ANSES Opinion
Request No 2021-SA-0019

The Director General

Maisons-Alfort, 22 January 2024

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on methods for assessing the health and environmental risks and socio-economic issues associated with plants obtained using certain new genomic techniques (NGTs)

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 22 January 2024 shall prevail.

On 28 January 2021, ANSES received a formal request from the Directorate General for Risk Prevention (DGPR) and the Directorate General for Food (DGAL) to undertake the following expert appraisal: “Request for an opinion on groundwork on methods for assessing the risks associated with the use of GMOs in food and feed”. After ANSES took over some of the missions of the High Council for Biotechnology (HCB) on 1 January 2022, the expert appraisal contract between the Agency and its commissioning ministries (May 2022) specified that the scope of this formal request had been broadened to include environmental and socio-economic aspects.

This collective expert appraisal work was undertaken within the scope of ANSES’ missions relating to biotechnologies, which include assessing the risks to the environment and public health associated with all biotechnological applications in the open environment and also assessing their socio-economic impacts. This opinion and the related expert appraisal report are intended to provide insights for the requesting parties and stakeholders in this scope, which covers some of the issues associated with the use of plants obtained using certain NGTs and their derived products. The other bodies that took over the HCB’s missions, i.e. the French Economic, Social and Environmental Council (CESE) and the National Consultative Ethics Committee (CCNE), were also consulted with regard to the issues associated with NGT plants, within the scope of their missions corresponding to societal and ethical issues respectively. The analyses and conclusions of this expert appraisal work should therefore be examined alongside the opinions of the other bodies consulted.

1. BACKGROUND AND PURPOSE OF THE REQUEST

New genomic techniques (NGTs) are a heterogeneous group of genome-modifying techniques that are based on a variety of mechanisms (mutations, insertions/deletions, gene silencing, etc.). Some of these techniques aim to modify a genetic sequence through precise and targeted action (site-directed or targeted mutagenesis), offering a very broad scope of application, particularly in the area of plant breeding. Like other genetic modification techniques, these NGTs can be used for a wide range of applications extending beyond plants. For example, in the area of medicinal products (whether human or veterinary), their targeting precision can bring considerable progress to gene therapy.

These genome-modifying techniques, especially those based on the CRISPR-Cas system, have developed very rapidly, and plant varieties obtained using these NGTs are already available on the market in certain countries, in particular the United States and Canada. No plants obtained using these NGTs are currently authorised on the European Union market.

Following an appeal filed by the *Confédération Paysanne* and other organisations with the French Council of State, which in turn referred questions to the Court of Justice of the European Union (CJEU) for a preliminary ruling, the CJEU's judgment of 25 July 2018 (Case C-528/16) concluded that "only organisms obtained by means of techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record" were excluded from the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. As a result, plants obtained using these NGTs were considered as falling within the current regulatory framework applying to genetically modified organisms (GMOs), particularly in terms of risk assessment.

On 29 April 2021, the European Commission published a study on NGTs, concluding that the current regulations on GMOs did not seem appropriate for plants obtained using certain NGTs. For other organisms (animals and micro-organisms), the Commission considered that efforts should be made to continue building up the necessary scientific knowledge and that products obtained using NGTs should be maintained within the scope of the current GMO regulations for the time being. The European Commission's study also pointed out legal uncertainties, the difficulty of implementing controls in particular, and the lack of flexibility in the current regulations. Moreover, it concluded that certain plants obtained using NGTs could be beneficial to society and contribute to a more resilient and sustainable food system as part of a "farm to fork" strategy. This study also highlighted a number of major issues, relating in particular to intellectual property, traceability, consumer information, the competitiveness of businesses and the agricultural sector, trade, and the acceptance of these products by society.

In its letter to the Portuguese Presidency of the Council of the European Union dated 29 April 2021, the Commission indicated that in light of the study's results, it intended to launch a legislative initiative for plants obtained through site-directed (or targeted) mutagenesis or cisgenesis. The idea was to adapt the current regulatory requirements in terms of risk assessment, authorisation procedures, labelling, and traceability, while maintaining a high level of protection for the environment and for human and animal health and taking account of the potential contribution of these plants and derived products to the sustainability of the food system. This intention took the form of a Proposal for a Regulation that was prepared by the Commission and published on 5 July 2023[1].

[1] https://food.ec.europa.eu/document/download/c03805a6-4dcc-42ce-959c-e4d609010fa3_en?filename=gmo_biotech_ngt_proposal_2023-411_en.pdf and https://food.ec.europa.eu/document/download/5a994ff5-153a-4886-a3cc-794512dce27a_en?filename=gmo_biotech_ngt_proposal_2023-411_annex_en.pdf (consulted on 12 October 2023)

In this context, the French Directorate General for Risk Prevention (DGPR) and Directorate General for Food (DGAL) submitted a formal request to ANSES for a scientific opinion within the scope of the Agency's missions, in preparation for the forthcoming European-level discussions.

In keeping with this scope, and in accordance with the expert appraisal contract, the two main objectives of the expert appraisal were established as follows:

- determine whether adaptations could be made to the current regulatory requirements for assessing the health and environmental risks associated with genetically modified plants when the assessment concerns plants obtained through site-directed (or targeted) mutagenesis;
- document and analyse the socio-economic issues associated with NGTs.

Concerning aspects relating to health and environmental risks, the scope of the formal request was limited to plants obtained through site-directed mutagenesis using the CRISPR-Cas system (see Section 3.1), which respectively are the type of application and the tool most commonly used or considered for use.

These two objectives were broken down into six sub-objectives (sub-objectives 1 to 4 for the first objective and sub-objectives 5 and 6 for the second objective):

- *Sub-objective 1*: review the current state of knowledge on the potential unintended on- and off-target effects on the plant's genome of site-directed mutagenesis using the CRISPR-Cas system;
- *Sub-objective 2*: determine specific requirements in terms of health and environmental risk assessment for plants obtained through site-directed mutagenesis using the CRISPR-Cas system;
- *Sub-objective 3*: for plants obtained through site-directed mutagenesis using the CRISPR-Cas system, determine which of the current regulatory requirements for the assessment of genetically modified plants can be waived;
- *Sub-objective 4*: depending on the progress made with regard to the above sub-objectives, determine how the current GMO assessment framework could be adapted for plants obtained through site-directed mutagenesis using the CRISPR-Cas system;
- *Sub-objective 5*: describe the sector or sectors concerned by the use of plants obtained using NGTs and products derived from these plants, from upstream to downstream in the value chain;
- *Sub-objective 6*: on this basis, document and analyse the associated socio-economic issues, firstly for the businesses and economic operators concerned, in particular in terms of competitiveness and capacity for innovation, and secondly, depending on the data available, for consumers and the supervisory authorities.

This expert appraisal had been initiated before the Commission's Proposal for a Regulation of 5 July 2023 was published. Following the publication of this proposal, and after the European Commission issued a technical note in October supporting the criteria for equivalence to conventional plants, ANSES decided to issue an internal request to analyse the criteria defining category 1 NGT plants as set out in Annex I of the regulation proposal. This analysis was carried out in parallel and was published on 21 December 2023 (ANSES 2023). Given the respective work timetables of the experts involved in addressing these different requests, the conclusions of this analysis have not been incorporated into the present expert appraisal,

which was carried out within the scope defined above and did not distinguish between category 1 and 2 NGT plants.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committees (CESs) on "Assessment of the biological risks in foods" (BIORISK, the lead CES), "Socio-economic analysis" (ASE), and "Biological risks for plant health" (SANTVEG). ANSES entrusted the expert appraisal to a Working Group (WG) on "New genomic techniques" set up after a public call for applications. The CES ASE was responsible for validating the work on economic and social sciences and the CES SANTVEG for validating the work on environmental aspects. The CES BIORISK was responsible for endorsing all the work.

The methodological and scientific aspects of the work were presented to the various CESs between May 2022 and December 2023. It was adopted by the CES BIORISK at its meeting on 11 December 2023.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the following website: https://dpi.sante.gouv.fr/.

Lastly, after it was validated by the CES BIORISK, the "NTG" WG adopted the following methodology in order to meet the sub-objectives set out in the previous section:

Sub-objective	Methodology
1	Analysis of systematic reviews available in the literature Systematic review of the literature on unintended effects on the tomato genome associated with the use of the CRISPR-Cas system Systematic review of the literature (2021-2023) on unintended effects on plant genomes associated with the use of the CRISPR-Cas system
2	Systematic review of the literature on the health and environmental risks associated with the use of the CRISPR-Cas system in plants Study of 12 representative examples of CRISPR-Cas being applied in plants
3	Analysis of the current assessment framework and its suitability for the assessment of plants obtained through site-directed mutagenesis
4	Construction of a decision tree
5	Description of the tomato, soft wheat, carrot and grapevine value chains

6	Systematic review of the literature on the socio-economic issues associated with NGT plants and products Hearings with stakeholders concerned with NGT plants and products Analysis of systematic reviews available in the literature on the impacts of plants obtained through transgenesis Analysis of the potential impacts of scenarios involving changes to the regulations on NGT plants and products

Part 3 of this opinion is a summary of the collective expert appraisal report, in which the methodology used, the analyses undertaken and the results obtained are detailed and explained.

3. ANALYSIS AND CONCLUSIONS OF THE WG AND THE CESs

3.1. Identification of the NGT applications most likely to lead to commercial varieties in the short term

In a report on current and future (worldwide) market applications of NGTs that was published by the European Commission's Joint Research Centre (JRC) in 2021, 426 commercial applications in plants were identified. In 2020, 17 of these were at the pre-marketing or marketing stage, including seven obtained using a CRISPR system. Furthermore, of the applications for which the genome-modifying tool was known (382 applications out of 426), 90.2% (305/382) involved a DNA double-strand break using a site-directed nuclease technique (CRISPR-Cas, transcription activator-like effector nucleases (TALEN), meganucleases or zinc-finger nucleases (ZFN)), with CRISPR-Cas accounting for 78.8% of cases. Without giving any figures, the JRC report states that a mechanism of deletion or insertion of a few base pairs, caused by the DNA repair process through the non-homologous end joining (NHEJ) cellular system, is used in the vast majority of cases. Two other reviews (Brinegar et al. 2017; Modrzejewski et al. 2019) confirm that the CRISPR-Cas system is the primary NGT used, in terms of both the number of applications published in the literature and the number of patents filed in the United States.

