Field Trials with GM Trees: A Step-by-Step Approach

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Abstract Field trials are an important step in the experimental research with and commercial development of genetically modified (GM) plants, including GM trees. Field trials with GM plants in the European Union (EU) are subject to authorisation granted on the basis of an environmental risk assessment (ERA). Data requirement for the ERA varies depending on the purpose of the field trial and the level of knowledge on the GM plant and its environmental impact. In the Netherlands a step-by-step approach has been developed for the categorisation of field trials. Under this approach the confinement of GM plants in a field trial can be gradually decreased and the scale of the trial increased in a step-wise manner at the same time that knowledge on the GM plant and its environmental interactions increases. Very few other countries seem to apply a similar classification of field trials. We argue that a formal step-by-step approach may be a helpful tool to facilitate the approval process for field trials of GM plants and the collection of relevant data/material for the ERA without compromising the environmental safety, and that this approach is also applicable to field trials with GM trees.

1 Introduction

Genetically modified (GM) plants, including trees, intended for commercial cultivation in the European Union (EU) shall be authorised according to Directive 2001/18/EC (EU 2001) or Regulation (EC) 1829/2003 (EU 2003). The latter Regulation is only applicable in case of plants also intended for food and/or feed

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use. Before an application for commercial growth can be filed, sufficient data should be obtained on the safety of the GM plant itself (e.g. molecular, phenotypic and agronomical characterisation) as well as on its interactions with the receiving environment, both intended and unintended. Data requirements are described in Directive 2001/18/EC and have been complemented by additional Guidance from the European Food Safety Authority (EFSA).

Such data can be obtained from various sources. However, to obtain reliable data on phenotypic and agronomic characteristics and on environmental interactions, field trials are a necessity. Indeed, plant behaviour and the plant's interaction with the environment can be only realistically studied in an open environment including the full set of biotic and abiotic interactions.

Field trials with GM plants can be performed with different objectives in mind: (1) scientific research or demonstration purposes, (2) regulatory purposes, (3) selection of the best lines for future commercial release (product development) or (4) variety registration of selected lines (in the EU).

(1) Field trials for scientific research are often small scale. They are generally meant to study the stability and expression of the trait and the phenotypic characteristics of the plant under field conditions or to demonstrate "proof of principle" of the introduced trait. Such field trials can also be done as part of biosafety research, to generate independent data on the biosafety of GM plants. GM plants cultivated in these field trials will not end up as a commercial product. The same accounts for trials for demonstration purposes. These trials are especially set up for the public, mainly for educational purposes. They are also often small scale.

(2) Trials for regulatory purposes are performed to generate data that are required to complete the environmental risk assessment of GM plants (potential effects on target and non-target organisms, the soil ecosystem, the abiotic environment or human and animal health, see Sect. 3) or the food and feed safety evaluations (compositional analysis, potential toxicity or allergenicity).

(3) Trials for product development are performed by breeders who need to evaluate new germplasm or new crosses for their agronomic performance (efficacy of the modification, yield, etc.). These trials can take many years, during which promising lines are selected and tested on a larger scale to be able to collect enough data on performance and for analyses.

(4) Field trials can also be performed for variety registration on a national or European catalogue and for breeder rights. Variety registration is a precondition for the certification and commercialisation in the European Union of seed of agricultural plant species, vegetable species and fruit trees (for more information, see http://ec.europa.eu/food/plant/plant_propagation_material/plant_variety_catalogues_databases/index_en.htm).

Except for field trials for scientific research or demonstration purposes, data and material to obtain these data are usually collected from trials in multiple locations, representing a range of growing conditions, and over multiple years.

In many cases GM plants that are tested in the field are not fully characterised from a molecular, phenotypic and agronomical viewpoint and/or there is incomplete knowledge on their interactions with the receiving environment. In this chapter we

discuss how these uncertainties could be taken into account during the risk assessment and how field trials with GM plants can be performed in an efficient way to obtain all relevant data/material without compromising the environmental safety. The approach towards field trials with GM plants of several EU member states, Canada and the USA is described and discussed with a special focus on field trials with GM trees.

