APPROVED: 17 October 2022

Public consultation on the updated scientific opinion on plants developed through cisgenesis and intragenesis

European Food Safety Authority (EFSA)

Abstract

The European Food Safety Authority (EFSA) carried out a public consultation to receive input from interested parties on the draft of the updated scientific opinion on plants developed through cisgenesis and intragenesis. This draft scientific opinion was prepared by the EFSA Genetically Modified Organisms (GMO) Panel, supported by an *ad hoc* Working Group on Cisgenesis and Intragenesis. The draft opinion was endorsed by the EFSA GMO Panel for public consultation on 5 May 2022. The written public consultation was open from 16 May 2022 until 27 June 2022. EFSA received comments from 11 interested parties from 5 countries and 10 anonymous contributors. EFSA and its GMO Panel wish to thank all stakeholders for their invaluable contributions. The present report contains the comments received and details how they have been considered for finalisation of the opinion. The final opinion was adopted at the GMO Panel Plenary meeting on 29 September 2022 and published in the EFSA Journal.

Keywords

cisgenesis, intragenesis, plants, genome editing, new genomic techniques, site-directed nucleases, risk assessment

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Over the last ten years, following the requests by the European Commission, the European Food Safety Authority (EFSA) has issued scientific opinions on plants obtained through certain new genomic techniques (NGTs). Among these, EFSA has published two opinions, one on site-directed nuclease (SDN)-1, SDN-2 and oligonucleotide directed mutagenesis (ODM)[1], and one on cisgenesis and intragenesis[2]. After the publication of the EFSA opinion on cisgenesis and intragenesis, an opinion on the safety assessment of plants developed through SDN-3 was also published[3]. In that document, EFSA was also envisaging the possibility to develop cisgenic and intragenic plants using SDN-3 techniques. These scientific opinions have focused on the potential risks associated to the new techniques, compared to conventional breeding techniques and established genomic techniques (EGTs)[4], and on the applicability of existing risk assessment guidance to plants produced with the NGTs under consideration.

The main conclusions of the abovementioned opinions, relevant to the present mandate, are the following:

- Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and transgenic plants.
- Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.
- The existing EFSA Guidance documents are sufficient and applicable in case of plants produced by cisgenesis and intragenesis, and sufficient and partially applicable in case of plants produced by SDN-1, SDN-2 and ODM techniques.
- There is a need for flexibility in the data requirements for the risk assessment, as on a case-by-case lesser amounts of data might be needed.
- SDN-3 opinion concludes that SDN-3 techniques can be used for cisgenesis/intragenesis.

While the scientific opinion on SDN-1, SDN-2 and ODM is very recent, dating from 2020, the cisgenesis/intragenesis and SDN-3 scientific opinions date from 2012. They take into account the techniques available at that time, notably *Agrobacterium*-mediated transformation and direct gene transfer, although several of the considerations therein are not linked to the use of a specific technique. Since 2012, several developments in terms of scientific knowledge and technologies have taken place. In particular, genome editing techniques, including SDN, can now also be used, alone or in combination with other techniques, to produce cisgenic and intragenic organisms, in addition to EGTs.

Against this background, the Commission would like EFSA to confirm whether the considerations and conclusions of EFSA scientific opinion on cisgenesis/intragenesis of 2012 are still applicable[5].

[1] EFSA GMO Panel. Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA Journal 2020;18(11):6299, 14 pp. https://doi.org/10.2903/j.efsa.2020.6299

[2] EFSA GMO Panel. Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA Journal 2012;10(2):2561. [33 pp.] doi:10.2903/j.efsa.2012.2561.

[3] EFSA GMO Panel. Scientific opinion addressing the safety assessment of plants developed using ZFN-3 and other SDNs with similar function. EFSA Journal 2012;10(10):2943. doi:10.2903/j.efsa.2012.2943.

[4] For the purpose of this document, established genomic techniques (EGTs) are those genomic techniques developed prior to 2001, when the existing GMO legislation was adopted, and used to obtain the GMOs authorised in the EU so far. EGTs include techniques such as Agrobacterium-mediated transformation and direct gene transfer.

[5] https://open.efsa.europa.eu/questions/EFSA-Q-2021-00361

1.1.2. Terms of reference

Building on previous work of EFSA, notably the abovementioned scientific opinions on SDN techniques and cisgenesis/intragenesis, the European Commission asks EFSA, in accordance with Article 29 of Regulation (EC) No 178/2002, to provide an updated scientific opinion on the safety and the risk assessment of plants developed through cisgenesis and intragenesis[6].

In particular, EFSA is requested to consider the current state-of-the-art and available knowledge on NGTs and:

1. Identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment.
2. Compare the above-mentioned risks with those associated to plants obtained by conventional plant breeding techniques and plants obtained with EGTs.
3. Determine whether the existing guidelines for risk assessment are applicable, fully or partially, and sufficient[7] to cisgenic and intragenic plants.
4. In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.

1.2. Rationale for the public consultation and brief summary of the outcome

In line with EFSA's policy on openness and transparency, and in order for EFSA to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key issues. Accordingly, the draft opinion together with its annexes was released for public consultation from 16 May 2022 until 27 June 2022 by means of an electronical comment submission tool together with the explanatory text on the EFSA website (See Appendix A). Comments were received from 11 interested parties from 5 countries and 10 anonymous contributors. Table 1 provides an overview on the interested parties that have submitted comments through the electronic submission. Federal Agency for Nature Conservation, Environment Agency Austria, Testbiotech, Association Française de Biotechnologies Végétales and two anonymous contributors uploaded supplementary files through the online tool.

[6] For the purpose of this mandate, the following definitions apply: cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species. (Adapted from Broothaerts, W., Jacchia, S., Angers, A., Petrillo, M., Querci, M., Savini, C., Van den Eede, G. and Emons, H., New Genomic Techniques: State-of-the-Art Review, EUR 30430 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-24696-1, doi:10.2760/710056, JRC121847).

[7] In the context of this mandate, "applicable" means "that can be used for the purpose", "fully applicable" means "that can be used in full", "partially applicable" means "that can be used only in part" and "sufficient" means "that does not need to be complemented".

Table 1: Overview on stakeholder comments received

Stakeholder	Category [a]	Country
Anonymous [b]	on behalf of affiliation/organisation	Belgium
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	National authority	France
Association Française de Biotechnologies Végétales	Non-Governmental Organization - NGO	France
CropLife Europe	Private sector, other	Belgium
Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	National authority	Austria
Federal Agency for Nature Conservation	National authority	Germany
Federal Office of Consumer Protection and Food Safety (BVL)	National authority	Germany
German Federal Institute for Risk Assessment (BfR)	National authority	Germany
International Seed Federation	Non-Governmental Organization - NGO	Switzerland
Reich Martin [c]	Private capacity	Germany
Sciensano - Service Biosafety & Biotechnology	Public institution	Belgium
Testbiotech	Non-Governmental Organization - NGO	Germany
Toussaint Erik [c]	Private capacity	
Union française des semenciers (UFS)	Private sector, other	France

[a] as specified by the commenter

[b] Ten comments received from anonymous contributors

[c] These stakeholders did not submit any comment.

2. Assessment of comments and use for finalisation of the opinion

The comments received were duly evaluated by the EFSA GMO Panel *ad hoc* working group (WG) on Cisgenesis and Intragenesis. Wherever appropriate, these comments were taken into account for finalisation of the draft opinion. Table 2 provides a detailed list with all comments received from interested parties together with EFSA responses and explanations how the comments were considered for finalisation of the draft opinion. Some comments, especially those suggesting editorial changes, have been directly addressed in the text of the opinion, if they were considered appropriate.

Table 2: Stakeholder comments and EFSA responses

Organisation Name	Chapter	Comment	EFSA response	Comment Number
Anonymous	-	-	No comment received	1
Anonymous	4 Conclusions	Line 792-796: we refer to our comments on line 747-748. The conclusions should be amended respectively. There would otherwise also be a contradiction with e.g. lines 715-717	The GMO Panel thanks for the comment. Please note that the conclusion section of the opinion has been rephrased to improve clarity of the text.	2

Anonymous	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	747-748: Crop Wild Relatives and some wild species belong to the 'breeders gene pool' for conventional breeding practices. Although these plants do not have a history of safe consumption as food and feed breeders have practices in place that allow to track specific genes known to influence traits of interest and concern in addition to characterizing more broadly the genetic landscape of new varieties. Importantly, although conventional breeding practices, such as cross or self-pollinating, reshuffle genetic allelic combinations to produce new progeny varieties, these breeding practices do not give rise to unfamiliar biosynthetic pathways that produce novel toxins. Therefore, plant breeders can fine tune their practices depending on the crop and specific known natural toxins inherent to that crop species, thereby ensuring a safe food supply (Trends in Food Science & Technology 100 (2020) 51'66). Subjecting conventional-like plants with cisgenic or intragenic elements to risk assessment requirements would be disproportionate in view of the same plants resulting from conventional breeding practices including the breeders' gene pool. Also, the Novel Food Regulation would be applicable for conventional-like NGT plants if the plant or a plant variety obtained by non-traditional propagating practices gives rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances (DE JONG, BERTOLOTTO AND DE SEZE (2018) FROM FARM TO FORK: THE REGULATORY STATUS OF NON-GMO PLANT INNOVATIONS UNDER CURRENT EU LAW VOL 16 ISSUE 6 Bioscience Law Reviews)	The GMO Panel thanks for the comment. The Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive and the current requirements under IR 503/2013 and EFSA guidances which still apply for the risk assessment of genome-edited plants. However, the section 3.4.1 explains that the risk assessment requirements for cisgenic and intragenic plants obtained through NGTs may vary, taking into account the scale of intervention in the genome and resulting changes in the genotype and phenotype.	3

Anonymous	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Line 715-717: should be part of the conclusion. If it is like a conventional plant the word 'most' should be deleted, because conventional plants are not subject to a risk assessment and it would be discriminatory to include risk assessment requirements for conventional like cisgenic or intragenic plants. From our perspective the following criteria and information requirements should be sufficient to identify for a regulator if a cisgenic or intragenic plant is conventional like (and with this shows a conventional-like rik profile) and should not require any risk assessment: Criteria: 1. there is no novel combination of genetic material (i.e. there is no stable insertion in the plant genome of one or more genes that are part of a designed genetic construct) or 2. the final plant product contains solely the stable insertion of inherited genetic material from sexually compatible plant species or 3. the genetic variation is the result of spontaneous or induced mutagenesis. To determine if a plant fulfills the criteria the following information is sufficient: ` Brief description of the NGT method used to develop the NGT-derived plant (specifically information if vector-derived or transgenic nucleic acid sequences have been introduced) ` Confirmation of the absence of vector-derived or transgenic nucleic acid sequences based on appropriate molecular analysis (if applicable) ` Information on the target gene and description of the intended genetic change(s) resulting from the application of the NGT and based on appropriate molecular analysis' ` Description of the changes in the plant phenotype resulting from the intended genetic change(s) due to the application of the NGT	The GMO Panel thanks for the comment. Regarding lines 715-717, the GMO Panel considers the text to be sufficiently clear. Moreover, please note that the Panel was not mandated to provide a set of criteria for risk assessment of cisgenic and intragenic plants.	4

Anonymous	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	3.2.2.1 elaborates on the possibility to also introduce 'cisfragments' in a targeted manner to either replace segments of a coding gene or to replace regulatory sequences like promotors. The same results can also be achieved by targeted mutagenesis and some of the outcomes of introducing a 'cisfragment' might not be distinguishable from a targeted mutation result. It would be worth elaborating on that as well. Even the introduction of 'intrafragments' might be indistinguishable from targeted edits via mutagenesis. Line 577: the term 'GM species' is unclear in this context and needs to be clarified	The GMO Panel thanks for the comment. Please note that the terms 'cisfragment' and 'intrafragment' have been removed from the text, which has been revised to improve clarity. Regarding line 577, the term 'GM' has been removed.	5
Anonymous	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 406: Since the term 'breeders gene pool' is a term defined by EFSA and not familiar to everybody we suggest to add the EFSA reference where it is defined (EFSA Journal 2012;10(2):2561 ' section 2.1) lines 424-426 / 442-445: The EFSA opinion has highlighted that in some cases similar products can be developed with different technologies and similar hazards can be associated to NGTs and conventionally bred plants (see also line 424-426 of this draft opinion and line 442-445). Nevertheless, EFSA has not considered the disproportionality of subjecting similar products, with similar risk profiles to different regulatory oversight and risk assessment requirements, just based on the breeding method. The EFSA scientific opinions (also the one of SDN-1/2) have mainly focused on the comparison of the plants developed with the NGTs with transgenic plants and on the applicability of the existing risk assessment guidance document for GM plants (see also our comments on 3.2.2.1 and 3.3.2.1 and 3.4.1) Line 450: We welcome the clarification that border sequences from Agrobacterium transformation can occur naturally. This is in line with the approach taken in the UK (https://www.gov.uk/government/publications/acre-	The GMO Panel thanks for the comment. Regarding line 406, the definition of the breeders' gene pool has been added in the footnote. Regarding lines 424-426/442-445, please note that defining which techniques and/or approaches should be regulated is not in the remit of the GMO Panel which operates within the boundaries of the GMO EU regulation. Regarding line 478, it is a citation of a previous document and the wording will not be modified. Regarding line 480, please note that the section 3.2.1.1 has been revised in order to clarify that this section refers to plants covered by EFSA 2012 opinion only.	6

		guidance-on-genetic-technologies-that-result-in-qualifying-higher-plants/technical-guidance-on-using-genetic-technologies-such-as-gene-editing-for-making-qualifying-higher-plants-for-research-trials) Line 478: the term 'illegitimate'is not a scientific term and we suggest to delete it and instead refer to NHEJ or homologous recombination. Line 480 only refers to 'new' risks, we suggest to also clarify that for some applications there are 'less risks' and with this less or no risk assessment requirements.		
Anonymous	3.1.3 NGTs relevant for this mandate	Line 360-361 replace DBS by DSB	The GMO Panel thanks for the comment. The text has been amended accordingly.	7

Anonymous	3.1.2 New Genomic Techniques (NGTs)	line 334-346: In this paragraph EFSA elaborates on the different delivery methods of the reagents that cause the alterations in the genome. If introduced by T-DNA transformation or other methods that involve the stable insertion of the reagents' DNA these 'transgenic' elements are in most cases eliminated in the final product. We suggest adding a paragraph that clarifies the delivery method does not make a difference in terms of risk assessment requirements of the final product, if it is verified that transgenic sequences were eliminated. The Commission has elaborated on the legal status in the context of an animal application (SANTE/E3/FSX/gk (2022)2439122, Letter from 22-04-2022).	The GMO Panel thanks for the comment. Since the GMO Panel was not mandated to describe the risks related to different delivery methods, it does not consider necessary to add the proposed sentences. Moreover, the text has been revised to improve clarity.	8

Anonymous	3.1.1 Established Genomic Techniques (EGTs)	Line 309-311: we welcome the clarification that random mutagenesis includes in vivo and in vitro mutagenesis and both can be considered EGTs. Nevertheless, using the term EGTs is confusing. According to the definition given in 3.1.1 line 313-314 it would be better to talk about transgenic techniques. The Commission questionnaire on NGTs differentiates between conventional plant breeding and classical mutagenesis while here conventional mutagenesis in principle should be understood as an EGT (https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques/public-consultation_en ; e.g. "...do not pose new hazards compared to plants produced with conventional, non-GM breeding techniques, or compared to classical mutagenesis techniques, which are considered as GMOs outside the scope of the legislation, and not subject to risk assessment."). We encourage EFSA and the Commission to use consistent terminology and definitions. The opinion also refers to natural processes (e.g. line 439): The opinion should therefore not only mention conventional breeding and EGTs but also natural processes next to conventional breeding and EGTs.	The GMO Panel thanks for the comment. Regarding the scope of the opinion, EFSA was mandated to compare plants obtained by NGTs to those obtained through conventional breeding and EGTs (ToR2), but not natural processes. Regarding the definition of EGTs, the term is broad and involves a variety of techniques; for this reason it was clarified that in the opinion we refer to those techniques that involve the transfer of genetic material to the host organism. Regarding lines 309-311, the mention to random mutagenesis is an example of techniques that are used, even though plants obtained through random mutagenesis are exempt from GMO legislation. The term 'EGT' was first used in the Explicatory Note on New Technologies in Agricultural Biotechnology (European Commission, 2017). The term does not have a legal definition.	9

Anonymous	2.4.2 Literature search	The statement 'none of the publications reported cisgenic or intragenic products developed by NGTs' seems to contradict line 592 where an example is mentioned	The GMO panel thanks for the comment. Additional text has been added in section 2.4.2 to clarify why certain publications were not retrieved by literature search.	10
Anonymous	1.4 Interpretation of Terms of Reference	Line: 135-137 mentioning the term 'transgenesis' in the context of genes from crossable species does not make sense and is confusing. It is also contradicting EFSA's own definition of the "breeders' gene pool".	The GMO panel thanks for the comment. The sentence has been amended to provide more clarity.	11
Federal Office of Consumer Protection and Food Safety (BVL)	4 Conclusions	794-796: For the sake of consistency, this sentence should be extended as following: 'With respect to the environmental risk assessment, all elements described in the current guidelines can apply to cisgenic/intragenic plants, however on a case-by-case basis, a lesser amount of data might be needed. 797-800: BVL strongly agrees on the conclusion of GMO Panel that more flexibility in the risk assessment of cisgenic/intragenic plants obtained through NGTs is needed. Establishment of some criteria for 'not applicability' may provide more clarity in the decision making process.	The GMO Panel thanks for the comment. Regarding lines 797-800, developping the criteria or categories of hazards/risks that would influence the risk assessment approach is not a part of the current mandate. Regarding lines 794-796, the text has been revised taking note of the comment.	12
Federal Office of Consumer Protection and Food Safety (BVL)	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	748-754: The BVL strongly agrees on that. 757-759: The construction of this sentence is very complex, please rephrase it to make it more readable and understandable. 763: Are there some criteria, how to decide whether the function and expression of an endogenous gene is modified 'profoundly" 765: Regarding 'continuum': This may pose the threat of asking more data for RA even it is not appropriate, e. g. 'for the sake of precaution'. To avoid this, it may be considered to categorize this continuum to simplify to some extent the decision making on RA regarding variation of requirements (e. g. if certain	The GMO Panel thanks for the comment. Regarding lines 757-759 and 763, the sentence has been revised to improve clarity. Regarding line 765, EFSA was not mandated to develop the criteria/categories for risk assessment of cisgenic and intergenic plants.	13

		criteria are fulfilled, the requirements on RA are reduced).		
Federal Office of Consumer Protection and Food Safety (BVL)	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	702-704: What does 'additional risk' assessment mean? It sounds contradictory to 'the requirements that aim to assess any potential hazard may not be relevant'. 719-722: The BVL strongly supports that in case of targeted insertion/modification the cisgenic and intragenic products will not present hazards associated with the unintentional disruption of other genes and/or regulatory elements in the recipient genome, and the requirements of the Regulation (EU) No 503/2013 and guidelines that aim at assessing these unintended effects will not be relevant.	The GMO Panel thanks for the comment. Regarding lines 702-704, the text has been revised to improve clarity.	14
Federal Office of Consumer Protection and Food Safety (BVL)	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Lines 658-660: Generally, BVL strongly supports the conclusion, that for risk assessment of food and feed derived from cisgenic and intragenic plants lesser amounts of event-specific data are needed. However, the case-by-case principle may be interpreted in the way, that every new plant product achieved by cisgenisis or intragenisis will be considered as a new case. Would it not be possible to envisage a categorization of hazards/risks, in which the reduction of amount of data is anchored? Within these categories the case-by-case principle can still be applied. 665-667: The BVL strongly supports the conclusion that in the case of a history of safe consumption of a donor plants as food and	The GMO Panel thanks for the comment. Developing the categories of hazards/risks that would influence the risk assessment approach is not a part of the current mandate.	15

		feed, certain parts of the comparative analysis, toxicity, allergenicity or nutritional assessment may not be necessary. Clarification of 'certain parts' may be helpful. 677-688: Probably, the case-by-case approach is too general and it might be helpful to describe the categories, in which requirements might be reduced in more detail'		
Federal Office of Consumer Protection and Food Safety (BVL)	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	How does EFSA define NEP (for cisgenesis) regarding its alteration? Which alterations (or their extent) should be considered to classify the expressed protein as 'new'?	The GMO Panel thanks for the comment. Any protein in which there is a change in primary structure (amino acid addition, deletion, substitution) would be considered a NEP.	16