Regarding the plant species concerned, the JRC report states that 38% are cereals, 17% are oil and fibre crops, 12% are vegetable crops, and 11% are tubers and root vegetables. NGTs are therefore applied to a wider variety of species than those obtained using transgenesis and authorized on the market under Regulation (EC) No 1829/2003 (maize, soya, rape, cotton and beetroot). Species that thus far have seldom or not at all been modified through transgenesis, such as banana, cocoa and chickpea, are among those identified in this report. Concerning the types of traits conferred to NGT plants, the three main ones are a change in the biochemical composition of the plant, tolerance to a biotic stress, and a change in plant yield and/or architecture. Herbicide tolerance, on the other hand, accounts for less than 7% of applications, whereas this is the most common type of trait in transgenic plants.

In order to identify the plants obtained using CRISPR-Cas (which appears to be the most widely used system) that are most likely to result in commercial varieties in the short term, as well as those that have already resulted in commercial varieties, the "NGT" WG compiled a database of applications developed for plants obtained using these techniques. The information was extracted from publications by Brinegar et al. (2017), Détain et al. (2022) and Modrzejewski et al. (2019), the JRC report (2021), international patent databases, and the

databases of the World Bank and the IMF (consulted on 21 December 2022); only plants obtained using the CRISPR-Cas system were included.

One hundred and twenty-one applications were identified. They concern a very wide variety of species, the most common of which are rice, tomato, and maize. Regarding the traits conferred to plants genetically modified using CRISPR-Cas, changes in biochemical composition make up the majority. These are followed by changes in plant architecture and/or improved yield, biotic stress tolerance, and breeding tools. Herbicide tolerance only accounts for 5% of the applications obtained using the CRISPR-Cas system.

Lastly, the experts in the "NTG" WG note that plants obtained through site-directed mutagenesis are starting to emerge on the market, in countries outside the European Union.

Considering that:

- **the CRISPR-Cas system appears, by far, to be the primary technique used in NGT applications likely to lead to commercial varieties in the short term;**
- **the CRISPR-Cas system is used for applications already available on the market outside the European Union;**
- **it appears that cisgenesis is still not widely used;**

the "NTG" WG chose to focus on plants obtained through site-directed (or targeted) mutagenesis using the CRISPR-Cas system for its expert appraisal work on health and environmental risks[2].

3.2. Site-directed mutagenesis using the CRISPR-Cas system

3.2.1. Description of the CRISPR-Cas system

The CRISPR-Cas system is a complex consisting of a Cas endonuclease enzyme capable of cutting DNA in addition to a strand of guide RNA whose sequence is complementary to that of the DNA targeted for mutation. The CRISPR-Cas system is used to create a DNA double-strand break at a specific site, thereby activating intracellular DNA repair mechanisms. As these mechanisms are more or less prone to error, changes in the genome can therefore occur. For example, point mutations or insertions/deletions of DNA fragments can occur at the targeted site, as can specific changes in the sequence of the targeted gene if a repair matrix is added by the breeder (the person or company developing the new variety). The CRISPR-Cas system can also be genetically engineered to produce new tools with a variety of applications. For example, modified CRISPR-Cas systems, with or without other proteins, can be used to produce DNA single-strand breaks. In this case, nickases (or nCas9) and applications such as base editing and prime editing make it possible to modify a single base, by enzymatic reaction or by reverse transcription of a specific guide RNA[3].

The CRISPR-Cas-based site-directed mutagenesis step is necessarily preceded by a step where the CRISPR-Cas system is delivered into the plant. Two types of approaches can be used by the breeder: those leading to stable expression and those leading to transient expression of the CRISPR-Cas system. Stable expression of CRISPR-Cas occurs when the

[2] This choice was set out in an amendment to the expert appraisal contract.

[3] CRISPR-Cas systems that do not cut DNA (dead Cas9 systems) can also be produced. Their use and applications have not been assessed in this report.

genetic material enabling expression of the guide RNAs and the Cas nuclease is integrated into the genome of the plant to be modified. Conversely, transient expression occurs when there is no integration of foreign genetic material into the plant genome, and when the guide RNAs and nuclease are not permanently expressed in the plant. Lastly, once the CRISPR-Cas system has been delivered and the targeted mutation is effective, additional excision, transgene segregation and backcrossing steps can be implemented by the breeder, enabling the CRISPR-Cas system to be eliminated from the plant genome in the event of stable expression.

3.2.2. Potential unintended effects on the genomes of plants modified using the CRISPR-Cas system

Although the specificity of the CRISPR-Cas system for its target sequence in the genome is regulated, on the one hand, by a specific 20-nucleotide protospacer sequence in the guide RNA (complementary to the target DNA sequence) and, on the other, by recognition of a defined protospacer-adjacent motif (PAM) sequence by the Cas protein, off-target genome cleavage by the Cas nuclease still remains possible. In some cases, on-target cuts by the CRISPR-Cas system can lead to unintended changes in the genome, such as larger deletions or insertions in large chromosomal regions. In both situations, these are considered unintended on- or off-target effects associated with the use of CRISPR-Cas.

In order to assess the nature and frequency of unintended on- and off-target effects on plant genomes, the WG first carried out a systematic review of the literature on unintended effects on the tomato genome over the period up to December 2022. Tomato was selected because of the multiple applications obtained using CRISPR-Cas in this species, and because it is unusual compared with authorised transgenic plants, i.e. cereals and oilseeds in particular, which have low water contents.

This assessment was then extended to all plants, by analysing systematic reviews already available in the scientific literature and by systematically reviewing original articles published over the 2021-2023 period, as these were not covered by the systematic reviews identified.

The systematic review carried out for tomato showed that a “biased” approach[4] (using bioinformatics tools to predict the regions that could be modified, followed by PCR amplification and sequencing analysis of these regions) had been used to screen for unintended effects in 58/61 of the articles analysed. Off-target effects were described in four of these publications. An unbiased approach (based on complete sequencing of the genome, but whose effectiveness in detecting off-target effects depends in particular on the sequencing depth) was used in four studies and no unintended effects were detected.

The analysis of the literature reviews that i) dealt with all plants, ii) had been published prior to this opinion, and iii) were assessed as being of very good quality according to the AMSTAR-2[5] assessment grid (Shea et al. 2017) showed:

- for Modrzejewski et al. (2019): a tendency to use a biased approach to identify off-target effects (211/228 studies), and the identification of 55 off-target effects for all the 1,738 sites analysed (i.e. a rate of 3%). The authors nonetheless emphasise the high level of heterogeneity observed between the studies, particularly in terms of the prediction and selection of the off-target sites to be studied (15 different bioinformatic

[4] When an approach is said to be “biased”, this is only because it is based on prior knowledge of the sites most likely to be modified, and not on the entire genome.
[5] AMSTAR: a measurement tool to assess the methodological quality of systematic reviews

prediction tools used), the detection method, and the modified species; they also note a lack of detailed information in several articles;

- for Modrzejewski et al. (2020): that the number of mismatches between the on-target sequence and a potentially off-target sequence appeared to be the most decisive factor in the occurrence of off-target effects; the likelihood of off-target effects decreased as the number of mismatches increased, with the probability of occurrence of off-target effects being virtually zero when there were more than four mismatches;
- for Sturme et al. (2022): a tendency to use a biased approach to identify off-target effects (97/107 studies), and the identification of off-target effects in 28 of the 107 publications selected. In particular, it was observed that off-target effects were identified when there were one to three mismatches between the off-target site and the guide RNA. One study (Arndell et al. 2019) also mentioned the insertion of transfer DNA which, according to the authors, is an important factor that should be taken into account in risk assessment.

Lastly, in the systematic review of original articles published over the 2021-2023 period, for all the plants for which applications of CRISPR-Cas were reported, 82 articles were selected mentioning screening for unintended effects on plant genomes. Of these 82 articles, 64 (78%) mentioned biased screening for unintended effects, 15 (18%) mentioned unbiased screening, and three (4%) mentioned a combination of biased and unbiased screening. The WG observed a higher proportion of articles using unbiased screening, compared with the review by Modrzejewski et al. (2019) (18% in the WG's analysis, versus 9/228, i.e. 3.4% in the review). The WG considers that this difference may be due to progress made in terms of sequencing techniques (which have become increasingly robust and powerful and less and less expensive, with higher sequencing depths). Of the 82 articles analysed, 28 (34%) described an unintended off-target effect. Eighteen (64%) of these effects were identified by biased screening, eight (29%) by unbiased screening, and two (7%) by using both approaches in parallel. Of the 837 sequences analysed for screening for unintended off-target effects using a biased approach (amplification and sequencing of the amplification products), only 60 showed an off-target mutation, i.e. 7% of the sequences analysed.

Although the types of unintended mutations observed were not often described in the articles analysed, the vast majority of the described cases involved short deletions or insertions (Jedličková et al. 2022; Liu et al. 2022; Narushima et al. 2022; Wang et al. 2021; You et al. 2022). Most of the unintended effects observed were off-target effects due to relatively non-specific guide RNAs, which in most cases had three or fewer mismatches with off-target sequences. The choice of the guide RNA sequence is therefore important for limiting these off-target effects, but it can prove complicated when sequence homologies are present in the genome. In addition, it is not always possible to choose between several guide RNAs for the targeted region, since the choice depends on the presence of a PAM sequence at the target site. Among the unintended effects identified, off-target insertions of unidentified origin (a 35 bp insertion in grapevine (Wang et al. 2021) and a DNA insertion, from the vector used, at the target site in soybean (Adachi et al. 2021)) were observed. Large deletions of 3200 bp and 1525 bp respectively were also observed in tomato and rice in the studies by Li R. et al. (2022) and Zhang et al. (2022).