2 Methodology and Data Requirement for the Environmental Risk Assessment of GM Plants

The purpose of the environmental risk assessment (ERA) is to assess if the introduction of a GM plant into the environment would have adverse effects (direct or indirect, immediate or delayed) on human and animal health and the environment. The ERA of GM plants is a comparative exercise, i.e. it involves generating, collecting and assessing information on a GM plant in order to determine its potential adverse impact relative to its non-GM comparator. The ERA should be carried out in a scientifically sound and transparent manner. It should also be carried out on a case-by-case basis, meaning that the required information may vary depending on the species of GM plants concerned, the introduced genes, their intended use(s) and the potential receiving environment(s), taking into account specific cultivation requirements and the presence of other genetically modified organisms (GMOs) in the environment.

Different national and international legal instruments have been established to regulate the environmental release of GM plants. Most of them follow the same general principles and methodology for the ERA. In the European Union (EU), these principles and methodology are laid down in Directive 2001/18/EC, in particular its Annex II. These principles and methodology are supplemented by guiding instruments, in particular Commission Decision 2002/623/EC (EC 2002) and the Guidance on the ERA of GM plants developed by the European Food Safety Authority (EFSA 2010). Supporting tools to assess the potential environmental risks associated with GMOs have also been developed at international level, in particular by the (OECD 2014) and in the frame of the Cartagena Protocol on Biosafety (CPB 2012).

In accordance with the above principles and methodology, an ERA should be conducted in six steps, in an integrated process and in an iterative manner, as follows (see also Fig. 1):

1. Problem formulation in which all important questions for the risk characterisation are identified. This step includes the identification of any characteristics of the GM plant which may cause adverse effects (hazards), of the nature of these effects, and of pathways of exposure through which the GM plant may adversely affect the environment;

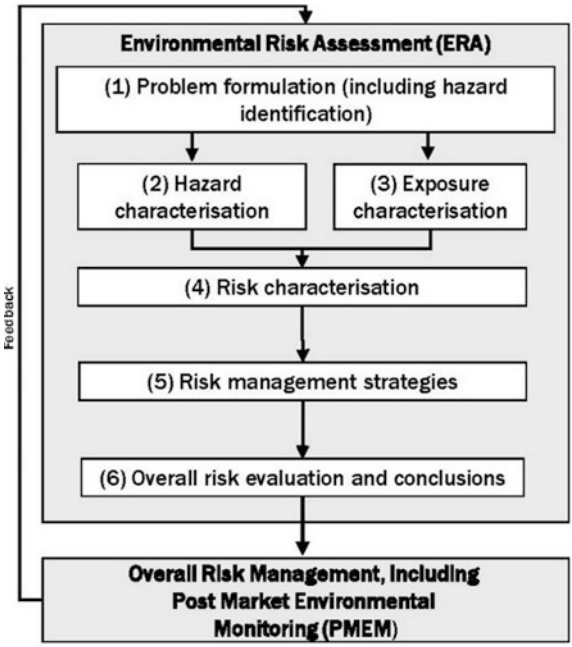

Fig. 1 Six steps in the environmental risk assessment (ERA) of GM plants (*Source* EFSA 2010)

2. Hazard characterisation, i.e. the evaluation of the potential consequences of each adverse effect;
3. Exposure characterisation, i.e. the evaluation of the likelihood of the occurrence of each identified potential adverse effect;
4. Risk characterisation, which is an estimation of the risk posed by each identified characteristic of the GM plant which has the potential to cause adverse effects;
5. Identification of management strategies to reduce potential identified risks associated with the GM plant to a level of no concern, and to address the uncertainties;
6. Evaluation of the overall risk of the GM plant, taking into account the results of the ERA and associated levels of uncertainty and the risk management strategies proposed.

Although quite some experience and knowledge has already been gained with (GM) crops worldwide, in many ERAs, both hazard and exposure are measured with a certain level of uncertainty. Uncertainty is an inherent and integral element of scientific analysis and risk assessment of any organism, whether it is GM or not. It can arise from: (i) lack of information, (ii) incomplete knowledge, and (iii) biological or experimental variability, for example due to inherent heterogeneity in the population being studied or to variations in the analytical assays (CPB 2012). Uncertainty resulting from lack of information includes, for example, information that is missing and data that are imprecise or inaccurate (e.g. due to study designs, model systems and analytical methods used to generate, evaluate and analyse the information).