Federal Office of Consumer Protection and Food Safety (BVL)	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 441: It might be helpful to substantiate what the wording "varying prevalence" means. It may become a key aspect for determination of a "risk profile" associated with interruption of endogenous sequences on the way to identify proportionate requirements for the risk assessment of certain techniques. In more detail, how to justify that the risk assessment of transgenic plants as a must-have takes into consideration the interruption of endogenous sequence in the light of this opinion's statement that 'insertional mutagenesis is known to occur naturally'? Is EFSA of the opinion that these requirements may not be mandatory for cisgenesis/intragenesis or not mandatory at least when the targeted insertion is used? Please clarify.	The GMO Panel thanks for the comment. Please note that the sentence in question is a quote from the previous opinion. Moreover, while in the conclusions it is stated that the NGTs minimise the risks related to alterations of the host genome, characterisation of the gene interruption is still required for risk assessment.	17
Federal Office of Consumer Protection and Food Safety (BVL)	1.4 Interpretation of Terms of Reference	Line 135: The integration of a reference to the mentioned old definition would be appreciated for comparability, since the current definition is given as a footnote. In fact, since the definitions play a crucial role for this mandate, it could be advisable to place them more prominently within the main text. The differences between these two definitions could be elaborated more clearly. Lines 139-141: Please clarify the phrasing ' it is not clear what is meant here. Lines 141 and 169: Please check the footnote reference ' should it be '6' instead of '5'?	The GMO panel thanks for the comment. The definitions have been added to the main text to highlight the difference between them. Regarding lines 139-141, the sentence has been deleted and the entire paragraph has been amended to improve clarity. Regarding lines 141 and 169, the footnotes have been revised.	18
International Seed Federation	4 Conclusions	Lines 792-796: we refer to our comments on lines 747-748. The conclusions should be amended respectively. There would otherwise also be a contradiction with e.g. lines 715-717;	The GMO Panel thanks for the comment. Please note that the conclusion section of the opinion has been rephrased to improve clarity of the text.	19

International Seed Federation	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	Lines 747-748: Crop Wild Relatives and some wild species belong to the 'breeders gene pool' for conventional breeding practices. Although these plants do not have a history of safe consumption as food and feed breeders have practices in place that allow tracking specific genes known to influence traits of interest and concern in addition to characterizing more broadly the genetic landscape of new varieties. Importantly, although conventional breeding practices, such as cross or self-pollinating, reshuffle genetic allelic combinations to produce new progeny varieties, these breeding practices do not give rise to unfamiliar biosynthetic pathways that produce novel toxins. Therefore, plant breeders can fine-tune their practices depending on the crop and specific known natural toxins inherent to that crop species, thereby ensuring a safe food supply (Trends in Food Science & Technology 100 (2020) 51'66). Subjecting conventional-like plants with cisgenic or intragenic elements to risk assessment requirements would be disproportionate in view of the same plants resulting from conventional breeding practices including the breeders' gene pool	The GMO Panel thanks for the comment and invites International Seed Federation to refer to the response to comment 3.	20

International Seed Federation	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Line 715-717: should be part of the conclusion. If it is like a conventional plant the word 'most' should be deleted because conventional plants are not subject to a risk assessment and it would be discriminatory to include risk assessment requirements for conventional like cisgenic or intragenic plants. From our perspective of the global seed industry, the following criteria and information requirements should be sufficient to identify for a regulator if a cisgenic or intragenic plant is conventional like and should not require any risk assessment. This approach is also followed by many regulatory agencies around the world: Criteria: 1. there is no novel combination of genetic material (i.e. there is no stable insertion in the plant genome of one or more genes that are part of a designed genetic construct) or 2. the final plant product contains solely the stable insertion of inherited genetic material from sexually compatible plant species or 3. the genetic variation is the result of spontaneous or induced mutagenesis. To determine if a plant fulfills the criteria the following information is sufficient: ` Brief description of the NGT method used to develop the NGT-derived plant (specifically information if vector-derived or transgenic nucleic acid sequences have been introduced) ` Confirmation of the absence of vector-derived or transgenic nucleic acid sequences based on appropriate molecular analysis (if applicable) ` Information on the target gene and description of the intended genetic change(s) resulting from the application of the NGT and based on appropriate molecular analysis' ` Description of the changes in the plant phenotype resulting from the intended genetic change(s) due to the application of the NGT	The GMO Panel thanks for the comment. Regarding 715-717, the GMO Panel considers the text to be sufficiently clear. Moreover, please note that the Panel was not mandated to provide a set of criteria for risk assessment of cisgenic and intragenic plants.	21

International Seed Federation	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	This section describes the possibility to also introduce 'cisfragments' in a targeted manner to either replace segments of a coding gene or to replace regulatory sequences like promotors. The same results can also be achieved by targeted mutagenesis and some of the outcomes of introducing a 'cisfragment' might not be distinguishable from a targeted mutation result. It would be worth elaborating on that as well. Even the introduction of 'intrafragments' might be indistinguishable from targeted edits via mutagenesis. Line 577: the meaning of 'GM species' is not really clear in this context and needs to be clarified. Line 577: the term 'GM species' is unclear in this context and needs to be clarified	The GMO Panel thanks for the comment. Please note that the terms 'cisfragment' and 'intrafragment' have been removed from the text, which has been revised to improve clarity. Regarding line 577, the term 'GM' has been removed.	22
International Seed Federation	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	lines 424-426 / 442-445: The EFSA opinion has highlighted that in some cases similar products can be developed with different technologies and similar hazards can be associated with NGTs and conventionally bred plants (see also lines 424-426 of this draft opinion and lines 442-445). Nevertheless, EFSA has not considered the disproportionality of subjecting similar products, with similar risk profiles to different regulatory oversight and risk assessment requirements, just based on the breeding method. This consideration should be added to this section as has important implications for using these products.	The GMO Panel thanks for the comment. Regarding lines 424-426/442-445, please note that defining which techniques and/or approaches should be regulated is not in the remit of the GMO Panel which operates within the boundaries of the GMO EU regulation.	23

International Seed Federation	3.1.2 New Genomic Techniques (NGTs)	lines 334-346: In this section, EFSA lists different delivery methods that cause modifications in the genome. If introduced by T-DNA transformation or other methods that involve the stable insertion of the reagents' DNA these 'transgenic' elements are in most cases eliminated in the final product. We suggest adding a paragraph that clarifies the delivery method does not make a difference in terms of risk assessment requirements of the final product if it is verified that transgenic sequences were eliminated. In many jurisdiction where policies in place on NGTs this approach is used as it is easy to submit experimental evidence showing the final product has no foreign DNA insert or sequences from the genome-editing tool construct.	The GMO Panel thanks for the comment. Since the GMO Panel was not mandated to describe the risks related to different delivery methods, it does not consider necessary to add the proposed sentences. Moreover, the text has been revised to improve clarity on this aspect. Under current regulation the applicant is required to submit evidence on the sequence of the inserted DNA, which will confirm the presence/absence of any foreign sequences.	24

Federal Agency for Nature Conservation	5 References	References from our comments that should be taken into account when revising the draft opinion at hand: Banakar et al (2019) Sci Rep 9:19902. https://doi.org/10.1038/s41598-019-55681-y. Boutin et al. (2022) The CRISPR Journal 5 (1), S. 19’30. DOI: 10.1089/crispr.2021.0120. Eckerstorfer et al. (2019) Frontiers in bioengineering and biotechnology 7, p. 319. DOI: 10.3389/fbioe.2019.00031. Eckerstorfer et al. (2021) BioTech 10 (3), S. 10. DOI: 10.3390/biotech10030010. Hahn & Nekrasov (2019) Plant Cell Rep 38 (4), S. 437’441. DOI: 10.1007/s00299-018-2355-9. Kapahnke et al. (2016) Cells 5 (4). DOI: 10.3390/cells5040045. Kannan et al. (2018) Plant Biotechnol J 16 (4), p. 856’866. DOI: 10.1111/pbi.12833. Kawall (2019) Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525. Kosicki et al. (2018) Nat. Biotechnol. https://doi.org/10.1038/nbt.4192. Mou et al. (2017) Genome biology 18 (1), p. 108. DOI: 10.1186/s13059-017-1237-8. Sharpe et al. (2017) Genome biology 18 (1), p. 109. DOI: 10.1186/s13059-017-1240-0. Sturme et al. (2022) ACS agricultural science & technology 2 (2), S. 192’201. DOI: 10.1021/acsagscitech.1c00270. Thomas et al. (2019) PLoS genetics 15, e1007994. DOI: 10.1371/journal.pgen.1007994. Zsögön et al. (2018) Nature biotechnology 36, p. 1211’1216. DOI: 10.1038/nbt.4272. Zünd et al. (2019) Monitoring of Spontaneous Populations of Genetically Modified Plant Species in the Environment - Experiences and Recommendations for the Design of a Monitoring Programme. Technical Report for EPA, ENCA, IGGMO.	The GMO Panel takes note of the list of references provided in the comment.	25

Federal Agency for Nature Conservation	4 Conclusions	Post-market environmental monitoring (PMEM) is not explicitly included in this draft scientific opinion. However, PMEM is an integral part of the approval procedure. Therefore, the adequacy of existing guidelines for the monitoring of plants developed by cis- and intragenesis should be covered in this issue, as well. PMEM builds on the environmental risk assessment (ERA). Its aims are to confirm the results of the ERA, to identify adverse effects that were not anticipated in the ERA and to detect cumulative, long-term effects. Due to the nature of the interplay of ERA and PMEM, it is important to analyze the adequacy of guidelines for the PMEM parallel to discussing potential risks of cis- and intragenic plants or even a potential decrease of the requirements for their risk assessment (cf. 546-547: 'As already proposed in the 2012 opinion (EFSA, GMO panel 2012a), targeted insertion of the cis- or intragenes should facilitate their risk assessment', 648: 'additional flexibility'). All steps of the tiered-based approval procedure need to be finished completely, before the next step can be undertaken. Aspects must not be shifted from the risk assessment into the PMEM. Cis- or intragenic plants can clearly pose severe risks that need to be investigated before approval. As these risks are case-specific, a generalized comparison with risks of other plants, either originating from conventional breeding or from EGT, cannot be justified. These risks may result from the nature and intended function of the introduced cisgenes themselves, or from the role they play in the new genetic context. In addition, the procedures used to produce cis- and intragenic plants entail additional risks. On-target and off-target unintended effects have already been widely described and recognized in the scientific literature. Therefore, cis- and intragenic plants must	The GMO Panel thanks for the comment. The Panel considers the assessment of PMEM as a part of environmental risk assessment, therefore all ERA-related considerations in this opinion also apply for PMEM.	26

		be subjected to a comprehensive and case-specific risk assessment so that their potential can be safely harnessed.		

Federal Agency for Nature Conservation	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	747-750: Amendment proposed: "...if the outcome of the molecular characterization, including gene expression analysis does not advise differently." Cf. also comment on lines 729-730. 757-761: In order to avoid misleading generalizations that would be inappropriate for a scientific opinion, such a statement should be reserved for the conclusion of the risk assessment of the specific plant and would, among others, require a comprehensive molecular characterization that, on the one hand, can thoroughly demonstrate technique-specific unintended on- and off-target effects, which have already been described frequently in the scientific literature (cf. e.g. comment on lines 650-653), and, on the other hand, actually excludes these in the result. The statement that a genetically modified plant could be safer than a conventionally bred one should be supported by substantiated literature data that addresses, for example, the potential "risk of linkage drag" to the specific objects of protection of the GMO risk assessment. Cf. comment on lines 443-445.	The GMO Panel thanks for the comment. Regarding lines 747-750, the Panel considers the current text sufficiently clear. It is implicit that risks identified through molecular characterisation will be followed up during risk analysis. Regarding lines 757-761, the GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods.	27

Federal Agency for Nature Conservation	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	700-702: Since modifications of gene expression might trigger further alterations in the plant transcriptome, it should be considered to include undirected gene expression analysis in these cases (cf. comm. on lines 729-730). 697-722: The whole section of this draft should be fundamentally revised since statements fail to acknowledge that process-specific effects of SDN at or near the target site have frequently been described in scientific literature, including large deletions or inversions (e.g. Mou et al. 2017), complex genomic rearrangements (Kosicki et al. 2018) and exon skipping which can result in the expression of altered proteins (Mou et al. 2017, Sharpe et al. 2017). Such effects can also be expected in off-target locations and must therefore be assessed genome-wide and evaluated on a case-by-case basis in a comprehensive molecular characterization before risk hypotheses can be tested and conclusions on biosafety can be made (for ref. see 3.3.1.1, lines 650-653 and section 5). 729-730: The environmental risk assessment is largely based on the formulation and assessment of risk hypotheses. However, to be able to sufficiently formulate and eventually evaluate these, a thorough assessment of the modified plant is needed beforehand. Therefore, 1. molecular characterization must take into account on-target and off-target unintended effects, ideally based on undirected analyses such as comprehensive sequencing strategies and, taking into account the flexibility in risk assessment mentioned above, transcriptomic analysis in plants with altered gene expression patterns, and 2. the phenotype of the plant should be assessed depending on the risk hypotheses based on intended and unintended changes including its composition and meaningful ecological parameters.	The GMO Panel thanks for the comment. Regarding lines 700-702, please note that the section 3.3.2.1 states that any specific needs for risk assessment shall be decided on a case-by-case basis. Regarding lines 697-722, the GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods. Moreover, the GMO Panel was not mandated to provide a comprehensive literature review on the SDN-based technology and its unintended effects. Regarding lines 729-730, please note that cisgenic and intragenic plants are subject to risk assessment under IR 503/2013, which include molecular and phenotypic characterisation.	28

Federal Agency for Nature Conservation	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	640-641: This example obviously refers to cisgenesis as defined in the scientific opinion from 2012, therefore, it should be made clear, that the conclusion is not fully valid for plants generated by means of intragenesis. 642-646: In light of new molecular and bioinformatic techniques and information available since 2012, and given the depth of intervention that can now be achieved by employing SDN and information available, such a conclusion is not appropriate (cf. comment on lines 512-516 and 650-653). 650-653: The draft focuses solely on potentially positive biosafety aspects of site-directed integration at the target site. It disregards the present and upcoming potentials and possibilities especially of SDN-1 and SDN-2 for deep genomic interventions (Eckerstorfer et al. 2019 and Kawall 2019), the possibility for unintended changes at and around the target site (e.g. Kapahnke et al. 2016, Kosicki et al. 2018, Mou et al. 2017, Sharpe et al. 2017 and Thomas et al. 2019) and possibly changes due to several steps involved in SDN interventions. Hence, the draft should be revised considering the relevant peer reviewed literature. Eckerstorfer et al. (2019) Frontiers in bioengineering and biotechnology 7, p. 319. DOI: 10.3389/fbioe.2019.00031 Mou et al. (2017) Genome biology 18 (1), p. 108. DOI: 10.1186/s13059-017-1237-8 Kapahnke et al. (2016) Cells 5 (4). DOI: 10.3390/cells5040045 Kawall (2019) Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525 Kosicki et al. (2018) Nat. Biotechnol. https://doi.org/10.1038/nbt.4192 Sharpe (2017) Genome biology 18 (1), p. 109. DOI: 10.1186/s13059-017-1240-0 Thomas et al. (2019) PLoS genetics 15, e1007994. DOI: 10.1371/journal.pgen.1007994	The GMO Panel thanks for the suggestion and takes note of the suggested references. Regarding lines 640-641 and 642-646, the text has been revised to improve clarity and stress that this section applies to the plants covered by the 2012 opinion. The GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods. Moreover, the GMO Panel was not mandated to provide a comprehensive literature review on the SDN-based technology. For this reason, the GMO Panel does not consider it necessary to provide a detailed discussion on the proposed references.	29

Federal Agency for Nature Conservation	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	612-613: Besides hazards 'related to the modifications of the pattern and/or level of expression of the endogenous protein', also those hazards are foreseeable that are related to unintended effects due to the use of certain types of SDN and must be assessed in a comprehensive molecular characterisation, ideally including a global sequencing strategy and transcriptome analysis (cf. comments on lines 618-620, 3.3.1.1, 650-653 and 3.3.2.1, 697-722). 616: Definition of 'NEP' missing. 618-620: This statement is clearly based on the hypothesis that neither off- and on-target effects (e.g. insertions, partial deletions and genome rearrangements), nor insertion of DNA from the SDN apparatus have occurred. However, those are broadly recognised by scientific literature (cf. comment on section 5). The absence of these effects can only be ensured by executing a comprehensive molecular characterisation (cf., e.g., comment on 729-730). Further, this draft and the corresponding EFSA opinions on site-directed nucleases (SDN1, -2 and oligonucleotide directed mutagenesis (ODM) and SDN-3 (EFSA 2020 and 2012)) consider that mutations deriving from conventional breeding, mutagenesis or SDNs are similar in principal. This disregards the fact that the genome becomes more accessible for changes by genome editing compared to conventional breeding (Kawall 2019). Also, intended and off-target effects of SDN do not occur at random positions but prevalently in sequences of high similarity to the target sequence and, hence, can alter several copies of a gene within a genome (e.g. Kannan et al. 2018). 622-625: Since neither rearrangement of genetic elements and partial insertion of coding sequences nor employment of SDN (cf. 577-579) have been considered, the conclusion should be reconsidered. See our	The GMO Panel thanks for the comment and takes note of the suggested references. Regarding lines 612-613 and 618-620, the GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods. To develop the opinion, the GMO panel did take into consideration review and opinion papers but paying particular attention to research papers that provided actual experimental data on off-target mutations. These papers provide evidence that the off-target mutations potentially generated by the application of SDN-based methods for genome editing are of the same type as those produced by conventional breeding including random mutagenesis. Regarding line 616, the explanation of the abbreviation has been added. Regarding lines 622-625, please note that the text has been modified to improve clarity and those lines are no longer included in the text.	30

		comments on 579-582 and 612-613. Kannan et al. (2018) Plant Biotechnol J 16 (4), p. 856’866. DOI: 10.1111/pbi.12833 Kawall (2019) Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525		

