**RISK ASSESSMENT OF LIVING MODIFIED TREES**

This guidance complements the Roadmap for Risk Assessment of LMOs giving emphasis to issues that are of particular relevance to the risk assessment of LM trees. As such, risk assessments of this type of LM plants also follow the general principles outlined in the Roadmap, but take into account the specific characteristics of perennial long-living trees outlined in this section of the present document.

**Background**

Forest biodiversity is a core area of work in the Convention on Biological Diversity. During its 8th and 9th session the Conference of the Parties dealt especially with transgenic trees.

In decision UNEP/CBD/COP/DEC/VIII/19 under “B. Other Matters” the COP laid down:

“*Recognizing* 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, as well as on the livelihoods of indigenous and local communities, and given the absence of reliable data and of capacity in some countries to undertake risk assessments and to evaluate those potential impacts, (…) *recommends* Parties to take a precautionary approach when addressing the issue of genetically modified trees”.

Two years later in decision UNEP/CBD/COP/DEC/IX/5 it was recommended to:

(s) “Authorize the release of genetically modified trees only after completion of studies in containment, including in greenhouse and confined field trials, in accordance with national legislation where existent, addressing long–term effects as well as thorough, comprehensive, science-based and transparent risk assessments to avoid possible negative environmental impacts on forest biological diversity; 2/

(t) Also consider the potential socio-economic impacts of genetically modified trees as well as their potential impact on the livelihoods of indigenous and local communities;

(u) Acknowledge the entitlement of Parties, in accordance with their domestic legislation, to suspend the release of genetically modified trees, in particular where risk assessment so advises or where adequate capacities to undertake such assessment is not available;

(v) Further engage to develop risk-assessment criteria specifically for genetically modified trees;

(w) Note the results of the Norway – Canada Workshops on Risk Assessment for emerging applications for Living Modified Organisms (UNEP/CBD/BS/COP-MOP/4/INF/13);

(x) Welcome the decision of the fourth meeting of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol to establish an Ad Hoc Technical Expert Group on Risk Assessment and Risk Management that is also mandated to address the issue of genetically modified trees;

(y) Collaborate with relevant organizations on guidance for risk assessment of genetically modified trees and guidance addressing potential negative and positive environmental and socio - economic impacts on the conservation and sustainable use of forest biodiversity associated with the use of genetically modified trees;

(z) Provide the available information and the scientific evidence regarding the overall effects of genetically modified trees on the conservation and sustainable use of biological diversity to the Executive Secretary for dissemination through the clearing-house mechanism;”

Given these recommendations and mandate and taking into account the topics identified in the previous intersessional periods as well as the outcome of the priority-setting exercise conducted in the Open-ended Online forum preceding the 3rd face-to-face meeting the AHTEG agreed to develop additional guidance for the environmental risk assessment of transgenic trees thus fulfilling the recommendations especially raised by COP IX.

**Introduction**

Trees and forests and their vast diversity in distribution, organismic networks, species and genotypes have significant ecological, economic, environmental, climatic and socio-economic values: forests and fruit trees/orchards provide important food supplies (for humans and animals); wood is an important raw material for building and construction, the pulp and paper industry, and energy production (incl. fire wood); sequestration of atmospheric carbon is an important function in mitigating climate change, air filtration, water and soil conservation as well as their role in local climate (micro climate), cloud formation and rain fall patterns (due to tree/forest transpiration) are important ecosystem functions and services. In addition forests are of high value for recreation and tourism and have cultural and spiritual significance.

31% of the total global land area or more than 4 billion ha are covered by forests. 1,2 billion of these are used for production of wood and non wood products. Additional 949 million ha are dedicated to multiple uses including soil and water conservation. Managed forests here also including commercial plantations are increasing and now comprise around 7% of the total forested area.. Accordingly forests trees especially those suited for plantations are in the focus of advanced breeding strategies including genetic engineering. (FAO 2010),

Fruit trees and forest tree species of economic interest grow in all different regions of the world from temperate to tropical climates. Usually tropical forests show higher species richness combined with a higher biomass production (per year). According to ecological theory the high biomass production is combined with nutrient poor soils (because of the high turnover which do not allow accumulating humus and nutrients)– meaning that there is a much higher sensitivity to disturbances and biotic or abiotic stress in tropical forests (Begon et al. 2006)

*The definition of a tree*

According to 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”. For FAO bamboos, palms and other woody plants if meeting the above criteria are included into the definition of a tree (FAO 2005). [[1]](#footnote-1) Tree plants occur in many different orders and families of plants. Most species of trees today are flowering plants belonging to the Angiosperms and Gymnosperms.