Although it may be impossible to identify all the uncertainties, the ERA shall include a description of the types of uncertainties encountered and considered during the different risk assessment steps. Their relative importance and their influence on the assessment outcome shall be described (EFSA 2009). Uncertainties originating from lack of information may be addressed by requesting further information on the specific issues of concern.

Addressing uncertainties originating from lack of information is especially relevant in the context of field trials and of the step-by-step approach foreseen in Directive 2001/18/EC. According to this Directive the containment of GMOs is reduced and the scale of field trials increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken. In this approach, it is anticipated that by progressively increasing knowledge on the characterisation and potential adverse effects of the GMO, uncertainties can be reduced.

It is recognised that an ERA can only be carried out based on scientific and technical data available at the time it is conducted. The ERA may not always result in definitive answers to all the questions considered because of lack of such data. Therefore appropriate risk management, including monitoring, has to be considered in accordance with the precautionary principle in order to prevent adverse effects on human health and the environment.

3 Information Requirements for the ERA

For each element of an ERA, information must be compiled. In the EU, the information requirements are specified in Annex III of Directive 2001/18/EC. It includes information relating to the recipient or parental organism(s), to the genetic modification, to the resulting GM plant (for instance factors that may affect its release, presence and persistence in the environment), to the characteristics of the release and of the receiving environment and to the interactions between the GM plant and the receiving environment. Relevant information can be obtained from a variety of sources such as scientific literature, expert opinions, monitoring reports, or experimental data obtained during or prior to the risk assessment process.

As stated in Directive 2001/18/EC, not all the information elements set out in the Annex III have to be delivered in every case. Only the particular subset of considerations which is appropriate to individual situations should be addressed (case-by-case). The level of detail required in response to each subset of considerations is likely to vary according, among other things, to the biology/ecology of the recipient organism, the intended use of the GM plant and its likely potential receiving environment, the (biological) containment of the trial, and the scale and duration of the environmental exposure (e.g. whether it is for field testing or for commercial use). Obviously, the level of detail required is lower for field trials than for an unconfined commercial release.

Defining the appropriate set of information to be provided for a specific ERA is not straightforward and can give rise to different interpretations amongst risk assessors and evaluators.

For small-scale field trials, especially at early experimental stages or in the early steps of environmental releases of GM plants that are conducted in a step-wise manner, limited information may be available to identify or characterise some of the potential hazards at the time the risk assessment is conducted. Whereas sufficient information will be generally available on the biology on the parent species and the characteristics and consequences of the genetic modification (from published data and laboratory experiments or from other sources), less information may be available with regards to the phenotypic and agronomic properties of the GM plant and its (un)anticipated interactions with the receiving environment. As mentioned above, in such cases the resulting uncertainties may be addressed through the implementation of risk management measures, aiming in particular at mitigating impacts on the receiving environment (e.g. spatial isolation, planting of non-GM border rows, prevention of pollen and seed dissemination). When additional information is requested in order to decrease uncertainty, one should keep in mind that this information should always be considered in terms of its relevance and contribution to the identification and evaluation of potential adverse effects of the GM plant in the context of its intended use(s), or how far it can affect the outcome of the risk assessment. Providing more information will not always contribute to reduce uncertainty. Data requirements should always consider the issue of "need to know" versus "nice to know". Only data useful for coming to a conclusion on the risk assessment should be generated, otherwise the study moves into more basic research (EFSA 2008) or may actually give rise to new uncertainties (CPB 2012).

For large scale or commercial environmental releases where the intent is widespread introduction of the GM plant in the environment, usually with few or no restrictions, the potential hazards should be characterised more completely, meaning that more detailed and comprehensive information should be considered for the risk assessment. Such information may be obtained from different sources including the scientific literature, laboratory studies, past risk assessments where relevant and also results from experimental field trials for the same or similar GMOs introduced in similar receiving environments.

Determining whether, when and how field studies should be set up to gather data to further inform the risk assessment of a (pre-)commercial release and contribute to reduce uncertainty is a complex matter.