Federal Agency for Nature Conservation	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	579-582: According to this definition, cisgenic plants could contain new metabolic pathways, e.g. produced by swapping only the substrate binding domains of corresponding enzymes, thus potentially enabling the production of secondary metabolites that are entirely new (or at least new to the host plant and maybe even to its "crossable species"). To our understanding this definition therefore would include plants, that even meet definitions of synthetic biology (cf. comment on Keywords). Hence, generalising conlusions such as those drawn under 3.2.2.2 (lines 622-625) and 3.3.2.1 (lines 715-717) should be taken with caution and might either have to be reconsidered or their context rephrased for clarity.	The GMO Panel thanks for the comment. Please note that section 3.2.2.1 has been revised to improve clarity. Moreover, EFSA has published two opinions on the accuracy of existing guidelines for the assessment of plants obtained through synthetic biology: https://doi.org/10.2903/j.efsa.2021.6301 and https://doi.org/10.2903/j.efsa.2022.7410 , which are more relevant to the modifications described in the comment.	31

Federal Agency for Nature Conservation	3.2.1.4 If there are new techniques/approaches, what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	561-562: SDN-2 like and SDN-3 approaches will enable and, in some cases of sought-after agronomic traits, almost certainly encourage breeders to expand the gene pool to include 'crossable species' which do not have a history of safe use and that have not previously been included in breeding (for reasons, i.a., laid out in lines 535-537 in the draft document). However, unintended effects could be highly unpredictable when cisgenes 'from a compatible wild species' are expressed in the genetic background of 'a cultivated one', which has experienced genetic separation due to the long history of breeding. On the opposite, attempts of de-novo domestication (Zsögön et al. 2018) should be considered as well because they could practically extend the gene pool of the breeder to plants that, since lacking a history of safe use, certainly should undergo a thorough risk assessment. Hence, the comparison with classical breeding, where the selection process still focuses, besides the intended trait, on the plant's overall performance and characteristics, should therefore at best be made with caution, especially with regard to potential risks. 562-566: The quote above (lines 554-555) correctly does not read "will", but "can optimize the genomic environment [...]". Although these assumptions might, "in some cases, be also true", the current and appropriate safety standards in biotechnology can only be met if off-target and on-target effects, as well as the insertion of additional DNA can be consistently ruled out or are thoroughly investigated. Zsögön et al. 2018: Nature biotechnology 36, p. 1211'1216. DOI: 10.1038/nbt.4272	The GMO Panel thanks for the comment. Regarding lines 561-562, as stated in section 3.1.2.4, use of SDN-3 minimises potential hazards related to changes in the host genome but does not remove the need for their risk assessment. Regarding lines 562-566, the molecular characterisation of the off-target and on-target effects, as well as the insertion of additional DNA is a part of the risk assessment of genetically modified plants.	32

Federal Agency for Nature Conservation	3.2.1.3 Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 opinion?	546-547: While new genomic techniques enable the targeted and potentially precise insertion of genetic material into the genome to obtain cis- or intragenic plants, these techniques do not act error-free. Both on- and off-target effects are common in targeted mutagenesis techniques and have to be assessed accordingly. Please include the work from Banakar et al. 2019, Boutin et al. 2022, Eckerstorfer et al. 2021, Hahn & Nekrasov 2019, Kosicki et al. 2018 and Sturme et al. 2022 into EFSA's considerations. Therefore, a thorough molecular characterization is an important tool for the risk assessment of cis- or intragenic plants. Banakar et al. 2019: Sci Rep 9:19902. DOI : 10.1038/s41598-019-55681-y Boutin et al. 2022: The CRISPR Journal 5 (1), p. 19'30. DOI: 10.1089/crispr.2021.0120 Eckerstorfer et al. 2021: BioTech 10 (3), p. 10. DOI: 10.3390/biotech10030010 Hahn & Nekrasov 2019 Plant Cell Rep 38 (4), p. 437'441. DOI: 10.1007/s00299-018-2355-9 Kosicki et al. 2018 Nat Biotechnol 36: p765'771. https://doi.org/10.1038/nbt.4192 Sturme et al. 2022: ACS agricultural science & technology 2 (2), p. 192'201: DOI: 10.1021/acsagscitech.1c00270	The GMO Panel thanks for the comment. To develop the opinion, the GMO Panel did take into consideration review and opinion papers but paying particular attention to research papers that provided actual experimental data on off-target mutations. These papers provide evidence that the off-target mutations potentially generated by the application of SDN-based methods for genome editing are of the same type as those produced by conventional breeding including random mutagenesis.	33

Federal Agency for Nature Conservation	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	485-488: Cis-and intragenesis use NGT and can efficiently target new species such as perennials. Also, cis-and intragenesis of non-crop plants may expand the scope of cultivation to include private gardens and semi-natural habitats. These organisms and areas have neither been assessed in ERA nor monitoring yet and suitable monitoring methods and concepts thus need to be developed. The current monitoring of GMP in Europe is not fit to cover new cultivation regions. The farmer questionnaires as part of the general surveillance e.g. focus on the farmers observations on their fields. It is not fit to provide data in other forms of cultivation e.g. in private gardens. Thus, adaptations or alternatives for the current monitoring have to be explored and established. The current monitoring of GMP authorized for food and feed focus on preventive measures such as HACCP. But, potential entry points into the environment such as e.g. transport routes should be also subjected to a scientific monitoring of environmental effects. Zünd et al. 2019 offer a conceptual basis and the VDI-guidelines provide standardized monitoring methods (www.vdi.eu/engineering/vdi-standards). 512-516: Assessing literature on plants only and restricting to full coding sequences weakens the analysis, since it neglects the possibility of extended stacking to reach much higher expression levels (cisgenesis) and recombination of genetic elements to achieve metabolic pathways new to the host plant which are, potentially, entirely new (resembling elements of synthetic biology through intragenesis, which is already common practice in microorganisms, e.g. yeast cells expressing certain food additives). In context of the guiding character that scientific opinions on techniques do have in risk assessment, restricting of the analysis to biotechnology	The GMO Panel thanks for the comment. Regarding lines 485-488, the GMO panel reminds that cisgenic and intragenis plants are still subject to GM regulations, including the required monitoring. For this reason, the Panel considers it unlikely that they will be cultivated in private capacity, e.g. in gardens, due to the requirement for monitoring and containment measures. Regarding lines 512-516, EFSA has published two opinions on the accuracy of existing guidelines for the assessment of plants obtained through synthetic biology: https://doi.org/10.2903/j.efsa.2021.6301 and https://doi.org/10.2903/j.efsa.2022.7410 , which are more relevant to the applications mentioned.	34

		applications that are already published and neglecting their potential in our view does not suit its purpose. Zünd et al. (2019) Technical Report for EPA, ENCA,IGGMO		

Federal Agency for Nature Conservation	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	434-436 and 480-481: The view expressed here disregards potential accumulation of off-target effects in similar genetic sequences ('off-target-effects') when applying state-of-the-art SDN. Hence, depending on the nature of the target sequence, more rather than less unintended functional deficiencies could occur in the plant in comparison to 'random' mutagenic events, which are expected to be more evenly distributed throughout the genome. Cf. comment on lines 650-653. 438-445: Transposons and retrotransposons are genetic elements that do not randomly insert into the genome, but have regions of higher insertion rate than others (e.g. Bourque et al., 2018). Comparing new genomic techniques and their results to evolutionary proven genetic elements is therefore not suitable. New genomic techniques can also target conserved genomic regions (Kawall 2019 and references therein) which is not possible with conventional breeding or EGT. Even though the plant's genome is not a fixed entity, new genomic techniques have the potential to introduce changes that access highly conserved genetic regions which can have potential adverse effects on the plant metabolism and its interactions with non-target organisms. 443-445: The approach of comparing SDN techniques with conventional breeding this draft and the EFSA Opinions on SDN are at least partially based upon is questionable, as it disregards that conventional breeding and genome editing take two distinct approaches to achieve new traits: one mainly phenotype-based and the other mainly genotype-based. Conventional breeding involves increasing genetic diversity in a first step and then narrowing it down by selection and backcrossing in the following steps, whereas most SDN applications attempt to achieve new traits in one step. Therefore, the idea of	The GMO Panel thanks for the comment. Regarding lines 434-436 and 480-481, please note that the assessment of unintended effects of genetic modifications is a part of molecular characterisation of the GM plants, including cisgenic and intragenic plants obtained by NGTs. Regarding lines 443-445, please note that the opinion states that cisgenic and intragenic plants may be considered similar to conventionally bred ones specifically in terms of the source of genetic material.	35

		on-target and off-target changes can only apply to SDN interventions, but not to conventional breeding, and must therefore be considered in the risk assessment.		

Federal Agency for Nature Conservation	1.4 Interpretation of Terms of Reference	The current update of EFSA's 2012 opinion is to be welcomed in principle because it is opportune. The mandate given by EU COM to EFSA comprises four relatively general tasks regarding the identification of potential risks and 'the risk assessment of plants developed through cisgenesis and intragenesis'. Even though it was requested to build on previous work of EFSA, i.e. mainly the EFSA opinions 2012a, 2012b and 2020 (cf. Table 2.) and to compare identified 'risks with those associated to plants obtained by conventional plant breeding techniques and plants obtained with [established genomic techniques]', the way in which the four tasks are divided into specific questions for the purpose of operationalisation leads to a lack of clarity in the answers, which seems neither appropriate to the initial request nor for a scientific opinion of guiding character for the risk assessment. This is partially due to the comparisons with the previous exercises that were focusing on different techniques, which did not consider state-of-the-art SDN and its potential technique-specific unintended effects known today and which the draft at hand still fails to acknowledge (cf. comment on 3.3.1.1, lines 650-653). This in turn leads to false assumptions in the generalised comparisons with conventional plants, which are not useful for answering questions of biosafety and risk assessment. In contrary existing risks of cis- and intragenesis are not sufficiently acknowledged. (cf. comment on 3.2.1.1, lines 443-445).	The GMO Panel thanks for the comment. Please note that the risks related to SDN techniques have been addressed by EFSA in its previous opinions (EFSA GMO Panel 2012b, 2020). Moreover, the sections 3.2.1.1 and 3.2.1.2 describe the risks related to cis- and intragenesis, and the further sections do not identify any new risks specific to cis- and intragenic plants obtained through NGTs. Please note that the opinion discusses plants and plant-derived products already covered by the EFSA 2012 opinion (mainly obtained by EGTs), and those not covered by the EFSA 2012 opinion. The GMO Panel considers this structure sufficiently clear and useful to address all potential products that could be achieved with the established and newly developed techniques.	36
Federal Agency for Nature Conservation	Keywords	Please add "Synthetic Biology" to the keywords. Cf. comment on 3.2.2.1, specifically on lines 579-582.	The GMO Panel considers the keywords' list exhaustive.	37

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	4 Conclusions	The chapter needs to be thoroughly revised with a view to the general and specific comments provided in our comments. Some conclusions directed to lessen the requirements for RA for cisgenic and intragenic plants obtained through NGTs ' particularly generalized conclusions which contradict the case-by-case approach which is recommended in the draft updated opinion at hands ' are not sufficiently justified in our opinion and need to be revised. See also the attached document with our compiled coments	The GMO Panel thanks for the comment. The literature search (2.4.2) and conclusion (4) sections of the opinion have been rephrased to improve clarity of the text. Regarding the general comments in the attached document, please note that the opinion does not recommend overall lesser evidence requirements for conducting a RA for cisgenic/intragenic plants, but rather a case-by-case approach. Regarding the literature search, please note that the literature retrieved after 2011 was not excluded from further consideration. Moreover, EFSA was not mandated to conduct a full literature search on cisgenesis and intragenesis, and the literature search served to inform the experts on current state of the art. The GMO Panel took note of the suggested publication and considers the conclusions still valid. Regarding the additional references, the experts are entitled to propose additional references that could contribute to developing the opinion. Finally, please note that the list of all retrieved and selected publications and patents is published as an annex together with the final version of the scientific opinion.	38

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	Line 753-754: It also needs to be taken into consideration whether the ex-pression of the cisgenic / intragenic construct is significantly different from its expression in the parental plant. A substantially increased expression could lead to effects which are not documented for the donor plant. Line 766-767: The discussion and the examples provided in sect. 3.3.2.1 should be revised with a view to the comments provided above. In addition the conclusion presented in the indicated lines doesn´t seem to be justified and should be revised as well.	The GMO Panel thanks for the comment. Regarding lines 753-754, this aspect has been discussed in section 3.3.2.1 of the opinion. Regarding lines 766-767, after the revision of the document, the GMO Panel considers the conclusion still valid.	39
Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Line 715f: Such plants may present risks if the transferred sequence ('cis-fragment') is derived from a plant which was not used in agriculture or food production previously and for which no history of safe use is available! This needs to be indicated in the text ' the general statement included in the draft updated opinion and the general reference to conventional plants is not ap-propriate! Line 722: Also any unintended modifications which are created by the insertion of the 'cisfragment' and which are linked to the intended trait need to be assessed properly.	The GMO Panel thanks for the comment. Regarding line 715, if the cisgenic/intergenic plant contains an allele from a species with no history of safe use, the GMO Panel would take that into account when applying the case-by-case approach for risk assessment. Regarding line 722, here only the unintended gene interruption is discussed, and not other unintended effects.	40

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Line 640-641: In case T-border sequences are part of the inserted construct, an assessment for the presence of unintended ORFs should be conducted since these elements create internal junctions in the 'insert'; a molecular characterisation should also be conducted for the presence of unintended in-sertions/deletions at / around the target site for SDN-3 modifications. Line 650ff: The conclusions that lesser data for RA might be needed can only be drawn on a case-by-case basis, not as a generalized statement for all SDN-3 constructs as implied by the draft updated opinion. In particular for such constructs / products an adequate molecular characterization needs to be provided to assess the presence of unremoved transgenic alterations and off-target modifications (Lema 2021). To characterize off-target modifications and other unintended changes the 10 step approach described in Ecker-storfer et al. (2019) should be used. Line 665: Lesser requirements for RA as indicated in the draft updated opin-ion could only apply if the expression of cisgenes and intragenes is not sig-nificantly different from the normal range of expression in closely related plants (see also Lines 700-702). Eckerstorfer, M. F., Grabowski, M., Lener, M., Engelhard, M., Simon, S., Dolezel, M., et al. (2021). Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. BioTech 10, 10. doi: 10.3390/biotech10030010 Lema, M. (2021). Regulatory Assessment of Off-Target Changes and Spurious DNA In-sertions in Gene-Edited Organisms for Agri-Food Use. Journal of Regulatory Science Vol. 9 No. 1 (2021): Special Issue on Genetically Modified Organisms.	The GMO Panel thanks for the comment. Regarding lines 640-641, if the insert contains T-border sequences and additional junctions are created, the potential of unintended ORFs will be assessed according to the current GM guidelines. Regarding line 650, the text of the opinion has been revised to further stress that the data requirements for risk assessment are applicable on a case-by-case basis. Moreover, The GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods. Regarding line 665, the Panel considers the term 'normal range of expression' not sufficiently specific, since expression levels vary depending on external conditions, developmental stage of the plant and many other factors. An explanation of the rationale for the proposed change is not sufficiently justified and the text will not be changed.	41

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	Line 616: Indicate what the abbreviation NEP is standing for (newly ex-pressed protein?).	The GMO Panel thanks for the comment. The meaning of the abbreviation has been explained in the text.	42
Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	Line 590ff: The discussion of the publication of Shi et al. (2017) exposes the flaws in the concept of 'cisfragments' and 'intrafragments' as introduced by the draft updated opinion. It is stated that this 'report is a clear example of a cisgenic approach used to create allelic variation for enhancing crop drought tolerance'. However, according to the definitions referred to in section 1.3 (Terms of Reference) and footnote 6 it is abundantly clear that this is wrong and misleading. The reported development is indeed an intragenic product combining promoter sequences from the maize GOS2 gene with the coding region of the maize ARGOS8 gene and needs to be characterized as such. The term 'cisfragments' is not helpful in this respect. It rather obfuscates the interpretation of the presented information as evidenced by the wording of Lines 600-601 of the draft updated opinion. The section therefore needs to be thoroughly revised to correct for that! Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., et al. (2017). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15, 207'216. doi: 10.1111/pbi.12603	The GMO Panel thanks for the comment. Please note that the terms 'cisfragment' and 'intrafragment' have been removed from the text, which has been revised to improve clarity.	43

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.2.1.4 If there are new techniques/approaches , what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	See attached doc for comments	The GMO Panel thanks for the comments. Please note that plants developed by NGTs are subject to GM regulations and as such undergo detailed molecular and phenotypic characterisation as a part of risk assessment. This characterisation includes any potential unintended effects. The Panel considers the present guidelines as sufficient and partially applicable for the risk assessment of cisgenic/intragenic plants developed by NGTs.	44
Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	Line 543: The draft updated opinion fails to mention the development of plants containing TLP regions (Ainley et al., 2013; Kumar et al., 2015 and 2016), which may also be used for the development of commercially relevant cisgenic / intragenic / transgenic plants (Eckerstorfer et al. 2021). Ainley, W. M., Sastry-Dent, L., Welter, M. E., Murray, M. G., Zeitler, B., Amora, R., et al. (2013). Trait stacking via targeted genome editing. Plant Biotechnol J 11, 1126'1134. doi: 10.1111/pbi.12107 Eckerstorfer, M. F., Grabowski, M., Lener, M., Engelhard, M., Simon, S., Dolezel, M., et al. (2021). Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. BioTech 10, 10. doi: 10.3390/biotech10030010 Kumar, S., AlAbed, D., Worden, A., Novak, S., Wu, H., Ausmus, C., et al. (2015). A modu-lar gene targeting system for	The GMO Panel was not mandated to provide neither a comprehensive literature review nor a horizon scan on the SDN-based technology or TLPs in particular. For this reason, the GMO Panel considers not to be necessary to include in the opinion the sections proposed in the comment on the TLPs.	45

		sequential transgene stacking in plants. J Biotechnol 207, 12’20. doi: 10.1016/j.jbiotec.2015.04.006 Kumar, S., Worden, A., Novak, S., Lee, R., and Petolino, J. F. (2016). A trait stacking sys-tem via intra-genomic homologous recombination. Planta 244, 1157’1166. doi: 10.1007/s00425-016-2595-2		