In conclusion, with regard to the molecular characterisation of plants obtained through site-directed mutagenesis using the CRISPR-Cas system (Figure 1), the WG recommends that:

- **the targeted regions(s) should be sequenced, the modification(s) obtained should be characterised, and an appropriate detection method should be provided by the applicant;**
- **whenever possible, the breeder should use guide RNAs with more than four mismatches with the non-targeted regions of the genome; if this is not possible, they should explain why;**
- **when the complete genome sequence of the species concerned is available and when resequencing of the modified plant's genome is feasible, unbiased screening should be carried out for unintended effects on the genome, combining long-read and short-read techniques, with a minimal coverage of 20 X;**
- **when resequencing is not feasible (for example, for polyploid plants or very large genomes) but a complete reference genome is available, biased screening should be carried out for any genome sequence that has four or fewer mismatches with the guide RNAs;**
- **when a complete reference genome is not available for the species concerned, screening for unintended effects should be carried out for any known homologous region;**
- **the absence of foreign DNA (including in the form of fragments) in the plant genome should be demonstrated, either by resequencing of the genome or by targeted sequencing or Southern blotting using probes specific to the plasmid or transfer DNA and the sequence corresponding to the CRISPR-Cas system used.**

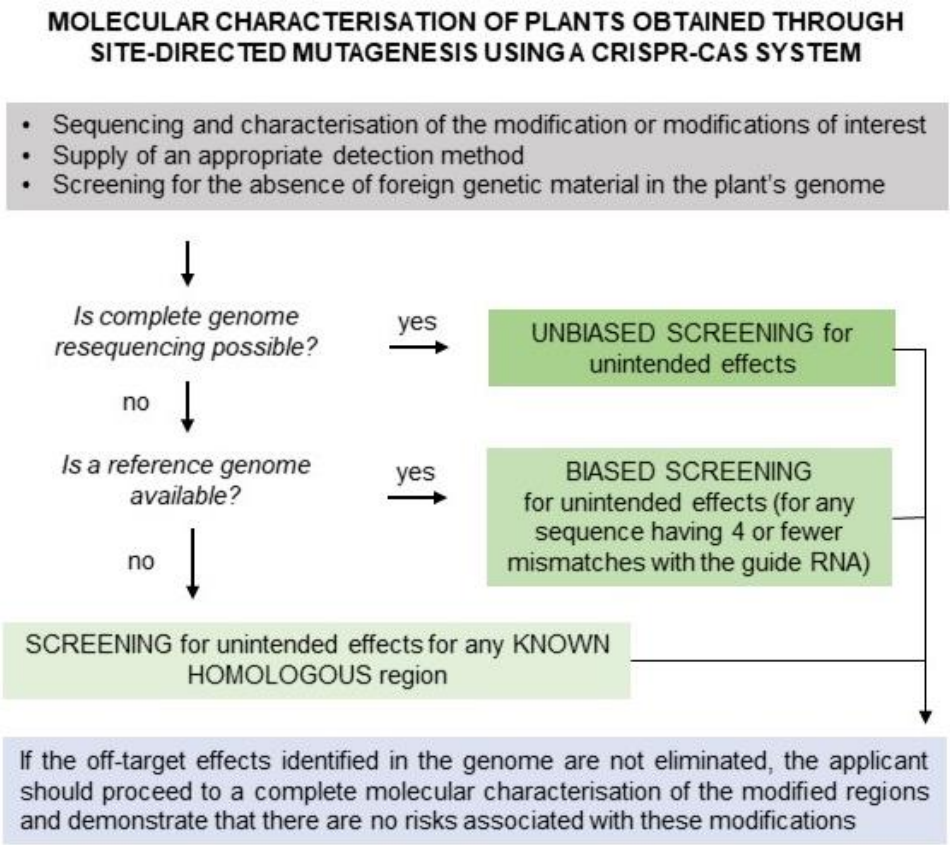

Figure 1. Recommendations for the molecular characterisation of plants obtained through site-directed mutagenesis using the CRISPR-Cas system.

3.3. Health and environmental risks associated with the use of plants obtained through site-directed mutagenesis using the CRISPR-Cas system

In order to identify the health and environmental risks associated with the use of plants obtained through site-directed mutagenesis using the CRISPR-Cas system, the WG successively analysed the risks covered by the current framework for assessing genetically modified plants, performed a systematic review of the literature on the health and environmental risks associated with the use of plants obtained in this way, and studied 12 cases considered to be representative of applications of the CRISPR-Cas system in plants intended for human consumption.

3.3.1. Analysis of the current assessment framework for genetically modified plants and its suitability for the assessment of plants obtained through site-directed mutagenesis using the CRISPR-Cas system

The framework in question is defined by the various regulatory texts applicable to GMOs (Directive 2001/18/EC of the European Parliament and of the Council, Regulation (EC) No 1829/2003 of the European Parliament and of the Council, Commission Implementing Regulation (EU) No 503/2013) and by the EFSA guidance documents (EFSA GMO Panel 2010; EFSA GMO Panel 2011a; EFSA GMO Panel 2011b; EFSA GMO Panel 2011c; EFSA GMO Panel 2015; EFSA GMO Panel 2017; EFSA GMO Panel 2019; EFSA GMO Panel 2023). In addition to identifying relevant risks for plants obtained through site-directed mutagenesis, the WG also assessed whether the current framework could be considered applicable to such plants.

For plants obtained through site-directed mutagenesis, **the WG considers that unexpected effects on the phenotype and agronomic characteristics of the modified plants are always possible, and that unexpected compositional changes in the plants or feed and food derived therefrom could also be observed, regardless of the modified trait.** Moreover, the WG underlines that some species intended for human consumption can naturally contain toxic or anti-nutritional substances (EFSA 2012). These substances should be taken into account in comparative analyses, and the WG considers that a 90-day toxicity study remains essential to identify a risk to human or animal health associated with the consumption of a genetically modified plant or products derived therefrom. The WG also stresses that it remains possible to generate new reading frames in a genome, particularly when a few base pairs are inserted into or deleted from one or more exons of a gene, and that the overall allergenicity of the plant can be modified. In addition, the WG considers that a nutritional study remains relevant when there are differences in the composition of plants obtained through site-directed mutagenesis.

Concerning environmental risks, the WG considers that the assessment of environmental risks as required under the current framework remains relevant for plants obtained through site-directed mutagenesis. In a context where the number of species concerned, the number of modified traits, and the number of applications could increase significantly in the short and medium term, the WG considers that this environmental risk assessment should take account of the potential long-term cumulative effects on the environment associated with an increase in the crop growing areas for authorised genetically modified plants and should consider the agro-environmental characteristics of their cultivation. Regarding the gene transfer to micro-organisms, the WG nevertheless considers that, since only plant genes are modified by site-

directed mutagenesis using CRISPR-Cas (without any introduction of bacterial genomes), the risk of transfer will be negligible.

Lastly, the WG's conclusions for each area of risk assessment, in terms of applicability, identified limitations, and recommendations, are set out in **Table 1**.

	Current requirements	Applicability according to the WG	Identified limitations and the WG's recommendations
Comparative analysis	1. Comparative analysis of agro-phenotypic characteristics and compositions between the genetically modified plant, an unmodified plant as genetically similar as possible, and six reference varieties, on at least eight sites 2. Comparative compositional analyses of processed products	1. Yes 2. Yes	• OECD guidance documents for compounds to be analysed not available for certain species
Toxicity	1. Repeated dose 28-day oral toxicity study in rodents of newly expressed proteins 2. Repeated dose 90-day oral toxicity study in rodents of the whole plant 3. Calculation of exposure to newly expressed proteins	1. No (except in specific cases) 2. Yes 3. No (except in specific cases)	• Low palatability of certain plant species for rodents • Difficulty ensuring the ingestion of controlled amounts of certain species (if high water content, for example)
Allergenicity	1. Analysis of the potential occurrence of new reading frames due to genetic modification of the plant 2. Analysis of the allergenicity of newly expressed proteins (including resistance to digestive proteolysis and heat denaturation) 3. Literature analysis on the allergenicity of the whole plant	1. Yes 2. No (except in specific cases) 3. Yes	• In general, adaptations can be made to take better account of species diversity, in particular by using LC/MS-MS[6] techniques
Nutritional assessment	1. Calculation of nutritional intakes in the event of consumption of the genetically modified plant 2. Nutritional study in the target animal	1. Yes 2. Yes	• Low palatability of certain plant species • Difficulty ensuring the ingestion of controlled amounts of certain species

[6] Liquid chromatography-tandem mass spectrometry

Environmental risks	Literature-based analysis of any direct or indirect, immediate or delayed risks to the environment associated with marketing authorisation	Yes	• In general, it would be necessary to take better account of the long-term cumulative risks and agro-environmental characteristics • Analysis of the risk of gene transfer to micro-organisms applicable but not very relevant

Table 1. Applicability and limitations of the current assessment framework for genetically modified plants, for plants obtained through site-directed mutagenesis using a CRISPR-Cas system.

The WG therefore considers that the current framework for assessing health and environmental risks is only partially suitable for plants obtained through site-directed (or targeted) mutagenesis. In particular, the WG considers that not all the requirements for newly expressed proteins contained in toxicity and allergenicity assessments can be directly transposed to the assessment of plants obtained through site-directed mutagenesis, and that the analysis of the risk of gene transfer to micro-organisms is not very relevant.

3.3.2. Systematic literature review

The literature search focused on both original articles and reviews. However, the 13 references selected were exclusively reviews, as no original articles could be identified that presented results relating to the health or environmental risks associated with plants obtained through site-directed mutagenesis. In addition, the reviews that were selected were only those containing a description and analysis of the potential risks associated with plants obtained using this technique.

Based on this systematic literature review, the WG notes that new applications, which have not been feasible using other breeding techniques, could emerge due to the use of NGTs. These include applications that involve multiplexing, or that target protected regions of the genome (for example, heterochromatin regions and genomic regions with low recombinogenicity) that can therefore not be reached with standard breeding methods. The WG also underlines that the CRISPR-Cas system could be applied to wild species, leading to *de novo* domesticated plants, without any history of safe use being available.

Concerning the risks associated with plants obtained through site-directed mutagenesis using CRISPR-Cas, the WG also notes that some of the known risks already associated with transgenic plants are also relevant for plants obtained through site-directed mutagenesis. Furthermore, the WG notes that the level of occurrence of these risks could be higher if the number of genetically modified plants appearing on the market and cultivated were to increase, in particular in terms of risks to the environment (differential use of certain herbicides or emergence of resistance in certain target pathogens or insects, for example).

Lastly, the WG agrees with the conclusions of several authors, who point to a new risk associated with the potential off-target effects of NGTs as well as to the possibility of pleiotropic effects (on several distinct traits). The WG also agrees that the possibility of pleiotropic effects or unintended changes in the composition of plants is increased in the event of multiplexing, which is commonly used at the research and development stages according to Kawall (2021).