First, environmental risk assessment for regulatory purposes is commonly organised through a tiered (step-wise) approach, whereby hazards are evaluated within different tiers that progress from worst-case scenario conditions framed in highly controlled laboratory environments to more realistic conditions in semi-field and subsequently field experiments (EFSA 2010). In this context the question whether field trials are always necessary or could be avoided when worst-case studies in the lab show the absence of a hazard with high enough certainty is subject to debate (Romeis et al. 2006; Lang et al. 2007).

Second, as mentioned in the previous section, adequate problem formulation is a critical step in the ERA process. If field trials must generate data useful for the risk assessment, their design should comply with a science-based hypothesis-driven approach focusing on appropriate assessment endpoints and relevant exposure pathways that can measure what is really at harm.

Third, field trial designs should be adequate to provide relevant and statistically valid data. This is not always evident to achieve in small-scale trials which rarely have the statistical power to detect low effect sizes due to their insufficient sample size. When sample size is low, meta-analysis may improve statistical power by combining several trials and assuming a common measure of effect size. Extrapolating the results of such trials is also an issue, since effects assessed at one scale, period or climate may not pertain at other scales, periods or climates, and since it is hardly conceivable (from time and resources reasons) to perform multiple testing tailored to all potential receiving environments relevant for the GM plant in question. Some models are available for making predictions for larger scales based on results obtained from smaller scale experiments ("scaling up of results") but their routine application to GMO risk assessment is still a matter of discussion (EFSA 2008).

Fourth, another important aspect is how to gather reliable data for the ERA from more or less confined field trials. Due to applied confinement measures, the interactions between the GM plant and its receiving environment are often limited. Results (including monitoring data) of these field trials may be therefore less informative with regard to the full potential of interactions that may occur between the GM plant and its receiving environment in large scale and unconfined trials and may not generate scientifically robust data.

To help applicants and risk evaluators determining when and how field trials and data gathering for an ERA should be performed, some countries have established specific procedures and requirements mainly based on a tiered (step-wise) approach. As an example, the approach as taken in the Netherlands is detailed in the next section.

4 A Step-by-Step Approach for the Design and Evaluation of Field Trials with GM Plants: The Dutch Model

In the Netherlands testing of GM crops in field trials is performed in a step-wise manner, based upon the step-by-step principle described in Directive 2001/18/EC which states that confinement of GMOs in field trials can be gradually decreased and the scale of the introduction can subsequently be increased in a step-wise manner, under the condition that the conclusion of the ERA of the former steps allows the next step.

In line with this principle, the Dutch Commission on Genetic Modification (COGEM) proposed a system of field trials in which GM plants can be tested in the

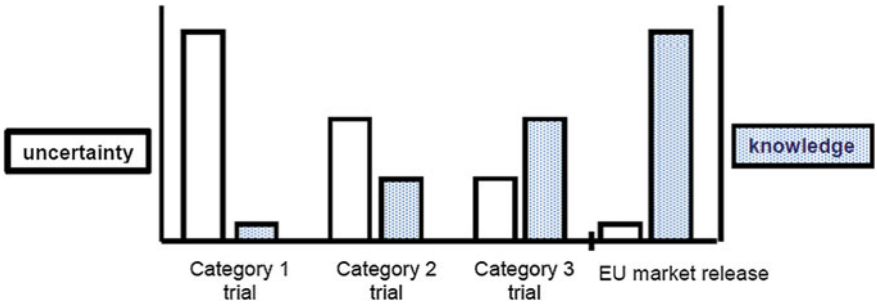

Fig. 2 Step-by-step approach for field trials with GM plants in the Netherlands. An increase in data results in a decrease of uncertainty. Due to this decreased uncertainty, the scale of the trials can increase and containment measures are no longer deemed necessary in category 2 and 3 trials (from COGEM 2008)

field on an increasing scale and with decreasing confinement measures on the condition that increasing knowledge on the GM plant and its environmental interactions is available (COGEM 2005, 2008). This system means to ensure that breeders can obtain sufficient data on their GM plants during the selection process in the field, without compromising the environmental safety of those trials. In general, the selection process of new plant varieties starts with a number of plants exhibiting the desired phenotype that are not fully characterised (phase 1). Thereafter a subset will be further tested and characterised (phase 2), resulting in one or two fully characterised events that will be commercialised (phase 3).