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 406/423ff: The draft update opinion defines cisgenes as "specific al-leles/genes present in the breeders' gene pool, without any change to the DNA sequence". We note that the 'breeder´s gene pool' due to modern breeding methods (e.g. wide crosses, hybrid rescue, etc.) is quite broad and will include genes / traits from crossable varieties or species that do not have a history of safe use in agriculture and food production. Therefore cisgenic products may also contain 'novel' traits which need to be fully assessed for their food safety and environmental safety. Thus the conclusion from the previous opinion (EFSA 2012) cited in the draft updated opinion (Line 423ff) needs to be put in context ' with respect to the novelty of the integrated cis-genes / intragenes and the possible effect of changed expression of these genes in the context of the cisgenic /intragenic plant (see above comment to Line 320-322 and the reference to Holme et al. (2020) provided in section 3.2.1.2). Line 450: The text mentions the likely presence of short T-border sequences in the cisgenic / intragenic plants, but fails to adequately discuss the conse-quences of the presence of such sequences: the notion that 'similar se-quences can be found in different plant species" is not considered relevant in this respect. This is rather deemed a flawed comparison (comparing apples with oranges) and not of specific relevance for the RA of individual specific cisgenic / intragenic plants and its outcome. Line 480-481: 'The EFSA GMO Panel does not identify new risks compared to what was identified in the 2012 opinion'. The draft updated opinion, how-ever, also fails to provide an improved discussions related to potential haz-ards due to (novel) cisgenes / intragenes and/or modified expression of such constructs. Thus the above conclusion should be revised based on an im-proved	The GMO Panel thanks for the comment. Regarding line 406, the opinion calls for a case-by-case approach, i.e. novelty of the introduced traits will be taken into account when conducting the risk assessment of cisgenic and intragenic plants. Regarding line 450, the presence and risks related to short T-DNA sequences was discussed in EFSA's 2012 opinion and the conclusions remain valid. Regarding line 480, please note that the section 3.2.1.1 has been revised in order to clarify that this section refers to plants covered by EFSA 2012 opinion only. Moreover, the GMO Panel has not identified any new hazards for the plants covered by the 2012 opinion which have not already been covered by the previous opinion.	46

		and more nuanced discussion of the mentioned aspects.		

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.1.2 New Genomic Techniques (NGTs)	Line 329-330: The draft updated opinion introduces target specificity as a distinctive characteristics of certain NGTs, however misses to provide an ad-equate discussion of the phenomenon throughout the document. Target specificity is neither an absolute nor the same for the many different existing SDN-tools. As reviewed previously (Eckerstorfer et al., 2021; European Commission. Joint Research Centre, 2021) different NGT approaches are associated with a different probability for off-target activity at other genomic loci than the target sequence for insertion of cisgenic or intragenic con-structs. Such off-target edits need to be taken into account during molecular characterisation of cisgenic / intragenic plants in addition to ensure that trans-genic constructs integrated for delivery of the tools for the SDN interventions are actually removed from the developed NGT plants (Lema, 2021). Eckerstorfer, M. F., Grabowski, M., Lener, M., Engelhard, M., Simon, S., Dolezel, M., et al. (2021). Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. BioTech 10, 10. doi: 10.3390/biotech10030010 European Commission. Joint Research Centre. (2021). New genomic techniques: state of the art review. Publications Office. Lema, M. (2021). Regulatory Assessment of Off-Target Changes and Spurious DNA In-sertions in Gene-Edited Organisms for Agri-Food Use. Journal of Regulatory Science Vol. 9 No. 1 (2021): Special Issue on Genetically Modified Organisms	The GMO Panel thanks for the comment and takes note of the proposed literature. The GMO Panel was not mandated to evaluate the target specificity of SDN tools, but rather to assess the applicability of the current guidelines on the assessment of cisgenic/intragenic plants. The GMO Panel reminds that the characterization of the unintended effects is part of the risk assessment for plants generated by SDN methods.	47

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	3.1.1 Established Genomic Techniques (EGTs)	Line 320-322: The potential consequences of the random integration on ex-pression are at least twofold: on the one hand consequences on the expres-sion of endogenous genomic plant genes at or near the integration site and on the other hand consequences for the expression of the integrated con-struct itself. The latter effect is well-known in the scientific literature (Atkinson & Halfon, 2014; Buchberger et al., 2019; Nagy-Staron et al., 2021) and needs to be fully taken into account during RA. Atkinson, T. J., and Halfon, M. S. (2014). Regulation of gene expression in the genomic context. Computational and Structural Biotechnology Journal 9, e201401001. doi: 10.5936/csbj.201401001 Buchberger, E., Reis, M., Lu, T.-H., and Posnien, N. (2019). Cloudy with a Chance of In-sights: Context Dependent Gene Regulation and Implications for Evolutionary Studies. Genes (Basel) 10. doi: 10.3390/genes10070492 Nagy-Staron, A., Tomasek, K., Caruso Carter, C., Sonnleitner, E., Kav'i', B., Paixão, T., et al. (2021). Local genetic context shapes the function of a gene regulatory network. Elife 10. doi: 10.7554/eLife.65993	The GMO panel thanks for the comment. Please note that the potential consequences mentioned in the comment are assessed as a part of the molecular characterisation of GM plants.	48

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	2.4.2 Literature search	Line 284 - 299: The presentation and discussion of the results of the literature search needs to be improved as indicated in the general comments: Why was relevant literature not retrieved during the search? - Which patents were identified as relevant and what is their relevance? - Are the patents describing the construction of crops modified to contain trait-landing pads (TLPs) and could these TPLs be used to integrate cisgenic / transgenic constructs?	The GMO Panel thanks for the comment. The text of the paragraph has been revised to improve clarity. None of the retrieved publications reported cisgenic/intragenic approaches obtained via NGTs. The GMO Panel highlights the fact that the success in this kind of searches depends on the presence of the relevant searchable terms in the text of the publication. If a publication describes a New Genomic Technique without mentioning any of the relevant terms (i.e. cisgenesis/cisgenic, intragenesis/intragenic etc.) the search fails to retrieve it. Therefore, experts have the possibility to add additional publications if they deem it necessary. The GMO Panel was not mandated to express an opinion on whether or not TLPs could be used to integrate cisgenic constructs.	49

Environment Agency Austria on behalf of the Austrian Federal Ministry for social affairs, health, care and consumer protection	1.4 Interpretation of Terms of Reference	Pls. see our general comments addressed to the overall draft updated opinion submitted in the attached document. Specific comments to ToR sections: Line 120ff and footnote 6: The provided definition is acknowledged; however it disregards the fact that the incorporated sequences likely comprise of sequences that are not cisgenic, e.g. integrated Agrobacterium T-Plasmid border sequences or other heterologous sequences that are co-integrated together with the cisgenic or intragenic construct (Wilson et al. 2006). The relevance of these sequences also needs to be characterised at a molecular level and needs to be taken into consideration during RA. The existence of such heterologous sequences in cisgenenic / intragenic plants is acknowledged in the draft updated opinion - section 3.2.1.2; Holme et al. (2012) and Miroshnichenko et al. (2020) - how-ever the consequences of the presence of such sequences of varying length are not sufficiently discussed. Holme, I. B., Wendt, T., and Holm, P. B. (2013). Intragenesis and cisgenesis as alterna-tives to transgenic crop development. Plant Biotechnol J 11, 395-407. doi: 10.1111/pbi.12055 Miroshnichenko, D., Timerbaev, V., Okuneva, A., Klementyeva, A., Sidorova, T., Pushin, A., et al. (2020). Enhancement of resistance to PVY in intragenic marker-free potato plants by RNAi-mediated silencing of eIF4E translation initiation factors. Springer Nether-lands.	The GMO panel thanks for the comment. Please note that the integration of non-cisgenic sequences is well-described in the EFSA opinion (2012a) for plants and derived products obtained through EGTs. For those products, no new risks have been identified. Plants obtained through NGTs should not contain those heterologous sequences. Please note that the description of inserted sequences is a part of the molecular characterization assessment, under the current regulation. For the replies to the points raised in the attached document, please refer to the reply to the comment 38.	50

Union française des semenciers (UFS)	4 Conclusions	The paragraph 792-796 must be developed to highlight that no risk assessment is needed. The conclusion of the 2012 Study remains valid and the rise of the NGT improves the technique. Then no risk assessment should be required too. The last paragraph (797-801) doesn't really fit with the statement written previously (711-717 and 792-796).	The GMO Panel thanks for the comment. Please note that the conclusion section of the opinion has been rephrased to improve clarity of the text. Regarding lines 792-796, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive, which means that they must be risk-assessed according to IR 503/2013.	51
Union française des semenciers (UFS)	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	It should be mentioned that crosses with crop wild relatives do not require any risk assessment and is considered as conventional breeding. A cross do not give rise to unfamiliar biosynthetic pathways while it shuffles all the genetic combinations.	The GMO Panel thanks for the comment. The Panel reiterates in the conclusions of the opinion that related to the source of the DNA, the cisgenic plants are similar to those obtained by crossing, but they make use of the same techniques as transgenesis and are subject to GM regulations and risk assessments. The GMO Panel do not consider necessary to add the proposed sentence.	52

Union française des semenciers (UFS)	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	UFS: Line 715-717 : This is the main conclusion of the study and should be part of the conclusion as well and could be written in a most straight forward way. If the risk are the same than conventional plant, then, risk assessment is not required.	The GMO Panel thanks for the comment. The conclusion section of the opinion has been rephrased to improve clarity of the text. Moreover, the cisgene/intragene products are considered GM and as such are subject to Regulation (EU) No 503/2013.	53
Union française des semenciers (UFS)	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	Line 577 : We wonder why the word 'GM species' is used. It should probably be replaced by another word.	The GMO Panel thanks for the comment. The term 'GM' has been removed.	54
Union française des semenciers (UFS)	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	line 485. We guess that 'Intragenic' should replace the word 'transgenic'	The GMO Panel thanks for the comment. The text has been amended accordingly.	55

Union française des semenciers (UFS)	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 406 The term Breeders' gene pool needs a definition to avoid misunderstandings. It has been previously defined in another EFSA study and the reference is needed (EFSA Journal 2012;10(2):2561 - section 2.1)) or the definition given. 422-427 : This conclusion from the 2012 EFSA study should be highlighted. The current study concludes again that cisgenesis and conventional breeding leads to similar risk. This statement is important and needs to be put forward. 478 : We propose to remove the word 'illegitimate' to avoid interpretation. A more scientific term could be use.	The GMO Panel thanks for the comment. Regarding line 406, the definition of the breeders' gene pool has been added in the footnote. Regarding lines 422-427, please note that the Conclusions section has been revised to improve clarity and better put forward the main message of the opinion. Regarding line 478, it is a citation of a previous document and the wording will not be modified.	56
Union française des semenciers (UFS)	3.1.3 NGTs relevant for this mandate	There is typing mistake at the line 360. 'DBS' should be replace by 'DSB'	The GMO Panel thanks for the comment. The text has been amended accordingly.	57

Union française des semenciers (UFS)	2.4.2 Literature search	Line 309 : we welcome the clarification that random mutagenesis includes in vivo and in vitro mutagenesis and both can be considered Established genetic technique. Line 313 : the use of the words 'Genetic alterations' doesn't bring clarity.	The GMO panel thanks for the comment. Regarding line 309 and the definition of EGTs, the term is broad and involves a variety of techniques; for this reason, it was clarified that in the opinion we refer to those techniques that involve the transfer of genetic material to the host organism. Please note that the mention to random mutagenesis is an example of techniques that are used, even though plants obtained through random mutagenesis are exempt from GMO legislation. Regarding line 313, the sentence has been modified to improve clarity.	58
Union française des semenciers (UFS)	1.4 Interpretation of Terms of Reference	Line 135-137. The 2012 EFSA study doesn't focus on transgenesis, this term should be removed as it can be confusing.	The GMO panel thanks for the comment. The sentence has been edited to improve clarity.	59

CropLife Europe	4 Conclusions	Line 782: We suggest to edit 'Targeted integrations potentially allow ", this more accurately reflects the lack of supporting literature. Line 792-796: We refer to our comments on line 747-748. The conclusions should be amended, capturing the statement in lines 715 to 717. We advocate for a non-discriminatory and proportionate system where similar products are treated in a similar manner by establishing a process whereby a regulator determines a plants' regulatory status on a case-by-case basis. From our perspective, the following criteria and information requirements should be sufficient to identify for a regulator if a cisgenic or intragenic plant requires any risk assessment: 1. there is no novel combination of genetic material (i.e. there is no stable insertion in the plant genome of one or more genes that are part of a designed genetic construct) or 2. the final plant product contains solely the stable insertion of inherited genetic material from sexually compatible plant species or 3. the genetic variation is the result of spontaneous or induced mutagenesis. To determine if a plant fulfills the criteria the following information is sufficient: ' Brief description of the NGT method used to develop the NGT-derived plant (specifically information if vector-derived or transgenic nucleic acid sequences have been introduced) ' Confirmation of the absence of vector-derived or transgenic nucleic acid sequences based on appropriate molecular analysis (if applicable) ' Information on the target gene and description of the intended genetic change(s) resulting from the application of the NGT and based on appropriate molecular analysis' ' Description of the changes in the plant phenotype resulting from the intended genetic change(s) due to the application of the NGT	The GMO panel thanks for the comment. Regarding line 782, targeted integrations in general are discussed, not only in the context of plant cisgenesis and intragenesis. Therefore, there is sufficient supporting literature for this statement. Regarding lines 792-796, the Panel takes note of the proposed criteria but would like to clarify that the Panel was not mandated to provide a complete list of all the requirements and criteria for risk assessment of cisgenic and intragenic plants.	60

CropLife Europe	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	Line 747-748: Crop Wild Relatives and some wild species belong to the 'breeders gene pool' for conventional breeding practices. Although these plants do not have a history of safe consumption as food and feed, breeders have practices in place that allow to track specific genes known to influence traits of interest and concern in addition to characterizing more broadly the genetic landscape of new varieties. Importantly, although conventional breeding practices, such as cross or self-pollinating, reshuffle genetic allelic combinations to produce new progeny varieties, these breeding practices do not give rise to unfamiliar biosynthetic pathways that produce novel toxins. Therefore, plant breeders can fine tune their practices depending on the crop and specific known natural toxins inherent to that crop species, thereby ensuring a safe food supply (Trends in Food Science & Technology 100 (2020) 51-66). Subjecting conventional-like plants with cisgenic or intragenic elements to risk assessment requirements would be disproportionate in view of the same plants resulting from conventional breeding practices including the breeders' gene pool. Line 753-754: EFSA indicates the mandatory requirement for a 90-day study may not be needed. Nevertheless, EFSA remains vague and general regarding other studies related to toxicity, allergenicity, etc, and it is not clear why only the 90-day study is specified. It would be helpful if EFSA considers including clear and detailed case studies, or more specifics on which studies may or may not be needed. This will provide clarity to applicants. Line 756: the statement 'the range of cisgenic and intragenic plant products has increased noticeably' is not supported by literature cited in the opinion and should be considered to be rephrased Line 759: should include (e.g. through an SDN2 like strategy), as there are multiple techniques	The GMO Panel thanks for the comment. Regarding lines 747-748, the Panel invites CropLife Europe to refer to the response to comment 3. Regarding lines 753-754, EFSA has not been mandated to develop specific criteria and case studies for the requirements for risk assessment of cisgenic and intragenic plants. Regarding lines 756 and 765, the text has been amended taking the comment into account. Regarding line 759, the GMO Panel considers the text accurate as it refers to a specific technique covered by the terms of reference.	61

		that could lead to the described change. Line 765: Suggest to delete 'extreme' to keep it factual.		

CropLife Europe	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Lines 690 - 693: It should be acknowledged that this can also be possible with conventional breeding Line 702-705: It is unclear why a change in the function or expression level of the endogenous protein would be a trigger for a need for an additional risk assessment. Such changes to endogenous proteins can happen through traditional breeding or natural processes, which do not require a risk assessment. Therefore, the function or expression level ranges of an endogenous protein can change continuously. Therefore, EFSA should clearly explain the rationale for this statement to ensure a clear understanding by applicants when an additional risk assessment would be needed or not, and what is meant with 'additional' risk assessment. Lines 706-709: Elaborate on 'differences' referred to. Line 715-717: Should be part of the conclusion. Suggest deleting 'most, if not all', since conventional plants are not subject to a risk assessment and it would be discriminatory to include risk assessment requirements comparable cisgenic plants. Line 725: conventional plants are not subject to an environmental risk assessment and it would be discriminatory to include risk assessment requirements comparable cisgenic plants Line 737: Aligned with our previous comments we recommend to add: 'plants and products. For some cisgenic plants, some or all risk assessment requirements would not be relevant.	The GMO panel thanks for the comment. Regarding lines 690-693, it is stated in the text of the opinion that some changes introduced by cisgenesis/intragenesis can also be obtained by conventional breeding and the GMO Panel considers the current text sufficiently clear. Regarding lines 702-705, please note that the sentence has been modified to improve clarity; the term 'additional' has been deleted. Regarding line 706-709, some examples have been added. Regarding comment to lines 715-717, the GMO Panel considers the text to be sufficiently clear. Regarding line 725, the cisgene/intragene products are considered GM and as such are subject to Regulation (EU) No 503/2013. Therefore, ERA is required. Regarding line 737, the text has been revised to clarify that only cis/intragenic plants obtained through NGT are considered there.	62

CropLife Europe	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Lines 637-684: We are concerned that the previous conclusion is missing that targeted insertion of the cisgene would generate similar plants (with similar risk) to those obtained through classical breeding. With the exception of the reference to less requirements in the molecular characterization, we regret that there is no similar science-based rationale in the food/feed or ERA. Line 648-649: We recommend for EFSA to include clarification on the proposed flexibility (e.g. when some data requirements would be only partially or not applicable at all). This is even more important for the next paragraph (Lines 650-654) when the risk associated with the development of cisgenic plants with SDN-3 technology may determine unnecessary the assessment of certain potential hazards. Line 657: We suggest editing the sentence since it is a past opinion: ".for risk assessment of food and feed from GM plants (EFSA, 2011) was, at that time, sufficient' and in the line 660 amend: plants it was envisaged that' Lines 660-661: refer to the conclusions of the 2012 indicating that "on a case-by-case basis, lesser amounts of event-specific data are needed.' In the subsequent sentences it is acknowledged that requirements in the guidance t have been enforced in IR 503/2013 together with additional requirements, but they may not be necessary for cisgenic plants. We agree that these requirements should not be necessary for plants which could be developed through conventional breeding and advocate for a clear indication in the conclusions. It is disproportionate to request a thorough risk assessment, incl. animal feeding studies to plants containing the same modification that could have been obtained via traditional breeding. EFSA should acknowledge that this would be sufficient grounds for a derogation from certain	The GMO Panel thanks for the comment. Regarding lines 637-684, the last paragraph of section 3.3.1 has been revised to clarify that ERA requirements' relevance is considered on a case-by-case basis. Regarding lines 648-649, the GMO Panel has not been mandated to propose any specific criteria for risk assessment or changes in regulation. Regarding lines 657 and 660, the comments refer to the quotes which cannot be edited. Regarding lines 660-661, the cisgene/intragene products are considered GM and as such are subject to Regulation (EU) No 503/2013. Therefore, risk assessment is required regardless of their similarity to plants obtained through conventional breeding, with a possibility of specific requirements to be defined case-by-case.	63

		requirements. Line 684: we disagree with the conclusion that guidance is fully applicable as the requirements should be defined on a case-by-case and will be disproportionate.		