*Short description what trees are engineered for*

Currently about 30 to 40 different tree species have been engineered to deliver changed characteristics due to inserted transgenes and have been field trialed (FAO 2004, Verwer et al. 2010). In the majority of cases these efforts are directed at the most commonly planted, commercial tree species The focus of forest or plantation tree genetic modification includes herbicide resistence, wood composition (eg lignin), growth rates and phenology (including flowering and fruiting) (Verwer et al. 2010), insect/pest tolerance, or abiotic stress tolerance whereas key aspects with fruit trees are resistence to pathogens and abiotic stress together with phenology. By far the most transformations and trials have been done with poplars (Canada Norway Workshop 2007), followed by eucalyptus and pine. Among fruit trees apples and papaya have received the most apprehension and field trial approvals (Gessler&Patocchi, 2007; Hanke & Flachowski 2010). Poplar is the only transgenic forest tree species planted on a commercial scale (in China, Ewald et al. 2006).) Two different types of fruit trees have been approved for commercialization (in the United States, virus resistant papaya (two different lines) and virus resistant plum, http://www.isb.vt.edu/search-petition-data.aspx).

*Uniqueness of trees*

Trees show unique characteristics compared to annual crop plants. They have a long lifespan and exhibit unique reproductive abilities. Together with long generation cycles and a late onset of building reproductive organs their vegetative phases where only vegatative propagation is possible may extend from one to several decades. Forest tree species like lime trees can live for several hundred years. For some living trees even an age of more than 4950 years is proven (Matyssek et al. 2010). High fecundity (reproduction capacity) together with seed dormancy, multiple and very effective distribution pathways of propagules, extended possibilities of vegetative reproduction and high viability are important aspects of their worldwide adaptive capacities and distribution. Forest trees are valued for their large biomass production and contribution to ecological and landscape architecture. Root systems are extensive and are inextricably enmeshed with mycorrhiza, symbiotic associations with fungi. In addition (forest) trees are involved in broad interactions with further organisms from decomposers to birds and wildlife. Given all these aspects quite often trees are described as constituting an ecosystem in themselves.

Breeding and cultivation of forest trees is a quite novel approach of resource utilisation (Campbell et al. 2003). In Europe, tree propagation and forest management commenced in the Middle Ages, but only since the 19th century, forest trees are being systematically adapted to the needs of wood production (Mathews & Campbell 2000). For this reason, even in commonly grown species, the level of domestication is still low.

The above description focuses on forest trees but fruit trees share quite a number of the mentioned characteristics with forest trees. Differences are found especially in the scale of domestication with forest trees being mainly undomesticated and, in the possible distribution pathways of seeds. Given the global production and trading with fruits a restriction of seed distribution via fruit consumption seems to be difficult except for some seedless cultivars of eg. lemon or orange trees

**scope of this Guidance**

This guidance focuses on perennial woody plants encompassing forest/plantation trees, and fruit trees used in transgenic approaches (see tree definition above).

The family of *Palmae* or *Aracaceae* (palm trees) and the herbaceous flowering banana plants as not being trees, and bamboos belonging to the family of true grasses (*Poaceae)* are not covered by this guidance.

Also not covered are so-called heritage trees, transgenic trees developed for the conservation or restoration of threatened forest trees. The application of transgenic technology to restore heritage trees aims for spread of the transgenes into endangered wild relatives (Merkle et al. 2007). Consequently a risk assessment has to focus on a number of different questions regarding exposure, spread and possible impact needing a seperate approach.

**Overarching issues in the risk assessment process**

**(***see “Overarching issues in the risk assessment process”**in**the Road Map)*

*Intentional and unintentional transboundary movement with reference to AIA requirements*

Trees display a special challenge in the context of the provisions of the Biosafety Protocol and the recommendations regarding conservation and development of forest biodiversity in the Convention on Biological Diversity setting the framework for the Protocol. According to the Protocol, the definition of a living organism in Art. 3 (h) refers to the capability of the biological entity to transfer or reproduce genetic material. Besides seeds the ability to sprout makes a tree capable to reproduce its genetic material under certain conditions. Thus all GM wood parts still capable of vegetative reproduction should be seen as living organisms. In this context there may be some overlap in the relation between "intentional transboundary movement for intentional introduction into the environment" (Art 7) as well as "unintentional transboundary movements" (Art. 17), e.g. in cases of LM tree releases on locations close to national borders as well as in cases of commercial approvals of LM trees. As a result, those cases of commercial and experimental release approvals by a Party should trigger the examination if the provisions of Art. 7 and/or Art.17 apply.