Following these 3 phases in the selection process of plant breeding, COGEM proposed a system in which three categories of field trials can be distinguished (Fig. 2) (Glandorf 2014).

The first category is meant to test the many uncharacterised plant lines in the start of the selection process. Due to the uncertainty related to the low level of characterisation of the plants, confinement measures can be applied such as isolation distances or removal of flower buds. The scale of the introduction is in this stage limited to maximum five locations of not more than one hectare per year. Thereafter, one can apply for the second category of field trials. This is only possible when there are sufficient data available to conclude, based on risk assessment, that potential adverse effects on human health and the environment are unlikely. This conclusion can be based on data obtained from the first category field trial, or based on data from other field trials or literature. This second category of field trials is meant—if applicable—to further characterise a subset of earlier tested plants which are of interest for the breeder. Based on the outcome of the risk assessment, the use of confinement measures to prevent outcrossing is considered no longer necessary. However, there still remains uncertainty since the GM plants are not fully characterised. Therefore the scale of the field trials is limited to a maximum of 10 ha per year.

The third and last category is meant for pre-commercial field trials with a single fully characterised event, which has proven in earlier trials in the Netherlands (or in

other EU countries) not to have adverse effects on human health and the environment. There is no limitation to the scale of the field trials and no confinement measures to prevent outcrossing are deemed necessary.

For each trial measures have to be taken to prevent spreading from GM material from the trial site. In addition, applicants have to comply with the Dutch coexistence distances between GM and non-GM plants.

GM plants should not necessarily be subject to all categories of field trials in a step-wise manner. For example, a trial with GM plants could be initiated directly in the second or third category based on information already available on the GM plant from other (EU) trials or from the literature. If the information is not considered sufficient, the trial will be classified as a lower category of field trial that fits with the level of GM plant characterisation. In that case also the maximum size and number of locations will be adjusted accordingly and confinement measures may apply if necessary. Alternatively, a trial with GM plants could be performed under category 1 only without any need to scale up, for example in case of scientific research without any intention to follow this up for commercial purposes. This applies also in cases where there is sufficient data available on the specific GM plant for category 2 but there is no need for a large size trial. Applicants are free to choose for a lower category, despite the fact that mandatory confinement measures may apply (category 1) or the size or number of location will be limited as a consequence of this choice (Glandorf 2014).

Data requirements and other requirements for each category of field trials are laid down in COGEM advices (COGEM 2005, 2008). Ideally, the scientific advice issued by COGEM for a field trial in a certain category indicates which specific data requirements are considered necessary for the next category of field trials so that applicants can gather the relevant data before applying for a next category of field trials.

Monitoring data are an important pillar in data gathering with regard to expected and potential unexpected effects of GM plants on the environment. A monitoring plan is therefore mandatory for all categories of field trials. Monitoring consists of general monitoring (phenotype, general agronomic features, any unexpected effect) and, depending on the crop/trait combination, specific monitoring. Examples are specific monitoring to study persistence in the soil of an antimicrobial protein expressed by GM apple trees, or to study a potential increase in frost tolerance in low amylose starch potatoes.

5 Approaches in Other Countries for the Evaluation of Field Trials with GM Plants

Several EU and non-EU countries having experience with field trials involving GM plants, including GM trees, were contacted to provide information on the way such field trials were assessed and managed depending on their purpose and the level of

knowledge on the GM plant and its environmental impact. Belgium, Finland, France, Romania, Spain, Sweden, the United Kingdom, as well as Canada and the USA provided some feedback based on the following questions:

1. Do you have in your country a similar system as the "Dutch system", in which field trials are upscaled in a step-wise manner? If so, do you have examples or documentation on this?
2. If such an approach is applied, do you indicate to the applicants which information is necessary to perform an upscaled field trial? If so, do you have examples or documentation on this?
3. If such an approach is not applied, do you use any other specific approach for dealing with experimental versus pre-commercial field trials, and for the gathering of biosafety data informing the environmental risk assessment? If so, do you have examples or documentation on this?