CropLife Europe	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	Line 612: 'modifications of the pattern and/or level of expression of the endogenous protein' is identified as a potential hazard. We note that this could also occur via mutations arising from the use of other breeding tools as well as through natural processes. Lines 615-617: We would welcome more details regarding the parameters that would establish whether it is considered a NEP or not, sincewe cannot think of applications in which a NEP would be used in cisgenesis. Line 621-622: This sentence is confusing as it states that the risk depends solely on exposure factors, while the basic principle of risk assessment and determining risk is a combination of exposure and hazard. When no hazard is present, the need for even conducting a risk assessment itself should be considered. This should either be clarified in Lines 621-622 or suggest the sentence to be removed.	The GMO Panel thanks for the comment. Regarding line 612, while change of expression patterns might be a result of mutations or natural processes, here we refer to the cases where mutations are directed at regulatory regions which aim at changing expression patterns. Regarding lines 615-617, any protein in which there is a change in primary structure (amino acid addition, deletion, substitution) would be considered a NEP. Regarding lines 621-622, they have been modified, taking note of the comment.	64

CropLife Europe	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	3.2.2.1 elaborates on the possibility to also introduce 'cisfragments' in a targeted manner to either replace segments of a coding gene or to replace regulatory sequences like promotors. The same results might also be achievable by targeted mutagenesis and some of the outcomes of introducing a 'cisfragment' might not be distinguishable from a targeted mutation result. This should be elaborated on as well. Also, the introduction of 'intrafragments' might be indistinguishable from targeted edits via mutagenesis. Instead, we proposed removing these two new terms and streamlining this section. It should be focused on the question, i.e. describe 'new' approaches that were not examined in the 2012 opinion that are considered to be relevant in scope. There is one such example in the last paragraph (lines 590-601), but the text should also point out that comparable outcomes are possible using other breeding/NGT approaches. Any exploration of other possible approaches in this section (e.g. paragraph lines 585-589) should be supported by relevant examples in the scientific literature. The sections that follow that use 'cisfragment' and 'intrafragment' would also require revision to remove these terms (3.2.2.2 and 3.3.2.1). The approaches referred to can still be discussed without the use of these terms, but in a more straightforward manner that does not introduce new unclear terminology. Line 591: An addition needs to be made to this sentence so that it reads: "through NGTs that are close to commercialization in the EU.' Line 592: The term 'cisfragment' is not used in the publication referred to.	The GMO panel thanks for the comment. Please note that the terms 'cisfragment' and 'intrafragment' have been removed from the text, which has been revised to improve clarity. Regarding lines 585-589, please note that the literature search did not retrieve publications on cisgenic/intragenic plants obtained through NGTs, nonetheless the experts and GMO panel members were able to identify potential products achievable with these techniques. Regarding line 591, the text has been amended accordingly. Please note that section 3.1.3 has been revised to clarify which NGTs are relevant for this mandate.	65

CropLife Europe	3.2.1.4 If there are new techniques/approaches , what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	As indicated in Line 163 we ask EFSA to consider amending the AQ4 as follows for simplification and clearance: If there are new techniques/approaches, what are the potential risks that they may arise as compared with those already covered in the 2012 opinion associated to plants obtained by conventional plan breeding techniques and plants obtained with EGTs . We want to flag that regardless the question is amended or not there is a missing last paragraph with EFSA general conclusions to this question. Based on the statements in lines 559-565 we would welcome a clear statement in line 567 that certain cisgenic plants obtained by this new techniques/approaches would pose similar risk than plants obtained through classical breeding.	The GMO Panel thanks for the comment. Regarding AQ4, the GMO panel considers the current text better suited to hold a continuous reference between the 2012 and the current opinions. Regarding line 567, the text of the section has been revised and similar risks related to alterations of the host genome have been discussed.	66

CropLife Europe	3.2.1.3 Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 opinion?	Lines 524-525: We suggest replacing 'been generalized and is now routine' with 'become more common'. Line 536: We suggest replacing 'is then very difficult' with 'can then be a lengthy and labour intensive process'. Line 538 : We suggest to delete the brackets in 'only'. Line 541: We suggest replacing 'will most likely' with 'may' unless the former can be supported by literature. Line 541-542: suggest to rephrase the last sentence since it seems speculative: 'Breeders will most likely use this technique rather than classical introgression by crossing and then back-crossing in the years to come'. In large part, global regulatory frameworks will determine how frequently this technique will be used by breeders globally even if it would be easier to do. Line 544: We suggest to add the following to the end of the line: " techniques have been proposed since 2012 to EFSA,"	The GMO Panel thanks for the comment. Regarding lines 524-5, the text has been modified to improve clarity. Regarding line 536, the current phrasing reflects the fact that the process is lengthy and labour-intensive, but on top of that eliminating the genetically linked genes might not be possible at all. For this reason, the sentence has not been amended. Regarding lines 538 and 541-542, the text has been amended accordingly. Regarding line 544, currently there are no cis/intragenic products on the global market, therefore the sentence has not been changed.	67

CropLife Europe	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	Section 2.4.2 indicates that no literature was found reporting cisgenic or intragenic products developed through the use of NGTs, and in this section scientific publications are reported. We suggest providing clarifications on the literature search, explaining why were these not found (according to 2.4.2) or were these a different search' It should also be clearly stated that the 'examples' cited are relevant to the previous (2012) opinion and not the modified definitions and new groups created in this updated draft opinion. Line 485: typo 'transgenic' should be 'intragenic'. Lines 503-504: CLE would suggest removing the speculative statement 'the insert is probably flanked by T-DNA sequences', if this information was not provided by the authors of the publication referred to.	The GMO Panel thanks for the comment. Regarding section 2.4.2, additional text has been added to explain why certain publications have not been retrieved through the literature search. Regarding line 485, the text has been amended accordingly. Regarding line 504, the publication does not explicitly discuss the present of T-DNA sequences in the construct but it can be inferred from the technique used from plant transformation i.e. Agrobacterium-mediated transformation, which results in introducing T-DNA sequences flanking the insert.	68

CropLife Europe	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 406: 'Breeders gene pool' is a term defined by EFSA, for clarity we suggest to add the EFSA reference where it is defined (EFSA Journal 2012;10(2):2561 ' section 2.1). Line 407: We suggest deleting the term 'exactly' since this is not mentioned in EFSA 2012 opinion and it is not always clear what the boundaries of a gene are but the term suggests that this is clear. Lines 424-426 / 442-445: The EFSA opinion highlighted that in some cases similar products can be developed with different technologies and similar hazards can be associated to NGTs and conventionally bred plants. Nevertheless, EFSA has not considered the disproportionality of subjecting similar products, with similar risk profiles to different levels of regulatory oversight and risk assessment requirements, just based on the breeding method. The EFSA scientific opinions (also the one of SDN-1/2) have mainly focused on the comparison of the plants developed with the NGTs with transgenic plants and on the applicability of the existing risk assessment guidance document for GM plants. Both the EU treaty and the GFL indicate that the measures taken in the EU need to be proportionate. It seems the wording suggests this key principle is not implemented in the GM area. Lines 449-450: Lines 449-450: We welcome the acknowledgement that border sequences from Agrobacterium transformation can occur naturally. There is stronger rationale for suggesting their presence in the genomes of current cultivated species and this has been recently acknowledged as well in the Technical guidance on using genetic technologies (such as gene-editing) for making 'qualifying higher plants' for research trials developed and published by the UK Government's ACRE to allow developers to self-determine whether a plant meets the criteria to be considered a	The GMO Panel thanks for the comment. Regarding line 406, the definition of the breeders' gene pool has been added in the footnote. Regarding line 407, the term 'exactly' has been removed. Regarding lines 424-426/ 442-445, The GMO Panel was not mandated to express an opinion on how cisgenic/intragenic plants should be risk assessed but rather to identify potential new risks and to assess the applicability of the current guidelines. Regarding lines 449-450, the GMO panel thanks for the comment. Regarding line 480, please note that the section 3.2.1.1 has been revised in order to clarify that this section refers to plants covered by EFSA 2012 opinion only.	69

		qualifying higher plant (QHP) for research trials. Line 480: We encourage EFSA to acknowledge in this sentence that for some applications there will be 'less risks'.		

CropLife Europe	3.1.3 NGTs relevant for this mandate	Lines-387-389: CLE welcomes the EFSA acknowledgement that the methodologies are in continuous evolution, and this is consistent with our comments in 3.1.1 We would encourage to EFSA to align with the views of several risk assessors worldwide moving towards more proportionate risk assessment paradigms based on the characteristics of the final product and not only to the development technique that have been used. Lines 360 - 361: typo, DBS should be DSB Line 377: Add reference supporting the statement 'it has been shown to allow insertions of exogenous sequence". It is also unclear why this is 'more relevant to this mandate'.	The GMO Panel thanks for the comment. Regarding lines 360-361, the text has been amended accordingly. Regarding line 377, a reference has been added. Prime editing is considered more relevant because it has been shown to be able to transfer longer fragments of DNA, which is in line with the new definition of cisgenesis/intragenesis. Regarding lines 387-389, the GMO Panel takes note of the comment.	70

CropLife Europe	3.1.2 New Genomic Techniques (NGTs)	Lines 334-338: In this paragraph EFSA elaborates on the different delivery methods of the reagents that cause the alterations in the genome. If introduced by T-DNA transformation or other methods that involve the stable insertion of the reagents' DNA, these intermediate 'transgenic' elements are in most cases eliminated in the final product. We suggest adding a paragraph that clarifies the delivery method does not make a difference in terms of risk assessment requirements of the final product, if it is verified that transgenic sequences were eliminated. The Commission has elaborated accordingly on the legal status in the context of an animal application (SANTE/E3/FSX/gk (2022)2439122, Letter from 22-04-2022). Line 325: Replace 'last 20 years' with 'since 2001' as this is the specific point in time used to define the EGT and NGT categories for the purposes of this opinion (see line 308) Line 332: We suggest to edit as 'In some NGTs, the reagents ". Line 338: We suggest to delete 'relative'. Line 340: We suggest to eliminate 'stable', as the 'components' in the paragraph above are not present any more in the final plant. Suggest to edit as 'Other NGT methods have been developed that do not involve the insertion" Lines 344-346: We suggest to edit as 'So-called 'DNA-free' NGT methods that do not involve the insertion of nucleic acid sequences have also been used, ... and to remove: "avoiding the use of any exogenous DNA sequence.'	The GMO Panel thanks for the comment. Regarding lines 334-338, the GMO Panel reminds that paragraph 3.1.2 is meant to introduce the NGTs and not to discuss the procedure to risk assess plants generated by NGTs, which is included in the current regulation. Regarding line 340, the GMO Panel considers the current text sufficiently clear, as it emphasizes the difference between stable and transient transformation methods; the text clarifies that the components originally inserted and no longer needed are absent from the final product. Regarding lines 325, 332, 338 and 344-346, the text has been amended taking the comment into account.	71

CropLife Europe	3.1.1 Established Genomic Techniques (EGTs)	CropLife Europe would like to raise our concern on the introduction and inconsistent and confusing use of the new term 'EGTs'. According to the definition given in lines 307-308 conventional breeding and classical mutagenesis can be considered EGTs as they were developed before 2001. But then in lines 312-314, the definition is restricted for the purposes of this opinion. It is our view that it would be clearer to replace EGT with the terminology 'transgenic techniques'. This would avoid capturing certain types of genome edited products where delivery techniques (e.g. Agrobacterium) have been used in their development. We further recommend deleting the sentence in lines 309-311, as random mutagenesis should not be categorised as an EGT (or as a 'transgenic technique'). Also we would like to highlight that a future-proof framework should not be tied to the use of specific techniques/methods because they will evolve and change faster than legislation making can keep up. On the contrary, focusing on the characteristics of the new plants is an opportunity to realign regulatory frameworks with sound risk analysis principles and align with the principle of proportionality included in the EU treaty and the general food law. Line 312: We suggest to replace 'includes' with 'may include'. Line 317: We suggest to replace 'exogenous DNA' with 'nucleic acid' (this is consistent with language used in the previous sentence). If 'exogenous DNA' is used, a clear definition should be provided. With definitions being modified and/or created for the purposes of this document, it is unclear what sources should be referred to for clarification of such terms. Line 320: It is unclear what is being referred to with 'all the above-mentioned EGT techniques'. Line 320: We suggest to replace 'exogenous' with 'nucleic acid'.	The GMO Panel thanks for the comment. Regarding lines 309-311, the mention to random mutagensis is an example of techniques that are used, even though plants obtained throuhg random mutagenesis are exempt from GMO legislation. The term 'EGT' was first used in the Explicatory Note on New Technologies in Agricultural Biotechnology (European Commission, 2017). The term does not have a legal definition. Regarding lines 321-314, The GMO Panel does not consider it appropriate to replace the term 'EGT' with 'transgenic techniques', as transgenesis can be achieved with various techniques. Regarding lines 312, 317 and 320, the Panel regards the text accurate and sufficiently clear. By 'above-mentioned techniques' we refer to the techniques that 'involve the transfer of genetic material to the host organism, using various strategies, such as Agrobacterium-mediated transformation, biolistic transformation or microinjection'. The definition of the exogenous DNA has been added to the glossary. Reference: European Commission, Directorate-General for Research and Innovation, New techniques in agricultural biotechnology, Publications Office, 2017, https://data.europa.eu/doi/10.2777/574498	72

CropLife Europe	2.4.2 Literature search	Lines 287-289 & Table 2 (Annex A): Search parameters include 'study design: cisgenesis, intragenesis' and the search did not report any cisgenic/intragenic products through the use of NGTs. This seems to indicate that researchers did not refer to cisgenesis/intragenesis when using NGTs, therefore publications will not appear in this search. Line 290: Question regarding the additional references not included in the initial literature search. Was it determined why those were not captured by the initial search and if the search could have been optimised to capture them?	The GMO Panel thanks for the comment and agrees with the explanation provided. The text has been amended to provide more clarity. Regarding line 290, the additional reference not included in the initial search was indeed not retrieved because it lacks terms related to cisgenesis.	73

CropLife Europe	1.4 Interpretation of Terms of Reference	General comments: CropLife Europe would like to emphasize that EFSA scientific opinions on NGT topics have mainly focused on the comparison of the plants developed with the NGTs and transgenic plants, and on the applicability of the existing risk assessment guidance documents for GM plants. Despite the fact that these opinions have highlighted that in some cases similar products can be developed with NGTs and conventional breeding tools, these opinions have not considered the disproportionality of subjecting similar products with similar risk profiles to different levels of regulatory oversight, just based on the breeding method. In regard to the draft updated opinion, we recommend maintaining consistent terminology throughout the document (e.g. techniques, methodologies, approaches, strategies). Lines 135-136 (also 144): Definition of cisgenesis in EFSA 2012 does not match with the current text where the definition of cisgenesis has been adapted from the reference cited in Note 6. For more clarity we would recommend including the former definition of cisgenesis in the footnotes also. Lines 135-137: The inclusion of 'transgenesis' and 'crossable species' in this sentence is not consistent with the definitions that apply to this mandate (as provided in footnote 6) and in the original opinion on cisgenesis/intragenesis (as provided on pages 13-14). Either 'transgenesis' or 'from a crossable species' should be deleted from this sentence. Line 144: Consider the addition of the following text for accurate description: '..aim at introducing a protein-coding gene from a crossable species into a plant) but considering as well the cases when the insertion is targeted and new potential' Line 163: Recommend EFSA to consider amending the AQ4 as follows: If there are new techniques/approaches, what are the	The GMO Panel thanks for the comment. The GMO Panel was not mandated to express an opinion on how cisgenic/intragenic plants should be risk assessed but rather to identify potential new risks and to assess the applicability of the current guidelines. The opinion has been checked for consistency in terminology. Regarding lines 135-136, both definitions of cisgenesis have been added to the main text. Regarding line 137, the sentence has been edited to improve clarity. Regarding line 144, the GMO panel considers the current text sufficiently clear. Regarding line 163, the GMO panel considers the current text better suited to hold a continuous reference between the 2012 and the current opinions.	74

		potential risks that they may arise as compared with those associated to plants obtained by conventional plan breeding techniques and plants obtained with EGTs.		