3.3.3. Case studies

To supplement its analysis, and in the absence of original articles on the health and environmental risks associated with plants obtained through site-directed mutagenesis, the WG carried out case studies based on an analysis of 12 plants that were identified among the applications most likely to enter the market in the short term and were selected to represent the diversity of applications, species, and modified traits.

In light of these case studies, the WG concludes that there are potential new health and environmental risks associated with plants obtained through site-directed mutagenesis using CRISPR-Cas, mainly due to:

- the production of new genotypes that cannot be obtained using other breeding techniques;
- new species and traits that can potentially be modified using CRISPR-Cas, compared with what has traditionally been observed for transgenic plants (modification of more invasive species, or easier modification of composition, for example);
- the potentially large increase in crop growing areas for varieties with the same modified trait.

The WG also points out that some of the known risks associated with genetically modified plants remain applicable for plants obtained through site-directed mutagenesis.

The main risks identified in these case studies are presented in **Table 2**.

	Identified risks	Examples of cases
Comparative analysis, plant composition	• Pleiotropic effects leading to a change in the agro-phenotypic properties or composition of the plant • In the event of multiplexing or if a transcription factor is targeted, increase in the risks associated with pleiotropic effects	• Herbicide-resistant potato • Reduced-gluten wheat
Toxicity, allergenicity, nutritional assessment	• In the event of a desired or unexpected change in composition, potential change in the toxicity, allergenicity or nutritional characteristics of the plant	• Tomato with high γ-aminobutyric acid (GABA) content
Environmental risks	• Risk of gene flow from edited genes to wild or cultivated populations • If a growing number of modified species are cultivated, increased risk of gene transfer to weed species, including invasive weed species • Change in interactions with animals consuming plants obtained using NGTs and with pollinating insects • Change in selection pressure potentially increasing the pathogenicity of certain biological hazards, particularly for long-growing crops • In the event of multiplexing, transfer of combinations of genes with unassessed epistasis	• Tomato with high GABA content • Smaller rice • Sage with reduced phenolic acid content • Grapevine resistant to grey mould • Switchgrass with increased tillering

Table 2. Health and environmental risks identified in the case studies as being associated with plants obtained through site-directed mutagenesis.

In particular, the WG notes that certain potential risks repeatedly emerged in these case studies. These include risks associated with an unexpected change in the composition of the plant, which could lead to nutritional, allergenicity or toxicity issues, or medium- and long-term environmental risks, such as the risk of gene flow from edited genes to compatible wild or cultivated populations (increased by the new diversity of potentially modified species), and risks associated with a change in interactions with animals (including insects) consuming or visiting plants obtained through site-directed mutagenesis. The WG stresses that all of these risks could become more frequent if the range of modified species increases. However, the WG also concludes that in some cases, the use of CRISPR-Cas for site-directed mutagenesis only enables known phenotypes to be replicated, by acting rapidly on one or a few well-described genes, and that it therefore has not identified any new risks to health or the environment.

3.3.4. Recommendations for assessing the identified risks

The WG recommends conducting a case-by-case assessment of the health and environmental risks associated with genetically modified plants obtained through site-directed mutagenesis using the CRISPR-Cas system, taking into account the characteristics of the genetic modification made and those of the resulting product, and analysing the consequences of the genetic modification in terms of agronomic, phenotypic and compositional characteristics and immunological, toxicological and nutritional aspects.

The WG recommends supplementing this assessment with an analysis of the literature extended to the modified gene or the new trait. For plant species which, following authorisation, would be newly cultivated in all or part of the country, the WG also recommends that, to supplement the required tests, the literature review should highlight, if available, articles relating to the environmental risks associated with the introduction or mass cultivation of these plants.

To take account of certain potential risks associated with the technical possibilities offered by the genomic modification of plants using CRISPR-Cas, the WG recommends in particular:

- **if the modification(s) made are intended to change the biochemical composition of the plant, analysing levels of the new compounds and of the compounds potentially affected by this modification, in parallel with the comparative compositional analysis;**
- **if the modification(s) made are intended to suppress or modulate one or more transcription factors, carrying out a bioinformatics analysis to identify the target genes of the transcription factor, followed by a comparative analysis of the transcription levels of the target genes identified;**
- **if the modified species has a known allergenic profile or appears to contain potentially allergenic substances, systematically using ELISA or LC-MS/MS to analyse the major allergens the least susceptible to environmental variations (nsLTP, cupins, trypsin inhibitors), supplemented by an analysis of gliadins and glutenins in the case of wheat, in parallel with the comparative compositional analysis;**

- **if the species in which the modification is made naturally expresses known toxic, genotoxic or anti-nutritional compounds, systematically analysing these compounds in parallel with the comparative compositional analysis.**

The WG also notes that, in some cases where a plant's genome is modified, the CRISPR-Cas system can be used to reproduce known mutations, either because they have already been induced with other systems, or because they are intended to replicate a known allele in another variety or in a related species. The WG recommends, when prior knowledge is available, i.e.

- **when the genetic modification(s) made are functionally similar at the molecular level to a modification that has been induced using other techniques, including random mutagenesis or standard breeding, and are already authorised on the market without any specific risks to health or the environment having been described OR when the genetic modification(s) made are naturally present in another species (homologous gene)**
- **AND when the genetic modification(s) made lead to a known phenotype whose safety to health and the environment has been demonstrated**

that the assessment procedure should be simplified and limited, after molecular characterisation, to a comparative compositional analysis of the plant (EFSA GMO Panel 2015), in order to rule out any unexpected pleiotropic effects on the said plant.

The CES SANTVEG recommends that the literature on environmental risks, when it is available for a plant that has a similar trait (and has been obtained through other breeding methods), should also be taken into account as far as possible in the assessment of plants obtained through site-directed mutagenesis.

Considering the lack of data on the medium- and long-term environmental risks associated with plants obtained through site-directed mutagenesis using the CRISPR-Cas system – in particular for long-growing species (in arboriculture, for example) and if the cultivation of this type of NGT plant and the potential direct and indirect cumulative effects, including on cultivation practices, are intensified – the WG recommends that a post-authorisation environmental risk monitoring plan should be set up by a body independent of the applicant, regardless of the assessment framework used. This monitoring plan should take account of the cumulative impacts related to the cultivation of different varieties obtained through site-directed mutagenesis and having the same modified trait, as well as the impact on cultivation practices of marketing authorisations for plants obtained through site-directed mutagenesis. In particular, it should contain:

- **for plants resistant to biotic stress, the monitoring of changes in the overcoming of resistance in the pests concerned;**
- **the dispersal of these plants in the environment;**
- **gene flow from these plants to any potentially compatible weeds or wild plants;**
- **an assessment of the impacts of the modified traits, specifically enabling the estimation of the volumes of inputs used.**

In the event of a confirmed negative environmental impact, the WG recommends that the results of the monitoring plan should lead to a review of the marketing authorisation.

3.4. Proposal for a framework for assessing the risks associated with the cultivation and use in food and feed of plants obtained through site-directed mutagenesis using the CRISPR-Cas system

Based on the results and conclusions presented in Sections 3.1 to 3.3, the WG is proposing a comprehensive, case-by-case assessment framework, which is presented in the form of a decision tree in **Figure 2**.

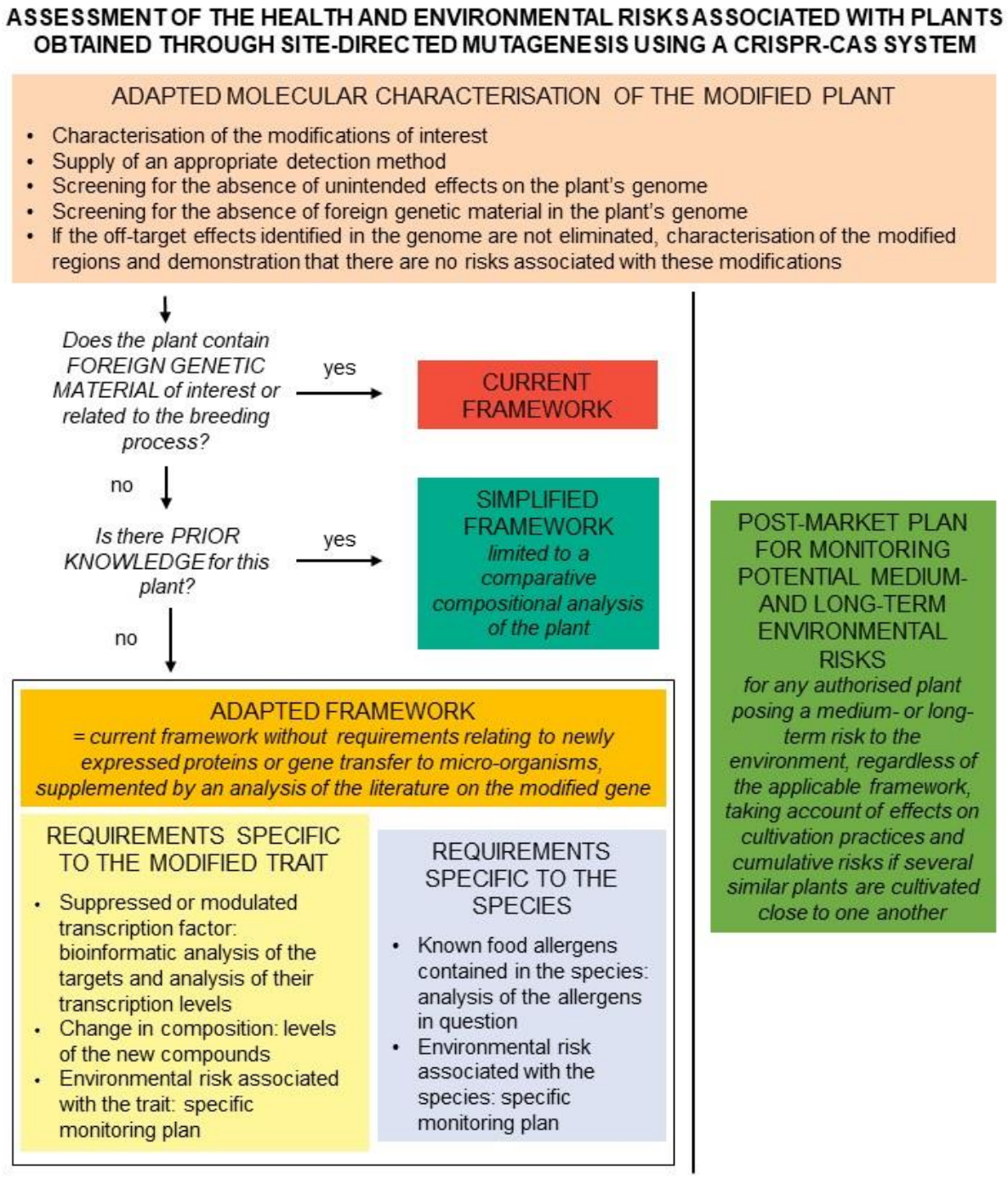

Figure 2. Decision tree for assessing the health and environmental risks associated with plants obtained through site-directed mutagenesis using a CRISPR-Cas system.