Belgium, Finland, Romania, Spain, Sweden, the United Kingdom and the USA indicated that they do not have a formal notification system similar as the Dutch system, in which field trials with GM crops or trees are—or can be—upscaled in a step-wise way. In these countries field trials are assessed on a case-by-case basis without making distinction between experimental and pre-commercial field trials, and without any pre-defined requirements on confinement measures, size or number of locations of the field trials. Belgium, Spain and Sweden reported cases where an authorisation for a small-scale research field trial was granted on the condition that specific additional studies are performed during the trial to increase knowledge on the potential environmental impacts of the GM plant. It was recognised however that small-scale trials may not always be adequate to provide scientifically robust data. Most countries indicated that management measures accompanying field trials were important to address uncertainties resulting from the risk assessment. It was noted that because such measures focus on minimising environmental exposure, they are rarely adequate to generate new data on the environmental impact of the GM plants, which could potentially be used if subsequent field trials with the same GM plants are considered.

France indicated that the High Council for Biotechnologies (HCB) has established in a recommendation an indicative classification of field trials in three categories corresponding to three stages of development of a GM plant. Indications are given on the level of detail of information expected by the HCB depending on the type of experimentation. This classification has no regulatory value. It is only indicative and does not prejudge the additional information that may be requested by the Ministry of Agriculture or the HCB during the examination of the application on a case-by-case basis.

In Canada the system is binary. An applicant can apply for a confined research field trial of plants with novel traits (PNTs) or an unconfined commercial release but there is no prescriptive "scale-up" procedure (CFIA 2014; Finstad et al. 2007). Confined field trials are limited in size as a risk management measure and terms and conditions are imposed. These terms and conditions provide for reproductive isolation of the plants within the trial from plants outside it, provide for physical

separation of plant material from the trial from food and feed supply chains, and mitigate persistence of the PNTs in the environment post-harvest. Developers can make a justification for larger size field trials based on their research, experimental design, the biology of the species in the trial or other relevant criteria. Applications for unconfined release usually follow several years of confined trials. Approvals are given following a thorough environmental safety assessment based on extensive high-quality, statistically sound data and/or valid scientific rationale provided by the applicant to demonstrate the environmental safety of the PNT.

6 Discussion: How a Step-by-Step Approach May Be Applied to Field Trials with GM Trees

The ERA of GM plants should be carried out based on the most complete set of available data relevant to the GM plant to be assessed. In that respect, a challenging aspect is whether and how experience gained from a field trial with a specific GM plant can be taken into account in the ERA of subsequent trials with the same or similar GMO plants. In the Netherlands, this aspect has been addressed through the application of a formal and binding step-by-step approach. Under this approach the confinement of GM plants in a field trial can be gradually decreased and the scale of the trial increased in a step-wise manner at the same time that knowledge on the GM plant and its environmental interactions increases.

Our survey, even if it provides only partial and indicative information, shows that very few other countries have applied specific procedures or approaches to assess and manage fields trials with GM plants depending on their purpose (experimental vs. pre-commercial) and the level of knowledge on the GM plant and its environmental impact. France reported the use of an approach similar to the Dutch one, although not being prescriptive. In Canada, environmental releases are categorised in "confined" or "unconfined" levels but without prescriptive "scale-up" procedure.

A formal step-by-step approach may provide several interesting features.

First, it provides clarification with regards to the necessary data requirements for the ERA. For research field trials involving uncharacterised GM plants, the risk assessment will be based on information relevant to the implementation and effectiveness of conditions of use (scale, duration, types of activities) and management measures to ensure risk mitigation, while allowing knowledge gaps with regards to the GM plant and its potential environmental impact (category 1 in the Dutch approach, or "confined" level in Canada). For pre-commercial environmental releases, the risk assessment will require exhaustive and scientifically sound information demonstrating the environmental safety of the GM plant (category 3 in the Dutch approach, or "unconfined" level in Canada).

Second, it can facilitate the collection of useful data for the ERA. Indeed considerably more information is necessary for the risk assessment of unconfined field

trials. Laboratory and greenhouse studies may be helpful in generating specific case-specific data for the risk assessment but, as mentioned earlier, such studies do not allow testing the GM plant against the full set of biotic and abiotic conditions. The information needed to feed the risk assessment may be difficult to collect from confined field trials (category 1) due to the application of strict confinement measures avoiding or limiting interactions between the GM plants and the environment. The necessary replication required to generate ecological effects data from confined field studies would also make such studies extremely difficult (Häggman et al. 2013). In that respect, the step-by-step approach allows for an intermediate phase (category 2 in the Dutch approach) between complete confinement and unconfinement in which relevant data can be collected for the ERA in open-field conditions. Examples are field trials without confinement measures, but with size limitations or spatial separation from sexual compatible species, in which interactions between the GM plant and the environment can be studied. Monitoring is an essential mean of generating data on those interactions.