German Federal Institute for Risk Assessment (BfR)	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	Paragraph 2: We propose to change the text according to the following sentence: "As all these cisgenic and transgenic products will be produced by targeted insertion/modification (e.g. via SDN3), they will not present additional hazards associated with the disruption of other genes and/or regulatory elements in the recipient genome compared to plants obtained by conventional plant breeding techniques and plants obtained with EGTs." Rationale: Off-target effects cannot be absolutely excluded. However, one would remove the absolute claim by adding the word "additional" and at the end the relative comparison to plants obtained by conventional plant breeding techniques and plants obtained with EGTs.	The GMO Panel thanks for the comment. The text has been revised taking the comment into account.	75
German Federal Institute for Risk Assessment (BfR)	Keywords	The German Federal Institute for Risk Assessment (BfR) would like to express its appreciation and support for the updated scientific opinion on plants developed through cisgenesis and intragenesis. The document is comprehensive in terms of cisgenesis/intragenesis and SDN-3 application and reflects the current state-of-the-art.	The GMO Panel thanks for the comment.	76
Testbiotech	4 Conclusions	As shown, the EFSA draft opinion does not address any of the relevant cases and specific risks caused by the technical processes. Therefore, the EFSA draft opinion cannot be seen as sufficient to derive final conclusions in regard to the TORs provided by the Commission. More specifically, some of the conclusions presented in the EFSA draft are flawed and misleading. Beyond that, there is an evident need for the introduction of more comprehensive methodologies to assess the risks of plants obtained from Old GE and New GE. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.	The GMO Panel thanks for the comment and takes note of the provided background text and cited references. After the revision of the document, the GMO Panel considers the conclusions still valid.	77

Testbiotech	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	The EFSA draft opinion cannot be seen as sufficient to derive to final conclusions in regard to TOR4. More specifically, some of the conclusions presented by EFSA in its draft are flawed and misleading (see 3.3.2.1). In regard to future guidelines, there is a need for the introduction of comprehensive methodology to assess changes in plant composition and phenotypic characteristics, which also makes use of 'omics' (genomics, transcriptomics, proteomics, metabolomics) and whole genome sequencing. In addition, the plants should be exposed to a sufficiently broad range of biotic and abiotic stressors to investigate the extent to which these factors impact plant composition, phenotypic characteristics and gene expression. In regard to toxicology, both the intended and unintended effects have to be considered. Apart from new proteins (peptides) which may be produced unintentionally, the emergence of other additional biologically active molecules (such as ncRNAs) and interactions with plant constituents, must also be considered. Furthermore, the impact on the immune system from the intestinal microbiome should, for example, also be taken into account. If applicants apply for approval to cultivate the plants, the guidelines should require the applicant to demonstrate that the plants cannot persist and propagate in the environment. Without introducing such 'cut off criteria', environmental risk assessment cannot be conclusive. In addition, food webs and interactions with non-target organisms as well as the soil bacteria must be assessed in detail, and safety demonstrated through experimental data (for more information, see Testbiotech, 2021). Finally, the risk manager should develop examination guidelines to assess potential benefits to ensure that the only NGT plants used in agriculture and food production are	The GMO Panel thanks for the comment and takes note of the provided background text and cited references. GMO Panels considers the current guidelines sufficient to establish the safety of the plants obtained through NGTs.	78

		those which are really necessary. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Based on its concept, EFSA includes (most) SDN-1, SDN-2 and SDN-3 applications in its opinion, and therefore plants considered to be 'synbio' (EFSA 2021 and 2022b) also appear to fall within its scope and will as such need to be fully considered. However, as previously shown, the EFSA draft opinion does not address these examples, relevant cases or any specific risks arising from the technical processes. Therefore, the draft opinion cannot be seen as sufficient to derive final conclusions in regard to TOR3. More specifically, some of the conclusions presented by EFSA in its draft are flawed and misleading. For example, EFSA suggests that if 'the targeted introduction/modification of a gene to obtain an allele already existing within the species' is used, 'these plants would not present new hazards as compared with conventional plants, and therefore most, if not all, risk assessment requirements would not be relevant.' As shown, the effects of NGT applications can present specific risks, which may extend in scale and quantity far beyond those already known from non-regulated breeding methods and/or Old GE. Without in-depth risk assessment, it is not possible to categorize NGT plants into specific 'risk profiles' in order to establish categories of plants which can be considered safe (Eckerstorfer 2021). It is completely inaccurate for EFSA to claim that NGTs do not pose a new scale and dimension of risk compared to plants derived from conventional breeding. Therefore, as with other regulated GE organisms, these plants must be assessed on a case-by-case basis to demonstrate safety as required by law. As shown, the risk assessment of these plants cannot be refined to the intended effects of the final products, it has to take into account unintended effects caused by the technical processes and the overall biological	The GMO Panel thanks for the comment. The text of the opinion has been revised to clarify which NGTs are relevant to the mandate (section 3.1.3). Considering the risks related to SDN techniques, please refer to previous EFSA's opinions (EFSA GMO Panel 2012b,2020). The conclusions of these opinions remain valid for cisgenic and intragenic plants obtained by these techniques. As for plants obtained through synthetic biology which are not in the scope of this opinion, EFSA has published two relevant opinions: https://doi.org/10.2903/j.efsa.2021.6301 and https://doi.org/10.2903/j.efsa.2022.7410.	79

		characteristics of the organisms. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Based on its concept, EFSA includes (most) SDN-1, SDN-2 and SDN-3 applications in its opinion, and therefore plants considered to be 'synbio', e.g. the newly domesticated tomato, also appear to fall within its scope and will as such need to be fully considered. However, the EFSA draft opinion does not address any of these examples or other relevant cases and specific risks arising from the technical processes. Therefore, the EFSA draft opinion cannot be regarded as sufficient to derive final conclusions for TOR3. Furthermore, it seems that flexibility, as discussed by EFSA, is already present in the current system: the data requirements depend to some extent on the type and trait in the application (such as herbicide resistance, insect toxicity, changes in nutritional composition). In regard to the 90-day feeding studies requirement, EFSA has so far been unable to put forward any other (better) methodology to assess the potential effects emerging from whole food and feed, such as combinatorial health effects. Therefore, the demand to abandon mandatory feeding studies is not underpinned by any sufficient alternatives needed to demonstrate safety of whole food and feed. Nevertheless, it can be agreed that risk assessment practice does indeed require higher standards and more reliable methodology. This is especially true when it comes to NGT plants with genetic and phenotypical changes which go beyond the GE plants assessed so far. As shown, Old GE as well as New GE can cause biological effects well beyond those which are known from non-regulated breeding methods, even if no additional genetic information is added to the gene pool of a species. These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change compared to those of non-regulated breeding	The GMO Panel thanks for the comment. The text has been revised to clarify which SDN techniques are in the scope of the opinion (section 3.1.3). The GMO Panel considers the current guidelines sufficient and partially applicable for risk assessment of food and feed derived from cisgenic and intragenic plants obtained through NGTs.	80

		methods. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	Similarly to SDN-2 and SDN-3 applications, SDN-1 also carries specific risks on several levels (see input to 3.2.1.4). All these effects can occur along with specific risks which may be new in scale and quality compared to non-regulated breeding methods and/or Old GE. Whatever the case, a lower frequency of genetic change (if compared to methods of random mutatgenesis) does not imply greater safety of the specific intended or unintended changes caused by NGT applications. Without in-depth risk assessment, it is not possible to categorize NGT plants according to specific 'risk profiles' and thus establish categories of plants which can be considered safe. In addition, the potential scale of exposure to many different (in terms of traits and / or species) NGT plants, which have not adapted via evolutionary processes, has to be taken into account when it comes to the assessment of their overall environmental impact. Furthermore, Barbour et al. (2022) show that a higher plant allelic diversity has an impact on different species within an experimental food web and may play a crucial role in the stability of ecosystems and food webs. These effects are not caused by the introduction of new genetic information into the gene pool of a species, but by changing the frequency of the allelic variants within a population. CRISPR/Cas applications in particular can be used to make gene variants within a population more uniform, i.e. the frequency of the abundance of different allelic variants can be reduced, the alleles can be changed or the respective gene (-family) can be blocked in its functions. Therefore, these effects are highly relevant for the effects caused by New GE applications on cisgenesis and intragenesis. Whatever the case, EFSA is completely wrong in	The GMO Panel thanks for the comment. The text has been revised to clarify which SDN techniques are in the scope of the opinion (section 3.1.3). To develop the opinions on the safety of plants developed by SDNs (EFSA GMO Panel 2012b, 2020), the GMO Panel did take into consideration review and opinion papers but paying particular attention to research papers that provided actual experimental data on off-target mutations. These papers provide evidence that the off-target mutations potentially generated by the application of SDN-based methods for genome editing are of the same type as those produced by other genetic modification techniques. Regarding the environmental impact of the cisgenic plants obtained by NGTs, the Panel considers the current environmental risk assessment sufficient.	81

		claiming that NGTs with 'cisfragments' and 'intrafragments' would not pose new risks compared to what was identified in the 2012 opinion. For references and further details see backgrounder uploaded under 1.4.		

Testbiotech	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	EFSA has introduced a new concept in this section by expanding the concept of 'cisgenesis' to SDN-1 applications. It proposes that a 'knocked-out' gene function typical for SDN-1 applications results in so-called 'cisfragements' that may already be within the gene pool of a species. This concept would have far-reaching implications: for example, Zsögön et al. (2018) show that the complexity of several introduced CRISPR/Cas-induced genetic changes results in a new quality of hazards and risks, even if no new genetic information is added to the gene pool of a species. In this case of 'de novo domestication', CRISPR/Cas9 is used to alter the genomes of wild species in such a way that some of their genes are modified to resemble domesticated ones. Such de novo domesticated plants still have some properties from wild species which were lost during plant breeding. While no new genes are added to the gene pool of the species, the plant composition and other biological characteristics of the plants may show pervasive changes that go beyond what was observed from previous GE. Therefore, plants altered with SDN-1 to achieve traits known from cultivated varieties, but which are now expressed in a new genetic background, cannot be equated to their conventional or natural counterparts, as the corresponding target gene(s) might have divergent functions or interactions in different genetic backgrounds (see Kawall, 2021, EFSA 2022). This example (Zsögön et al., 2018) also shows that the dissecting of gene linkages does not mean greater safety or predictability of the biological characteristics of the resulting plants. This example again shows that a lower frequency of genetic changes (if compared to methods of random mutagenesis) in no way implies greater safety of the specific intended or unintended changes arising from	The GMO Panel thanks for the comment. The text has been revised to clarify which SDN techniques are in the scope of the opinion (section 3.1.3). Moreover, plants obtained by SDNs are subject to GM risk assessment for possible unintended effects, by the assessment of studies including phenotypic and the compositional analysis of the GM plant, as laid down on IR 503/2013 and EFSA guidances. The GMO Panel considers the current regulations sufficient to assess the safety of cisgenic/intragenic plants, including the unintended effects of the genetic modifications.	82

		NGT applications (Kawall 2021). For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.2.1.4 If there are new techniques/approaches, what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	EFSA is completely wrong in suggesting that these plants do not carry any more risks than those identified in the 2012 opinion. Very generally, it is important to understand that a lower frequency of genetic change (if, for example, compared to methods used in random mutagenesis) does not imply greater safety of the specific intended or unintended changes arising from NGT applications. SDN-2 and SDN-3 applications carry specific risks on several levels: (i) in many cases, the application requires a multistep process, including the production of transgenic plants in a first step. Potential risks associated with these processes were not sufficiently addressed in the 2012 opinion; (ii) tools such as CRISPR/Cas can, to varying degrees, escape the natural mechanisms of genome organisation. Therefore, the intended and unintended changes, the site of the integration, the patterns of genetic change and the resulting effects (that may come with risks) can exceed the effects already known from non-regulated breeding methods and Old GE; (iii) SDN-2 and SDN-3 processes cause specific unintended effects (often in the targeted genomic region, but also off-target) such as indels, larger genomic changes and unintended insertion of transgenes that would otherwise have been unlikely occur; (iv) if genetic linkages are separated or alleles of specific genes are made uniform (lowering the variety and frequency of the genetic variety in the population) this may cause genomic effects or biological phenomena which impact plant health, ecosystems and food safety. The same is true, if genetic information, which is within the gene pool of the species, is introduced into a new genetic background. All these effects can carry specific risks which may be new in scale and quality compared to	The GMO Panel thanks for the comment and takes note of the attached references. To develop the opinion, the GMO panel did take into consideration review and opinion papers but paying particular attention to research papers that provided actual experimental data on off-target mutations. These papers provide evidences that the off-target mutations potentially generated by the application of SDN-based methods for genome editing are of the same type as those produced by conventional breeding including random mutagenesis.	83

		non-regulated breeding methods or Old GE. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.2.1.3 Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 opinion?	EFSA points out that genes or gene variants (alleles) from wild relatives might become introgressed into domesticated varieties, as the use of new GE techniques, facilitates the transfer of isolated DNA sequences. However, EFSA does not give the 'full picture' of existing publications or of the effects that may be caused by these approaches which can, for example, involve multiplexing, i.e. targeting several genes at once within a single application. Although it is true, that gene linkage may be avoided with NGTs, this does not mean greater safety or predictability of the biological characteristics of the resulting plants. On the contrary, this may be associated with thus far not experienced or unexpected biological effects that may deserve the specific attention of the risk assessor. Whenever additional genetic information is added to the gene pool of a species, the processes used for technical insertion of DNA can cause intended or unintended effects that extend far beyond what is already known from non-regulated breeding methods. Such effects may comprise epigenetic regulation, disruption of genes, new position effects, open reading frames, the unintended introduction of additional genes, changes in gene expression and genomic interactions which can involve plant constituents, plant composition and agronomic characteristics. In addition, it has to be expected that the frequency and variety of genetic information which is present in the populations will be changed, thus also affecting the biological functions of what may be considered to be 'keystone genes'. These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change as well as their quality compared to those of non-regulated breeding methods. Therefore, the intended and unintended effects have to be assessed on a case-by-case basis	The GMO Panel takes note of the comment. The GMO Panel considers the current legislation sufficient to assess the safety of cisgenic/intragenic plants, including the unintended effects of the genetic modifications.	84

		to demonstrate safety as required by law. For references and further details see backgrounder uploaded under 1.4.		

Testbiotech	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	Recent research shows that - contrary to what was assumed by EFSA (2012) - the emergence of mutations is not completely random but influenced by gene regulation and genome organisation. Relevant factors that impact the likelihood of mutations are, for example, the composition of base pairs, histone modification and the status of chromatin. Gene regulation and genome organisation causes 'essential' genes to mutate less frequently than others also have a substantial impact on the likelihood of repair mechanisms in response to DNA damage. As a result, the occurrence of mutations is not simply dependent on random processes followed by selection. Rather, gene regulation and genome organisation act as 'flexible safety barriers' in the evolution of plants. These findings are relevant for both Old GE ('EGTs') and New GE ('NGTs') and the resulting plants or products. Furthermore, Barbour et al. (2022) show that a higher plant allelic diversity has an impact on different species within an experimental food web, and may play a crucial role in the stability of ecosystems and food webs. These effects are not caused by the introduction of new genetic information into the gene pool of a species, but by changing the frequency of the allelic variants within a population. Therefore, these effects are highly relevant to the effects caused by cisgenesis. All in all, both Old GE and New GE can be the cause biological effects which extend beyond those known from non-regulated breeding methods, even if no additional genetic information is added to the gene pool of a species. These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change and their resulting biological characteristics compared to those of non-regulated breeding methods. Therefore,	The GMO Panel thanks for the comment and takes note of the suggested references. Plants obtained by SDNs are subject to GM risk assessment for possible unintended effects, by the assessment of studies including phenotypic and the compositional analysis of the GM plant, as laid down on IR 503/2013 and EFSA guidances. The GMO Panel considers the current legislation sufficient to assess the safety of cisgenic/intragenic plants, including the unintended effects of the genetic modifications. Regarding the environmental impact of the cisgenic plants obtained by NGTs, the Panel considers the current environmental risk assessment sufficent.	85

		these effects have to be assessed on a case-by-case basis to demonstrate safety as required by law. For references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	This section again shows that the TOR1 question needs a comprehensive answer, without relying mainly on previous opinions and EFSA assumptions or on EFSA conflating TOR1 with TOR2: it is evident that even when the previous EFSA opinion on cisgenic plants was published in 2012, the EFSA findings and conclusions were not sufficiently backed by the science. For example, it was known (and also confirmed more recently) that insertional mutagenesis caused by transposons and retrotransposons is based on specific mechanisms which can also impact the sites of insertion and, in addition, many of these elements are integrated and 'domesticated' as regulatory elements into the plants' genome. Whatever the case may be, the mechanisms and results of these naturally occurring phenomena cannot be equated to the technical processes for the technical insertion of genes, such as biolistic methods and usage of Agrobacterium tumefaciens. For example, Yue et al. (2022), identified specific larger and smaller insertions as well as deletions caused by the biolistic method of gene insertion into papaya. In conclusion, the processes used for the technical insertion of DNA can cause effects which are different in their scale, in the sites and in the patterns of the genetic change as well as their biological characteristics compared to those of non-regulated breeding methods or natural processes. Such effects may concern epigenetic regulation, the disruption of genes, position effects, open reading frames, the unintended introduction of additional genes, changes in gene expression and genomic interactions which can involve plant constituents, plant composition and agronomic characteristics. Therefore, these effects have to be assessed on a case-by-case basis to demonstrate safety of the plants as required by law. For	The GMO Panel thanks for the comment. The Panel has considered the hazards associated to cisgenic/intragenic plants that were already covered by the EFSA (2012) opinion and those that were not covered. Please note that the characterization of the unintended effects caused by the techniques used is part of the molecular characterization step of the risk assessment, which is a requirement laid down in IR 503/2013 and EFSA guidances.	86

		references and further details see backgrounder of Testbiotech, uploaded under 1.4.		

Testbiotech	3.1.2 New Genomic Techniques (NGTs)	The EFSA approach not only includes plants into which genes have been transferred and introduced into the cells, but also those generated with new genetic engineering techniques (New GE or NGT) using site-directed nucleases without the introduction of additional DNA (SDN-1). It seems that the plants have been divided into two groups: those with changes that add new genetic information to the gene pool of the species and those which do not (see 3.2.2.1.). However, it remains unclear as to how such conclusions can be drawn if the gene pool of a (potentially cross-able) species indeed comprises the genetic variants introduced by technical means into specific varieties. It appears that the inclusion of SDN-1 and also SDN-2 plants extends beyond the EU Commission TORs dealing with cisgenesis (intragenesis) based on the transferal and introduction of additional gene sequences (which may, therefore, include SDN-3 plants only). If this is the accepted approach, it would require the integration in the opinion of all relevant findings in regard to intended and unintended effects caused by SDN-processes. It should be born in mind that EFSA has never provided a full and comprehensive overview in any of its previous reports. For example, the EFSA (2020) opinion on SDN-1 plants explicitly states that no comprehensive literature research was conducted on this issue. In addition, several publications highlight the risks inherent to SDN technology that are not referenced in the draft opinion. As long as EFSA simply continues to reiterate its position that NGTs pose no new risks, regardless of whether they are compared to conventional breeding or transgenic plants, the whole opinion is just an empty shell which fails to answer the TOR1 question, and can only provide flawed and highly misleading	The GMO Panel thanks for the comment. The text in paragraph 3.1.2 has been amended to improve clarity on the techniques considered relevant for cisgenesis/intragenesis. The GMO Panel was not mandated to discuss the unintended effects caused by the SDN processes. The GMO Panel reminds that the characterization of the unintended effects caused by the SDN process, which is part of the molecular characterization step of the risk assessment, is a requirement laid down in IR 503/2013 and EFSA guidances and it is still considered necessary for plants generated via SDN-based methods.	87

		conclusions. For references and further details see backgrounder of Testbiotech uploaded under 1.4..		

