Annex D. Soil Functions

A consideration for the environmental risk/safety assessment of a transgenic plant is the evaluation of the potential for the plant to have adverse effects on soil microbial communities responsible for soil processes and their soil functions, relative to the comparator.

Concepts and terms

Soil functions, such as soil quality, primarily depend on biotic factors including soil flora and fauna and their abundance and composition. Fauna ranging from micro- through meso- and macro- to megafauna and the associated soil processes are critical to maintain soil quality. This environmental consideration focuses on the interactions of transgenic plants with soil micro-organisms, and the potential effects on soil quality, soil biogeochemical cycling, or other microbe-mediated soil processes when soil microbes are adversely affected. Considerations in Annex C (Organisms (Animals)) can be applied to higher soil fauna, such as arthropods and nematodes.

Soil quality has many definitions depending on the context, national legal frameworks, and the soil science community. Soil quality reflects, *inter alia*, the potential of the soil to sustain plant growth and the above-ground ecosystem by providing nutrients and minerals, by providing microbial factors involved in plant health (e.g. absence of pathogens or presence of antagonists of plant pathogens), and by safeguarding microbial functional diversity. The requirements for soil quality may be different in different ecosystems.

Biogeochemical cycling processes have important ecological functions for the maintenance of soil quality. Examples of such processes are mineralisation, nitrification, carbon (C)-cycling, nitrogen (N)-fixation, soil respiration, decomposition of organic matter, and humification. It is widely recognised that microbial communities in soils, which are known to be important for biogeochemical cycling processes, vary considerably both temporally and spatially. Biogeochemical cycling processes are relatively robust to changes in soil microbial community structure (abundance and diversity of species) due to redundancy in microbial community function.

Problem formulation

For this consideration, below is a simple example that illustrates the approach for planning an environmental risk/safety assessment. It includes a discussion of assessment endpoints, potential adverse effects, and a linear pathway to harm with corresponding risk hypotheses and information elements to illustrate the approach. As previously indicated in the document (section 1.2.6), the process is often more complex.

(a) Determination of assessment endpoints

Soil microbial communities are very complex, often characterised by high microbial diversity (Tiedje et al., 1999; Roesch-Luiz et al., 2007; Fierer and Lennon, 2011), and in constant flux in response to several factors (Leitner, Aaron and Jodi, 2021). The occurrence and abundance of soil micro-organisms are affected by 1) soil characteristics like organic matter content, nutrient content, and moisture capacity; 2) typical physico-chemical factors such as temperature, pH, redox potential and physical soil structure;

and 3) influences caused by human activities like crop rotation, soil management practices and chemical control methods. Soils are heterogeneous and significant variation in microbial populations is expected in soil, including in agricultural fields.

Plants have impacts on soil micro-organisms with which they interact. These interactions may then affect soil microbial communities. Such a change could also affect soil processes underlying soil quality.

One example of an assessment endpoint for soil functions is the quality of soil.

(b) Identification of potential adverse effects on the assessment endpoints

The identification of potential adverse effects of the transgenic plant on soil functions considers the characteristics of the transgenic plant linked to the genetic modification, including the novel gene product(s), and the potential receiving environments.

A transgenic plant may express a gene product or produce a new metabolite based on the expression of a gene product. This may cause adverse effects on soil functions in different ways, for example by affecting the diversity of microbial species and/or soil microbial communities, or by affecting biogeochemical cycling processes. Plant growth and health may be compromised by impaired soil functions.

Potential impacts of a transgenic plant on soil quality – and more generally on soil functions – via crop management practices are taken into account in Annex F (Crop Management Practices).

Other impacts of a transgenic plant on soil quality – and more generally on soil functions – may occur due to a potential plant to micro-organisms gene transfer (for example in the case of antibiotic resistance genes), whose corresponding risk assessment may be required in some jurisdictions but will not be elaborated further in this document.

Consideration of the mechanism of action of the newly introduced trait and the characteristics of the transgenic plant relative to its comparator aids in identifying potential adverse effects on soil functions. An example of an adverse effect on the environment according to the assessment endpoint identified above is reduction of soil quality.