For any new plant obtained through site-directed mutagenesis, the comprehensive assessment framework proposed by the WG provides for a **molecular characterisation of**

the modified plant, including a characterisation of the modified site, screening for unintended effects on the plant genome, and screening for the absence of foreign genetic material potentially introduced during the processing stage, according to the procedures described in Section 3.2.2. Furthermore, if unintended effects on the genome are identified and their elimination is not demonstrated, the WG recommends that the region concerned by the unintended effect should be characterised and that the absence of risks associated with the unintended modification should be demonstrated by the applicant.

If the absence of foreign genetic material in a plant obtained using NGTs cannot be demonstrated, in particular following a phase of stable expression of the CRISPR-Cas system in the plant in order to induce the desired mutation, **the WG recommends that the plant should be assessed according to the current assessment framework**, i.e. according to the provisions of Directive 2001/18/EC, Regulation (EC) No 1829/2003, and Implementing Regulation (EU) No 503/2013, according to their respective scopes.

If the absence of foreign genetic material is demonstrated and the applicant can provide prior knowledge (see Section 3.3.4), the WG recommends a simplified assessment framework limited to a comparative compositional analysis of the plant.

If the absence of foreign genetic material is demonstrated but the applicant cannot provide prior knowledge, the WG recommends that an adapted framework should be put into place. This would correspond to the current assessment framework for genetically modified plants, with the exception of the requirements relating to the expression of a new protein and the requirements relating to the risk of gene transfer to micro-organisms (see Section 3.3.1), but it would be supplemented by specific requirements relating to the species or the modified trait, in accordance with the procedures described in Section 3.3.4.

Lastly, the WG recommends implementing a post-authorisation environmental risk monitoring plan, throughout the duration of the authorisation, taking account of the cumulative impacts related to the cultivation of different varieties obtained through site-directed mutagenesis and having the same modified trait, as well as the impact on cultivation practices of marketing authorisations for plants obtained through site-directed mutagenesis.

3.5. Socio-economic issues associated with NGT plants and products: multiple sectors and stakeholders

The introduction of NGT plants and products could have impacts for the agricultural sectors concerned in France, from upstream to downstream in the value chain. The WG identified the various areas of activity and stakeholders potentially concerned with NGT plants and their derived products by describing four agricultural sectors (tomato, soft wheat, carrot, and grapevine), representing various possible applications of NGTs and different technical and economic situations in terms of plant development, production, marketing, and consumption in France. The socio-economic issues associated with NGT plants and products for these different French sectors and stakeholders were then analysed through a systematic literature review. This literature review was supplemented by an analysis of the stakeholders' positions, based on the existing literature on the controversies surrounding NGT plants and on hearings with stakeholders[7]. On this basis, the WG analysed the potential socio-economic implications of amending or not amending the regulations concerning plants obtained using NGTs according to various possible scenarios.

[7] The stakeholders interviewed are listed in the collective expert appraisal report.

3.5.1. Description of the agricultural sectors potentially concerned with NGT plants and products

Describing the agricultural sectors potentially concerned with NGT plants and products helped the WG identify the different types of stakeholders involved in these sectors and understand the issues they are facing due to the introduction of these plants and products. However, given the current absence of NGT plants and products in Europe, no analysis of these innovations' impacts on the sectors potentially concerned could be carried out.

The sectors for the varieties selected for the case studies (tomato, soft wheat, carrot, and grapevine) comprise six stages: plant breeding, seed and plant production, variety production, agri-food processing, variety or product distribution, and consumption (**Figure 3**).

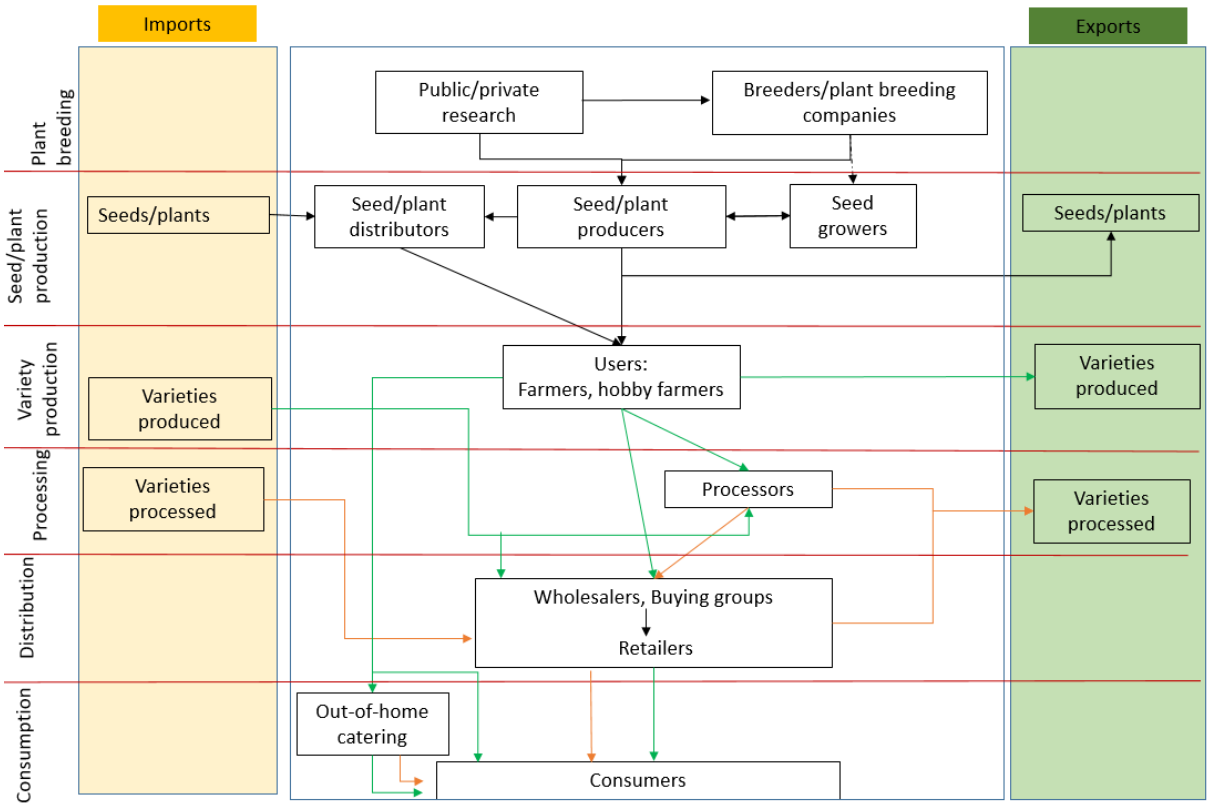

Figure 3. Conceptual diagram of the value chain for the selected varieties. The arrows show the links between the different groups of stakeholders from the upstream stage of variety production to the last link in the value chain. The black arrows represent transactions involving seeds, the green arrows transactions involving products derived directly from seeds, and the orange arrows transactions involving processed products.

Plant breeding is a dynamic stage for all four sectors in France, although to a lesser extent for the carrot sector. This dynamism does not always translate into dynamic production. Indeed, in recent years, tomato production has been on the decline, soft wheat production has remained static, grape production has fluctuated with no clear trend, and carrot production, the sector where plant breeding is the least developed, has increased. All these trends should be considered in light of the strategic choices made by producers (the choice of high quality for tomato), as well as weather conditions, prices, and diseases (for grapevine).

The four agricultural sectors are not equally dependent on international trade. The tomato and carrot sectors depend on imports to meet demand. Both of those sectors import twice as much

as they export. Although the carrot sector imports almost exclusively from the European Union, the tomato sector depends mainly on countries outside the European Union. Morocco alone accounts for 66% of the tomatoes imported into France by volume.

The French grape sector is integrated differently into international trade depending on whether the grapes used are table or wine grapes. High volumes of table grapes are imported, while imports of wine grapes are non-existent, according to the various institutions that record trade. Nevertheless, the wine sector is highly integrated into international trade, exporting more than it imports; the wines imported come primarily from Italy, a member of the European Union.

The soft-wheat sector is very well integrated into international trade and plays a highly favourable role in France's trade balance. Exports of soft wheat are 79 times greater than imports.

Given the specific characteristics of each agricultural sector described, it is likely that the introduction of NGT plants and products into the European Union would not affect them in the same way. The effects could be non-negligible for sectors that are highly integrated into international trade. Sectors that depend on imports, such as tomato and carrot, could be encouraged to use NGT varieties to become more competitive. As for sectors that play a major role in France's trade balance, such as the soft-wheat and grapevine sectors, the introduction of NGT plants and products could enable them to maintain or even gain market share. Lastly, agricultural sectors that are highly dependent on countries outside the European Union would be affected to a greater or lesser extent according to the regulations on NGT plants.

Despite the advantages conferred by the various characteristics of plants obtained using NGTs as highlighted in the potential applications, the adoption of these innovations in various sectors could require changes in specifications, particularly for organic farming. This could lead to potential difficulties associated with the coexistence of the NGT, conventional and organic sectors.

3.5.2. Socio-economic issues associated with NGT plants and products

Figure 4 shows the main points that should be considered with regard to the economic and social impacts associated with the introduction of NGT plants and products (right-hand side of the figure). The available socio-economic literature is fairly limited and is largely made up of position papers focusing on the aims of these innovations instead of their impacts. Few empirical studies have been carried out to date. A few articles based on survey data have identified the impacts perceived by various types of stakeholders to a certain extent, but little work has been undertaken quantifying the actual effects of decisions to regulate NGT plants and products on seed prices, agricultural and food prices, costs and gains for sectors, or the economic risks for the various types of stakeholders. All quantitative assessments are therefore very partial.