Testbiotech	3.1.1 Established Genomic Techniques (EGTs)	Using the expression 'EGTs' (as introduced by the Commission in its terms of reference) confuses the differences between regulated genetic engineering techniques (transgenic plants) and random mutagenesis as well as hybridisation techniques. If 'EGT' is used, it should be made clear which plants are genetically engineered (GE), and thus regulated, and which plants do not have to undergo the mandatory approval process and are, therefore, are non-regulated. Furthermore, EFSA states 'with all the above-mentioned EGT techniques, the exogenous sequence integrates randomly at one or several positions in the genome, with potential consequences on the expression patterns.' As a 'stand-alone finding' this sentence should be put into context. The random integration of additional genes may have many effects, and the EFSA should therefore also address other effects, e.g. epigenetic effects, the disruption of genes, genomic position effects, new and unintended open reading frames, the unintended introduction of additional genes and genomic interactions (including changes in gene expression) which may concern the plant constituents, changes in plant composition and agronomic characteristics of the plants. For references and further details see backgrounder of Testbiotech uploaded under 1.4..	The GMO Panel thanks for the comment. Please note that in section 3.1.1 it is specified that here we refer to the alterations that involve the transfer of the genetic material to the host organism, and plants obtained by these EGTs are considered GM and subject to all relevant regulations. Please note that 'EGT' is not a legal term and does not have a legal definition. As for the unintended effects, the risk assessment of GM plants includes molecular characterisation, which adresses the effects of random integration of the insert.	88

Testbiotech	2.4.1 Problem formulation	According to EFSA, a literature review was conducted along the lines of specific criteria. While Annex 1 presents some of the criteria used for the selection of the references, there appears to be no information available on which of the references were ultimately deemed relevant. Instead, most references included in the draft opinion are simply previous EFSA opinions; only very few publications were referenced, and they appear to have been chosen more or less arbitrarily. Another point is that the methodology appears to have changed (!) during the writing process so as to add some further references not included in the initial literature search results. Consequently, unless there is more transparency regarding the outcome of the research, the EFSA findings cannot be assessed and no conclusions can be drawn. This also means that the results cannot be compared to the outcomes of other research, or to reports. SDN-1 applications are included in the opinion if they cause 'cisfragments' and 'intrafragments'. We would assume that such plants can, for example, be found in a report of the JRC report. Beyond that, numerous reports and publications were published within last years which appear to create the impression that plants derived from NGTs will soon be brought to the market. Furthermore, there are several publications dealing with the TOR1 question in regard to the risks of the technology and the resulting organisms. It is not clear whether EFSA took note of these findings. Consequently, we expect EFSA to substantially improve its methodology as well as to provide full transparency on its findings and on its selected/ rejected sources. For references and further details see backgrounder of Testbiotech (uploaded under 1.4.).	The GMO Panel decided to search for publications reporting cisgenic/intragenic products obtained with EGTs and NGTs. All the retrieved publications deal with cisgenic/intragenic products obtained with EGTs. The majority of those publications were not referenced in the opinion because they describe products that are already covered in the 2012 opinion. Nevertheless, some publications are referenced, as an example to demonstrate that reports regarding cisgenic/intragenic approaches obtained with EGTs continue to be published, even after 2012, confirming the validity of the 2012 opinion. Shi et al., 2017 describes products obtained by NGTs, and it was added by the GMO panel at a later stage, because it was not retrieved from the initial search (due to the lack of searchable terms in the text of the publication). The GMO Panel highlights the difficulty of finding publications if the text of the publications does not include any of the relevant terms used for the search. Therefore, the Panel members have the possibility to add publications not included in the initial search, if they deem it necessary. Please note that a list of retrieved publications will be published together with the final version of the opinion.	89

Testbiotech	1.4 Interpretation of Terms of Reference	EFSA has fragmented and partially misinterpreted the questions posed by the Commission. TOR1 very generally asks 'to identify potential risks that plants obtained by cisgenic and intragenic approaches ' .' From our perspective, a much broader, unbiased survey would be needed to fulfill this requirement, and thus come to reliable conclusions. The EFSA approach of conflating TOR1 with TOR2 is suffering from its former opinions, as the methodology and accuracy of these previous findings and assumptions has not been sufficiently analyzed. This leads to confusion, because previous EFSA opinions were failing to deal with the risks inherent to the processes of new genomic techniques (NGT) comprehensively. For example, by referring to its previous opinions in the introduction, EFSA (2022a) reiterates that 'Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and transgenic plants.' From the outset it appears EFSA has concluded that NGTs do not pose any new risks, regardless of whether they are compared to regulated technologies or non-regulated breeding methods. However, directly afterwards it states that 'Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.' EFSA now appears to be assuming that two different comparisons should be made: one comparison between the risks of plants derived from cisgenesis and conventional breeding and the other between intragenic plants and transgenic plants. The question arises as to why a more specific comparison should be made for cisgenic and intragenic plants, if, on the other hand, all NGTs can just be collectively thrown into one category (which is apparently would be wrong). EFSA should avoid such confusion and	The GMO Panel thanks for the comment. The panel has deemed it appropriate to address ToR1 and ToR2 together, as both required an analysis of the potential risks posed by cisgenic/intragenic plants. The mandate requests to identify risks of cisgenic/intragenic plants, therefore the Panel addressed plants and plants-derived products achieved with techniques already covered in the EFSA (2012) opinion and with those not covered in that opinion. The GMO Panel considers the structure of the opinion sufficiently clear.	90

		answer the EU Commission question (TOR1) without fragmentation or conflation with TOR2. For references and further details see backgrounder of Testbiotech.		

Association Française de Biotechnologies Végétales	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Lines 711-717, 757-761, Page 20 You mention that use of a cisgene to reproduce an existing allele will create a plant that is as safe, if not safer, than its existing conventional counterpart. You even suggest that 'most, if not all, risk assessment requirements would not be relevant.' For AFBV, this specific example of using a cisgene to reproduce an existing allele or a targeted modification (SDN-2 or 3) to reproduce an existing allele represents a category of plants that should be excluded from GMO legislation. In such a case a simple confirmation that the cisgenic modification corresponds to an existing allele should suffice and no risk assessment evaluation as such should be requested. Do you agree' In countries such as Canada, the United States or the UK a consultation / confirmation mechanism has been put in place for developers to confirm the status of plants obtained through NGTs. Would EFSA agree that such consultation mechanism is desirable and would it so recommend it to the Commission?	The GMO Panel thanks for the comment. Please note that defining which techniques and/or approaches should be regulated or not regulated is not in the remit of the GMO Panel, which operates within the boundaries of the GMO EU regulation.	91

Association Française de Biotechnologies Végétales	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Lines 646-649, Page 19 You indicate that 'although the case-by-case principle is still present in the additional guidance and regulatory documents published since 2012, the additional flexibility recommended in the EFSA 2012 opinion has not yet been introduced, and therefore this recommendation also remains valid'. How should the case-by-case flexibility be introduced? For instance, would it require in your view modification or elimination of Regulation 503/2013? What specific data packages would be required specifically for cisgenic plants and intragenic plants? Line 667, Page 19 We suggest changing the end of the sentence to read 'may not be necessary in the case of transgenic plants and not necessary at all in the case of cisgenic plants".	The GMO Panel thanks for the comment. Regarding lines 646-649, the GMO Panel was not mandated to provide a comprehensive list of the studies required or not for the risk assessment of cisgenic and intragenic plants obtained by NGTs, nor to replace the current requirements under IR 503/2013. Regarding line 667, an explanation of the rationale for the proposed change is not sufficiently justified. Therefore, the proposed changes have not been integrated in the text of the opinion.	92

Association Française de Biotechnologies Végétales	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	Lines 573-596 - You mention that the targeted insertion of less than a complete gene was not considered in the 2012 opinion. In this 2022 opinion you state, line 579, that: 'When ... intrafragment'. You further explain the advantages of precisely introducing fragments as opposed to complete genes (line 585): 'The possibility ` protein'. Our understanding is that in all these cases you are describing both insertions and replacement of sequences (SDN-3 and SDN-2 type approaches) and that the insertion of cisfragments and intragments can be multiple. In relation to the 1st example (line 592) you cite from Shi et al. 2017, the cisfragment (GOS2 constitutive promoter) is inserted. In the 2nd example (line 594) the GOS2 constitutive promoter replaced the native promoter (SDN-2 approach). Please confirm that both types enter in the category you described (insertion or replacement of a cisfragment). Line 584 - As you have introduced the concept of cisfragments and as a cisgenic plant may often contain multiple cisgenes at a single locus or single cisgenes at multiple loci, would you consider differently a plant having multiple but different targeted cisfragments from one having multiple identical targeted cisfragments? Our view is that if each cisfragment is a single continuous sequence, a plant having two or more different cisfragments would be considered a cisgenic plant. Could you please confirm the status? Lines 598- 601 - Your commentary regarding the examples of Shi et al. indicates that the insertion in one case of the native maize GOS2 constitutive promoter (a cisfragment) and the replacement in the second case of the native corn promoter with the GOS2 constitutive promoter (a cisfragment) lead to the conclusion that both examples constitute a clear example of cisgenic approach used to create allelic variation for	The GMO Panel thanks for the comment. Regarding lines 573-596, the text of the opinion has been revised and the terms 'cisfragment' and 'intrafragment' are no longer used. Regarding line 584, the definition of cisgenesis/intragenesis refers to the nature of the introduced sequence, independently of the number of sequences introduced. Regarding lines 598-601, the text has been amended to improve clarity. The GMO panel considers that 'when the insertion of fragments occurs within a host gene, the end result leads to the formation of a rearranged gene and, as such, should be considered intragenic'. Both examples from Shi et al 2017 belong to the same category.	93

		enhancing crop drought tolerance (lines 600-601). Could you please confirm whether both examples constitute a cisgenic plant as indicated on line 581?		

Association Française de Biotechnologies Végétales	3.2.1.4 If there are new techniques/approaches, what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	Lines 559-566, Page 17 Asked to comment regarding potential risks associated with new techniques/approaches that did not exist in 2012, you respond: 'Therefore, the production of cisgenic plants by 'SDN-2 like' or SDN-3 approaches could minimize the hazards related to the introduced DNA and trait, as these already exist in the gene pool of the breeder and, on this aspect, would be similar to plants obtained through classical breeding. In addition, use of SDN-2 like or SDN-3 approaches to produce cisgenic plants would minimize both the potential alterations to the host genome observed during random integration through EGTs and would avoid the possible linkage drag effect when using classical breeding techniques of gene introgression.' It would appear from your analysis that even fewer hazards exist in these examples than producing the same plant through conventional breeding and, in fact, you suggest at line 716, page 20 that risk assessment requirements would not be relevant. AFBV agrees and has proposed that such cisgenic plants should be excluded from the GMO legislation and be treated from a regulatory standpoint the same way as their conventional counterparts, see the enclosed document. Line 565-566, Page 17 You mention that: 'These conclusions could, in some case, be also true for intragenic plants'. Could you elaborate on this topic, i.e., are such targeted intragenic plants to be distinguished from other randomly-inserted intragenic plants such that they should be assimilated to cisgenic plants (insertion of the intragene at a defined site)?	The GMO Panel thanks for the comment and takes note of the attached document. Regarding lines 559-566, the sentence states that cisgenesis obtained through NGT could minimize the hazards related to the introduced trait, as it is already present in the breeder's gene pool, and the hazards related to alteration of the genome, due to the targeted insertion. Please note that defining which techniques and/or approaches that should be regulated or not regulated is not in the remit of the GMO Panel which operates within the boundaries of the GMO EU regulation. Regarding lines 565-566, the sentence has been modified to improve clarity.	94

Association Française de Biotechnologies Végétales	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 400, Page 13 and Note 6, Page 5 - In Section 3.2.1.1. you revisit your 2012 assessment of the risks that cisgenic/intragenic plants in light of accumulated knowledge since 2012 and you mention the 2012 definition of cisgenesis as the introduction in a plant of "specific alleles/genes present in the breeders' gene pool, without any change to the DNA sequence". In this definition, the cisgene corresponds exactly to the native gene including introns, 5' and 3' UTRs, and flanking native promoter and terminator in the normal sense orientation; and intragenesis as the introduction in a given plant of genetic elements "created by recombining genetic elements such as promoters, coding sequences and terminators of different genes within the breeder's gene pool" for that particular plant species. On Page 5, Note 6 it is mentioned that for purposes of the mandate the following definitions apply: cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species. This definition is adapted from Broothaerts et al., 2021 - doi:10.2760/710056, JRC121847 and has been slightly expanded to cover sequences present in a sexually compatible species. The use of the term 'sequences' in the definitions of the current mandate as opposed to 'alleles/genes' in the 2012 cisgenesis definition and 'genetic elements' in the 2012 intragenesis definition might create confusion. Could you specify what is meant by the term 'sequence' compared to 'allele/gene' or 'genetic element'? Please also confirm whether the phrase 'already present in the species or in a sexually compatible	The GMO Panel thanks for the comment. Please note that the text in section 1.4 has been improved for clarity, with both definitions explained in the text. A footnote with the definition of 'Breeders gene pool' has been added. The definition of cisgenesis from the 2012 EFSA opinion is linked to the concept of 'gene', intended as a protein-coding gene, and its promoter, introns, terminator. With the development of new technologies, cisgenesis can be achieved with any sequence, therefore the term 'sequence' is broader and it refers to any genetic element, not only genes coding for proteins. The sentence 'already present in the species or in a sexually compatible species' has the same meaning as 'within the breeder's gene pool for that particular plant species'.	95

		species' (Note 6) has the same meaning as 'within the breeder's gene pool for that particular plant species' (Line 406).		
Association Française de Biotechnologies Végétales	1.4 Interpretation of Terms of Reference	Line 135, Page 5 The comma after definition should be removed so that the sentence makes sense.	The GMO panel thanks for the comment. The text has been amended accordingly.	96

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	5 References	Mostly limited to EFSA opinion papers. Please see also 3.2.2.1 above and general conclusion from readers below. This text is rather repetitive and refers too often to the two EFSA opinions published in 2012 (https://doi.org/10.2903/j.efsa.2012.2561; https://www.efsa.europa.eu/it/efsajournal/pub/2943) that it means to update. This is not disturbing as such, but it still provokes a pitfall in terms of precise references included to support some of the statements made, which are sometimes missing. In this respect, this reader was a little perplex about the choice of restraining the literature search to the WOS core collection instead of applying the full database of WOS which would have also included (non-grey) publications such as book chapters, conference proceedings and institutional opinions and reports which are often not exhaustively covered by the other databases applied (see Tables 3 and 4, Annex A).	The GMO Panel thanks for the comment. The book chapters and conference proceedings were included in the literature review. However, only the sources containing original findings fulfilled the inclusion criteria.	97
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	4 Conclusions	L770 : ` remain ` replaced by " remained"	The GMO Panel considers the current wording accurate.	98
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	L757-759 : Sentence unclear, needs reorganisation. New proposed text : On the one hand, plants could be produced where the cisgene, corresponding to an already existing allele in the genetic pool of the breeder, would be targeted to the corresponding endogenous gene (through an SDN2-like strategy).	The GMO Panel thanks for the comment. The text has been amended accordingly.	99

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	L642 : "flexibility" must be defined	The GMO Panel thanks for the comment. Since the term in question is a quote from the EFSA 2012, its meaning is not elaborated on in the current opinion.	100
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	L616 : NEP not defined = Newly Expressed Protein ` L616 : case-by-case must be defined 621-625 : Conclusion in apparent contradiction with preceding statement in L614-617 where the need for a case-by-case evaluation is advocated for.	The GMO Panel thanks for the comment. Regarding line 616, the explanation of the abbreviation has been added. Regarding line 616, the Panel considers the preceding sentences sufficient to explain the factors to be taken into account when evaluating the hazards related to NEPs (altered expression, modification of the protein sequence etc.). Regarding lines 621-625, please note that section 3.2.2.2 has been revised to improve clarity of the text.	101

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	L583 : The definition of intragenesis should be more precise. A possible text would be ` When the fragment to be introduced results from the combination of different sequences from a crossable species, without any accompanying linkage drag (Araki and Ishii, 2015), the plant will be considered an intragenic plant ` M. Araki, T. Ishii (2015) Towards social acceptance of plant breeding by genome editing Trends in Plant Science 20 (3): 145-149. https://doi.org/10.1016/j.tplants.2015.01.010	The GMO Panel has developed this opinion by strictly adhering to the terms of reference, which included the definitions of cisgenesis and intragenesis.	102

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	Details for performing such a docking approach should be provided in the guidelines because of the multiplicity of docking programs that are currently available as web servers or can be downloaded for personal use (mainly on Unix machine). In addition, it has been shown that various proteins from pathogenic or commensal bacteria (microbiota), can generate apparently functional immunotoxic peptides (capable of causing inflammatory reactions in patients with celiac disease). If such bacteria or neighboring bacteria were used as source microorganisms for proteins expressed in GMPs, the problem of their safety would arise even more acutely. 3- The assessment of adjuvancity is hardly documented in EFSA's guidelines. In the absence of details, the petitioners merely look for total and global sequence identities that proteins expressed in GMPs might share with toxins. This search is carried out using the FASTA algorithm and most often uses the non-redundant NCBI protein bank. In this regard, there is a definite lack of a bank of well-defined toxins, updated periodically, as are the allergen banks AllergenOnline or Compare, that could be used for this purpose.	The GMO Panel thanks ANSES for the comment. The issues raised here are also relevant for GM plants develop by established technologies. The aspects related to celiac disease have been addressed by EFSA GMO Panel in its guidance from 2017 on allergenicity and on the more recent scientific opinion on development needs for the allergenicity and protein safety assessment from 2022 and Vriz et al 2021. Furthermore, and in relation to the docking approach, please note that an EFSA procurement is ongoing to develop a software tool for HLA-DQ modelling. The tool is now published and up for public comments. The tools can be found here: https://r4eu.efsa.europa.eu/app/predq In relation to adjuvancity, it is definitely an area of protein safety that will require further research. Once more predictive tools are available, these will be incorporated into the overall safety assessment of proteins. The EFSA GMO Panel also takes note about the need to establish databases for toxins.	103