(c) Identification of plausible pathways to harm, formulation of risk hypotheses, and identification of information elements relevant to evaluating the risk hypotheses

In this section, a plausible pathway to harm is postulated. For each step of the postulated pathway to harm, a corresponding risk hypothesis is formulated that will enable the risk assessor to determine whether the pathway is likely to occur. Once it is shown that any part of the pathway is highly unlikely to occur, one does not need to continue evaluating the subsequent steps in the pathway and can conclude that the specific pathway to harm is unlikely to occur. In addition, examples of information elements that can be used to evaluate the risk hypotheses are given along with their rationales.

Postulated pathway leading to a reduction in soil quality

Soil micro-organisms and/or microbial communities can be exposed to a gene product or new metabolite produced by a transgenic plant via root exudation or by leaching from plant parts that are shed onto or into the soil. If the gene product or new metabolite has the capability to directly affect certain soil micro-organisms and/or microbial communities (e.g. a transgenic plant with a disease resistance trait), this may lead to changes in biogeochemical processes and in the end could lead to an altered soil quality.

One example of a postulated pathway to harm for this adverse effect is shown in the first column of Table A D.1. Risk hypotheses for each step of the pathway are formulated in the second column and the third column provides examples of information elements for evaluating the hypotheses.

Table A D.1. Postulated pathway leading to a reduction in soil quality, corresponding risk hypotheses, and relevant information elements

Pathway steps	Risk hypotheses	Examples of information elements
In relation to the comparator, the transgenic plant produces a new substance that has antimicrobial properties against certain soil micro-organisms	In relation to the comparator, the transgenic plant does not produce a new substance that has antimicrobial properties against certain soil micro-organisms	The intended function of the DNA sequences of the transgene in the transgenic plants are to produce an antimicrobial protein or metabolite
The new substance is released into the soil	The new substance is not released into the soil	Level and pattern of expression of the novel substance in the transgenic plant Soil stability and fate of novel substance in the soil
Abundance and diversity of the soil micro-organisms affected by the new substance are reduced	Abundance and diversity of the soil micro-organisms affected by the new substance are not reduced	Population dynamics of soil micro-organisms
Soil quality due to microbial activity in the soil is affected, e.g. reduced	Soil quality due to soil micro-organisms is not affected	Effects on processes such as for example ammonification
Key soil processes due to activities of beneficial soil micro-organisms are persistently disrupted	Soil processes due to activities of beneficial soil micro-organisms are not persistently disrupted	Role of micro-organisms in disrupted biogeochemical processes Functional redundancy among soil micro-organisms Length of time to soil processes recovery
Soil property is persistently reduced by the transgene in the cultivation of the transgenic plant		

It is important to note that examples of information elements in this table are intended to illustrate the types of information that can be used in evaluating a risk hypothesis, i.e. to determine whether particular pathway steps are likely to occur. However, for any step there might be other information that could be relevant. Rationales for how such information elements may be used to evaluate the risk hypotheses include:

- The DNA sequences introduced into the transgenic plant, any novel proteins or metabolites produced in the transgenic plant, and the antimicrobial properties of the novel gene product/metabolite, which inform the potential for the transgenic plant to produce novel anti-microbial substances;
- The level and pattern of expression of the novel substance in the transgenic plant and its stability and fate in the soil (e.g. rapidly degraded or persistent), which informs the level and duration of exposure of the soil micro-organisms to the novel substance;
- The changes in microbial activity in the soil related to soil processes (e.g. ammonification), which provide insight on the level of impact of these changes to the soil quality;
- The role of the micro-organisms in disrupted biogeochemical processes including supporting plant growth, the functional redundancy among soil micro-organisms and the length of time of soil processes recovery, which informs the likelihood and magnitude of the impact on key biogeochemical processes due to persistent disruption of activities of beneficial soil micro-organisms.

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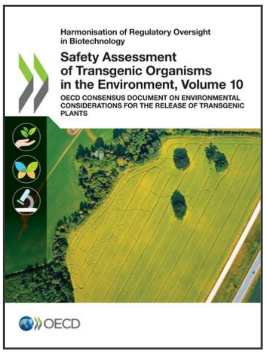

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