As NGT plant varieties are not currently available on the European market, the analyses are prospective and aim to assess the possible economic and social impacts that would result from stakeholders' strategies and public regulatory choices. Assessment of these possible impacts presupposes that the NGT plants in question have been granted marketing authorisation (and therefore undergone a health or environmental risk assessment if they are not considered equivalent to conventional plants). The abundant literature on GMOs obtained through transgenesis can provide benchmarks and illustrate certain economic mechanisms. **However, most of the data from the economic literature available on plants and products obtained**

using NGTs should be considered as assumptions that have yet to be confirmed rather than as proven results.

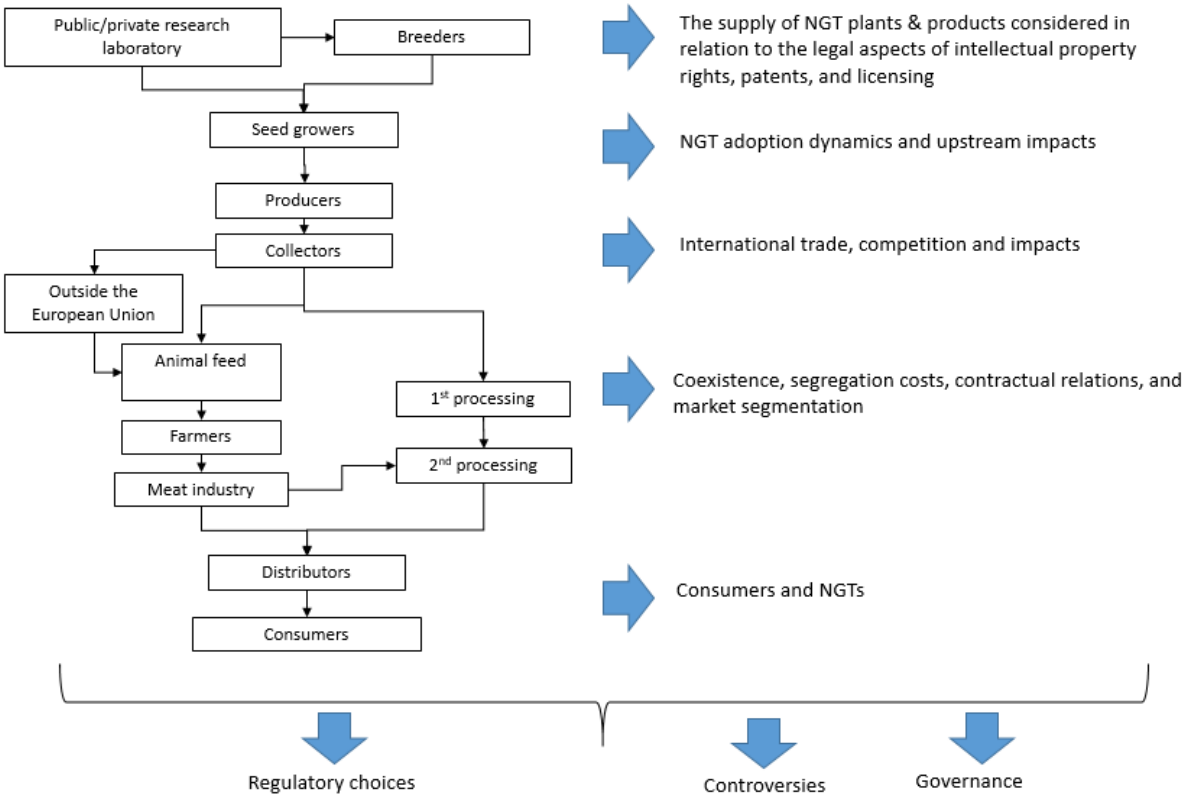

Figure 4. Conceptual diagram of the analysis of the socio-economic issues associated with NGT plants and products.

3.5.2.1. The supply of plants and products obtained using NGTs considered in relation to the legal aspects of intellectual property rights, patents, and licensing

An analysis of the regulatory landscape for plants shows that, even though the regulations on GMOs (including NGTs) have no direct impact on the patentability of plants and products obtained through these techniques, the supply of such plants and products may nevertheless be indirectly impacted by the regulatory situation. Indeed, changes in the regulations can influence patenting decisions, depending on whether they are perceived as flexible or rigid by biotechnology companies.

As far as plants are concerned, there are two forms of industrial property rights that apply in Europe: plant variety rights (also known as plant breeders' rights), which confer rights only for products, allowing breeders to reproduce processes to obtain other marketable varieties; and patents, which apply to products and processes and whose use requires the negotiation of a licence with the holder. In Europe, a plant variety can only be protected by plant variety rights (and not by a patent), unlike in the United States where, for example, a plant variety can be protected (including simultaneously) by both forms of rights. The scope of patent regulations also varies from one legal system to another. Exceptions applied in the European regulations, for example, protect the natural traits of plants by including a disclaimer in patents.

According to the literature, several solutions have been put forward to regulate the development of patents for NGTs. These range from specific patent models (patent pools, clearinghouses, licensing pledges, open-source patents) to a legislative reform of the system, with various possible options: patent abandonment or in-depth revision of or adjustments to patent law. The advantages and uses of these different types of patents (in the development phase) depend on the types of stakeholders/companies (small breeders, large biotechnology companies, etc.) developing these technologies.

The regulations on intellectual property associated with patents for plant breeding are a major issue that should be considered in connection with possible changes to the GMO regulations. Assuming that the objective would be to provide opportunities for dynamics in breeding innovation by limiting imbalances between stakeholders in terms of value-sharing, the WG stresses the importance of adapting the current regulatory framework with regard to intellectual property rights.

3.5.2.2. NGT adoption dynamics and upstream impacts in the sectors

Compared with the other available breeding methods, most scientific publications consider that NGTs more precisely target the traits to be developed and are more likely to succeed in the research and development (R&D) phases. This means that NGTs could be used to develop varieties likely to reach the end market at a lower cost and more quickly than plants obtained through transgenesis. As a result, (i) the profiles of companies involved in R&D appear, at this stage, to be more diversified for NGTs than for transgenic plants, with the significant involvement of small and medium-sized companies and public bodies; (ii) NGTs would reduce the market size needed to ensure profitable investments for those who use these techniques; and (iii) NGTs would allow for more diversified breeding innovations in terms of the desired traits and species covered.

The plant breeding industry has undergone major concentration at international level, to which company strategies developed for transgenesis applications have contributed. For plants obtained using NGTs, the question is to what extent their characteristics (precision in the selection of desired traits, lower development costs, ease of use, etc.) could amplify the process of concentration in the plant breeding and seed sector, or on the contrary would contribute to reducing barriers to entry into these markets. At this point in time, this question cannot be answered based on the available literature. The consequences that should be studied in particular include effects on the market power of plant breeding companies, the sharing of value within sectors, and seed prices for producers.

The literature analysed also emphasises that the economic impacts associated with NGT plants and products will depend heavily on the regulatory choices made at European level. By affecting the economic trade-offs of the various types of stakeholders, these will have a direct influence on incentives to develop and adopt these techniques. These regulatory choices may also influence the degree of concentration in the sector as well as R&D investment and location decisions, and will affect the ability of European companies to operate on international markets.

To what extent the characteristics of site-directed mutagenesis technology could amplify the process of concentration in the plant breeding and seed sector or, on the contrary, would contribute to reducing barriers to entry into these markets and would increase the involvement of small and medium-sized biotechnology companies, or even public research bodies, remains an open question. It is nonetheless clear that the

impact of the development of NGT plants on the concentration of the plant breeding and seed sector is a major issue. The public authorities should be vigilant with regard to this issue, in the event that changes are made to the GMO regulations, and should be alert to any abuses of dominant market positions.

3.5.2.3. International trade, competition and impacts

Based on the body of literature analysed, publications on international trade, competition and impacts focus mainly on the implications of differences between regulatory frameworks for NGT plants and products. The threat of unpredictable and restrictive trade environments has been highlighted, showing that these can lead to the establishment of trade barriers and have an impact on competition. Although there have been no documented cases of disruption to international trade in crops or products obtained through genome editing, the experience of GMOs obtained through transgenesis is frequently cited as an early warning sign of such tension.

Scenario-based studies show that regulatory differences could affect trade and the competitiveness of EU farmers on world markets using NGTs. These studies offer insights into future issues and opportunities for international trade in products derived from NGT plants. **However, as few such studies have been carried out, the insights they offer should be interpreted with caution. Opportunities related to trade barriers caused by differences in regulatory choices (for protecting national or European economic activities, encouraging the development of alternative technologies, etc.) have not been studied.**

3.5.2.4. Coexistence, segregation costs, contractual relations, and market segmentation

The economic impacts of NGT plants being introduced into sectors will depend on the nature of the innovative traits.

The first type of innovation corresponds to breeding innovations aimed at increasing the effectiveness and/or efficiency of agricultural and agro-industrial production. Here, the economic interests of producers and the upstream stages of value chains will be decisive in the development and adoption of these innovations. In the presence of GMO regulations, based on strict rules of product coexistence and segregation, the additional costs associated with these rules are likely to be higher than the gains for stakeholders in the sector, due to the likely devaluation of products by consumers (as NGT products do not provide consumers with more advantages than conventional products, suppliers of NGT products will only be able to enter the market at a lower price than for conventional products). As with products obtained through transgenesis, this could be an obstacle to the development of these breeding innovations in the human food sector.

The second type of breeding innovation fits into companies' product differentiation strategies (allergen-free products, different sensory and nutritional properties, etc.). The aim here is to capitalise on the fact that some consumers will be willing to pay for these distinctive characteristics. This type of strategy could be feasible, even in the event of restrictive regulations on coexistence, because traceability and segregation requirements would be imposed, in any case, for reasons of commercial credibility with regard to consumers. However, this would require that breeding objectives be defined in a coordinated way between sector stakeholders, based on contractual relations between seed growers, producers, and

companies marketing final products. Given the additional costs and the complexity of the coordination arrangements to be put in place, the distinctive characteristic would need to be significantly valued by consumers as a prerequisite.