Third, a step-by-step approach is applicable to all plants, including GM trees. It has been helpful as a tool to aid the approval process for GM trees, as illustrated by the following examples in the Netherlands.

The first example is a small-scale category 1 trial with GM poplar (*Populus* × *canescens*) for bioethanol production, grown in a short rotation coppice (COGEM 2010a). The tree was modified with the *ccr* gene, coding for cinnamoyl coenzymeA reductase, resulting in low lignin content, and a marker gene *hpt*, coding for hygromycin resistance. No flowering was expected. The application complied with the criteria for a category 1 trial. To prevent spreading of the poplar outside the field location, removal of inflorescence, root suckers and falling branches was required. Monitoring on root suckers should take place for at least two years.

An example of a category 2 trial is a small-scale field trial with flowering, scab-resistant apple trees (*Malus pumila*) (COGEM 2010b). The trees were modified with a resistance gene *HcrVf2* obtained from apple (*M. floribunda*), which is already present in commercial apple varieties and natural apple populations. The absence of vector backbone sequences was confirmed. The field plot was located 150 m from any apple tree and 500 m from commercial apple orchards. This distance was not considered enough to prevent outcrossing. However, among others, an important consideration in the ERA was that the *HcrV* gene is already present in commercial apple varieties and in natural apple populations. Therefore no environmental risks were foreseen as a result of outcrossing and no (additional) confinement measures were necessary.

The risk assessment of GM trees can already rely on existing knowledge on the biology of the corresponding non-GM tree species and their interaction with the receiving environment (see e.g. OECD 2014). This is especially true for intensively managed systems such as plantation forest trees. Additionally, commercial application of fruit trees or other woody perennial species may also provide useful information relevant to the ERA (Häggman et al. 2013). Many genes used to genetically modify trees and their resulting traits are not new and experience exists with regard to the assessment of those traits. In that respect the compilation of

existing information performed in the frame of the EU COST Action FP0905 on the biosafety of forest transgenic trees (Fladung et al. 2012) will be of great value. As mentioned before, laboratory or greenhouse studies may also be a valuable tool in generating data on ecological effects.

With the application of a step-by-step approach, the ERA of GM trees might benefit from additional data generated from unconfined field trials that would complement already existing information and studies about the host, the genetic modification and the receiving environment. When designing field trials with GM trees one should however cope with some safety and methodological considerations specific to GM trees. There are some differences between trees and crops, for example their longevity and ability to disperse, that are no new aspects in the ERA of GM plants but may need more emphasis in the ERA of field trials with GM trees, in particular trees for plantation forests (Aguilera et al. 2013). Trees are generally perennial, woody, long lived species with long life cycles taking several years to reach sexual maturity and commence reproduction. When mature they can produce large amounts of seed and pollen that can disperse over long distances (Hoenicka and Fladung 2006). The choice of appropriate non-GM comparators may be also more limited. All these considerations may require some changes in data types, collection and field design. For example, extremely long field trials with these long lived species may be challenging. Although it seems difficult to avoid field trials with GM trees to generate data on possible unexpected/unintended effects resulting from the genetic modification, useful data may be obtained in field trials with non-GM trees of the same species exhibiting natural variation in the trait of interest.

As indicated by Aguilera et al. (2013) and Fladung et al. (2012) further discussions are still needed on the types of studies required for providing safety data to be used in the risk assessment of GM trees. Further clarifications may also be necessary with regards to the current EFSA guidance documents (that might not be readily applicable to GM trees) and the guidance developed under the Cartagena Protocol (that does not differentiate between confined and unconfined field trials). However, it is broadly recognised that field trials are important to collect data that are relevant to the specific characteristics of GM trees. A step-by-step approach involving a categorisation of field trials, such as the one developed in the Netherlands, provides a useful mean to frame and facilitate the design, evaluation and regulation of these field trials.

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