Summary

The decision concerning the classification of plants produced by new plant breeding techniques and derived products as genetically modified organisms (GMO) or non-GMO results in consequences relevant for the legislation and the market. To date, there is no general consensus concerning definition or interpretation of the status of these plants.

Following the discussion of practical consequences of cisgenesis, intragenesis, zinc-finger nucleases (ZFN) and oligonucleotide-directed mutagenesis (ODM) techniques, and agroinfiltration in a previous study, this study focuses on the discussion of RNA-dependent DNA methylation (RdDM), reverse breeding and grafting (on GM rootstock). Challenges for regulators, and practical consequences resulting from the limitations of the current GMO regulatory framework when applied to plants produced by new plant breeding techniques have been identified.

In RdDM small RNA molecules lead to methylation of specific DNA sequences and thereby alter gene expression. These epigenetic effects may be achieved through stable insertion of a construct or by transient expression. For reverse breeding plants are transformed with a construct for RNA interference (RNAi) which leads to suppression of meiotic recombination. The progeny results from segregation (negative segregants) and therefore does not contain the construct. When grafting non-GM plant tissue onto GM rootstock, the grafted plant benefits from the molecules expressed in the rootstock and transferred to the upper part of the plant. The non-GM part of the plant does not contain the transgene.

It is challenging to assess to which extent potential risks of the plants and derived products produced through new techniques have to be evaluated. For some of the new techniques (RdDM, reverse breeding, ZFN and ODM) the database to draw final conclusions is currently not sufficient. Cisgenesis, intragenesis, agroinfiltration and grafting make use of well-established methods for plant transformation; risk assessment should therefore follow the current guidance documents for GM plants. When the gene has already been within the compatible gene pool and/or has been part of the usual diet of humans and animals, the data requirements for the relevant elements concerning the risk assessment (e.g. plant-to-plant gene transfer, toxicology) may be reduced. Other elements like the transgenerational inheritance of the epigenetic effects induced by RdDM and thus the stability of the trait have to be evaluated on a solid database, which is currently not available. The same applies to the accuracy and efficiency of site-directed mutagenesis methods. For these techniques further research is required. Reverse breeding and RdDM open up questions concerning the status of negative segregants. When the intermediate GM plants have been produced by using standard plant transformation methods unintended effects due to the genetic modification cannot be excluded. These potential effects should be taken into consideration when recommending risk

assessment procedures. Finally, the general question of whether the effector molecules produced by the application of new techniques, e.g. siRNAs, pose risks when ingested by humans and animals to date has not been researched sufficiently. The same applies to effects on other organisms (e.g. insects, nematodes) that are exposed to such molecules, including their potential transmission. In conclusion, for all new plant breeding techniques core elements of the current risk assessment requirements for GM plants are mandatory.

In addition to the characterisation of the plants under investigation, the different techniques pose various challenges to regulators, in particular concerning currently existing labelling regimes for GMOs. The commercialised plants and/or their products should, by technical definition, not contain any foreign sequences and thus cannot be identified using standard molecular methods. Consequently, the application of the techniques frequently does not leave unequivocally detectable traces in the final product, or the changes to the genome cannot be distinguished from naturally occurring ones. Moreover, in many cases the resulting plants are phenotypically similar to traditionally bred ones. In case of grafting the change induced by the transgene harboured in the GM rootstock cannot at all or not reliably be detected in the product. Whenever there is no change to the genome of the plant, detection and traceability on this basis are seriously hampered. Consequently, labelling according to the currently established threshold for food and feed is not possible, and therefore the regulatory framework has to be reconsidered if labelling and thus information on the use of the new techniques is to be ensured.