A third type of innovation involves breeding innovations intended to meet environmental, health and/or societal issues (e.g. restoring biodiversity), when there is not necessarily any economic incentive to adopt them. The private costs of these environmental or health innovations can exceed the anticipated private benefits. If net collective benefits (economic cost/benefit or environmental risk/benefit ratio) were proven, this would raise the question of possible public support to encourage their adoption.

While certain characteristics of NGT varieties (increased yields, allergen-free products, different sensory and nutritional properties, etc.) may encourage sector stakeholders to develop them, this is not necessarily the case for some innovations which, while responding to environmental and climate concerns, do not generate either productivity gains or growth in demand (additional willingness to pay for these characteristics on the part of consumers in particular). **In this context, given the health, environmental and social concerns that these innovations could raise, public intervention, in particular support for public research, would be decisive in guaranteeing the capacity to develop innovations with a view to making the European agricultural and food system more sustainable.**

3.5.2.5. Consumers and NGTs

Concerning consumer behaviours, the literature consulted shows that, even though genetically engineered food products appear to be less well accepted and appreciated by consumers than conventional products, there is some heterogeneity in perceptions between different consumer profiles and between countries, even within the European Union. Although some studies show that consumers with good knowledge of technologies are the most averse and tend to reject them, others underline that the information available on biotechnologies and on differences between transgenesis and NGTs could cause a shift in perspective for some consumers, from the rejection to the acceptance of food products derived from them, especially since NGT products are associated with lower prices. Furthermore, the studies conducted do not make it clear whether consumers appreciate NGTs differently depending on the potential benefits (productivity, environment, health) they bring to food products or production processes. However, since NGT products are not currently available to consumers, the decisions and behaviours observed remain declarative (intention rather than action). Lastly, none of the studies assumed that NGT products may be untraceable or unlabelled. In this sense, there is still some uncertainty as to how consumers would respond if there was no tracing and labelling through to the final product in some or all of the food derived from plants obtained using NGTs.

In general, consumers' acceptability of and willingness to pay for NGT food products position these foods between GMO products and conventional farming products, which themselves are less well perceived than organic farming products. Further studies, which should be more precise in terms of the characteristics of the products on offer and the information disseminated in particular, would be necessary to gain a better understanding of the mechanisms of acceptance and rejection. The issue of the intensity of this information could also be raised.

One of the expectations of consumers is that they be informed about the nature of the products they are offered, particularly in terms of the technologies used for plant breeding. This concern should therefore be taken into account with a view to increasing the overall transparency regarding these products. Requiring that the applicant provide

a detection method when applying for marketing authorisation for a variety obtained through NGTs could help ensure the traceability of the products derived from these plants.

Agricultural sectors (organic in particular) wanting to highlight the non-NGT nature of their products could develop a specific voluntary labelling system. However, this could require the strengthening of documentary traceability, already in place in sectors with quality labels, and would most certainly result in an increase in product monitoring costs for both agricultural sectors and the control authorities, especially when no standardised analytical detection methods are available. Seed labels, mentioning the technology used, would be an essential requirement for traceability.

3.5.2.6. Regulatory choices

The publications analysed highlight four major characteristics of NGTs that should be taken into account when discussing the issues associated with regulatory choices: (i) the difficulty of tracing NGTs in the resulting organisms (and products) using the current analytical methods, which raises the question of the conditions and procedures for controls distinguishing between products on markets; (ii) the decentralisation of knowledge and uses made possible by easier access to technology, which could potentially open up the market to new stakeholders but could thereby increase the risks run (for example, the risk of uncontrolled off-target modifications), if these stakeholders have less experience than the traditional stakeholders on the breeding market; (iii) uncertainties surrounding off-target modifications, which require a combination of *ex-ante* regulatory procedures (prior to the deployment of innovations), defining the scope of NGTs, and *ex-post* procedures (following their deployment, where applicable), based on liability and compensation rules in the event of unexpected effects; (iv) rapid advances in knowledge and plant breeding technologies, which risk causing certain regulations to rapidly become obsolete and would make it necessary to put in place appropriate modes of governance.

The first trade-off discussed in several articles concerns the choice between process-based and product-based regulations. In the first case, the technologies used in the breeding process are what determine the marketing authorisation procedure for the new variety. In the second case, the legislation is based on the product: the specific characteristics of the new variety are what determine the authorisation procedure (on a case-by-case basis). The two regulatory frameworks have different properties: product-based regulations are more flexible because they can be applied to any technology, whereas process-based regulations have to be adjusted every time a new technology is introduced.

The second trade-off concerns the possibility of applying different rules according to the technology's level of modification of the initial genome by establishing, for example, exemptions for products obtained using SDN-1 or even SDN-2 techniques (these products could be treated as conventional products) and by maintaining process-based regulations for SDN-3[8] products including transgenes.

[8] SDN-1 editing is when the results obtained are point mutations or insertions/deletions of DNA fragments (generally a few base pairs), when no DNA sequence is added to act as a repair matrix. When a DNA sequence is added as a repair matrix, the results obtained are either a modification of the sequence of one or more genes (this is known as SDN-2 editing, as the matrix is not integrated into the genome) or the integration of this sequence into the genome (this is known as SDN-3 editing).

The third trade-off concerns the role of *ex-ante* regulations (e.g. rules on crop coexistence) and liability rules in the event of *ex-post* damage. An important point here, in the event that NGT varieties are newly introduced onto the market, is the interaction between products derived from them and products complying with specifications that exclude these technologies, such as specifications for organic farming. This means that how products derived from NGT varieties are identified (detection and labelling) on markets and how they coexist with products derived from non-NGT varieties (which will influence the cost of segregation, control and preservation of product identity) will be decisive. To date, there has been no specific analysis of this point in the economic literature on NGTs, but the parallel with GMOs obtained through transgenesis could provide food for thought.

3.5.2.7. Positioning of stakeholders and governance of NGT controversies

An analysis of the controversies surrounding NGTs shows that not everyone agrees on the framework that needs to be established to address the various issued raised: as the debate often focuses on technical, risk and effectiveness aspects, it ignores issues associated with the systemic context, intellectual property, market dynamics, justice and equity, and ethical issues. As a result, one of the visible areas of tension is that observed between the different agricultural systems and aims: on the one hand, a vision that sees technological innovation as a guarantee of greater precision, yields and economic benefits; on the other, a vision that criticises this system, arguing that it is not in line with social and environmental issues, that it relies too heavily on monoculture and pesticides, and that it mobilises – as was the case when GMOs and synthetic biology were originally being developed – an entire "economy of promises". One of the criticisms voiced in some of the hearings held (see Table 10 in the report) is that NGTs can only resolve certain symptoms of climate change and ecological problems but are not capable of resolving their root causes. The analysis of the controversies surrounding NGTs also highlights the issue of choice. Studies show that consumers prefer that genetic modification be made visible, whether through traceability and/or labelling.

The hearings held when this formal request was being addressed show that there are many areas of tension and uncertainty. This is consistent with the results of the literature showing that different audiences – be they consumers, farmers, or other stakeholders – do not form homogeneous groups and that placing the "public" in the position of a receiver of information is likely to cause their values, criticisms, arguments, choices and policy questions (particularly when they relate to systemic issues) to be disregarded. The questions raised by the stakeholders interviewed covered topics such as the following: (i) the allocation of costs associated with a possible health or contamination problem and/or the downgrading of a batch of organic products due to NGT products, (ii) the implications arising from the profusion of new terms, such as "NGTs", "NBTs", and genome editing – and the parallel disappearance of terms such as "GMOs" – in terms of access to debates for different audiences, (iii) the potential consequences of the development of NGT plants for market diversification/concentration, and (iv) arrangements for the potential labelling of NGT products and those for coexistence between different farming systems. If the current regulations are revised, the indicators used to draw the line between GMOs and NGTs (and to determine "equivalence" between conventional products and NGT products) are likely to become a hotly contested issue.

The WG's analysis of these controversies identified several areas of tension. The technology of site-directed mutagenesis raises a new controversial issue, concerning

whether or not there is a line between "GMO" and "NGT" technologies and what indicators should be used to draw this line and determine whether there is any "equivalence" between conventional products and products obtained using site-directed mutagenesis (a NGT). The debates on regulatory changes raise potential issues of "path dependency", where the decisions taken today could limit the room for manoeuvre available in the future. On the one hand, the current decision to not use site-directed mutagenesis technology can be seen as limiting capacities for action in the event that future changes to farming practices and production methods alone are unable to adequately meet climate and environmental concerns. On the other hand, the use of site-directed mutagenesis technology can be seen as opposing the current agricultural and food system's necessary shift towards a more sustainable agro-ecological model. The role of technology, and in this case of genetic engineering, in establishing an agro-ecological model for European agriculture is at the heart of these debates.

In this context, the question of how to ensure that objections and their foundations can be expressed in public debate, based on scientific arguments, and how to overcome them, is crucial. While there seems to be a consensus on the need for public dialogue, the way in which this dialogue should be organised and conducted, if it is to be fruitful and contribute to overcoming these objections, is not as clear. As the analysis of the conditions and procedures for governance of these controversies goes beyond the scope of this formal request, further work should be devoted to it.

3.5.2.8. Regulatory scenarios applying to plants and products obtained using certain NGTs and associated socio-economic issues

Possible changes to the GMO regulations, and the issue of determining whether or not varieties obtained through transgenesis and those obtained through site-directed mutagenesis should be considered in the same way, can be approached from different angles. First of all, they can be analysed in terms of the impacts they could have on i) incentives encouraging sector stakeholders to develop and use varieties obtained through site-directed mutagenesis, ii) the choices given to consumers, and iii) more generally the advantages and disadvantages, particularly economic, for the various types of stakeholders.

However, beyond the short-term effects of the possible options available, changes to the regulations also raise questions with regard to the longer-term dynamics of the agricultural and food system and the role, for example, that genetic engineering-based breeding innovation should play in it, in terms of changes in farming practices in an agro-ecological model for European agriculture, the need to rethink patent and licensing regulations in light of the development of site-directed mutagenesis technology, and the role of public research bodies in guaranteeing that breeding innovations address sustainability concerns. These are important questions, but they go beyond the scope of this formal request. They should nonetheless be analysed and discussed at length in subsequent studies, especially as they lie at the root of many controversies.

The WG has therefore limited itself to examining scenarios that are "feasible" in the short term, by analysing the possible economic impacts. It has nevertheless attempted to link this analysis to the controversies identified and thus consider it in relation to some longer-term issues.