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Advances in the detection and quantification of peptides by tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS), currently make it possible to dose with very high precision the peptides generated by protein fragmentation. These techniques use labeled peptides as internal standards, which makes it possible to accurately measure trace amounts of proteins expressed in GMPs. The guidelines should include these experimental approaches in the obligation for the petitioner to ensure that proteins corresponding to potential RFOs are not expressed. The immunotoxicity assessment shall be carried out by looking for immunotoxic peptides that proteins expressed in GMPs could release as a result of digestive proteolysis. EFSA's 2017 Guidelines on Allergenicity Assessment call for the search for exact identities with proven immunotoxic peptides (determined experimentally) and to take into account a certain degree of degeneration that allows multiple amino acid replacements at certain positions of 9-mer immunotoxic peptides. On the other hand, a limited number of mismatches (mismatches) are tolerated. The guidelines also provide a list of genuine immunotoxic peptides used as controls. In case of suspicion, the guidelines recommend using a molecular docking approach of putative peptides to the HLA-DQ2 and HLA-DQ8 basket, but without further details.	The EFSA GMO Panel thanks for the comment. The EFSA GMO Panel guidance on allergenicity of 2017 defines in detail, for the first time, how to perform the safety assessment of proteins regarding their capacity to cause celiac disease. In the future, also advances in mass spectrometry together with better models of in vitro digestion will be powerful tools for the safety assessment of proteins. In relation to the details on the molecular docking, please note that an EFSA procurement is ongoing to develop a software tool for HLA-DQ modelling. The tool is now published and up for public comments till April/May 2023. The tools can be found here: https://r4eu.efsa.europa.eu/app/predq	104
ANSES (French Agency for Food, Environmental and Occupational	3.1.3 NGTs relevant for this mandate	L360-361 : Misspelling of DSB corrected	The GMO Panel thanks for the comment. The text has been amended accordingly.	105

Health & Safety)				
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	2.4.2 Literature search	L287 : Some exclusion criteria too harsh thereby risking overlooking some relevant literature. L306 : 'novel' added	The GMO Panel thanks for the comment. The GMO Panel considers the exclusion criteria appropriate and useful to retrieve the relevant literature. Regarding line 306, the text has been amended accordingly.	106
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	2.4.1 Problem formulation	My main concern about the determination of risk assessment, especially for humans, deals with the possibility of generating unintentional changes to the genome, especially ORFs in the edges of inserts, that can arise during transgenesis, intragenesis and cisgenesis. It is essential to ensure that proteins or protein fragments corresponding to ORFs are effectively expressed in the GMPs and do not have allergenic, toxic/immunotoxic (celiac disease) or adjuvant properties. This is a problem that has remained unresolved in the guidelines drawn up so far. Indeed, these guidelines do mention the need to identify the potential ORFs generated by conventional techniques and to ensure that the proteins or fragments of putative proteins corresponding to these ORFs are devoid of allergenic, toxic/immunotoxic or adjuvant properties, but do not make specific recommendations to ensure that 1) putative proteins or protein fragments are actually expressed in the GMP and, 2) the assessment of immunotoxicity and 3) the assessment of the adjuvant potency of proteins.	The GMO Panel thanks for the comment. The GMO Panel was not mandated to express an opinion on specific recommendations for risk assessment, but rather to assess the applicability of the current guidelines regarding cisgenic/intragenic products. The text of the opinion clarifies that cisgenic/intragenic products do not pose new hazards as compared to the ones described in the 2012 opinion. The GMO Panel considers the current guidelines sufficient to assess the risks, including the formation of ORFs.	107

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	2.3 EFSA opinion on SDN-3	L247 : ‘ , secondly, ‘ (the two commas added) L248 : ‘ modification techniques, ‘ (comma added) L260 : Double Strand Break (DSB) added. The text is rather imprecise, with some abbreviations not spelled out at first use L263 : HR’ needs to be spelled out as it is used for the first and only time in the text . L264 : NHEJ = Non Homologous End-Joining to be added (first time the abbreviation is used)	The GMO panel thanks for the comment. Regarding lines 247,248,260,263 and 264, the text has been amended accordingly.	108
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	1.4 Interpretation of Terms of Reference	L135 : A comma in excess deleted. L185 : ‘are’ repeated twice, one deleted	The GMO Panel thanks for the comment. The text has been amended accordingly.	109

ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	Keywords	the literature search strings (choice of keywords) applied (Tables 6 and 7, Annex A) as well as those used in the search for patents in Espacenet and Google patents (Tables 8 and 9, Annex A) seems not as comprehensive as required. For instance, the Latin names of several key species are missing or just limited to one species in a given genus, even if 'any' is added in Tables 8 and 9 of Annex A. This can risk overlooking other related species within the same genus where relevant literature may be available (typical examples are with pea, fababean where the genus Vicia is not even mentioned). Also, several important crops are simply not listed (walnut or cotton, to name but two), and there are some misspellings in the scientific names of plants searched for (i.e. Cicer arietrinum instead of Cicer arietinum). For an easier assessment, I am appending annotated files of both the Draft Opinion and of Annex A for perusal.	The GMO Panel notes that no files of an annotated opinion or annex were appended to this comment. Please note that the output of the EFSA literature searches has been annexed to the final scientific opinion for information. Risk assessment considerations for cisgenic and intragenic plants developed by established and new genomic techniques were discussed in the opinion based on the outcome of the searches, the past cases considered in 2012 EFSA Opinion, and the experts knowledge elicitation on new potential developments. Regarding the misspelled name of the species, EFSA has conducted an internal check, and the misspelling did not have any effect on the results.	110
Sciensano - Service Biosafety & Biotechnology	4 Conclusions	Overall we agree with the answers to the formulated questions and the conclusions of the updated EFSA Opinion on Plants Developed through Cisgenesis and Intragenesis. We did identify some issues that may need some further clarification or need to be nuanced (see comments on specific sections).	The GMO Panel thanks for the comment.	111

Sciensano - Service Biosafety & Biotechnology	3.4.1 Which aspect (if any) of existing guidelines should be Updated, adapted or complemented?	Line 757-759: What is meant with 'where the cisgene, ', would be targeted to the corresponding endogenous gene'? This should be formulated more clearly. Please revise the sentence. Editorials: Line 757: 'On one hand' should be 'On the one hand' Line 759: SDN2 like' should be 'SDN2-like'	The GMO Panel thanks for the comment. Regarding lines 757-759, the text has been revised taking note of the comment.	112
Sciensano - Service Biosafety & Biotechnology	3.3.2.1 Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient to these new products?	Line 701: What is meant with 'tissue pattern" Line 718: same comment as on line 618. We consider 'will be' a too strong phrasing, and propose: 'As all these cisgenic and intragenic products will most likely be produced by targeted insertion/modification,". Editorials: Line 732-733: Please replace 'A need for flexibility may therefore be needed' by 'Therefore, flexibility may be needed" (to avoid double use of 'need') Line 735: 'in the present guidance' should be 'by the present guidance'	The GMO Panel thanks for the comment. Regarding line 701, the text has been amended, with a more general term 'expression pattern' which refers to expression in different tissues and over various developmental stages. Regarding line 718, in this section we discuss NGT products, which are all developed through targeted insertion/modification. Regarding lines 732-733 and 735, the text has been amended accordingly.	113

Sciensano - Service Biosafety & Biotechnology	3.3.1.1 Are the conclusions raised in the EFSA 2012 on the applicability of the existing guidelines still valid, taking into account the new guidelines published and the information made ...	Line 647-649: states: 'Moreover, although the case-by-case principle is still present in the additional guidance and regulatory documents published since 2012, the additional flexibility recommended in the EFSA 2012 opinion has not been introduced, and therefore this recommendation also remains valid.' We propose to the delete the first part of the sentence, referring to the presence of the case-by-case principles in the EFSA guidance and regulatory documents. The case-by-case principle is a general principle of risk assessment that should always be applicable and not only because it 'is still present' in EU documents published since 2012. Further, we consider that the issue of 'case-by-case' is more accurately addressed in paragraph 655-673. We therefore propose to delete lines 645-649 which actually repeats the information of paragraph 655-673. Line 682-683: What is meant with 'relative to the requirements of the Implementing Regulation'? Editorials: Title 3.3.1.1 'Are the conclusions raised in the EFSA 2012' should be 'Are the conclusions of the EFSA 2012 opinion' Line 643: 'no new data has been' should be 'no new data have been' Line 669: 'implementing Regulation' should be 'Implementing Regulation' Line 680: 'remains' should be 'remain'	The GMO Panel thanks for the comment. Regarding lines 645-649, the GMO panel believes that the text is needed to emphasize an aspect of the conclusion of the 2012 opinion. Regarding title 3.3.1.1 and lines 643, 669 and 680, the text has been amended accordingly. Regarding lines 682-683, the text has been modified to improve clarity.	114

Sciensano - Service Biosafety & Biotechnology	3.2.2.2 What could be the risks that those products could pose to humans, animals and the environment, as compared with the risks associated with plants obtained by conventional plant breeding ...	Line 612: What is meant with 'the pattern of expression'? Please use terminology of EFSA guidance documents to refer to certain issues. Line 616: Please spell NEP full-out (as first and only time used in text) Line 618: states 'As all these cisgenic and intragenic products will be produced by targeted insertion/modification, ". We consider 'will be' a too strong phrasing, and propose: 'As all these cisgenic and intragenic products will most likely be produced by targeted insertion/modification,".	The GMO Panel thanks for the comment. Regarding line 612, the pattern of expression means the expression levels of a set of genes, in different tissues and over various developmental states. Regarding line 616, the text has been amended accordingly. Regarding line 618, since the section 3.2.2.2 refers only to cisgenic/intragenic products obtained through NGTs, and all described NGTs are based on targeted insertion/modification, the text will not be modified.	115

Sciensano - Service Biosafety & Biotechnology	3.2.2.1 What are the new products that could be obtained using new approaches, in particular with the use of SDNs, that could give rise to cisgenic/intragenic plants according to the definition ...	Line 573-577: same comment as the one for lines 137-141 Line 577: We propose to delete 'GM' as the gene pool of the plant species is considered here and not solely the genome of the GM plant. Line 580: What is meant with 'as a continuous sequence'? Line 587 & 612 & 784: What is meant with 'the pattern of expression'? Please use terminology of EFSA guidance documents to refer to certain issues. Line 591: 'Concerning the new products' is in contradiction to what is said in the previous sentence that the Panel is not aware of any products. We propose deleting 'Concerning the new products, as defined before', and to start the sentence as follows: 'As reported above, one publication'' Editorials: Line 573: 'site directed' should be 'site-directed' Line 593: 'CRISPR-Cas mediated' should be 'CRISPR-Cas-mediated' Line 596: 'gene edited' should be 'gene-edited' Line 600: 'clear example of cisgenic approach' should be 'clear example of a cisgenic approach'	The GMO Panel thanks for the comment. Regarding lines 573-577 and 600, the text has been revised to improve clarity. Regarding lines 573, 593 and 596, the text has been amended accordingly. Regarding line 577, the term 'GM' has been removed. Regarding line 580, the text has been revised and the term 'single and continuous' has been replaced by 'intact and continuous', meaning that there were no modifications or rearrangements. Regarding lines 587 & 612 & 784, the pattern of expression is understood as expression levels of a set of genes in different tissues or over different developmental stages. Regarding line 591, it has been clarified that no cisgenic/intragenic products are close to commercialisation in the EU.	116

Sciensano - Service Biosafety & Biotechnology	3.2.1.4 If there are new techniques/approaches , what are the potential risks that may arise as compared with those already covered in the 2012 opinion?	The title gives the impression that new risks for cis/intragenesis plants were identified and are covered in the 2012 opinion, which is not the case. We propose as title: 'If there are new techniques/approaches, may these lead to new (not yet identified) potential risks"	The GMO Panel thanks for the comment and considers the current text sufficiently clear.	117
Sciensano - Service Biosafety & Biotechnology	3.2.1.3 Are there new techniques/approaches developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 opinion?	Editorials: Line 533: 'new obtained' should be 'newly obtained' Line 543: 'If no new applications " should be 'While no new applications " Line 545: 'should facilitate " should be 'could facilitate" (there is no certainty here)	The GMO Panel thanks for the comment. Regarding lines 533 and 543, the text has been amended accordingly. Regarding line 545, it reflects an expectation/speculation rather than certainty and will not be amended.	118
Sciensano - Service Biosafety & Biotechnology	3.2.1.2 Is there new information available that could impact on the risks assessment of the products included in the EFSA 2012 opinion?	The title gives the impression that risk assessments of cisgenic/transgenic products have been done and are described in EFSA 2012 opinion, which is not the case. We propose as title: 'Is there new information available that could alter the conclusion on risks of the EFSA 2012 opinion?' Editorials: Line 485: ' from cisgenic or transgenic plants' should be 'from cisgenic or intragenic plants' Line 515: 'new data has" should be 'new data have"	The GMO Panel thanks for the comment. The GMO Panel considers the current title sufficiently clear. The title refers to the assessment of all potential products obtained with methods described in the 2012 Opinion. The text of the paragraph clarifies that further. Regarding lines 485 and 515, the text has been amended accordingly.	119

Sciensano - Service Biosafety & Biotechnology	3.2.1.1 What are the risks that cisgenic/intragenic plants could pose to humans, animals, and the environment, that were identified in the 2012 cisgenesis opinion?	Line 409: Does 'this document' refer to the updated opinion or to the one of 2012? This is not clear, please clarify.	The GMO Panel thanks for the comment. The text has been amended accordingly to clarify that it refers to the 2012 opinion.	120
Sciensano - Service Biosafety & Biotechnology	3.1.3 NGTs relevant for this mandate	Editorials: Line 360 & 361: 'DBS' should be 'DSB' Line 361 (& 650 &789): 'Site-directed' should be 'site-directed'	The GMO Panel thanks for the comment. The text has been amended accordingly.	121
Sciensano - Service Biosafety & Biotechnology	3.1.2 New Genomic Techniques (NGTs)	Line 338-339: What is meant with 'the relative transgenes'? We propose: " they are no longer needed, and they the relative transgenes are usually segregated" Editorial: Line 335 'micro particles' should be 'microparticles'	The GMO Panel thanks for the comment. Regarding lines 335 and 338-9, the text has been amended accordingly.	122
Sciensano - Service Biosafety & Biotechnology	3.1.1 Established Genomic Techniques (EGTs)	Line 312-315: The term EGT refers to techniques and not to the alterations these techniques can introduce. We propose: 'While the term 'EGT' is broad, here we refer to genetic techniques that involve the transfer of genetic material to the host organism, using various strategies, such as Agrobacterium-mediated transformation, biolistic transformation or microinjection.' Editorial: Line 320 'EGT techniques' should be 'EGTs'	The GMO Panel thanks for the comment. Regarding lines 312-315 and 320, the text has been amended taking the comment into account.	123
Sciensano - Service Biosafety & Biotechnology	2.2 EFSA opinion on Cisgenesis and Intragenesis	Editorial: Line 223 'Cisgenesis' should be 'cisgenesis'	The GMO panel thanks for the comment. The text has been amended accordingly.	124

Sciensano - Service Biosafety & Biotechnology	1.4 Interpretation of Terms of Reference	Line 137-141: We find these sentences unclearly formulated. First off, 'changes' (rather understood as point mutations) do not lead to cisgenic/intragenic plants; second 'When these sequences/changes are already present in a crossable species " does not clearly refer to the 'natural' presence of these sequences/changes (i.e. it could also refer to transgenes present in a crossable species). We propose to change the sentences as follows: 'The new developments of site-directed modification of genomes offer the possibility to target the insertion of new sequences or introduce changes sequences at specific loci in the genome. When these sequences are native to a crossable species, these type of modifications could also be considered as cisgenic/transgenic modification in light of the definition given in the framework of this mandate'. Line 141: It is unclear to what 'these two definitions' refers to. No second definition is given (yet). We propose to change the sentence in line 141-147 to 'Therefore, in delivering its opinion, EFSA chose to address potential cisgenesis/intragenesis products already covered " Line 142-143: The sentence gives the impression that cis/intragenesis products exist, which is not the case. We propose to refer to 'potential products' (see also comment on line 141 for text proposal). Line 148: same comment as on lines 142-143 Editorials: Line 134: 'addressed' should be 'addresses' Line 137: 'site directed' should be 'site-directed' Line 185: 'Are the existing guidelines for risk assessment are applicable, " should be 'Are the existing guidelines for risk assessment applicable, "	The GMO Panel thanks for the comment. Regarding lines 137-141, the sentence has been deleted and the entire paragraph has been edited to improve clarity. Regarding line 141, the two definitions have been added to the main text. Regarding lines 142-143 and 148, the text also refers to cisgenic/intragenic products that have already been developed and reported, therefore the GMO Panel decided not to add the term 'potential'. Regarding line 134, the text has been amended. Regarding line 137 and 185, the text has been amended accordingly.	125
Anonymous	-	-	No comment received	126

Abbreviations

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats

DSB Double strand break

EC European Commission

EFSA European Food Safety Agency

EGT Established Genomic Technique

ERA Environmental Risk Assessment

EU European Union

GM Genetic Modification / Genetically Modified

GMO Genetically Modified Organism

IR Implementing Regulation

NEP Newly Expressed Protein

NGO Non-Governmental Organization

NGT New Genomic Technique

ODM oligonucleotide-directed mutagenesis

RA Risk Assessment

SynBio synthetic biology

SDN site-directed nucleases

ToR Terms of Reference

WG Working Group

Appendix A – Explanatory note on the EFSA website for the public consultation

EFSA's Nutrition and Food Innovation Unit (NIF) has launched a public consultation on a draft updated scientific opinion on plants developed through cisgenesis and intragenesis. The updated draft opinion has been developed upon an EC request to confirm whether the considerations and conclusions of EFSA's Scientific Opinion on "the safety assessment of plants developed through cisgenesis and intragenesis", published in 2012, are still applicable.

Interested parties are invited to submit their comments by the indicated deadline.

Additional data or files to support the comments may be submitted using the relevant function in the digital form and naming the file in a way to make it easy to link to the relevant comment.

All comments will be considered, so long as they:

• are submitted by the closing date of the consultation;

• are finalised (comments in 'draft' status will not be accepted);

• are presented according to the instructions and relevant function in the tool (regrettably, we cannot accept comments sent by email);

We will not consider any comments that contain, personal accusations, irrelevant or offensive statements or material.

Copyright-cleared contributions:

Persons or organizations participating in a public consultation of EFSA are responsible for ensuring that they hold all the rights necessary for their submissions and subsequent publication by EFSA. Comments should inter alia be copyright-cleared considering EFSA's transparency policy and practice to publish all submissions. In case the submission reproduces third-party content in the form of charts, graphs or images, the required prior permissions of the right holder(s) should have been obtained by the public consultation respondent.

Publication of contributions:

Third-party comments will be made public in their original form without delay after the closing date of the consultation and may be reused by EFSA in a different context. The outcome of the consultation will be made public in conjunction with the publication of the relevant scientific output.

Contributions submitted by individuals in a personal capacity will be published indicating the author's first and family name unless the respondent has requested anonymity. Contributions submitted on behalf of an organisation will be attributed to the organization in question.

More information on the processing of personal data are available in the Privacy Statement.

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