely potential receiving environment(s) *(see "Step 1", "Points to consider (f) and (g)"," Step 2","Points to consider (b), (d) (f) and (g) and (h)"," Step 3","Points to consider (a) and (e) in the Roadmap)*

Rationale

The identification and characterisation of likely potential receiving environment(s) may be dependent on the species in question, their habitats, the traits and modified characteristics and its mechanisms for dispersal. With some trees the intensity of management in the receiving environment is likely to be less than for annual plants. Given that the domestication level of some forest trees may be low and trees can often survive without human intervention, the dispersal of propagative material (e.g. seeds, branches) may lead to persistence and spread of the LM tree in question. Therefore, the potential for dispersal of propagative material into environments other than the intended receiving environment is an important consideration during the risk assessment.

Points to consider

(a) Environments which offer the potential for seeds and/or vegetative propagules to establish;
(b) Degree of management of these environments;
(c) Presence and proximity of species including in orchards and gardens in the receiving environment with which the LM tree may hybridize;
(d) Occurrence of protected areas according to national legislation, centres of origin and genetic diversity or ecologically sensitive regions nearby
(e) Water tables and water sheds in or linked to the potential receiving environment and the potential impact of the LM tree compared to that of non-LM comparators;
(f) Changes in landscape patterns (e.g. because of new plantations or aforestation);
(g) Ecosystem functions and services of the potential receiving environment;
(h) Relevant components of food webs and multi-trophic effects;
(i) Sensitivity of the receiving environment to human changes (e.g. climate changes)]

Exposure of the ecosystem to LM trees and potential consequences *(see "Step 2", "Points to consider (e) to (h)", and "Step 3" in the Roadmap)*

Rationale

As trees may be relatively undisturbed for much of their life cycle they may engage in a variety of ecological interactions, such as providing habitat for other organisms and functioning as part of complex and elaborate food webs. In determining the likelihood of an adverse effect to occur, an assessment of the exposure to the LM tree should take into account the expected duration of the trees' presence in the receiving environment together with the transgenic traits and the intended use (e.g. processing, trade routes) as well as dispersal mechanisms. Given the late onset of reproductive maturity of a number of tree species, pollen and seed production may not be available for the duration of field trials.

A number of species (including some trees) under exploration as bioenergy crops have the potential of becoming invasive[15] (Gordon et al. 2011) which could greatly increase the exposure of the environment to these trees. Genetic modification has been proposed as a strategy to mitigate the potential invasiveness of new bioenergy crops (Kausch et al, 2010).

Points to consider

(a) Duration of the presence of the LMO trees in the receiving environment and their impact;
(b) Persistence and long-term effect of the LM trees in the environment including potential for the non-LMO and LM species to be invasive;
(c) Possible impacts from the modified trait on invasive characteristics;
(d) Long-term interactions with other organisms including in the food webs.
(e) Possible impacts of the modified trait on loss of biodiversity and ecosystem stability.

Risk management strategies *(see "Step 4", "Point to consider (d)" and "Step 5" in the Roadmap)*

Rationale

Risk management strategies designed for LM trees will depend on the result of the risk assessment, and may vary depending on the LM tree and conditions under which it is grown. When the recommendations of the risk assessment include measures for limiting or preventing dispersal of forest or plantation LM trees, strategies that may be used include delaying or avoiding flowering (e.g. fast-growing trees for pulp or biomass/bioenergy production being cut before reaching the reproductive phase) and bioconfinement (e.g. induction of male sterility or flower ablation). While complete flower ablation would not be desirable for many fruit or horticultural tree species, male sterility may be appropriate in some species (e.g. apples) where pollen from a different variety (which could be non-LMO) is usually required. It is noted, however, that male sterility approaches would have no effect on potential dispersal by seed. Where applications involve genetic modification of only the rootstock in grafted trees, dispersal may be managed by ensuring that the rootstocks do not produce shoots or flowers (Stegemann and Bock 2009).

Points to consider

(a) Results of the risk assessment;
(b) Type and intended use of the LM tree;
(c) Degree and type of management (e.g. grafting of fruit trees, rotation period of forest trees);
(d) Specific effects and risks of any containment strategy achieved through the use of modern biotechnology.

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[15] The Convention on Biological Diversity (CBD) defines the term "invasive alien species" as "species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity." Since this document is not limited to consideration of alien species, the following definition is being used for "invasive species": "a species whose introduction and/or spread threaten biological diversity." This definition was chosen in consideration that the Cartagena Protocol on Biosafety is a protocol to the CBD. [This footnote may be supplemented according to the text referenced in: (i) Committee on the Biological Confinement of Genetically Engineered Organisms, N. R. C. (2004): Biological Confinement of Genetically Engineered Organisms (Washington, D.C., National Academies Press) and (ii) Committee on Environmental Effects of Transgenic Plants, N. R. C. (2002): Environmental effects of transgenic plants: the scope and adequacy of regulation (Washington, D.C., National Academies Press).]

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