The scenarios considered range from the status quo (current GMO regulations) to a scenario where the current regulations are revised, and therefore from a situation of low probability of development of breeding innovations obtained through site-directed mutagenesis technology to a situation where this probability would be much higher.

To summarise, concerning the possible economic impacts of various regulatory scenarios: changes in the regulations – based on the distinction between plants obtained through site-directed mutagenesis, which would be subject to regulatory provisions similar to those for plants obtained through conventional breeding, and plants that would continue to be subject to the current GMO regulations – could therefore lead to different impacts depending on whether a breeding innovation is in one situation or the other.

The first point concerns the criteria that would be used to make this distinction. These criteria could play an important role if they are not too restrictive for biotechnology companies. They would facilitate access to the market for breeding innovations for plants subject to regulatory provisions similar to those for conventionally bred plants; they would also limit the development of innovations subject to the current regulations. By choosing the criteria to be used, public policymakers can guide the dynamics of innovation in a direction expected by the community. It should be noted that in terms of risk assessment, the WG's proposal set out in the first part of this conclusion is to maintain a risk assessment for obtaining marketing authorisation, even if this assessment is simplified, for varieties similar to conventionally bred plants. The WG recommends a case-by-case approach, without exempting either type of NGT plant from a risk assessment. Furthermore, by requiring the establishment of an environmental impact monitoring system, this proposal aims to ensure that the regulatory choices made can be revised in the event of unanticipated negative effects on the environment.

The second point concerns the sector-wide effects of potential changes to the regulations that would consider plants obtained through site-directed mutagenesis as conventional varieties. In this case, the regulations on conventional varieties would exempt the sectors concerned from the rules on crop segregation, coexistence, and labelling, thus creating a favourable context for their development in Europe. This regulatory approach would reinforce the effects of lower R&D costs made possible by site-directed mutagenesis technology. On the one hand, considering NGT plants as conventional varieties would make it possible to use this technology, together with other levers for action, for breeding innovations with agronomic and/or environmental benefits. It would also allow for a degree of harmonisation with the regulations in place outside of Europe, which would limit import tensions and enable European companies to operate on international NGT markets. On the other hand, such changes to the GMO regulations could have a major impact on non-NGT production systems such as organic farming.

3.6. Conclusions of the WG and the CESs

The WG and the CESs[9] consider that the current framework for assessing the health and environmental risks associated with genetically modified plants is only partially suited to the assessment of plants obtained through site-directed mutagenesis using the CRISPR-Cas system. They recommend that a specific assessment should be carried out on a case-by-case basis (Figure 2). In addition, the WG and the CESs consider that

[9] WG on "New genomic techniques", CESs on "Assessment of the biological risks in foods", "Socio-economic analysis", and "Biological risks for plant health".

a comprehensive monitoring plan should be implemented for each marketing authorisation (MA) decision.

This post-MA monitoring plan should enable environmental and socio-economic data to be gathered on the *in situ* impacts of authorised NGT plants and products. From a socio-economic point of view, it should help to control the effects of the development of NGT plants, particularly on the market power and degree of concentration of biotechnology companies and the plant breeding sector, by paying attention to potential abuse of dominant market positions. The definition and implementation of such a comprehensive plan should involve all stakeholders, within a transparent and democratic framework.

Given the technical (detection of NGT plants), economic and social uncertainties identified in this report and the controversies surrounding the development of NGT plants, this monitoring plan will have to be based on a system that ensures the traceability and control of NGT plants and products and informs the public about their characteristics.

To conclude, the WG and the CESs underline that this work has highlighted some major socio-economic issues arising from the existence of NGT plants and products. These issues show that decisions concerning the development and regulation of future breeding innovations obtained using NGTs are societal choices that cannot be based solely on scientific and socio-economic arguments. The WG and the CESs consider that these societal choices should be managed using a structured and democratic approach.

4. ANSES CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) received a formal request to study the specific characteristics of plants obtained using certain new genomic techniques (NGTs), in particular site-directed mutagenesis, and to propose an appropriate health and environmental risk assessment framework, based on the current framework for assessing GMO plants. In addition to the potential risks associated with these modified plants, and in line with the scope of its missions which had been extended in January 2022, ANSES also analysed the socio-economic issues for various stakeholders associated with the development of these plants and their derived products. This work was commissioned against a backdrop of intense legislative activity at European level aimed at adapting the system that had been applicable to GMOs since the early 2000s to the regulation of these new techniques as they apply to plants. Indeed, although the European Commission's current work is focusing only on plants, the Agency emphasises that, just like transgenesis, NGTs are used in a variety of fields (therapies, vaccines, etc.).

This expert appraisal was initiated before the European regulation proposal was published. Therefore, it does not take into account the proposed system for distinguishing between two categories of NGT plants, the first of which is considered equivalent to plants obtained using standard techniques, with a view to applying different requirements to them. After the regulation proposal was published in July 2023, ANSES issued an internal request to analyse the criteria defining category 1 NGT plants within a short time frame. This work concluded that the definitions and scopes in the regulation proposal needed to be clarified. It identified limitations in the scientific rationale for the equivalence criteria, while noting that "for NGTs, the

equivalence criteria approach extends the dividing line between plants subject to assessment and those not subject to assessment" characterising the GMO regulations (ANSES 2023).

Concerning the assessment of the health and environmental risks associated with plants obtained through site-directed mutagenesis using the CRISPR-Cas system, ANSES endorses the experts' conclusion that the current risk characterisation and assessment framework is only partially suitable; it refers to the proposals made on these topics in the body of the opinion.

ANSES also endorses the conclusions and recommendations of the WG and the CESs in favour of a case-by-case assessment of the health and environmental risks associated with plants obtained through site-directed mutagenesis using the CRISPR-Cas system. Furthermore, it notes the possibility, raised by the experts, of a simplified assessment for genetically modified plants for which prior knowledge demonstrates a lower level of risk.

The Agency points out that the current framework for GMOs under the legislation resulting from Directive 2001/18/EC makes a distinction between plants subject to and those not subject to prior assessment, which is based on the technique used and not on the nature of the plant obtained. The experts recommend an *ex-ante* risk assessment that takes account of both the technique used and the characteristics of the plant thus obtained. This is one of the policy decisions that need to be made in light of all the issues posed by GMOs in general, which are growing in number with the emergence of NGTs.

ANSES agrees with the experts that some of the risks identified for plants obtained through site-directed mutagenesis are similar to those already identified for plants obtained through transgenesis, but that exposure to these risks could increase with the development of site-directed mutagenesis applications and the size of the market for these plants, especially since work on widely distributed plants, not currently subject to transgenesis, is currently in progress.

As such, ANSES stresses the importance of post-market monitoring and considers that it should play a greater role. It therefore endorses the recommendation for a comprehensive system for monitoring the use of plants obtained using NGTs once they have been placed on the market, from an environmental and socio-economic standpoint, for example by monitoring changes in cultivation practices. Due to the nature and variety of the applicable requirements, such a system may require a combination of several different tools: risk monitoring plans, initiatives such as observatories of practices, and the monitoring of results by health and environmental agencies. Such monitoring would fill the knowledge gap in the area of NGT plants and products and would enable corrective action to be taken in the event that undesirable effects are identified following cultivation or marketing.

To conclude this part on risk assessment, ANSES considers that it will be important, once European regulatory requirements have been defined, for precise risk assessment guidelines to be drawn up in order to avoid or limit differences in interpretation from one country to another. For its part, it intends to work with EFSA to develop shared guidelines.

Regarding the socio-economic issues associated with NGT plants and products, ANSES also endorses the results of the work carried out by the WG and the CESs. It points out the usefulness of the value chain diagram used to carry out the corresponding analysis and would like to thank the stakeholders who so readily agreed to participate in the hearings conducted by the experts to provide input for this part of the expert appraisal.

The Agency underlines the wide variety of motivations that can lead to the development of breeding innovations. These are divided into three main groups in the opinion: increasing the effectiveness or efficiency of agricultural and agro-industrial production, implementing product differentiation strategies, and responding to health, environmental or societal concerns. Every innovation can fall into one or more of these groups. All these motivations could be dealt with differently in future legislation and regulations. To do so, it would be necessary to examine the best way to achieve the main objectives, taking account of the health, environmental and social concerns that these different types of innovations are likely to raise and considering the respective roles of public research and market mechanisms.

ANSES would also like to mention the importance of adapting the current regulatory framework for intellectual property rights so that it is better suited to these breeding innovations. Moreover, it stresses the need to take account of the concerns of the various groups of stakeholders involved, including consumers, who expect to be informed about the nature of the products they are offered. Some expectations downstream in the value chain also entail major constraints for the upstream stages, particularly in terms of traceability and detectability.

Given the scarcity of available data, as there are currently very few of these innovations on the worldwide market, the Agency underlines the need to carry out scientific research to better characterise the socio-economic issues associated with NGT plants and products.

Lastly, the work carried out on the socio-economic aspects shows that the controversies surrounding NGT plants and products extend beyond the scientific and technical issues that may be associated with them and cover much broader concerns relating to agricultural production models and the role of genomic technologies in an agro-ecological transition. These concerns go beyond the field of health and safety. Each of the scenarios analysed for regulatory changes is based on choices that go beyond the sole issue of health risk and that may potentially generate very different economic and social impacts for the various stakeholders involved. Therefore, ANSES considers that the corresponding issues should be discussed within institutions responsible for these issues, such as the CESE and the CCNE, and then, of course, in parliamentary bodies.

This very diversity in terms of issues and concerns is leading ANSES to recommend that, for future decisions, the issue of health risks, including as part of a holistic approach to health, should be considered as a necessary but not sufficient factor in the structural choices that are made.

Pr Benoit Vallet

KEY WORDS

Nouvelles techniques génomiques, nouvelles techniques de sélection, organismes génétiquement modifiés, plantes, mutagénèse dirigée, CRISPR-Cas, évaluation des risques, analyse socio-économique

New genomic techniques, new breeding techniques, genetically modified organisms, plants, site-directed mutagenesis, CRISPR-Cas, risk assessment, socio-economic analysis

SUGGESTED CITATION

ANSES (2023). Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the assessment of the health and environmental risks and socio-economic issues associated with plants obtained using certain new genomic techniques (Request No 2021-SA-0019). Maisons-Alfort: ANSES, 31 p.