**Planning Phase of a Risk Assessment of transgenic trees**

**The Comparative approach - aspects of implementation**

Rationale *(see “Planning Phase of the Risk Assessment”, “The choice of comparators” in the Roadmap)*

Trees as long-living species with unique adaptive and reproductive capacities and low degrees of domestication do not easily fit into an assessment based on a comparative approach developed for annual crops with high degree of domestication. The extensive network of interactions with other organisms changing over the lifetime of the tree and the adaptive capabilities regarding abiotic and biotic impacts and stresses present great challenges.

In sustainable forestry the use of regional provenances is regarded as being of special importance because of higher plant vigour, better adaptive capabilites and consequently better performances (Hubert & Cundall 2006). 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……”[[2]](#footnote-2) .

Thus a comprehensive planning phase is needed to define what are suitable comparisons. However, given the long lifespan of trees and their capacity for spatial spread into natural ecosystems, the always limited predictive power of any environmental risk assessment should be taken into account.

*Points to consider*

1. Needed body of data of glasshouse experimentation as a basis for assessment and a prerequisite to field trials;
2. field trial design and length;
3. choice of regional provenances or ecotypes inside their natural range as conventional counterparts;
4. Recommendations regarding exposure to multiple stresses (biotic and abiotic) and over time to detect unexpected effects that are either delayed or stress depended/specific.

**CONDUCTING The risk assessment**

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

*Rationale*

To accelerate breeding and multiplication approaches in trees clonal and vegetative propagation has been developed including tissue culture and micropropagation (Giri et al. 2004). Embryogenic tissues turned out to be the most promising in regenerating plantlets (Frankenhuyzen & Beardmore 2004). Somaclonal variation in propagative and clonal tissue culture is very common (Rani & Raina 2000) Consequently clonal fidelity is an important consideration in micropropagation of trees. Transformation protocols are mainly based on Agrobacterium tumefaciens and to a lesser extent on ballistic methods (Frankenhuyzen & Beardmore 2004, Harfouche et al. 2011). These, as well as transformation related tissue culture, are sources for the introduction of mutational variation. Back-crossing has been suggested to reduce the presence of transformation induced mutations, which is a greater challenge for trees than for annual crop plants (Flachowsky et al. 2009).

*Points to consider*

1. Propagation method used;
2. Transformation methods;
3. Degree or number of back-crossing.

**Genetic and phenotypic characterisation and stability** *(see “Step 1”, “Point to consider (d) and (e)” in the Roadmap)*

*Rationale*

During a trees lifespan these organisms experience multiple abiotic and biotic impacts and environmental changes with plasticity in genomic and phenotypic reactions considered to play an important role in such adaptive responses. In addition transgene instability including gene silencing and highly variable expression levels during the long lifespan of trees have to be considered. (Ahuja 2009; Harfouche etal. 2011). There is evidence that gene/environment interactions play an important role (Strauss et al. 2004) Consequently stability of transgenes are of concern especially when transgenic approaches are used for containment strategies (eg male sterility or ablation of floral organs).

*Points to consider*

1. Genetic rearrangements or other changes over time;
2. Interaction with genes of the host genome and gene/environment interactions in space and time;
3. Variability of expression levels, including gene silencing over time;
4. Influence on and stability of phenotypic characteristics over time;
5. Altered or unstable ability to respond to biotic and abiotic stresses.
6. Changed interaction with other organisms, and changed or reduced ability to maintain role and function in ecosystem.

**Receiving environment(s)** *(see “Step 1”, “Points to consider (f) and (g)” in the Roadmap)*

*Rationale*

The identification and characterisation of receiving environment(s) may be a difficult task depending on the species in question and the individual case. Given the often low domestication level of LM forest trees and the possible outreach of propagation material into similar and other nearby habitats may offer quite easy possibilies to persist and proliferate.

*Points to consider*

1. nearby landscapes e.g., forests which offer the potential for seeds and/or vegetative propagules to establish
2. Presence and proximity of species in the receiving environment with which the LM tree may hybridize;
3. ;
4. Hybridising species and their spatial distribution in the receiving environment;
5. Degree of management of these forests;
6. Orchards, gardens and fruit production with hybridising cultivars in the receiving environment;
7. Occurrence and frequency of individual organisms or feral groups of trees
8. Occurrence of protected areas nearby;
9. Impacts on water tables and water sheds in or linked to the potential receiving environment;
10. Degree of change in landscape architecture ( eg because of new plantations);
11. Ecosystem function and services of potential receiving environment.

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

*Rationale*

Trees have developed a multitude of ways to reproduce and distribute via seeds and vegatative propagules. Propagules are often designed to spread far and wid, (eg. by wind, but also by water, animals, insects) with large amounts of pollen and seed per individual. (e.g. Williams 2010) Seeds inside fruits may travel as commodities around the globe and be released at the place of consumption being road margins, railway roads or touristic areas, as well as local gardens[[3]](#footnote-3).

*Points to consider*

1. Pollen dispersal:
   * 1. Pollen viability and pollination specifics;
     2. Possible spatial pollen distribution;
     3. Timing of pollen production vs. receptivity of female flowers
     4. Mechanism developed in some species to ensure selfing.
2. Seed dispersal:
3. Seed dormancy and viability;
4. Abiotic distribution (wind, water, floods etc.);
5. Biotic distribution via animals including humans e.g., seed dispersal via commodity fruits;
6. Vegetative dispersal (including via exported or imported wood/branches);

**Exposure** *(see “Step 1”, “Points to consider (e)to (h)” in the Roadmap)*

*Rationale*

Trees as ecosystems in themselves are constituting individuals with complex organismic interactions, provide habitats and are part to complex and elaborate food webs. Exposure assessment for LM trees needs a broadened analysis over time and space taking also into account processing and trade routes. A number of tree species under exploration as transgenic bioenergy plantation trees are assessed to have the potential of becoming invasive (Gordon et al. 2011) possibly extending time and space of exposure. In addition the potential for vegetative propagation is an overall characteristic of the different forest and fruit trees under consideration opening the possibility of branches or root parts with living tissue to establish new plant individuals. Extended dispersal pathways especially with fruits incorporating seeds have to be analysed because of trading as food and feed.[[4]](#footnote-4)

*Points to consider*

1. Persistence (e.g. life span);
2. Potential of the LM tree to become invasive;
3. Interactions/Food webs:
4. with symbiotic microorganisms/mycorrhiza
5. with soil organisms including decomposers and pest organisms;
6. with pest organisms (eg. viruses, bacteria, fungi);
7. with above ground invertebrates (including predators and pests);
8. with birds;
9. with wildlife.
10. With humans (e.g. via pollen inhalation or sawdust)

**Management strategies** *(see “Step 4”, “Point to consider (d)” and “Step 5” in the Roadmap)*

*Rationale*

Manangement strategies for forest or plantation trees encompass transgenic confinement approaches like induction of male sterility or flower ablation or rotation schemes with fast-growing species being cut before reaching the reproductive phase. The management of fruit trees/orchards will be quite different because of different practices of tree propagation and improvement eg via grafting. Whereas the prolonging of the juvenile phase to prevent the onset of flowering may be an aim with plantations trees, early flowering is an important target with fruit trees (Flachowsky et al. 2009) .

*Points to consider*

1. Rotation period;
2. Degree and type of management;
3. Evaluation of management strategies;
4. Monitoring results of field trials.

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1. A similar definition can be found in Henderson´s Dictionary of Biological Terms 1992. A tree is “ a woody perennial plant which has a single main trunk at least 7,5 cm in diameter at 1,3m height, a definitely crown of foliage and a height of at least 4 m. (…) [↑](#footnote-ref-1)
2. Resolution 1 of the Ministerial Conference on the Protection of Forests in Europe, Helsinki 1993 [↑](#footnote-ref-2)
3. . The OECD Working Group on Harmonization of Regulatory Oversight has published consensus documents on the biology of 13 species of trees 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> [↑](#footnote-ref-3)
4. See also the relevant OECD biology documents on trees [↑](#footnote-ref-4)